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INTRODUCTION

Much evidence has been accumulating recently that the problem of interstratification in clays is more complex than had been supposed. Early investigations concentrated on the idea of a completely random interstratification (see Weaver 1956), and undoubtedly the phenomena can often be fairly satisfactorily analysed on this assumption.

Methods have however been developed (Méring 1949, MacEwan 1956, 1958, Cesari, Morelli and Favretto 1965, Kakinoki and Komura 1952, 1954) for analysing more general types of mixtures (an analysis which was implicit in the original treatment of Hendricks and Teller (1942), but not then applied), and these developments have led to the realisation that “non-random” sequences are quite common.

MacEwan (1955) found such a non-random sequence in a clay from Worcestershire, England, and also on a re-examination of Byström’s (1956) results on the Ordovician bentonites from Kinnekulle, Sweden. Sato, Oinuma and Kobayashi (1965) found a similar sequence in mixed-layer illite-montmorillonites from volcanic tuff in the Sorachi coal fields.

MacEwan (1956) has pointed out that simple sequences may be defined by a series of coefficients. For a two-layer mixture of layers A and B these are $p_A$, $p_B$, $p_{AA}$, $p_{AB}$, $p_{BA}$, $p_{BB}$. Here $p_A$ is the probability of occurrence of layer A; $p_{AA}$ the probability that A succeeds A, etc. Similar coefficients have been defined by Cesari et al. (1965) and by Kakinoki and Komura (1952).

Several of the clays hitherto studied are characterised by the property that $p_{BB} = 0$, where B is the more hydrated layer. This is true of MacEwan’s results and those of Kakinoki and Komura (1952). Sato (1965) has plotted the results obtained by various investigators against $p_{AA}$ and $p_{BB}$, and showed that there is a tendency to group around the $p_{BB} = 0$ axis.

Now $p_{BB} = 0$ corresponds to the non-occurrence of neighbouring B-layers; it is in fact one of the nearest possible approaches to an alternating sequence with $p_B < \frac{1}{2}$ (with $p_B = \frac{1}{2}$, the pure alternation $ABAB \ldots$ occurs; with $p_B > \frac{1}{2}$, $p_{BB} = 0$ is impossible). To define such structures, Sato (1965) has used the concept of the “Reichweite,” $g$, which is the range of interaction between neighbouring layers. The present case corresponds to $g = 1$, i.e. next-neighbour interaction. Sato suggests that the common occurrence of structures with $p_{BB} = 0$ is dependent on the interlayer energy.
Fig 1. Calculated curves of the mixing function $\Phi(S)$ for 10/15·5 Å mixtures with $p_A = 0·4$ and values of $p_{AA}$ varying from 0·6 to 1·0. The values attached to the curves are $10p_A$ and $10p_{AA}$ (in that order). These curves do not include the completely random case, for which $p_A = p_{AA}$. The scales give other possible interpretations in terms of spacing.
INTERSTRATIFICATION IN SOIL CLAYS

MORE COMPLEX INTERSTRATIFICATIONS

We have by no means exhausted the possible types of interstratification by considering on the one hand the "purely random" structures, and on the other those in which $p_{BB} = 0$. For the case of $g < 1$, we have in fact the following possible sub-cases:

1. Separate crystals: $p_{AA} p_{BB} = 1$
2. Partial segregation of crystal types
   1. $p_{AA} > p_A$
   2. $p_{BB} > p_B$
3. "Purely random" case
   $p_A = p_{AA} = p_{BA}$
   $p_B = p_{BB} = p_{AB}$
4. Partial alternation
   $p_A > p_{AA} > 0$, or $> 1$ if $p_A > p_B$
5. Greatest tendency to alternation
   $p_{AA} = 0$, or $1 - \frac{p_B}{p_A}$ if $p_A > p_B$
   (in the latter case, $p_{BB} = 0$)

The interpretation of such cases can be facilitated by a series of curves of the mixing function $\phi$, not only for various values of $p_A$, but also for various values of $p_{AA}$ (which determines $p_{AB}$ etc.). Such calculations have been made by MacEwan, Ramirez and Ruiz Amil (1967). Figure 1 shows one set of curves of the series, for $d_A = 10\AA$, $d_B = 12.7\AA$, $p_A = 0.4$, and values of $p_{AA}$ from 0.6 to 1.0. From such curves, a series of curves of peak position movements can be constructed, of which Figure 2 is an example. Similar curves have been published by Cesari et al. (1965). It emerges from such figures that on departing from the purely random structure in either direction, in that of increasing segregation, or of alternation of layers, the type of X-ray diffraction obtained tends to approximate rather rapidly to that which is given by the pure case, that is to say either completely segregated layers, or purely alternating layers. This is true even if the proportion of layers is such that the purely alternating case is impossible. Departures from these pure cases are shown particularly by changes in intensity, and secondly by shifts in position of the peaks; but it is likely that in practice quite a number of minerals have been classified as purely alternating on the basis of the X-ray diagrams when in reality they should be considered to be partially random with a small value of $p_{AA}$. In particular, the occurrence of a low-angle reflexion does not indicate necessarily that the structure is alternating, though it has been interpreted in this way in the past. Such a reflexion does indicate a very low value for $p_{BB}$, though it can occur even if $p_A \neq 0.5$.

STRUCTURES WITH LONGER-RANGE INTERACTION

It is not necessary to suppose that all the soil minerals in nature have $g$ between 0 and 1, but the investigation of more complex cases will be impeded by the difficulty, at present, of recognising them with certainly.
Fig. 2. Curves of peak migration for 10 Å mixtures. The A series is the low spacing, the B the high spacing, and M is the alternating series. Note that there is a marked tendency to shift from A or B to M at about the value where $p_A = p_{AA}$.

These curves are plotted for constant values of $p_A$, the values being indicated by the figures attached to the curves. These values are multiplied by 10 for convenience; for instance, the curve labelled "5" corresponds to $p_A = 0.5$. The scale at the left gives $p_{AA}$, also multiplied by 10. Dotted lines correspond to regions where the peak becomes diffuse or disappears, and a shaded region to a broadened peak or series of peaks. An alternating structure ABAB... always has $p_A = 0.5, p_{AA} = 0$, i.e., such structures occur along the lower line, at the terminations of the curves labelled "5". Such structures are indicated by "M".
The direct Fourier-transform method (MacEwan 1956) provides in principle a means of recognising such structures, but uncertainties in its application make this difficult in practice. Structures of this sort are characterised by the fact that the six coefficients \( p_r, p_{rs} \) are not sufficient to enable the frequency of occurrence of all sequences of layers to be deduced from them. Thus a quite common type of sequence is the regular one of type \( AABAAB \ldots \). This cannot be represented by any series of coefficients of type \( p_r, p_{rs} \). For clearly \( p_A = 2/3, p_B = 1/3, p_{AA} = \frac{1}{2}, p_{AB} = \frac{1}{2}, p_{BA} = 1, p_{BB} = 0 \). From this we deduce the probability of the sequence \( AAB \) (or \( ABA \) or \( BAA \)) to be as follows

\[
P_A P_{AA} P_{AB} + P_A P_{AB} P_{BA} + P_B P_{BA} P_{AA} = (2/3) \cdot \frac{1}{2} + (2/3) \cdot \frac{1}{2} + (1/3) \cdot 1 \cdot \frac{1}{2} = (1/6) + (1/3) + (1/6) = 2/3.
\]

But the probability of sequence \( AAB \) is clearly 1, since the crystal is a regular one having an \( AAB \) unit cell. The coefficients above in fact correspond to a different type of structure, a random and not a regular one. The structure in question is determined by coefficients which specify the probability of sequences of three layers, not of two.

This structure was a completely regular one, but it is possible also to have random structures which are determined by next-nearest-neighbour interactions. We are at present contemplating the calculation of diffraction effects due to such structures.

**ILLITIC INTERSTRATIFICATION**

Meanwhile, it is interesting to consider the effects due to one particular example of a more complicated interstratification. This is the illitic, or what has been termed “zoned” type of interstratification by Vivaldi and MacEwan (1957, 1961). These authors propose to recognise three main types of interstratification, in clays, namely “regular”, “random” and “zoned”. In the latter there is a principal component, which in the case of illite is mica, and which gives rise to the main X-ray reflexion. The first order however has a “tail” extending towards lower angles, due presumably to irregular interpolation of the second component, which in the case of illite will be vermiculitic or montmorillonitic. This type of interstratification is common in intermediate minerals, especially in soil clays.

It is difficult to suggest an adequate model for calculating diffraction effects from this type of interstratification. The author, with A. Ruiz Amil, has been working on the following lines.

Instead of defining the interstratification by probability coefficients which define sequences of layers such as \( AB \) (for \( g = 1 \)), or \( AAB \) (for \( g = 2 \)), we suppose that some layers are hydrated (or otherwise altered, e.g. chloritised), and that the distance between such layers is determined by a distribution function. More exactly, if between the altered layers there are \( v \) unaltered layers, we suppose that the quantity \( v \) is defined, on the average (over various crystallites) by a distribution function \( \mathcal{F}(v) \). This function can be of any type; it can for instance rise to a maximum for a certain value of \( v \), and then drop rapidly to zero for higher values, or it can (more probably perhaps) follow a Gaussian type curve.
This means that instead of supposing that there exists a certain probability that an altered layer succeeds an unaltered layer (or vice versa)—a supposition that makes no difference of principle between altered and unaltered layers—we suppose that there is a certain probability for a given separation to exist between altered layers, i.e. that the altered layers repel each other.

**SUMMARY OF CALCULATIONS FOR THE ILLITIC CASE**

Let there be a sequence of probabilities \( p_1, p_2, \ldots, p_r, \ldots, p_m \). These are the probabilities of encountering an uninterrupted sequence of \( r - 1 \) layers of type \( B \) (unaltered) preceded or succeeded by one layer of type \( A \) (altered). For instance \( p_7 \) is the probability of encountering a sequence of the type \( ABBB BBB \). These coefficients define a maximum number of successive layers \( B, N_{max} \), which can not be exceeded.

The calculations are then as follows.

1. The following table of coefficients is formed

\[
\begin{align*}
\Sigma_z &= p_z \\
\Sigma_{z-1} &= p_z + p_{z-1} \\
&\vdots \\
\Sigma_2 &= p_z + p_{z-1} + \ldots + p_2 \\
\Sigma_1 &= p_z + p_{z-1} + \ldots + p_2 + p_1 = 1
\end{align*}
\]

2. The total number of layers per unit is calculated and is

\[
p_1 + 2p_2 + 3p_3 + \ldots + mp_m = n
\]

This is the common denominator of all the probability values. It takes this form because \( p_r \) represents a sequence of \( r \) layers.

3. A table of coefficients is formed for the probabilities of encountering sequences in various orders of \( r \) layers of type \( B \) and \( s \) layers of type \( A \). The total number of layers in each of these sequences is \( m = r + s \). These coefficients are called \( \pi_{rs} \). The sum of all the \( \pi \)'s for a given value of \( m \) is 1. Thus, for example, for the value \( m = 4 \) we obtain

\[
\pi_{40} + \pi_{31} + \pi_{22} + \pi_{13} + \pi_{04} = 1
\]

To calculate a coefficient such as \( \pi_{52} \) we proceed as follows (the procedure cannot be fully justified here). Add 1 to \( m \) and \( s \), giving 8 and 3. Then calculate all the permutations of three figures (except 0) summing to 8. One obtains

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These combinations of numbers are the sub-indices of the coefficients which must be multiplied together to obtain the corresponding probabilities (dividing by \( n \) in each case). For example, the combination 422 gives

\[
\frac{1}{n} (\Sigma_4 \cdot p_2 \cdot \Sigma_2)
\]

and 251 gives

\[
\frac{1}{n} (\Sigma_2 \cdot p_5 \cdot \Sigma_1)
\]

To take a more general case, the combination 4215213 would give the coefficient.
The value of \( n \) for a given combination of \( r \) and \( s \) is obtained by summing all the coefficients we have just described. They correspond to the different numbers of ways in which a sequence of \( r \) layers of type \( A \) and \( s \) layers of type \( B \) may be established. To take an example, the value of \( n_{22} \) is given by a sum of 21 separate values.

The reason why a \( \Sigma \) appears at the beginning and end of each series is that there the sequence is "open-ended", i.e. the \( A \) layer may be preceded (or followed) by any number of \( B \) layers equal to or greater than the value required for the particular sequence.

There is one exception to the above rule, when \( s = 0 \), in which case \( r = m \) and

\[
\pi_{m0} = \frac{n - \Sigma_1 - \Sigma_2 - \ldots - \Sigma_m}{n}
\]

The value \( \pi_{rs} \) obtained in this way is expressed as \( \sigma(R) \), where \( R = rd_B + sd_A \), where \( d_B \) and \( d_A \) are the spacings corresponding to the layers \( B \) and \( A \). This means that \( R \) is the total spacing corresponding to \( r \) \( B \) layers and \( s \) \( A \) layers.

From the values \( \sigma(R) \), modified values \( \sigma'(R) \), are calculated by multiplying by a weight function depending on a supposed crystallite size distribution \( \psi(N) \), in the manner described by MacEwan (1958). From the values of \( \sigma'(R) \), a Fourier series is calculated

\[
F(S) = \sum R \sigma'(R) \cos(2\pi R \cdot S),
\]

where \( S \) is a reciprocal co-ordinate.

Calculation of the coefficients for the higher values of \( m \) gets exceedingly laborious, and an electronic calculator is essential. These calculations have been programmed for the IBM 7070 machine of the "Instituto de Cálculo Electrónico", and we are very grateful for their aid.

**RESULTS**

A sufficient number of curves has not yet been calculated to allow a very adequate comparison with actual diffraction patterns, but the present model does allow certain observed features to be reproduced.

Figure 3 shows some examples of illitic diffraction patterns observed by Burst (1958). Case 1 is one in which the diagram is almost normal with possibly a slight modification of \((002)\) and \((003)\) intensities. In case 2 there is a very marked tail to \((001)\), and \((002)\) and \((003)\) are markedly affected. The third curve shows a highly irregular type with very diffuse reflexions.

Samples of our calculations are shown in Figure 4, and they show a trace of tailing to \((001)\) and marked modification of the intensities. It should be noted that in Figure 4 the mixing function is shown, not the complete diffraction function. Thus for a normally crystallised mica, whatever the structure of the layers, all the peaks would be exactly equal in height. In fact, the layer structure does not figure at all in this calculation.
Fig. 3. Diffractometer curves for three types of illite from Burst (1958). Curves 1, 2 and 3 show progressively increasing alteration. Note the increase in "tailing" of (001), and the marked intensity changes in (002) and (003).

though of course it influences Burst’s curves, and is responsible for the low values of (002). Our (002) in case 7 (Figure 4), which is weak anyway, would be still further reduced by the layer structure factor. A noteworthy feature of our results therefore is that a very moderate degree of interlayering may markedly affect the intensities of the basal reflexions, though the effect on shape and position is less marked. In the cases illustrated, the most probable number of unaltered layers intervening between hydrated layers was 12. The distribution was a Gaussian one, going practically to zero for 3 layers.

Fig. 4. Sample calculations of mixing function \( \Phi \), for a gaussian hydration distribution with maximum at \( p_x = 15 \) and a Gaussian grain size distribution with maximum at 13 layers. In no. 6 the mixtures are of 10 Å and 14 Å layers, in no. 7, 10 and 12.6 Å.
It appears that, to account for the very marked weakening and broadening shown in Curve 3 of Figure 3, some still more complex type of interstratification must be introduced, probably consisting of layers which are hydrated to various degrees. The Japanese acid clays studied by Sudo and Hayashi (1956) represent a still more extreme case.

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Cesari, M., Morelli, G. L. and Favretto, L. (1965) — On the determination of the type of stacking in mixed-layer minerals (Private publ. by AGIP and SNAM).


**SUMMARY**

Interstratified clay minerals may be (1) "completely random", i.e., with no influence of a layer on its successor; or (2) partially random, defined, in the case of a two-layer mixture by six coefficients, \( p_r, p_s \). The latter case is more common than was suspected, and is probably due to inter-layer interactions.

A more general case is when non-nearest-neighbour interactions occur. The type of interstratification found in illitic materials may correspond to this general case. A possible mathematical approach is to suppose that such structures are defined by a distribution probability function of distances between altered layers, and not by nearest-neighbour interactions. A method for calculating the diffraction to be expected in such a case is described.

**RÉSUMÉ**

Les minéraux d’argile interstratifiés peuvent être (1) entièrement au hasard, c’est-à-dire, sans l’influence d’une couche sur son successeur; ou (2) partiellement au hasard, définis, dans le cas d’un mélange de deux couches par six coefficients \( p_r, p_s \). Ce dernier cas est plus commun qu’on ne le pensait, et est probablement dû à des interactions parmi les couches.

Le cas le plus général est celui où ont lieu des interactions avec le voisin qui n’est pas le plus proche. Le type d’interstratification trouvé dans les matériaux illitiques correspond peut-être à ce cas général. Une approche
mathématique possible est de supposer que de telles structures sont définies par une fonction de probabilité de distribution des distances entre les couches transformées, et non par des interactions avec le voisin le plus proche. Une méthode pour calculer la diffraction attendue dans ce cas est décrite.

ZUSAMMENFASSUNG

Intergeschichtete Tonminerale können als (1) "vollkommen zufällig", d.h. ohne Einfluss einer Schicht auf die darauf folgende; oder (2) teilweise zufällig, bezeichnet werden, was im Falle einer zwei-schichtigen Mischung von sechs Koeffizienten \( p_v \), \( p_r \), bestimmt wird. Der letztere Fall kommt öfter vor als angenommen wurde und ist wahrscheinlich die Folge einer interschichtlichen Aufeinanderwirkung.

Ein häufiger Fall ist das Aufeinanderwirken, nicht angrenzender Schichten. Der Interschichtungstyp, der in illitischen Materialien gefunden wird, mag mit diesen Umständen übereinstimmen. Eine mögliche mathematische Annäherungsmethode wäre die Annahme, dass solche Strukturen durch eine Verbreitungsmöglichkeit-Funktion der Entfernungen zwischen veränderten Schichten definiert sind und nicht durch nächst-nachbarliche Aufeinanderwirkungen. Eine Methode zur Errechnung der Diffraction, die in einer solchen Fall erwartet werden kann, wird hiermit beschrieben.
CRYSTAL STRUCTURE OF MICA MINERALS

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Division of Soils, Adelaide, Australia

This paper will be a brief review of the current knowledge of the structural crystallography of well-crystallized mica minerals. It is not directly concerned with poorly crystalline micaceous clay minerals nor with the surfaces of well-crystallized micas. The reason for this is that the normal x-ray diffraction data of single crystals essentially contains information about atomic arrangements which are ordered over thousands of unit cells, and cannot easily be made to yield information about mistakes in a regular crystal structure (such as occur in surfaces or in disordered crystals such as clay minerals). Structural crystallography has advanced enormously in the last ten years due to the impact of large computers with the result that the detailed information now available about the crystal chemistry of quite complex silicate minerals is much greater than would have been thought possible previously.

Careful refinement of the mica structures by several workers has yielded general principles concerned with the "real" structures of these minerals which are probably applicable to micaceous clay minerals and therefore of considerable interest to soil scientists.

The geometric or stylized mica structure found in most textbooks is based on the analysis by Jackson and West (1933) of the dioctahedral mica, muscovite. This structure was subsequently refined by Radoslovich (1960)—the first modern structure analysis of a mica—with the following major results:

1. The surface network of oxygens does not have hexagonal symmetry. If we consider the triads of oxygens which make up the tetrahedral bases in the stylized mica model, then to adjust these anions to their true positions in muscovite the main displacement required is a rotation of each triad about an axis through its centre (and normal to the layers) of approximately 13°. Since the triads are also linked through the shared or bridging oxygens this means that neighbouring triads must turn through +13° and -13° respectively. The effect of such an adjustment on each hexagonal hole in the layer surface is that three alternate oxygens move in towards the centre of the hole, whilst the remaining three move away, but of course towards the centre of the neighbouring holes. When two such surface holes are opposed to enclose the interlayer cation (e.g. K+) the K+ can only be 6- and not 12-coordinated, roughly within an octahedron of oxygens, with K-O bonds of about 2.8 Å.

2. In muscovite the surface oxygens are not strictly coplanar; each triad is slightly tilted. This is now known to be a general feature of dioctahedral layer silicates, as discussed by Takéuchi (1966).
3. The monoclinic angle does not only depend on the stagger within the octahedral layer. There is a further displacement of $K^+$ (away from their positions centrally in the surface holes) which increases the monoclinic angle beyond $\beta = \cos^{-1}\left(-\frac{a}{3c}\right)$.

4. The forbidden 061 reflections with “1” odd can now be accounted for by the displacement of the tetrahedral groups (from their “ideal” positions) due to muscovite being dioctahedral.

5. The octahedral layers conform strictly to Pauling’s Rules, in having shortened shared edges by counter-rotations of triads making up the faces of the octahedra parallel to the sheets, and in being “squashed” down normal to the sheets.

6. The octahedral cations are in a strictly ordered arrangement; two thirds of the sites are fully occupied by $Al$ and the remaining sites are completely vacant.

7. The structural refinement of muscovite strongly suggested that, on the basis of the T-O bondlengths, the $Al$ does not occupy all possible tetrahedral sites with an equal probability. It was, in fact, claimed that half the sites were occupied only by $Si$ whereas the statistical occupancy of the other sites is $Si_{2}Al_{2}$. This result was apparently confirmed by the less accurate refinement of the muscovite structure by electron-diffraction by Zviagin and Mischenko (1960).

The subsequent refinements of mica structures have confirmed these results as being generally true for these minerals with the possible exception of the ordering of tetrahedral cations. In 1963 Burnham and Radoslovich carried out a full three-dimensional refinement of $2M_{1}$ paragonite and $2M_{2}$ muscovite which had been formed naturally in mutual equilibrium. This was the first structure analysis of two similar coexisting minerals from the same handspecimen. It was hoped that this would allow a detailed evaluation of any variations in tetrahedral $Al/Si$ distribution resulting from the change in $K/Na$ ratio in the interlayer cation region. Re-examination of the muscovite structure assumed critical importance after Gatineau (1963) presented results based on the least squares refinement of intensities of Radoslovich (1960) which yield parameters for muscovite which differed from those reported originally—particularly with respect to the cation distribution within the tetrahedral layers.

Least squares refinement of anisotropic thermal models (using about 600 reflections in each case) reduced the discrepancy factor, $R$, to $3.4\%$ (weighted) for both minerals. No attempt was made to locate hydrogen and the refinement was not biased by any postulated tetrahedral cation distribution, both crystallographically distinct sites being treated as $Si^{4+}$ initially.

As an indication of the precision of this refinement the occupancy of the interlayer cation sites, as determined by microprobe analysis and by the structure analysis, may be compared.

<table>
<thead>
<tr>
<th>Microprobe:</th>
<th>Paragonite ($K_{0.15} Na_{0.85}$)</th>
<th>Muscovite ($K_{0.05} Na_{0.95}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure:</td>
<td>$K_{0.25} Na_{0.75}$</td>
<td>$K_{0.04} Na_{0.96}$</td>
</tr>
<tr>
<td></td>
<td>$\pm 0.02 \pm 0.02$</td>
<td>$\pm 0.02 \pm 0.02$</td>
</tr>
</tbody>
</table>
### Table I

**INTERATOMIC DISTANCES (Å) IN 2M¹ MUSCOVITE (Mu₂₆) AND PARAGONITE (Mu₁₅) AND 3T MUSCOVITE**

<table>
<thead>
<tr>
<th>Atom Pair</th>
<th>Muscovite</th>
<th>Paragonite</th>
<th>3T Muscovite</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T₃ tetrahedron</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₃-O₆ (apical)</td>
<td>1.642 ± 0.004</td>
<td>1.648 ± 0.002</td>
<td>1.609 ± 0.014</td>
</tr>
<tr>
<td>T₃-O₈</td>
<td>1.645 ± 0.004</td>
<td>1.655 ± 0.004</td>
<td>1.606 ± 0.025</td>
</tr>
<tr>
<td>T₃-O₄</td>
<td>1.643 ± 0.004</td>
<td>1.642 ± 0.004</td>
<td>1.608 ± 0.024</td>
</tr>
<tr>
<td>T₃-O₅</td>
<td>1.649 ± 0.004</td>
<td>1.664 ± 0.003</td>
<td>1.633 ± 0.017</td>
</tr>
<tr>
<td>Mean T₃-O</td>
<td>1.645</td>
<td>1.652</td>
<td>1.657 ± 0.010</td>
</tr>
<tr>
<td>O₆-O₅</td>
<td>2.694 ± 0.005</td>
<td>2.706 ± 0.004</td>
<td>2.650 ± 0.023</td>
</tr>
<tr>
<td>O₆-O₄</td>
<td>2.725 ± 0.005</td>
<td>2.720 ± 0.004</td>
<td>2.799 ± 0.024</td>
</tr>
<tr>
<td>O₆-O₆</td>
<td>2.701 ± 0.005</td>
<td>2.709 ± 0.004</td>
<td>2.628 ± 0.018</td>
</tr>
<tr>
<td>O₆-O₇</td>
<td>2.696 ± 0.005</td>
<td>2.707 ± 0.005</td>
<td>2.838 ± 0.026</td>
</tr>
<tr>
<td>O₆-O₈</td>
<td>2.654 ± 0.005</td>
<td>2.685 ± 0.005</td>
<td>2.657 ± 0.034</td>
</tr>
<tr>
<td>O₆-O₉</td>
<td>2.639 ± 0.005</td>
<td>2.656 ± 0.005</td>
<td>2.643 ± 0.028</td>
</tr>
<tr>
<td>Mean O-O</td>
<td>2.685</td>
<td>2.697</td>
<td>2.703 ± 0.016</td>
</tr>
<tr>
<td><strong>T₂ tetrahedron</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₂-O₆ (apical)</td>
<td>1.644 ± 0.004</td>
<td>1.652 ± 0.003</td>
<td>1.636 ± 0.013</td>
</tr>
<tr>
<td>T₂-O₈</td>
<td>1.648 ± 0.004</td>
<td>1.656 ± 0.004</td>
<td>1.552 ± 0.022</td>
</tr>
<tr>
<td>T₂-O₄</td>
<td>1.644 ± 0.004</td>
<td>1.653 ± 0.003</td>
<td>1.610 ± 0.028</td>
</tr>
<tr>
<td>T₂-O₅</td>
<td>1.645 ± 0.004</td>
<td>1.644 ± 0.004</td>
<td>1.684 ± 0.020</td>
</tr>
<tr>
<td>Mean T₂-O</td>
<td>1.645</td>
<td>1.651</td>
<td>1.620 ± 0.010</td>
</tr>
<tr>
<td>O₆-O₅</td>
<td>2.702 ± 0.005</td>
<td>2.709 ± 0.005</td>
<td>2.581 ± 0.019</td>
</tr>
<tr>
<td>O₆-O₄</td>
<td>2.726 ± 0.005</td>
<td>2.726 ± 0.005</td>
<td>2.773 ± 0.022</td>
</tr>
<tr>
<td>O₆-O₆</td>
<td>2.699 ± 0.005</td>
<td>2.707 ± 0.005</td>
<td>2.688 ± 0.020</td>
</tr>
<tr>
<td>O₆-O₇</td>
<td>2.694 ± 0.005</td>
<td>2.677 ± 0.005</td>
<td>2.496 ± 0.025</td>
</tr>
<tr>
<td>O₆-O₈</td>
<td>2.647 ± 0.005</td>
<td>2.650 ± 0.005</td>
<td>2.611 ± 0.034</td>
</tr>
<tr>
<td>O₆-O₉</td>
<td>2.695 ± 0.005</td>
<td>2.709 ± 0.005</td>
<td>2.714 ± 0.027</td>
</tr>
<tr>
<td>Mean O-O</td>
<td>2.686</td>
<td>2.696</td>
<td>2.644 ± 0.015</td>
</tr>
<tr>
<td><strong>Al octahedron</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-O₆</td>
<td>1.943 ± 0.004</td>
<td>1.933 ± 0.002</td>
<td>1.998 ± 0.016</td>
</tr>
<tr>
<td>Al-O₇</td>
<td>1.920 ± 0.004</td>
<td>1.914 ± 0.002</td>
<td>1.973 ± 0.016</td>
</tr>
<tr>
<td>Al-O₈</td>
<td>1.917 ± 0.004</td>
<td>1.906 ± 0.004</td>
<td>1.973 ± 0.016</td>
</tr>
<tr>
<td>Al-O₉</td>
<td>1.906 ± 0.004</td>
<td>1.938 ± 0.004</td>
<td>1.998 ± 0.016</td>
</tr>
<tr>
<td>Al-OH</td>
<td>1.907 ± 0.004</td>
<td>1.891 ± 0.004</td>
<td>1.939 ± 0.017</td>
</tr>
<tr>
<td>Al-OH'</td>
<td>1.907 ± 0.004</td>
<td>1.899 ± 0.004</td>
<td>1.939 ± 0.017</td>
</tr>
<tr>
<td>Mean Al-O</td>
<td>1.923</td>
<td>1.913</td>
<td>1.970 ± 0.007</td>
</tr>
<tr>
<td>Mean of 9 unshared O-O</td>
<td>2.824</td>
<td>2.807</td>
<td>2.964 ± 0.013</td>
</tr>
<tr>
<td>Mean of 3 shared O-O</td>
<td>2.420</td>
<td>2.417</td>
<td>2.462 ± 0.009</td>
</tr>
</tbody>
</table>
A comparison of final atomic coordinations shows that the two crystallographically independent tetrahedral cations are coplanar in both structures, as are the two apical oxygens, and also two of the three basal triad oxygens. The different z-coordinate of the third basal oxygen, however, causes all the tetrahedral basal planes to be slightly tilted. The equivalent isotropic temperature factors, B, are remarkably similar, atom for atom, in the two structures and the equality of the temperature factors for all four tetrahedral cations \((0.65, 0.65, 0.62, 0.63)\) strongly suggests that the \(\text{Al}/\text{Si}\) distribution is identical in all four positions.

The important interatomic distances are listed in Table 1. The \(T_1-O\) and \(T_2-O\) distances demonstrate conclusively that the distribution of tetrahedral cations is disordered and the same in both tetrahedra in both structures. In muscovite the two crystallographically distinct tetrahedra are identical within the precision of the determination. The two tetrahedra of paragonite, although having identical average interatomic distances, individually are somewhat distorted. Comparison of interatomic distances in the \(\text{Al}\) octahedra shows that this layer is practically unaffected by the change of \(\text{K}/\text{Na}\) ratio in the interlayer cation position. These octahedra show no unusual distortions attributable to "stresses" arising in the tetrahedral layers or due to the presence of interlayer alkalis; and distortions from ideality are primarily due to octahedral edge-sharing.

The effective alkali coordination is six rather than twelve (Figure 1),...
and the average of the six alkali-oxygen distances reflects the change in the $K/Na$ ratio.

What changes do take place, then, in the mica framework when $Na$ is substituted for $K$? The primary adjustment is in the surface oxygen network to accommodate more $Na$ and less $K$ and is seen in significant changes in some $O_{\text{surface}}-O_{\text{surface}}$ distances. Simultaneous decrease in the interlayer separation results in a minor increase in only three of the six distinct $O_{\text{surface}}-O_{\text{apical}}$ distances.

The recent study of coexisting muscovites and paragonites by Zen and Albee (1964) shows that the solvus is extremely asymmetric and the solubility of muscovite in paragonite very limited. From a structural point of view the asymmetric solid solution is easily explained by considering the variation in the average alkali-oxygen interatomic distances with changing $K/Na$ atomic ratio. During the structure analysis it was assumed that the $K$ and $Na$ atoms are truly disordered in both structures. This in fact is supported by a study of the apparent r.m.s. displacements of the surface oxygens which are principally directed towards the alkali rather than normal to the sheet. Variations in the average alkali-oxygen distances would furthermore suggest that in view of the minor changes in the mica framework between $Mu_{66}$ and $Mu_{15}$ the structural differences between $Mu_{66}$ and $Mu_{100}$ will be negligible.
More recently Güven and Burnham (1965) have refined the crystal structure of 3T muscovite seeking to answer the question of whether the 2M₁ and 3T muscovite modifications are polytypes or not, i.e. do they possess truly equivalent layers? The optical and chemical data for this specimen showed that its composition is very close to that of the end-member muscovite. The final refinement, which gave an \( R \) of 3·3\% (weighted), led to the interatomic distances listed in Table 1. Comparing the tetrahedral bondlengths with those given by Smith and Bailey (1963), the \( T_2 \) tetrahedra would appear to have pure SiO₄ composition whilst the \( T_1 \) tetrahedra would contain about 35\% Al. The octahedral distances compared with the values given by Donney et al (1964) indicate that the \( Al_1 \) octahedron contains about 30-35\% Mg, Fe whereas the \( Al_2 \) octahedron has no noticeable isomorphous replacement. In the chemical formula calculated by Axelrod and Grimaldi (1949) appropriate site occupancies were assigned as:

\[
Al(Al_{0.83}Me_{0.17})_2(Si_{0.555}Al_{0.445})_2(OH_{1.98}F_{0.03})O_{10}(K_{0.90}A_{0.08}) \quad \text{where} \quad Me=(Fe^{3+}_{0.222}Fe^{2+}_{0.222}Mg_{0.506}Ti_{0.058}) \quad \text{and} \quad A=(Ca_{0.125}Na_{0.750}Ba_{0.125}).
\]

If we compare 3T muscovite with 2\( V /I \) muscovites there is a noticeable partial ordering in both the tetrahedral and octahedral positions in the 3T, with Al restricted to \( T_1 \) tetrahedron and Fe, Mg, Ti to the \( Al_1 \) octahedron. This is confirmed by significant differences in the octahedral and tetrahedral cation isotropic temperature factors which are \( B = 0\cdot24, 1\cdot14 \) (octahedral) and \( B = 0\cdot99, 0\cdot40 \) (tetrahedral). In the 3T structure there is also a corrugation of the surface of the basal oxygens, but the corrugation is less than for the 2\( M_1 \) muscovite.

It is worthwhile re-emphasising, as Bailey (1966) has done, the problem of obtaining sufficient precision to prove statistically whether an observed difference between two mean bondlengths is real, i.e. greater than three standard deviation units. Bailey considers that it is of scientific interest to detect ordering where the compositions of the two tetrahedra are respectively \( Si_1 Al_1 \) and \( Si_2 Al_2 \). For this purpose the standard error of the mean of 4 \( (T-O) \) distances must be \( < 0\cdot009\AA \). The values in the literature for \( r (T-O) \) for layer silicates range from 0·003\AA{} to 0·040\AA{}. It is worth pointing out that this sort of precision can only be attained by using single crystal diffractometers and the most modern computer programs and facilities, as was in fact the case for the 2\( M_1 \) and 3T muscovite and paragonite structures discussed so far.

Bailey (1966) has also discussed at some length the problem that the space-group in which one is refining the structure simply may not allow for the complete ordering to be observed. It is quite feasible that the symmetry of the tetrahedral Si and Al atoms could be lower than for the rest of the structure and that this will not be detected by the present methods. The ordering of the Si and Al tetrahedral positions constitutes only a minor perturbation on the major features of the structure, even considering the distortions which occur in the coordination around the larger Al and smaller Si. The fact is that the extra diffraction effects which would establish the lower symmetry and allow the ordering to be determined
experimentally may be so weak that nothing can be done with them. Shirozu and Bailey (1966) pointed out that this is probably the case for a parent 1M mica from which a 2-layered ordered vermiculite structure was developed. The vermiculite structure has tetrahedral ordering, whereas the mica structure shows no deviation from the $C\ 2/m$ symmetry which prohibits ordering.

I have so far discussed only ordering throughout the single crystal, which is the kind detected by conventional structure analysis. There is, however, still the possibility of short-range ordering, which is much more difficult to detect. Gatineau (1964) has attempted to obtain further information about the so-called "real" structure of muscovite by the admittedly difficult analysis on non-Bragg reflections or diffuse scattering. His work, which has been summarised by Brown (1965), is the only study reported so far of short-range order in layer silicates and is particularly interesting for that reason. Gatineau concluded that the substitutions of $Al$ for $Si$ tend to occur in rows in which every atom is substituted (Figure 2); these rows favour $[10]$, $[11]$ or $[1\bar{1}]$ directions in the plane of the layer, which are at $120^\circ$ to each other. The crystal is then divided into domains each of which is characterized by the directions of these rows. As shown in Figure 2 the rows of substitutions are grouped together into bands that contain equal numbers of rows of entirely $Al$ and entirely $Si$ atoms, so that the overall ratio of $Al/Si$ is 1:1. This ratio

![Figure 2](image-url)

Fig. 2.—Probable arrangements of lines of substitutions in the different sequences possible; note the tendency for the substitution rows to form zigzags about the centre of each sequence (after Gatineau, 1964).
is reduced to 3:1 by adding bands of equal size that have no Al substitution at all. The charge balance around each K locally is preserved by opposing substituted and unsubstituted bands across the interlayer region.

This is Gatineau's interpretation of his experimental data. It is certainly highly desirable to obtain information about short-range ordering, and Gatineau (1964) has made a serious and worthwhile attempt to do this for muscovite. Before we accept his scheme of ordering as valid (and therefore applicable to other studies) some difficulties should be noted. Experimentally the measurement and correction of the intensity data for non-Bragg reflections is usually subject to substantial errors; and the interpretation of this via Patterson functions requires quite a few mathematical approximations. The ordered distribution arrived at is, moreover, unusual in that it joins pairs of Al's through bridging oxygens. This is in direct contradiction to the empirical rules developed by Loewenstein (1954) and apparently valid for most silicates so far. Furthermore the long-range tetrahedral ordering found in other layer silicates alternates pure Si with partially substituted Si (e.g. Güven and Burnham, 1965; Shirozu and Bailey, 1966). This is also the partially-ordered form predicted by Fujii (1967) from a general theoretical study of order-disorder phenomena.

In summary, long-range tetrahedral ordering has been positively demonstrated in at least one mica; it may have been masked by the symmetry conditions imposed in some other analyses. Short-range tetrahedral ordering may well be expected to occur but satisfactory experimental data are not easily obtained, and the interpretation of these data is in some respects inconsistent with observed long-range ordering and theoretical studies on ordering phenomena.

REFERENCES


SUMMARY

The crystal structure of muscovite was determined in 1933, and refined by partial three-dimensional methods in 1960. This refinement seemed to point clearly to a partly ordered arrangement of Si, Al cations in the tetrahedral sites.

Further work in several laboratories since then has made this feature of the muscovite structure rather suspect. Gatineau has since shown that the 1960 refinement was not carried on sufficiently until it had properly converged, and that when the full power of modern computers is used on the original data then a completely disordered tetrahedral arrangement results, on the average. Burnham and Radoslovich have refined simultaneously the structures of coexisting muscovite and paragonite, giving some of the most precise structural information available on silicate minerals. These refinements also show complete disordering of the tetrahedral cations.

RéSUMÉ

La structure cristalline de la muscovite a été déterminée en 1933, et raffinée par des méthodes partielles à trois dimensions en 1960. Ce raffinement a semblé indiquer nettement une disposition en partie ordonnée des cations Si, Al, dans les emplacements tétraédraux.

Du travail supplémentaire en plusieurs laboratoires depuis cela, a rendu cet aspect de la structure muscovitique assez suspecte. Gatineau a plus récemment montré que le raffinement de 1960 n’a pas été suffisamment continué jusqu’à une bonne convergence, et que lorsqu’on utilise toute la force des computeurs modernes sur les données originelles, alors, il en résulte, en moyen, une disposition tétraédrale complètement désordonnée. Burnham et Radoslovich ont raffiné simultanément les structures de la muscovite et de la paragonite en coexistence, en donnant des renseignements de structure des minéraux silicatés des plus exacts dont on dispose. Ces raffinements montrent, eux aussi, un désordre complet des cations tétraédraux.

ZUSAMMENFASSUNG


Burnham und Radoslovich haben gleichzeitig die Strukturen von co-existierenden Muscovit und Paragonit verfeinert und gaben eine der genauesten strukturellen Informationen über Silikatmineralien welche derzeit vorliegen. Diese Verfeinerungen zeigen komplette Unordnung der tetraedrischen Kationen.
SOME PROBLEMS OF STUDYING REGULAR MIXED-LAYER MINERALS

B. P. GRADUSOV
Dokuchaev Soil Institute, Moscow, U.S.S.R.

A number of regular mixed-layer minerals have been found in soils (Johnson 1964, Gradusov 1967a,b,c) the study of which has a common interest to soil science, geology and clay mineralogy.

One of the main problems in the study of mixed-layer minerals is the establishment of differences between vermiculite and montmorillonite packets. Of particular interest is Walker's suggestion (1958) that vermiculite and montmorillonite packets may be distinguished by means of the $0.6$ eq charge. This enables labile $2:1$ packets, regularly interstratified, to be identified objectively on the basis of their behaviour on $(Mg + glycerol)$ saturation.

Two minerals have been investigated by this method: (1) Sample No. 0-5, separated from Zerevinsk asbestos rock in the South Urals; (2) Sample No. 799, from Lower Permian rocks of Kumolinsk muld, in Kazakhstan. Both minerals are trioctahedral as shown by the value of $1.53$ to $1.54$ Å for $d_{100}$ on the powder diagram. The air-dry Mg-saturated mineral from Kazakhstan and the mineral from the South Urals have periodicities of $28.9$ Å and $28.6$ Å respectively (Fig. 1a-d and Table 3). After heating at $600°$, both minerals collapse to $23.5-24.0$ Å. Solvation with ethylene glycol increases the $d_{100}$ of the mineral from Kazakhstan to $31.1$ Å and that of the mineral from the Urals to $30.5$ Å. This evidence is sufficient to consider both minerals of regular mixed-layer chlorite-montmorillonite or chlorite-vermiculite type. The difference in $2:1$ packets of these minerals is clearly seen from their behaviour after glycerol treatment: the $d_{100}$ of the Kazakhstan mineral increased to $32$ Å, while the South Urals mineral did not swell. According to Walker the $2:1$ packet in the Kazakhstan mineral would belong to montmorillonite, and in the S.-Ural mineral to vermiculite type.

These data as well as Fourier transforms (MacEwan, 1956; Dyakonov, 1962) of ethylene glycol-treated minerals from South Urals (Fig. 2b) show that it consists of two packets. One of them occurs at $14$ Å, the other at $16$ Å, in the proportions of $1:1$. Their interstratification is of the ABAB ... type, confirmed by the agreement in $F_i$ and $F_o$ ($R = 13\%$ and $15\%$). The one-dimensional Fourier synthesis for both minerals treated with ethylene glycol (Fig. 3a,b) demonstrates all components of the proposed structure.

Recently another regular chlorite-vermiculite was detected in asbestos from Kazakhstan (Gradusov, 1967, 1966).

The author has also investigated the well-known mineral “corrensite”
Fig. 1.—X-ray diffractograms.

a—chlorite-vermiculite from Zerevtinsk, the South Urals; b—the same, glycerol treated; c—chlorite-montmorillonite from Kazakhstan, Mg-saturated, air-dry; d—the same, glycerol treated; e—mineral from Zaisersweiher, air-dry; f—the same, Mg + glycerol; g—the same, heated at 300°; h—mineral from Göttingen, air-dry; i—the same, Mg + glycerol; j—rectorite from Dagestan, natural $d_{001} = 24.7\ A$; k—rectorite from Dagestan, natural $d_{001} = 22.0\ A$; l—rectorite from Dagestan, natural $d_{001} = 21.75\ A$; m—tosudite from Uskot (the Crimea), air-dry; n—the same, $K + 300°$; o—the same, $K + 400°$; p—the same, randomly interstratified.
Fig. 2.—Fourier transforms.

a—reectorite from Dagestan, natural $d_{001} = 22.0 \, \text{Å}$; b—chlorite-vermiculite from Zerevinsk (the Urals), ethylene-glycol treated.

from Zaisersweiher and the “corrensite” from Göttingen (Lippmann, 1954, 1956). Some of the data obtained are given in Fig. 1,e-i. Because of its collapse on heating the mineral from Zaisersweiher cannot be regarded as a regular interstratification of chlorite and “swelling chlorite”. Furthermore the swelling of both minerals when $Mg$-saturated and glycerol
Fig. 3.—Electron density distribution curves.

a—chlorite-vermiculite from Zerevtinsk (the South Urals), ethylene-glycol treated; b—chlorite-montmorillonite from Kazakhstan, ethylene-glycol treated; c—rectorite from Dagestan, air-dry with $d_{001} = 24.7 \text{ A}$; d—the same, heated at 600°; e—rectorite from Dagestan, air-dry, $d_{001} = 22.0 \text{ A}$. 
treated shows that they belong to the chlorite-montmorillonite type, accord­
ing to the criteria given above for distinguishing between montmorillonite
and vermiculite packets. It follows from this that the term “corrensite”
(i.e. as a mineral consisting of chlorite and “swelling-chlorite” packets)
should not be associated with the mineral from Zaisersweiher. Indeed, since
the term “corrensite” is applied to different regular mixed-layer minerals
this name is normally not included in clay minerals nomenclatures and
classifications.

Korolev (1962) has suggested that hydroxonium cations are inserted
between rigidly bound packets of the rectorite from Dagestan whereas
Brown and Weir (1963) found it to have a high Na content.

The character of the curve at Z/0 from the one-dimensional Fourier
synthesis for natural and heated rectorite from Dagestan with $d_{001} = 24·7$ Å (Fig. 3,c,d) gives rise to a supposition that metal cations are
present between rigidly bound packets. The content of $Na_2O$ in this mineral
is 2·75 per cent on an oven-dry basis. Ca-saturated sample contains 2·70
per cent of $Na_2O$. It is clear that practically all Na is found between mica-
like packets. This component of rectorite should be regarded structurally
as a paragonite layer. Greene-Kelly’s Li-test (1953) showed that the labile
2:1 packet of the mineral belongs to the beidellite type.

There is a natural form of the Dagestan rectorite for which $d_{001} =

\begin{center}
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{I-R absorption spectra. \(a\)—natural rectorite from Dagestan, \(d_{001} = 24·7\) Å; \(b\)—the same, rectorite from Dagestan, natural with \(d_{001} = 22·0\) Å; \(c\)—rectorite from Dagestan, natural with \(d_{001} = 21·75\) Å; \(d\)—NH$_4$-saturated rectorite.}
\end{figure}
\end{center}
22·0 A. It is thought (Korolev and Ivkin, 1966) that the mica-like packets of this mineral are attached to each other by oxoniums and that the labile 2:1 packets contain Na and one layer of water molecules. X-ray data (Fig. 1,k) and their Fourier transform (Fig. 2,a) show that the mineral contains the interstratified ABAB . . . packets with heights of 9·5 and 12 Å in air-dry state. The Z/2 peak on the electron density distribution curve reflects the presence of one layer of water molecules (Fig. 3,e). Chemical analysis of this mineral has given only 3·23 per cent of Na₂O, while the Na-saturated mineral contains 3·95 per cent of Na₂O. The discrepancy of about 0·7 per cent Na₂O suggests that some other cation must be present in the labile 2:1 packet. Infrared spectra of this mineral showed adsorption bands characteristic of the NH₄-group which were absent in the mineral described above (Fig. 4,a,b). This suggests that there are NH₄-ions in the interspacings of rectorite with d₀₀₁ = 22·0 Å. Moreover, a band characteristic of the NH₄ group has also been found on the IR-spectrum of rectorite with d₀₀₁ = 21·75 Å separated from a hand specimen of the Dagestan mineral (Table 1, Fig. 1,1) by means of a binocular microscope.

Thus, the Dagestan rectorite represents a regular alternation of two kinds of packets—paragonite and beidellite. In some cases interlayer spacings are filled with Ca and 2 layers of water molecules, in other cases—with one water molecule layer and probably with NH₄.

Kodama (1966) found that rectorite from Pakistan contains beidellite packets. For a rectorite with a muscovite packet, the beidellite nature of the other packet was established by Gradusov and Chizhikova (1967).

A rectorite containing muscovite and montmorillonite-type packets has been observed recently in some Karelian soils (Gradusov, 1967a).

A proposal has been made to return the term “alushtite” for regular dioctahedral chlorite-montmorillonites (Korolev, 1965). The author examined the rock and the mineral from Uskot (in the Crimea). The rock contained a mineral similar to tosudite from Privetnoe (Frank-Kamenetsky, 1963), mica-montmorillonite mixed-layer mineral, chlorite and a kaolinite mineral (Fig. 1,m, Table 3). In different samples there are found similar but not identical minerals of the tosudite type, varying in quantity of montmorillonite packets and in the degree of regularity of the packet interstratification (Fig. 1,p).

The unusually strong effect of heating at 600° on the d₀₀₁ collapse is probably due to destruction of the binding layers (Korolev, 1965). It is seen from Fig. 1,n,o that already at 300-400° the d₀₀₂ of the K-saturated mineral is 11·5 Å. It is doubtful that this strong contraction of the mineral at such a low temperature is related to destruction of a gibbsite-like layer.

The slow decline in the intensity at the left side of the peak and the maxima at 10·5 Å suggest that phases containing more than 50 per cent of 2:1 packets are present.

As the sample from Uskot is not a monomineral and the regular dioctahedral chlorite-montmorillonite mineral is a less perfect analogue of tosudite from Privetnoe it is unnecessary to return the term “alushtite”.
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<td>21.88</td>
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</table>

\( d_{001} \) average: 21.75.
A mineral similar to tosudite has been described in taiga soils on Upper Cambrian rocks of Irkutsk region (Gradusov, 1967). On the basis of the data given above a classification is suggested of regular mixed-layer ABAB . . . minerals and their diagnostics by Mg-saturated forms in three states (Table 2).

### Table 2

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<th>Interstratifying packets</th>
<th>d₀₀₁, Å</th>
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<th>g</th>
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<td>Vermiculite/chlorite²</td>
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<td>28·5</td>
<td>23·7</td>
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<tr>
<td>Montmorillonite/chlorite³</td>
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<td>32·0</td>
<td>24·0</td>
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<tr>
<td>Diocotahedral</td>
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<td>27·7</td>
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<tr>
<td>Beidellite/paragonite⁵</td>
<td>24·4</td>
<td>27·4</td>
<td>19·3</td>
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</tr>
<tr>
<td>Montmorillonite/chlorite(sudoite)⁶</td>
<td>28·9</td>
<td>31·5</td>
<td>23·1</td>
<td></td>
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</tbody>
</table>

¹ From the <0·001 mm soil fraction (Eastern Sayan).
² From asbestos, Zerevtinsk (the South Urals).
³ From the rock Nr 799 (Kazakhstan).
⁴ Kuli-Kolon (Tadjikistan).
⁵ Dagestan.
⁶ Uskot (the Crimea), the nature of the montmorillonite packets was not established.
⁷ a — air-dry; g — glycerol treated.

The problem of determining the type of interlayings of labile 2:1 packets in regular mixed-layer minerals is a very important one which can be successfully resolved by the study of different cation-saturated mineral forms. Experimentally determined values of d₀₀₁ for various cation-saturated forms of some regular mixed-layer minerals are given in Table 3. The technique of their preparation has been described previously (Gradusov and Chizhikova, 1967).

### Table 3

<table>
<thead>
<tr>
<th>Mineral state</th>
<th>Rectorite, Dagestan</th>
<th>Tosudite, Uskot (the Crimea)</th>
<th>Chlorite-vermiculite, the South Urals</th>
<th>Chlorite-montmorillonite, Kazakhstan</th>
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<tr>
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<tr>
<td>Na</td>
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<td>K</td>
<td>22·0</td>
<td>26·6</td>
<td>24·6</td>
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The problem of determining the type of interlayings of labile 2:1 packets in regular mixed-layer minerals is a very important one which can be successfully resolved by the study of different cation-saturated mineral forms. Experimentally determined values of d₀₀₁ for various cation-saturated forms of some regular mixed-layer minerals are given in Table 3. The technique of their preparation has been described previously (Gradusov and Chizhikova, 1967).
ACKNOWLEDGMENTS

The author wishes to thank G. Harder, F. Lippmann, D. D. Kotelnikov, O. B. Beiseev and G. P. Barsanov for supplying the mineral samples and N. P. Chizhikova and L. S. Travnikova for their assistance during the performance of this study.

REFERENCES


SUMMARY

Two regular mixed-layer minerals are described: chlorite-montmorillonite and chlorite-vermiculite.

The rectorite from Dagestan consists of paragonite and beidellite packets, the interlayer regions being occupied by Ca in one case, and probably by NH4 or (NH4 + Na) in the other. Tosudite from Uskot (the Crimea) is a less perfect analogue of the mineral from Privetnoe and is contaminated with several other clay minerals. “Corrensites” from Zaisersweiher and Göttingen are regular chlorite-montmorillonites. A classification and diagnostic indications for regular mixed-layer minerals are suggested.

RÉSUMÉ

Deux minéraux réguliers à couche mixte sont décrits: la chlorite-montmorillonite et la chlorite-vermiculite.

La rectorite de Dagestan comprend des paquets de paragonite et de beidellite, les régions entre couches étant remplies de Ca dans l’un des cas,
et probablement de $NH_4$ ou de $(NH_4+Na)$ dans l’autre. La tosudite d’Uskot (dans la Crimée) est un analogue moins parfait du minéral qui se trouve à Privetnoe, étant contaminé par quelques autres minéraux argileux. Les “corrensites” de Zaisersweiher et de Göttingen sont des chlorite-montmorillonites réguliers. On suggère une classification et des indications diagnostiques pour les minéraux réguliers à couche mixte.

**ZUSAMMENFASSUNG**

Zwei reguläre Minerale gemischter Schichten werden beschrieben: Chlorit-Montmorillonit und Chlorit-Vermikulit.


“Corrensit” von Zaisersweiher und Göttingen sind reguläre Chlorit-Montmorillonite.

Eine Klassifizierung und diagnostische Kennzeichnung der regulären Minerale gemischter Schichten werden vorgeschlagen.
CLAY MINERALOGY OF SOME ACID-SULFATE SOILS ON THE GUINEA COAST

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Fayetteville, Arkansas, U.S.A.

The coastal soils of the Republic of Guinea in west Africa are developed in sediments that have been transported from interior uplands by fresh water streams and subsequently deposited in a littoral zone. Here the influence of salt water tides has created a chemical environment much in contrast to that existing in the source area. An abundance of potassium and other ions in the encroaching sea water may contribute to diagenetic changes in soil clay minerals. Extreme acidity associated with acid-sulfate (cat clay) soils may also affect clay-forming processes or alterations. These processes may be further affected by an abundance of organic matter and organically-derived silica present in the soils. This paper reports the results of mineralogical investigations of four Guinea coastal soils and considers the possible affects of sea water on the clay mineral assemblage.

GENERAL DESCRIPTION OF SOILS

The soils investigated are clays developed under conditions of periodic salt water intrusion and fresh water flooding in a tropical area that has distinct wet and dry seasons. This environment favours the accumulation of sulfate and organic matter and leads to varying degrees of salinity. Sulfate from the sea water is precipitated in the sulfide form under reducing conditions. Subsequent oxidation of iron sulfides causes the highly acid conditions that are associated with cat-clay soils. Chemical changes involved have been described in detail by Van Beers (1962) and further elaborated with respect to Guinea soils in an earlier paper (Horn, Hall, Chapman, and Wiggins, 1967).

The four soils reported upon herein include one from the Kapatchez area and one from Koba on the coastal plains north of Conakry and two south of Conakry on Kakossa Island. The chemical properties of these soils are given in Table 1.

MINERALOGY

Sand fraction—The sand fraction (50 to 200 μ) composes a minor part of the soil. Particles in this size fraction are predominantly quartz. The angularity of these particles and the presence of feldspars in the silt
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<th>Silt %</th>
<th>Clay %</th>
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<th>Organic Matter 1:1 %</th>
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<th>K</th>
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<th>Na</th>
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fraction suggests rapid removal from the source area and a relatively short period of transportation. Appreciable amounts of sponge spicules, diatoms, spores, and other resistant organic debris also occur (Figure 1).

Fig. 1.—Sponge spicule fragments (needle shaped objects with axial canals) and diatoms (round, dark) are organically derived siliceous materials that make up appreciable portions of the silt and sand fractions of the Guinea coastal soils. Platy particles are mostly muscovite. (Approx. 40X).

Silt fraction—The silt fraction (2 to 50 μ) composes about 15 to 30% of these soils. Coarse silts (20 to 50 μ) are composed nearly entirely of quartz with trace amounts of feldspars. The fine (2 to 5 μ) and medium silts (5 to 20 μ) also contain large quantities of organic debris that is resistant to $H_2O_2$ digestion. This siliceous material makes up 40 to 60% of the silts. Inorganic portions are composed mainly of quartz, kaolinite, micas (mostly muscovite but some biotite), together with small amounts of feldspars, and gibbsite. Approximate compositions of the fine silts are given in Table 2.

Clay fraction—Compositions of the coarse clays (0·2 to 2 μ) and fine clays (< 0·2 μ) in each of the four soils are summarized in Table 2. Coarse clay is somewhat more abundant than fine clay. The X-ray patterns, obtained from diffractometer scans of oriented samples using copper K-
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**Table 2: Mineralogical Properties**

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</table>

**Kakossa #11**

†**** = >50%, *** = 16 to 50%, ** = 5 to 15% and * = <5%.
alpha radiation, are generally similar for all of the clay samples. The patterns in Figure 2 are typical.

An abundance of well crystallized kaolinite in the clays of all soils is indicated by very strong sharp peaks at 7.1Å and 3.55Å that disappear upon heating to 550°C. In addition to kaolinite, mica (illite), identified by the 10Å peak in the Mg-saturated sample, and montmorillonite, identified by the 18Å peak in glycerol-solvated samples, are present in roughly equal amounts in the coarse clays.

The presence of gibbsite, identified by a 4.83Å X-ray diffraction peak is of interest since it is reflective of the intense weathering prevailing in the Guinea uplands. It is most abundant in the fine silts amounting to as much as 10 to 25% of the sample. In the coarse clays it is present in small amounts (< 5%). It is absent in the fine clays.

There are minor amounts of vermiculite or perhaps interstratified chlorite and vermiculite or montmorillonite in the fine silt and coarse clays of the Kapatchez and Koba soils. This is suggested by 14Å peaks of
potassium saturated samples that survive a 550°C heat treatment. Broadening of the 14A peak toward the 10A peak suggests interstratification. Very small peaks at 30 to 32A, visible in X-ray patterns from Mg-saturated samples suggest the presence of interstratified chlorite/montmorillonite. The 14A peaks are most obvious in X-ray patterns of the coarse clays from upper soil horizons.

The fine clays are chiefly kaolinite and montmorillonite with possibly small amounts of chlorite.

**DISCUSSION**

The effects of sea water on detrital sediments have been rather extensively investigated by mineralogists as shown in a comprehensive review by Keller (1962). For the most part, these studies have been concerned with near offshore sedimentation. Furthermore, inferences made about clay diagenesis from the clay mineralogy of offshore sediments and marine sediments in the geologic column and, from experimental evidences taken from the incubation of standard clays in natural or artificial sea water, have been for assumed or controlled pH values of 7-5 to 8-5.

Only recently, Lynn and Whittig (1966) have called attention to the possible effects of an acid marine environment on clay mineral diagenesis. Environments of this type are associated with cat clay, or acid-sulfate soil formation and are known to be quite extensive in the world today (Moorman, 1963). It is likely that similar conditions existed in various places throughout much of geologic history and thus may be of great significance to the mineralogy of marine shales as well as of present-day soils.

Little information exists in the literature on the clay mineralogy of the acid-sulfate soils themselves. In addition to the study by Lynn and Whittig of California cat-clay soils, Herbillon et al. (1966) have reported analyses of the clay mineralogy of acid-sulfate soils that occur in the Mekong Delta of Vietnam.

The composition of the Vietnam soils appears to be much the same as that of the soils reported upon in this paper. Soil clays in the acid sulfate soils of Guinea and Vietnam are highly kaolinitic whereas those of the California coast contain only small amounts of kaolinite. This is probably due to the differences in the weathering regime and the sediments in the source area.

Another aspect of clay formation in an acid-sulfate environment is the possible role of organically-derived silica in silicate clay formation. As noted earlier an abundance of opaline silica in the form of sponge spicules, diatoms, etc., exists in the Guinea soils. Phleger and Bradshaw (1966) have pointed out that sedimentary particles of organic origin are abundant in the sediments of marine marshes.

The availability of such silica, particularly at the alkaline pH values existing prior to acid-sulfate soil formation, may contribute substantially
to the diagenetic formation of silicate clay minerals in coastal soils. The illite and montmorillonite present in the Guinea soils are likely to have formed under these conditions.

Conversely, the extremely acid conditions existing in acid-sulfate soils probably favour preservation of kaolinite and deter further illite and montmorillonite formation. Information about these suspected processes may be obtained from timed experiments currently under way in our laboratory using artificial sea water, controlled pH values from pH 4.0 to 7.8, kaolinite, and diatomaceous earth as a source of silica, and aluminium chloride as a source of Al. Data from these experiments were not available at the time of this writing.

ACKNOWLEDGMENTS

Samples of Guinea soils were made available for this study by Harza Engineering Company International, Chicago, Illinois.

REFERENCES


SUMMARY

Acid-sulfate (cat clay) soils on the coast of the Republic of Guinea are developed in sediments deposited in a marine environment. Their clay mineralogy is dominated by well crystallized kaolinite. A highly alkaline pH system and an abundance of potassium in encroaching sea water probably accounts for the presence of smaller amounts of illite and montmorillonite. An abundance of organically derived silica (sponge spicules, diatoms, etc.) in the soils may contribute silica to silicate clay formation in the alkaline environment. Upon oxidation of sulfides, following drainage or lowering water tables, a strongly acid condition develops which probably deters further diagenetic changes and favours preservation of kaolinite and gibbsite present in the original sediments. It is noted that an acid marine e
environment associated with acid-sulfate soils may have been important throughout much of geologic history in determining the clay mineralogy of marine shales as well as present day soils.

RéSUMÉ

Les sols de sulfate acide (argile à chats) de la République de Guinée se développent en sédiments déposés dans un milieu marin. Leur minéralogie argileuse est dominée par de la kaolinite bien cristallisée. Un système de pH fortement alcalin et une abondance de potassium dans l'eau de mer, qui se propage dans les sols, expliquent probablement la présence des quantités plus petites d'illite et de montmorillonite. Une abondance de silice tirée organiquement (spicules d'éponge, diatomées, etc.) dans les sols, contribue à la formation d'argile silicatée dans le milieu alcalin. Lors de l'oxydation des sulfides, après le drainage ou un abaissement des plans d'eau, il se développe une condition fortement acide qui probablement détourne les changements diagenétiques supplémentaires et favorise la préservation de la kaolinite et de la gibbsite qui se trouvent dans les sédiments originels. On remarque qu'un milieu acide marin, associé aux sols de sulfate acide, a peut-être été important dans l'histoire géologique en déterminant la minéralogie argileuse des schistes marins aussi bien que des sols d'aujourd'hui.

ZUSAMMENFASSUNG

MINERALOGY AND WEATHERING OF THE CLAYS IN ORTHIC PODZOLS AND OTHER PODZOLIC SOILS IN CANADA

J. E. BRYDON, H. KODAMA AND G. J. ROSS

Soil Research Institute, Canada Department of Agriculture, Ottawa, Ontario

The majority of soils of Eastern Canada have developed upon soil materials which have been either deposited directly by the continental glacier or have been reworked by the action of fresh or marine water. A variety of minerals has been inherited (Allen and Johns, 1960) by the soils depending upon the source rock or sediment. In some cases the minerals are relatively fresh (Brydon and Patry, 1961) whereas in others they have been altered drastically prior to glaciation (Forman and Brydon, 1961). In Eastern Canada these soil materials have been exposed for periods of 10,000 years or less.

This paper presents a summary of clay mineral data obtained in a study of a number of soils by the Soil Research Institute. These data along with others obtained previously will be used to draw several conclusions on the stability of the various clay minerals and the weathering mechanisms prevailing in Podzolic soils.

MATERIALS AND METHODS

The various soils have been described in a number of Canadian Soil Survey Reports and grouped according to Great Soil Groups by McKeague and Day (1966). Major horizons were sampled and chemical analysis of representative soils have been published by McKeague and Day (1966).

Aliquots of less than 1 micron clay were taken following the pipette sampling for particle-size distribution. Prior to dispersion, the soils received \( H_2O_2 \) treatment to remove organic matter, \( HCl \) to remove carbonate and the citrate-dithionite treatment (Mehra and Jackson, 1960) to remove free iron oxides. The treated soil was flocculated with \( NaCl \) and washed by centrifuging until dispersal occurred. The less than 1 micron clay in the aliquots was flocculated with \( CaCl_2 \), washed until chloride-free and freeze-dried. In order to make oriented specimens, 20 mg of dry clay was suspended in 1 ml \( H_2O \), the suspension was stirred with a tissue grinder and then was transferred to 37.5 mm x 25 mm microscope slides where the clay was allowed to settle and dry in air. X-ray diffraction patterns were obtained on a Philips diffractometer with proportional counter and pulse height analyser with the sample in the air-dry condition as well as after glycerol sorption and heat treatments at 500°C and 700°C for \( \frac{1}{2} \) hour under the following conditions: radiation \( Fe \)-filtered \( Co \) (\( \lambda = 1.790A \)); scanning speed—1°/min; slits 1°—0.012°—1°.

1 Contribution No. 245.
Semi-quantitative analysis of the clay minerals was carried out using the principles outlined first by Johns et al. (1954) and elaborated by Sudo et al. (1961). Chlorite and kaolinite were estimated by the intensity of the 7Å peak and in some cases are reported together in the tables.

Certain specific specimens were treated for 1 hour in hot 2 N HCl to remove orthochlorites and to isolate kaolinite on the X-ray diffraction patterns (Sudo et al., 1961). Smectite and vermiculite were identified by the usual techniques but were estimated along with their inter-stratified complexes with mica by the increase in intensity of the 10Å peak on heating at 700°C.

RESULTS

Podzols, Humic Podzols and Podzo Regosols

The data for the soils in these Great Groups are shown in Table 1. Also included are the data for the < 0.2 micron and 2-0-2 micron clays from two Podzols described in detail by McKeague (1965). There was a considerable variation in the amounts of the minerals in the C horizons although in most cases mica predominated. Only two of the C horizons showed a definite 18Å peak when glycerol-solvated but some showed ill-defined diffraction bands in the 14 to 19 Å region of the spectrum in addition to a 14Å chlorite plus vermiculite peak. In the latter cases, because of the variety of possibilities, the enhancement of the 10Å peak on heating is indicated in the table as vermiculite-smectite. The C horizons of four soils gave diffraction patterns with a very broad diffraction band at 3-5Å characteristic of amorphous material and also gave an indication of a poorly defined 14Å mineral which had properties of interstratified vermiculite-chlorite. Thus, these clays seemed to resemble the material found in the early stage of weathering in certain soils derived from volcanic ash in Japan (Yoshinaga and Aomine, 1962).

The most striking feature of the clays from the Ae horizons was the absence of chlorite. A strong smectite component without vermiculite was present in 9 of the 15 samples while vermiculite or a complicated mixed-layer structure was present in addition to smectite in the other six samples. One of the samples, the Ste Agathe, showed an amorphous material-14Å mineral mixture similar to its corresponding C horizon and to three other C horizon clay samples. However, this Ae clay showed a poorly defined 18Å smectite peak which was not shown by the C horizon clay.

Based on the assumption that the Ae horizons were derived from material identical to the material present in the C horizon, the data show that inherited orthochlorite has disappeared in the Ae horizon of all Podzols and there was a decrease in the mica content from the C horizon to the Ae in the majority of cases. There does not appear to be any increase in the kaolinite content of the Ae over that of the C horizons. It is difficult to estimate kaolinite in the presence of chlorite by X-ray diffraction and, while kaolinite can be identified by acid dissolution of the chlorite, the partial dissolution of the associated mica may cause an overestimation of kaolinite. Since most of the kaolinite contents of the Ae horizons (where
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| **Humic Podzol** |       |       |       |       |       |       |
| Tuadook I Ac | 1   | 0     | 1   |       |       | 4   |
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| Thorn I Ac   | 5   | 0     | 1   | 1     |       | 1   |
|              C   | 3   | *     | 2   | 2     |       | 2   |
| Richibucto II Ac | 3   | 0    | 1   | 2     |       | 2   |
|              C   | 2   | *     | 3   | 2     |       | 2   |
| Millar Ac    | 3   | 0     | 1   | 2     |       | 2   |
|              C   | 3   | 0     | 1   | 2     |       | 2   |
| Kingsville Ac | 2   | 0     | 1   |       |       | 4   |
|             C   | 3   | *     | 1   |       |       | 3   |

| **Podzo Regosol** |       |       |       |       |       |       |
| Richibucto I Ac | 2   | 0     | 0   | 4     |       | 4   |
|              C   | 2   | *     | 3   | 2     |       | 2   |
| Kingsport Ac   | 3   | 0     | 1   |       |       | 3   |
|              C   | 3   | *     | 1   |       |       | 3   |

* Identifiable.
- Not identifiable because of overlapping peaks or interstratification.
** McKeague (1965).
† Based upon the following ranges as per cent. of the total phyllosilicate content:-

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the chlorite was absent) were low, the kaolinite contents of the C horizons
must have been low also if we assume that kaolinite would be as stable
in the other profiles as it was in the Millar, Tormentine, and Queens. The
kaolinite in the latter two was estimated after acid treatment. The low
kaolinite contents of most of the Ae horizons and the similarity of the
amounts in the other Ae and C horizons suggest that the neoformation
of kaolinite has been minimal or zero.

Orthochlorites were present in many of the B horizon clays along with
a complex combination of mica, vermiculite, kaolinite and pedogenic
chlorite whereas an 18A reflection was absent in every case. Reliable
quantitative estimation of the constituents of this kind of mixture is
difficult to obtain and the data have not been included in Table 1.

Gray Brown Podzolic and Gray Wooded Soils

The data for these soils (Table 2) show the same variability of C
horizon materials as was found in the Podzols. Chlorite was absent in two

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**Table 2**

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* Identifiable but amount not estimated.
- Not identifiable because of overlapping peaks or interstratification.
†† The values indicate the same percentage ranges as in Table 1.
profiles whereas kaolinite was shown to be absent in one. An 18Å smectite reflection was shown by a number of the C horizon clays but was only distinct enough in 2 of them to allow estimation.

The Ae horizons of these soils showed a number of differences from those of the Podzols. First, chlorite was found in the Ae horizon clays of all of the soils containing it in the C horizon. Second, a distinct, quantitatively measurable 18Å reflection was shown by only one Ae horizon clay and that was also present in the C horizon.

The data show that in the majority of these soils, mica and chlorite tend to decrease while vermiculite-smectite increases on going from the C to the Ae horizon. In the Podzols, on the contrary, chlorite was absent and a distinct smectite reflection was found in the Ae horizons.

**X-ray diffraction patterns**

Many of the C horizon clays as well as some of the shales of Eastern Canada consist of well-ordered mica and chlorite (Allen and Johns, 1960; Brydon, 1958), an example of which is shown by the diffraction patterns

![X-ray diffraction patterns](image)
of the Holmesville C horizon clay (Fig. 1). In the other C horizon clays the degree of disorder of the mica and the chlorite varied from the well-ordered minerals in the Holmesville to the amorphous material-14A mineral mixture found in the Gibraltar, Ste Agathe, Bayswater and Aspotogan C horizons.

X-ray diffraction patterns of the Ae horizon clay of the Holmesville (Fig. 1) is representative of the Podzols. The 001 reflection is strong and sharp and the higher order reflections, though weaker, have a high degree of rationality. Thus, the degree of interstratification with mica layers was low in this sample. The others in Table 1 varied considerably and included no 18Å/n higher orders in the Tormentine, poor rationality in the Millar, average rationality in the Johnville and good rationality in the Tuadook I. In addition, there was a small, very broad reflection at 28Å (24Å when air-dry) (Fig. 1) which has been identified as being similar to the long-spacing mineral of Sudo et al. (1960). The variety of types

![Fig. 2.—X-ray diffraction patterns of oriented specimens of the < 1 micron clay fraction from the Ae and C horizons of the Saugeen soil, an orthic Gray Brown Podzolic.](image)
CLAYS IN ORTHIC PODZOLS

and degrees of interstratification of phyllosilicates in the Ae horizons of five Podzols have been studied by Kodama and Brydon (1968).

The diffraction patterns of the Saugeen clays (Fig. 2) are representative of the kind and distribution of minerals in the Gray Brown Podzolic and Gray Wooded Soils. The mica and chlorite tended to be more disordered and there were more expanding layer components in the Ae than in the C horizon of these soils. These were revealed as smectite-mica, hydrous mica and interstratified chlorite-vermiculite reflections. There was no distinctive difference between the clays of the Ae and the C horizon in these podzolic soils.

DISCUSSION

It is generally considered (Droste et al., 1962; Schwertmann, 1964; Jackson, 1965) that dioctahedral clay mica and trioctahedral chlorite become progressively more altered toward the surface of soils of the Temperate Zone. The weathering products are commonly hydrous mica, vermiculite, smectite and soil chlorite (Schwertmann, 1964). In the soils developed on the younger tills, vermiculite associated with mica is commonly found in surface horizons (Jackson, 1965). Our data for the Gray Brown Podzolic and Gray Wooded soils are in line with these previous findings.

A different situation emerges from a consideration of the “true” Podzols. The enrichment of the Ae horizon in SiO$_2$ has been amply documented (Muir, 1961) but the mineralogical changes have become elucidated only recently. There is abundant evidence of the formation of smectite in the Ae horizon (Brown and Jackson, 1958; Forman and Brydon, 1961; Gjems, 1963; Franzmeier and Whiteside, 1963; McKeague, 1965; Ross and Mortland, 1966; Kodama and Brydon, 1968). Brown and Jackson (1958) showed that the smectite from a Wisconsin Podzol collapsed to 10A readily. Gjems (1963) concluded that the expanding-layer mineral from a Norwegian Podzol had properties intermediate between vermiculite and montmorillonite, and Ross and Mortland (1966) identified the smectite from Michigan as a beidellite. Kodama and Brydon (1968) have shown that the expanding-layer components were interstratifications of smectite, vermiculite and mica. By means of one-dimensional Fourier syntheses the proportions of the components and their degree of randomness of stacking were found to vary among the five Ae horizon clays. In the clays reported here, the variation in rationality of the 18A/n reflections may be interpreted also as a variation in proportion and degree of randomness, and it is conceivable that a similar relationship may exist in the clays investigated by the other workers. However, the development in the Ae horizon of an 18A smectite-rich clay showing no impedance to collapse appears to be characteristic of Podzols.

A second noteworthy feature of Podzols is the apparent absence of kaolinite formation in the Ae horizon. There is no evidence of kaolinite formation in the data in Table 1 and none was reported in the seven references cited above.
A third feature of "true" Podzols which seems to differentiate them from the Brown Forest, Gray Brown Podzolic, and Gray Wooded soils is the sharp mineralogical change at the boundary between the Ae and B horizons. In the Podzols, orthochlorites were found in the B horizon clays but not in the Ae, the B horizon clays showed no 18A reflections, and there was no pedogenic chlorite in the Ae horizon clays. On the other hand, the clays from the B horizons of the Gray Brown Podzolic and Gray Wooded soils were not distinctly different from the respective Ae horizons.

The cause of the persistence of orthochlorite in the B horizon and its disappearance in the Ae has been considered by Brydon and Ross (1966). By analogy with the behaviour of orthochlorites in very dilute acids, it was considered that iron and aluminium hydroxide coatings in the B horizon of Podzols prevent the rapid decomposition of chlorites by impeding the uptake of $H$ and the release of $Mg$ and $Fe$. It is generally held that free iron and aluminium are efficiently removed from the Podzol Ae during podzolization (Muir, 1961; Rode, 1964; McKeague, 1965). Direct evidence for this in Canadian Podzols is afforded by the symmetrical smectite reflection at 10A or less upon heating the specimens (Fig. 1) which is an indication of the absence of interlayer aluminium hydroxide (Brydon and Kodama, 1966). Without the protection of the iron and aluminium hydroxide coatings, the trioctahedral chlorite is thought to be liable to rapid decomposition. On the basis of the completeness of the decomposition of the chlorites in concentrated $HCl$ Ross (1967) and the failure to form a partially altered chlorite or a trioctahedral vermiculite (Kodama and Brydon, 1968), the decomposition of chlorite in the Podzol Ae is considered more likely to yield an amorphous aluminosilicate than a smectite by partial alteration.

Undoubtedly, the smectite in the Ae horizon can originate in a number of ways. One is by inheritance; for example, the Kingsport soil in Table 1 and the Alberta Podzol described by Pawluk (1960). A second is by synthesis from the amorphous aluminosilicate and/or the soluble constituent elements (Jackson, 1965). The third possible origin of the smectite is as a direct alteration product of mica (Jackson, 1965; Ross and Mortland, 1966; Kodama and Brydon, 1968). Potassium may be partially removed from each layer giving mica-smectite interstratification in the a-b directions or it may be selectively removed entirely from certain layers (the "preferential weathering plane" of Jackson et al., 1952) giving interstratification in the c axis direction. Long-spacing periodicities (28-32A) may be built up. Several examples of this type of interstratification have been considered by Sudo et al. (1960) and an indication was shown by the Holmesville Ae in Fig. 1.

In an attempt to distinguish the "true" podzol from the soils with marked clay accumulation in the B horizon, the term "lessivage" has been introduced by Duchaufour. Considerable discussion has taken place in the Russian literature (e.g. Fridland, 1958; Parfenova and Yarilova, 1960; Rode, 1964) regarding the practical distinction between podzolization (the decomposition of the clay minerals in the Ae horizon and the
translocation of the products to the B horizon) and lessivage (translocation of clay from the Ae to the B horizon without breakdown). Chemical composition and particle-size distribution have commonly been used in the characterization (Muir 1961, Rode 1964). From the present work, it can be concluded that mineralogical differentiation can also be used. Furthermore, the results of Kodama and Brydon (1968) suggest that, within the Podzol Group, the proportion of expanding layers and the randomness of stacking of the component layers in the interstratified clays of the Ae horizons may be useful in determining the degree of podzolization.

REFERENCES

SUMMARY
The clays from the C horizons ranged from well-ordered mica and chlorite mixtures to a mixture of amorphous material and a poorly crystallized “14A mineral”. Smectite was not a common constituent of the C horizons.

Examination of the clays from the Ae horizon of Podzols showed that: 1. the major mineral was a well-crystallized smectite with varying degrees
and kinds of mica interstratification; 2. orthochlorites had completely disappeared from the Ae horizon clays yet were present in the lower horizons. This was considered to be due to the protection of iron and aluminium hydroxides in the B horizons and to the removal of this protection in the Ae by the podzolization process; 3. in the majority of soils there was a decrease in mica from the C horizon to the Ae horizon. Part of this change was thought to take place by removal of K along an entire plane preferentially rather than randomly thereby producing smectite-mica mixed layer minerals; 4. there was no evidence of major kaolinite formation in the Ae horizon of Podzols.

The clays in the Gray Brown Podzolic and Gray Wooded soils did not show distinctive horizon differentiation of clay mineralogy. Chlorite persisted in the Ae horizons; and there was a gradual increase in hydrated-layer minerals from the C horizon to the Ae.

Résumé

L'argile des horizons C contenait une variété de minéraux se déployant depuis des micas bien ordonnés et mélanges de chlorites jusqu'à un mélange de matière amorphe et d'un minéral mal cristallisé. Les smectites ne furent pas communément retrouvées dans les horizons C.

L'examen de l'argile de l'horizon Ae des podzols révéla (1) que le minéral le plus abondant était une smectite bien cristallisée et possédant divers degrés et modes d'empilement des feuillets micacés, (2) que les orthochlorites avaient complètement disparu de l'argile des horizons Ae tout en étant présentes dans les horizons inférieurs. Ce phénomène serait dû, à notre avis, d'une part à l'effet protecteur des hydroxides de fer et d'aluminium dans les horizons B et, d'autre part, à l'absence de cet effet dans l'horizon Ae résultant du procédé de podzolisation, (3) que dans la plupart des sols examinés, il y avait une diminution des micas à partir de l'horizon C vers l'horizon Ae. Ce phénomène se produit, à notre avis, grâce en partie au lessivage du potassium suivant une couche horizontale et selon un mode de préférence plutôt que de hasard; il se produit ainsi des minéraux en feuillets mélangés de smectites et de micas. enfin, (4) qu'on ne retrouve aucune trace importante de cuolisation dans l'horizon Ae des podzols.

Zusammenfassung

Die Tone von den C-Horizonten erstreckten sich von den gut geordneten Glimmer- und Chloritmischungen bis zu einer Mischung von amorphen Material und einem schwach kristallisierten "14A mineral". Smectit war nicht ein allgemeiner Bestandteil der C-Horizonte.
Eine Untersuchung der Tone aus dem Ae-Horizont des Podsols zeigte, dass:

1) Das Hauptmineral war ein gut kristallisierter Smectit mit unterschiedlichen Gradern und Arten von Glimmer-Interstratifikation.
2) Orthochlorite waren vollständig vom Ae-Horizont verschwunden, Tone waren jedoch in den niedrigen Horizonten vertreten. Dieses war als die Ursache für die Beschützung von Eisen- und Aluminiumhydroxyden in den B-Horizonten und für die Beseitigung dieser Beschützung in den Ae durch den Podsolisationsprozess angesehen.
4) Im Ae-Horizont von Podsolen war kein Anzeichen von größerer Kaolinitbildung vorhanden.

Die Tone aus graubraunen Podsolen und grauen Waldböden zeigten keine ausgeprägte Horizontdifferenzation in der Tonmineralogie. Chlorite verharrten in den Ae-Horizonten; es war ein allmählicher Anstieg an hydrierten Schichten von Mineralien vom C-Horizont zum Ae hin.
THE TITANIUM CONTENT OF SOILS AND CLAYS OF SICILY

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INTRODUCTION

The occurrence of titanium as a normal component of rocks and soils is well-known. Higher titanium contents are found in basic eruptive rocks and especially in basaltic, granitic and syenitic rocks. The first broad investigation of the distribution of titanium in soils was that of Geilmann (1920) who stated that the general diffusion of this element through soils occurred in quantities strictly related to the nature of parent materials. Lateritic and tropical soils show the highest titanium content (Seelye et al. 1938). Further detailed investigations by Robinson and Holmes (1924), Joffe and Pugh (1934) and Salminen (1938) were particularly directed to the study of the vertical distribution of titanium in some typical soil profiles, and the study of the titanium content of the clay fractions of soils. The results showed that titanium in soils is present mainly in the tetravalent form (in the minerals ilmenite, titanite and rutile) and seldom in the more mobile trivalent form. In general the titanium content of soils varies remarkably with depth, showing a slight mobility. It seems moreover that the element accumulates in the clay fractions as a colloidal compound or as a clay mineral constituent.

More recent investigations include those by Ching Kwei Lee (1943) on the soils of China, by Sherman (1952) on Hawaiian soils, by Karim and Khan (1955) on Pakistan soils and by Dobritskaya (1960) on several Russian soils. The titanium distribution in soils has been proposed as an index for soil classification (Karim 1953).

We found very few references to the titanium content of Italian soils. There is some preliminary work by Comel (1953), an investigation by Pieruccini (1951) on some sediments of the Apennines and research by Bottini and Polesello (1954) on available and total titanium content of a series of Italian soils.

The Institute of Agricultural Chemistry of Palermo University has been interested for some time in the distribution of trace elements in Italian soils (Averna, Petronici et al. 1960, Lotti et al. 1966), and as an extension of this work we have determined the total titanium (and iron) content in a group of soils and clays of Sicily. The distribution of titanium and iron in some typical soil profiles was also estimated.

MATERIALS AND METHODS

The investigations were carried out on 70 Sicilian soils, sampled in the arable horizon, dried and passed through a 2 mm sieve.
The soils were derived from alluvial and saline sediments, from pliocene and miocene clays, from triassic limestones, from "argille scagliose" and from volcanic basaltic sediments of Mount Etna and Mount Lauro. The clay fractions less than 2 μ were separated from the soils by simple dispersion in distilled water.

### Table 1
TITANIUM AND IRON CONTENT OF SICILIAN SOILS

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<th>Fe/Ti</th>
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</table>

|     | **Triassic soils**     |      |       |       |       |
| 51  | Monreale               | 7.6  | 0.79  | 3.34  | 4.2   |
| 52  | Sciacca                | 8.2  | 0.63  | 3.73  | 5.9   |
| 53  | Misilmeri              | 7.5  | 0.73  | 3.84  | 5.2   |
| 54  | Pizzo Cervo            | 7.8  | 0.76  | 3.60  | 4.7   |
| 55  | Cinisi                 | 6.2  | 0.61  | 2.65  | 4.3   |
| 56  | Castellammare del Golfo| 7.7  | 0.65  | 3.75  | 5.7   |
| 57  | Monte Inici            | 7.8  | 0.56  | 2.74  | 4.9   |
| 58  | Borgetto               | 7.8  | 0.60  | 3.98  | 6.6   |
| 59  | Sagana                 | 7.8  | 0.56  | 3.47  | 6.2   |
| 60  | S. Stefano Quisquina   | 7.2  | 0.79  | 3.65  | 4.6   |

|     | **Volcanic soils**     |      |       |       |       |
| 61  | Trecastagni            | 6.2  | 1.31  | 6.88  | 5.2   |
| 62  | Randazzo               | 7.1  | 1.24  | 7.55  | 6.1   |
| 63  | Adriano                | 7.5  | 1.26  | 7.12  | 5.6   |
| 64  | Fornazzo               | 5.9  | 1.00  | 6.60  | 5.0   |
| 65  | Nicolosi               | 7.0  | 1.23  | 7.66  | 6.2   |
| 66  | Biancavilla            | 6.8  | 1.22  | 6.17  | 5.0   |
| 67  | S. Maria Licodia       | 7.1  | 1.34  | 5.84  | 4.3   |
| 68  | Bronte                 | 6.8  | 1.10  | 4.84  | 4.4   |
| 69  | Linguaglossa           | 7.6  | 1.38  | 6.10  | 4.4   |
| 70  | Buccheri               | 6.5  | 1.37  | 4.65  | 3.4   |

The soil profiles examined were: a profile of saline soil, two profiles of “terra rossas”, three profiles of brown soils on various parent materials (miocene clayey sediments, basalt and “argille scaglieise”) and a profile of mediterranean black soil.

Titanium and iron were determined after solution of the soil with hydrofluoric acid. The dry residue was then dissolved in dilute sulphuric acid and the iron in a suitable aliquot of the sample solution was deter-
mined by Margueritte's method, using permanganate titration. The titanium was then determined colorimetrically in the same solution by hydrogen peroxide oxidation (Weller 1898), reading the extinction of the yellow solutions at 420 nm against a calibration curve. The curve was prepared from standard titanium solutions, obtained from potassium titanium oxalate, and containing from 0.5 to 5 mg of titanium.

The cation exchange capacity (C.E.C.) of clays was determined on 1 g of material by a rapid complexometric method (Cecconi and Polesello 1955).

RESULTS AND DISCUSSION

The results of the titanium and iron determinations in soils of Sicily

<table>
<thead>
<tr>
<th>No.</th>
<th>Locality</th>
<th>Clay %</th>
<th>Analysis of the clay fraction</th>
<th>C.E.C. m.e./100g</th>
<th>Ti in clay</th>
<th>Ti in soil</th>
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</table>
overlying various parent materials are summarized in Table 1, together with the values of pH and Fe/Ti ratios.

These data show first of all that there are not very marked differences

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<tr>
<th>Soil profiles</th>
<th>Analysis of soil</th>
<th>Analysis of clay</th>
<th>Ti in clay</th>
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<td>Ti % Fe % Fe/Ti</td>
<td>%</td>
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<td>Red earth (Belmonte Mezzagno)</td>
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<td>6.3</td>
<td>0.69  5.66 8.2  1.5</td>
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<td>cm.  20 — 40</td>
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<td>7.6</td>
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</tr>
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<td>0.76  6.69 8.8  1.4</td>
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<td>cm.  80 — 100</td>
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<td>0.83  3.83 4.6  1.5</td>
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<td>Brown soil on basalt (Monte Lauro)</td>
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<td>7.3</td>
<td>0.98  9.77 9.9  0.9</td>
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<td>1.03  10.10 9.8  0.8</td>
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<td>0.77  3.66 4.7  1.6</td>
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<td>0.67  5.93 8.8  1.3</td>
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<td>cm.  40 — 60</td>
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<td>0.70  6.99 9.9  1.1</td>
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<td>cm.  60 — 80</td>
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<td>5.1</td>
<td>0.86  6.40 7.4  1.4</td>
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<td>5.4</td>
<td>0.88  6.44 7.5  1.5</td>
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</table>
in the titanium content of each group of soils except for the volcanic soils. The highest titanium content occurs—as others have already noted—in volcanic soils (on basalts and andesitic basalts), where it reaches a mean value of $1.21 \pm 0.07\%$, followed by the soils on triassic limestones ($0.66 \pm 0.06\%$), those on alluvial sediments ($0.53 \pm 0.08\%$), the saline soils ($0.52 \pm 0.03\%$), the soils derived from miocene clayey sediments ($0.41 \pm 0.07\%$), from pliocene sediments ($0.34 \pm 0.07\%$) and from “argille scagliose” ($0.34 \pm 0.06\%$). The iron content varies with the titanium content, as is clearly shown from the values of $Fe/Ti$ ratios, which move within narrow limits and vary between 4.6 and 6.9 for most of the soils.

An examination of the data reported in Table 2, representing the titanium and iron content of the clay fractions separated from a group of Sicilian soils, clearly indicates that clay fractions always show higher titanium content than the respective soils. This appears to be in agreement with the observations of Joffe and Pugh (1934) and indicates that titanium may be concentrated in the more dispersed fractions of the soil. This is presumably related to the slow discharge of the element from the insoluble compounds of the soil to a colloidal form during weathering.

The ratio of $Ti$ in clay to $Ti$ in soil was higher than 1 in all soils, varying between 1.1 and 1.7, except in the clay fractions separated from volcanic soils, where it was lower than 1. This means that in slightly weathered volcanic soils, titanium is present mainly in the mineral form in the well crystallized sandy fraction.

Our results show no relation between titanium content and cation-exchange capacity of soils.

Finally, Table 3 shows the results of the determinations of titanium (and iron) contents of some typical soil profiles of Sicily and of their clay fractions.

These results show that in the profiles of saline soil and of "terra rossas" the titanium content remains practically unchanged or shows a slight increase with the depth. In the brown soil profile originating on "argille scagliose" the titanium does not vary with depth, whereas in the profiles of the soils formed on miocene clayey sediments, slight increase of titanium occurs progressively in the lower horizons.

In the profile of the mediterranean black soil the accumulation of titanium in the lower horizons is more pronounced, though smaller than that described by Dobritskaya (1960) for podzolic soil profiles.

The clay fractions separated from lower horizons of the soils also show titanium contents equal or slightly higher than those in the clay fractions of the surface horizons.

In this case also, except in the basaltic soils, the values of the ratio $Ti$ in clay to $Ti$ in soil are higher than 1 varying between 1.1 and 1.9.

The present study has shown that titanium is widely distributed in soils of Sicily and is present in considerable quantities. Furthermore, the element shows a slight but not insignificant mobility, and thus there exists the possibility of its immobilization in the illuvial horizons.
TITANIUM CONTENT OF SOILS

REFERENCES


Ching Kwei Lee (1943) — Chemical characteristics of the great soil groups of China. Soil Sci. 55, 343-349.


Weller, P. H. (1898) — Some further applications of hydrogen peroxide to quantitative analysis. J. Am. chem. Soc. 20, 513-520.

SUMMARY

Titanium contents of the soils and clays of Sicily have been studied as part of a general survey of the distribution of minor elements in Italian soils.

The soils examined were developed from alluvial saline and recent sediments, from pliocene and miocene deposits, from "argille scagliose", from triassic limestones and from volcanic rocks.

The results show that titanium is widely distributed in Sicilian soils and has a mean content of 0.58 ± 0.18%. Clay fractions contain more titanium than the respective soils. The highest titanium content was found in the volcanic soils.
Further investigations on the vertical distribution of titanium in some typical soil profiles of Sicily have indicated that this element usually has a slight tendency to accumulate in the lower horizons.

**RéSUMÉ**

Dans le cadre des recherches sur la distribution des micro-éléments dans les sols italiens, les auteurs ont déterminé la teneur en titane des sols et des argiles de la Sicile.

Les sols examinés étaient développés de sédiments alluviaux et salins, de dépôts du Pliocène et du miocène, d’"argille scagliose", de calcaire du Trias et de roches volcaniques.

Les résultats obtenus ont montré que le titane est largement répandu dans les sols siciliens dont le contenu moyen pour cent est 0,58 ± 0,18. La fraction argileuse présente plus de titane dans les sols respectifs et la quantité plus élevée en titane se trouve dans les sols volcaniques.

Les investigations ultérieures sur la distribution verticale du titane des quelques profils typiques des sols de la Sicile ont indiqué qu’en général cet élément a une petite tendance à s’accumuler dans les horizons inférieurs.

**ZUSAMMENFASSUNG**

In Rahmen von Untersuchungen über die Verteilung von Spurenelementen in italienischen Böden haben die Verfasser den Titaniumgehalt von Böden und Tonen in Sizilien bestimmt.

Die untersuchten Böden haben sich auf rezenten Alluvial und Salzsedimenten, auf Sedimenten vom Pliozän und Miozän, auf "argille scagliose", auf triassischen Kalkgestein und auf vulkanischen Felsen gebildet.

Die erhaltenen Ergebnisse zeigen eine weite Verteilung des Titanium in sizilianischen Böden, mit einem mittleren Prozentgehalt von 0,58 ± 0,18 %. Die Tonfraktionen enthalten grossere Mengen von Titanium als die entsprechenden Böden und die vulkanischen Böden zeigen den höchsten Titaniumgehalt.

Weitere Untersuchungen über die Verteilung des Titaniuns mit der Tiefe in einigen Bodenprofilen Siciliens haben angedeutet dass das Element im allgemeinen eine leichte Tendenz zur Anhäufung in den tieferen Hori­

zonten zeigt.
OCCURRENCE OF CLAY MINERALS IN SOILS
AS RELATED TO FERTILITY

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The occurrence of clay minerals and their accessories in the principal soil types of the U.S.S.R., has been established by X-ray, thermal and chemical analyses and electronic microscopy of the <0.001 mm fraction. A description of the clay minerals in many soils of the U.S.S.R. follows below.

CLAY MINERALOGY

1. Soddy-podzolic soils on morainic and blanket loams. Hydromicas, vermiculite, chlorite and montmorillonite minerals in various ratios; non-silicate amorphous sesquioxides. Note that in these soils the hydromicas often form the main component, the occurrence of secondary minerals is irregular, and the coarse-fraction minerals are partly destroyed. The podzolic horizon is depleted, but the illuvial horizon is enriched with highly dispersed minerals and sesquioxides.

2. Soddy-podzolic soils on old massive-crystalline or well-drained sedimentary rocks. Hydromicas, kaolinite, montmorillonite minerals, chlorite, vermiculite and minerals of mixed-layer structure. Secondary minerals are distributed unevenly down the profile.

3. Grey forest soils on blanket loams. Montmorillonite minerals, hydromicas, vermiculite, sometimes with a little kaolinite, chlorite and mixed-layer minerals. There is an uneven distribution of secondary minerals down the profile.

4. Grey forest soils on granite eluvium. Hydromicas, kaolinite, montmorillonite minerals, vermiculite, and mixed-layer minerals.

5. Chernozems on blanket and loess-like loams. Montmorillonite minerals and hydromicas, sometimes a little chlorite. In these soils the distribution of secondary minerals down the profile is fairly even.


7. Alkaline soils (solonetz), of steppe and semi-desert zones, on loams and clays. Hydromicas, montmorillonite minerals, sometimes with a little kaolinite and chlorite. In the upper horizon more hydromica is found than montmorillonite, but in the solonetz horizon montmorillonite predominates. Distribution of secondary minerals down the profile is uneven.
8. Degraded solonetz (solod) among solonetz of steppe and semi-desert zones on loams and clays. Montmorillonite minerals, hydromicas, amorphous substances. The secondary minerals are more abundant in the upper layers of the profile, but their distribution down the profile is uneven.

9. Grey desert soils (sierozems) on loess-like loams and on loess. Hydromicas, montmorillonite minerals and palygorskite. The hydromicas are often dominant in the upper part of the profile.


11. Brown forest soils on loams and clays. Montmorillonite minerals, hydromicas, mixed-layer minerals, kaolinite, goethite and gibbsite. The highly-dispersed secondary minerals are unevenly distributed down the profile.

12. Brown forest soils on old massive-crystalline rocks. Montmorillonite minerals, hydromicas, kaolinite, with a little goethite and gibbsite. The secondary minerals are unevenly distributed down the profile.

13. Yellow soils on clay shales. Kaolinite, montmorillonite and hydromica minerals, together with goethite and gibbsite. The distribution of secondary minerals down the profile is often uneven.

14. Red soils on eluvium of andesite-basalts and other basic and intermediate rocks. Kaolinite minerals, with considerable goethite and gibbsite. The primary minerals are either badly weathered or absent.

15. Red soils on eluvium of granites and other acid rocks. Kaolinite, hydromicas and montmorillonites, with some goethite and gibbsite.


17. Lateritic soils on eluvium of basic and intermediate rocks. Kaolinite, goethite and gibbsite. The last two sometimes are dominant. The primary minerals are destroyed.

18. Lateritic soils on eluvium of granites and other acid rocks. Hydromicas, kaolinite with a little goethite and gibbsite. Some of the primary minerals are destroyed.


Relation of Clay Mineralogy to Soil Formation

Diectahedral and trioctahedral hydromicas occur most commonly in these soils, followed by minerals with an expanding lattice, such as montmorillonite and vermiculite. Kaolinite minerals (including kaolinite and halloysite) are also widely distributed, and are abundant in the soils of the humid tropics and subtropics (i.e. in red soils and laterites). Goethite and gibbsite also occur abundantly in these soils, though the goethite occurs chiefly on soils formed on basalts and andesite-basalts. Mixed-layer minerals observed include illite-montmorillonite, illite-vermiculite and illite-
kaolinite. Amorphous iron and aluminium hydroxides and fine-grained quartz are observed in nearly all of the soils.

There is no close relationship between soil type and observed clay mineralogy because the latter is also determined by many factors including climate, parent material, age, vegetation, topography, erosion, irrigation and human occupation. The particular influence of one or other of these factors can, however, be illustrated by the data given above.

The role of climate may be demonstrated by comparing mineralogical associations in soddy-podzolic and red soils. The former contain mostly hydromicas, sometimes montmorillonite and a little kaolinite. Red soils, developed on basalts, predominantly contain kaolinite and considerable amounts of gibbsite and goethite. Hydromicas and montmorillonite are usually absent from red soils and laterites.

In order to demonstrate the role of parent material in the formation of clay minerals, let us consider the red soils developed on basalts, granites and recent Quaternary deposits. The red soils on basalts predominantly contain kaolinite minerals and are especially high in goethite and gibbsite. There are no hydromicas in them. The red soils on granite, conversely, have considerable amounts of hydromicas in addition to kaolinite. Gibbsite is present in small amounts but there is no goethite because granites are low in iron. The red soils on recent Quaternary deposits contain much hydromica and montmorillonite, some kaolinite, and practically no gibbsite or goethite.

The abundance of kaolinite in red soils as against soddy-podzolics is explained by the abundance of warmth and moisture in the tropics and subtropics which contribute to the vigorous weathering of minerals and leaching of silica.

The absence of precise coincidence of mineralogical associations with soil types stems from the fact that clay minerals are the product of the same primary minerals: feldspars, muscovite, biotite, chlorite, amphiboles, pyroxenes. The stage-by-stage transformations of these minerals may be schematically presented as follows:

1. feldspars \(\rightarrow\) their complete destruction \(\rightarrow\) synthesis of sericite \(\rightarrow\) dioctahedral hydromica \(\rightarrow\) montmorillonite and kaolinite. This process may be accompanied by the formation of goethite, gibbsite, amorphous silica, and at the last stage of weathering, gibbsite, amorphous aluminium hydroxide and silica.

2. muscovite \(\rightarrow\) dioctahedral hydromica \(\rightarrow\) montmorillonite, kaolinite \(\rightarrow\) gibbsite, amorphous aluminium hydroxide and amorphous silica.

3. biotite \(\rightarrow\) trioctahedral hydromica \(\rightarrow\) vermiculite \(\rightarrow\) kaolinite \(\rightarrow\) gibbsite. This is accompanied by the formation of goethite and amorphous iron and aluminium hydroxides.

4. pyroxenes and other chain-structure minerals yield palygorskite.

Besides the above schemes, synthesis of clay minerals from the detritus of primary and secondary minerals is possible. Thus, amorphous silica and aluminium hydroxide are known to yield allophanes which in turn may
yield montmorillonite. Allophanes and montmorillonite are also formed from igneous amorphous substances, while amorphous iron and aluminium hydroxides respectively give rise to goethite and gibbsite.

The formation of clay minerals through structure simplification is not so complex as synthesis. The latter does occur in soils, though slowly. The formation of mixed-layer minerals is apparent in view of the incessant migration of colloids. In addition, the parent minerals are often a mixture of different, irregularly alternating minerals. The minerals themselves may form at different rates depending on local environment.

The data on mineralogical associations in soils may reveal their physico-chemical properties (hydrophility, exchange capacity, cohesion, water-permeability and fertility). Likewise the availability of essential plant nutrient elements is related to the resistance to weathering of different minerals. For example plants obtain potassium from soil solutions from the prior breakdown of micas and hydromicas; iron from amorphous iron hydroxide and goethite; magnesium from biotites. The strength of the chemical bonds within the mineral determines the availability of particular elements, and on this basis we may broadly classify minerals in three groups, according to their ability of "immediate supply, short-term reserve or potential reserve" of nutrients.

For minerals forming part of the "immediate supply" of nutritional elements, the cations are exchangeable cations, or alternatively the minerals themselves are soluble salts. Interlayer cations in hydromicas and micas are an example of nutrients supplied from a "short-term reserve mineral" in a soil. The primary minerals such as feldspars, pyroxenes and amphiboles are part of the "potential reserve" of nutrient elements in soils. These concepts will be increasingly important in future soil technology.

**Summary**

Associations of clay minerals in the principal soil types of the U.S.S.R. and schemes of transformation and synthesis of minerals are dwelt upon. The role of different factors, including climate, parent material, age, vegetation and human occupation in the formation of minerals is discussed.

According to the strength of the bonds between nutrient elements and minerals and the subsequent availability of cations to plants, the minerals are divided into three groups according to their ability of "immediate supply, short-term reserve or potential reserve" of nutrients.

**Résumé**

On a étudié les associations des minéraux des argiles dans les principaux types de sol dans l'U.R.S.S. et des systèmes de transformation et de synthèse de minéraux. Le rôle de différent facteurs: le climat, les matériaux d'origine, l'âge, la végétation, l'anthroposéquence, dans la formation de minéraux est éclairci.

D’après la force de connexions entre les éléments de cendre et les minéraux et la disponibilité des cations aux plantes, les cations sont
divisés en trois groupes: la réserve la plus immédiate, la réserve immédiate, et la réserve potentielle.

ZUSAMMENFASSUNG


MINERALOGICAL AND CHEMICAL CHARACTERISTICS OF A GLEY SOIL FROM NORTH-EAST SCOTLAND

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Studies on the mineralogy of soil clays in relation to soil formation and classification are usually based on total mineral composition of the clay separated from the various horizons of the profile (see, for example, Jackson 1959, Mitchell 1962, Zaydelman and Ogleznev 1965, Mitchell et al. 1964). However, only a few attempts have been made to analyse for, and to seek relationships between, the minerals present in certain morphological features within horizons upon which field differentiation at certain categorical levels is based (Brown 1953, 1954). In this investigation an attempt has been made to correlate specific features of a gley soil to mineralogy and chemistry.

I. MATERIALS AND METHODS

A surface-water gley soil developed on varved lacustrine clay (Tipperty Association — Glentworth and Muir, 1962) exhibiting a strongly gley and seasonally waterlogged A\textsubscript{lg} horizon immediately beneath the surface horizon was selected. The intensity of gleying diminished with depth and the red-brown colour of the parent material was better expressed in the B\textsubscript{g} and C\textsubscript{g} horizons. Large cleavages with grey surface coatings are features of these lower horizons and similar but thinner coatings cover parts of the coarse prismatic structural peds. Ochreous mottles are also characteristic of the surfaces of the structural units of the B\textsubscript{g} horizon, indicating an oxidation stage. In the C\textsubscript{g} horizon such mottles are much less evident since anaerobic conditions prevail throughout the greater part of the year and thus the reduction phase of the gleying process predominates. Consequently, sampling was confined to the C\textsubscript{g} horizon—in particular to the grey coatings representing the first observable stage of gleying and to the underlying red-brown material which is virtually the undifferentiated parent material. The marked colour difference between these two materials permitted their effective separation.

The particle size distribution within the two samples was determined by the pipette method (Piper 1950) using O IN NaOH. The clay fraction (<1.4 \( \mu \)) was obtained by dispersing the soil with ammonia according to the method of Mackenzie (1956). Before treatment with 6% hydrogen
peroxide to remove organic matter the clays were saturated with ammonia using neutral normal ammonium acetate to prevent the formation of calcium oxalate (Farmer and Mitchell 1963). After washing with water, the clays were again NH₄-saturated and then equilibrated at 56% relative humidity for four days.

The proportion of poorly ordered alumino-silicate in the clays was determined by treatment with Na₂CO₃ solution (Follett et al. 1965a) and this was followed by assessment of the "free" iron oxide content using the dithionite procedure of Mitchell and Mackenzie (1954). The fluoride exchange test of Fieldes and Perrott (1966) in which OH⁻ pass into solution through the action of F⁻ was carried out on the original clays and on the residues after completion of these treatments. Suspensions of 20 mg clay per ml of 1·0 M sodium fluoride were used and the pH, which increased due to OH⁻ ion release, was measured at intervals for thirty minutes.

The X-ray diffraction patterns of the clays were obtained with a modified Debye-Scherrer powder camera, and the electron micrographs with an A.E.I. EM 6 instrument. The differential thermal curves were determined in nitrogen with a controlled-atmosphere differential thermal apparatus. The infra-red absorption spectra were obtained from potassium bromide pressed discs using a Grubb Parsons S4 double-beam spectrometer equipped with a grating for the 3 μ region and a sodium chloride prism for the 5-16 μ region. The micro method of Mackenzie (1951) was used to determine the cation-exchange capacity of the clays and the specific surface area was measured by the nitrogen absorption tehnique of Brunauer et al. (1938).

II. RESULTS AND INTERPRETATION
(a) Mechanical Analysis

The mechanical analyses of the red-brown and grey materials are similar as far as the sand, silt and clay fractions are concerned, coming within the limits of the soil textural class of sandy clay. The particle size distribution within the clay fraction of the two samples is, however, significantly different (Table 1).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Red-brown clay</th>
<th>Gleyed clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0·3 μ</td>
<td>47·3</td>
<td>25·6</td>
</tr>
<tr>
<td>0·3—0·5 μ</td>
<td>9·7</td>
<td>17·7</td>
</tr>
<tr>
<td>0·5—0·8 μ</td>
<td>15·0</td>
<td>17·0</td>
</tr>
<tr>
<td>0·8—1·2 μ</td>
<td>8·2</td>
<td>9·2</td>
</tr>
<tr>
<td>1·2—2 μ</td>
<td>19·8</td>
<td>30·5</td>
</tr>
</tbody>
</table>

In particular, the clay fraction of the gleyed material has only 25% of the particles < 0·3 μ as compared with 50% in the red-brown. Characteri-
zation of the fine clay fraction, therefore, appears essential to the elucidation of the gleying process in this soil.

Since fine clay may be lost in normal centrifuging and because the Tipperty Association clay samples are finely particulate and consist of hydrous mica, high speed centrifuging (12,500 r.p.m.) was adopted in the sodium carbonate and dithionite treatments.

(b) Chemical Treatments

The total chemical analyses of the red-brown and grey samples (Table 2) show that they have the same silica to alumina ratio (3.1:1) but the former contain twice as much ferric oxide. The ferrous oxide content is virtually the same for both. The slightly higher potassium content (4.36%) of the grey material is consistent with the lower content of finely particulate clay. In the fluoride-exchange tests the pH rose in 30 minutes from 7.4 to 8.8 for the original red-brown and to 9.05 for the gleyed material; the corresponding figure for Fithian illite was 7.95. The differences are shown in the curves of $OH^-$ ion concentration against time given in Figure 1 (curves a, b and c).

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>TOTAL CHEMICAL ANALYSIS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red-brown clay</td>
</tr>
<tr>
<td>$SiO_2$</td>
<td>43.72</td>
</tr>
<tr>
<td>$Al_2O_3$</td>
<td>23.82</td>
</tr>
<tr>
<td>$Fe_2O_3$</td>
<td>8.10</td>
</tr>
<tr>
<td>$FeO$</td>
<td>1.33</td>
</tr>
<tr>
<td>$TiO_2$</td>
<td>1.47</td>
</tr>
<tr>
<td>$MnO_2$</td>
<td>0.00</td>
</tr>
<tr>
<td>$CaO$</td>
<td>0.65</td>
</tr>
<tr>
<td>$MgO$</td>
<td>4.06</td>
</tr>
<tr>
<td>$Na_2O$</td>
<td>0.27</td>
</tr>
<tr>
<td>$K_2O$</td>
<td>3.72</td>
</tr>
<tr>
<td>$H_2O$ (105°C)</td>
<td>4.26</td>
</tr>
<tr>
<td>$H_2O$ (300°C)</td>
<td>1.30</td>
</tr>
<tr>
<td>L.O.I. (1000°C)</td>
<td>7.22</td>
</tr>
<tr>
<td></td>
<td>99.92</td>
</tr>
</tbody>
</table>

The amounts of silica and alumina extracted by three cold $Na_2CO_3$ treatments and seven subsequent hot carbonate digestions on the steam bath are given in Table 3. The gleyed material contains about twice as much silica extractable by cold sodium carbonate as the red-brown sample but the amount of “readily soluble” alumina (2.3%) is the same. The residues from this treatment gave, in the fluoride-exchange test, a pH rise to only 7.8 in 30 minutes for both types of clay (Figure 1, curve d). Digestion with hot sodium carbonate (following treatment with cold sodium carbonate) removes about 14% and 15.5% of silica and alumina, respectively, from both samples implying that the treatment has affected the same component in both. This “less readily soluble” alumino-silicate fraction is
larger than that extracted with cold sodium carbonate and it is slightly less siliceous ($SiO_2:Al_2O_3 = 2.9:1$) than the total clay. The residues from the hot sodium carbonate treatment again gave, in the fluoride-exchange tests, increases of $pH$ to 7.8 in 30 minutes for both types of clay.

Following treatment with cold and hot sodium carbonate solution three dithionite treatments were required to bleach the red-brown clay; the gleyed sample was similarly treated (Table 4). The $SiO_2:Al_2O_3$ ratios of the residues are 3.0:1 and 3.2:1 for the red-brown and grey clays, respectively, and this is consistent with the higher quartz content of the latter.
TABLE 3
PROPORTIONS OF TOTAL SILICA AND ALUMINA EXTRACTED BY COLD AND HOT SODIUM CARBONATE SOLUTION (RELATIVE TO COMPOSITION OF TOTAL CLAY)

<table>
<thead>
<tr>
<th>Clay Type</th>
<th>Cold SiO₂ %</th>
<th>Cold Al₂O₃ %</th>
<th>SiO₂/Al₂O₃</th>
<th>Hot SiO₂ %</th>
<th>Hot Al₂O₃ %</th>
<th>SiO₂/Al₂O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-brown clay</td>
<td>1.9</td>
<td>2.3</td>
<td>2.6</td>
<td>13.6</td>
<td>15.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Gleyed clay</td>
<td>3.4</td>
<td>2.3</td>
<td>4.4</td>
<td>14.6</td>
<td>15.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

From 2 to 5% Fe₂O₃ in the samples is unaffected by the dithionite treatment and presumably is retained in the clay mineral lattice. The aluminosilicate removed with the dithionite extractable iron oxide from the undifferentiated clay has a SiO₂:Al₂O₃ ratio of 5.5:1 whereas that from the gleyed material is much more aluminous (SiO₂:Al₂O₃ — 0.8:1), and the composition of the two extracts corresponds approximately to Fe₁₅Al₂Si₅-O₃₅ and Fe₆Al₁₂Si₅-O₃₇ for the red-brown and gleyed material respectively. In the fluoride-exchange tests, the pH rose to 7.8 and 7.9 in 30 minutes for the residues from the dithionite extraction of the red-brown and gleyed material, respectively.

TABLE 4
PROPORTIONS OF TOTAL SILICA, ALUMINA AND FERRIC OXIDE EXTRACTED BY THREE DITHIONITE TREATMENTS (RELATIVE TO COMPOSITION OF TOTAL CLAY) AND COMPOSITION OF RESIDUE AFTER TREATMENT

<table>
<thead>
<tr>
<th>Clay Type</th>
<th>Na₂S₂O₄ extract SiO₂ %</th>
<th>Na₂S₂O₄ extract Al₂O₃ %</th>
<th>Na₂S₂O₄ extract Fe₂O₃ %</th>
<th>Residue Al₂O₃ %</th>
<th>Residue Fe₂O₃ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-brown clay</td>
<td>4.6</td>
<td>2.6</td>
<td>82.3</td>
<td>44.6</td>
<td>25.0</td>
</tr>
<tr>
<td>Gleyed clay</td>
<td>2.2</td>
<td>8.2</td>
<td>27.5</td>
<td>44.5</td>
<td>23.8</td>
</tr>
</tbody>
</table>

(c) Instrumental Techniques
Randomly orientated Ca-saturated specimens and glycerol-treated pressure aggregates of the two samples before and after treatment with alkali and dithionite were examined by X-ray diffraction using filtered Co Kα radiation. No changes were observed in the basal reflections upon treatment with glycerol. Dioctahedral mica is the dominant mineral in the red-brown clay but some trioctahedral mica is also present. The dioctahedral is indicated by sharp basal reflections and relatively strong reflections at 1.99-2.00 Å in addition to an 006 reflection at 1.5 Å. The trioctahedral component manifests itself in an 006 reflection at 1.534-1.535 Å. There is a small amount of well-crystallized kaolinite. The principal accessory minerals are quartz and hematite; a sharp reflection at 3.033 Å can probably be attributed to a low-plagioclase felspar. The crystalline clay mineral content of the gleyed material is more or less identical except that it contains slightly more quartz and the reflections arising from hematite are absent. The
diffraction pattern of the residue from the red-brown clay treated with alkali-dithionite indicates the complete removal of hematite. However, there is no evidence from this or from the diffraction pattern of the gleyed material that the chemical pretreatment has affected the crystalline clay minerals.

The differential thermal curves substantiate the hydrous mica character of the samples and since there is no evidence of an endothermic peak in the region of 300°C, neither goethite nor lepidocrocite are present.

Electron microscopy indicates that the red-brown and gleyed clays consist of finely particulate and, in general, discrete irregularly-shaped particles. The only distinguishing features between the two clays are the particles labelled H and A (Figure 2a). These are found most frequently in the red-brown clay and only occasionally in the gleyed clay; they are not observable in the alkali-dithionite treated clays. The morphological features labelled H (Figures 2a, b) occur for the most part as fragmentary and eroded particles, but where erosion is negligible or nil they exhibit sharp hexagonal edges (Friend 1966, Mackenzie and Meldau 1967). Most of these particles, even the eroded, give strong single-crystal electron diffraction patterns consisting of a hexagonal array of spots (Figure 2c). Such patterns are consistent with that for a hematite crystal lying with 001 parallel to the beam. The spotty rings on the pattern arise from hydrous mica flakes underlying the hematite crystal.

The feature marked A (Figure 2a) appears to be an aggregate of tiny acicular crystals and could represent a more advanced stage of erosion of a hematite crystal. Judging from the generally eroded appearance of the hematite crystals dissolution of iron oxide is taking place even in the
Characteristics of a gley soil

Fig. 2.—Electron micrographs of (a) red-brown clay showing hydrous mica and hematite crystals; (b) well-shaped but eroded hematite crystal, and (c) electron diffraction pattern of hematite crystal.

Apparently ungleyed material. There was no indication of electron dense granules such as those described by Follett et al. (1965 b) and considered to be a ferruginous phase.

The infra-red absorption spectra of the clays provide further evidence
of the close similarity in mineralogical composition. In addition to the slightly higher quartz content of the gleyed material there is indication of a small amount of disordered alumino-silicate. The $< 0.3 \mu$ fraction from both samples was also examined and gave identical spectra—quartz was absent and the kaolin content was less than that in the total clay.

(d) Specific Surface Area

The slightly higher content of finely particulate material in the red-brown clay compared to that of the gleyed clay is reflected in the specific surface area values which are 41 and 38 m$^2$/g, respectively, and following alkali-dithionite treatment increase to 67 and 62 m$^2$/g, respectively. The increase could be associated simply with improved dispersion. However, exposure of approximately 1% suspensions of the original clays to a high intensity ultrasonic field (Kerry Vibrason, titanium velocity transformer, 20 KC/S, power 100 watts) for ten minutes resulted in specific surface areas of 75 and 63 m$^2$/g for the red-brown and gleyed clays, suggesting that this treatment reduced the degree of aggregation. This was confirmed by electron microscopy. Sharpening of the infra-red spectrum substantiated the improvement in dispersion but gave no indication of an increase in the amount of amorphous material. Ultrasonic treatment, therefore, apparently did not induce a breakdown of the crystalline clay minerals and it is assumed that the specific surface areas following this treatment represent ultimate dispersion. Thus the results would indicate that alkali-dithionite treatment not only improves dispersion but also removes, particularly from the red-brown clay, material of relatively high specific surface area.

(e) Cation-exchange Capacity

Table 5 gives the cation-exchange capacity of the clays, both the original values and those after various treatments. Values for the red-brown clay, irrespective of treatment, are invariably higher than those for the corresponding gleyed material and this consistent difference can most probably be accounted for by the higher content of very finely particulate material in the red-brown clay. Treatment with hot Na$_2$CO$_3$ solution removes alumino-silicate of higher exchange capacity. However, treatment with dithionite is accompanied by an increase in exchange capacity, presumably since this involves essentially the removal of hematite which has a very low exchange value.

| TABLE 5
| CATION-EXCHANGE CAPACITY BEFORE AND AFTER VARIOUS TREATMENTS (me/100g) |
|---------------------------------|------------------|------------------|
| Untreated                       | 30·0             | 28·9             |
| After hot Na$_2$CO$_3$ treatment | 26·7             | 26·1             |
| After Na$_2$CO$_3$ + Na$_2$S$_2$O$_4$ treatment | 28·4             | 27·2             |
| After ultrasonic treatment      | 31·9             | 31·3             |
III. DISCUSSION AND CONCLUSIONS

The gleying process in the simplest terms involves the reduction of ferric iron to the ferrous state under anaerobic conditions induced by waterlogging. The latter is leached from the profile by lateral or ground water in either ionic form or as part of an organic complex (Bloomfield 1950, 1951, Zaydelman and Ogleznev 1965). However, certain bacteria capable of reducing ferric compounds in various media have been isolated from soil and grass and have been shown to dissolve and reduce not only “free” ferric oxide but also other insoluble ferric compounds (Bromfield 1954). The depth from which the samples were taken for this investigation (approximately 4 feet) is such that bacterial action is most likely to be the principal agent in the gleying process.

The X-ray and DTA results gave no indication of the presence of hydrous iron oxides. Hematite was the only crystalline iron oxide identified but as the dithionite extracts contained both silica and alumina, part of the “free” iron oxide may be associated with these two components. Between 2 and 5% oxide is unaffected by the gleying process and must be an integral part of the clay mineral lattice. The amount of ferrous oxide in the two samples is low and virtually the same (Table 2) and while part of this may be sorbed on the clays it is more likely that this component is also fixed in the clay mineral lattice.

The dissolution of hematite accounts, in part, for this loss of finely particulate material during gleying since the largest particles of hematite observed were about 0.5 μ but most were fragments less than half that size. Even allowing that the “free” iron oxide is exclusively in the form of finely particulate hematite this would account for < 30% of the fine fraction of the undifferentiated clay. X-ray diffraction results indicate that gleying produces no observable changes in the crystalline clay mineral complement. This is not altogether surprising as hydrous mica is the principal clay mineral present and there is considerable evidence from studies on temperate soils to suggest that this clay mineral, if not an end product, is at least a very stable stage in pedological weathering (Mitchell 1962). The finest fraction of both samples, from infra-red absorption evidence, is devoid of quartz and low in kaolin. Thus, from instrumental techniques, there is no evidence that crystalline clay minerals, irrespective of particle size, are removed by gleying from the Cg horizon of the profile.

The results from chemical dissolution techniques point to the presence of a small amount of readily soluble aluminosilicate and since the cold carbonate extract from the gleyed sample is relatively high in silica, aluminous material has presumably been removed from a poorly ordered component during the gleying process. Since the amount and composition of the aluminosilicate removed by hot sodium carbonate digestion for the red-brown and gleyed material is similar this component must be unaffected by gleying.

Although the ultimate analyses of the residues following dithionite treatment are similar, the composition of the extracts is markedly different, that from the gleyed sample containing much more aluminous material than that
from the relatively undifferentiated parent material clay. The gleying process therefore appears to involve principally iron oxide and alumina. From electron microscopy there is evidence of eroded hematite crystals, but as mentioned above (Follett et al. 1965b) no indication of a ferruginous phase. However, cation-exchange capacity and surface area results support the concept that, like the "readily soluble" and "less readily soluble" aluminosilicate fractions, part of this dithionite-extractable material may also be present as a coating on crystalline components. The iron oxide in this coating together with the "free" hematite is removed from the system by the gleying process. The latter, however, only accounts for part of the finely particulate material lost through this process. The aluminous material extracted by cold sodium carbonate may make up the remainder. The fluoride-exchange tests, in which \( \text{OH}^- \) ions pass into solution through the action of \( \text{F}^- \) ions, indicate that this type of chemical reactivity is closely associated with the material removed by cold sodium carbonate. The rate of reaction in the residues was only of the order of that of the hydrous mica clay mineral itself. In the original clays, the greater reactivity of the gleyed material may reflect the loss of the finely particulate components in the gleying process, exposing previously inaccessible active sites.

The results of this investigation have a practical significance inasmuch as the poor internal drainage of the profile has induced in certain horizons, general, and in others, localized intense, gleying. Although this has apparently brought about only minor changes in the bulk mineralogy there is evidence of significant differences in surface properties.

IV. Acknowledgments

The authors wish to thank Dr. V. C. Farmer for the infra-red absorption determinations.

V. References

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Bloomfield, C. B. (1951) — *J. Soil Sci.* 2, 196-211.
CHARACTERISTICS OF A GLEY SOIL

Summary

Certain morphological features of a surface-water, non-calcareous gley soil have been studied in relation to mineralogical and chemical composition. Chemical dissolution techniques were employed and the effects of these on pedologically unweathered material and on completely gleyed material have been followed by differential thermal analyses, X-ray diffraction, infra-red absorption and electron-microscopic examination. Measurements have also been made on specific surface area, cation-exchange capacity and reactivity to fluoride ion. The results indicate that although the gley process has induced only minor changes in the bulk mineralogy it has produced significant changes in the surface properties.

Zusammenfassung

Es wurden gewisse morphologische Eigenschaften eines Oberflächenbewässerten, nichtkalkhaltigen Gleys in Beziehung zur mineralogischen und chemischen Zusammensetzung studiert. Chemische Auflösungsmethoden wurden angewendet und deren Wirkungen auf die pedologisch-unverwitterten und die vollkommen vergleyten Stoffe wurden durch verschiedenartige Wärmeanalysen, Röntgen-Diffraktion, infrarote Absorption und durch elektronenmikroskopische Untersuchungen verfolgt. Es wurden auch Messungen der spezifischen Oberfläche, der Kation-Austauschkapazität und der Reaktivität zu Fluorid-ion unternommen.

Die Ergebnisse zeigen, dass, obwohl der Vergleyungs-Prozess nur wenige Umgestaltungen in der totalen Mineralogie veranlasste,—er doch wichtige Veränderungen in den Oberflächen-Eigenschaften erzeugte.
CONSIDÉRATIONS SUR LES NOTIONS DE STABILITÉ ET D'INSTABILITÉ DES MINÉRAUX EN FONCTION DES CONDITIONS DU MILIEU; ESSAI DE CLASSIFICATION DES "SYSTÈMES D'AGRESSION"

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INTRODUCTION

Dans les études relatives aux problèmes d'altération, on fait beaucoup état en général des questions de "stabilité" ou de "fragilité" des minéraux. C'est ainsi que divers auteurs, en s'appuyant sur l'examen de nombreuses formations exogènes, sont arrivés à proposer des échelles de résistance ou de persistance (Goldich, 1938, Pettijohn, 1941, Jackson et al. 1948, 1952). De telles séquences sont d'ailleurs devenues classiques, notamment dans le cas des silicates, car elles peuvent à première vue être explicitées à partir de simples considérations structurales: degré de liaison des tétraèdres de silice, taux de substitution Si-Al en positions tétraédriques, degré de basicité (cf. Grüner, 1950, Keller, 1954 entre autres). De ce fait, on a été amené, plus ou moins implicitement jusqu'ici, à considérer la stabilité comme une caractéristique intrinsèque des minéraux.

En réalité, diverses séries d'expériences effectuées depuis quelques temps, à relativement basse température, montrent incontestablement qu'en présence de certaines conditions, l'évolution de minéraux réputés très stables, comme la muscovite par exemple, se révèle possible tandis que celle de constituants réputés plus sensibles, tels les plagioclases calciques, apparaît difficile, si ce n'est même pratiquement impossible.

Il ressort ainsi de ces quelques éléments que la notion de résistance ou de stabilité d'un minéral semble dépendre non seulement de caractéristiques structurales, mais encore de facteurs variés, et en particulier de la forme d'agression. On ne peut donc plus continuer à la considérer comme une donnée absolue et immuable; d'où la nécessité d'envisager maintenant les problèmes d'altération en fonction de ce qu'on peut appeler le système "minéral—structure d'agression".

C'est ce que nous allons tenter de justifier à partir d'un certain nombre d'exemples qui permettront de définir quelques archétypes que l'on pourra considérer ensuite comme des modèles de référence.

Auparavant, il faut considérer brièvement la constitution cristallochimique des minéraux et en présenter les types fondamentaux. On peut, à cet effet, distinguer 2 grands ensembles :

— D'une part, les "espèces chimiques cristallisées" (sels classiques, oxydes, hydroxydes ...) qui sont des composés simples, à structure de type
ionique et pour lesquels, dans tout l’assemblage, il n’existe qu’un type de liaison entre le cation et l’anion (anion simple ou oxyanion). De tels composés peuvent être le siège, vis à vis du milieu ambiant, de réactions de décomposition de plusieurs sortes : phénomènes de dissolution ou réactions de double décomposition lorsqu’ils sont solubles dans l’eau, processus de solubilisation sous l’influence d’agents divers : acides, bases . . . lorsqu’ils sont insolubles. Mais, dans tous les cas, ils réagissent de façon simple, c’est-à-dire avec dissociation stoechiométrique des anions et cations, et sans laisser de carcasse solide mal définie à l’issue de l’attaque.

— A l’opposé, il existe un second ensemble, formé d’espèces connues uniquement à l’état solide cristallisé et qu’on peut, de ce fait, désigner sous le terme général d’“espèces réticulaires” ; il s’agit notamment des phosphates, borates . . . et surtout des silicates, qui ont une constitution complexe et une structure mettant en jeu plusieurs liaisons différentes par leur nature ; dans les silicates en particulier, la carcasse est constituée par un assemblage de tétraèdres de silice (ou Al) enchainant d’autres polyèdres d’anions (octaèdres d’aluminium, fer, magnésium . . .) et pouvant emprisonner encore des éléments à l’état de cations simples (cations alcalins ou alcalino-terreux).

On peut distinguer ainsi, au sein des structures caractérisant les espèces réticulaires, 3 principaux types d’éléments :
— des anions condensés et polynucléaires : ce sont les divers anions silicatés ou alumino-silicatés.
— des anions structuraux, qui sont à la base des agencements octaédriques : Al, Fe(III), Fe(II), Mg, Ti(IV).
— enfin, des cations compensateurs de charge tels les cations basiques : Na, K, et Ca.

Or, de tels édifices réticulaires, qui sont toujours insolubles, réagissent aux conditions du milieu suivant des schémas qui ne peuvent s’exprimer en termes classiques, comme cela a été fait dans le cas précédent. Il faut donc examiner le problème avec plus de détail, et ce d’autant, que les silicates par exemple forment les constituants majeurs de la lithosphère.

A l’heure actuelle, l’évolution des “espèces réticulaires” semble pouvoir se concevoir grâce au développement, grosso modo, de 3 types de réactions :
1) Réactions physicochimiques classiques comportant essentiellement la mise en Œuvre de protons (hydrolyse et attaque acidolytique).
2) Réactions provoquées par la présence de réactifs solubles amenant, soit par déplacement, soit par précipitation, la concentration d’un ou de plusieurs éléments en dessous du seuil où il y a équilibre entre le solide et la solution.
3) Réactions du même type, mais résultant de l’intervention de corps ayant une structure rigide de réception (racines, résines, minéraux).

I. RÉACTIONS CLASSIQUES DE L’ALTÉRATION (AVEC MISE EN ŒUVRE DE PROTONS)

Ces réactions sont, en général, les seules dont on fasse état habituellement dans les études sur la décomposition des minéraux ; aussi sont-elles,
à première vue, les mieux connues. Nous les passerons donc simplement en revue dans cette note, en mettant uniquement l’accent sur les principales directions de l’évolution.

On peut envisager dans ce cas, 2 formes d’attaque :
— L’attaque en conditions acides.
— L’attaque par de l’eau pure (hydrolyse).

a) Décomposition acidolytique

Les anions qui provoquent un abaissement du pH du milieu et qui fournissent en même temps un vecteur susceptible d’entrainer les éléments passés en solution, constituent en effet toute une catégorie d’agents d’agression.* Mais, pour pouvoir se développer convenablement un tel mode d’action implique, soit une concentration en protons relativement importante au départ (évolution en système fermé), soit dans le cas plus général de solutions diluées, une circulation du liquide d’altération de façon à déplacer continuellement la réaction (évolution en système ouvert).

Quoiqu’il en soit, dans ces conditions, tous les éléments solubles dans le milieu d’attaque, c’est-à-dire l’ensemble des cations (structuraux et compensateurs) quitteront alors plus ou moins rapidement la carcasse du minéral, alors que le squelette anionique, relativement dégradé, persistera en général.

Le cas le plus typique de ce genre d’action différentielle est celui représenté par l’altération expérimentale des silicates phylliteux en présence de solutions acides (Murata, 1946, Méring, 1949, Brindley et Youell, 1951, Gastuche et al., Arvieu et Chaussidon, 1964, Polzer et Hem, 1965 ...). Ces différents auteurs ont constaté que, suivant les règles d’une cinétique chimique relativement simple, les couches octaédriques des montmorillonites, chlorites, kaolinites, micas ... se trouvent solubilisées alors que s’accumule sur place un résidu enrichi en silice, qui semble même, dans certains cas, se souvenir de la structure primitive (cf. Méring, loc. cit).

Les recherches dues à Pedro, 1964, Pedro et Bitar, 1966, Pedro et Iniguez, 1967, qui comportaient toutes une étude de l’action de l’acide acétique sur différentes roches en drainage continu, ont d’ailleurs permis de vérifier ce bilan à un niveau plus global et de le situer, pédogénétiquement, dans l’optique d’une évolution de type podzolisante. Toutefois, lorsqu’il y a juxtaposition de minéraux de fragilité inégale, le résultat peut se présenter un peu différemment car la solubilisation des édifices les plus sensibles à la présence d’un milieu acide protège en quelque sorte les autres constituants, les sesquioxydes par exemple, qui auraient normalement dû être évacués dans de telles conditions (cf. Pedro et Bitar, loc. cit.).

Cette dernière remarque montre ainsi que, même pour des conditions d’altération définies, il n’existe pas un seul type de comportement caractérisant un minéral donné; tout dépend encore une fois du “système”, qui est le siège de l’évolution, défini dans son ensemble.

b) Découpage hydrolytique

C'est le cas le plus général à la surface du globe et, de ce fait, le plus étudié. Nous avons vu que les diverses espèces réticulaires étaient insolubles dans l'eau au sens strict du terme; toutefois, si l'on renouvelle constamment, ou même fréquemment, l'eau au contact des minéraux (et ceci est indispensable dans ce cas), on peut mettre en évidence une certaine solubilisation. Celle-ci résulte essentiellement d'un phénomène d'hydrolyse, puisque ce sont les ions de l'eau et en particulier les protons qui sont à l'origine de la rupture d'un certain nombre de liaisons : les éléments les moins tenus, tels les cations compensateurs de charge (alcalins ...), passent alors en solution et servent en quelque sorte de vecteurs à d'autres éléments, comme la silice, qui présentent un comportement anionique; seuls s'accumuleront donc sur place les cations structuraux des sesquioxydes qui, à la limite, comme dans la latéritisation par exemple, seront les constituants essentiels du résidu d'altération (cf. Pedro, 1964, Pedro et Berrier, 1966). Le bilan de l'hydrolyse s'établit ainsi d'une façon fort différente de celui résultant d'une attaque acidolytique.

Il faut ajouter enfin que les modifications du pH sont également susceptibles de provoquer la mise en solution de certains cations, notamment ceux présentant plusieurs degrés d'oxydation, et de participer ainsi d'une manière différentielle à l'altération des minéraux plus ou moins complexes.

En définitive, ces formes classiques de l'altération interviennent toujours sur 2 des 3 catégories d'éléments constituant les espèces réticulaires : cations structuraux et compensateurs dans le cas de l'attaque acidolytique (évolution podzolisante), anions silicatés et cations compensateurs pour la décomposition hydrolytique (évolution siallitisante et latéritisante). Mais ne pourrait-on pas aussi imaginer des processus d'attaque encore plus spécifiques, c'est-à-dire agissant soit sur une seule catégorie d'éléments constitutifs, soit même sur un élément bien déterminé. C'est ce que nous allons envisager en nous basant sur les 2 modèles réactionnels suivants.

II. Réactions provoquées sous l'Effet de Composés Chimiques Divers

Un certain nombre de composés chimiques, lorsqu'ils se présentent à l'état dissous, sont susceptibles d'être à l'origine de réactions de dégradation des minéraux par suite de l'établissement, dans le milieu, d'un gradient élevé vis-à-vis d'un type d'élément. Ce gradient, qui provoque un déplacement continu de l'équilibre dans le milieu de réaction, peut alors réserver,

— soit de la présence d'une certaine concentration en un ion donné (utilisation de sels solubles et réactions du type "échange");

— soit de la formation de composés peu ionisés qui peuvent d'ailleurs être solubles (complexes) ou insolubles (précipités classiques ou édifices réticulaires de néogénèse).

Aussi, ces réactifs d'attaque, apparemment inoffensifs, sont-ils capables de provoquer des actions moins globales et plus spécifiques que celles
résultant de l’hydrolyse et de l’acidolyse. Mais on assiste alors toujours à une sorte de compétition entre la force d’extraction propre au réactif vis-à-vis de tel ou tel élément et la force de rétention caractérisant la liaison de ces mêmes éléments au sein du réseau des minéraux. Nous allons d’ailleurs nous en rendre compte tout de suite à travers les données résultant d’un certain nombre d’études expérimentales et portant respectivement sur les 3 catégories d’éléments.

a) Comportement des cations structuraux

Des essais sur l’extraction sélective des éléments des sesquioxydes ont été tentés récemment à partir de roches aluminosilicatées et en utilisant des réactifs classiques, tels que l’”aluminon” en ce qui concerne Al et le “ferri cyanure de potassium” pour Fe. Ils n’ont abouti à aucune extraction des ions en question; c’est plutôt le réactif qui s’est fixé sur le minéral : il y a donc, dans ce cas, adsorption et non agressivité. Ce comportement se généralise d’ailleurs à d’autres composés moins spécifiques, les acides phénoliques en particulier (acides pyrogalliques).

Mais, d’un autre côté, l’utilisation de sels classiques dont les anions sont “actifs”, tels les phosphates, s’est révélée efficace dans la dégradation des oxydes et hydroxydes cristallisés de fer et d’aluminium, qui sont pourtant stables dans les conditions normales de la surface du globe. En effet, mis au contact de diverses solutions phosphatées, ces minéraux peuvent être aisément solubilisés avec formation correlative d’espèces cristallines, telles la variscite AlPO$_4$, 2H$_2$O, la strengite FePO$_4$, 2H$_2$O, la barandite (AlFe)PO$_4$, 2H$_2$O ou encore la tarakanite (palmité) (cf. Haseman et al., 1950, Kittrick et Jackson, 1956, Bache, 1964), qui apparaissent, dans ces conditions, comme étant beaucoup moins fragiles que les constituants de départ.

Ces différentes observations donnent alors à penser que l’action d’un réactif en solution n’est pas uniquement caractérisée par son “affinité” pour l’un des éléments constitutants les minéraux. Si celui-ci est, par exemple, fortement lié au sein d’une structure complexe (Al d’un silicate), ce sont les anions du réactif qui seront fixés; dans les édifices plus simples, type oxydes ou hydroxydes par contre, ce sont les cations du minéral qui peuvent être extraits aisément.

On voit ainsi s’esquisser un principe général qui ne pourra toutefois être formulé que lorsque d’autres preuves expérimentales auront permis de mieux circonscrire les mécanismes en cause.

b) Comportement des cations compensateurs de charge (K, Na, Ca)

Nous envisageons, ici, uniquement le cas des cations compensateurs qui sont fixes, c’est-à-dire non échangeables dans les conditions habituelles et pour lesquels il existe une liaison assez forte entre eux et la carcasse réticulaire du minéral; les édifices tels que les smectites (montmorillonite), les vermiculites... pour le groupe des silicates phylliteux ou encore les zéolites et dans une certaine mesure les feldspathoides dans le groupe des silicates tridimensionnels, sont donc exclus de cet examen.

La réaction avec les micas, triocédriques (phlogopite, biotite . . .) et même dioctédriques (muscovite), apparaît dans certaines conditions comme étant particulièrement nette; il y a, en effet, extraction d’une large fraction des ions $K$ interfilaire qui viennent précipiter dans le milieu extérieur, alors que le cation des réactifs utilisés ($Na$) se place entre les feuillets. Par contre, les autres éléments des minéraux sont peu atteints au cours de cette évolution qui est ainsi typiquement “vermiculitisante”.

Inversement, dans les mêmes conditions, la réaction avec les feldspaths potassiques ne se produit pas; c’est même le contraire qui arrive, puisque c’est le réactif qui a tendance à se fixer à la surface du minéral. A ce sujet, il faut d’ailleurs rappeler que le cobaltinitrite, par exemple, est utilisé couramment en pétrographie comme colorant spécifique de l’orthose (microcline).

A la lueur de ces résultats, on remarque ainsi que la muscovite se révèle plus vulnérable que les feldspaths potassiques en présence de réactifs précipitant le potassium, alors qu’elle est donnée généralement comme aussi stable et même, dans une certaine mesure, plus stable que l’orthose au sein de la biosphère (hydrolyse).

On peut rattacher aux modèles précédents les effets résultant de l’emploi de sels classiques à cations “actifs”, qui sont susceptibles de provoquer à chaud un échange partiel des cations compensateurs par une sorte de double décomposition.

Dans le cas des phyllites micacées, l’action de sels d’acides forts, peu hydrolysables, tels que les chlorures ou nitrates de $Na$, $Mg$, $Ca$, $Ba$ et $Ni$, se révèle sélective et permet ainsi l’extraction des ions $K$ interfilaire des espèces triocédriques sans toucher pratiquement aux feuillets; il s’agit donc, là encore, d’une évolution “vermiculitisante”. (Barshad, 1948, Cail­lère et al., 1949, Mortland, 1958, Besson et al., 1966a, Robert, 1968).

Dans le cas des feldspaths potassiques, le résultat de l’extraction obtenue en présence de telles conditions est quasiment nul; mais en ce qui concerne les plagioclases. Robert (1968) note que des sels comme les chlorures de potassium ou d’ammonium permettent déjà un certain remplacement des ions $Na$ et $Ca$ du réseau (cf. aussi Nash et Marshall, 1956).

Ces diverses réactions, qui nécessitent toujours une certaine concentration au sein de la solution ($M$ ou dans certains cas $M/10$), semblent résulter d’une simple action de masse et aboutissent dans tous les cas à un nouvel état d’équilibre : minéral-solution. C’est la raison pour laquelle
on est, ici, obligé de renouveler la solution d'altération si l'on veut que les phénomènes provoqués expérimentalement aient un rendement raisonnable.

Naturellement, il est encore possible d'utiliser, comme réactifs d'attaque, d'autres sels tel l'acétate de magnésium, par exemple, qui s'hydrolyse aisément en solution; mais on obtient alors une action moins spécifique, étant donné qu'elle porte en même temps sur le squelette silicaté (cf. Besson et al., 1966a).

Nous sommes donc amenés de cette façon à nous arrêter quelque peu devant ce nouveau type d'effet.

c) Dégradation des carcasses anioniques (squelettes silicatés)

Une dégradation de ce genre sera susceptible de se réaliser, lorsqu'au sein du milieu d'attaque, on pourra imaginer la formation d'une liaison "cation structural—anion silicaté" qui soit plus forte que n'importe quelle liaison du minéral initial. C'est ce qui se produirait par exemple, lorsqu'on fait évoluer à chaud divers solides : quartz, feldspaths... au sein de solutions de sels, comme l'acétate de magnésium, c'est-à-dire de composés susceptibles de s'hydrolyser très lentement en libérant un hydroxyde de type brucitique. Hénin et Robichet avaient constaté, dès 1954, que la simple ébullition en ballon de verre de solutions d'acétate de magnésium conduisait à l'apparition de phyllites magnésiennes de néogénèse, du type antigorite notamment, qui s'étaient édifiées en utilisant la silice du verre constituant le récipient. Des expériences du même genre, réalisées en présence d'orthose et de quartz, ont montré depuis qu'il en était toujours de même avec corrosion des minéraux silicatés et formation in situ d'antigorite (Caillère et al., 1963-64).

Ainsi, dans les conditions expérimentales de ces divers essais, la liaison Si—O—Mg apparaît être beaucoup plus stable que les autres liaisons du milieu et en particulier que la liaison Si—O—Si de la carcasse structurale du minéral de départ. On est donc bien, encore ici, en présence d'un cas où des minéraux dits résistants comme le quartz et dans une certaine mesure les feldspaths alcalins, semblent vulnérables alors que des espèces réputées fragiles comme celles constituant les serpentines sont, au contraire, parfaitement stables.

En conclusion, les différents exemples proposés au cours de cette revue consacrée au second grand modèle de dégradation, montrent une fois de plus que la stabilité minéralogique est une notion essentiellement contingente.

L'étude des phénomènes d'altération effectuée dans l'optique qui vient d'être indiquée, nous amène à envisager maintenant, pour un système "minéral — structure d'agression", deux modes possibles d'évolution, suivant d'une part le pouvoir d'extraction du réactif et d'autre part la force de liaison du cation considéré au sein du minéral; la réaction éventuelle peut se produire, en effet,

— ou bien, dans le sens : minéral → solution (milieu agressif et dégradation du cristal);

— ou bien, au contraire, dans le sens : solution → minéral (milieu inactif et adsorption par le minéral).
Mais, cette dernière observation nous conduit logiquement plus loin et nous amène ainsi à poser, au cours d’une troisième partie, le problème de la structure du milieu de réception.

III. RéACTIONS AVEC INTERVENTION DE CORPS AYANT UNE STRUCTURE RIGIDE DE RÉCEPTION

Le modèle de ce genre d’action est fourni par les “résines” synthétiques; on peut y adjoindre aussi ceux relatifs aux “racines” des organismes végétaux et aux “composés humiques” du sol, qui sont toutefois plus difficiles à analyser.

Les résines catióniques sont des corps dont l’anion, qui est un haut polymère soluble, c’est-à-dire une sorte de réseau macro-moléculaire présentant des groupes fonctionnels caractéristiques, est neutralisé par un certain nombre de cations simples, faiblement liés et s’échangeant de ce fait aisément avec d’autres cations du milieu. De telles résines ont en général un pouvoir de fixation relativement élevé et comme, en outre, elles jouent sur le plan physicochimique le même rôle que le renouvellement des solutions dans les réactions classiques, elles peuvent être finalement considérées comme des agents efficaces quant à la dégradation sélective des minéraux.


Enfin, les composés organiques polymérisés du sol, tels l’humus et les matières humiques, doivent vraisemblablement avoir une action du même type. C’est ainsi que récemment, Juste et Delas (1967), complétant les premiers travaux de Duchaufour (1964), ont montré que Fe²⁺, Al³⁺, Ca²⁺, Mg²⁺ ou Cu⁺ étaient, par exemple, plus ou moins complexes par des acides humiques extrait de sol. Toutefois, la spécificité est alors beaucoup plus difficile à prévoir puisque l’évolution dépend aussi de l’intervention d’une flore microbienne extrêmement variée et variable (cf. Bloomfield, 1951, Bétrémieux, 1951).

Mais, le meilleur exemple de ce type de phénomènes, se déroulant par l’intermédiaire d’une seconde phase condensée, est dû à l’existence de certains édifices minéraux qui sont susceptibles d’agir directement sur le milieu en voie d’évolution. On peut d’ailleurs concevoir leur intervention de deux façons:

— La première est une façon que nous dirons “passive”, la présence d’un minéral déterminé “protégeant” en quelque sorte son voisin de la
SUR LES NOTIONS DES “SYSTÈMES D’AGRESSION” 87
dégradation. Ce type d’interaction semble avoir été indiqué, il y a déjà longtemps, par Lapparent (1909) : lors de la séricitisation des plagioclases, la biotite reste en effet intacte, si la roche contient des feldspaths potassiques qui fournissent les ions K, nécessaires à l’édification de la séricite, en se décomposant ; par contre, en l’absence d’orthose, la biotite se dégrade aisément en libérant du potassium et en se transformant alors en chlorite.
Mais, on peut aussi rattacher à ce dernier modèle les diverses réactions qui découlent de l’apparition de certains précipités au cours des processus de décomposition et en particulier de l’individualisation d’hydroxydes métalliques dans le milieu. C’est d’ailleurs ce que nous avons envisagé dans le paragraphe précédent lors de l’étude de l’altération du quartz et de différents silicates en présence d’acétate de magnésium et qui résulte essentiellement d’une libération lente et continue de Mg(OH)₂ au sein de la solution (cf. Caillère et al., 1963-1964; loc. cit.). La fixation de la silice, qui découle dans ce cas de la formation de véritables liaisons de type Si—O—Mg, est alors infiniment plus efficace quant à l’épuration des eaux, comme l’ont montré notamment les travaux de Betz et al., 1940 (loc. cit.).

CONCLUSION
Les différents problèmes qui viennent d’être évoqués montrent bien ainsi que la notion d’ “altérabilité” des minéraux ne peut réellement se définir que vis-à-vis du milieu d’évolution, c’est-à-dire par rapport à un ensemble de conditions et de facteurs qui déterminent ce que nous avons appelé la “structure d’agression”. Nous avons vu en effet que des constituants silicatés, comme le quartz par exemple, n’étaient stables que dans le cas de certaines évolutions (hydrolyse en particulier), alors qu’ils pouvaient devenir fragiles dans d’autres conditions (milieu magnésien.
notamment). Par contre, c’est l’inverse qui semble se produire pour d’autres minéraux (cas de l’antigorite).

Mais, en même temps, nous avons été amenés à constater que, suivant le type de l’évolution, le résidu de l’altération pouvait avoir une structure et une composition extrêmement différentes. C’est ce qui se produit ainsi avec les micas biotites ; on sait, par exemple, que l’extraction spécifique du potassium conduit à une vermiculite vraie, tandis que l’attaque en milieu acide aboutit à une sorte de gel siliceux.

Naturellement, de tels exemples devront à l’avenir être étendus à d’autres minéraux. C’est d’ailleurs là une nécessité absolue si on veut améliorer nos connaissances relatives aux processus de décomposition. Ce n’est, en effet, que dans la mesure où ces modèles seront appliqués systématiquement à l’ensemble du monde minéral, que les phénomènes d’altération pourront faire l’objet d’une classification rationnelle qui permettra alors, dans chacun des cas, de circonscrire le champ des interprétations.

Note: Les termes de ”dissolution” et de ”solubilisation” ont été employés dans cette Note conformément aux définitions préconisées par l’Académie des Sciences — Comité du langage scientifique (Comptes rendus Paris, 1967, 264 généralités, p. 79).

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RÉSUMÉ

La stabilité d'un minéral ne peut pas être considérée en pédologie comme une donnée fixe, mais doit toujours se définir en fonction des conditions du milieu, c'est-à-dire de ce que l'on se propose d'appeler le "système d'agression".

En partant de cette conception, les auteurs montrent que l'on peut concevoir 3 formes de réactions:

— réactions classiques avec mise en solution (attaque hydrolytique ou acido lytique)
— réactions spécifiques mises en jeu au contact de réactifs solubles, mais formant des composés peu ionisés (précipités ou complexes)
— réactions par l'intermédiaire d'agents d'agression ayant une structure rigide de réception (racines, résines, minéraux en voie de formation).

Un certain nombre d'exemples relatifs aux comportements et aux modes d'évolution de divers édifices cristallins dans le milieu naturel sont finalement présentés pour étayer de telles considérations.
ZUSAMMENFASSUNG

Die Stabilität eines Minerals kann in der Bodenkunde nicht als ein ständiger Wert betrachtet werden, sondern muss immer in Hinsicht auf die Milieuverhältnisse bestimmt sein, ein Milieu, welches man als „Angriffssystem“ bezeichnen könnte.

Von diesem Standpunkt aus haben die Autoren gezeigt, dass man sich drei Reaktionsformen vorstellen kann:

— klassische Reaktionen mit Auflösungen (hydrolytische oder azidolithische Einwirkung)
— spezifische Reaktionen bei Kontakt mit auflöslichen Reagenzien, die aber wenig ionisierte Verbindungen aufweisen (niedergeschlagen oder komplex)
— Reaktionen durch Angriffsagenten, die eine stabile Empfangsstruktur besitzen (Wurzeln, Austauschresinen, Mineralien während der Bildung).

Eine gewisse Anzahl von Beispielen vom Verhalten und der Evolutionsart der verschiedenen Kristallstrukturen in natürlichem Milieu befürworten diese Forschungen.

SUMMARY

In pedology, the stability of a mineral cannot be considered invariant but must always be defined in terms of the environmental conditions, namely as a function of what it is here proposed should be called the "aggression system".

On the basis of this conception, the authors have suggested three kinds of reactions:

(a) the classical reactions with specific solubilisation (hydrolytic or acidolytic attack)
(b) the specific reactions involving soluble reagents and the formation of weakly ionized compounds (precipitates or complexes)
(c) the reactions carried out through the intermediary of aggression agents with rigid structure (roots, resins, minerals at formation stage).

Some examples relative to the behaviour and modes of evolution of various crystalline silicates under natural conditions are given by the authors to substantiate the above considerations.
TRANSFORMATION DES MINÉRAUX ARGILEUX PAR LA PODZOLISATION

T. S. ZVEREVA

Institute d'Agrophysique, Leningrad

Dans ce travail nous exposons les résultats des examens de la transformation des minéraux argileux de certains sols formés dans la zone podzolique sur des matériaux à contenu divers des calcaires, ce qui détermine le divers degré d'intensité du processus pédogénétique zonal. Nous avons choisi trois groupes des sols. Le premier groupe comprend les rendzines formés sur l'éluvium du calcaire et sur la moraine calcaire. Les minéraux dominants de ces roches sont la calcite et la dolomite; le quartz et les feldspaths sont moins fréquents; les micas sont rares. L'ilite est déterminée de la fraction limoneuse. Le rendzine podzolique et le sol dermo-podzolique moyen forment le deuxième groupe.

Dans leur roche génératrice les carbonates jouent un rôle subordonné, le quartz et les feldspaths forment la part principale et les micas sont rares. L'ilite est un minéral prédominant de la fraction limoneuse. La kaolinite, la chlorite y déterminent. Le podzol sur le sable de dune appartient au troisième groupe. Dans ce sol, le quartz et les feldspaths sont les minéraux prédominants et les micas sont très rares. L'ilite et la chlorite sont présentes dans la fraction < 0,001 mm. L'analyse minéralogique des sols et de leurs roches génératrices témoinque que la composition des aluminosilicates y est la même et les différences ne sont que de nature quantitative.

La texture des sols montre qu'ils ont été formés sur des matériaux comparativement uniformes. Les faits examinés plus haut permettent de considérer les sols étudiés comme les stades divers de l'évolution de la podzolisation.

Le Tableau 1 donne les résultats de certaines analyses de ces sols. Le Tableau 2 représente le bilan de la détermination de la composition minéralogique de la fraction < 0,001 mm au moyen des méthodes suivantes: radiométrique, thermique et par le microscope électronique.

Les données de la micromorphologie, de la texture des sols, des analyses chimiques et minéralogiques des sols et de leurs fractions limoneuses ont permis de constater les régularités suivantes de la transformation des minéraux dans ces sols. La formation des sols sur l'éluvium du calcaire et sur la moraine calcaire locale s'accompagne de la destruction des carbonates et l'apparition dans la solution du sol de Ca++ et Mg++. Comme la stabilité de la dolomite est plus grande que celle de la calcite, la dolomite se conserve dans le sol plus longtemps et donne Mg++ dans la solution.

La transformation des minéraux argileux dans les rendzines se passe
TABLEAU 1
LES ANALYSES DES SOLS

<table>
<thead>
<tr>
<th>Sols et roches</th>
<th>Horizons</th>
<th>Profondeur, cm</th>
<th>Teneur en fraction &lt;0.001 mm, %</th>
<th>Humus (d'après Tiurin) %</th>
<th>pH</th>
<th>Cations adsorbés (d'après Gedroz), m.e.</th>
<th>L'analyse globale des sols (% par rapport du sol séché au feu)</th>
<th>L'analyse globale des fractions &lt;0.001 (% par rapport de la fraction séchée au feu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Ca</strong>++ <strong>Mg</strong>++ <strong>H</strong>+</td>
<td><strong>SiO</strong>₂</td>
<td><strong>Al₂O</strong>₃</td>
</tr>
<tr>
<td>Rendzine sur l'éluvium du calcaire</td>
<td>A₁</td>
<td>0-12</td>
<td>35</td>
<td>13,7</td>
<td>6,7</td>
<td>56,6</td>
<td>2,9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>*C</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rendzine sur la moraine calcaire locale</td>
<td>A₁</td>
<td>0-10</td>
<td>21</td>
<td>6,9</td>
<td>6,5</td>
<td>16,2</td>
<td>13,2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B(BC)</td>
<td>25-30</td>
<td>23</td>
<td>-</td>
<td>6,9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>*C</td>
<td>70-75</td>
<td>25</td>
<td>-</td>
<td>7,8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rendzine podzolique sur la moraine calcaire</td>
<td>A₁</td>
<td>0-10</td>
<td>12</td>
<td>2,1</td>
<td>4,2</td>
<td>10,2</td>
<td>1,4</td>
<td>8,3</td>
</tr>
<tr>
<td></td>
<td>A₂</td>
<td>35-40</td>
<td>11</td>
<td>0,8</td>
<td>5,0</td>
<td>2,7</td>
<td>0,9</td>
<td>1,9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>50-55</td>
<td>22</td>
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<td>10,0</td>
<td>1,8</td>
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<tr>
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<td>110-115</td>
<td>19</td>
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<td>7,2</td>
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<td>Derno-podzolique moyen sur la moraine calcaire</td>
<td>A₁</td>
<td>0-10</td>
<td>13</td>
<td>5,4</td>
<td>5,0</td>
<td>8,5</td>
<td>2,5</td>
<td>2,6</td>
</tr>
<tr>
<td></td>
<td>A₂</td>
<td>45-50</td>
<td>7</td>
<td>-</td>
<td>6,0</td>
<td>2,2</td>
<td>0,6</td>
<td>0,2</td>
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<td></td>
<td>B</td>
<td>60-65</td>
<td>18</td>
<td>-</td>
<td>6,3</td>
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<td>2,8</td>
<td>0,1</td>
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<tr>
<td></td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Derno-podzolique intensif sur la moraine calcaire</td>
<td>A₁</td>
<td>0-7</td>
<td>9</td>
<td>4,1</td>
<td>3,8</td>
<td>0,9</td>
<td>0,5</td>
<td>0,7</td>
</tr>
<tr>
<td></td>
<td>A₂</td>
<td>31-36</td>
<td>5</td>
<td>-</td>
<td>4,6</td>
<td>0,6</td>
<td>0,2</td>
<td>0,7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>85-90</td>
<td>15</td>
<td>-</td>
<td>5,2</td>
<td>3,0</td>
<td>2,2</td>
<td>0,6</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>120-125</td>
<td>14</td>
<td>-</td>
<td>6,2</td>
<td>4,8</td>
<td>1,7</td>
<td>0,1</td>
</tr>
<tr>
<td>Derno-podzolique intensif sur la moraine sans calcaire</td>
<td>A₁</td>
<td>0-9</td>
<td>13</td>
<td>2,6</td>
<td>4,2</td>
<td>2,7</td>
<td>1,8</td>
<td>5,9</td>
</tr>
<tr>
<td></td>
<td>A₂</td>
<td>11-16</td>
<td>5</td>
<td>1,8</td>
<td>4,2</td>
<td>0,9</td>
<td>0,7</td>
<td>3,3</td>
</tr>
<tr>
<td></td>
<td>A₃</td>
<td>37-42</td>
<td>8</td>
<td>-</td>
<td>4,1</td>
<td>0,7</td>
<td>0,5</td>
<td>0,1</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>50-55</td>
<td>22</td>
<td>-</td>
<td>4,2</td>
<td>2,8</td>
<td>1,6</td>
<td>1,7</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>120-125</td>
<td>14</td>
<td>-</td>
<td>5,1</td>
<td>2,5</td>
<td>0,6</td>
<td>0,5</td>
</tr>
<tr>
<td>Podzol sur le sable</td>
<td>A₁</td>
<td>17-21</td>
<td>2</td>
<td>0,2</td>
<td>3,9</td>
<td>2,5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>35-40</td>
<td>1</td>
<td>-</td>
<td>4,3</td>
<td>0,5</td>
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<td>-</td>
</tr>
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<td>BC</td>
<td>55-60</td>
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<td>-</td>
<td>5,2</td>
<td>-</td>
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</tr>
</tbody>
</table>
### TABLEAU 2
**DISTRIBUTION DES MINÉRAUX ARGILEUX EN SOLS**

<table>
<thead>
<tr>
<th>Sols et roches</th>
<th>Horizon</th>
<th>Minéraux argileux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rendzine sur l'éluvium de calcaire</td>
<td>A1</td>
<td>Vermiculite, illite, vermiculite-chlorite, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>A1,C</td>
<td>Illite, chlorite-vermiculite, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>*C</td>
<td>Illite</td>
</tr>
<tr>
<td></td>
<td>*le calcaire</td>
<td></td>
</tr>
<tr>
<td>Rendzine sur la moraine calcaire</td>
<td>A1</td>
<td>Illite, vermiculite, vermiculite-chloritizable, kaolinite</td>
</tr>
<tr>
<td></td>
<td>B(C)</td>
<td>Illite, vermiculite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>*C</td>
<td>Illite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>*le calcaire</td>
<td>Illite</td>
</tr>
<tr>
<td>Rendzine podzolique</td>
<td>A1</td>
<td>Vermiculite, illite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>A1,B</td>
<td>Illite, vermiculite, vermiculite-chloritizable, kaolinite</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Illite, vermiculite, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>Illite, chlorite, kaolinite</td>
</tr>
<tr>
<td>Derno-podzolique moyen sur la moraine calcaire</td>
<td>A1</td>
<td>Illite, vermiculite, illite-vermiculite, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>A1,B</td>
<td>Illite, vermiculite, illite-vermiculite, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Illite, vermiculite, illite-vermiculite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>Illite, chlorite, kaolinite</td>
</tr>
<tr>
<td>Derno-podzolique moyen, sur la moraine sans calcaire</td>
<td>A1</td>
<td>Illite, vermiculite, vermiculite-chloritizable, kaolinite</td>
</tr>
<tr>
<td></td>
<td>A1,B</td>
<td>Illite, vermiculite, vermiculite-chloritizable, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Illite, vermiculite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>Illite, chlorite, kaolinite</td>
</tr>
<tr>
<td>Derno-podzolique intensif sur la moraine sans calcaire</td>
<td>A1</td>
<td>Illite, vermiculite, kaolinite</td>
</tr>
<tr>
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<td>A1,B</td>
<td>Illite, vermiculite, vermiculite-chloritizable, kaolinite</td>
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<tr>
<td></td>
<td>B</td>
<td>Illite, vermiculite, vermiculite-chloritizable, chlorite, kaolinite</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>Illite, chlorite, kaolinite</td>
</tr>
<tr>
<td>Podzol sur le sable</td>
<td>A2</td>
<td>Montmorillonite, illite-montmorillonite, illite, kaolinite?</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Illite, illite-montmorillonite chlorite, allophane?, kaolinite?</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>Illite, chlorite</td>
</tr>
</tbody>
</table>

* On a analysé la composition de fraction <0,001 mm du résidu du calcaire dans le 5% HCl.
dans le milieu neutre ou faiblement acide devant la concentration considérable de \( Ca^{++} \) et \( Mg^{++} \) dans la solution, devant la saturation du complexe adsorbant de ces cations et le lessivage de \( Ca^{++} \) du profil des sols. La part argileuse des sols est faiblement mobile dû à une grande quantité des électrolytes. Dans les rendzines se manifestent deux zones qui se distinguent par la conversion des minéraux argileux. La première touche à la roche génératrice du sol (l’hor. A1 C) et se caractérise évidemment par une concentration considérable de \( Mg^{++} \) dans la solution du sol. Ces ions pénètrent dans le réseau des illites en remplaçant le \( K^+ \) et forment les couches de brucite dans les espaces intercouches de l’illite. Ces derniers se transforment de ce fait en chlorite. Comme les minéraux primaires ne sont pas abondants ils ne peuvent pas être la source des quantités considérables de la chlorite.

La deuxième zone coïncide avec l’horizon A1 et se caractérise par le pH plus acide, par le lessivage des bases plus intensif et évidemment par la moins grande concentration de \( Mg^{++} \) dans la solution du sol que la première. La chlorite dans cette zone n’est pas stable, elle perd une part du \( Mg^{++} \) de la couche de brucite et se convertit en minéral intermédiaire, la chlorite-vermiculite et en vermiculite. Dans ces conditions l’illite se transforme en vermiculite en remplaçant \( K^+ \) par \( Mg^{++} \). Cette conversion se traduit par le schéma suivant:

\[
\text{illite} \rightarrow \text{chlorite} \rightarrow \text{vermiculite}
\]

\[
\text{illite} \rightarrow \text{vermiculite}.
\]

La rendzine sur la moraine calcaire a le pH plus acide et le lessivage des bases plus grand que les rendzines sur l’éluvium des calcaires, mais la saturation des bases du complexe adsorbant est déjà grande et la concentration de \( Mg^{++} \) dans la solution du sol est aussi grande. La part argileuse du sol n’est pas mobile. La transformation des minéraux argileux dans la rendzine sur la moraine calcaire s’exprime par le remplacement du \( K^+ \) de l’illite par \( Mg^{++} \) dans la solution du sol et le complexe adsorbant, ce qui amène la conversion de l’illite en vermiculite, comme dans la deuxième zone des rendzines sur l’éluvium de calcaire. La pédogénèse sur les roches à petit contenu des carbonates, ainsi que sur les roches sans carbonates est semblable. Elle se caractérise par la destruction des minéraux primaires et par le lessivage des produits de cette destruction à la part basse, où se forme l’horizon illuvial. La puissance de cet horizon dépend de l’intensité du lessivage des bases.

Dans la rendzine podzolique et dans le sol derno-podzolique moyen sur la moraine calcaire on distingue trois zones de la transformation des minéraux argileux. La première (l’hor. BC) se caractérise par la faible influence de la pédogénèse. Le pH de cette zone est neutre ou faiblement alcalin, la solution du sol et le complexe adsorbant ont des quantités considérables des bases. Dans ces conditions l’illite et la chlorite sont stables. La transformation de l’illite en chlorite, comme dans la rendzine, est possible. Un pH acide, une apparition de \( H^+ \) adsorbant, un lessivage considérable des minéraux argileux et des produits de destruction des minéraux primaires sont typiques pour la deuxième zone. La transformation de l’illite en
La transformation des minéraux argileux

La vermiculite est possible (comme dans la rendzine sur la moraine calcaire), mais la part majeure de ce minéral y est apportée de la zone haute. La chlorite est stable dans cette zone. La troisième zone (les hor. A₁–A₂B) se caractérise par un pH fortement acide. Le rôle de H⁺ dans le complexe adsorbant est considérable. Tous les éléments, à l'exception de SiO₂ qui s'y accumule comparativement, se lessivent. Dans ces conditions l'illicite perd son K⁺ intensivement et se convertit en vermiculite; ce minéral est différent de la vermiculite des rendzines. Le premier se forme au pH acide et en présence des quantités considérables d'hydrates d'aluminium et de fer libérés. Le deuxième paraît au pH neutre ou faiblement acide à une grande concentration de Ca⁺⁺ et Mg⁺⁺ dans la solution de sol. La chlorite perd dans cette zone une part du Mg⁺⁺ et se transforme en vermiculite chloritizable. Le changement des relations $\frac{SiO₂}{K₂O}$ et $\frac{SiO₂}{MgO}$ à la composition chimique des fractions < 0,001 mm de ces sols démontre la modification intensive de l'illicite et de la chlorite. La troisième zone se caractérise par le lessivage intensif de matériaux limoneux. Dans les sols étudiés les minéraux argileux peuvent se former des minéraux primaires, ce qui se réalise dans l'apparition de la kaolinite par suite de l'altération des feldspaths, et dans l'apparition de l'illicite par suite de la conversion des micas. Ainsi, la modification de la composition chimique et minéralogique de la fraction < 0,001 mm des sols de la rendzine podzolique et du sol derno-podzolique moyen, se fait sous l'influence des processus suivants: la néoformation de l'illicite et de la kaolinite des minéraux primaires, la conversion par stades des minéraux argileux selon le schéma

$$\text{illite} \rightarrow \text{vermiculite}$$
$$\text{chlorite} \rightarrow \text{vermiculite chloritizable}$$

et le lessivage des matériaux limoneux. La conversion la plus intense des minéraux argileux a lieu dans l'horizon A₁ de la rendzine podzolique.

Les sols derno-podzoliques moyen et derno-podzoliques intensif sur la moraine sans calcaire ont quatre zones de la transformation des minéraux argileux. La première zone (l'hor.BC) se caractérise par un pH acide et par une prédominance de Ca⁺⁺⁺ Mg⁺⁺ sur H⁺ dans le complexe adsorbant. Dans ces conditions la chlorite et l'illicite sont stables. La deuxième zone (l'hor.B) a un pH acide, mais dans le complexe adsorbant Ca⁺⁺⁺ Mg⁺⁺ prédominent sur H⁺. Dans cette zone a lieu l'apparition d'une quantité considérable des minéraux argileux et à juger par relations $\frac{SiO₂}{K₂O}$ et $\frac{SiO₂}{MgO}$ dans la composition chimique de la fraction < 0,001 mm, la transformation intensive des illites en vermiculite et de la chlorite en vermiculite chloritizable (dans le sol derno-podzolique moyen). L'apparition du vermiculite chloritizable dans la deuxième zone du sol derno-podzolique intensif s'explique par la néoformation de ce minéral du vermiculite et par son transfert de la zone haute. La troisième zone (les hor.Aₙ>p₀, A₂B) se caractérise par un pH fortement acide et par une augmentation du rôle de H⁺ dans le
complex adsorbant. On note le lessivage du matériel limoneux. L’accroissement des relations $\frac{SiO_2}{K_2O} \text{et} \frac{SiO_2}{MgO}$ dans la composition chimique des fractions $< 0,001 \text{mm}$ montre les dimensions de la transformation de l’illite et de la chlorite. L’apparition de la vermiculite chloritizable est le processus typique de la troisième zone du sol derno-podzolique intensif; elle est possible dans les conditions des quantités considérables des sesquioxydes mobiles et de la substitution fréquente de l’humidification par séchage. Dans le sol derno-podzolique moyen, on suppose la néoformation de la vermiculite chloritizable de la vermiculite, mais cette formation est possible par l’alternation de la chlorite. La quatrième zone se caractérise par le pH le plus acide et par une prédominance considérable de $H^+$ sur $Ca^{++}$ $+ Mg^{++}$ dans le complexe adsorbant. Dans ces conditions tous les éléments se transfèrent à l’exception de $SiO_2$ qui s’accumule comparativement. L’appauvrissement par la fraction limoneuse est intensif. Dans cette zone les minéraux argileux perdent $K$, $Mg$ et $Al$ à juger par les relations $\frac{SiO_2, SiO_2, SiO_2}{K_2O, MgO, Al_2O_3}$ dans la composition chimique de la fraction $< 0,001 \text{mm}$. Or, dans les sols derno-podzolique moyen et derno-podzolique intensif ont lieu les processus suivants: la néoformation de l’illite et de la kaolinite des minéraux primaires, la conversion des minéraux argileux selon le schéma:

-  illite → vermiculite → vermiculite chloritizable
-  chlorite → vermiculite chloritizable → vermiculite

et le lessivage du matériel limoneux dans l’horizon illuvial.

Dans cette recherche le podzol est la stade la plus mûr de la podzolisation. Il se caractérise par la grande prédominance des minéraux primaires sur les secondaires dans le profil du sol.

Le quartz et les feldspaths sont prédominants parmi les minéraux primaires. La source des minéraux argileux est les aluminosilicates des couches, mais ils sont très peu nombreux. Les feldspaths au cours de l’altération et de la pédogénèse se décomposent jusqu’aux oxydes. Le pH très acide de l’hor. $A_2$ assure le lessivage intensif de tous éléments y compris $Si$ et $Al$. Dans l’horizon illuvial s’effectue l’accumulation des sesquioxydes et se créent les conditions dans lesquelles la synthèse des aluminosilicates, secondaire (allophane) est possible.

Les minéraux argileux se forment des primaires dans le profil de ce sol. Ils sont peu nombreux et se transforment très intensivement. On note deux zones dans ce sol. La zone haute (l’hor. $A_2$) se caractérise par la conversion de l’illite et de la chlorite en mortmorillonite. Les matériaux amorphes sont transférés dans la zone basse. Cette zone se caractérise par l’altération des minéraux argileux moins intense. Dans la zone basse s’accumulent les argiles transférés du haut et c’est là que se forment les conditions pour la cristallisation des matériaux amorphes. L’illite se transforme en minéraux intermédiaires l’illite-montmorillonite. La chlorite est stable.
Enfin on peut faire la conclusion de l’altération des minéraux argileux dans les sols d’âge divers relatif.

L’illite se transforme en chlorite, en minéraux intermédiaires la chlorite-vermiculite quand le grand contenu des carbonates entrave la podzolisation (stade des rendzines). Le développement de la pédogénèse y est accompagné de la baisse du pH, du lessivage des Ca²⁺ et Mg²⁺ du complexe adsorbant et de la substitution de ces cations par H⁺. Dans ces conditions, l’illite perd K⁺ et se transforme en vermiculite qui se distingue de la vermiculite des rendzines. L’intensité de ce changement dépend du degré de l’acidité du sol. Dans le sol derno-podzolique intensif apparait une grande quantité des sesquioxydes mobiles et la vermiculite est convertie en vermiculite chloritizable. Le podzol est caractérisé par le plus fort lessivage du K⁺ : du réseau de l’illite, qui est suivi de la formation de la montmorillonite et éventuellement de la kaolinite.


La plupart des chercheurs des podzols montrent la présence de l’illite, des minéraux intermédiaires de l’illite-montmorillonite et de la montmorillonite.

Or, l’analyse des résultats obtenus par les chercheurs cités ci-dessus indique que la transformation des minéraux argileux observée dans ce travail reflète la direction de l’évolution des sols de grand territoire.

Note. On a analysé la composition de fraction < 0,001 mm du résidu du calcaire insoluble dans le 5% HCl.

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RÉSUMÉ
Nous avons soumis à l'examen des sols de zone podzolique (depuis la rendzine jusqu’au podzol). Ces sols ont été analysés comme les stades diverses d'évolution pédologique.
L'illite prédomine dans les roches génératrices des sols. Ce minéral se transforme en chlorite, en minéraux intermédiaires la chlorite-vermiculite et en vermiculite quand le grand contenu des carbonates entrave la podzolisation (stade des rendzines). Le développement de la pédogénèse y est accompagné de la baisse du pH, du lessivage des Ca++ et Mg++ du complexe adsorbant et de la substitution de ces cations par H+. Dans ces conditions, l'illite perd K+ et se transforme en vermiculite qui se distingue de la vermiculite des rendzines. L'intensité de ce changement dépend du degré de l'acidité du sol. Dans le sol derno-podzolique intensif apparaît une grande quantité des sesquioxydes mobiles et la vermiculite est convertie en vermiculite chloritizable. Le podzol est caractérisé par le plus fort lessivage du K+ du réseau de l'illite, qui est suivi par la formation de la montmorillonite et éventuellement de la kaolinite.

Dans les sols examinés la chlorite change plus fort que l'illite. Elle perd une part de Mg++ de la couche de brucite et se transforme d'abord en vermiculite chloritizable et ensuite en vermiculite. Le stade du podzol se caractérise par la transformation de la chlorite en montmorillonite.

ZUSAMMENFASSUNG
Wir haben Böden der podsolischen Zone (von der Rendzina bis zum Podsol) untersucht. Diese Böden sind auf ihre podsolischen Entwicklungsphasen analysiert worden.


In den untersuchten Böden verändert sich des Chlorid mehr als der Illit. Ein Teil von Mg++ in der Bruzitschicht geht verloren und verwandelt sich erst in einen Chlorid-Vermikulit und dann in Vermikulit. Die Podsolphase ist durch die Umwandlung des Chlorids in Montmorillonit charakterisiert.

SUMMARY
We have submitted for examination soils of the podsolic zone (from rendzina to podsol). These soils have been analysed at the various stages in pedological evolution.

Illite predominates in the rocks which produce the soils. This mineral is transformed into chlorite, into the intermediary minerals chlorite-vermiculite,
and into vermiculite when a large carbonate content prevents podsolization (rendzina stage). Soil development is accompanied there by a lowering of the pH value, leaching of the calcium and magnesium ions of the adsorbing complex, and the substitution of these cations by hydrogen ions. In these conditions, illite loses potassium ions and is changed into vermiculite, which is distinguishable from rendzina vermiculite. The intensity of this change depends on the degree of acidity in the soil. In intensive derno-podsolic soil, a great quantity of mobile sesquioxides appears and vermiculite is changed into chloritizable vermiculite. Podsols are characterized by the heaviest leaching of potassium ions from the illite lattice which is followed by the formation of montmorillonite and possibly of kaolinite.

In the soils examined, chlorite shows a more marked change than illite. It loses part of the magnesium ions from the layer of brucite and changes first into chloritizable vermiculite and then into vermiculite. The podsol stage is characterised by the transformation of chlorite into montmorillonite.
CHARACTERIZATION OF CLAY MINERALS BY INFRARED SPECTROSCOPY

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The infrared spectrum of a clay provides information which is largely complementary to that given by X-ray and thermal methods. A pure mineral species can often be more quickly and more fully defined in structure and composition by its infrared spectrum than by any other single technique; interpretation of features of the spectrum can yield information which cannot readily be obtained by other means. The power of infrared methods in clay studies can, however, be considerably increased by following changes in the spectrum when the clay is subjected to various chemical and physical treatments. Thermal treatment, deuteration, and oxidation-reduction reactions can all contribute to the interpretation of infrared spectra.

Thus changes in spectrum following thermal treatment of clays can identify reactions which give features on differential thermal analysis and thermogravimetric curves. Such studies can differentiate absorption bands of structural OH from those of adsorbed water, and can distinguish species of OH differing in thermal stability.

Water in clay samples is readily replaced by D$_2$O on flushing with D$_2$O vapour at room temperature. Structural OH bands and water absorption bands can then be examined free from mutual interference, and information obtained on the accessibility of NH$_4^+$ in clays. At higher temperatures, structural OH can exchange hydrogen atoms for deuterium of D$_2$O, so that their accessibility and reactivity can be assessed by this means.

The vibrations of OH groups coordinated to iron atoms are affected by the valency of iron, and so can serve as an indicator of valency. Thus oxidation-reduction reactions involving iron in octahedral sites of layer silicates can be followed in the infrared spectrum, and the spectral changes induced can assist in characterizing clay species.

The potentiality of these techniques is obvious; their application depends on the availability of suitable apparatus and procedures. It is the purpose of this paper to describe some convenient procedures in use at the Macaulay Institute, and to illustrate their application in clay mineral studies in progress there.

TECHNIQUES

(a) Sample preparation

The KBr pressed disk technique is perhaps the most generally useful method of sample preparation in clay studies. It is, however, inapplicable in...
investigations concerning the effect of exchangeable cations on clay mineral properties, as exchange between the potassium of the KBr and the cation initially on the clay is inevitable. Self-supporting films of smectites (the montmorillonite-saponite group) are ideal subjects for infrared studies. Such films were first used by Serratosa (1960) and Fripiat et al. (1960); their potentialities are well illustrated by the work of Mortland et al. (1963), Russell and Farmer (1964), Russell (1965) and Farmer and Mortland (1966). The preparation of high quality films is now a routine procedure. Smectites saturated with appropriate cations are best stored as salt-free freeze-dried powders. Such powder (10 mg) is readily dispersed in 1 ml water, and evaporation of this dispersion on to thin polyethylene film, held flat on a glass plate by the capillary action of a drop of water, yields a one-inch diameter film. This film is separated from the polyethylene by drawing the plastic sheet over a sharp edge. Vermiculite films of very high optical quality can be obtained by evaporating dispersions of propylammonium vermiculite prepared as described by Walker and Garrett (1967); such films are sufficiently stable to permit soaking in normal salt solutions to introduce the desired exchangeable cation, and subsequent water washing to remove excess salt. The very high degree of parallelism of the separate vermiculite lamellae which make up these films makes diffusion of gases and liquids into them slow, so that results obtained with them are probably intermediate between those which would be obtained with large single crystals on the one hand, and with separate clay-size crystals on the other. Porous vermiculite films more representative of the properties of clay-size vermiculites can be obtained from dispersions of vermiculite saturated with the common inorganic cations, and these dispersions can be obtained from dispersions of propylammonium vermiculite by washing on the centrifuge with appropriate salt solutions, followed by thorough water washing to remove excess salt. These suspensions require treatment with an ultrasonic probe or a high speed homogenizer to reduce particle size sufficiently to avoid excessive light scattering in the vermiculite films. The Ultra-turrax homogeniser (Janke and Kunkel KG, Staufen, W. Germany) is particularly effective.

The preparation of self-supporting films by evaporation of aqueous suspensions is restricted to minerals of the smectite and vermiculite groups. Films of kaolinite and illite have insufficient cohesion and can only be handled when supported on windows transparent to infrared radiation. Such window materials are often too reactive or too limited in transmission for the purpose in hand. Thus sodium chloride windows are sensitive to high humidities, and will generally convert the clay film to the sodium-saturated form. Silver chloride windows fuse at 450°C, tend to react with base metals, and soon lose transmission after prolonged exposure to light. Zinc sulphide oxidizes on heating in air. The high reflectivity of both silver chloride and zinc sulphide reduces the transmitted radiation. Windows of quartz or thin glass (microscope slides) are suitably unreactive, but limit study to the spectral region (2-5 μ) in which OH stretching vibrations occur.

Evaporation of aqueous suspensions of hydrated oxide gels and of
allophanes yields films which shrink and crack as they dry. Many soil clays contain these components in amounts sufficient to forbid the formation of satisfactory films. An alternative technique of forming self-supporting films has been found to be applicable to co-precipitated hydrated silica-aluminas. This technique, which involves spreading the finely dispersed freeze-dried powders between two polished steel faces, and pressing them at about 10,000 lb./sq. in., was developed in the study of the surface properties of anhydrous silica, silica-alumina catalysts, and zeolites (McDonald, 1958; Angell and Schaffer, 1965). But films prepared from the highly hydrated oxides of interest in soil studies are not always mechanically stable when heated, and it is difficult to prepare specimens thin enough to study regions of the spectrum where absorption is strong.

(b) Vacuum cells

Thermal treatment of clays to remove adsorbed water, or selectively to decompose their components, is a simple procedure; the main problem that arises is the examination of the product under conditions which exclude the readsoption of water. In most commercial infrared spectrometers, it is not permissible to examine samples maintained at temperatures much above 100°C, as thermal radiation from the sample distorts the absorption spectrum. It therefore often becomes necessary to cool and examine the sample in vacuum to prevent exposure to atmospheric moisture. Exclusion of atmospheric moisture is also essential in the examination of reactive deuterated samples.

For both thermal treatment and deuteration studies, the cell described by Angell and Schaffer (1965) has been found particularly convenient. Two or three samples can be treated simultaneously in this cell, so that in comparative studies all samples receive identical treatment. Its sole disadvantage lies in the fact that highly oriented films can be observed only at perpendicular incidence, so that absorption bands associated with dipole oscillations perpendicular to the clay sheets, and so perpendicular to the film surface, can escape detection. In an alternative cell design (Granquist and Kennedy, 1967) sample orientation relative to the incident beam can be adjusted, but this cell has a longer path length, and only one sample can be treated at a time.

(c) Thermal treatment in KBr pressed disks

The use of self-supporting films is limited to clays with appropriate physical properties. Powder dispersions on windows require material of small particle size (< 1 µ), and a deposition technique which avoids coagulation of these small particles, to give good transmission properties at the shorter wavelengths (near 3 µ) where OH stretching absorption bands occur. Grinding to achieve a suitable particle size fraction is always a dangerous, if unavoidable, procedure (Farmer, 1964; Tuddenham and Lyon, 1960); quantitative transformation of small samples into a suitable form for study as window-supported dispersions is seldom possible, and always uncertain. For such materials, the KBr pressed-disk technique has considerable advantages, as microgram quantities of material can be quan-
titatively handled and examined by this means, and particle-size require-
ments are less stringent because of the good match in refractive index
between the minerals and the surrounding KBr. Even with minerals which
have good film-forming characteristics, the pressed disk technique is often
the most convenient way of studying the strongest bands in the spectrum.

Suitably prepared KBr pressed disks are sufficiently porous to permit
the slow diffusion of water vapour into and out of them (Farmer, 1966).
It is therefore possible to drive off adsorbed water by heating the disk
overnight in an oven at 100-200°C and to record the spectrum before
atmospheric water is readorsorbed. Provided the clay complex is saturated
with a cation such as ammonium or potassium, and structural hydroxyl
absorption is not too weak, 100°C is generally sufficient (Farmer and
Russell, 1967). It is also possible to follow thermal decomposition of clay
minerals by examining in KBr disks a series of samples heated to succes-
sively higher temperatures. Where amounts of material are limited, there
would clearly be an advantage if thermal decomposition could be followed
on a single sample incorporated in a KBr disk. This procedure has, in fact,
been successfully applied at temperatures up to 300°C (Farmer, 1966) and
recent trials have indicated that thermal decompositions generally proceed
normally in KBr disks at temperatures up to 700°C. Experiments have not
been carried out beyond the fusion point of KBr (730°C). The pressed
disks often become opaque on heating at higher temperatures, but full
transparency was readily recovered by repressing the disk. In general, the
temperatures of decomposition of clay minerals in disks, following heating
for 16 hours, did not differ markedly from those observed when the
powdered mineral is heated alone in air.

Selective decomposition of different species of OH in a single mineral
was observed in a celadonite. It has been established from a study of
synthetic and natural celadonites that in this mineral the OH stretching
frequency is determined solely by the octahedral ions to which the OH
group is coordinated, provided substitution of aluminium for silicon is
low (Farmer, Russell, Ahlrichs, and Velde. 1967). In the celadonite used
in the present study, four discrete OH stretching frequencies were detect-
able, corresponding to OH coordinated to the following octahedral ion
pairs: Fe²⁺Fe³⁺  - 3534 cm⁻¹; MgFe²⁺  - 3557 cm⁻¹; Fe⁴⁺Al  - 3577
cm⁻¹; and MgAl  - 3602 cm⁻¹. By 400°C, the two lower frequency bands,
corresponding to OH groups coordinated to a ferric ion, were largely lost,
but the two higher frequency bands were not affected. Dehydroxylation was
complete for all species of OH at 500°C.

In one instance, clear evidence was obtained for interaction between the
KBr and the mineral. The brucite interlayer of the chlorite, pennine, was
found to decompose completely at 500°C in KBr disks, but this layer did
not begin to decompose till 600°C when the mineral was heated alone. In
both instances, OH groups of the phlogopite layer were retained up to
700°C, but for the mineral heated alone these absorbed at 3679 cm⁻¹,
whereas in the presence of KBr they absorbed principally at 3710 cm⁻¹.
A frequency near 3679 cm⁻¹ is characteristic of OH in the talc structure,
whereas a frequency near 3710 cm\(^{-1}\) is characteristic of \(OH\) in phlogopite. The higher frequency of the latter has been shown to be due to the electrostatic field of the potassium ions which are positioned directly above the protons of the lattice \(OH\) in phlogopite (Farmer and Russell, 1966). The results with the chlorite therefore indicate that potassium ions from the potassium bromide migrate into the interlayer space of the chlorite as the brucite layer decomposes. The infrared spectrum of montmorillonite dehydroxylated in a \(KBr\) disk showed significant differences from the normal pattern of the dehydroxylate, suggesting that here, too, some interaction with the \(KBr\) may have occurred.

**APPLICATIONS**

Problems in which some of these techniques have found application have frequently arisen in the course of collaborative studies initiated by colleagues at the Macaulay Institute, R. C. Mackenzie, B. D. Mitchell, W. A. Mitchell and M. J. Wilson, as well as by scientists at other centres. Published work involving infrared investigations of clays at this Institute has recently been reviewed (Farmer and Russell, 1967), and it is proposed here to illustrate their application in current investigations which have yielded new and interesting results.

(a) *Deuteration studies*

At room temperature interlayer water in smectites can readily be replaced by \(D_2O\), and exchange can occur between interlayer \(D_2O\) and hydrated \(NH_4^+\) to yield \(ND_4^+\). \(NH_4^+\) trapped in the interlayer space of collapsed vermiculites might be expected to be inaccessible to \(D_2O\), and this has been tested with films of vermiculite prepared from the Loch Scye soil vermiculite described by Aitken (1965). These films were prepared in the propylammonium form, and converted to the \(NH_4^+\) form by washing with \(NH_4Cl\) solution. Repeated flushing with \(D_2O\) at room temperature failed to convert 56% of the \(NH_4^+\) to \(ND_4^+\). Warming the film to 350°C in \(D_2O\) vapour induced further exchange between \(D_2O\) and \(NH_4^+\), but exchange between \(ND_4^+\) and lattice \(OH\) was then also detectable. It is interesting to compare these results with those obtained by the procedure of Alexiades and Jackson (1966) for estimating the vermiculite content of minerals. This indicated that after potassium saturation and drying at 100°C, 77% of the potassium resisted exchange with ammonium ions. It is clear from this work that estimates of the “vermiculite” content of minerals are dependent on the conditions used and the method of study.

\(NH_4^+\) in beidellite and saponite readily exchanged with \(D_2O\), but a significant proportion of \(NH_4^+\) in Wyoming montmorillonite (12%) and hectorite (23%) resisted exchange with \(D_2O\) at room temperature. Alexiades and Jackson (1966) obtained very similar figures for the “vermiculite” content of hectorite and montmorillonite by their procedure. These results support the suggestion that the stable \(NH_4^+\) formed when Na-saturated montmorillonite is treated with ammonia gas is strongly adsorbed on inaccessible sites (Russell, 1965).

Exchange between interlayer \(D_2O\) and the lattice \(OH\) of expanding
layer silicates has been mainly studied using ammonium saturated species since the $ND_4^+$ formed provides a source of deuterium in the interlayer

![Diagram](image)

Fig. 1.—$OH$ vibrations of (a) fresh green biotite, (b) weathered, golden biotite, (c) weathered biotite, partially dehydroxylated at 400°C and (d) vermiculite, prepared from fresh green biotite, and oxidized with $H_2O_2$. Spectra were obtained in 12 mm. $KBr$ disks, using the sample weights indicated. For spectra (a), (b) and (d), disks were heated to 200°C to reduce the adsorbed water content, but for spectrum (c), the disk was heated to 400°C to effect dehydroxylation.
space at elevated temperatures, when interlayer $D_2O$ is largely lost, and the mineral collapses to a 10 Å spacing. No exchange with lattice $OH$ has been detected at room temperature, but a weak $OD$ vibration first appears after treatment at 100°C. In contrast, the hydroxyl of interlayer $Mg(OH)_2$ and $Al(OH)_3$ formed in montmorillonite by the technique of Slaughter and Milne (1960), undergoes essentially complete exchange with $D_2O$ at 100-150°C. On exposing such a preparation to air humidity, interlayer $D_2O$ is replaced by $H_2O$, and the $OD$ vibrations of the interlayer hydroxides can then be observed entirely free from interference by water absorption bands, and relatively free from interference by lattice $OD$ absorption. This study has shown that the absorption bands of the interlayers in these synthetic chlorite-like preparations are considerably weaker, and occur at higher frequencies, than the corresponding bands of natural chlorite minerals. The highest frequency observed for the brucite layer in natural chlorites is in pennine, where it gives a strong broad band at 3638 cm$^{-1}$ with a shoulder at 3500 cm$^{-1}$. In montmorillonite, interlayer $Mg(OH)_2$ gives only the single maximum at 3710 cm$^{-1}$ observed by Russell (1965); interlayer $Al(OH)_3$ has its maximum absorption at 3680 cm$^{-1}$, with a low frequency shoulder which does not extend much below 3580 cm$^{-1}$.

Complete or nearly complete replacement of lattice $OH$ by $OD$ groups in beidellite, montmorillonite, saponite and hectorite is only achieved after repeated treatment at temperatures of 350°C or higher. The temperature of treatment is, however, limited by the tendency of $NH_4$-saturated smectites to lose their expanding properties (Russell and Farmer, 1964). Comparison of the spectra of the deuterated forms of these minerals with the spectra of the normal proton-containing species permits identification of absorption bands arising from $OH$ vibrations. The results (Farmer, Russell, Ahlrichs and Velde, 1967) support previous suggestions (Farmer and Russell, 1964, 1967) that the bending vibrations of $OH$ groups coordinated to the octahedral ion pairs $AlAl$, $MgAl$, and $Fe^3-Al$ give distinct absorption bands in the 800-950 cm$^{-1}$ region of the spectra of dioctahedral minerals. This assignment is a powerful tool which gives direct information on the octahedral occupancy of montmorillonites, and on the oxidation state of iron in the octahedral layer.

In saponite and hectorite, the study of deuterated samples has indicated that a band at 655 cm$^{-1}$, previously assigned to an $Si-O$ in-plane vibration (Farmer, 1958), is in fact the $OH$ bending vibration. This assignment is consistent with the results of neutron scattering studies on these minerals (Naumann et al., 1966).

(b) Oxidized biotites and vermiculites

Iron in fresh biotites is predominantly in the ferrous form, but natural weathering processes in soils largely oxidize this iron to the ferric state, with or without accompanying vermiculitization of the biotite (Walker, 1949). Where the ferrous content of the original biotite is high, the valency change must lead to a considerable excess of positive charge in the octahedral layer, and it has been suggested that this is compensated by ejection of ferric ions from the octahedral layer (Walker, 1949). This suggestion is supported
by the common association of goethite (Walker, 1949) and hematite (Rimsaite, 1967a, b) with oxidized biotites, but it is difficult to confirm the hypothesis by chemical analysis, as there are uncertainties in the calculation of structural formulae of biotites (Rimsaite, 1967a), and these uncertainties are further increased by partial vermiculitization and chloritization of oxidized biotites (Rimsaite, 1967b).

Ejection of iron from the octahedral layer leads to an increase in the number of unfilled sites; $OH$ groups associated with such vacancies are coordinated to only two octahedral cations, and give distinctive infrared absorption bands, which lie at frequencies lower than the bands of $OH$ groups associated with three octahedral cations (Serratosa and Bradley, 1958; Vedder, 1964). Comparison of the spectra of oxidized and unoxidized portions (golden and green respectively) of a single crystal, previously studied by Rimsaite (1967b), confirms the presence of greatly increased numbers of vacancies in the oxidized portion, as indicated by the development of a strong band at $3550\text{ cm}^{-1}$ (Fig. 1a and b). $OH$ groups associated with vacancies are lost by dehydroxylation in the $KBr$ disk at $400-450^\circ C$, and it is then possible to see (Fig. 1c) a weak residual band of $OH$ groups associated with filled sites at $3643\text{ cm}^{-1}$. This frequency can be ascribed to $OH$ associated with the grouping $Mg_2Fe^3+$. The principal band of the unoxidized biotite at $3658\text{ cm}^{-1}$ can be ascribed to $OH$ associated with $Mg^2Fe^3+, MgFe^2+, Fe^3+$ groupings, and the loss of this band is consistent with oxidation of ferrous to ferric ions.

Refluxing with $BaCl_2$ solutions proved effective in removing interlayer potassium from the fresh biotite without significant oxidation, as indicated by the infrared spectrum. Air oxidation of the vermiculite formed resulted in partial oxidation (loss of the $3658\text{ cm}^{-1}$ band) without creation of many new vacancies. Oxidation with $H_2O_2$ or $Br_2$ caused a marked colour change from green to golden, and in these samples the vacancy band at $3550\text{ cm}^{-1}$ is strongly developed, as in the naturally oxidized biotite (Fig. 1d).

Ferric ions ejected from the octahedral layer of oxidized biotites and vermiculites would be expected to be initially trapped in the interlayer spaces as hydrated ferric oxides or oxy-cations. Treatment of bromine-oxidized vermiculite with hydrazine to reduce these interlayer ferric species to ferrous forms permitted the extraction of 1.6% Fe from the vermiculite under mild conditions ($NaCl$ solutions containing 1:10 phenanthroline at $pH$ 4). The unoxidized vermiculite yielded only one tenth the amount of Fe under these conditions.

Infrared study of the thermal behaviour of oxidized biotites and vermiculites (Fig. 1) indicates that considerable loss of lattice $OH$ occurs before loss of adsorbed molecular water is complete. Lattice $OH$ in altered phlogopites of low iron content is considerably more stable, and infrared studies at this Institute have served to determine the point on thermogravimetric curves at which loss of molecular water is complete and loss of lattice $OH$ begins. This information is essential to the calculation of structural formulae for altered phlogopites from the results of chemical analysis (Newman, 1967).
SPECTROSCOPY OF CLAY MINERALS

CONCLUSIONS

The application of infrared spectroscopy to mineral studies is essentially an empirical science, in which theory guides interpretation and points to profitable fields of investigation, but can seldom predict details of the spectra of novel structures. The development of such a science is necessarily slow, as it is dependent on experience gained from a wide range of minerals and this in turn is dependent on convenient and appropriate apparatus and techniques of study.

The first exploratory investigation of mineral spectra was made over sixty years ago by Coblentz under conditions of intimidating difficulty. Developments in sample handling techniques, infrared spectrometers, and ancillary equipment have now rendered the process of obtaining high quality mineral spectra over an ever-widening range of frequencies a rapid and often routine procedure, and a body of knowledge is being built up which makes the study of such spectra increasingly profitable.

It is hoped that this paper will make some convenient techniques more widely known to soil mineralogists, and suggest possible fields in which they can be employed.

ACKNOWLEDGMENTS

The authors are indebted to Dr. J. H. Y. Rimsaite (Geological Survey of Canada) and Dr. A. C. D. Newman (Rothamsted Experimental Station) for samples of well characterized biotites, and to Mrs. K. Law and Mr. A. R. Fraser for able technical assistance.

REFERENCES

The potential contribution of infrared spectroscopy to the characterization of clay minerals is often limited by technical considerations. Recent advances in techniques of sample preparation and handling are described which permit the study of clay mineral spectra under vacuum or controlled-atmosphere conditions after heat treatment at temperatures up to 1000°C. The use of KBr pressed disks to follow the thermal behaviour of a single small sample in the range 100 to 700°C is illustrated. Examples of the application of these techniques are given; these include studies of the accessibility and reactivity of ammonium ions and hydroxyl groups in clay minerals, and evidence for the development of octahedral vacancies in biotite and vermiculite consequent upon the oxidation of octahedral ferrous to ferric ions.

SUMMARY

The potential contribution of infrared spectroscopy to the characterization of clay minerals is often limited by technical considerations. Recent advances in techniques of sample preparation and handling are described which permit the study of clay mineral spectra under vacuum or controlled-atmosphere conditions after heat treatment at temperatures up to 1000°C. The use of KBr pressed disks to follow the thermal behaviour of a single small sample in the range 100 to 700°C is illustrated. Examples of the application of these techniques are given; these include studies of the accessibility and reactivity of ammonium ions and hydroxyl groups in clay minerals, and evidence for the development of octahedral vacancies in biotite and vermiculite consequent upon the oxidation of octahedral ferrous to ferric ions.

RÉSUMÉ

La contribution potentielle de la spectroscopie infra-rouge à la caractérisation des minéraux des argiles est souvent limitée par des considérations techniques. Des progrès récents dans les techniques de la préparation et du traitement des échantillons sont décrits, qui permettent l'étude des spectres des minéraux d'argiles sous des conditions de vacuum ou d'atmosphère contrôlée, après avoir été soumis à la chaleur jusqu'à 1000°C. L'emploi de disques pressés KBr pour observer le comportement d'un seul petit échantillon dans la zone de 100 à 700°C est illustré. Des exemples de l'application de ces techniques sont indiqués; parmi ceux-ci sont inclues des études de l'accessibilité et de la réactivité des ions d'ammonium et des groupes hydroxyles dans les minéraux des argiles, et des évidences du développement de vides dans la biotite et la vermiculite comme suite de l'oxydation des ions octaédriques ferreux en ions ferriques.

ZUSAMMENFASSUNG

WEATHERING AND CLAY MINERALOGICAL CHARACTERISTICS OF VOLCANIC ASHES AND PUMICES IN JAPAN

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In general the formation of clay minerals in soils is governed by numerous factors. In other words, it is related to all soil forming factors (parent material, climate, vegetation, relief, and age). The problems have been repeatedly discussed by many investigators (Fieldes and Swindale 1954, Gorbunov 1963, Jackson and Sherman 1953, Mitchell et al. 1964, Rich and Thomas 1960, Schuylenborgh and Sänger 1950), but some of them have not yet been settled. This is also applicable to weathering and its products of volcanic ash soils in spite of considerable attention being paid to their pedogenesis and clay mineralogy. The object of the present paper is to offer some information on weathering products of volcanic ashes and pumices in Japan.

I. A Relation between Allophane and Imogolite

Amorphous substances hitherto regarded as allophane can be subdivided into two groups, i.e. allophane and imogolite (Aomine and Miyauchi 1965, Miyauchi and Aomine 1966, Yoshinaga and Aomine 1962). The latter is characterized by a more ordered structure than amorphous allophane, the occurrence of a sharp endothermic reaction at about 420°C in its differential thermogram, and its thready shape in electron micrographs. According to Aomine and his collaborators, thready particles of volcanic ash soils and pumices are not always imogolite.

In order to study the relation between allophane and imogolite, fine clay fractions (< 0.2 μ in e.s.d.) of a Humic Allophane soil found at the north-north-eastern slope of Volcano Unzen from which mica-hornblende andesitic ash was ejected were examined by clay mineralogical techniques. Coarse clay fractions (< 2 μ in e.s.d.) consist mainly of allophane, gibbsite (4-7%), amorphous free iron (several %), Al-vermiculite, illite, kaolin minerals, cristobalite, glass, and quartz. The separation of imogolite and allophane from fine clay fractions followed the method of Yoshinaga and Aomine (1962). For convenience the fraction dispersed at pH 10 was called the allophane fraction and the fraction dispersed only at pH 3.5 the imogolite fraction. The proportion of imogolite increased with depth, whereas allophane decreased. Only the imogolite fraction of the A11 horizon showed the above-mentioned characteristics of typical imogolite.
Fig. 1.—X-ray diffractograms of the imogolite (Im.-fr.) and allophane (All.-fr.) fractions (<0.2 μ in e.s.d.) separated from the A11 horizon (Shimabara near Volcano Unzen).

Remark: Mg-G-20°C = After glycerol solvation of Mg-clay at room temperature.
SHIMABARA—AB

Im.-fr.
K-500°C
K-300°C
K-20°C
Mg-G-20°C
All.-fr
K-500°C
K-300°C
K-20°C
Mg-G-20°C

Fig. 2.—X-ray diffractograms of the imogolite (Im.-fr.) and allophane (All.-fr.) fractions (<0.2 μ in e.s.d.) separated from the AB horizon (Shimabara near Volcano Unzen).

Remark: Mg-G-20°C = After glycerol solvation of Mg-clay at room temperature.
In other words, reflections at 14 and 8Å were fairly distinct and reflection at 18Å appeared after heating to 300°C, indicating that imogolites A and B coexist in the fraction. Since the allophane fraction also gave a weak reflection at 14Å which did not shift after Na-citrate treatment, the reflection may be due to admixture of imogolite A or to the occurrence of a phase intermediate to allophane and imogolite. Both fractions of the

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**Fig. 3.**—Differential thermograms of the imogolite (Im.-fr.) and allophane (All.-fr.) fractions (<0.2 μ in e.s.d.).
Fig. 4.—Infrared spectra of the imogolite and allophane fractions (<0.2 μ in e.s.d.).

Horizons other than the A11 horizon showed little or no difference in X-ray diffraction patterns, differential thermograms, and infrared spectra, as has been given in Figures 2, 3, and 4. Silica-alumina and silica-sesquioxide ratios of both fractions closely resembled each other, though there is little difference in water-alumina ratios.

However, electron micrographs of both fractions show a remarkable difference in shape of particles, i.e. thready particles are much more abundant in the imogolite fractions than in the allophane fractions (Figures 5 and 6).

Since even small amounts of the imogolite fraction in the A11 horizon gave prominent features of imogolite, and since clay mineralogical characteristics of both fractions of the lower horizons were almost the same except for their shape, it is reasonable to propose that there is a phase intermediate to allophane and imogolite rather than to say that the separation of imogolite from allophane was incomplete.
II. FORMATION OF AMORPHOUS MATERIALS FROM GLASS AND PUMICE

The mechanisms for allophane formation are probably manifold, as has been stated by Mitchell et al. (1964) and Fieldes (1966). The following are examples showing the formation of imogolite and allophane under Japanese conditions. In some pumices with varying degrees of weathering, the decreasing rate of glasses was the greatest as weathering proceeds, and the next was plagioclases (Kanno 1961). Fieldes (1966) also stated that glasses have predominantly random structures and hydrous aluminosilicate clays formed from them are likely to have initially random structures corresponding to allophane irrespective of whether or not complete dissolution and reprecipitation occurs. These statements indicate that glasses are important as a source for allophane.

From electron micrographs (Figure 7) it may be assumed that thready particles have been directly formed from glass-like particles, though the particles have not yet been identified as glasses. If thready particles are regarded as imogolite, it would seem that imogolite has not passed through the allophane stage, but has been formed directly from glasses. However, the thready particles would not be imogolite, but would be amorphous particles intermediate to allophane and imogolite, because it is doubtful
that ordered imogolite would be formed earlier than amorphous allophane. According to Miyauchi and Aomine (1966), filmy, gel-like substances in pumice beds are identified as imogolite. This indicates that imogolite has been formed by reprecipitation of aluminium and silica released from glasses.

Infrared spectra of silt and clay ($<2 \mu$ in e.s.d.) fractions separated from half-weathered pumices were compared with those of silica gels, alumina gels, and glasses. The silt and clay fractions were amorphous to X-rays. The latter contain a considerable amount of thready particles. Figures 8 and 9 indicate that main absorption bands of clay separates shifted to longer wavelengths with increasing alumina content, as has been described by Mitchell et al. (1964), and that infrared spectra of silt fractions closely resemble those of glasses. Absorption bands at 10-11 and 12.57 $\mu$ due to SiOH and Si-O-Si linkages could not be detected in the clay separates. It is assumed from Figures 8 and 9 that there is a possibility that glasses in pumices and ashes are changed into allophane through desilication, hydration, and replacement of Si-O-Al linkages for Si-O-Si linkages, and that the degree of shift in absorption maxima near 10 $\mu$ to longer wavelengths due to Si-O linkages may be regarded as an index of allophanization of glasses dependent on the coordination numbers of aluminium (Leonard et al. 1964).
Fig. 7.—Electron micrographs of clay fractions (<2 μ in e.s.d.) showing that thready particles are directly formed from glass-like particles. Right of the upper part, part of a glass-like particle strongly weathers. Left of the upper part, thready particles develop around a glass-like particle. The lower part, thready particles strongly develop around glass-like particles. (Scale = 1 μ)

It is noteworthy that there are absorption bands at 7·1 μ in the clay separates of Humic Allophane soils and pumices in Japan. These bands did
Fig. 8.—Infrared spectra in the NaCl region of the silt (20-2 μ in e.s.d.) and clay (<2 μ in e.s.d.) fractions separated from pumices.

Remarks: Si-G, silica gels; Gl(c), clay-sized volcanic glasses (n = 1-50); S13, silt fraction of the Sakurajima pumice; S16, silt fraction of the Akagi pumice (so-called Kanumatsuchi); C8, clay fraction (SiO₂/Al₂O₃ = 1-60) of the Aso pumice; C4, clay fraction (SiO₂/Al₂O₃ = 1-24) of the Nantai pumice (so-called Upper Kanumatsuchi); Al-G, alumina gels.

not disappear by deferration treatment (Mehra and Jackson 1960), but disappeared by dissolution treatment of allophane (Hashimoto and Jackson 1960). Infrared spectra of freshly-precipitated iron silicate (SiO₂ : Fe₂O₃ : H₂O(+)) = 2-59 : 1 : 2-10) indicated that intensities of absorption bands at 7-14 μ remarkably decreased after heating to 300°C. Further investigations are required to solve the question whether the bands near 7-1 μ are due to iron silicates or hisingerite (DeMumbrum and Chester 1964).
Fig. 9.—Infrared spectra in the KBr region of the silt (20-2 μ in e.s.d.) and clay (<2 μ in e.s.d.) fractions separated from pumices.
Remarks: S3, silt fraction of the Nantai pumice (so-called Imaichitsuchi). The rest is the same as given in Fig. 8.
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SUMMARY

In Humic Allophane soils and pumices, of all primary minerals, volcanic glasses are the most important minerals for allophane formation. In case of the lower horizons where amounts of the imogolite fraction are greater than those of the allophane fraction, clay mineralogical characteristics of both fractions, except for particle shape in electron micrographs, closely resembled each other. This suggests that there is an intermediate phase between allophane and imogolite. Electron micrographs of clay separates of a Humic Allophane soil showed that thready particles may have been directly formed from glass-like particles. It is doubtful whether these particles are imogolite. They are probably the above-mentioned intermediate phase. Allophanization of glasses proceeds through desilication, hydration, and replacement of Si-O-Al linkages for Si-O-Si linkages and depends on the degree of shift in absorption maxima near 10 μ to longer wavelengths. Further investigations are required to solve the question whether absorption bands, near 7 μ, of clay separates of Humic Allophane soils and pumices are due to iron silicates or hisingerite.
Dans les sols allophane humiques et dans les pones, de tous les minéraux primaires, les verres volcaniques sont les minéraux les plus importants pour la formation allophane. Dans le cas des horizons inférieurs, où les quantités de la fraction imogolite sont plus élevées que celles de la fraction allophane, les caractéristiques minéralogiques de l'argile des deux fractions, sauf la forme des particules dans les électron-micrographes, se ressemblent beaucoup. Ceci suggère qu'il y a une phase intermédiaire entre l'allopehane et l'imogolite. Les électron-micrographes des composants d'argile d'un sol allophane humique montraient que les particules en forme de fil auraient pu être formées directement de particules ressemblant à du verre. On doute que ces particules soient imogolites. Elles sont probablement la phase intermédiaire sus-mentionnée, entre l'allopehane et l'imogolite. L'allopehanisation des verres prend place en passant par la desilication, l'hydration et le remplacement des liens Si-O-Al par des liens Si-O-Si, et dépend des degrés de mouvement des maxima d'absorption proches de 10 μ vers des longueurs d'ondes plus longues.

D'autres recherches sont nécessaires pour découvrir si les bandes d'absorption proches de 7 μ des composants argileux de sols allophane humiques et des pones, sont dus à des silicates de fer ou à l'hisingérite.

ZUSAMMENFASSUNG

DIFFERENTIAL FORMATION OF ALLOPHANE, “IMOGOLITE” AND GIBBSITE IN THE KITAKAMI PUMICE BED

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I. INTRODUCTION

The gel film in a weathered pumice bed in the Kitakami district was first found by Shioiri (1934) and described as allophane. Emphasis was placed on the similarity between the gel film and a fine-clay fraction of glassy volcanic ash soil in the chemical composition and staining reaction. This observation has recently been extended by Miyauchi and Aomine (1966) to identification of “imogolite”—a distinct, poorly-crystalline, aluminium silicate (Yoshinaga and Aomine, 1962)—in both the gel film and the latter clay fraction. Prior to this investigation, Kanno (1959) and Kanno et al. (1960) concluded from their studies that the gel film consists of poorly crystalline progenitor of montmorillonite.

The present study deals primarily with the formation and inter-relationships of allophane, “imogolite” and gibbsite in the same pumice bed. These three are important products in the weathering of volcanic ejecta under the temperate and humid climatic conditions. The coarse texture of the pumice makes it possible to evaluate the differential formation in relation to the differences in the micro-environment and heterogeneous nature of the parent material.

TABLE 1
DESCRIPTION OF MURASAKINO PROFILE

<table>
<thead>
<tr>
<th>Layer</th>
<th>Origin</th>
<th>Depth</th>
<th>Colour</th>
<th>Carbon content %</th>
<th>pH</th>
<th>H₂O</th>
<th>n KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Volcanic ash</td>
<td>0–15</td>
<td>Dark reddish brown (5 YR 3/2)</td>
<td>3-20</td>
<td>4-8</td>
<td>4-0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Volcanic ash</td>
<td>15–75</td>
<td>Reddish Yellow (7-5 YR 6/8)</td>
<td>0-75</td>
<td>5-5</td>
<td>4-2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pumice</td>
<td>75–125</td>
<td>Reddish yellow-yellowish red (5 YR 5-5/8)</td>
<td>0-64</td>
<td>6-0</td>
<td>5-5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pumice</td>
<td>125–250</td>
<td>Reddish yellow (7-5 YR 7-5/8)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Volcanic ash</td>
<td>250–267</td>
<td>Light brown (7-5 YR 6-4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Location: Murasakino, Itoyono-machi, Kitakami-shi, Iwate-ken (Longitude, 141°08′E., Latitude, 39°19′N.).
Climate: Mean annual temperature, 9.5°C; annual precipitation, 1275 mm.
Vegetation: Mixed forest (Rhus trichocarpa, Quercus serrata, Potos densiflora, Miscanthus sinensis and Sasa).

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II. PROFILE DESCRIPTION

A brief description of the Murasakino profile summarized from that given by Honya and Sekiya (1964) is given in Table 1. The core sample for the present study was taken from the upper half of the fourth layer. The mineralogy of the upper volcanic ash soils was studied by Masui et al. (1966). Their results showed that the ashes are lithologically alkalic andesite which has been well weathered. Layer silicates, mainly vermiculite-$Al$ chlorite intergrades with 10 and 7 Å minerals, dominate in the weathering product.

III. OCCURRENCE OF WEATHERING PRODUCTS

(a) Allophane

Fig. 1 shows, in a generalized and schematic way, the occurrence of the weathering products in the pumice bed. The pumice is weathered fairly

![Diagram](image-url)

*Fig. 1.—Occurrence of weathering products in Kitakami pumice bed. A; allophane. Gb; gibbsite. Hu; humic substances. I; "imogolite". Mn; manganese oxides.*
well, and its mechanical composition determined after light crushing with a wooden pestle and a dispersion has about 10 and 40% of the total in less than 2 and 2-20 μ fractions, respectively. Allophane dominates in these fractions together with about 10% of Na$_2$S$_2$O$_4$-NaHCO$_3$-Na citrate.

Fig. 2.—X-ray diffraction patterns; (1) allophane, less than 0.5 μ fraction from pumice, after Na$_2$S$_2$O$_4$-NaHCO$_3$-Na citrate treatment, Mg-saturation, parallel orientation and air-drying. (2) “imogolite” from gel film, after Na$_2$S$_2$O$_4$-NaHCO$_3$-Na citrate treatment, parallel (a) and random (b) orientation and air-drying.
extractable oxides. Allophane, here, is nearly X-ray amorphous (Fig. 2, 1), and is soluble in a hot 0·5 \( \text{N} \) \( \text{NaOH} \) giving an \( \text{Si/Al} \) ratio of 1/1·17. After dispersion, fine granules and poorly defined threads appear in the electron microscope (Fig. 3a).

The pumice shows a typical reticular structure consisting largely of glass shards. The feldspar of labradorite composition, hypersthene and augite appear as phenocrysts. A very small amount of fine grained quartz was identified in the fine-sand fraction separated from the crushed pumice.

As the carbon replica indicates, “subgrains” of a few to ten microns diameter appear in the fresh section of the weathered pumice (Fig. 3b). The allophane granules seen in the dispersed state (Fig. 3a) are found in the crust, concentric to the apparently unwealhered core of “subgrains” as well as in the interstices.

(b) “Imogolite”

The gel films, semi-translucent to translucent, appear to be exclusively filling the interstices of the pumice grains or covering their surface (Fig. 1). Black filamentous roots also develop in the interstices throughout the pumic layer, often protruding the gel films. These penetrating roots could not be traced to the upper volcanic ash layer, and hence, to the present vegetation (Personal communication, Dr. N. Miyauchi). The content of the gel film in the core sample amounted to about 8 to 10% by weight. Its main constituent is “imogolite” as reported by Miyauchi and Aomine (1966). A mechanically disintegrated and acid dispersed specimen shows a marked planar orientation in the X-ray analysis (Fig. 2, 2a) and dissolution in hot 0·5 \( \text{N} \) \( \text{NaOH} \) solution yielded an \( \text{Si/Al} \) ratio of 1/1·7. The slightly higher value than that expected for an ideal “imogolite”, 1/2 (Wada, 1967), is probably due to the presence of small pumice grains in the film (Fig. 3g). Fine but well-defined threads appear in the electron microscope (Fig. 3c).

The matrix of the gel film is almost transparent, and shows an anomalous double refraction (Fig. 3h). This double refraction was noted by the former investigators (Kanno et al., 1960; Miyauchi and Aomine, 1966), but its anomalous nature has not been well understood. Its appearance is not similar from place to place and does not necessarily depend on the thickness of the gel film. The most marked effect is observed at its fringe, particularly viewing along the direction parallel to the film surface, where a parallel extinction and hence, an oblique illumination occur along possible structure lines. Single flakes, ca. 0·3 x 0·2 mm, cut from the gel films always give a typical powder pattern of “imogolite” in a rotation X-ray camera, irrespective of the presence or absence of the double refraction and of their orientation, so the colours are truly anomalous.

The carbon replica of the gel film shows that the “imogolite” threads run parallel to its surface (Fig. 3d). Further, the fissures appearing on the film indicate the presence of a planar net structure consisting of twisted “imogolite” threads (Fig. 3e). Similar electron micrographs have recently been obtained at higher magnifications by Yoshinaga et al. (1968). The development and stacking of such planar nets can cause the anomalous
Fig. 3.—Electron micrographs, x 6,670; (a) allophane, less than 0.5 μ fraction from pumice, after Na₂SO₄-NaHCO₃-Na citrate treatment and acid dispersion, (b) fresh section of weathered pumice (carbon replica), (c) “imogolite” from gel film, after Na₂SO₄-NaHCO₃-Na citrate treatment and acid dispersion, (d) and (e) surface of gel film (carbon replica), and (f) fresh section of gibbsite concretion (carbon replica). Light micrographs, x 27; gel film, plain light (g) and nicols crossed (h).
double refraction described above, while the twisting of the "imogolite" threads accounts for the lack of the orientation effect in the single-flake X-ray analysis.

In the gel film, many pumice grains of various size are entrapped (Fig. 3g). In addition, the accumulation of humic substances with marked pigmentation (Fig. 1; $H_u$) occurs associating with reddish brown to purple red granules of biological origin and mycelial filaments (Fig. 3g). As Fig. 3d illustrates, the microbiotic activity on the gel film is also evidenced.

![X-ray diffraction patterns](image)

*Fig. 4.*—X-ray diffraction patterns of white concretions (a) and associated black portions including rock fragment and manganese concentration (b), after crushing in an agate mortar, random orientation and air-drying.
on the carbon replica, where a number of microbe colonies remain despite the HF treatment. On the other hand, many colourless, filamentous striations appear throughout the gel film. These evidently have no relationship to the double refraction of the gel matrix and show similarities in appearance to the coloured mycelial filaments. If a biological origin is admitted, gel formation must have proceeded under the fairly strong biotic activity as well as under the leaching activity of the water moving downward from the pumice surface.

(c) Gibbsite

Few but prominent white concretions mainly appear on some rock fragments with surface concentrations of black manganese oxides (Fig. 1; Mn). The X-ray analyses show that the white concretions are nearly pure gibbsite together with a small amount of an unidentified 10A component (Fig. 4a), which disappears from the X-ray pattern on heating at 100°C. The X-ray pattern of the associated black portions including the manganese concentrations and rock fragments indicates the presence of a chlorite, quartz and feldspar (Fig. 4b). The amount of finely divided quartz is much higher than that of the feldspar, whereas the reverse situation exists in the pumice. No obvious difference between the two is seen in the mineral composition of the heavy fine-sand fraction.

The replica indicates that the gibbsite concretion consists of densely packed and peculiarly warped rods (Fig. 3f). The similar morphology was reported both for macroscopic and microscopic aggregates of gibbsite by Ladding (1961) and Wada and Aomine (1966), respectively. The rods show cleavage both parallel and perpendicular to the long axis, suggesting an association of the bundles which consist of small and very thin platelets of gibbsite.

Genetic Implications

The foregoing observations may illustrate the following pattern of weathering in the Kitakami pumice bed under the cool, temperate and humid climatic conditions. The weathering commences in the differentiation of the weathered crusts from the unweathered cores within the pumice "subgrains" that exist in the pumice grain. Formation of allophane with the Si/Al ratio considerably higher than 1/2, proceeds in the crusts and interstices between the "subgrains". The outward diffusion of the leached solution from the pumice grain, and the reprecipitation of Si and Al at the ratio of 1/2, result in the formation of the networks of "imogolite" threads somewhat concentric to the exposed pumice surface. Subtle changes in the balance between the basic cations and silicate ions in the leached solution and in the moisture regime may control the differential formation of allophane and "imogolite". The gibbsite forms separately, mostly in the rock fragments and associated portions. An exact mechanism of the formation of the gibbsite concretion is not known, although a similar occurrence of gibbsite has been reported in the volcanic sand and gravel layers at Kurioshibaru, Kumamoto (Wada and Aomine, 1966).

The differential formation of halloysite and allophane in the macro-
scopic scale in the weathering of volcanic ash and pumice under different circumstances was previously studied and interpreted in terms of the transition from one stage to another in a weathering sequence, volcanic glass, feldspar—allophane—halloysite (Aomine and Wada, 1962). More generally, the occurrence of a variety of the products in weathered volcanic ash was interpreted in a similar way. The present observation indicates a parallel formation of allophane and "imogolite" from the pumice, and a lack of sequential relationship between either of these two and gibbsite. The same process could occur in a microscopic or submicroscopic scale in the weathering of finely comminuted volcanic ash.

ACKNOWLEDGMENTS

The authors wish to acknowledge with thanks the guidance of Dr. H. Shirozu, Kyushu University, for the single-flake X-ray analysis, Dr. H. Yotsumoto, Japan Electron Optics Laboratory Co. Ltd., for the preparation of carbon replicas and the assistance of Dr. N. Miyauchi, Kagoshima University, for the sample collection and vegetation study.

REFERENCES


SUMMARY

The weathering of the Kitakami pumice bed displays a variety of products. Within the pumice grains, fine granules of allophane with the Si/Al ratio, 1/1-17 appear in the crust concentric to the unweathered core of micron-size "subgrains" of the pumice and their interstices. "Imogolite" appears exclusively as macroscopic gel films covering the surface of the pumice grains or filling their interstices. The gel films show a double refraction, but their flakes, ca. 0.3 x 0.2 mm, always give a typical powder pattern of "imogolite", irrespective of the presence or absence of the double refraction and their orientation. The development and stacking of a net structure of twisting "imogolite" threads occurs parallel to the film surface. Few white concretions appear on the rock fragments with some black surface concentrations rich in manganese oxides. The concretions consist of peculiarly warped, micron-size rods of gibbsite.

The differential formation of the weathering products in the Kitakami pumice bed was interpreted in terms of the differences in the micro environ-
ment and the heterogeneous nature of the parent material. The same process could occur on a microscopic scale in the weathering of finely comminuted volcanic ash.

RÉSUMÉ

L’altération du lit de ponce du Kitakami montre une variété de produits. Parmi les grains de ponce, de fins granules d’allophane avec un rapport de \( \text{Si}/\text{Al} \) de 1 à 1.17 apparaissent sur la croûte autour d’un monolithe de sol intact de “sous-grains” de la taille de microns de la ponce et des interstices. L’“imogolite” apparaît exclusivement sous forme de pellicules de gel microscopiques recouvrant la surface de grains de ponce ou comblant les interstices. Les pellicules de gel montrent une réfraction double, mais leurs flocons (0.3 x 0.2 mm) pulvérisés, donnent un motif typique, sans rapport avec la présence ou l’absence de double réfraction ou de leur orientation. Le développement et l’empilement d’un réseau de filets sinueux d’imogolite apparaît parallèlement à la pellicule de surface. Quelques concrétions couleur de neige apparaissent surtout sur les morceaux de la roche associées à une concentration noire riche d’oxydes de manganèse. Les concrétions consistent en paillettes de gibbsite de quelques microns, particulièrement déformées.

La formation différentielle des produits d’altération du lit de ponce du Kitakami a été interprétée en termes de différences du micro-environnement et de la nature hétérogène de la roche-mère. La même processus pouvait se produire à une échelle microscopique lors de l’altération de la cendre volcanique finement pulvérisée.

ZUSAMMENFASSUNG


Die Differentiale Bildung der Verwitterungsprodukte in der Kitakami Bimsstein-Schicht wurde ausgelegt als Ausdruck der Unterschiede in der Mikroumwelt und der heterogenen Beschaffenheit des Urmaterials. Derselbe Prozess könnte sich in einem mikroskopischen Massstab in der Verwitterung fein-lockerer, vulkanischer Asche ereignen.
Allophane has been the subject of a number of soil studies in New Zealand. This paper describes the trends of some of these studies with particular reference to the nature of the allophanic materials and aspects of their significance with respect to pedogenesis and soil classification.

Recognition of Allophane in Rocks and Soils

Allophane obtained from fissures in rocks was named by Stromeyer and Hausmann (1816). It was reported as a soil constituent by Seki (1913) who, from chemical and petrographic evidence, deduced its presence in Japanese volcanic ash soils. From similar evidence its presence in New Zealand volcanic ash soils was reported by Henderson and Ongley (1923) and by Taylor (1933). The properties of geological allophane were fully described in a modern sense by Ross and Kerr (1934) who used optical, X-ray diffraction, thermal dehydration, and chemical methods. They concluded that allophane is an amorphous material, commonly associated with halloysite, with no definite chemical composition, and no crystal structure, and that the name should include all mutual solid solutions of silica, alumina, water, and minor amounts of bases even though the amounts of these components may differ.

High amounts of allophane in six different New Zealand volcanic ash soils were found by Birrell and Fieldes (1952) using X-ray, differential thermal, electron-micrographic, and chemical methods.

Soil Classes Containing Allophane

The above mentioned techniques, together with infrared absorption (Adler, 1950; Fieldes et al., 1956) and refined measurements of surface properties, were increasingly used in the investigation of allophanic soils from 1950 onward. In Japan, Aomine and Yoshinaga (1955) showed that certain ando soils from volcanic ash had clay fractions composed predominantly of allophane. In New Zealand, Birrell and Fieldes (1952), and Fieldes (1953, 1955) showed that all soils included both in yellow-brown pumice soils derived from rhyolitic volcanic ash, and in yellow-brown loams derived from rhyolitic ash and from andesitic ash, similarly, had clay fractions composed predominantly of allophane. The ability to obtain allophane from soils in a form relatively free from crystalline material, and the high proportions of agriculturally important soils derived from volcanic ash in Japan and New Zealand, encouraged study of soil allophane in
<table>
<thead>
<tr>
<th>Soil Type Name*</th>
<th>Soil Class and Parent Rock</th>
<th>Mean Annual Rainfall (cm)</th>
<th>Average Mean Temp. (°C)</th>
<th>Allophane %</th>
<th>Clay % less than 2 micron, other than allophane</th>
<th>Nature of dominant clay, other than dissoluble allophane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Templeton</td>
<td>Recent soil from greywacke alluvium</td>
<td>64</td>
<td>11</td>
<td>3</td>
<td>13</td>
<td>Micaceous</td>
</tr>
<tr>
<td>Matapiro</td>
<td>Yellow-grey earth from siltstone</td>
<td>84</td>
<td>12</td>
<td>6</td>
<td>15</td>
<td>Illite</td>
</tr>
<tr>
<td>Porirua</td>
<td>Yellow-grey to yellow-brown earth from wind-blown sand</td>
<td>102</td>
<td>11</td>
<td>5</td>
<td>11</td>
<td>Micaceous</td>
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<tr>
<td>Paremata</td>
<td>Yellow-grey to yellow-brown earth from greywacke</td>
<td>107</td>
<td>11</td>
<td>7</td>
<td>32</td>
<td>Illite, Kaolin</td>
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<tr>
<td>Tekapo</td>
<td>High country yellow-brown earth from greywacke</td>
<td>58</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>Micaceous</td>
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<td>Puketeraki</td>
<td>High country yellow-brown earth from schist</td>
<td>127</td>
<td>8</td>
<td>10</td>
<td>35</td>
<td>Clay-vermiculite</td>
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<tr>
<td>Judgeford</td>
<td>Central yellow-brown earth from loess plus volcanic ash</td>
<td>114</td>
<td>12</td>
<td>10</td>
<td>19</td>
<td>Clay-vermiculite</td>
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<td>Taupo</td>
<td>Yellow-brown pumice soil from rhyolitic ash</td>
<td>127</td>
<td>12</td>
<td>17</td>
<td>17</td>
<td>Hydrous feldspars and glasses</td>
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<td>Egmont</td>
<td>Yellow-brown loam from andesitic ash</td>
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<td>25</td>
<td>Hydrous feldspars and glasses</td>
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<tr>
<td>Papakauri</td>
<td>Red loam from basalt scoria</td>
<td>158</td>
<td>14</td>
<td>46</td>
<td>16</td>
<td>Gibbsite</td>
</tr>
</tbody>
</table>

* These soils are more fully described in "Soils of New Zealand" (N.Z. Soil Bureau, in press).
both countries at an early date. Allophane was also recognised as a dominant clay constituent of certain weakly developed New Zealand red loams and brown loams formed from basalt (Fieldes, 1953; Fieldes and Swindale, 1954). Mitchell and Farmer (1962) reported allophane in soils formed from more basic rocks and high amounts of allophanic material have been found in New Zealand soils formed from ultrabasic rocks.

In the same paper they described infrared absorption methods for estimating amounts of allophanic soil constituents in the presence of crystalline clay constituents. Methods such as these have shown that most New Zealand soils, with the exception of peaty and highly siliceous soils, contain appreciable amounts of allophane. Results for a dissolution technique (Hashimoto and Jackson, 1958) are shown in Table 1.

**Forms of Allophane**

(a) Allophane A and B

Fieldes (1955) studied a sequence of New Zealand soils derived from volcanic ash of increasing age from yellow-brown pumice soils through yellow-brown loams to brown granular clays whose corresponding mineral colloid sequence (for particles less than 2 microns) advanced from allophane B through allophane A to metahalloysite. Allophane A resembled geological allophane but allophane B in the younger soils appeared to have a higher proportion of very small particles (about 50 to 10\(\text{A}\)) and showed infrared evidence of discrete silica, that is not present in allophane A. Unlike kaolins, in which \(\text{Si}\) and \(\text{Al}\) are closely linked, and which, like allophane A, give sharp exothermic DTA peaks near 900°C due to formation of mullite, allophane B gives no such peaks, and it was therefore suggested that the alumina and silica in allophane B were discrete.

Miyauchi and Aomine (1964) questioned the presence of allophane B in corresponding Japanese soils, because the finest particles separated from such soils had properties of allophane A. They attributed the infrared evidence of discrete silica in the coarser fractions to cristobalite. Fieldes and Furkert (1966) who reconsidered this viewpoint for a range of New Zealand soils, confirmed allophane A in the finest particles of some of these soils, but found insufficient cristobalite in coarse fractions (0-2 to 2 microns) to account for the magnitude of the infrared effect mentioned. They considered that the evidence confirmed the presence of discrete amorphous silica in the coarse clays, and in some of the fine clays (less than 0-2 microns) of the soils. They suggested that some effects of amorphous silica could be derived from domain zones of vitreous silica in volcanic glass, or from related hydrous silica zones in hydrous glass. The evidence of the latter paper and of that of Miyauchi and Aomine (1964) indicate that coarse fractions of the clays are likely to include amorphous hydrous derivatives of volcanic glass or feldspars, which fit the definition of allophane as amorphous hydrous aluminosilicate, and which have surface properties like allophane A. It was proposed by Fieldes and Furkert (1966) that the term allophane B be retained generically for this kind of material, thus recognising the possibility of association with crystalline minerals and
also the possibility of differences between hydrous derivatives of feldspars, hydrous glasses and vitreous glasses and allowing for the possible presence of discrete amorphous hydrous oxides of aluminium and silicon.

At the present stage of definition therefore both allophane \( A \) and allophane \( B \) are essentially amorphous. Allophane \( A \) gives a characteristic high temperature exothermic reaction on heating but no evidence that it is composed of other known discrete materials. Allophane \( B \) does not give a high temperature exothermic reaction but may contain one or more known discrete materials.

(b) Hydrous feldspars

As indicated above, clay-size particles of hydrous feldspar are likely to have surface properties similar to allophane. The term “hydrous feldspar” was used for hypothetical clay-size particles of amorphous hydrous feldspar included in material not dissolved by the action of sodium hydroxide solution on clay fractions of volcanic soils (Fieldes, 1962). Fieldes and Furkert (1966) showed that particles formed by prolonged grinding of feldspar have X-ray, differential thermal, infrared, and cation-exchange properties which justify regarding them as allophane or allophanic.

(c) Hydrous glasses

Fieldes and Furkert (1966) have shown that, for reasons like those discussed for hydrous feldspars, clay-size hydrous glass particles may be regarded as allophanic.

(d) Hydrogels and Xerogels

In soils containing much allophane there are very great differences in physical properties between those soils that are permanently moist and those that are periodically dry. The former, for instance, are of greasy consistence while the latter are friable (Fieldes, 1966). It is considered that fragments of soil allophane are initially essentially gel-like. In the greasy hydrous gel condition the degree of cross-linking of hydrous silica and alumina is limited because water keeps the structures open. Upon drying the gels shrink isometrically and increasing condensation and cross-linking results in compact xerogel particles which are hard and friable but retain allophanic surface properties.

(e) Imogolite

Imogolite, a fibrous amorphous aluminosilicate with many properties of allophane, has been separated from certain Japanese volcanic ash soils after acid dispersions, by Yoshinaga and Aomine (1962). According to Dr. Claridge (pers. comm.) there is evidence that fibrous allophanic material can be obtained in clays from certain New Zealand volcanic ash soils by acid dispersion following alkali dispersion.

REATIONS WITH SODIUM FLUORIDE

Egawa et al. (1960) described the release of \( \text{OH} \) from allophane by fluoride and Huang and Jackson (1965) have elucidated the mechanism
of reaction of neutral fluoride solution with soil constituents including allophane. Fieldes and Perrott (1966) described an application of the reaction to provide a field test for allophane in which 0.2 g of soil reacts with a drop of 1M NaF on phenolphthalein-treated paper, the resulting effect being red, pink or colourless for amounts of allophane above 7%, 5-7%, and below 5% respectively.

At the time of writing, the latter test is being examined by soil workers in a number of countries. Dr. K. Norrish, Mr. B. Tucker and Mr. J. G. Pickering (pers. comm.) have tested a range of mineral specimens, finding that some (but not all) specimens labelled, for instance, "gibbsite" or "halloysite", give a pink or red test. Since some specimens of these minerals do not produce colours, it seems likely that those that do contain impurities or imperfections which could justify their description as allophane.

The method has been tested also by officers of the United States Department of Agriculture who, in a preliminary report, confirmed the semi-quantitative nature of results of the test when applied to soils known to contain amorphous aluminosilicates. They considered that it failed to distinguish between ando soils and B horizons of podzols, and pointed out that different results were obtained from different batches of sodium fluoride, possibly due to differences in content of silicofluoride (Dr. G.

<table>
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<th>Table 2</th>
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<tr>
<td><strong>AVERAGE VALUES OF pH AT 20°C OF 1g SOIL IN 50 ml 1M NaF AFTER 1 HOUR, FOR ALLOPHANIC INTRAZONAL SOILS (TAYLOR, 1948)</strong></td>
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<tr>
<td></td>
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<tr>
<td><strong>Average mean annual rainfall (cm)</strong></td>
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<tr>
<td>Yellow-brown pumice soils from rhyolitic ash</td>
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<td>Yellow-brown loams from andesitic ash</td>
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<tr>
<td>Red and brown loams from basalt (weakly developed)</td>
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<table>
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<th>Table 3</th>
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</thead>
<tbody>
<tr>
<td><strong>AVERAGE VALUES OF pH AT 20°C OF 1g SOIL IN 50 ml 1M NaF AFTER 1 HOUR, FOR MODAL ZONAL SOILS (TAYLOR, 1948) DERIVED FROM SILICEOUS ROCKS</strong></td>
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<tr>
<td></td>
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<tr>
<td><strong>Average mean annual rainfall (cm)</strong></td>
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<td></td>
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<tr>
<td>Brown-grey earths</td>
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<tr>
<td>Yellow-grey earth</td>
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<tr>
<td>Yellow-brown earths (strongly weathered)</td>
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<tr>
<td>Podzols</td>
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</tbody>
</table>
Smith, pers. comm.). The basis of a tentative laboratory test suggested by Fieldes and Perrott (1966), in which amounts of allophane are judged from pH of 1 g soil treated with 50 ml 1M NaF for a period of 2 minutes, is incorporated in the supplement to the Soil Classification System (7th Approximation) (Soil Survey Staff, 1967) as a test for "soil domination by amorphous material".

Fieldes and Perrott (personal communication) have considered the significance of pH values obtained by treating 1 g samples of a wide range of air-dried soils for 1 hour in 50 ml 1M NaF at 20°C. Their results are summarised for allophanic soils from volcanic ash and from basalt in Table 2, and for zonal soils from siliceous rocks in Table 3.

It may be seen from Table 2 that release of OH by NaF from allophanic soils results in B horizon pH values approaching 11. Where organic matter tends to be high in the horizons, as in the case of the yellow-brown pumice soils which have turfy topsoils, H released from the exchange complex lowers pH so that the pH spread between A and B horizons becomes considerable, and of course much wider than the spread of pH obtained by water or by 1M KCl. The spread so obtained may be regarded as a manifestation of a podzolising process, and this appears to be confirmed by the extremely high SiO₂:Al₂O₃ ratios exhibited by inorganic clay fractions of the top half-inch of yellow-brown pumice soils and some yellow-brown loams.

Results for zonal soils derived from comparable siliceous rocks arranged in a sequence of increasing rainfall are shown in Table 3. For the soils, temperature, and therefore weathering intensity, also increases with increasing rainfall. The increasing spread of pH between A and B horizons again appears to be evidence of increasing podzolisation and the higher values in the B horizons of more weathered and more podzolised soils appears to be evidence of formation of allophane-like material by the podzolising process.

Table 4 lists characteristic pH values given by high country soils of

<p>| TABLE 4 |
| VALUES OF pH AT 20°C OF 1g SOIL IN 50 ml 1M NaF AFTER 1 HOUR, FOR MODAL HIGH COUNTRY ZONAL SOILS (TAYLOR et al., 1959) |</p>
<table>
<thead>
<tr>
<th>Mean annual rainfall (cm)</th>
<th>pH Horizon A</th>
<th>pH Horizon B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conroy soil (Brown-grey earth)</td>
<td>33</td>
<td>7.60</td>
</tr>
<tr>
<td>Tekapo soil (High country yellow-grey earth?)</td>
<td>58</td>
<td>10.35</td>
</tr>
<tr>
<td>Puketeraki soil (High country yellow-brown earth)</td>
<td>127</td>
<td>8.75</td>
</tr>
</tbody>
</table>

*In this soil, because of low rainfall, small amounts of free CaCO₃ appear in the subsoil. CaCO₃ reacts with NaF contributing to alkaline pH values.
different rainfall. Due to their altitudes the average mean temperatures of these soils are much lower than those of the yellow-grey earths, yellow-brown earths and podzols of Table 3. The soils of Table 4 may thus be considered to constitute a high country sequence. The very high pH values given by the B horizon of the Puketeraki soil and by the A and B horizons of the Tekapo soil with NaF treatment are evidence of the development of allophane-like material by the soil process in high country zonal soils even at an annual rainfall of 23 inches. The magnitude of this effect (pH 10.35 to 11.00) in the case of the Tekapo soil (cf. the yellow-grey earth of Table 3, also with rainfall of 23 inches, pH 7.50 to 8.50) probably demonstrates the greater effectiveness of moisture in producing and maintaining allophane at the lower temperatures of the high country soils. This question is dealt with by Pohlen (personal communication), who has also suggested the name "High country yellow-grey earth", not previously used, for high country soils with rainfall characteristic of the yellow-grey earth zone. The concept appears acceptable and may have considerable significance. With increasing rainfall in the Puketeraki soil, what may be evidence of increasing podzolisation is exhibited in the increased spread of pH between A and B horizons.

**Significance of Structural Randomness in Pedogenesis**

Fieldes (1966) has shown that the formation of allophane from volcanic ash is due to structural randomness of volcanic glass and imperfect structures of component feldspars. He demonstrated that structural randomness is an essential feature of allophane which can be produced by any soil process leading to randomness. Thus allophane is expected, and occurs, in soils derived from glacial rock flour in which randomness is induced by excessive comminution of minerals. Allophane is also expected and found in soils from rapidly weathering basic minerals, and also in subsoils of podzols where randomness is induced by rapid precipitation. Allophane tends to persist in permanently moist conditions where it remains gel-like and water-filled so that it is prevented from assuming more orderly crystal-line aluminosilicate structures.

**Conclusions**

Allophane in soils, first recognised in clays of volcanic ash soils, is now known to be produced by weathering of volcanic ash, of basic rocks, of ultrabasic rocks, by the grinding of minerals, and by the podzolising process. Accumulation of related material is favoured by the soil process of the high-country soils.

Appreciable amounts of allophane appear to be present in most mineral soils in New Zealand and in spite of the difficulties in dealing with such random-structured amorphous material, its high reactivity, as indicated by its reaction with NaF, makes it clear that its possible effects on physical and chemical behaviour deserve attention for the majority of soils.
REFERENCES


SUMMARY

Published work on allophane in New Zealand is reviewed, and different forms of allophane are considered. The significance of various aspects of a recently proposed sodium fluoride field test for allophane is also considered, and some views of users in two other countries are given. Some allophane is present in most mineral soils in New Zealand. The material of the B horizons of podzols has allophanic activity. A modified laboratory version of the sodium fluoride method, depending on the range of pH developed between the A and B horizons of any soil, indicates the extent of the podzolising effect. This pH range, which is characteristic for the principal soil classes, gives evidence of the operation of a podzolising process in some degree in many New Zealand mineral soils.

RÉSUMÉ

Un compte rendu est fait de la littérature sur l’allophane en Nouvelle-Zélande, et les différentes formes d’allophane sont considérées. La signification de différents aspects d’un essai de fluorure de sodium au champ récemment proposé est considéré aussi, et le point de vue de deux usagers.
dans différents pays est indiqué. Une certaine quantité d'allopiane est présente dans la plupart des sols minéraux en Nouvelle-Zélande. Le matériel des horizons B de podzols a une activité allophanique. Une version modifiée de laboratoire de la méthode de fluo statue de sodium qui dépend de la zone de pH développée entre les horizons A et B de n'importe quel sol indique le degré de l'effet podzolisateur. Cette zone de pH, qui est caractéristique des principales catégories de sol, fait preuve de la présence d'un processus podzolisateur à un certain degré dans beaucoup de sols minéraux de la Nouvelle-Zélande.

ZUSAMMENFASSUNG

HUMUS ACIDS AND THEIR ORGANO-MINERAL DERIVATIVES IN SOIL

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Much research has been devoted to the study of the nature of humus acids, yielding data on their elementary composition, the content and character of their functional groups, and the probable components of the nucleus of the molecule.

The major advances made over the last decade have been summarized in a number of monographs and review papers (Alexandrova 1962, Kononova 1963, Manskay 1963). These data suggest that humus substances in soil consist of a double component polycarboxylic acid system containing nitrogen. The system is formed during the humification of plant residues and consists of a group of humic acids and a group of fulvic acids.

The use of the genetic research method makes it possible to observe the changes in humus acids during the humification of plant residues. This is of great interest for the further study of the nature of humus acids and the mechanism of the formation of their double-component system.

A series of experiments was conducted on the humification of various plant materials, oak and clover leaves, and grass roots, under laboratory conditions, i.e. in quartz sand at a temperature of 22-25°C and a moisture content equal to 60% of the maximum water-holding capacity of the decaying material. Humus substances were extracted from the decaying plant residues by treatment of the material with 0.02 N KOH solution performed three times at 3, 15, 30, 90 and 180 days after the beginning of humification. The alkaline extracts were clarified by use of centrifugation and ceramic filters. Humic acids were precipitated at pH 1, dialysed and dried at 25-30°C. In the preparations, the following were determined; infrared spectra, elementary composition, the amount of carboxyl groups (determined by titration with barium hydroxide) and phenolic hydroxyl groups (estimated by quantitative acetylation). Particle size was determined by filtration on “Sephadex” gel.

Figure 1 shows the infrared spectra of humic acid preparations extracted from humifying clover and oak leaves. All the spectra are of the same type and have well distinguished absorption bands which are characteristic of carboxyl and phenolic hydroxyl groups.

From the data given in Table 1 it is clear that the elementary composition of humic acids is primarily conditioned by the chemical composition of humifying plant residues. The most carbonaceous humic acids were observed to form from clover leaves, and they contain the greatest amount
of nitrogen. Humic acids which formed during humification of oak leaves and grass roots were less carbonaceous. The latter contain the least nitrogen. Humic acid preparations extracted from decaying roots were of similar elementary composition over the whole observation period. Their relative carbon content gradually decreased at the expense of the amount of oxygen and nitrogen. The C:H ratio also decreased.

Other regular features were characteristic of the humic acid preparations extracted from humifying clover leaves. The total carbon content of the preparations remained practically constant, but was highest in those obtained from the initial plant residues. During the humification process the content of hydrogen decreased markedly and the amount of oxygen increased. The C:H ratio increased only at the last observation period.

In humic acid preparations from oak leaves the amount of carbon decreased appreciably during the first period of humification, but remained stable thereafter.

Thus, it was impossible to deduce much about the carbonization of humic acids and the process of their humification during the 180 days of the experiment. Variations of elementary composition are due first of all to the chemical composition of the plant residues, and also to the different

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**Fig. 1.**—Infra-red spectra of humic acids
1—Clover leaves—3 days, 2—the same—30 days, 3—180 days; 4—oak leaves 30 days, 5—the same 180 days of humification.
## Table I

**Elementary composition and functional groups of humic acids, extracted from humifying plant residues**

<table>
<thead>
<tr>
<th>Plant residues</th>
<th>Time of humification (days)</th>
<th>Elementary composition (% on oven-dry ash-free basis)</th>
<th>Functional groups (m-equiv./100 g oven-dry ash-free basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>Clover leaves</td>
<td>0</td>
<td>62.6</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>58.7</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>57.1</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>57.4</td>
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<td></td>
<td>90</td>
<td>57.8</td>
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</tr>
<tr>
<td></td>
<td>180</td>
<td>58.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Oak leaves</td>
<td>0</td>
<td>55.3</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>56.2</td>
<td>5.7</td>
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<td></td>
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<td>51.9</td>
<td>5.0</td>
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<tr>
<td></td>
<td>90</td>
<td>50.7</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>51.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Roots of grasses (Gramineae)</td>
<td>0</td>
<td>54.6</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>57.5</td>
<td>5.0</td>
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</tr>
<tr>
<td></td>
<td>180</td>
<td>55.6</td>
<td>5.1</td>
</tr>
</tbody>
</table>
rates of humification of the various components (protein, tannin, lignin) of these residues.

The functional groups show more definite regularities. The total amount of carboxyl and phenolic hydroxyl groups varied from 518-574 m-equiv./100 g in humic acids extracted from humifying roots, to 702-773 m-equiv./100 g for humic acids from humifying oak leaves. Their dynamics in the humification process were as follows: in the humic acid preparations extracted from oak leaves their amount increases noticeably; in the preparations from clover leaves and roots the total amount of these groups varied slightly. In the root preparations a decrease in the total amount of carboxyl and phenolic hydroxyl groups was observed. Evidently the total amount of functional groups in humic acids that are in the process of formation is also due to a great extent to the chemical composition of humifying plant residues; their total content does not change markedly during the first stage of humification. At the same time, from the initial stages of humification of all plant residues one may observe a definite increase in the content of carboxyl groups with a simultaneous decrease of hydroxyl content. This process of carboxylation is especially strongly pronounced for preparations extracted from leaves of clover and oak.

Similar evidence is obtained from measurements of the cation exchange capacity of the plant residues at pH 7. As is well known, at this pH the hydrogen of carboxyl groups is replaced.

<table>
<thead>
<tr>
<th>Plant residues</th>
<th>Time of humification (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Clover leaves</td>
<td>58</td>
</tr>
<tr>
<td>Oak leaves</td>
<td>40</td>
</tr>
<tr>
<td>Roots of grasses (Gramineae)</td>
<td>36</td>
</tr>
</tbody>
</table>

In Table 2, data are given on the dynamics of cation exchange capacity determined by saturation of the humifying plant residues by a buffered solution of CaCl₂ at pH 7. In all samples cation exchange capacity increases from 36-58 m-equiv./100 g in initial residues, to 75 in roots which humify very slowly, 230 in oak leaves and 437 m-equiv./100 g in clover leaves.

These data suggest that one of the characteristic reactions during the humification of plant residues is the carboxylation of various high molecular products of the hydrolytic breakdown of protein, tannin and lignin, which takes place as a result of a continuous slow biochemical oxidation of the material. This process of carboxylation determines the formation of a
system of high molecular weight carbon acids which we classify as humus acids.

The identity of this class of compounds is in the carboxylation of all its components and its internal differences are due to different chemical composition, different conditions and stages of humification of plant residues.

Elution of a solution of Na-humate from Sephadex G-75 gel with borate buffer at pH 10 showed that, throughout the humification period, the preparations are products of high molecular weight (Figure 2). At the same time it is evident that at an early stage of humification, humic acids are less homogeneous as to their particle size. At the end of the observation period the amount of the homogeneous, high molecular weight fraction apparently increases.

The data obtained suggest that by humification, together with carboxylation, an inter-condensation of the high molecular weight products of the hydrolytic breakdown takes place. In what way does the double component system of humus matter form? In previous work it was proved that it is possible to detect, at any stage of the humification of plant residues, a double component system of humus acids, which splits easily when interacting with the mineral part of the soil containing non-siliceous sesquioxides and the exchangeable cations, calcium, magnesium and hydrogen.

The less soluble and less dispersible part of the system quickly passes into a solid phase giving water-insoluble compounds of the humic acid type and their organo-mineral derivatives—alkaline earth humates, and aluminium and iron humic complex salts. The more soluble and more dispersible part of the system forms the fulvic acid group and moves...
gradually down the profile. It also forms organo-mineral derivatives (Alexandrova 1966a, 1966b).

Our published results of investigations over many years, showed that it is reasonable to distinguish three groups of organo-mineral derivatives of humus acids: heteropolar salts, complex salts and adsorption complexes (Alexandrova 1960, 1962).

Heteropolar salts of humus acids are formed by ion binding with cations of strong bases. The mechanism of their formation is an exchange reaction between the hydrogen of a functional group of humus acid and the corresponding cations:

\[
\text{hum} \quad \frac{(COO-H^+)_n}{\frac{(O-H^+)_m}{\frac{(O-Me^+)_m}{\text{hum} + Me^+ \to H^+}}}
\]

(\text{Me} = \text{Ca}^{2+}, \text{Mg}^{2+}, \text{Na}^+, \text{K}^+).

The most characteristic property of any humate or fulvate is the reversibility of the exchange reaction and the possibility of cation migration in ionic form. The properties of this group of compounds are studied well enough. One must keep in mind that pure humates and fulvates of strong bases are rarely present in soil. Usually more complex derivatives, complex heteropolar salts, are formed.

Complex salts are formed when covalent or coordination linkage takes place between the ion and the humus acid molecule. The main complex-forming ions in soil are iron and aluminium. One may prove experimentally that iron and aluminium, in their interaction with humus acids, displace hydrogen from a functional group and give complex salts in which the metal does not behave as a cation (Alexandrova 1960, Schnitzer and Skinner 1963, 1964).

We give the following scheme of their formation:

\[
\text{hum} \quad \frac{(COO-H^+)_n}{\frac{(O-H^+)_m}{\frac{(O-Me^+)_m}{\text{hum} + \text{Me}^{t} \to H^+;}}}
\]

(\text{Me}^{t} = \text{Fe(OH)}^{2+}, \text{Fe(OH)}^{3+}, \text{Al}^{3+}, \text{Al(OH)}^{3+}, \text{Al(OH)}^{2+})

This group of compounds is able to undergo exchange reactions with cations of strong bases at the expense of the hydrogen of that part of the functional groups which remains undisplaced by the complex-forming ion; the cation exchange capacity of these complex salts is always low in comparison with the initial humus acids (Figure 3). The second characteristic property of this group of compounds is the dual nature of the aluminium contained in them, one part of which is fixed strongly and the other behaves as a cation and retains its capacity for exchange reactions. Taking into consideration these peculiarities we name this group of organo-mineral derivatives "complex-heteropolar salts". The complex nature of these compounds is proved by the potentiometric titration curves and the presence of the pH effect (Figure 4). It is most clearly expressed in solutions of humus
acids and their corresponding iron or aluminium salts, not purified by dialysis. In dialyzed complex salts the potentiometric titration curves have a less well-marked pH effect, which may be observed only in the pH region from 7 to 10. One may evidently suppose that the main type of interaction of humus acids with sesquioxides is really an exchange reaction between the hydrogen of a carboxyl group and a cation: \( \text{Fe(OH)}^{2+}, \text{Fe(OH)}_2^{+}, \text{Al(OH)}^{2+}, \text{Al(OH)}_2^{+} \). For phenolic hydroxyl groups the coordination bonds with these cations is impossible. At any rate whether there is a possibility of considering this group of compounds as belonging to chelates is not yet clear and their structure is not clear either.

The nature of adsorption complexes of humus acids with clay minerals and non-siliceous forms of sesquioxides is multiform and is not yet completely studied.

As shown in the investigations of Alexandrova (1960) and Levashkevich (1966) humus substances (humus acids, their heteropolar and complex-heteropolar salts) can form soluble and insoluble complexes with non-siliceous forms of sesquioxides. The essential mechanism of their formation is adsorption of humus substances on sesquioxide gel, resulting in the formation of organo-mineral complexes of very variable composition which always have the ability to exchange cations. Besides adsorption, there are chemical forms of binding with the formation of complex compounds in the surface part of the film. Clay humus complexes form, by coalescence, humus films on the surface of the crystal lattice of clay minerals by means of intermolecular forms of binding.
Fig. 4.—Potentiometric titration curves of humus acids and their Fe- and Al-humus salts.

I. Humic acids:
- 1,4—initial solution, $2 \cdot 6 \times 10^{-3}H^+$
- 2,5—Fe-humic salt, $3 \cdot 3 \times 10^{-2}mMFe^{3+}$
- 3,6—Al-humic salt, $3 \cdot 7 \times 10^{-2}mMAl^{3+}$

II. Fulvic acids:
- 1,4—initial solution, $8 \cdot 1 \times 10^{-3}H^+$
- 2,5—Fe-fulvic salt, $3 \cdot 3 \times 10^{-2}mMFe^{3+}$
- 3,6—Al-fulvic salt, $3 \cdot 7 \times 10^{-2}mMAl^{3+}$

1, 2, 3—after dialysis; 4, 5, 6—before dialysis.

REFERENCES


SUMMARY

The study of the composition of humic acids extracted from the humification products of vegetable residues, showed that variations in elementary composition and the total amount of functional groups in the first stages of humification is conditioned by the chemical composition of plant residues.
The characteristic humification reactions are carboxylation of the high molecular products of hydrolytic breakdown of protein, tannin and lignin, and their inter-condensation. The double-component system of humus substances, consisting of humic acid and fulvic acid, forms by breakdown of the high molecular weight products of humification when they interact with the mineral part of the soil.

The less soluble and less dispersible substances remain in the solid soil phase at the place of formation, giving humic acids and their organo-mineral compounds. The more dispersible and more soluble compounds form fulvic acids and migrate down the soil profile, also giving a number of organo-mineral derivatives.

The principal organo-mineral derivatives of humus acids in the soil are:
1. heteropolar salts with cations of strong bases, forming as a result of exchange reactions between hydrogen of carboxyl and phenolic hydroxyl groups,
2. complex-heteropolar salts with iron and aluminium, forming also as a result of exchange reactions with hydrogen of carboxyl and possibly with phenolic hydroxyl groups, having a residual cation exchange capacity and
3. adsorption complexes of humus acids with clay minerals and non-siliceous forms of sesquioxides.

Résumé

En étudiant la composition des acides humiques provenant des produits d’humification des débris végétaux, on a montré que la composition élémentaire et la quantité globale des groupes fonctionnels, aux premiers stades de l’humification, sont conditionnées par la composition des débris végétaux.

Les réactions caractéristiques d’humification sont la carboxylation des produits à poids moléculaire élevé provenant de la décomposition hydrolytique des protéines (tannin et lignine) et leur condensation mutuelle. Les substances humiques de composition double (acide humique et acide fulvique) sont formées par la décomposition des produits d’humification à poids moléculaire élevé en réaction avec la partie minérale du sol.

Les substances moins solubles et qui se dispersent moins facilement restent dans la fraction solide sur le lieu de formation, cédant des acides humiques et leurs composés minéraux organiques. Les composés plus faciles à disperser et plus solubles produisent des acides fulviques et descendent dans la pente, donnant en même temps un nombre de dérivés minéraux organiques.

Les principaux dérivés minéraux organiques des acides humiques dans le sol sont:
1. des sels hétéropolaires avec des cations de bases fortes résultant des réactions d’échange entre l’hydrogène du carboxyle et les groupes hydroxyles phénoliques,
2. des sels hétéropolaires complexes avec du fer et de l’aluminium résultant aussi des réactions d’échange avec l’hydrogène du carboxyle et peut-être aussi avec les groupes hydroxyles phénoliques qui ont la capacité d’échanger les cations des résidus,
3. des complexes d’acides humiques avec des minéraux argileux et des formes de sesquioxides non-siliceux.
ZUSAMMENFASSUNG

Die Untersuchung der Zusammensetzung aus Humifizierungsprodukten pflanzlicher Rückstände entzogener Huminsäuren ergab, dass Variationen in der elementaren Zusammensetzung und Gesamtmenge funktioneller Gruppen in den ersten Stadien der Humusbildung durch die chemische Komposition der Pflanzenrückstände bedingt sind.


Die weniger löslich und weniger zerstreubaren Substanzen verbleiben in der soliden Bodenphase auf dem Bildungsraum und ergeben Huminsäuren und deren mineralorganische Verbindungen. Die zerstreubarereren und löslicheren Verbindungen bilden Fulvosäuren und wandern in die Bodenprofile ab, wobei sie ebenso eine Anzahl mineralorganischarer Abweichungen ergeben.

Die hauptsächlichen mineralorganischen Abweichungen der Huminsäuren im Boden sind: (1) Heteropolare Salze mit starkbasigen Kationen, die sich als ein Ergebnis der Austauschreaktionen zwischen Karboxylwasserstoff und phänolischen Hydroxylgruppen bilden; (2) Komplexe heteropolare Salze mit Eisen und Aluminium, die sich ebenso als ein Ergebnis der Austauschreaktionen mit Karboxylwasserstoff und möglicherweise phänolischen Hydroxylgruppen bilden und eine rückständige Kationsaustauschkapazität haben; und (3) Adsorptionskomplexe von Huminsäuren mit Tonmineralen und nicht kieselhaltigen Formen von Sesquioxiden.
I. INTRODUCTION

At any time the organic matter in soil (SOM) consists of materials in various stages of humification or decomposition and some separation of these stages based on differences in density and size is possible (Greenland and Ford 1964). A whole soil extracted by various reagents would therefore be expected to contain a mixture of humic acids which could differ quite markedly because they are derived from a range of starting materials. If the soil was fractionated the properties of the acids extracted from the fractions might also give an insight into the nature of humification since comminution of soil organic matter is part of the humification process. The present paper describes the differences occurring with decreasing size of organic matter within the one sample of soil, as revealed by the infrared spectra of humic acids extracted successively by various reagents, the yield of fulvic and humic acids and the molecular weight distributions of certain of the humic acids.

II. EXPERIMENTAL

Soil samples were taken to a depth of 2.5-3" from a red-brown earth (RBE) that had been under pasture for about 14 years. The pasture was sown to phalaris, subterranean clover, lucerne and Wimmera rye grass in the proportion 6:12:2:1. The principal clay minerals in the soil are kaolinite and illite.

Other samples were taken from a lateritic podzolic (LPz) soil (Wongan loamy sand) that had been under subterranean clover for four years. The principal clay mineral in this soil is kaolinite. The samples were taken during summer when there was no growth. The soils were air-dried and passed through a 1 mm sieve. The fractionation of the soils followed the scheme given below.

Average particle size was determined by direct measurement using the light microscope for coarse fractions. The size of the fine fraction was calculated from the BET (nitrogen adsorption) surface area assuming the clay platelets were discs with a diameter to thickness ratio of 10:1.

Extractions were carried out in the absence of air by successive treatments with 0.1M pyrophosphate (pH 7.0), cold 0.5N NaOH, followed by hot 0.5N NaOH, each for a 24 hour period. This procedure (Posner 1966) produces humic acids with about 1% ash. Sulphuric acid was used
Soil (2 kg in 5 l water) repeated stirring for ½ hr periods

Coarse plant remains float to surface and removed (Fr. 1)

½ hr stirring, sediment for 4 min, collecting particles to a depth of 30 cm

Soil residue

Combined supernatants of 1st and 2nd sedimentations

Sediment

(Supernatant Particles Sedimenting at < 16·5 cm/hr) Residue

Combined supernatants of 3rd, 4th and 5th sedimentations

Sediment

(Supernatant Particles Sedimenting at < 16·5 cm/hr) Residue

Soil residue

Resuspended in water. Layers rich in O.M. (L) settled on top of coarse mineral components

Combined supernatants of 1st and 2nd sedimentations

Residue

Combined supernatants of 3rd, 4th and 5th sedimentations

Residue

Combined supernatants of 3rd, 4th and 5th sedimentations

L fractions combined and passed through 100 mesh sieve

Residue

Sieved material

Fr. $L_1^1$ coarse O.M. but denser and darker than Fr. 1

Treat with petroleum ether/bromoform (40/60) to give Frs., $L_1^1 > 120$ mesh; $L_1^2 > 120 - 200$ mesh; $L_1^3 < 200$ mesh; $L_2^1 > 120$ mesh; $L_2^2 > 120 - 200$ mesh; $L_2^3 < 200$ mesh.

$L_1^1$ fractions still have a strong visual resemblance to plant material but darker than Fr. 1.

Floating Fr. $L_1^2$

Residue contained 5% O.M. which appears as dark particles among mineral particles Fr. $L_1^3$. 

Fractions $L_1$ and $L_2$ passed through 120 and 200 mesh sieves and then treated with petroleum ether/bromoform (40/60) to give Frs., $L_1^1 > 120$ mesh; $L_1^2 > 120 - 200$ mesh; $L_1^3 < 200$ mesh; $L_2^1 > 120$ mesh; $L_2^2 > 120 - 200$ mesh; $L_2^3 < 200$ mesh.
Fig. 1.—The infrared spectra of humic acid from RBE.

A Spruce lignin
B Pyrophosphate extracted humic acid from Fr. 1 (coarse plant remains).
Base-lines used for optical densities are shown in this spectrum. Base line for 1660 cm\(^{-1}\) band calculated on the assumption that the bands for 1720 cm\(^{-1}\) are symmetrical and that the contributions of their optical densities to 1660 cm\(^{-1}\) are additive.
C Cold NaOH extracted humic acid from Fr. 1
D Hot NaOH extracted humic acid from Fr. 1
E Hot NaOH extracted humic acid from Fr. A1 (finest clay)
in the isolation of the humic acid. Supernatant solutions and the unex-
tractable residues were adjusted to pH 7 before drying for carbon deter-
mination.

Organic matter was analysed according to the Walkley-Black (1934)
procedure assuming a 77.5% recovery of carbon and that the organic
matter contained 50% carbon.

Infrared spectra were recorded on a Perkin-Elmer 337 spectrophotometer using a 0.8 mg sample of humic acid in a 1.0 g KCl disc, 13 mm
in diameter (Theng, Wake and Posner 1966). The optical densities were
calculated from three determinations using the base lines shown in Figure
1.

To obtain the molecular weight distributions, two humic acids, one
extracted from the coarse organic matter (RBE Fr. one) and the other
from the clay fraction (RBE Fr. Al) were fractionated by dialysis at
pH 8.5-9 using Visking 20/32, Millipore 10, 50, 100 µ and Sartorius
200 and 275 µ membranes. The molecular weights of the fractions were
determined from osmotic pressure measurements in Pinner-Stabin osmo-
meters using U.M. 2 Diaplex membranes (Wake and Posner 1967). The
humic acids were dissolved in a buffer containing 0.02N NaHCO₃, 0.3N
KCl and 0.04% sec-octyl alcohol. A full account of the procedure will be
published elsewhere.

III. RESULTS AND DISCUSSION
(a) Infrared Spectra

Some typical spectra of humic acids are given in Figure 1 to illustrate
the differences between the extractants, soil fractions and lignin. Spectra
were obtained for all humic acids and the data assembled in histogram
form for key wavelengths as in Figure 2. The particle size of the fraction
decreases from left to right of the abscissa of the histogram and so does
the organic matter content of all fractions except RBE L₁².

The band assignments are taken from the work of Čeh and Hadži
(1956), Farmer and Morrison (1959), Wagner and Stevenson (1965) and
Theng and Posner (1967).

Comparison of the spectra given in Figure 1 and the data in Figure 2
shows that the humic acids extracted with NaOH are similar to lignin.
The fraction extracted with the cold reagent showed the closest resemblance
because of the greatest optical densities of the 1500 (aromatic $C=\text{C}$),
1460 and 1420 cm⁻¹ bands which are characteristic of lignin from different
sources. The latter bands are attributed to $\text{CH}_3$ and or $\text{CH}_2$ in-plane
deformation and possibly derive from the methyl of methoxyl groups. The
similarity of the humic acid to lignin decreases as the particle size of the
soil fraction decreases and becomes less plant-like in appearance.

The humic acids extracted by hot NaOH have the greatest aliphatic
$C-H$ content; a similar result was obtained from a whole soil extract
(Theng, Wake and Posner 1967).

The pyrophosphate humic acid has little resemblance to lignin. It is
in the highest state of oxidation as indicated by the greatest intensity of
EXTRACTION OF ORGANIC MATTER

Lateritic Podzolic (1.2% O.M.)

Red Brown Earth (5.9% O.M.)

<table>
<thead>
<tr>
<th>Particle Size (μ)</th>
<th>L_1</th>
<th>L_2</th>
<th>B_2</th>
<th>A_2</th>
<th>A_1</th>
<th>B_1</th>
</tr>
</thead>
<tbody>
<tr>
<td>x76</td>
<td>830</td>
<td>678</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>x66</td>
<td>65x20</td>
<td>0.39</td>
<td>0.33</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

O.M. Content (%): 79.4 61.5 16.0 9.0 7.1 3.9 3.3

% of Total Soil O.M. in Fraction: 14.9 3.9 3.3 6.6 12.4 21.8 3.3

O.M. Contents Expressed on Oven Basis

\[
\begin{align*}
\text{0.1M Pyrophosphate (pH 7.0)} & \\
\text{0.5N Cold NaOH} & \\
\text{0.5N Hot NaOH} & \\
\end{align*}
\]

Fig. 2.—The relationship between optical density for humic acids extracted from soil fractions of differing size.
the 1720 cm\(^{-1}\) band (carboxyl and carbonyl) and the lowest intensity of the 2925 (aliphatic C — H) and 1500 cm\(^{-1}\) bands. Similar conclusions for carboxyl groups have been drawn from direct titration and for the 1400 cm\(^{-1}\) band of the COO⁻ titrated to pH 11 (Theng, Wake and Posner 1967).

The aromatic C = C of lignin and simple aromatic compounds shows two absorption bands, one at 1610 cm\(^{-1}\) and the other at 1500 cm\(^{-1}\), the latter being the stronger. This relative intensity is not shown by humic acid because of the presence of a second band at 1610 cm\(^{-1}\). Although the extinction coefficients per group are unlikely to be similar, the difference between the optical densities at 1610 and 1500 cm\(^{-1}\) are plotted in Figure 2. This could give some guide to the presence of \(\beta\)-diketone groups, which appear to be present in largest amount in the pyrophosphate extracted humic acids, again indicating a greater state of oxidation of these acids.

The optical densities of the 1720 cm\(^{-1}\) band for the pyrophosphate humic acid show little systematic change with fraction for both soils and a fall in the 2925 and 1500 cm\(^{-1}\) bands for the heavier soil.

There is an increase in the 1720 cm\(^{-1}\) band and a fall in the 1500 cm\(^{-1}\) band with comminution for the NaOH-extracted acids. The aliphatic C — H content (2925 cm\(^{-1}\)) of these acids passes through a maximum with comminution which is more noticeable for the heavier soil. As degradation takes place the components containing a larger number of aliphatic C — H groups become more oxidised and hence more soluble in NaOH. A state will however be reached where oxidation causes a reduction in the total C — H content and it therefore passes through a maximum.

The infrared spectra of the humic acids show a trend with comminution of the organic matter which indicates an increase in the state of oxidation either through an increase in the carbonyl content of the acid and or a reduction in its aliphatic and aromatic character. Such changes may result from the conversion of a particular type of group to another with or without loss of organic material. The relative importance of these two processes could only be determined by the preparation of an organic matter balance sheet. Similar changes in the carbonyl group to that reported here have been observed by Visser (1964) and Kumada and Aizawa (1958).

While there is little qualitative difference between soils there is an indication that the increase in oxidation state of the organic matter with comminution is greater for the heavier soil. For example, the 1720 cm\(^{-1}\) band and the difference between the 1610 and 1500 cm\(^{-1}\) band rises more rapidly for the heavier soil, while the fall of the 1500 cm\(^{-1}\) band is more rapid. The median values of the intensities of these bands are about the same although the intensity of 2925 cm\(^{-1}\) band is considerably greater for the humic acids from the light soil. The difference between the soils could be due to a more ready leaching of the most highly oxidised forms of the organic matter from the light soil.
(b) Yield of Extracted Organic Matter

There appears to be little difference between the yields of humic acid extracted either by hot or cold NaOH and between the two soils (Table 1). There is an indication that the yield of the fulvic acid fraction might be slightly greater than the humic acid fraction from the hot NaOH extractant. For the heavier soil (RBE) the pyrophosphate extract increases continu-

<table>
<thead>
<tr>
<th>Soil Fraction</th>
<th>Longest Dimension (µ)</th>
<th>Yield (%)*</th>
<th>Cold NaOH</th>
<th>Hot NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H</td>
<td>F</td>
<td>H</td>
</tr>
<tr>
<td>RBE</td>
<td>1</td>
<td>2350</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>(L_1)</td>
<td>760</td>
<td>2.2</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>(L_2)</td>
<td>236</td>
<td>8.1</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>(L_3)</td>
<td>54</td>
<td>7.8</td>
<td>5.5</td>
</tr>
<tr>
<td>B_2</td>
<td>0.58</td>
<td>10.4</td>
<td>14.0</td>
<td>9.2</td>
</tr>
<tr>
<td>A_1</td>
<td>0.19</td>
<td>13.0</td>
<td>9.0</td>
<td>12.2</td>
</tr>
</tbody>
</table>

| LPz           | 1                     | 830       | 1.0       | 1.6      | 12.4     | 10.4     | 14.9     | 15.0     |
|               | \(L_1\)               | 678       | 11.4      | 5.0      | 10.4     | 12.6     | 12.2     | 14.4     |
|               | \(L_2\)               | 65        | 19.6      | 4.3      | 15.7     | 14.3     | 9.2      | 10.4     |
| B_2           | 0.39                  | 8.4       | 6.0      | 12.6     | 16.3     | 10.0     | 12.6     |
| A_2           | 0.33                  | 4.5       | 5.5      | 15.6     | 14.9     | 13.9     | 14.9     |
| A_1           | 0.25                  | 5.7       | 16.7     | 10.8     | 17.4     | 14.3     | 15.0     |
| B_1           | 0.25                  | 2.8       | 16.5     | 7.6      | 17.0     | 8.4      | 15.8     |

* expressed as % of \((\text{yield of humic}+\text{fulvic}+\text{unextracted SOM})\) as determined by Walkley-Black Method].

ously for both the humic and fulvic acid fractions (Table 1) which are comparable in amount. The increase in pyrophosphate extractable humic acid is more rapid for the lighter soil (LPz) than the heavier one but then passes through a maximum. The yield of fulvic acid fraction for this extract from the light soil increases only slowly with comminution and then more rapidly for the finest soil fractions.

These results together with the infrared spectra suggest that the organic matter changes with comminution from a pyrophosphate insoluble form to a pyrophosphate soluble fulvic acid and a more highly oxidised pyrophosphate extractable humic acid both of which result largely from a decrease in the humins.

(c) Molecular Weight Distribution

The virtual absence of the highest molecular weight components, i.e. \(> 15,000\) for the coarse organic matter (RBE Fraction 1) humic acid should be noted (Figure 3), although the number averages for the whole acids are similar.
Molecular Weight of Fraction x 10^{-3}

Fig. 3.—Molecular weight distribution curve of cold 0.5N NaOH humic acids from the Red Brown Earth.

O Fr. I Coarse plant remains
• Fr. A1 Finest clay fraction

IV. ACKNOWLEDGMENTS

The authors are grateful to A. Beamish and M. Baltinas for carrying out the extraction and carbon analyses.

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EXTRACTION OF ORGANIC MATTER


**Summary**

Two soils were fractionated into different particle size ranges. Each fraction was extracted successively with 0.1M pyrophosphate (*pH* 7), cold 0.5N NaOH and then hot 0.5N NaOH.

The infrared spectra of the humic acids were determined and the optical densities of the salient bands in the spectra compared. The humic acids extracted by pyrophosphate were in the greatest state of oxidation with the least resemblance to lignin. The acid extracted with cold NaOH had the greatest resemblance to lignin.

The infrared spectra taken together with the yield of fulvic and humic acid showed that the state of oxidation of the organic matter increased with comminution and that the organic matter in the medium-textured soil (RBE) was less oxidized than that in the light soil (LPz).

Molecular weight distributions are reported for two of the samples of humic acid.

**Résumé**

Des prélèvements d'un sol léger et d'un de texture moyenne, ont été divisés en fractions suivant la grandeur des particules. Chaque fraction a été extraite successivement avec 0.1M pyrophosphate (*pH* 7), 0.5N NaOH froid et ensuite 0.5N NaOH chaud.

Les spectres infra-rouges des acides humiques ont été déterminés et on a fait la comparaison des densités optiques des bandes saillantes des spectres. Les acides humiques extrait par le pyrophosphate ont été les plus oxydés et ont eu la moindre ressemblance avec le lignin. L'acide extrait par le NaOH froid a eu la plus grande ressemblance avec le lignin.

Les spectres infra-rouges considérés avec le rendement des acides fulvique et humique montrent que le niveau d'oxydation de la matière organique augmente avec le meublement et que la matière organique dans le sol de texture moyenne est moins oxydée que celle dans le sol léger.

On rapporte les distributions des poids moléculaires pour deux des prélèvements d'acide humique.

**Zusammenfassung**

Proben von leichten Böden und Böden mittlerer Gefüge wurden in verschiedenen Korngrössen-Anordnungen fraktioniert. Jede Fraktion wurde nacheinander mit 0.1M Pyrophosphat (*pH* 7), kaltem 0.5N NaOH und dann heissen 0.5N NaOH extrahiert.

Die infraroten Spektren der Humussäuren wurden festgestellt und die optischen Dichten der prominenten Bänder in den Spektren verglichen.
Die mit Pyrophosphat extrahierten Humussäuren waren im höchsten Oxydationszustand, und ähnelten Lignin am wenigsten. Die mit kaltem \textit{NaOH} extrahierte Säure war Lignin am ähnlichsten.

Die infraroten Spektra, zusammen mit dem Ertrag der Fulvo- und Humus-Säuren, weisen auf, dass der Oxydationszustand der organischen Substanz mit Verfeinerung zunimmt, und dass die organische Substanz im Boden mittleren Gefüges weniger oxydiert ist als im leichten Boden.

Molekulargewichtsverteilungen sind für zwei der Humussäureproben mitgeteilt.
In the Japanese Archipelago extending from the cold temperate zone to the humid subtropical zone, there are several kinds of zonal and intrazonal soils such as Podzols, Brown Forest soils, Red-Yellow soils, Peat soils, and Humic Allophane soils (including Altered Humic Allophane soils) in addition to Alluvial soils under rice and upland crop cultivation. Since classification problems on some of them are still under controversy, it is of importance to characterize the nature of the humus as well as that of the clay fractions to settle the problems. Therefore, in recent years Japanese pedologists have paid increasing attention to the nature of the humus of the above-mentioned genetic soil types. The object of the present paper is to offer general information on the nature of humus of Humic Allophane soils (including Altered Humic Allophane soils), Red-Yellow soils and Peat soils. In the present study the method of Tiurin (1951) was used for fine earths (less than 0.25 mm in e.s.d.) as an aid in characterizing the nature of the humus. The aromatic nature of humic acids was determined by elementary analysis, X-ray diffraction, infrared and light adsorption spectroscopy. The procedure for preparing the specimens of humic acids followed that of Kononova (1963) and the details are given by Tokudome and Kanno (1965). The fractional composition of the humus was obtained by the method of Tiurin (1951) according to the scheme outlined below.

**Humic Allophane Soils (Volcanic Ash Soils)**

The amounts of humus in the soils (several up to 40%) are much greater than any other terrestrial soil in Japan. Its main source is grasses, especially *Miscanthus sinensis*, which is a solfatara plant of wild grasslands. The group composition of humus is shown in Fig. 1. The humic acid (Ch)/fulvic acid (Cf) ratios ranged from 0.4 in young members to 1.2 in well-developed members. The ratios in well-developed members in the subboreal zone (southern Hokkaido and northern Honshu), the humid subtropical zone (central Japan), and the humid subtropical zone (southwestern Honshu, Shikoku, and Kyushu) are 0.9-1.2, 0.8-1.0, 0.8-1.9, respectively. This suggests that the nature of the humus of intrazonal Humic Allophane soils has been influenced by zonal characteristics within the limits of definite conditions. Furthermore, the younger the soil, the greater is the proportion of fulvic acid.

The fractional composition of the humus is characterized by the most
A SCHEME FOR OBTAINING FRACTIONAL COMPOSITION OF HUMUS BY THE METHOD OF I. V. TIURIN (1951)

AIR-DRIED SOIL SAMPLE (0.25 mm in c.s.d.)

EXTRACTION WITH AN ALCOHOL-BENZENE (1:1) MIXTURE
(A = bitumen)

EXTR. WITH 0.1N NaOH
(D = fr. [la + i] + Na₂SO₄
-soluble fr.)

EXTR. WITH 0.5 H₂SO₄
(C = Na₂SO₄-soluble fr. + fr. la)

EXTR. WITH N Na₂SO₄
(B = Na₂SO₄-soluble fr.)

EXTR. WITH 0.1N NaOH
(E = fr. la + i + 2)

EXTR. WITH 0.5N H₂SO₄
(F = H₂SO₄-hydrolyzable fr.)

EXTR. WITH 0.1N NaOH
(G = NaOH-soluble fr.)

EXTR. WITH N H₂SO₄
(H = H₂SO₄-hydrolyzable fr.)

EXTR. WITH 0.1N NaOH
(I = NaOH-soluble fr.)

RESIDUE (Humin)(L)

Remarks:
1. With respect to the separation of humic acids from fulvic acids in fractions D, E, and K, the nearly complete flocculation of humic acids was obtained at pH 2 or below after the addition of adequate amounts of H₂SO₄.
2. The amount of each fraction was proportionally allotted as per cent of total organic carbon. Bitumen = A; fr. 1a = C-B; fr. 1 = D-(C); fr. 2 = E-(D-B); fr. 3 = G+I; H₂SO₄-hydrolyzable fr. = F+H; Humin or residue = L.

Mobile fractions 1a and 1 loosely bound with sesquioxides and allophane, and by the scarcity of fraction 2 bound with calcium and of humins. Fraction 3 strongly bound with allophane and sesquioxides increased with depth.

Prominent features of the humus are governed by the nature of highly
condensed humic acids which is reflected in the elementary composition, X-ray diffractograms, optical densities, and infrared spectrograms. Ten humic acid specimens are relatively high in carbon content (56 to 60%), high in C/H and C/N ratios and low in Δ log K(log K 400 με - log K 600 με) (Kumada, 1955) (average 0.536). X-ray diffractograms of humic acids (Fig. 2) show that all but one of the specimens have broad peaks at 3.5 Å caused by the 002 reflection of an amorphous carbon-like structure. Sample No. 4 does not show any reflection near 3.5 Å, but has a halo near 4.1 Å which has been named γ-band by Kasatochkin and Zil'berbrand (1956). The γ-band caused by chain carbon structures is predominant in fulvic acids of Humic Allophane soils irrespective of the bioclimatic conditions (Tokudome and Kanno, 1965).

Infrared spectrograms of humic acids (Fig. 3) show that the aromatic nature is indicated by the occurrence of the aromatic C = C group (near 6.2 μ) and the aromatic C – H group (at 3.25 μ). Absorption bands at 3.4 and 7.2 μ are caused by the aliphatic C – H group and its deformations, respectively. Absorption bands at 5.8 and 8.2 μ caused by the C = O stretching vibrations of carboxyl (or carbonyl) groups and the C – O stretching vibrations of ethers, esters, organic acids and others, respectively, are seen in Fig. 3. These data indicate that the humic acids are characterized by being predominantly aromatic.
Fig. 2.—X-ray diffractograms of humic acids (CoKα).
Humic Allophane soils: 1, Shimabara (A_{11} horizon), Kyushu. 2, Kodonbaru (A_{11} horizon), Kyushu. 3, Chiba (A horizon), Honshu. 4, Ono (A horizon), Hokkaido.
Altered Humic Allophane soils: 5, Makinohara (IIA horizon), Honshu. 6, Shinshiro (IIIA_{11} horizon), Honshu. 7, Chikugo (Ojima) (II layer), Kyushu.
Red-Yellow soils: 8, Makinohara (Haibara) (A horizon), Honshu. 9, Matsuyama (Pleistocene) (A horizon), Shikoku. 10, Ogi (A horizon), Kyushu. 11, Omura (A horizon), Kyushu.
Peat soils: 12, Hassamu (low peat, 20-45 cm), Hokkaido. 13, Shinshinotsu (high peat, 10-14 cm), Hokkaido. 14, Hassamu (low peat, 0-20 cm), Hokkaido.

**ALTERED HUMIC ALLOPHANE SOILS**

Dark-coloured soils (so-called “Kuroboku soils”), which closely resemble Humic Allophane soils in morphology and in some chemical characteristics, are found in Pleistocene terrace lands of central Honshu and Kyushu. There is a controversy among Japanese pedologists on their pedogenesis and taxonomic position. Some pedologists (for example, Matsui et al. 1963) believe that one of the essential factors for the formation of humus-rich horizons is not the action of allophane, but poorly-drained or meadow-like conditions in the past. On the other hand, Kanno (1965) is of the opinion that they are derived from a mixture of old
Fig. 3.—Infrared absorption spectra of humic acids separated from Humic Allophane soils and Altered Humic Allophane soils. The sample numbers are the same as given in Fig. 2.
volcanic ash and Pleistocene sediments, and that allophane would be responsible for the formation of such humus-rich horizons. In the present paper Kanno has tentatively named “Kuroboku soils” Altered Humic Allophane soils.

The humus of nine Altered Humic Allophane soils is characterized by relatively high \(Ch/Cf\) ratios (average 3·14) (Fig. 1). The fractional composition of the humus closely resembles that of well-developed Humic Allophane soils. The elementary composition and optical densities (average 0·524) of the nine humic acids also resemble those of humic acids in the Humic Allophane soils. X-ray diffractograms (Fig. 2) and infrared spectrograms (Fig. 3) reflect these features, indicating that the humic acids have a relatively high degree of aromaticity.

**Red-Yellow Soils**

The group composition of the humus is given in Fig. 1. Considerable amounts of organic matter (2-7%) have accumulated on the surface and the \(Ch/Cf\) ratios (0·4-0·8) of the humus must be distinguished from those of normal and Altered Humic Allophane soils. The fractional composition of the humus is characterized by the predominance of fraction 1 and humins and by the scarcity of fraction 2. The humic acids of Red-Yellow soils have a low degree of aromaticity. Eleven humic acid specimens have relatively low carbon contents and low \(C/H\) ratios whereas the nitrogen and hydrogen contents are relatively high. Optical densities expressed as \(\Delta \log K\) (average 0·707) are relatively low. The occurrence of the \(\gamma\)-band (Fig. 2), and of the aliphatic \(C - H\) group at 3·4 \(\mu\), the aromatic \(C = C\) with chain carbon structures at 6·6 \(\mu\) (Orlov and Zub 1963), and the aliphatic \(C - H\) deformations near 7·1 \(\mu\) (Fig. 4) indicates that the humic acids are predominantly aliphatic in nature.

**Peat Soils**

Ponomareva and Nikolaeva (1961) devised a method which is more satisfactory for the study of the peaty soil organic matter. When their method is compared with the method of Tiurin (1951), both methods are almost the same in respect of the fractionation of humic substances. Consequently, the use of Tiurin’s method with 0·1 N \(NaOH\) as an extractant is permissible.

In Japan, Peat soils are widely developed in Hokkaido, whereas only small patches of peaty lands are found in Honshu and Kyushu. The group composition of humus is shown in Fig. 1. The \(Ch/Cf\) ratios of the humus of the uppermost layers (ignition loss = 30-50%) and the immediately lower layers (ignition loss = 65-95%) were 1·2-2·8 and 2·9-5·8 respectively, suggesting that inorganic substances are able to promote the humification of peat. The humic substances have a relatively high content of bitumen (average 10%).

The elementary composition, optical densities (average \(\Delta \log K\) = 0·781), X-ray diffractograms (Fig. 2), and infrared spectrograms (Fig. 4)
Fig. 4.—Infrared absorption spectra of humic acids separated from Red-Yellow soils and Peat soils. The sample numbers are the same as given in Fig. 2.
indicate that the humic acids of Peat soils closely resemble those of Red-Yellow soils.

**DISCUSSION AND CONCLUSIONS**

The group compositions of the humus of the soils examined can be differentiated from one another (Fig. 1). According to Zonn's criterion (Zonn 1962), Red-Yellow soils belong to the fulvate-humate group \((Ch/Cf\) ratios = 0.5-0.7), whereas the other soils belong to the humate group \((Ch/Cf\) ratios = > 0.7). The humic acids of normal and Altered Humic Allophane soils can be distinguished from those of Red-Yellow soils and Peat soils by their aromatic nature. The \(Ch/Cf\) ratios of Humic Allophane soils have certain geographical regularities. For example, the ratios for the volcanic ash soils of Kamchatka (Sokolov and Targulian 1962, Zonn et al. 1963) and Indonesia excluding high elevations (Tan 1966) are less than 1. In Japan the ratios generally increase from the north to the south. This view may be supported by the data of Adachi (1966) and Oba (1965) with some modification. The above statement suggests that the accumulation of humic acids in volcanic ash soils reaches a maximum under humid subtropical conditions where the formation of allophane is most favourable. Furthermore, it is assumed that the alteration of fulvic acids to humic acids may be accelerated by the catalytic action of allophane (Kyuma and Kawaguchi 1964).

The forming process of the humus of Altered Humic Allophane soils is quite different from that of geographically associated Red-Yellow soils, but closely resembles that of Humic Allophane soils. If Altered Humic Allophane soils were of the hydromorphic origin, their humic acids would not have such a high degree of aromaticity. This has been shown by the nature of humus of Peat soils.

The fact that the natures of the humus in Red-Yellow soils closely resemble one another irrespective of parent material suggests that the bioclimatic conditions play an important role in the formation and the nature of the humus. This is supported by the resemblances in the nature of the humus in the Red-Yellow soils of Japan, Yellow-Brown Forest soils of Central China (Wu Zhi Hua 1964), and some Krasnozems of the USSR and South China (Bel'chikova 1951, Kononova 1963, Niu Tzi Ven 1961, Sabashvili 1954, Yü and Pie 1962, Zonn and Li 1958). However, the \(Ch/Cf\) ratios of Red-Yellow soils are higher than those of some Lateritic soils in South China (Niu Tzi Ven 1961, Tu Men-Jao 1961, Yü and Pie 1962).

In general, it is thought that the humification of organic matter in Peat soils has been accelerated under aerobic conditions prevailing in woody peat soils, or by an inflow or a fall of inorganic materials and is characterized by the abundant accumulation of weakly condensed humic acids and the weak formation of fulvic acids (Nikonov et al. 1960, Kuwano 1961, Ponomareva and Nikolaeva 1961, Shoji and Matsumi 1962). The above statement agrees with the data obtained by this study.
ACKNOWLEDGEMENTS


REFERENCES


The present paper deals with the nature of the humus of Humic Allophane soils, Altered Humic Allophane soils, Red-Yellow soils, and Peat soils in Japan. The results obtained are summarized as follows:

1. The humus contents (8.05-38.74%) and the $Ch/Cf$ ratios (0.80-1.74, averaged 1.15) of fifteen Humic Allophane soils increased with the increase of the allophane content. The humus composition was characterized by the predominance of fraction 1 and low contents of fraction 2 and humins. The humic acids have a high degree of aromaticity.

2. Altered Humic Allophane soils should be distinguished from Humic Allophane soils by the predominance of $Al$-vermiculite and kaolin minerals in their clay fractions. The $Ch/Cf$ ratios (averaged 3.14) of nine Altered Humic Allophane soils are higher than those of Humic Allophane soils, but the aromatic nature of the humic acids is almost the same as that of Humic Allophane soils.

3. The humus contents and the $Ch/Cf$ ratios of twelve Red-Yellow soils were about 4.6% and 0.63 respectively. The humic acids had a low degree of aromaticity. The $Ch/Cf$ ratios ranging from 3 to 6 of six Peat soils increased with increasing the organic matter contents. The humus of Peat soils is characterized by a relatively high content of bitumen and a relatively low degree of aromaticity of the humic acids.

The above-mentioned features of humic acids are consistent with the data for the elementary composition, optical densities, X-ray diffraction, and infrared spectroscopy.

RÉSUMÉ

Cette communication a pour sujet la nature de l’humus des sols humiques à allophane, des sols humiques modifiés à allophane, des sols rouge-jaunes, des sols tourbeux au Japon. Les résultats obtenus sont résumés ci-dessous:

1. La teneur en humus (8.05 - 38.74%) et les rapports $Ch/Cf$ (0.80 - 1.74, moyenne 1.15) de quinze sols humiques à allophane a augmenté avec l’augmentation du contenu d’allophane. La composition de l’humus a été caractérisée par la prédominance de la fraction 1 et la basse teneur de la fraction 2 et des humines. Les acides humiques ont un haut degré d’aromaticité.

2. Les sols humiques modifiés à allophane doivent être distingués des sols humiques à allophane par la prédominance des minéraux à $Al$-vermiculite et à kaolin dans leurs pourcentages d’argile. Les rapports $Ch/Cf$ (moyenne 3.14) de ces sols sont plus grands que ceux des sols humiques à allophane, mais la nature aromatique des acides humiques est presque la même que celle des sols humiques à allophane.
3. La teneur en humus et les rapports \(Ch/Cf\) de douze sols rouge-jaunes ont été environ 4,6% et 0,63 respectivement. Les acides humiques ont donné un degré bas d'aromaticité. Les rapports \(Ch/Cf\) s'étendant de 3 à 6, de six sols tourbeux ont augmenté avec l'augmentation du contenu de la matière organique. L'humus des sols tourbeux est caractérisé par un contenu relativement haut de bitume et un degré relativement bas d'aromaticité des acides humiques.

Les traits des acides humiques mentionnés ci-dessus sont conséquents avec la donnée de la composition élémentaire, la densité optique, la diffusion du rayon X et la spectroscopie infra-rouge.

**ZUSAMMENFASSUNG**

Die vorliegende Arbeit befasst sich mit der charakteristischen Beschaffenheit des Humus in den Humusallophanböden, veränderten Humusallophanböden, rotgelben Böden und Torfböden in Japan. Die dabei erzielten Ergebnisse sind folgendermassen zusammengefasst:


2. Veränderte Humusallophanböden sollten von den Humusallophanböden durch das Vorherrschen von \(Al\)-Vermikutit und Kaolin-Mineralien in ihren Tonbestandteilen unterschieden werden. Die \(Ch/Cj\) Verhältnisse (Durchschnitt 3-14) von diesen Böden sind höher als die der Humusallophanböden, aber die aromatische Beschaffenheit der Humussäuren ist der den Humusallophanböden fast gleichwertig.

3. Der Humusgehalt und die \(Ch/Cf\) Verhältnisse im Bezug auf zwölf rotgelbe Böden waren ungefähr 4-6% resp. 0-63. Die Humussäuren ergeben einen niedrigen Aromatizitätsgrad. Die \(Ch/Cj\) Verhältnisse für sechs Torfböden lagen zwischen 3 und 6 und nahmen bei zunehmendem Gehalt an organischer Substanz höhere Werte an. Humus der Torfböden ist durch einen verhältnismässig hohen Bitumen- (Erdpech-) Gehalt und einen verhältnismässig niedrigen Aromatizitätsgrad der Humussäuren gekennzeichnet.

Die oben erwähnten Eigenschaften der Humussäuren sind übereinstimmend mit den Angaben für die Grundzusammensetzung, optische Dichte, Röntgendiffraktion und Infrarote-Spektroskopie.
ÜBER DIE BETEILIGUNG VON PHENOLEN AM AUFBAU VON HUMINSÄUREN

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Untersuchungen zur Beantwortung der Frage, welchen Ursprung die am Aufbau von Huminsäuren beteiligten Phenole haben, ergeben verschiedene Möglichkeiten. Eine Möglichkeit besteht darin, dass sich die mit den abgestorbenen Pflanzen in den Boden gelangenden Phenole, von denen das Lignin mengenmässig den Hauptteil darstellt, an der Bildung der Huminsäuren beteiligen. Eine weitere Möglichkeit ist die Synthese von Phenolen aus aliphatischen Verbindungen, die über den Stoffwechsel der Mikroorganismen verläuft.


Das Lignin verschiedener Pflanzen ist je nach Species unterschiedlich zusammengesetzt. Das Lignin der Nadelhölzer ist vornehmlich ein Poly-
merisat des Coniferylalkohols, in dem nur einige p-Cumaralkoholeinheiten enthalten sind. Das Lignin der Laubhölzer ist ein Mischpolymerisat von Coniferyl- und Syringyl-alkohol. Im Lignin der Gramineen ist als dritter Baustein in grösserer Menge p-Cumaralkohol enthalten.

Das Strukturschema des Coniferenlignins von Freudenberg et al. (1964a und b) lässt erkennen, dass die Coniferylalkoholeinheiten über verschiedenartige C-C- oder C-O-Bindungen dreidimensional verknüpft sind (Abb. 1).

Die Bildung der Lignine in den verschiedenen Pflanzen erfolgt durch eine dehydrierende Polymerisation von Gemischen unterschiedlicher Zusammensetzung der Ligninbausteine. Dabei werden die Lignine zwischen den
Zellulosefibrillen der Zellwand eingelagert und sind chemisch mit der Zellulose verbunden.


**Tabelle 1**

<table>
<thead>
<tr>
<th>markiert in</th>
<th>Coniferylalkohol polymerisiert</th>
<th>Coniferyl- (markiert)+ p-Cumar- + Sinapin-alkohol, polymerisiert</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁴CH=CH-CH₂OH</td>
<td>3,5</td>
<td>4,0</td>
</tr>
<tr>
<td>-CH=¹⁴CH-CH₂OH</td>
<td>2,6</td>
<td>2,5</td>
</tr>
<tr>
<td>-CH=CH-¹⁴CH₂OH</td>
<td>4,4</td>
<td>4,5</td>
</tr>
<tr>
<td>-O-¹⁴CH₃</td>
<td>4,5</td>
<td>3,8</td>
</tr>
<tr>
<td>¹⁴C₁₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋افة</td>
<td>14</td>
<td>22</td>
</tr>
</tbody>
</table>

* Die Zahlen geben die prozentuale Abspaltung der markierten Kohlenstoffatome als Kohlendioxid innerhalb von 10 Tage wieder (nach Haider (1965)).

Bei den beiden Polymerisaten I und II fällt auf, dass die Abspaltung der Kohlenstoffatome aus der C₆-Seitenkette und die aus den Methoxylgruppen verhältnismässig gering ist. Dagegen ist die Abspaltung von Kohlendioxid aus den Ringen durch die Mikroorganismen stärker.

Die Einbaurate der markierten Gruppen in Myzelbestandteile ergibt ein ähnliches Bild wie die Abspaltung als Kohlendioxid. Auch hier sind die Werte für die Seitenketten wesentlich niedriger als die für die Ringe. Erst wenn man die Werte für das abgespaltene aktive Kohlendioxid mit dem für den Einbau in das Myzel gemeinsam berücksichtigt, erhält man einen
Uberblick über die Verwertung der verschiedenen Kohlenstoffatome. Lässt man die Versuche länger als 10 Tage laufen, nimmt nach ungefähr 14 Tagen die Abspaltung der Kohlenstoffatome aus den Seitenketten und die aus den Methoxylgruppen zu, während hingegen die Bildung von aktivem Kohlendioxid aus den Ringen zurücktritt.

Untersucht man Mischpolymerisate, bei denen Syringyl- oder p-Cumar-alkohol markiert sind, so stellt man fest, dass die Ringatome der Syringylalkoholeinheiten stärker abgebaut werden als die der Coniferylalkoholeinheiten. Dagegen werden die Ringe der p-Cumar-alkoholeinheiten weniger abgebaut als die der Coniferylalkoholeinheiten.


Im Anfangsstadium des Wachstums von Epicoccum nigrum treten vornehmlich Orsellinsäure und Cresorsellinsäure sowie Orcin und 2,4-Dihydroxytoluol auf. Wahrscheinlich bildet der Pilz nur Orsellinsäure und Cresorsellinsäure aus aliphatischen Vorstufen. Durch Decarboxylierung, Einführung weiterer Hydroxylgruppen und Oxydation von Methylgruppen zu Carboxylgruppen entstehen andere Phenole aus diesen beiden Säuren (Abb. 2).

Je mehr die Bildung der Huminsäuren in der Kulturlösung fortschreitet, umso weniger sind die Phenole in freier Form nachweisbar. Die autoxydablen 2,3,5-, 2,4,5- und 3,4,5-Trihydroxytoluole sind für die Bildung der Huminsäuren von Bedeutung. Diese werden bereits durch den Luftsauerstoff, bei dem in dem Medium herrschenden pH von 6 bis 8 zu Chinonen oxydiert und können andere Phenole nucleophil addieren (Musso et al. 1965). Die dabei entstehenden Dimerisationsprodukte werden erneut durch Luftsauerstoff oxydiert und polymerisiert. Im Medium vorhandene Aminosauren oder Peptide reagieren ebenfalls nucleophil mit diesen Chinonen und werden dadurch addiert.

Phenole, wie Ferula-, Kaffee-, Vanillin- oder Protocatechu-säure, welche dem Nährmedium zugesetzt werden, erhöhen die Ausbeute an Huminsäuren beträchtlich. Durch den Pilz erfolgt dabei eine Umwandlung der zugesetzten Phenole (Abb. 3).

Der Pilz hat die Möglichkeit, auch zugesetzte Phenole (Abb. 3) durch

\[ \text{Abb. 2: Umwandlung von Orsellinsäure und Cresorsellinsäure durch} \]
\[ \text{Epicoccum nigrum (Haider und Martin 1967).} \]


LITERATUR


AUFBAU VON HUMINSÄUREN


Lim, S. U. (1965)—Beiträge zur Aufklärung der Zusammenhänge zwischen dem mikrobiellen Abbau des Lignins und der Bildung von Humusstoffen. Diss. Universität Bonn, 89 S.


ZUSAMMENFASSUNG

Die am Aufbau der Huminsäuren beteiligten Phenole können aus dem Lignin der Pflanzen stammen oder durch mikrobielle Synthese entstanden sein.

Der mikrobielle Abbau von "synthetischen" Ligninen aus spezifisch markierten Bausteinen ergibt, dass die Ringspaltung eine wichtige Reaktion im Verlauf der Umsetzung von Lignin durch Mikroorganismen ist.

Dadurch entstehen auch größere Spaltstücke des Lignins.

Aus Huminsäuren, die über den Stoffwechsel einiger Mikroorganismen gebildet werden, lassen sich bei der reduktiven Spaltung Resorcindervate nachweisen. Für *Epicoccum nigrum* wurde die Biosynthese von Orsellinsäure und Cresorsellinsäure aus aliphatischen Vorstufen aufgezeigt; diese beiden Säuren werden durch den Pilz in etwa 20 verschiedene Phenole überführt. Die Bildung von Huminsäuren in den Kulturlösungen und Myzelfen tritt erst dann auf, wenn sich autooxydable Hydroxyhydrochinon- oder Pyrogallol-derivate gebildet haben.
Kleinere Spaltstücke des Lignins werden mit in die Pilz-Huminsäuren eingepolymerisiert. Dabei erfolgt durch Abbau der Seitenketten, Demethyllierung und Einführung weiterer Hydroxylgruppen eine Umwandlung dieser Phenole. In diesem Fall werden die Ringe kaum abgebaut.

RéSUMÉ

Les phénols qui participent à l’agrégation des acides humiques peuvent provenir soit de la lignine des plantes ou avoir été créés par la synthèse microbienne.

On a trouvé que pour la désagrégation microbienne des lignines "synthétiques" des éléments marqués spécifiques le clivage de cercles est une réaction importante au cours de la transformation de la lignine par les micro-organismes. Ceci produit aussi des fragments plus grande de clivage des lignines.

Les acides humiques produits par le métabolisme de quelques micro-organismes donnent, après le clivage réductif, des dérivés de résorcinol. Pour l’Epicoccum nigrum on a pu observer la biosynthèse des acides orsellique et cresorsellique des précurseurs aliphatiques; ces deux acides ont été transformés à l’aide du fungus en 20 phénols différents. La formation des acides humiques dans les solutions de cultures et les mycéliums se produit seulement lorsque les dérivés auto-oxydables de hydroxyhydroquinone ou pyrogallol ont été formés.

Des fragments plus petits de la lignine clivée sont polymérisés dans les acides humiques fongiques. La transformation de ces phénols se produit par la désagrégation des formes latérales, la déméthylisation et l’introduction d’autres groupes hydroxyliques. Dans ce cas le noyau est à peine désagrégé.

SUMMARY

Phenols participating on the formation of humic acids can originate from the lignin of plants or be formed by microbial synthesis.

For the microbial degradation of “synthetic” lignins from specifically labelled units it was shown that the cleavage of the ring is an important reaction during the transformation of lignin by microorganisms. In the same way larger degradation products of lignin are also formed. From humic acids, which are synthesized through the metabolism of some microorganisms, resorcinol derivatives could be identified after reductive cleavage. In the case of Epicoccum nigrum the biosynthesis of orsellinic and cresorsellinic acid from aliphatic precursors was observed; these two acids were transformed by the fungus in about 20 phenols. The formation of humic acids in the culture media and the mycelium of this organism occurs only then, if autooxidizable hydroxyhydroquinone or pyrogallol derivatives are formed.

The smaller lignin degradation products are polymerized into the fungal humic acids. This occurs by a transformation of these phenols through degradation of the side chain, demethylation and introduction of hydroxyl groups. In this case the benzene nucleus is scarcely degraded.
EFFECT OF TIME OF SAMPLING AND CROPPING SEQUENCES ON THE CARBOHYDRATES IN RED BROWN EARTHS

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INTRODUCTION

Plant residues added to the soil are rich in carbohydrates and serve as a substrate for macro- and micro-organisms which in turn contribute other carbohydrates to the soil. Thus the carbohydrates or at least a portion of them must be subject to rapid turnover. It is not surprising therefore that the polysaccharide preparations isolated from soils are complex and varied. Hexoses, pentoses and uronic acids have been identified in most polysaccharide preparations but deoxy-hexoses, hexosamines and amino acids have been detected occasionally (Greenland and Oades, 1968).

Separation of the extracted polysaccharides on charged cellulosics has yielded fractions rich in uronic acids (Müller, Mehta and Deuel, 1960; Roulet et al., 1963; Thomas, 1963; and Barker et al., 1965). Different proportions of neutral sugars have also been noted after such fractionations. Electrophoresis and estimations of molecular weights of the polysaccharides have invariably indicated a range of charges and sizes.

Some of the differences reported may be due to technique, particularly different extraction procedures whereas others may be associated with soil type. Such factors have not been examined systematically. Little is known about the effect of time of year or the cropping sequence on soil carbohydrates although Oades (1967a) showed that differences in total carbohydrate content throughout the year were sufficiently small to make them difficult to detect and that differences in amounts of carbohydrates present in a soil under different cropping sequences were similar to the changes in amounts of other organic materials. This paper reports fractionations of the carbohydrates in two red brown earths and changes associated with the time of sampling and the agronomic history of the site from which the sample was taken. Caustic soda was used to extract polysaccharides since, despite considerable efforts to obtain quantitative non-degradative extraction procedures for soil carbohydrates, it still appears to be the most efficient single extractant (Swincer, Oades and Greenland 1967).

SOILS

Composite samples consisting of eight Coile samples per 0.1 ha were obtained from the permanent rotation trial on the Urrbrae fine sandy loam at the Waite Institute and from areas with known cropping histories on the
Belalie clay loam near Jamestown, South Australia. Both soils are red brown earths (Piper 1938). Relevant data are shown in Table 1.

METHODS

Subsamples were immediately cooled to 0° and stored at -15°. When required, samples were dried in a stream of warm air (about 35°) before thorough drying in vacuo over \( P_2O_5 \) for 2 days at 35°.

Carbon was determined using a Fisher carbon induction furnace. Total carbohydrates were estimated by acid hydrolysis, charcoal clarification of hydrolysates and the use of anthrone (Oades 1967a). Partly decomposed plant remains (subsequently referred to as “light-fraction”) were removed from soils by the densimetric procedure of Greenland and Ford (1964) using 0·1% (w/v) Aerosol OT (sodium dioctyl sulphosuccinate, Cyanamid, U.S.A.) in Nemagon (1, 2-dibromo-3-chloropropane, Shell Chemical Proprietary Ltd.) as the heavy liquid. Nemagon has a density of 2·06. After removal of the light fraction, carbohydrates were extracted by shaking in 0·2N \( \text{NaOH} \) for 16 hr at 25° with a 5:1 solution to soil ratio. The suspension was centrifuged, the supernatant passed upwards through a column of \( \text{H}^+ \) Dowex 50, 20-30 mesh. The eluted fulvic acid solution was clarified using Polyclar AT (polyvinyl pyrrolidone, E.I. Noble, Melbourne), and the molecular weight distribution of polysaccharides in the clarified fulvic acid extract was obtained by gel filtration on Sephadex G-25 and Biogel P-100 (Swincer, Oades and Greenland, 1967). Amino acids, amino sugars, uronic acids and neutral sugars were determined in each molecular weight fraction by the methods listed below.

Amino acids—alkaline hydrolysis followed by ninhydrin determination using glutamic acid as a standard (Hirs, Moore and Stein, 1956).

Amino sugars—8N \( \text{HCl} \) hydrolysis (Swann and Balazs, 1965) followed by purification on \( \text{H}^+ \) Amberlite IR 120 (Mueller and Herranen, 1955) and colorimetric determination of the amino sugars by the method of Allison and Smith (1965).

Uronic acids—by the carbazole colorimetric method of Bitter and Muir (1962).

Neutral sugars—by an anthrone procedure (Oades 1967a) and individual monosaccharides by gas-liquid chromatography of the derived alditol acetates (Oades, 1967b).

RESULTS AND DISCUSSION

Amounts of carbohydrate in the soil and light fraction separates

The cropping history, organic carbon and carbohydrate contents of the soils are shown in Table 1. The organic carbon content in the surface horizon of the soil under old pasture (sample 1) was 2·8% compared with 1·8% in the soil cropped continuously with wheat (sample 3). The six samples (5 to 10) taken throughout the year from the soil under the six course rotation 4P2W contained about 2% organic carbon. The carbohydrate contents of these soils estimated by anthrone after acid hydrolysis...
### Table 1

AMOUNTS OF ORGANIC CARBON AND CARBOHYDRATES IN RED BROWN EARTHS

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Depth (cm)</th>
<th>Rotation</th>
<th>Sampling date (month and year)</th>
<th>Organic carbon % soil</th>
<th>Carbohydrate % soil</th>
<th>Carbohydrate in light fraction % total carbohydrate in soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-6</td>
<td>old pasture</td>
<td>9/66</td>
<td>2.8</td>
<td>0.58</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>9-15</td>
<td>old pasture</td>
<td>9/66</td>
<td>1.8</td>
<td>0.26</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>0-6</td>
<td>wheat</td>
<td>9/66</td>
<td>1.8</td>
<td>0.27</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>0-6</td>
<td>wheat</td>
<td>9/66</td>
<td>1.9</td>
<td>0.18</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>0-6</td>
<td>4P2W</td>
<td>5/66</td>
<td>2.0</td>
<td>0.34</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>0-6</td>
<td>4P2W</td>
<td>7/66</td>
<td>2.4</td>
<td>0.40</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>0-6</td>
<td>4P2W</td>
<td>2/67</td>
<td>2.1</td>
<td>0.42</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>0-6</td>
<td>4P2W</td>
<td>5/67</td>
<td>2.1</td>
<td>0.41</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>0-6</td>
<td>4P2W</td>
<td>7/66</td>
<td>2.1</td>
<td>0.31</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>0-6</td>
<td>4P2W</td>
<td>7/66</td>
<td>1.1</td>
<td>0.12</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>0-6</td>
<td>4PW</td>
<td>7/66</td>
<td>0.6</td>
<td>0.58</td>
<td>32</td>
</tr>
<tr>
<td>12</td>
<td>9-15</td>
<td>4PW</td>
<td>7/66</td>
<td>0.6</td>
<td>0.15</td>
<td>9</td>
</tr>
</tbody>
</table>

Urrbrae fine sandy loam

Belalie clay loam

P = pasture, W = wheat. Sample 5 was obtained just after the 4 year old pasture was ploughed. Samples 6-10 thus represent a sequence during the growing wheat crop to the next autumn ploughing. Samples 11 and 12 were in the first year wheat after 4 years pasture, and samples 13 and 14 in second year wheat after 4 years pasture.

showed a similar trend: 0.58% under old pasture and 0.27% in the continuously cropped soil. The carbohydrate content increased slightly 0.34-0.41% in the 12 months May, 1966, to May, 1967, during which period a wheat crop was grown and harvested. In all the surface samples from the Urrbrae soil about two fifths of the soil carbohydrate was present in the light fraction. This material contained 43% of the soil carbohydrate in the soil under old pasture, slightly less under other cropping systems and only about 30% of the carbohydrate in the Belalie soils investigated. Gas-liquid chromatography of the acetylated reduced neutral sugars showed a high proportion of glucose. The relative amounts of the different neutral sugars present showed that the carbohydrate composition of the light fraction was intermediate between that of plant material and the soil. Of the organic carbon, 5.2 to 7.3% was present as carbohydrate in the Urrbrae soils while the proportions were 9.5 to 9.9% in the Belalie soils. The fact that the proportion of organic matter in the form of carbohydrate
is dependent on soil type but not cropping history or season has been reported previously by Oades (1967a).

**Extraction and size distribution of polysaccharides**

The amounts of carbohydrate in humin, humic acid and fulvic acid fractions are shown in Table 2. Carbohydrate materials were most efficiently extracted from the soil cropped continuously with wheat and the subsurface soil under old pasture from which 45% was removed. The polysaccharides extracted from these soils contained little material with molecular weight greater than 100,000. The proportion of low molecular weight material was correspondingly large and a large part of the carbohydrates was associated with humic materials. Caustic soda was slightly less efficient as a carbohydrate extractant in the Belalie samples probably due to the higher clay contents. There was a trend showing an increased extraction of carbohydrate during the winter months (samples 6 and 7) in the 4P2W rotation with maximum extraction of 38% in September. This fell to 30% in the following May (sample 10). Thus both season and cropping sequence appear to affect the efficiency of extraction of carbohydrates from these soils by caustic soda, maximum extraction occurring in soils under cultivation during the winter months, i.e. the period of most active plant and microbial growth under a Mediterranean type of climate.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbohydrate after removal of light fraction % soil</th>
<th>Expressed as % of carbohydrate in soil after removal of light fraction</th>
<th>Carbohydrate not extracted by NaOH i.e. humin fraction</th>
<th>Extracted carbohydrate associated with humic materials</th>
<th>Extracted carbohydrate in fulvic acid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbohydrate extracted by NaOH &lt; 4000 M.W.</td>
<td>Extracted carbohydrate &gt; 100,000 M.W.</td>
<td>4000 to 100,000 M.W.</td>
<td>&lt; 4000 M.W.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.33</td>
<td>65.5</td>
<td>7.7</td>
<td>4.1</td>
<td>12.8</td>
</tr>
<tr>
<td>2</td>
<td>0.19</td>
<td>55.0</td>
<td>15.4</td>
<td>2.9</td>
<td>13.0</td>
</tr>
<tr>
<td>3</td>
<td>0.17</td>
<td>55.0</td>
<td>23.3</td>
<td>3.6</td>
<td>9.7</td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>61.0</td>
<td>11.5</td>
<td>2.9</td>
<td>13.3</td>
</tr>
<tr>
<td>5</td>
<td>0.21</td>
<td>64.8</td>
<td>10.2</td>
<td>3.3</td>
<td>9.9</td>
</tr>
<tr>
<td>6</td>
<td>0.19</td>
<td>63.6</td>
<td>1.8</td>
<td>3.7</td>
<td>15.1</td>
</tr>
<tr>
<td>7</td>
<td>0.22</td>
<td>62.0</td>
<td>6.2</td>
<td>3.2</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>0.25</td>
<td>67.1</td>
<td>10.7</td>
<td>3.2</td>
<td>9.3</td>
</tr>
<tr>
<td>9</td>
<td>0.27</td>
<td>68.3</td>
<td>10.6</td>
<td>2.5</td>
<td>8.6</td>
</tr>
<tr>
<td>10</td>
<td>0.26</td>
<td>69.9</td>
<td>9.1</td>
<td>2.7</td>
<td>8.4</td>
</tr>
<tr>
<td>11</td>
<td>0.22</td>
<td>69.1</td>
<td>7.7</td>
<td>3.6</td>
<td>10.3</td>
</tr>
<tr>
<td>12</td>
<td>0.11</td>
<td>65.0</td>
<td>7.9</td>
<td>2.8</td>
<td>9.5</td>
</tr>
<tr>
<td>13</td>
<td>0.40</td>
<td>76.0</td>
<td>9.2</td>
<td>2.1</td>
<td>6.7</td>
</tr>
<tr>
<td>14</td>
<td>0.14</td>
<td>69.0</td>
<td>13.5</td>
<td>1.7</td>
<td>5.6</td>
</tr>
</tbody>
</table>
The fulvic acid fraction from the soil under old pasture contained the largest proportion of carbohydrate with molecular weight greater than 100,000 (according to gel filtration). The soil cropped continuously with wheat, yielded a smaller proportion of big polymers. The Belalie soil after a 4PW cropping sequence also contained a higher proportion of large polymers compared with the 4P2W sequence (3·6 to 2·1% respectively). This increase in the proportion of large polymers under the influence of pasture may bear some relation to the increase in stability of aggregates under pasture compared with continuous cropping. It may also be suggested that it is the strongly sorbed carbohydrates that are involved in aggregate stabilization. In the soil under pasture 0·22% remained after extraction but only 0·09% in the heavily cropped soil.

Of the Urrbrae samples taken throughout the year on the 4P2W rotation, the July and September samples (6 and 7) were obviously different. They contained a higher proportion of carbohydrate extractable by 0·2N NaOH and less of the extracted carbohydrate was associated with humic materials. Most of the additional carbohydrate extracted in these samples compared with samples 5 and 8 to 10 was in the medium and low molecular weight cuts as obtained by gel filtration (4,000-100,000 and < 4,000 molecular weight). It is possible that during the summer when these soils dry out and microbial and plant activity is minimal, these polymers become associated with humic materials and/or are sorbed on mineral colloid surfaces and are thus less easily extractable.

Composition of the extracted polymers

The relative composition of the three molecular weight fractions are shown in Table 3. Most of the uronic acids were in polymers greater than 4,000 molecular weight. About one uronic acid moiety per twenty or more neutral sugars was present in the <4,000 molecular weight materials. The ratio was about one to two and one to three or four in the medium and high molecular weight fractions respectively. Thus the larger polymers were particularly rich in carboxyl groups of uronic acids. Such groups have a pK value of 3·2 (Swincer, Oades and Greenland 1967). Müller, Mehta and Deuel (1960) obtained a small uronic acid rich fraction using charged cellulose. It may be that the polymers with molecular weight > 4,000 can be further fractionated on the basis of charge to give purer polyuronides.

There were no marked changes in the uronic acid composition of the polymers from different soils or from the same soil sampled throughout the year except in the low molecular weight fraction where a higher proportion of uronic acids occurred in samples taken during winter and spring. Thus although there was an increase in the amount of uronic acids extracted into the larger polymers in the fulvic acid fraction in samples 6 and 7, there was also an increase in the amount of neutral sugars so that the composition of these materials remained similar throughout the year and was not affected by the cropping regime.

The proportion of amino sugars to neutral sugars in the three molecular weight fractions was almost constant in any one soil and they
<table>
<thead>
<tr>
<th>Sample</th>
<th>&gt;100,000 Molecular weight fraction</th>
<th>4,000 — 100,000 Molecular weight fraction</th>
<th>&lt;4,000 Molecular weight fraction</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ratio on weight to weight basis</td>
<td>Ratio on weight to weight basis</td>
<td>Ratio on weight to weight basis</td>
</tr>
<tr>
<td></td>
<td>neutral sugar</td>
<td>uronic acid</td>
<td>amino sugar</td>
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<tr>
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<td>27</td>
<td>13</td>
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<tr>
<td>2</td>
<td>100</td>
<td>36</td>
<td>18</td>
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<td>14</td>
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<td>25</td>
<td>17</td>
</tr>
</tbody>
</table>
CARBOHYDRATES IN RED-BROWN EARTHS

represented from 10 to 20% of the amounts of neutral sugars. In the medium and low molecular weight fractions, there was a tendency for the proportion of amino sugars to be high during the winter months (samples 6 and 7). This was in spite of the increased amount of other materials extracted into the fulvic fraction in these two samples.

The amino acid compositions of the three molecular weight fractions were quite different. The amino acids were concentrated in the low molecular weight fractions and in some of these samples there were similar amounts of amino acids and carbohydrate. As with amino sugars there was an increase in the proportion of amino acids during the winter (samples 6 and 7) but this was maintained into the summer (samples 8 and 9). This trend was not obvious in the other molecular weight fractions.

CONCLUSIONS

The cropping history of the soil had an effect on the total amount of carbohydrate present in the soil and the extractability of that carbohydrate by 0-2N NaOH. With the soil under old pasture a higher proportion of the extracted polysaccharide was high molecular weight material and a lower proportion was associated with humic materials. No clear cut differences were apparent in the composition of the polysaccharides extracted from a soil under old pasture compared with a soil cropped continuously with wheat.

A seasonal effect on soil carbohydrates was also evident. Samples taken during winter contained a higher proportion of carbohydrate extractable by 0-2N NaOH. This extra material extracted by NaOH during the winter was of < 100,000 molecular weight and was richer in amino acids and charged sugars than the rest of the extracted polymers. A smaller proportion of it was associated with humic materials. It is possible that this material represents the soluble portion of the microbial population. During summer it may be metabolised and incorporated into more complex insoluble materials or may be sorbed on organic and inorganic colloids and become difficult to extract.

Because of the lack of information regarding errors in the results due to soil sampling variability, some of these conclusions require confirmation. However, the trends described suggest a basis for further investigations.

The composition of the extracted polymers was dependent on molecular size. Uronic acids were concentrated in the polysaccharides of molecular weight > 4,000 with the highest proportion relative to neutral sugars in the polymers of molecular weight 4,000-100,000. Conversely amino acids were concentrated in the lower molecular weight fractions, particularly the < 4,000 fraction where equivalent amounts of neutral sugars and amino acids were present. The proportion of amino sugars present was relatively constant. Differences in the proportions of neutral sugars were also obvious between molecular size fractions. Galactose was the predominant sugar in the high molecular weight fractions with noticeable proportions of rhamnose and fucose. Hexoses, in particular glucose were predominant in the smaller polymers.
Acknowledgments

G. D. Swincer wishes to thank the Commonwealth Wheat Industry Research Council for a Senior Studentship. Other financial support was received from the South Australian Wheat Industry Committee.

References


rest of the year) were of molecular weight less than 100,000 according to gel filtration on Sephadex and Biogel. They were richer in amino acids and amino sugars than the remainder of the extracted materials.

The composition of the extracted materials depended on their size. Large polymers were rich in uronic acids which were most concentrated in the 4,000-100,000 molecular weight fraction. In the < 4,000 molecular weight range uronic acids were in low concentration and approximately equivalent amounts of amino acids and neutral sugars were present. The ratio of neutral sugars to amino sugars was between 5:1 and 20:1 in all molecular weight fractions.

RÉSUMÉ

On retira des restes de plantes en partie décomposées de deux sols rouges-bruns sous différents régimes de culture et à différentes époques de l’année par le moyen de flottaison sur un liquide lourd. Ce traitement retira aux alentours de deux cinquièmes du total des hydrates de carbone dans le sol. Des hydrates de carbone furent encore extraits du sol par la soude caustique qui s’avéra plus efficace sur un sol qui fut soumis sans interruption à des moissons de blé que sur un sol où des pâtures furent comprises dans les séquences de récoltes. L’extraction maximum sur un sol donné se présenta pendant les mois d’hiver. Les polymères supplémentaires extraits en hiver (par comparaison avec le reste de l’année) eurent un poids moléculaire de moins de 100,000 d’après la filtration sur gel Sephadex et Biogel. Leur teneur en amino acides et en sucres aminés fut plus riche que celle du reste de la matière extraite.

La composition des matières extraites dépendit de leur taille. De longs polymères furent riches en acides uroniques qui furent les plus concentrés dans la fraction 4,000-100,000 de poids moléculaire. Dans la gamme < 4,000 de poids moléculaire, les acides uroniques firent preuve d’une concentration basse et des quantités un peu près équivalentes d’amino acides et de sucres neutres furent présentes. Le rapport entre les sucres neutres et les sucres aminés fut de l’ordre de 5 : 1 à 20 : 1 dans toutes les fractions de poids moléculaire.

ZUSAMMENFASSUNG

weniger als 100.000 bestimmt nach Gel-Filtrierung auf Sephadex und Biogel. Sie waren reicher an Aminosäure und Aminozucker als der Rest der entzogenen Stoffe.

STUDIES ON THE POLYSACCHARIDE CONSTITUENTS OF AN ACID EXTRACT OF A FENLAND MUCK SOIL

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Chemistry Department, University of Birmingham, England

INTRODUCTION

Previous reports from this department (Barker et al., 1965; Finch et al., 1966; Barker et al., 1967) have described the isolation and some of the properties of soil polysaccharides in the acid extract of a Fenland muck soil, but have been largely confined to the highest molecular weight fraction. We now describe the fractionation of the total polysaccharide extract, and the characterisation of some of the fractions obtained.

EXPERIMENTAL

Extraction and purification

The neutralisation of an acid extract of a Fenland muck soil to yield a supernatant solution A and a precipitate B and subsequent fractionation of these preparations on Sephadex G-100 has been described by Finch et al. (1966).

These fractions obtained by molecular sieve chromatography were further treated as shown in the flow sheet (Figure 1). All fractions except AF and BF which represent brown fulvic materials were refractionated on columns (5.5 x 100 cm) of Sephadex G-100 to obtain materials of narrower molecular weight distribution for the present work. A small portion of fraction A2 was electrodialysed at 200v for 8 to 9 hr. The freeze-dried product A2E, was obtained in >90% yield and was free of brown materials.

Small scale ion exchange chromatography of polysaccharide fractions

Solutions (5 mg freeze-dried material in 0.5 ml) of the polysaccharide fractions in tris-HCl buffer, pH 9.1 (tris-(hydroxymethyl)-aminomethane, 49.9 g per l., conc. HCl 4.82 ml per l., ionic strength 0.05) were applied to columns (1.1 x 35 cm) of Whatman DEAE cellulose DE-52 which had previously been equilibrated with the tris buffer. Elution into 2.5 ml fractions was carried out with a linear gradient of 0 to 1 M NaCl (200 ml) in the buffer. The fractions were analysed for total sugar content by the procedure of Dubois et al. (1956). Fraction Ia was obtained by fractionation of I using a linear gradient of 0.005-0.05 ionic strength tris-HCl buffer. Fraction Ia was eluted at 0.005 ionic strength. It was dialysed, freeze-dried and retained for sugar analysis.
Fenland muck soil
\[ \text{neutralised with NaHCO}_3 \]
\[ 0.6N \text{ H}_2\text{SO}_4 \]

Supernatant A
Sephadex G-100
A1 A2 A3 AF

electro-dialysis

A2E

Refractionation on Sephadex G-100

A1 A2 A3

Precipitate B
redissolved in acid Sephadex G-100
B1 B2 B3 BF

Large scale ion exchange chromatography on DEAE cellulose DE-52, elution by NaCl in tris-HCl buffer

ON

0.25M

0.25M

0.3M NaCl

I (23%) IIa (28%) IIb (40%) III (1.7%)

Small scale ion exchange chromatography on DEAE cellulose DE-52, tris-HCl buffer elution

Ia (ionic strength 0.005)

Per cent figures in brackets represent the recoveries in each fraction of the sample (B2) applied to the column.

Fig. 1.—Flow sheet for the extraction and purification of polysaccharides from a Fenland muck soil.

**Large scale ion exchange chromatography of fraction B2**

Polysaccharide B2 (872 mg) was stirred for 6 hr with tris-HCl \(-1\% (v/v)\) \(N, N\)-dimethylformamide (150 ml) at 20\(^\circ\) and the resulting turbid solution clarified by centrifugation. The clear pale yellow supernatant (96\% recovery) was applied to a column (4·0 x 62 cm) of Whatman DEAE cellulose DE-52 previously equilibrated with the buffer, and eluted successively with 825 ml 0 M, 1,425 ml 0·25 M and 700 ml 0·35 M solutions of NaCl in the buffers. The eluted fractions (25 ml) were analysed for total sugar content (Dubois et al., 1956) and brown colour (\(E_{400}^{1cm}\)). Polysaccharide containing fractions obtained with each concentration of salt were pooled, dialysed against several changes of distilled water over a period of 1 week, and freeze-dried.
Investigation of the homogeneity of fractions I, IIa and IIb obtained by ion exchange chromatography

Solutions (ca. 0.5% w/v) of the fractions in a phosphate buffer (0.15 M NaCl, 0.0067 M KH₂PO₄, 0.0133 M Na₂HPO₄, pH 7.0) were examined in the analytical ultracentrifuge (Spinco Model E, courtesy of Dr. G. A. Gilbert) at 59,780 r.p.m.

Solutions of the polysaccharide fractions in 0.0333, 0.0500 and 0.0667 M sodium tetraborate (fraction I), in pH 8.2 (ionic strength 0.1) barbiturate-sodium chloride (Long, 1961a; fractions IIa and IIb), and in pH 7.0 (ionic strength 0.1) phosphate (Long, 1961b; fraction IIa) were examined by free boundary electrophoresis using a Zeiss Model 35 Free Solution Electrophoresis Apparatus.

Analysis of polysaccharide fractions for amino acids and sugars

Freeze-dried polysaccharide fractions were dried exhaustively over P₂O₅ in vacuo, before analysis.

Amino acid analysis

Samples of fractions I, IIa and IIb together with norleucine as an internal standard were oxidised with performic acid solution for 3 hr at 0° (Bidmead and Ley, 1958), and hydrolysed for 24 hr at 110° under nitrogen with 6N HCl. Amino acids were determined in the hydrolysates using a Technicon Autoanalyser (Technicon Instruments Company, Chertsey, England) and the buffer system of Spackman et al. (1958).

Sugar analysis

Polysaccharide fractions I, Ia, IIa and IIb were hydrolysed in sealed tubes under nitrogen by N H₂SO₄ at 100° for 3.75 hr. After hydrolysis 2, 3, 6-tri-O-methyl-D-glucose was added as an internal standard and the solutions passed successively down columns of Amberlite IR-120 (H⁺) and Amberlite IR-4B (OH⁻). The eluates were concentrated to dryness in vacuo and the sugar alcohol acetates prepared by the method of Crowell and Burnett (1967). Solutions of the alditol acetates in methylene dichloride were analysed by gas-liquid chromatography using a Pye Argon apparatus with a Pye standard glass column packed with 3% ECNSS-m on Gas-Chrom Q, 100 to 120 mesh (Applied Science Laboratories Inc. Penn. U.S.A.) at 185° (Sawardeker et al., 1965). Peak areas for the sugar derivatives were obtained by planimetry and the amounts of sugars calculated from the relative responses compared with the internal standard.

The Amberlite IR-4B (OH⁻) columns were each washed with 2 N ammonium hydroxide and the eluates analysed for uronic acid using D-glucurone as a standard (Bitter and Muir, 1962).

RESULTS

Small scale anion exchange chromatography

Fractions A1, A2, A3, A2E and B2 all showed four peaks which were eluted at salt concentrations of 0, 0.13, 0.37 and 0.54 M (fractions A1-A3), and of 0, 0.07, 0.22 and 0.33 M (A2E and B2). The relative
proportions of the four peaks were similar for each of these two groups of polysaccharide fractions. The component of second lowest negative charge was minor and poorly resolved from that eluted at 0 M sodium chloride. This minor component disappeared on incorporating 8 M urea or 1% (v/v) N, N-dimethylformamide into the buffer system (see Figure 2).

![Anion-exchange chromatography](image)

**Fig. 2.**—Anion-exchange chromatography on DEAE cellulose DE-52 of polysaccharide fraction A2E in pH 9.1 tris-hydrochloric acid buffer (ionic strength 0.05) alone (-----) and containing 8 M urea (-x-).

**Large scale anion exchange chromatography**

Two separate polysaccharide-containing fractions were eluted at 0.25 M sodium chloride concentration, the first containing a very small amount of brown material (maximum OD 400 m/\(\mu\) = 0.04) and the second a larger amount (maximum OD 400 m/\(\mu\) = 0.15). No UV absorbing material was present in polysaccharide eluted at 0 M and 0.35 M sodium chloride. The amounts of the different fractions obtained are shown in Figure 1. The total recovery was 92%.

**Homogeneity of fractions I, IIa and IIb**

In the ultracentrifuge all fractions exhibited single broad peaks, indicative of extreme polydispersity, which were broader in the series IIa<IIb<IIIB. The uncorrected sedimentation constants at 20° were IIa, 1.867 ± 0.004; IIb, 1.55 ± 0.07; IIIB, 1.59 ± 0.13.

During electrophoresis fraction I showed the presence of two negatively charged components in all three buffers, but the separation was poor in 0.05 M sodium tetra-borate. A sample of I was examined by small-scale anion-exchange chromatography (as described above) using a linear gradient of 0.005-0.05 ionic strength tris-hydrochloric acid buffer. A sharp fraction was eluted immediately at 0.005 ionic strength (designated Ia)
followed by several more negatively charged components eluted over the range 0.025-0.05 ionic strength.

On free boundary electrophoresis in barbiturate and phosphate fraction IIa was homogeneous, apart from a small hump on the leading edges of the boundaries which was attributed to contamination by fraction IIb (ca. 5%). The mean ascending electrophoretic mobilities observed were $-3.54 \times 10^{-5}$ (phosphate) and $-3.95 \times 10^{-5}$ cm$^2$ volt$^{-1}$ sec$^{-1}$ (barbiturate).

Free boundary electrophoresis of fraction IIb in phosphate buffer revealed two negatively charged components with mobilities of $-12.6$ and $-10.3$ (ascending) and of $-12.2$ and $-6.95$ (descending) cm$^2$ volt$^{-1}$ sec$^{-1}$. The faster peak comprised ca. 30% of the total. The ascending and descending patterns were not enantiographic, the leading component being very much sharper in the ascending boundary. Molecular-sieve chromatography of IIb on Biogel P-300 (V. A. Howe and Co. Ltd., London) revealed single peaks of polysaccharide (assayed by the procedure of Dubois et al., 1956) and brown material ($E_{100}^{	ext{cm}}$). No separation was apparent when the experiment was performed in 0.2 M sodium chloride, but elution with pH 2.1 hydrochloric acid-potassium chloride buffer ($\text{HCl} \ 1.23 \text{ ml per l, } \text{KCl} \ 12.98 \text{ g per l, ionic strength } 0.2$) brought about a distinct separation of the two components, the brown material exhibiting the lower molecular size.

**Amino acid and sugar composition of fractions I, Ia, IIa and IIb**

**Amino acids**

The proportions of the amino acids which could be identified from their elution positions amounted to 0.99% (I), 0.82% (IIa) and 1.62% (IIb) of the polysaccharides on a weight basis. The most abundant products were glycine, aspartic acid, threonine, serine, glutamic acid, alanine, and an unidentified component (eluted just after ammonia). Traces of tyrosine and phenylalanine were present in fraction IIb only. Two peaks appearing on the chromatograms are valine were attributed to glucosamine and galactosamine, and correspond to amino sugar contents of ca. 2% in each polysaccharide fraction, assuming a 50% loss on hydrolysis (Sowden, 1959).

**TABLE 1**

<table>
<thead>
<tr>
<th>Anhydro Sugar Contents (Mole Percent) of Polysaccharide Fractions in Terms of the Total Amount Recovered for Each Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Anhydrorhamnose</td>
</tr>
<tr>
<td>Anhydrofucose</td>
</tr>
<tr>
<td>Anhydroarabinose</td>
</tr>
<tr>
<td>Anhydroxylose</td>
</tr>
<tr>
<td>Anhydromannose</td>
</tr>
<tr>
<td>Anhydrogalactose</td>
</tr>
<tr>
<td>Anhydroglucose</td>
</tr>
<tr>
<td>Uronic acid (as glucuronolactone)</td>
</tr>
</tbody>
</table>
Sugars

The results of the sugar analyses are presented in Table I. Chromatographs showed no peaks of measurable area other than those for the sugars listed. The reproducibility of repeated measurements on a single hydrolysate was found to be ca. 10%. The total recoveries of anhydro sugars amounted to 30.0% (Ia), 28.5% (I), 33.1% (Ha) and 14.8% (IIb) by weight.

Discussion

Physico-chemical properties of the fractions

The close similarity of the anion-exchange chromatograms for the polysaccharide fractions of different molecular sizes implies that the components of different charge densities possessed broad and similar molecular weight distributions. This was supported by the broad uncharacteristic polysaccharide elution record obtained on molecular-sieve chromatography of the crude extracts on Sephadex G-100, and also by the similarity of the sedimentation constants of the different charged components separated by anion-exchange chromatography.

The different elution positions of the charged components for fractions A1-A3 and for fractions A2E and B2 might be attributed to the fact that the latter (A2E and B2) were subjected to an extra dialysis step during isolation. However, the dialysis appeared to release anionic, rather than cationic, species since the charged components of A2E and B2 were eluted at lower sodium chloride concentrations than in the cases of A1-A3. The disappearance of the minor charged component eluted from DEAE cellulose DE-52 at 0.07M salt concentration when the buffer contained 8M urea (A2E) or 1% (v/v) N,N-dimethylformamide (B2) suggested that this component was a complex formed by hydrogen bonding, possibly between the buffer cation and the most highly charged polysaccharide material. [The abundance of this material was increased when 8M urea was included in the eluant (Figure 2)].

The elution of two polysaccharide fractions at 0.25M sodium chloride concentration was attributed to non-linearity of the salt gradient during the small-scale experiments; more recent conductivity measurement have tended to confirm this. The inhomogeneity of Fraction I revealed by electrophoresis and anion exchange chromatography conflicts with previous results (Finch et al. 1966) for a similar fraction, however different conditions of anion-exchange chromatography were used in the two cases during isolation. The sharpening of the leading ascending boundary observed during free-boundary electrophoresis of fraction IIb probably arose because this boundary was moving from solution to pure buffer, a region of higher conductivity and therefore lower potential gradient. The relative magnitudes of the electrophoretic mobilities of the peaks may have been due to pH effects, hydrodynamic effects, or to the existence of complexes. It was not possible to identify the fast component with the small amount of brown material present.

Two explanations are possible for the ability of pH 2.1 buffer to bring
about a separation of the polysaccharide and brown constituents of fraction IIb during molecular-sieve chromatography on Biogel P-300. The two constituents may have been bound together by an acid-labile bond, or, less likely, the low pH may have effected a drastic reduction in molecular size of the brown constituent. The effect of acid in promoting a separation of polysaccharide from brown material, which has been demonstrated previously (Barker et al. 1965; Barker et al., 1967; Dubach et al., 1964), suggested an acetal type of linkage, which has been indicated by other work (Bolker, 1963; Brownell, 1965) on the nature of the carbohydrate-lignin bond in wood. However, Kringstadt and Ellefsen (1964) isolated a lignin containing hemicellulose from spruce-wood in which the majority of the polysaccharide-lignin bonds were stable to 0·1 N sulphuric acid at 100°.

Monosaccharide composition

The low recoveries of sugars after acid hydrolysis of the polysaccharide fractions might be attributed to degradation during hydrolysis, or to the presence of non-sugar organic components. Barker et al. (1967) reported evidence for the presence of an unidentified acid component in the most highly charged polysaccharide fraction obtained by anion-exchange chromatography of purified polysaccharide extract A1.

The large difference between the uronic acid contents of fractions IIa and IIb is in accord with their separation by anion-exchange chromatography in the same buffer solution, and also with their different electrophoretic mobilities.

The neutral sugar composition was similar for fractions IIa and IIb but different for fraction Ia, with fraction I (which contained ca. 50% Ia) occupying an intermediate position. Fraction Ia had a high glucose content, which may indicate that it contained cellulose, but this would not normally be soluble in 0·6 N H$_2$SO$_4$ at 5°.

On the basis of sugar compositions, it may be concluded that polysaccharide fractions IIa and IIb are the products of endo- and exo-cellular microbial synthesis. This conclusion has been reached by a number of workers (see Mehta et al., 1961), but it must be extended to account for the isolation of apparently discrete fractions by anion-exchange chromatography (Müller et al., 1960; Barker et al., 1965). The number and composition of polysaccharide fractions which can be isolated from a particular soil may be a reflection of the population of the dominant synthetic microbial species present.

ACKNOWLEDGMENTS

The authors thank Mr. R. L. Jones for technical assistance, and also the Science Research Council for the provision of a Research Fellowship to one of us. (P.F.)
SUMMARY

An acid extract of a Fenland muck soil was separated into polysaccharide fractions of different molecular size, and a brown fraction, by molecular-sieve chromatography on Sephadex G-100.

Five polysaccharide fractions showed similar patterns on anion-exchange chromatography, and one fraction was resolved into three components by stepwise elution from DEAE cellulose with salt solutions of increasing concentrations. On examination by ultra-centrifugation and by free-boundary electrophoresis, one of the components was homogeneous under the conditions used. The least highly charged component was further resolved into two fractions by anion-exchange chromatography.

Four polysaccharide components were analysed for neutral sugar constituents by acid hydrolysis, preparation of the sugar alditol acetates and quantitative gas-liquid chromatography. The composition of one of the components suggested that it might contain cellulose. The origin of the other polysaccharide components is discussed briefly.
fraction fut réduite en trois composants par élutions successives sur cellulose DEAE au moyen de solutions salines de concentrations croissantes. Examiné par ultra-centrifugation et par électrophorèse libre un des composants se révéla de nature homogène, sous les conditions utilisées. Le composant le moins chargé fut réduit encore en deux fractions au moyen de la chromatographie par échange anionique.

On analysa quatre composants de polysaccharides par hydrolyse acide pour obtenir des glucides neutres dont on prépara les acetates d’alditol pour ensuite séparer ceux-ci par chromatographie en phase gazeuse. La composition d’un des composants laissait croire qu’il contenait de la cellulose.

Les origines des trois autres composants polysaccharidiques sont brièvement examinées.

ZUSAMMENFASSUNG

Ein Säureextrakt aus einer Fenlanderde setzte sich als Polysaccharidfraktionen verschiedener Molekulargewichte und als eine braune Fraktion ab, und zwar nach Gelfiltrierung von Sephadex G-100.


Weiterhin wird der Ursprung der anderen Polysaccharidenkomponenten besprochen.
RATES OF ORGANIC MATTER DECOMPOSITION MEASURED BY WARBURG RESPIRATORY EXPERIMENTS

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INTRODUCTION

The Warburg respirometer (Umbreit, Burris and Stauffer 1949) is widely used to measure soil respiration over short periods. Its advantages for this purpose are its accuracy, and the possibility of determining respiratory quotients, and the immediate effects of added substrate or inhibitor (Rovira 1953).

Soil respiratory rates are generally related to the amount of soil organic matter (Bunt and Rovira 1955). Jensen (1936) found that there was a low correlation between the plate count and respiratory activity but a high correlation with the total count. There appears to have been little attempt, however, to relate rates to the total soil populations, animal and microbial, to the productivity of the plant regime or to the turnover of the soil organic cycle.

The present studies were initiated in conjunction with a biological survey of a wide range of New Zealand soils with the purpose of obtaining some measure of biological activity.

METHODS

There are two alternatives in carrying out laboratory experiments. The first is to relate the experimental conditions as nearly as possible to field conditions. The second is to treat the experiment as an arbitrary test and to standardise the conditions of the test. The second alternative was followed.

The soil was initially tested with as little physical change as possible and in the shortest time possible after collection. Readings were taken for a period of about 3 hours in the morning of the first day (I). Then 3 ml of sterile distilled water was added uniformly with a pipette to the 3 to 4 g (wet weight) of soil in the reaction flask and a second series of readings of about 3 hours was made in the afternoon of the first day (II). A similar series of readings was carried out on the morning of the second day (III) and then 0.5 ml of 1% (w/v) glucose solution added by pipette and a further series of readings was taken in the afternoon of the second day (IV). This completed the experiment. The experiments were carried out at 24°C, a compromise between a temperature required to give an adequate reading in the time and one not too remote, in most cases, from field
conditions. It was found that there was a relatively steady rate of respiration following the addition of glucose in the first three hours although after this period there tended to be a more marked response, doubtless due to the multiplication of the population (Drobnik 1960). In one experiment (b), a fifth reading was taken on the morning of the third day and in this case 0·5 ml of 2% lactose was added in place of glucose.

Five separate topsoil samples (2-10 cm depth) were collected from each site and a composite sample prepared from them. The soil was not sieved or air dried and the experiments were carried out as soon as possible after sampling. Moisture was determined by oven drying at 105°C. Total bacterial populations were determined by plating from ten-fold serial dilutions on glucose (0·5%)-tryptone (0·5%)-yeast extract (0·4%) agar incubated at 24°C. Level of aerogenic fermenters was estimated as M.P.N. from McGrady’s Tables (Pochon and Tardieux 1962) on the basis of acid and gas production in tubes of glucose-tryptone broth. 5 tubes being inoculated for each dilution level, except in the third experiment where only a single tube was inoculated. Earthworm populations were determined by hand sorting of soil blocks taken from the sites. (Sears et al 1965). Experiments were in duplicate for each site.

Results were calculated initially as the evolution of CO₂/g dry weight of soil but because of the direct relationship to the amount of soil organic matter it was generally expressed as CO₂/g organic matter or organic C. Organic carbon was determined for the first two experiments by a modification of the Walkley and Black method and for the third by a manometric method using an induction furnace.

### Table 1

**Respiratory Activity and Bacterial Populations of Three Grassland Plots, of Low, Medium and High Productivity on Kairanga Silt Loam (cf. Plots 1, 4, 7, Sears et al. 1965)**

**Sampled March 1964 at Grasslands Division, D.S.I.R., Palmerston North (40° 23' S 175° 37' E).**

<table>
<thead>
<tr>
<th>Plots</th>
<th>Low producing</th>
<th>Medium producing</th>
<th>High producing</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6·4</td>
<td>6·4</td>
<td>6·5</td>
</tr>
<tr>
<td>% Moisture (d.w.)</td>
<td>15</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>% Organic C</td>
<td>1·2</td>
<td>1·5</td>
<td>2·0</td>
</tr>
<tr>
<td>Annual net loss</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>org. C kg/ha</td>
<td>120</td>
<td>3,200</td>
<td>11,100</td>
</tr>
<tr>
<td>Respiratory Activity μl CO₂/g Org. C/hr. at 24°C (R.Q.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Basal Rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II, III</td>
<td>171 (0·68)</td>
<td>178 (0·70)</td>
<td>175 (0·73)</td>
</tr>
<tr>
<td>Zymogenic Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>188 (0·81)</td>
<td>220 (0·62)</td>
<td>316 (1·29)</td>
</tr>
<tr>
<td>Bacterial Populations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plate count</td>
<td>3</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Fermenters</td>
<td>2·5</td>
<td>7</td>
<td>600</td>
</tr>
<tr>
<td>× 10⁹/g wet wt.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The measurements on the soil, unamended or with the simple addition of water, are referred to as the basal respiratory rate, while the response to glucose is referred to as the zymogenic response.

RESULTS

(a) Same soil under pasture grasses of greatly different productivity

The three experimental plots sampled were established on a recent alluvial soil of initially very low organic content and maintained under different pasture regimes. In one, the soil was topdressed with phosphate and a high producing ryegrass-white clover pasture was established. From clippings of the herbage of this ungrazed pasture 80% was returned to the soil. In the second, the soil was not topdressed and the clippings of herbage were not returned to the soil. It had no fertilisers added and consisted of a medium producing sward of ryegrass, white clover, browntop and flatweeds. The third plot, which was topdressed with phosphate but had no return of clippings, was a low producing sward of browntop.

The primary purpose of this experiment was to measure pasture production and nitrogen fixation in the soil (Sears et al., 1965), but the Warburg measurements give some indication of the pattern of organic decomposition. Table 1 shows that despite great differences in productivity, the different plots show no significant difference in the basal rates (calculated as $\mu l CO_2/g Org. C$), but there is a much higher zymogenic response associated with a higher R.Q. in the high producing plot in which the clippings were returned to the soil. Dilution counts showed that this plot also had a higher bacterial population (total plate count) and in particular a much higher

<table>
<thead>
<tr>
<th>pH</th>
<th>Control Soil</th>
<th>Irrigated Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.0</td>
<td>5.75</td>
</tr>
<tr>
<td>% Moisture (d.w.)</td>
<td>33</td>
<td>43</td>
</tr>
<tr>
<td>% Organic C</td>
<td>5.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Respiratory Activity $\mu l CO_2/g Org. C/hr.$ at 24°C (R.Q.)

<table>
<thead>
<tr>
<th>Mean Basal Rate</th>
<th>Control</th>
<th>Irrigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, II, III</td>
<td>109</td>
<td>132</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Zymogenic Response</th>
<th>Control</th>
<th>Irrigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>162</td>
<td>348</td>
</tr>
<tr>
<td>V</td>
<td>153</td>
<td>458</td>
</tr>
</tbody>
</table>

Bacterial Populations

<table>
<thead>
<tr>
<th>Total plate count</th>
<th>Control</th>
<th>Irrigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\times 10^6/g$ wet wt.</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Fermenters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\times 10^7/g$ wet wt.</td>
<td>35</td>
<td>1200</td>
</tr>
</tbody>
</table>
population of aerogenic fermenters (*Enterobacter* = *Aerobacter*). Earthworm populations recorded by Sears *et al.* (1965) showed a similar pattern.

(b) *Pasture irrigated with whey*

In New Zealand a number of dairy factories producing casein, dispose of the residual whey by using it to irrigate grazed pastures. As a consequence these pastures receive, amongst other whey ingredients, about 100,000 kg of lactose per hectare per year. Whey irrigation effects little change in the population of soil arthropods or the amount of fungal mycelium but the yeast and bacterial populations and their micro-predators, the protozoa, are greatly stimulated and there are higher earthworm populations (see Table 2). Aerogenic fermenters increase greatly and these include *N*-fixing and lactose-fermenting *Enterobacter* (= *Aerobacter*) and *Clostridium* (di Menna 1966).

Initially there is no great difference in the basal respiratory rate of the irrigated and unirrigated soil but the irrigated soil may show a more marked response to the addition of water. There is however a marked difference to the addition of nutrient, in this case lactose. The irrigated soil shows a much greater response associated with a higher R.Q. and this difference persists for at least twenty-four hours (Experiment V).

(c) *Same soil under forest, pasture and cultivation*

The chief difference, biologically, between the soil subjected to prolonged cultivation and that under forest or pasture is in the animal population which is very greatly depressed in the cultivated soil and which differs in species composition between the exotic pasture and the native forest (see Table 3). No earthworms were recovered from the present cultivated site when sampled, whereas the pasture had a very high popu-

### Table 3

**Differences in topsoil respiratory activity and bacterial populations of the same soil type under different plant regimes on Patumahoe clay loam, near Pukekohe (37° 13' S 174° 53' E).**

<table>
<thead>
<tr>
<th></th>
<th>Cultivated Soil</th>
<th>Pasture Soil (Established pasture)</th>
<th>Forest Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>5·9</td>
<td>5·5</td>
<td>5·1</td>
</tr>
<tr>
<td><strong>% Moisture (d.w.)</strong></td>
<td>28</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td><strong>% Organic C</strong></td>
<td>2·6</td>
<td>6·0</td>
<td>7·2</td>
</tr>
</tbody>
</table>

| Mean basal rate     | 131 (0·71)      | 69 (0·82)                         | 95 (0·71)   |
| Zymogenic response  | 173 (0·71)      | 97 (1·23)                         | 103 (0·89)  |

<table>
<thead>
<tr>
<th>Bacterial Population</th>
<th>Total plate count</th>
<th>Fermenters</th>
<th>Fermenters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \times 10^6/g ) wet wt.</td>
<td>( \times 10^3/g ) wet wt.</td>
<td>( \times 10^3/g ) wet wt.</td>
</tr>
<tr>
<td>Cultivated Soil</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Pasture Soil</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Forest Soil</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
lation. This difference is associated with a difference in the return of organic matter to the soil which is of the order of several thousand kg/ha/year under forest or pasture but only several hundred for the cultivated soil. There is less difference in the size of the bacterial populations, though the composition differs.

Because of the lower return of organic matter, the cultivated soil has a lower organic content. However the mean basal respiratory rate calculated as $\mu l \text{CO}_2/g \text{ Org. C}$ is higher than the rate of both the forest soil and the pasture soil. None shows a great response to glucose although the pasture soil has subsequently a high R.Q.

**DISCUSSION AND CONCLUSIONS**

The data summarised in this paper will be published in greater detail elsewhere. The conclusions which emerge are, first, that the basal respiratory rate determined by Warburg measurements is only a partial measurement of the total soil respiration and that comparisons between different soils based simply on Warburg measurements are not necessarily valid comparisons of different rates of soil metabolism. Secondly, a possible explanation of this discrepancy may lie in differences in the magnitude of the zymogenic responses of different soils, and that differences in the zymogenic responses of different soils may reflect significant differences in their soil metabolism. Thirdly, that such differences in zymogenic response appear to be related in some cases, at least, to differences in the animal and microbial populations on the one hand and differences in the type and productivity of the vegetation on the other.

The differences in zymogenic response appear to be related, in some cases at least (a, b), to the quantity of fresh organic substrate added to the soil and the population of aerogenic fermenters, an obviously zymogenic population, does appear to be related in these cases to such differences as suggested by the high R.Q. In the artificial situation of whey irrigation (b), they appear to be of direct importance in the breakdown of the added lactose and, apart from the associated population of micropredators, appear to be the only population other than yeasts that is greatly stimulated.

In more natural situations however the position is more complex. It has been found that, in a New Zealand pasture soil, the weight of earthworms is directly related to pasture productivity and that for every 1000 kg dry matter herbage produced, there is an earthworm biomass of 170 kg wet weight (Waters 1955). The turnover of animal tissue in the soil contributes a very readily accessible source of microbial substrate—in the case of non-predator mortality—and since the seasonal fluctuations in soil animal biomass may be great this could be an important contribution to the overall zymogenic metabolism and also to the soil metabolism in general. It does appear that, in these experiments, the zymogenic response is most marked in those soils which have the largest populations of soil animals particularly earthworms, and it is least evident in soils which have low animal populations.

In the main, therefore, this data would support the views of Jenkinson
of a distinction between a rapid zymogenic response to freshly added plant material and a much slower turnover of older organic matter. It would seem that this difference is associated with qualitative as well as quantitative differences in the soil populations, both microbial and animal, and thus the data could be considered as supporting, generally, the classical thesis of Winogradsky (1949), and his distinction between an autochthonous population associated with soil organic matter and a zymogenous population associated with freshly incorporated nutrients.

ACKNOWLEDGEMENTS

We are indebted to Dr. R. H. Jackman, Grassland Division, D.S.I.R., J. D. Cowie and G. E. Orbell, of Soil Bureau, for assistance in collecting soil samples and for information relating to the soils sampled, and to L. C. Blakemore, of Soil Bureau, for chemical analyses.

REFERENCES

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SUMMARY

Two types of respiratory activity have been measured; respiration of unamended soil, called the basal respiratory rate, which may be inhibited by moisture deficiency or excess; and response to glucose, called the zymogenic response.

Experiments were carried out on grassland plots under different levels of productivity, the same soil under different types of vegetation and a whey irrigated pasture soil.

The basal respiratory rate does not appear to be directly related to the productivity of the soil and is not, therefore, a valid reflection of the total soil metabolism. It is related to the amount of the soil organic matter or available substrate, the kind of vegetation or organic cycle, and to the moisture regime to which the soil is subject.

There appear to be significant differences in the magnitude of the zymogenic response of different soils and it is suggested that such differences, which appear to be related to differences in animal, particularly earthworm, and microbial populations, may be important in assessing the metabolism of soil organic matter.
ORGANIC MATTER BY WARburg EXPERIMENTS

RÉSUMÉ

On a mesuré deux types d'activité respiratoires: respiration du sol à l'état brut, appelée régime de respiration de base, qui peut être inhibé par l'absence ou l'excès d'humidité, et la réponse à la glucose, appelée réponse zymogénique.

Des expériences ont été entreprises sur des parcelles de prairie sous différents niveaux de productivité, sur le même sol sous différents types de végétation et sur un sol de prairie irrigué au petit lait.

Le régime de respiration de base ne paraît pas être directement lié au rendement du sol et n'est pas, par conséquent, une image valable du métabolisme total du sol. Il est lié à la quantité de matière organique du sol ou des substrats disponibles, au type de végétation ou de cycle organique et au régime d'humidité auquel le sol est soumis.

Il semble qu'il existe des différences importantes dans l'amplitude de la réponse zymogénique des différents sols et on suggère que de telles différences, qui semblent être liées à des différences de populations animales, surtout le ver de terre, et microbiennes, puissent être importantes pour l'estimation du métabolisme de la matière organique du sol.

ZUSAMMENFASSUNG


Die folgenden Versuche sind durchgeführt worden: in Grasland-Parzellen mit verschiedener Ertragsfähigkeit, in denselben Boden aber unter verschiedenen Vegetationstypen und in molkenirrigiertem Weideboden.


Es bestehen bedeutende Unterschiede im Ausmass der Zymase-Reaktion der verschiedenen Böden und man kann annehmen, dass solche Verschiedenheiten von den Unterschieden in tierischer besonders Regenwurm- und mikrobiischer Besiedlung abhängen scheinen, und sehr wichtig sind, um den Stoffwechsel der organischen Substanz des Bodens zu bewerten.
MACRO- AND MICROMORPHOLOGICAL CHANGES IN TOPSOILS UNDER INTENSIFIED GRASSLAND MANAGEMENT

BERYL C. BARRATT

Soil Bureau, Department of Scientific and Industrial Research, Lower Hutt, New Zealand

The soils of temperate latitudes have been changing ever since their native forest cover was first removed. These changes have been accelerated in recent years, however, owing to intensified grazing and hoof action, the use of chemical fertilisers, heavy machinery, herbicides and other treatments.

Topsoils are generally the first parts of soil profiles to respond to changes in land use. Their detailed morphological study, with the aid of the microscope, is a means of assessing the effects of differences in land use. Until recently, however, detailed comparisons have been handicapped by the absence of suitable nomenclature for either humus forms under grassland, or their microfabrics.

The nomenclature of both humus forms and their microfabrics has now been revised (Barratt, 1964 and in prep.). In this paper it is applied to a characteristic sequence of topsoils, developed under native forests and pastures in New Zealand and in England.

THE REVISED NOMENCLATURE

Classification of Humus Forms

Humus forms may be defined as the group of O and A horizons in which organic matter is concentrated. They are divisible into two main groups: mors and mulls.

In mors, organic residues accumulate, predominantly above the soil surface, but show progressive decomposition with increasing proximity to the soil. This is reflected in the development of L, F and H horizons, which may be equated with the O1, upper O2 and lower O2 horizons respectively of Taylor and Pohlen (1962). In mulls, most organic residues are comminuted to colloidal size and are intimately mixed with clay. L and F horizons tend to be thin and there is no true H horizon. Mulls and mors are subdivided, mainly according to horizon thickness and structure. Strongly and weakly granular, massive, fine (i.e. finely granular) and laminated mulls are recognised, and granular, massive and laminated mors. Root matted variants of these humus forms occur, as well as intergrades such as lenticular mull and nullised mor, in which originally leafy, laminated horizons appear to have been disturbed. Composite intergrades also occur, including mors over mulls. A series of intergrades is recognised, termed...
mor-like mulls. These are mulls, very high in organic matter and low in clay, that are difficult to distinguish from mors in the field. It is possible, however, to determine their nature microscopically. Mor-like mulls are subdivided into the same structural subclasses as mulls.

Peat develops by the accumulation of organic residues under wet conditions to form virtually the entire soil profile. It is generally distinguished from mor by its great thickness.

Classification of Microfabrics

Each horizon of a humus form may contain one or more different microfabrics. These microfabrics have recently been reclassified (Barratt, in prep.).

Five main classes of microfabric are recognised: lithiskels, consisting essentially of mineral particles of sand and silt size; humiskels, of undecomposed organic matter; humicols, of strongly decomposed organic matter; mullicols, of intimately associated clay and organic colloid; and argillicols, of clay-sized mineral particles.

The five classes are subdivided, where necessary, according to the kinds of components they contain into: lignic (woody), mycetic (fungal), silicic (with quartz grains), calcitic with calcite or dolomite grains), feldspathic (with feldspar grains) etc. Additional names are coined by suffixing an appropriate word root by -ic.

The classes are subdivided according to microstructure into very coarse to very fine size subclasses based upon U.S. D.A. particle size gradings for sand (Soil Survey Staff, 1951), with the addition of an extremely fine size subclass for silt-sized particles. They are also subdivided into massive, single grain, blocky, pelleted, vesicular and expanded shape subclasses, which permit the addition of new descriptive terms when required.

Application of the Revised Nomenclature

The revised classifications are applied to topsoil sequences under forest and under pasture, on some New Zealand southern and central yellow-brown earths (moderately and strongly enleached fulvic soils), on English brown earths and on related soils as follows.

Humus Forms of Soils under Native Forest

Humus forms developed under the native dicotylous and podocarp-dicotylous forests of New Zealand include strongly granular mulls and laminated mors over mulls (Fig. 1a and Barratt, 1968). It is also believed, from unpublished observations, that corresponding variations in humus form occur under deciduous forests in England. Despite great differences in their litter horizons, however, the forest humus forms resemble each other in their A₄ horizons, all of which appear to have strongly granular structure, high porosity and mullicol microfabrics that contain abundant plant remains, i.e. mullicols with folic or lignic humiskels (Fig. 1g).

Marked differences are seen in the humus forms where native forests have been replaced by pasture. The humus forms under pasture also differ according to whether or not the soils are topdressed with lime and fertilisers.
whether soil macrofauna including certain kinds of earthworm are present or absent and according to the levels of grazing and hoof action experienced.

Fig. 1.—Humus forms and microfabrics developed in yellow-brown earths, brown earths and related soils under native forest and grassland, arranged in a sequence according to intensity of grassland management.
Humus Forms under Untopdressed Pastures without Topsoil-Casting Earthworms

In Great Britain, extensive areas of unttopdressed rough pasture occupy the uplands and moorlands which are situated mainly in the north and west of the country. These areas have been lightly grazed for centuries without topdressing and in many places carry as few as one sheep to four or more acres (Ellison, 1953). Where the pastures are grown on brown earths of low base status, they are characteristically dominated by fibrous shallow rooting grasses such as *Agrostis* spp. (browntop or bent), and *Nardus stricta* (matgrass).

Crompton (1953) has drawn attention to the development, in these grassland topsoils of matted conditions which have been examined in more detail by Barratt (1960). The characteristic humus form is a laminated mor (Fig. 1b). It consists of three distinct organic horizons which reflect increasing decomposition of plant residues from the surface, downwards, as follows:

- $O_1$ or *L* horizons are leafy, laminated, with little evidence of decomposition and their characteristic microfabric is a coarse folic humiskel (Fig. 1h).
- $O_2$ or *F* horizons resemble *L* horizons but tend to be softened and stained, with associated fungal mycelium. Their characteristic microfabric is a folic humiskel, mycetic phase.
- $O_2$ or *H* horizons consist of strongly decomposed and finely comminuted material which is unrecognisable to the unaided eye. They generally contain mesofauna such as mites and Collenbola which produce very fine faecal pellets and pelleted humicol microfabrics (Figure 1j).

In brown earth profiles the laminated mors are in most places underlain by weakly granular mulls, with microfabrics that are intergrades between mullicols and argillicols (Fig. 1m).

In New Zealand where the practice of aerial topdressing reaches even into steep hill country, unttopdressed introduced pastures are now difficult to locate. Peaty mors have been reported under low-producing pastures of *paspalum*, browntop or Yorkshire fog (Taylor, 1954), but now even the fibrous, sod-bound condition which he describes on somewhat better pastures has a limited distribution. It is probably represented by thin, leafy and matted and by finely granular mulls, developed, respectively, in yellow-brown earths such as Wingate silt loam at Taita Experimental Station and in Korokoro silt loam (Barratt, 1965). In each of the examples cited, the dominant microfabric is a pelleted mullicol (Fig. 1j).

Humus forms exhibiting some accumulation of organic residues at the soil surface are also found under native tussock grasslands and under subsequent unttopdressed pastures on yellow-brown earths such as the Waikawhi and Craigieburn silt loams (Barratt, 1968). These humus forms are laminated fine mulls (Fig. 1c) with *L + F* horizons an inch or more in thickness, containing loose, relatively undecomposed leafy residues and humiskel microfabrics. These horizons are underlain by $A_1$ horizons with finely granular structure and pelleted mullicol microfabrics.
In England, soils with laminated mors under pasture are generally deficient in earthworms, even where there is pedological evidence that earthworms were previously more active (Crompton, 1953). Two species of earthworms that have been shown to disappear with progressive mat formation are *Lumbricus terrestris* L. and *Allobophora caliginosa* (Wood, 1966); and in New Zealand pastures where soil conditions are appropriate but where exotic earthworms are still absent from the district, the mat has been destroyed experimentally, by introducing *A. caliginosa* (Stockdill, 1966). These species are known to be active in the formation, in the topsoil, of burrows and casts (Guild, 1955), which are prominent features of granular mulls.

Where leafy, root matted or finely granular mulls occur, as in the Taita and Wingate soils, soil fauna appear active enough to prevent accumulation of organic residues at the soil surface to form laminated mors. However, they seem insufficiently active to incorporate organic matter to appreciable depths. Instead, it is mixed intimately with clay in discrete faecal pellets, to form thin, dark topsoils with pelleted mullicol microfabrics.

In most of the examples examined, the fauna producing this pelleted microfabric appears to consist mostly of small earthworms.

**Humus Forms under Topdressed Pastures without Topsoil-Casting Earthworms**

Laminated mors have developed in two English experimental hayfields (Barratt, 1960), which for up to 100 years have received topdressings that include acid ammonium sulphate. A similar result has also been reported in some experimental plots on a New Zealand golf course (Metson and Gibbs, 1946). In these examples, mor development probably results from the unfavourable effect of extreme acidity on earthworms, which has been demonstrated by Satchell (1955). Other treatments such as herbicides and insecticides, which happen also to be toxic to earthworms might therefore be expected to give a similar result (Van Rhee, 1963; Kelsey and Arlidge, 1968).

Laminated mors and related mull-mor intergrades also occur under topdressed pastures where earthworms appear to be limited by soil physical conditions. They are found, for example, in some areas of Taupo sandy loam and in related yellow-brown pumice soils (R. L. Nielson, pers. comm.), although in Taupo sandy silt a pH level as high as 5.5 has been recorded in the topsoil, even under fernland and scrub. The limiting soil factors are unknown, but they are unlikely to be specific to earthworms, since, according to Lee (N.Z. Soil Bureau, in prep.), no soil fauna are present in the pumice soil at a depth of only 4 in. from the soil surface.

In Northern England, laminated mors have also been discovered in soils of heavy texture sown down to pasture after open cast coal mining operations.
Humus Forms under Untopdressed Pastures with Topsoil-Casting Earthworms

When topsoils under untopdressed pastures and containing topsoil casting earthworms are compared with topsoils under native forest, three general trends are observed:

1. The amount of organic matter concentrated at the soil surface is decreased;
2. The strength of granular structure development is decreased;
3. Interpedal porosity is decreased.

In addition, the sward is characteristically thin with a weakly branched rooting system. Stock carrying capacity tends to be low, and a low population of soil fauna, including topsoil casting earthworms, is supported.

The characteristic humus form is a weakly to moderately granular mull (Fig. 1d). Its A_1 horizons contain microfabrics that are mullical-argillicol intergrades (Fig. 1m), demonstrating that the organic regime is rather weakly impressed upon the soil.

Similar humus forms have also developed in untopdressed long term experimental hayfields after repeated cropping (Barratt, 1960).

Humus Forms under Topdressed Pastures with Topsoil-Casting Earthworms

With lime and phosphate topdressing, topsoils tend to become darker, thicker—penetrating deeply into B horizons along earthworm channels—and to possess more strongly granular structures and higher porosities than untopdressed topsoils. They support stronger growths of herbage, with more strongly branched rooting systems, larger numbers of grazing stock and larger numbers of soil fauna, including earthworms.

The characteristic humus form is a strongly granular mull (Fig. 1e) with a vesicular mullicol microfabric (Fig. 1k), consisting of abundant earthworm casts that contain very fine, intimately associated particles of organic and mineral matter. Similar humus forms have also developed in response to topdressings of lime and basic slag in long term experimental hayfields (Barratt, 1960).

Humus Forms under Topdressed Pastures with Topsoil-Casting Earthworms and Intensive Hoof Action

Although both granular soil structure and soil porosity increase after topdressing, they decrease again at the surface of soils under intensive hoof action, particularly after the soil has become thoroughly wet (Gradwell, 1960).

The characteristic humus form is a composite, platy over granular mull (Fig. 1f) and platiness at the surface is reflected in a platy or blocky mullicol microfabric (Fig. 11). It should also be noted that platy structure has also developed in cropped soils in Eire in response to treatment with herbicides (Bulfin, 1966), and after longterm cultivation of brown granular loams in market gardens at Pupekohe, New Zealand. In the examples quoted,
platy structure under pasture appears to result from the destruction of cast granular structure by hoof action, but platy structure in the cropped soils could reflect a decrease in soil faunal activity as a result of chemical treatments.

**Conclusions**

The revised classifications provide a means of assessing changes in topsoils that occur with intensified land use.

Under deciduous forests on brown earths and under broadleaf or broadleaf-podocarp forest and native tussock grasses on yellow-brown earths, accumulations of plant residues towards the soil surfaces are demonstrated. These are brought about by regular additions of litter to the soil surface, and the accumulations are regarded as normal.

Under pasture, however, litter accumulations leading to the development of laminated mor appear to be both undesirable and avoidable. Mors under pasture can be converted to mulls by topdressing but only, it appears, in the presence of topsoil casting earthworms. Very acid fertilisers, which eliminate earthworms, promote the development of laminated mor. Topdressing pastures with lime or basic slag in the presence of topsoil casting earthworms greatly improves soil structure, promoting the development of strongly granular mull. The structural improvement associated with topdressing is, however, likely to be offset in the future by more intensive grazing and hoof action, promoting the development of platy structures towards the soil surface. This poses a further management problem.

**References**


Crompton, E. (1953)—Agriculture, Lond. 60, 308-308.


Revised classifications of humus forms and their characteristic horizon microfabrics are used to assess morphologically the sequence of changes that occur in response to increasingly intensified grassland management, in English brown earths, New Zealand fulvic soils (yellow-brown earths) and closely related soils.

Under native forests, humus forms include strongly granular mulls and laminated mors over mulls but their A horizons are alike in their porous, strongly granular structures and mullicol, folic or lignic humiskel microfabric complexes.

Under pastures, in the absence of topsoil casting earthworms, laminated mors develop, with humiskel and pelleted humicol microfabrics, but where such earthworms are present, mors are replaced by mulls.

Mulls under untopdressed pastures of low nutrient status are weakly granular, with mullicol-argillicol microfabrics, but under topdressed pastures, mulls are thick, and strongly granular, with vesicular mullicol microfabrics.

Under puddled pasture land produced by intensive animal treading, mulls develop platy structures towards their surfaces, with platy mullicol microfabrics.

Résumé
Des classifications révisées de formes d’humus et leurs microstructures d’horizon caractéristiques sont employées pour évaluer morphologiquement la séquence de changements qui ont lieu comme résultat d’une régie intensifiée de prairie dans les sols bruns en Angleterre, des sols fulviques en Nouvelle-Zélande (sols bruns-jaunâtres) et les sols apparentés de prés.

Sous les forêts indigènes, les formes d’humus comprennent des mulls fortement granulaires et des mors stratifiés au dessus des mulls, mais leurs horizons A se ressemblent dans leurs structures poreuses, fortement granulaires et dans leurs microstructures mullicoles, foliques ou ligniques humiskeles.

Sous les pâturages, dans l'absence de vers de terre pour produire une couche arable, des mors stratifiés se développent avec humiskel et des microstructures humicoles en boules, mais dans les endroits où il y a des vers de terre, les mors sont remplacés par des mulls.

Les mulls sous des pâturages sans fumure de surface et d’un niveau nutritif bas sont faiblement granulaires, et de microstructures mullicoles vesciculaires.

Sous des pâturages compactés produits par le piétinement intensif d’animaux les mulls développent des structures feuilletées vers leurs surfaces, avec des microstructures mullicoles feuilletées.

Zusammenfassung
Verbesserte Klassifizierungen der Humusformen und ihrer charakteristischen Horizont-Mikrogefüge sind benutzt worden, um die Reihenfolge der Veränderungen einzuschätzen, die infolge von vermehrter intensivierter
Weidewirtschaft in englischen Braunerden, Neuseeland-Fulvosaure-Böden und eng damit verwandten Böden auftreten.

Unter einheimischen Wäldern treten stark gekörnte (granulierte) "Mulle" in Humusformen und blättriger "Mor" (Auflegehumus) über den "Mullen" auf—aber ihre A-Horizonte sind ähnlich in ihren porösen, stark gekörnten Strukturen und ihren "mullicol", folisch oder lignisch "humiskel" Mikrogefügen.

Unter Weiden entwickelt sich bei Abwesenheit von Regenwürmern blättrige "Mor" mit "Humiskel" und gekörnten "humicol" Mikrogefügen. Aber da, wo solche Regenwürmer vorhanden sind, sind "Mulle" anstelle von "Mor" vertreten.

"Mulle" unter angedüngten Weiden in niedrigem Nährstoffzustand sind schwach gekörnt (granuliert) mit "mullicol-argillicol" Mikrogefügen; aber unter gedüngten Weiden sind die "Mulle" dick und stark granuliert mit vesikulären "mullicol" Mikrogefügen.

Unter matschigem Weideland, hervorgerufen durch intensives Betrampeln von Tieren, entwickeln "Mulle" blättrige Strukturen zur Oberfläche hin mit blättrigen "mullicol" Mikrogefügen.
In the boreal forest zone of Eurasia among major soil types—podzolic soil, soddy-calcareous soil (rendzina), grey and brown forest soils, taiga frozen soils and others—besides "normal" humus profiles, there are developed, under distinct ecological conditions, soils with two characteristic maxima of humus accumulation in the profile, the first maximum at the surface, the other at some depth, in the middle of the profile or lower.

The formation of the second humus horizon may take place in different ways. In some cases it is formed by infiltration of organic matter or by transfer of organomineral compounds from the first humus horizon, in others it is formed by the preservation of stable humus components at a certain depth—the remains of a thicker humus horizon or even a peculiar burial of the humus stratum.

Soils with two humus horizons have only occasionally been studied by pedologists, e.g. in determining diagnostic features of systematic units within some types of forest soils. However, soils with complex humus horizons may be of great interest from genetic, geochemical and paleogeographical points of view. These soil investigations provide an opportunity for better understanding the principles of the process of humus formation and of the interaction of organic and mineral substances in the soil profile. In the study of geochemical problems of organic matter the process occurring in soils with a complex humus profile should also be considered. The inventory of organic and organomineral compounds of soils, their fixing and concentration in the upper humus horizon, and in the lower one, and also their migration out of the soil profile are of great theoretical and practical importance. Owing to their specific nature, second humus horizons may be of some importance in the migration of chemical elements, particularly the rare metals. Biological turnover of substances in a forest growing on soils with an active humus illuvial process will be characterized by several peculiarities. It is essential to determine the distribution of areas of soil with relic humus horizons in order to solve a number of paleogeographic problems and to study the history of development of the soil covering individual areas.

In agricultural production, soils with complex humus profiles, due to their specific features, require the elaboration of special agricultural practices. For soils characterized by great mobility of the organic compounds
taking part in humus migration from the soil surface to the second humus horizon it is essential to develop methods of artificially fixing organic matter in the arable layer.

Among forest soils with complex humus profiles, podzolic soils with illuvial humus horizons and illuvial humus ferruginous horizons are well known and most widespread. These soils are mainly characterized by light mechanical composition—sandy and sandy loam soils; with heavier mechanical composition the formation of the second horizon under other suitable conditions takes place only where the soil substratum is stony or in the presence of sandy inclusions. These soils are widespread in forest-tundra and in northern taiga. There soil formation is favoured by the fulvoacidic nature of the humus substances, their high removal rate, mobility of sesquioxides, and excessive soil moisture. Permafrost also promotes the illuvial-humus process, but in the taiga of Central Siberia it is weakly developed due to the dry climate of the region.

Illuvial humus soil genesis is reported in the works of Ivanova and Polynseyeva (1936) and of Ponomareva (1951). Both papers deal with soils of the Kola Peninsula and Northern Karelia. Kreida (1962) notes that in the broken ground of the Kola Peninsula illuvial humus podzols at the bottom of slopes with excessive moisture are replaced by illuvial humus ferruginous podzols on drier, elevated land. The occurrence of illuvial humus ferruginous podzols increases in the direction from northern forest-tundra to the southern one and then in the northern taiga subzone. In southern regions of the forest zone, soils with an illuvial humus horizon may be found everywhere up to the forest-steppe zone, but they are especially widespread in the more humid western and extreme eastern soil provinces. In the ecological series of soils on elevated areas of light mechanical composition these soils are always considered to be extremely "wet". They often surround high bogs and transitional swamps with soft ground water. Their formation is characterized by the presence of soft ground water, regular profile re-moistening and sharp variations of oxidation-reduction potential. There is a certain relation between the depth of the upper boundary of the soil saturation zone and the distance of migration of organic matter within the profile. Illuvial humus horizons with the deepest location (60-70 cm from the surface) are typical of soils of the south-western provinces of the forest zone. There may be found also soils with humus illuviation still lower down the profile; in such profiles the accumulation is not the direct result of the illuvial humus process, but is due to the presence of impermeable layers obviously blocking migration of organic matter through the soil mantle. As a rule, illuvial humus horizons in the north and in the south of the forest zone are characterized by their division into two sub-horizons: the upper one with maximum concentrations of alumina and organic matter and the lower one with a predominance of ferric oxide. Where humus horizon formation occurs in the lower part of the soil profile the elementary composition of horizons is very unsteady because of a compact ground layer, permafrost or other mechanical factors.
Table 1 gives some elementary analytical data, characterizing soils with a developed illuvial-humus process. Profile 35—north taiga peaty humus podzolic sandy loam soil, taken from the north of the Arkhangelsk region. The accumulation of organic matter caused by humus tongues takes place in this soil at the upper boundary of lime detritus, not deeply deposited in subsoils. Profile 3 is a typical strongly podzolic illuvial humus ferruginous soil, formed under spruce forest with *Hylocomium, Dicranum* and *Rhytidiadelphus* on red coarse boulder loam of the Devonian plateau in the Leningrad region.

Profile 10 is a soddy strongly podzolic soil from the Briansk region, with illuvial humus and ferruginous horizons on ancient alluvial sands, closely underlain by cretaceous red sands. The vegetation is light mixed coniferous and broad-leaved forest with gramineous cover (thick turf).

In forests of the southern provinces, humus illuvial horizons are often of a relic nature, are not active and contain relatively inert organic matter. The illuvial humus process is inactive at present. Table 5 gives a group composition of organic matter of various kinds of illuvial humus soils.

In soddy-calcareous soils, stony soils in particular, irrespective of their provincial peculiarities in the development of leaching and podzolization, the formation of a humus illuvial horizon is often observed. The second humus maximum accompanies the illuvial clay red-brown horizon. Migration and accumulation of humus takes place in weakly leached soils at a neutral reaction of the medium. With further progress of carbonate leaching the red-brown horizon of clay and humus accumulation moves down the profile, probably in the form of organomineral compounds, following the descent of the upper boundary of effervescence. The accumulation of humus in the illuvial horizon of soddy-calcareous soils will never be equivalent to the quantity in podzolic illuvial humus soils, the process of illuviation in soddy-calcareous soils being quite different: it is connected with lessivage. The peculiarities of soddy-calcareous soils characterized by the presence of the second humus horizon are described in the works of Ponomareva and Miasnikova (1954) and Gagarina and Khantulev (1961).

Table 2 gives analytical data on three soddy-calcareous soils of the Izhora hills in the Leningrad region, characterized by various rates of leaching and podzolization (profiles 2, 3, 4). Profile 28 is a soddy-calcareous podzolized soil of the northern taiga subzone in the Arkhangelsk region.

Among frozen taiga soils of the plain and mountainous region of Eastern Siberia there may be observed soils characterized by the presence of a second humus horizon over a permafrost layer. But they are less widespread, as may be expected, considering the general character of bioclimatic features there. The absence of soil leaching ensures the accumulation of exchangeable bases and neutralizing of reaction. Only very acidic and skeletal soils, e.g. under thick moss cover, possess a more or less formed illuvial humus horizon over a permafrost layer. Its organic matter content of fulvo-acidic nature may reach 3-4%. The illuvial humus horizon is located either on a permafrost layer directly or, if there is water overlying the permafrost layer, it occurs above both the soil layer at 0°C tem-
<table>
<thead>
<tr>
<th>Profile No.</th>
<th>Genetic horizon</th>
<th>Depth cm</th>
<th>pH water</th>
<th>pH salt</th>
<th>Exchangeable acidity by Sokolov, m-equiv./100g</th>
<th>Absorbed bases, m-equiv./100g</th>
<th>Content of particles with diameter (µm)</th>
<th>Total soil composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0—4</td>
<td>5.1</td>
<td>4.5</td>
<td>77.6*</td>
<td>3.3</td>
<td>38.5</td>
<td>22.9/100</td>
<td>35</td>
</tr>
<tr>
<td>A&lt;sub&gt;T&lt;/sub&gt;</td>
<td>4—14</td>
<td>5.2</td>
<td>4.4</td>
<td>75.1*</td>
<td>0.7</td>
<td>36.1</td>
<td>27/100</td>
<td>35</td>
</tr>
<tr>
<td>A&lt;sub&gt;T&lt;/sub&gt;</td>
<td>14—18</td>
<td>5.4</td>
<td>4.6</td>
<td>40.8*</td>
<td>0.7</td>
<td>35.3</td>
<td>26.2/100</td>
<td>35</td>
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<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>18—24</td>
<td>5.3</td>
<td>4.3</td>
<td>0.5</td>
<td>—</td>
<td>2.6</td>
<td>3.6/100</td>
<td>35</td>
</tr>
<tr>
<td>B</td>
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<td>6.6</td>
<td>5.0</td>
<td>4.4</td>
<td>—</td>
<td>31.8</td>
<td>17.4/100</td>
<td>35</td>
</tr>
<tr>
<td>C</td>
<td>40—50</td>
<td>7.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—/100</td>
<td>35</td>
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<td>0—4</td>
<td>4.1</td>
<td>3.9</td>
<td>—</td>
<td>31.1</td>
<td>8.2</td>
<td>4.6/100</td>
<td>78</td>
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<td>3—9</td>
<td>3.9</td>
<td>3.4</td>
<td>—</td>
<td>30.4</td>
<td>6.7</td>
<td>5.0/3.9</td>
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<td>4—16</td>
<td>3.9</td>
<td>3.5</td>
<td>1.7</td>
<td>1.0</td>
<td>0.9</td>
<td>0.04</td>
<td>0.6</td>
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<td>0.04</td>
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<td>4.5</td>
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<td>0.2</td>
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<td>5.0</td>
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<td>0.1</td>
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<td>30/50</td>
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<td>0.2</td>
<td>—</td>
<td>—</td>
<td>—/100</td>
<td>12</td>
</tr>
<tr>
<td>C</td>
<td>100—117</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—/100</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 1: Podzolic Soils with a Second Humus Horizon.
TABLE 1 (Continued)

I'll
Profile
No.

Genetic
horizon

Depth
cm

water

salt

Humus
by
Tiurin,

"/

10

A0At
AiA 2
A2
B
B,
B,

c,
C,
* Losses by ignition.

0—4
4 S
10—20
27—37
40—50
58—68
80—90
110—120

4-5
4-7
51
5-6
5-6
5-7
5-5
5-5

3-6
3-8
4-2
41
4 6
4-7
4-3
4-3

0

7-5
2-3
0 3
1-4
1-4
0-3

—
—

Exchangeable
acidity
by Sok olov,
m-equi\ /100g
Al
H
0-3
01
002
004
002
002
002
004

1-3
10
0-4
1-1
0-5
0-3
0-3
0-2

Absorbed
bases,
m-equiv/100g
Ca
1-8
0-9
0-4
10
10
0-8
2-6
2-2

Mg
10
0 6
0-4
0-8
0-7
0-5
0 9
0-6

Content of
particl :s
with
diameter (%)
< V < 10/i
10
6
2
5
4
3
9
8

17
15
7
9
S
7
II
10

Total soil
composition

%
SiOz
87
94
94
92
91
92
90
91

=
-Z

RjO,
5
4
4
6
7
6
7
6

s

-r

Z
X

X
-

N

0
Z

-


# Table 2

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<tr>
<th>Profile No.</th>
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<th>Depth cm</th>
<th>pH water</th>
<th>pH salt</th>
<th>Humus by Tiurin, %</th>
<th>absorbed Ca + Mg m-equiv/100g</th>
<th>% saturation ( CLY (%))</th>
<th>Clay (CLY)</th>
<th>Composition of Soil</th>
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<td>78</td>
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<td>R₂O₃</td>
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<td>6.0</td>
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<td>Depth cm</td>
<td>( pH )</td>
<td>( pH ) water</td>
<td>( pH ) salt</td>
<td>Humus by Tuurin %</td>
<td>Absorbed Ca m-equiv/100g</td>
<td>Absorbed Mg m-equiv/100g</td>
<td>% saturation (% sat.)</td>
<td>Clay composite Soil Clay fraction %</td>
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<tr>
<td>------------</td>
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<td>76</td>
<td>5</td>
<td>82</td>
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*Table 2 (Continued)*
perature and the zone of complete saturation. In the latter case illuvial humus frozen soils resemble the typical illuvial humus subzones mentioned above: they mainly differ in weaker podzolization of the frozen soils.

Soils with humus illuvial horizons under the permafrost layer are found rarely among grey forest soils or even among frozen meadow chernozemic soils. Such horizons are of a relic nature, their humus having a peculiar composition, and they are often located at a depth of 1.5-2 metres below the permafrost layer. In the Amur basin and to the south from it there occur peculiar soils, termed forest and meadow podbels. These soils are characterized by a greatly developed process of lessivage. It has resulted in thick illuvial clay horizons, characterized by considerable organic matter accumulation.

Table 3 gives some analytical data, characterizing soils of Siberia having the second humus horizon. Profile 55—a mountain frozen-taiga podzolic soil of the Borschevochny mountain ridge in the Transbaikalian area; profile 79—a grey forest non-podzolized soil also from the Transbaikalian area; and profile 63—forest podbel from Southern Primorje.

Quite another genesis is typical of the soils with second humus horizons which are described below. In the Eastern European plains at the boundary of forest and forest-steppe zones of Eurasia, especially in "opolie"—islands of black earth beyond the northern forest-steppe boundary—there is an occurrence of grey forest soils, distinguished by heterogeneity of the humus profile. To the west of the Gorky meridian in Vladimir, Meshchovsk and Briansk opolies ("islands") and others to the west, soils with a dark and thick humus profile are quite usual; they are considered to be dark grey forest soils and even chernozem soils. Their humus profile consists of two layers: a lighter upper layer 20-30 cm thick and a darker layer directly under the upper one. From the ecological point of view the presence of such soils beyond the northern forest-steppe boundary is a considerable departure from principles of zonality and does not correspond to modern natural conditions in this area of the forest soils.

The investigations show that these thick layers of black earth have a polygenetic profile, composed of genetically unrelated horizons, and that the upper humus horizon belongs to the modern light grey soil, while the lower horizon is relic and belongs to late glacial black meadow or chernozem soil. Soils with a polygenetic profile may be illustrated by profile 8 from the Briansk opolic ("islands") (Table 4).

The rolling plains of Povolzhje and Priuralje are characterized by extremely peculiar soddy-podzolic and grey forest soils with a dark horizon below the characteristic podzolic horizon. They are found even in Vladimir opolic, and they are more widespread to the east. The area of their distribution goes from the Urals to the Enisei river as a wide strip across the Western Siberian plain. In Siberia they are known as secondary podzolic soils.

Table 4 gives some data on these soils (profiles 6 and 7), for samples taken from the Kirov region. Though the soils have been formed in a coniferous forest with a moss cover they are regarded as a subtype of
### Table 3
**FROZEN AND LATE THAWING SOILS WITH ILLUVIAL HUMUS HORIZON**

<table>
<thead>
<tr>
<th>Profile No.</th>
<th>Depth cm</th>
<th>pH</th>
<th>Humus Exchangeable bases</th>
<th>% removal</th>
<th>Clay removal</th>
<th>Total composition</th>
<th>SiO₂</th>
<th>R₂O₃</th>
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- **Humus Exchangeable bases:**
  - Tiurin, %
  - Ca, m-equiv 100g-
  - Mg

- **% removal:**
  - Ca, %
  - Mg, %

- **Clay removal (μA):**
  - %

- **Total composition of ignited soil:**
  - SiO₂
  - R₂O₃

**Notes:**
- **SiO₂:**
- **R₂O₃:**

**Permafrost Zone:**
- **2-4:**
- **6-12:**
- **20-30:**
- **40-50:**
- **70-80:**
- **100-110:**
- **120-130:**
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<th>( \text{pH} )</th>
<th>Water-soluble salts</th>
<th>Absorbed bases, m-equiv/100g</th>
<th>Hydrolytic acidity, m-equiv/100g</th>
<th>Content of particles with diameter &lt;1μ</th>
<th>&lt;10μ</th>
<th>SiO₂ / %</th>
<th>Al₂O₃ / %</th>
<th>Fe₂O₃ / %</th>
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<td>( C )</td>
<td>Group content of ( C ) in ( % ) from the total content</td>
<td>( N )</td>
<td>Solution in alcohol-benzene</td>
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<td>Fulvic acid</td>
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<td>51.4</td>
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<td>11</td>
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<td>16.5</td>
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<td></td>
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<td>9</td>
<td>—</td>
<td>68.8</td>
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Table 5 (Continued)

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<th>C</th>
<th>N</th>
<th>Solution Extracts of NaOH</th>
<th>Extracts of Na$_2$P$_2$O$_5$</th>
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<td>3</td>
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<td>27.2</td>
<td>1.05</td>
<td>26</td>
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<td>A$_1$A$_2$</td>
<td>4—16</td>
<td>0.9</td>
<td>0.05</td>
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<td>6.8</td>
<td>3.1</td>
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<tr>
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<td>A$_2$</td>
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<td>11</td>
<td>9.7</td>
<td>24.0</td>
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<tr>
<td></td>
<td>B</td>
<td>25—39</td>
<td>2-3</td>
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</tr>
<tr>
<td></td>
<td>B</td>
<td>24—34</td>
<td>2-6</td>
<td>—</td>
<td>—</td>
<td>32-9</td>
<td>2-4</td>
<td>5-1</td>
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</table>
light grey forest soils, but not podzolic ones. Though the second humus horizon is distinguished by its dark colour, its humus percentage is no greater than up or down the soil profile. Certain regularities are observed in the distribution of soils with a second humus horizon according to topography: they are found on smooth non-erosive watershed divides and interfluvial plateaus. The productive capacity of soils with two humus horizons is greater than those with one such horizon; their plough layer is darker and more humified, and the harvest is greater.

Most scientists consider the soils described above to be of a secondary nature and their second humus horizon to be relic. Due to climatic changes a plant succession occurred; herbaceous plants were replaced by woody plants; this intensified podzolization and caused degradation of the black earth.

Quite opposite ideas however may be found in certain literature. Some scientists think that the formation of the second humus horizon may be connected with a modern process of humus migration, but the principles of this process are not clear.

We consider the second humus horizon of the soils described above to be relic. The group composition of the organic matter of the dark horizon is quite specific (Table 5) and proves its residual and metamorphic nature. The association of this horizon only with specific topographic features also proves its ancient origin.

Considering all the above, it is possible to conclude that soils with complex humus profiles are widespread in the boreal forest zone. The nature of the second humus horizon may be variable. It influences the productive value and fertility of various soils. There are several processes of soil formation with complex humus profiles. The second humus horizons may be active, of modern origin, or residual.

Further study of these soils is essential for theory and practice.

REFERENCES


Ponomareva, V. V. (1951)—To the knowledge of humus illuvial podzol formation process. Leningrad. Uchenye zapiski LGU, seria biologicheskaja. Vyp. 27.


SUMMARY

Under specific ecological conditions there occur in the forest zone, besides soils with “normal” humus profiles, soils with two maxima of humus accumulation.

The lower humus horizon is formed by the infiltration of mobile organic
matter, by the transfer of organomineral compounds or as the result of the preservation of stable humus components at a certain depth.

Results of physical and chemical analyses are given for soils with complex humus profiles from among podzols, soddy-calcareous types, frozen and late-thawing soils and grey forest soils. The implications with relation to the mode of formation of such profiles are discussed.

The investigation of soils with complex humus profiles makes for a better understanding of the principles of humus formation processes and of the interaction of organic matter and minerals in the soil profile. The determination of the areas of soils with two humus horizons of various origins is essential for solving geochemical, paleogeographical and paleopedological problems. Soils with two humus horizons should be specially treated in agricultural production.

RéSUMÉ
Dans des conditions écologiques spécifiques de la zone forestière, on trouve des sols ayant 2 maxima d’accumulation de l’humus, ainsi que des sols à profils d’humus “normaux”.

L’horizon humifère inférieur est formé par l’infiltration de matière organique mobile, par le déplacement de composés organo-minéraux, ou à la suite de la conservation de composés d’humus stabilisé à une certaine profondeur.

On fournit les résultats des analyses physiques et chimiques pour les sols ayant des profils d’humus complexes venant de sols podzoliques, calcaires-gazonnés, de sols gelés et qui dégèlent tard, et des sols gris forestiers. On discute aussi ce que ces résultats impliquent par rapport au mode de formation de tels profils.


ZUSAMMENFASSUNG
Bei bestimmten ökologischen Bedingungen, sind in den Waldzonen, außer Böden mit “normalen” Humusprofilen, Böden mit zwei Maximalen der Humusanspeicherung vorzufinden.

Der tiefere Humus Horizont wird durch Infiltration der beweglichen organischen Substanz gebildet, zu Folge des Überganges der organisch-mineralen Verbindungen, oder er ergibt sich aus der Erhaltung stabiler Humus Verbindungen in einer gewissen Tiefe.

Ergebnisse der physikalischen und chemischen Analysen sind für Böden mit komplizierten Humusprofilen aus Podsolen, schlammig-kalkigen
Typen, gefrorenen, und spät-tauenden Böden gegeben. Die Verwicklungen im Bezug auf die Art der Formierung derartiger Profile wurden besprochen.

INTRODUCTION

This paper reports studies on three aspects of organic matter formation and decomposition in pumice soils beneath Pinus radiata plantations in central North Island, New Zealand. They are the decomposition of litter, soil 'bleaching' and organic matter content of buried soils.

These forests have been planted in the last 30-40 years on uplands with a heath type natural vegetation. The first crop has grown remarkably quickly (Will, 1966) without any evidence of growth having been restricted by inadequate nutrition although appreciable quantities of nutrients are removed in logs as thinnings and at clearfelling at 30-35 years.

The soils are recent and have been developed on rhyolitic sands and gravels: their natural fertility is low and soil organic matter is the major constituent able to retain cycling nutrients and prevent severe leaching losses by the 50-60 in. annual rainfall. Cycling of nutrients is also greater than is usually found in coniferous forests (Will, 1959, 1964). While organic matter can provide nutrient retention capacity, agricultural experience has shown that a very stable form of organic matter can accumulate in these soils making considerable quantities of nutrients unavailable to plants (Jackman, 1964a, b).

DECOMPOSITION OF LITTER

Trees add organic material to the soil from parts that fall to the ground and small roots that decay. Nothing is known about root decomposition under P. radiata in New Zealand, but studies have shown that there can be an annual litterfall of up to 10,000 lb an acre. This is made up of a regular needlefall amounting to approximately 4,000 lb a year, plus a seasonal fall of pollen cones and in older stands intermittent falls of dead trees, branches and female cones, associated with storms (Will, 1959).

As the needlefall, which has the highest nutrient content and comprises the greater part of the litterfall in most years, is relatively constant and uniform over the soil surface, it undoubtedly has a large influence on processes of litter decomposition and the supply of organic matter to the soil.

In a recent study the decomposition of freshly fallen needles has been...
followed for 6½ years. Fifty-one bags made of 1 mm mesh nylon were each filled with 50g of air-dry needles and placed on the forest floor under a mature stand of trees. They were allowed to become incorporated in the litter layer and buried by subsequent litterfall. Three bags were lifted at each of 17 sampling times over the 6½ year period. Dry matter and N, P, K, Ca, Mg contents were determined.

The rate of dry matter loss and changes in nutrient composition are shown in Fig. 1. During the first three years the dry weight of litter in the bags dropped steadily to about one-third of that present at the start. During the same period some nutrient contents varied markedly; while K content dropped sharply, other nutrients were not lost to the same extent and the conservation of N during decomposition resulted in a steady rise in concentration as dry matter was lost.

After three years there was little further change in the quantity and chemical composition of the litter in the bags and it also showed no further signs of physical breakdown. In contrast the litter surrounding the bags

Fig. 1.—Changes in dry matter and nutrient content of Pinus radiata needle litter in nylon mesh bags on the forest floor.
became more and more fragmented, until after six years it consisted almost entirely of faecal pellets (Fig. 2). The size of the pellets and their absence in the litter bags suggest that later stages of decomposition depend on the activity of the larger soil fauna. Although after six years litter in the bags differed so greatly in appearance from that to which larger fauna had access, chemical compositions were very similar (Table 1).

![Fig. 2. - Pinus radiata litter after six years on forest floor. Left: natural conditions; Right: confined in nylon bag.](image)

This study suggests that the chemical composition of needle litter under *P. radiata* reaches equilibrium after about three years and thereafter changes little until it is incorporated in the *A*₁ horizon. While the chemical composition remains static, physical breakdown by the larger soil fauna continues until the litter is entirely in the form of faecal pellets. Observations have shown that the fauna which cause this breakdown also, in passing it through their gut, distribute it below the soil surface.

**Soil ‘Bleaching’**

Normally the *A*₁ horizon of a pumice soil under *P. radiata* is black due to the presence of organic matter, but some years ago it was noticed that patches of greyish-white soil occur within this horizon and sometimes patches merge to form a complete horizon of light grey soil just below the surface of the mineral soil (Fig. 3). Superficially the appearance is like the *A*₂ horizon of a podzol but investigations have shown that the light colour is due to loss of organic matter leaving the light coloured mineral particles exposed and to the presence of large masses of fungal hyphae.

This ‘bleached’ soil is held together by the hyphae and is very difficult to wet. A study with soil moisture blocks showed that re-wetting after rain
<table>
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<th>Within bag</th>
<th>Below bag</th>
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<td>0.99, 1.07, 1.20</td>
<td>1.12, 1.10, 1.25</td>
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<td>P</td>
<td>0.032, 0.032, 0.037</td>
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<td>0.04, 0.04, 0.04</td>
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<td>Ca</td>
<td>0.21, 0.38, 0.35</td>
<td>0.26, 0.38, 0.26</td>
<td>0.23, *, 0.33</td>
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<tr>
<td>Mg</td>
<td>0.04, 0.06, 0.05</td>
<td>0.05, 0.06, 0.05</td>
<td>0.04, *, 0.06</td>
</tr>
</tbody>
</table>

* Insufficient sample for full analysis.
is slow compared with normal soil (Will, 1962), and when crumbled onto water it will float. Not only is it difficult to wet but its moisture content is always lower than normal. On one occasion six pairs of samples were taken

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Fig. 3.—Pumice topsoil under Pinus radiata showing patches of 'bleaching' particularly on left hand side.

---

—one each from 'bleached' soil and from adjacent normal soil at approximately the same depth, 2-6 in. The six 'bleached' samples had an average moisture content of 17.5% (range 12.0-27.0%) and the normal soil samples an average of 48.3% (range 34.0-59.5%). These sandy pumice soils owe their high water holding capacity to the vesicular nature of the mineral particles and it seems that the presence of large masses of hyphae limit water movement to these storage sites.

Chemical changes that 'bleaching' brings are well illustrated by the comparisons in Table 2.

Such severe drops in nutrient content and nutrient retaining capacity stimulated interest in 'bleaching', so studies of the pattern and progress of bleaching are being made. Points of 'bleaching' first appear near the roots of young stands that have closed canopy and started to build up a litter layer. The extent of 'bleached' soil fluctuates during the life of a tree crop and in some stands at least is greatest when the tree canopy is dense and the litter layer deep. Subsequent thinning of the stand reduces litterfall and
# Table 2

**Chemical Analysis of 'Bleached' and Normal Pumice Topsoils, P. radiata Forest**

<table>
<thead>
<tr>
<th></th>
<th>Field Moisture Content %</th>
<th>C %</th>
<th>N %</th>
<th>me. %</th>
<th>C.E.C.*</th>
<th>T.E.B.*</th>
<th>Citric Soluble P (ppm)</th>
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<td>Centre of 'bleached' zone</td>
<td>8</td>
<td>2.5</td>
<td>11</td>
<td>3.6</td>
<td>0.2</td>
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<td>240</td>
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<td>Margin of 'bleached' zone</td>
<td>11</td>
<td>4.5</td>
<td>17</td>
<td>6.7</td>
<td>1.7</td>
<td></td>
<td>310</td>
</tr>
<tr>
<td>Just outside 'bleached' zone</td>
<td>19</td>
<td>5.7</td>
<td>24</td>
<td>9.2</td>
<td>4.6</td>
<td></td>
<td>510</td>
</tr>
<tr>
<td>Normal black soil</td>
<td>23</td>
<td>6.9</td>
<td>38</td>
<td>11.2</td>
<td>7.2</td>
<td></td>
<td>530</td>
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</tbody>
</table>

* C.E.C. Cation Exchange Capacity; T.E.B. Total Exchangeable Bases.
promotes a more rapid litter breakdown. This apparently results in the rehumification of some ‘bleached’ areas.

After a crop of trees is clearfelled, fungal activity in the ‘bleached’ areas apparently ceases and increased activity by soil fauna results in their disappearance within 3 to 4 years. Within a short time the next tree crop reaches the age when points of new ‘bleaching’ may be seen in proximity to roots.

‘Bleaching’ has been found in plantations of other species of *Pinus* and Douglas fir but it never reaches the proportions seen under *P. radiata* forest. Soil ‘bleaching’ under a lone *P. radiata* tree in a plantation of *P. ponderosa* was found to be confined to the soil under the canopy spread of the *P. radiata* tree. Similar fungal activity has been found under *P. radiata* growing on coastal sands (Thornton et al., 1956): there the fungus was identified as a basidiomycete.

The association of ‘bleaching’ with roots (particularly those of *P. radiata*), the significant losses of nutrients, and the end of fungal activity when the trees are felled, all suggest that the fungal action is not that of a saprophyte but that it may well be the action of an efficient mycorrhizal fungus mobilising nutrients for tree use.

**Organic Matter Content of Buried Soils**

Volcanic activity has been intermittent in the central region of the North Island, New Zealand, for several thousand years, so that the present soil profile is made up of a series of aerially deposited ash beds, almost all of which are rhyolite. Some of the lower beds were once the surface long enough for vegetation to become established and a soil to be formed (Healy et al., 1964). Over large areas of the planted exotic forests tree roots are able to penetrate to and proliferate in one or more of these fossil soils which have been buried for many centuries.

The chemical composition of two such fossil soils, as they exist in the central part of Kaingaroa Forest (the upper one has been buried since at least A.D. 130), has been studied and some analyses are given in Table 3. These show that appreciable quantities of organic matter still remain although the soil is free draining at all times. Pot trials used to assess the quantities of nutrients available to *P. radiata* seedlings showed that in contrast to *K, Ca* and *Mg* negligible amounts of *N* and *P* can be taken up from the two buried soils. However *N* and *P* are available for plant growth from the present topsoil. The organic matter remaining in the buried soils seems of such a stable form that material kept for even four years in a glasshouse at higher temperatures and well watered, does not decompose to release *N* or *P*. The figures in Table 3 show that the *C/N* ratios are not unfavourable for the release of *N* but the greater part of the *P* present is in an organic form.

It is known that the main weathering product in these soils is allophane and that this can form stable complexes with organic matter (Jackman, 1964a). Therefore it is probable that the organic matter remaining in the buried soils consists almost entirely of such complexes. This raises the
<table>
<thead>
<tr>
<th>Depth Below Present Surface (ft)</th>
<th>C %</th>
<th>N %</th>
<th>C/N</th>
<th>Truog</th>
<th>(a) $N\text{H}_2\text{SO}_4$ (ignited)</th>
<th>(b) $N\text{H}_2\text{SO}_4$ (air dry)</th>
<th>Organic (difference) a-b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present topsoil</td>
<td>0.0-0.6</td>
<td>4.2</td>
<td>0.23</td>
<td>18</td>
<td>2.0</td>
<td>39.8</td>
<td>12.9</td>
</tr>
<tr>
<td>Buried topsoil I</td>
<td>5.5-6.5</td>
<td>0.8</td>
<td>0.06</td>
<td>13</td>
<td>0.02</td>
<td>17.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Buried topsoil II</td>
<td>7.5-8.5</td>
<td>1.6</td>
<td>0.09</td>
<td>18</td>
<td>0.01</td>
<td>22.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>

(a) Normal sulphuric acid extraction following ignition.
(b) Normal sulphuric acid extraction on air-dry sample.
question whether similar stable forms of organic matter will gradually accumulate in the present radiata pine-forest topsoil. Indications at present are that they will not because the investigations into soil 'bleaching' reported in the previous section show that fungi associated with *P. radiata* roots are able to break down any organic matter present almost completely, thus releasing the nutrients. However, it does seem that the buried soils, lying well below the surface as they do, are unsuitable environments for these fungi. It has been observed that mycorrhizal roots occur mainly in the top 6-12 in. of soil and are rarely found below 2-3 feet.

**REFERENCES**


Jackman, R. H. (1964b) — II. Rates of mineralisation of organic matter and the supply of available nutrients *ibid.*, 7, 472-479.


**SUMMARY**

The chemical composition of *Pinus radiata* needle litter reaches equilibrium after about three years on the forest floor. The final stages of physical breakdown and movement into the soil are dependent on ingestion by larger soil fauna but this does not cause any further changes in mineral content.

Intense fungal activity just below the surface of the mineral soil can result in 'bleached' areas from which the greater part of the organic matter and nutrient content have been removed. This condition may be due to efficient mycorrhizal action mobilising nutrients for tree use.

In fossil pumiceous soils lying several feet below the present surface no such fungal activity occurs and trees are apparently unable to extract any appreciable quantities of *N* or *P* from the very stable allophane-organic matter complexes that are present.

**RÉSUMÉ**

La composition chimique de la litière aiguilleuse forestière atteint son équilibre après avoir couvert pendant environ trois années la litière de la forêt. Les phases finales de décomposition physique et d'intégration dans le
sol dépendent de l’ingestion de plus larges faunes du sol, ceci toutefois sans amener d’autres changements de teneur en minéraux.

Une activité fongeuse intense juste en-dessous de la surface du sol minéral peut avoir comme résultat des zones "blanchies" desquelles ont été enlevées la plus grande partie de la matière organique et la teneur en substances nutritives. Cette condition peut résulter de l’action efficace du mycorhize en mobilisant les nutriments afin de les rendre utilisable par les arbres.

Dans les sols fossiles à ponce, à plusieurs mètres en-dessous de la surface actuelle, cette activité fongeuse ne se produit pas, et il semble que les arbres ne peuvent pas extraire de quantités importantes de N ou de P des complexes très stables de matière allophane-organique qui s’y trouvent.

**ZUSAMMENFASSUNG**


Intensive Pilzaktivität gerade unter der Oberfläche des Mineralbodens kann "ausgelaugte" Gebiete hervorbringen, von denen der grössere Teil der organischen Substanz und des Nährstoffgehaltes entnommen wurden. Diese Bedingungen können auf die wirksame Mykorrhiza-Aktion zurückgeführt werden, welche Nährstoffe für die Baumnutzung mobilisiert.

In den innerhalb einiger Metern unter der jetzigen Oberfläche liegenden fossilien, bimssteinhaltigen Böden tritt keine solche Pilzaktivität auf. Die Bäume sind offenbar unfähig, irgendwelche merkbaren Stickstoff- oder Phosphormengen aus den vorhandenen, sehr stabilen, allophan-organischen Substanzkomplexen zu extrahieren.
STUDIES ON THE HUMIFICATION OF PLANT TISSUE

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INTRODUCTION

Detailed studies by Simonart and Mayaudon (Simonart 1964) have shown that carbon-14 from labelled rice and ryegrass tissues and their components, as well as carbon-14 from labelled microorganisms, becomes incorporated into humus when incubated in soil. Later work by Sørensen (1965) showed how the amounts of carbon-14 from labelled glucose and labelled cellulose incorporated in different fractions of soil organic matter varied with the length of the incubation period.

Complex enzyme systems are required in order to transform monomeric and polymeric organic compounds into humic materials; such systems are too complex to be studied at present. However, useful information on humus biosynthesis can be obtained by isolating and characterising products formed at different stages during the humification process.

Gel filtration has already been used by Barker et al. (1967), Chahal and Mortensen (1966), Dubach et al. (1964), Finch et al. (1968, 1966), Söchtig (1966 and references therein) to fractionate soil organic matter extracts. As far as the authors are aware, the gel filtration technique has not previously been applied to studies of the humidification of plant materials in the absence of soil organic matter.

This paper will describe our approach to a study of the changes in composition and molecular size which can take place in the water soluble products from humifying ryegrass tissue.

EXPERIMENTAL

Preparation of Incubation Columns

Sand, passed through a ten mesh sieve, was stirred in chromic acid, in water, in concentrated hydrochloric acid and finally in distilled water until the supernatant was acid free. Italian ryegrass S22 (Lolium multiflorum Lam.) was freeze dried, ground in a hammer mill, then intimately mixed (10 g) with the washed and dried sand (290 g) and supported on glass wool over a porcelain disc in a glass column (23 x 3-8 cm). The column was sealed at the top with a rubber bung perforated with glass air inlet and outlet tubes. The bottom of the column was drawn out, and clipped pvc tubing attached for sampling. Several such sand-grass columns were moistened to field capacity with a mineral salts solution, pH 6-9 (Phillips and Traxler 1963). Air, bubbled through a 2N sodium hydroxide scrubber system, then through water, was passed over these columns and through
50 percent sulphuric acid; the carbon dioxide trapped in 0.5N sodium hydroxide was measured by titration.

**Sampling of Water Soluble Column Eluates**

On first sampling, each column was exhaustively extracted by eluting with 30 ml and then with 20 ml aliquots of mineral salts solution until the optical density values (at 400mμ) of the eluates reached a limiting value. Some columns were subsequently extracted partially every seventh day by eluting each column with mineral salts solution (30 ml). The first aliquot (30 ml) eluted during each exhaustive extraction and all samples from the partial extractions were clarified by centrifugation and optical density (400 mμ) and pH values and the ultraviolet spectra of the supernatants recorded. All liquid eluted during the first sampling of a column was combined, centrifuged, freeze dried, suspended in distilled water and exhaustively dialysed in rotating dialysis tubing against distilled water at 5°C. The retentate was freeze dried.

Nine columns were incubated at room temperature. One, containing sand (290 g) and mineral salts solution, served as a blank; it was partially extracted every seventh day. Of the eight columns which contained rye-grass, one was left open to the air and extracted only once (exhaustively) after 14 days. A second column was extracted twice (exhaustively each time) after 2 hours and after 142 days. The remaining six columns were first extracted exhaustively after n days (n = 7, 23, 37, 65, 93 and 142, respectively) and the subsequent partial extractions of n = 7-93 were carried out every seventh day as described.

**Carbon Dioxide Evolution**

The carbon, collected as carbon dioxide after 139 days in the case of the column extracted after 2 hours and 142 days, amounted to 36 percent of the carbon content of the rye-grass. (The carbon content of the rye-grass

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Optical Density and pH Values of Extracts in Mineral Salts Solution from Humifying Rye Grass (L. Multiflorum Lam.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation period</strong> (days)</td>
<td>2 hours</td>
</tr>
<tr>
<td>Optical density values at 400 mμ</td>
<td></td>
</tr>
<tr>
<td>(i) of first extract</td>
<td>4.95</td>
</tr>
<tr>
<td>(ii) of recovered retentate (1 mg/ml in 0.1M borax)</td>
<td>0.55</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>(i) of first extract</td>
<td>5.60</td>
</tr>
<tr>
<td>(ii) of extract taken after 142 days incubation</td>
<td>7.52</td>
</tr>
</tbody>
</table>
Fig. 1.—(a) Optical density values and (b) pH values of extracts in mineral salts solution from ryegrass *L. multiflorum* Lam. incubated in sand columns.
was estimated by combustion after the powdered, freeze dried foliage had been dried in vacuo at 60°C in the presence of phosphorus pentoxide). From columns first sampled after 7, 23, 37, 65, 93 and 142 days, approximately 20, 17, 24, 31 and 52 percent of the ryegrass carbon was evolved.

**Optical Density of Eluates**

The optical density values (samples were diluted where necessary and the values obtained by multiplying the optical density by the appropriate dilution factors) of the first 30 ml aliquot eluted from each of seven columns and of the freeze dried retentates from the first exhaustive extractions are given in Table 1. Figure 1a presents the optical density values of the successive partial extracts of 3 columns. The changes in optical density for columns first extracted after 37 and 93 days were similar to those for the column first extracted after 65 days (Figure 1a).

**pH of Eluates**

The pH values for the first extracts from seven columns and for the partial extracts taken after 142 days are given in Table 1. Figure 1b presents the pH trends for the successive extractions of columns. The pattern when \( n = 37 \) resembled that for \( n = 23 \) if the value for the first point on the graph (\( n = 23 \)) is omitted.

**Ultraviolet Spectra**

The u.v. spectra of all extracts showed increasing absorption at the lower wavelengths with \( \lambda_{\text{max.}} \) values varying from 210 m\( \mu \) in the pH range 7 to 8, to 235 m\( \mu \) in the pH range 3 to 4. There was evidence for some u.v. absorption at 275 m\( \mu \).

**Gel Filtration of Retentates after Dialysis**

Urea solution (5-88M) was an efficient solvent for the freeze dried dialysis retentates (solubility approx. 0.7 per cent w/v). Sephadex G-100 (Pharmacia, Uppsala, Sweden), preswollen in 5-88M urea, was packed in a column (60 x 1.8 cm) which was then calibrated for void volume (36-5 ml) with Blue Dextran M2,000 (Pharmacia) and for low molecular weight compounds with glucose (elution volume 127.5 ml) and guaiacol (elution volume 130.5 ml). Urea (5-88M) was used as eluant in all cases. Retentates (10 mg) dissolved in 5-88M urea (2 ml) were passed through the column and the fractions collected (1-5 ml) analysed for total sugars (Dubois et al. 1956), colour (optical density values at 400 m\( \mu \)), and for phenolic compounds by a modification of the method of Keith et al. (1958). [To a sample (0.5 ml) add 0.5 ml Folin reagent (British Drug Houses Ltd.) in distilled water (1:1 v/v) and 1 ml 20 percent aqueous sodium carbonate. Shake the mixture and after 2 hours measure the optical density at 660 m\( \mu \). Guaiacol (2-20y) in 5-88M urea gave a linear concentration-optical density relationship by this method.]

The gel filtration experiments (Figure 2a) showed that saccharides of intermediate molecular sizes and lower concentrations of monosaccharides
Fig. 2.—Fractionation of retentates from humifying ryegrass on Sephadex G-100 and on DEAE cellulose DE52. (a) Distribution of total sugar. (b) Distribution of coloured organic matter. (c) Fractionation on DE52 cellulose of retentate from ryegrass incubated for 142 days and eluted in the void volume from a column of Sephadex G-100.
were extracted after incubation of the ryegrass for 2 hours. The total saccharide concentration decreased during the first week \( (n = 7, \text{ Figure 2a}) \) and polysaccharides of high molecular size were formed. Polysaccharides were the major carbohydrate constituents eluted after 23 days \( (n = 23, \text{ Figure 2a}) \) and longer periods of incubation.

Since the elution patterns for coloured and for phenolic components were almost superimposable, only the optical density values at 400 nm are plotted in Figure 2b. The retentate from ryegrass incubated for 2 hours contained little coloured organic matter. However, the proportions of coloured components (and phenolic materials) eluted in the void volume increased as the time to the first sampling of the columns increased.

**Anion Exchange Chromatography**

The retentate of the extract from an incubation column extracted after 142 days (which had been extracted earlier after 2 hours) was chromatographed on Sephadex G-100 and the material eluted in the void volume was dialysed against distilled water, freeze dried (yield 1-2 mg), dissolved in 5-8M urea (0-5 ml) and eluted with a 0 to 1M sodium chloride gradient in 5-8M urea through a Whatman DEAE Cellulose DE52 (H. Reeve Angel and Co., London, England) in the chloride form. Fractions (1-5 ml) were analysed for colour, saccharides and phenolic compounds as above. The coloured materials and a major polysaccharide and phenolic component were eluted at 0-4-0-5M salt concentration. A major phenolic component and a major polysaccharide component (Figure 2c) were eluted at 0-8 and 1-0M sodium chloride concentrations, respectively. Small amounts of phenolic constituents were eluted at zero and at 0-5M sodium chloride concentrations.

**Hydrolysis with \( 1N \) Sulphuric Acid**

The retentates from the columns first sampled after 14 and 65 days incubation were hydrolysed and analysed for monosaccharides according to techniques described by Finch et al. (1966). By paper chromatography arabinose, galactose, glucose, mannose and xylose were tentatively identified in both hydrolysates; rhamnose was apparent only in the hydrolysate of the sample incubated for 14 days. Work is in hand to determine the monosaccharides of all retentates by gas phase chromatography (Finch et al. 1968).

**Hydrolysis with \( 2N \) Hydrochloric Acid**

The retentate (30 mg) from the extract taken after 14 days' incubation was refluxed with \( 2N \) hydrochloric acid (10 ml) for 3-5 hours. The hydrolysate was saturated with sodium chloride, extracted with ethyl acetate (3 x 10 ml) and the extracts were combined and evaporated to dryness in vacuo at 30°C to yield approx. 0-4 mg of product. More retentate (30 mg) in \( 2N \) hydrochloric acid at room temperature was extracted immediately (as above). Extracts, in 70 percent ethanol, were spotted alongside phenol standards on mixed thin layer plates of silica gel G (E. Merck AG, Darmstadt, Germany) and MN300 cellulose (Macherey,
Nagel and Co., 516, Düren, Germany), steamed and irrigated with toluene-ethyl formate-formic acid (5:4:1 v/v/v) (Van Sumere et al. 1965). Four spots, fluorescent in ultraviolet light, with Rf values of 0·43, 0·48, 0·57 and 0·78 respectively were detected. These components were absent from the unhydrolysed extract; when recovered from preparative thin layer chromatograms by ethyl acetate extraction they were present in amounts too small for chemical characterisation.

**Liberation of Amino Acids**

Retentates (10 mg) were each oxidised with performic acid (10 ml) (Hirs, 1956), hydrolysed with 6N hydrochloric acid (2 ml) under nitrogen in sealed tubes for 24 hours at 110°C and analysed for amino acids by means of a Technicon Autoanalyser (Technicon Instruments Co. Ltd., London, England). Full results are listed by Standley (1968). As many as 16 common amino acids were found in the retentates.

**DISCUSSION**

Most changes, occurring in the incubation columns which had not been extracted, were complete after 65 days: the recovery of carbon dioxide after this time was minimal; the fractionation patterns for the retentates incubated for 65, 93 and 142 days were almost superimposable; the pH values of first extracts taken on the three occasions mentioned were similar.

Sand in the columns provided a free draining medium to support the ryegrass and the mineral salts solution supplied nitrogen, phosphorus, potassium and magnesium necessary for microbial activity. The loss (52 percent) of carbon from the ryegrass in the column first extracted after 142 days compares favourably with the amount (55 percent) lost from ryegrass incubated for 128 days in rifle peat columns (Hayes and Mortensson, 1963).

The optical density values of retentates rose as the duration of incubation extended (Table 1) indicating that humification is accompanied by the synthesis of brown products of increasing molecular size. The low optical density of the retentate recovered after 142 days suggests that the brown material synthesised in that column had become of too high a molecular size to be readily soluble in water. Periodic fluctuation in the optical density values of successive partial extracts suggests that after partly humified products had been removed from the columns humification recommenced (see Figure 1a).

The low pH values for early first extracts indicate that acids were released from the ryegrass into the extractant; such acids were probably neutralised or metabolised by microorganisms because the pH of later first extracts (Table 1) increased. Ryegrass partially extracted for several weeks gave extracts of pH 3·5-4·5: conditions in the columns may have approached those associated with mor formation (Russell, 1961).

Solvents used in the fractionation of soil organic matter by workers mentioned in the introduction dissolved our retentates incompletely. Our
success with 5-88M urea may be attributed to its ability to break hydrogen bonded complexes (Shifin and Steers, 1967; Stevenson and Stevenson, 1967); indeed, some of the sugar components from retentates recovered after 2 hours and 7 days incubation had elution volumes from Sephadex G-100 corresponding to those of glucose (Figure 2a) although the retentates did not pass through dialysis tubing. The rapid loss of saccharide from retentates as humification proceeded agrees with the findings of Simonart (1964) that after seven days 80 percent of labelled glucose can be recovered as radioactive carbon dioxide during incubation experiments in soil. Saccharides in retentates formed after 23 days gave elution patterns similar to those in later samples indicating that polysaccharide became complexed in a brown humus polymer resistant to microbial decomposition.

The elementary composition of the retentates (C, H, N and ash, Standley, 1968) and the monosaccharides and amino acids released by hydrolysis are similar to those found by Hardisson and Robert-Gero (1966) and Finch et al. (1966) in soil organic matter.

ACKNOWLEDGMENTS

We are indebted to Esso Research Ltd., Abingdon, Berkshire, England, for granting one of us (J.S.) an Esso Research Scholarship. We thank Dr. Finch for helpful discussions.

REFERENCES

SUMMARY

*Lolium multiflorum* Lam. (Italian ryegrass S22) was incubated for 20 weeks in 8 sand columns irrigated with a mineral salts solution (*pH* 6·9) and the carbon dioxide evolved was measured. The 8 columns were exhaustively extracted after 2 hours, 7, 14, 23, 37, 65, 93 and 142 days, respectively, and the non-dialysable extracts were freeze-dried. After the initial extraction each column (except that first sampled after 14 days) was partially extracted every seventh day. The optical density at 400 m\(_\mu\), the *pH* and u.v. spectra of all extracts were studied. Optical density and *pH* values tended to increase as the time to the first exhaustive extraction was increased, but the *pH* values of the successive partial extracts tended to fall.

Non-dialysable extracts, in 5·88M urea, were chromatographed on Sephadex G-100 and the materials of high molecular sizes separated were fractionated into several components when eluted with a salt gradient through DEAE cellulose DE52 (anion exchange). Saccharide components with intermediate molecular sizes predominated in the earlier extracts. Later browner extracts had higher molecular sizes, were more phenolic, and contained lower total saccharide.

RÉSUMÉ

*Le Lolium multiflorum* Lam. (ray-grass Italien S22) fut humifié pendant vingt semaines dans 8 colonnes de sable irriguées par une solution de sels minéraux (*pH* 6·9) et le bioxide de carbone résultant fut mesuré. Le contenu des 8 colonnes fut lavé la première fois avec la solution ci-dessus nommée après deux heures, 7, 14, 23, 37, 65, 93 et 142 jours respectivement et les extraits non-dialysables furent lyophilisés. Après la première extraction chaque colonne (à l'exception de celle qui fut lavée après 14 jours) fut soumise à une extraction partielle tous les huit jours. La densité optique mesurée à 400 m\(_\mu\), les valeurs de *pH* et le spectre dans l'U.V. furent examinés pour tous les extraits. La densité optique et les valeurs de *pH* avaient tendance à croître à mesure que la période de temps avant la première extraction augmentait, mais les valeurs de *pH* pour les extractions partielles successives tendaient à diminuer.

Les extraits non-dialysables dans une solution d'urée 5·88M furent fractionnés au moyen du passage sur gel de Sephadex G-100 et les matières de grand volume moléculaire furent séparées en plusieurs composants par élution de cellulose DEAE DE52 (forme anionique) en utilisant une solution saline de concentration croissante.

Les composants sacchariques de volume moléculaire intermédiaire prédominèrent dans les premiers extraits. Les extraits plus sombres obtenus des extractions ultérieures étaient de volume moléculaire supérieur et de caractère plus phénolique, moins saccharique.
**Zusammenfassung**


Le fractionnement de la matière organique par la méthode de Tyurin (1940) a mené à l’obtention de résultats très utiles tant au point de vue pédologique qu’au point de vue agronomique.

En tenant compte d’un tel fait et étant donné que cette méthode a été rarement appliquée aux sols des régions tropicales et notamment qu’elle n’a jamais été appliquée aux sols ferrallitiques typiques, nous avons jugé très opportun d’essayer cette méthode d’étude de la matière organique sur ces sols, ce qui fait l’objet de cette communication.


Dans le fractionnement effectué, la technique utilisée a été celle établie par le laboratoire des sols de l’École Nationale des Eaux et Forêts de Nancy (France), en utilisant le NaOH 0,1 N comme réactif d’extraction et le H₂SO₄ 0,5 N comme agent de floculation (Duchaufour 1960). Outre les fractions de Tyurin (F₁, F₂, H₁, H₂ et H₃) nous avons déterminé aussi la fraction désignée couramment sous le nom de humines (Hm). À cet effet, le résidu des échantillons de sol après l’extraction de H₁ a été traité avec une solution chaude de soude caustique. Nous avons aussi tâché de réunir des informations plus minutieuses en ce qui concerne la constitution de la matière organique des sols mis en étude.

Le quantitatif de chacune des différentes fractions est exprimé en pourcentage de carbone, le carbone étant déterminé par la méthode d’oxydation par voie humide avec le K₂Cr₂O₇ en suivant la technique de Springer et Klee (1954).

Le Tableau 1 montre les teneurs trouvées dans le sol pour les différentes fractions tout aussi bien que leur distribution dans le profil. On peut alors constater que le quantitatif de chacune des fractions est toujours assez réduit, comme également celui de la matière organique totale. En outre, on peut constater aussi que toutes les fractions humiques diminuent régulièrement dans le profil, se comportant de façon semblable à la matière organique totale. La fraction F₁ est celle qui diminue le plus lentement, la teneur du niveau 8/10-20/23 cm correspondant encore à 80,5/95.8 % de la teneur du niveau 0-8/10 cm et celle du niveau 46/63-90/100 cm se situant aux environs des 20 % (Tableau 2). En ce qui concerne les fractions restantes, au niveau 8/10-20/23 cm, il y a déjà moins de 50 % de la teneur corre-
**TABLEAU 1**

Teneurs des différentes fractions humiques exprimées en C % par rapport à la terre fine sèche à 105 °C

<table>
<thead>
<tr>
<th>Profils</th>
<th>Profondeur (cm)</th>
<th>C total %</th>
<th>Humus C %</th>
<th>Acides fulviques C %</th>
<th>Acides humiques C %</th>
<th>Humines C %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H&lt;sub&gt;1&lt;/sub&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>P99/56</td>
<td>0—10</td>
<td>1.595</td>
<td>1.052</td>
<td>0.127</td>
<td>0.155</td>
<td>0.417</td>
</tr>
<tr>
<td>(Hb27)</td>
<td>10—20</td>
<td>0.808</td>
<td>0.532</td>
<td>0.120</td>
<td>0.065</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>20—46</td>
<td>0.350</td>
<td>0.169</td>
<td>0.065</td>
<td>0.034</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>46—90</td>
<td>0.106</td>
<td>0.052</td>
<td>0.023</td>
<td>0.014</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>90—145</td>
<td>0.089</td>
<td>0.038</td>
<td>0.023</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**TABLEAU 2**

Pourcentages des différentes fractions humiques exprimés en carbone, ramenés à 100 par rapport au carbone du matériel organique respectif du niveau de surface

<table>
<thead>
<tr>
<th>Profils</th>
<th>Profondeur (cm)</th>
<th>C total %</th>
<th>Acides fulviques</th>
<th>Acides humiques</th>
<th>Humines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>P99/56</td>
<td>0—10</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>(Hb27)</td>
<td>10—20</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>20—46</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>46—90</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
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<td></td>
<td>90—145</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**R. PINTO RICARDO**
MATIÈRE ORGANIQUE DES SOLS FERRALLITIQUES 259

spondante au niveau superficiel, et inférieurement la proportion devient bien moindre—à profondeur supérieure à 100 cm elle n’arrive pas à représenter 5 % de la teneur qui est constatée à la surface. Les résultats obtenus montrent, ainsi, que la matière organique s’accumule aux niveaux superficiels et qu’il n’existe pas d’illuviation des substances humiques.

En ce qui concerne la constitution de l’humus (Tableau 3) (nous considérons l’humus comme l’ensemble des acides fulviques, acides humiques et humines) on constate qu’il existe une certaine variation avec la profondeur. Au sol superficiel (0-20/23 cm) les acides fulviques représentent moins de 50 % des substances humiques (21,9 à 42,6 %); inférieurement leurs proportions varient entre 58,6 et 71,1 %. À une profondeur de plus

<table>
<thead>
<tr>
<th>Profils</th>
<th>Profondeur (cm)</th>
<th>Acides fulviques (C%)</th>
<th>Acides humiques (C%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F_1^+F_2$</td>
<td>$F_1$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(1)$</td>
<td>$(2)$</td>
</tr>
<tr>
<td>P99/56</td>
<td>0—10</td>
<td>26·8</td>
<td>45·0</td>
</tr>
<tr>
<td>(Hb27)</td>
<td>10—20</td>
<td>34·8</td>
<td>64·9</td>
</tr>
<tr>
<td></td>
<td>20—46</td>
<td>58·6</td>
<td>65·7</td>
</tr>
<tr>
<td></td>
<td>46—90</td>
<td>71·1</td>
<td>62·2</td>
</tr>
<tr>
<td></td>
<td>90—145</td>
<td>68·4</td>
<td>88·5</td>
</tr>
<tr>
<td>P211/56</td>
<td>0—8</td>
<td>21·9</td>
<td>39·2</td>
</tr>
<tr>
<td>(Hb28)</td>
<td>8—23</td>
<td>41·1</td>
<td>53·1</td>
</tr>
<tr>
<td></td>
<td>23—38</td>
<td>66·2</td>
<td>69·1</td>
</tr>
<tr>
<td></td>
<td>38—63</td>
<td>60·9</td>
<td>69·6</td>
</tr>
<tr>
<td></td>
<td>63—90</td>
<td>68·2</td>
<td>75·6</td>
</tr>
<tr>
<td></td>
<td>90—120</td>
<td>67·2</td>
<td>94·6</td>
</tr>
<tr>
<td>P104/56</td>
<td>0—8</td>
<td>29·2</td>
<td>35·0</td>
</tr>
<tr>
<td>(Hb33)</td>
<td>8—20</td>
<td>42·6</td>
<td>50·5</td>
</tr>
<tr>
<td></td>
<td>20—50</td>
<td>67·8</td>
<td>46·2</td>
</tr>
<tr>
<td></td>
<td>50—100</td>
<td>65·5</td>
<td>40·8</td>
</tr>
</tbody>
</table>

(1)—Par rapport à l’humus.
(2)—Par rapport à l’ensemble des acides fulviques.
(3)—Par rapport à l’ensemble des acides humiques.

de 20 cm, les acides fulviques constituent donc la fraction dominante, soit en considérant séparément les acides humiques et les humines, soit même quand on considère les deux fractions conjointement. Au niveau 8/10-20/23 cm, en prenant alors seulement comme base les valeurs individuelles des trois fractions, les acides fulviques ont tendance à être aussi la fraction prédominante. Au niveau superficiel (0-8/10 cm), sont les acides humiques, toutefois, qui représentent la fraction la plus importante. D’ailleurs, nous pouvons dire qu’il y a une augmentation dans la proportion des acides fulv-
ques en profondeur, tandis que celle des acides humiques diminue. En ce qui concerne les humines, seule une petite variation est observée et il est à remarquer aussi qu'elles constituent tout au long du profil une proportion appréciable de l'humus (entre 21,3 et 35,1 %). Relativement à la variation de la proportion des acides fulviques et acides humiques, nous pouvons arriver à la même conclusion en partant des valeurs du rapport acides fulviques/acides humiques (Tableau 4). En effet, tel rapport augmente avec la profondeur, étant inférieur à 1,0 à peine au niveau superficiel ou aux deux premiers niveaux.

Quant à la nature de la fraction fulvique (Tableau 3), exception faite au niveau 0-8/10 cm, nous pouvons dire que les acides fulviques libres (F1) tendent à prédominer et en général s'accentuent davantage selon la profondeur.

Les acides humiques (Tableau 3) sont généralement constitués d'une façon prédominante par les acides humiques libres (H1). Cette fraction existe à tous les niveaux du profil et nous pouvons même dire que sa proportion, relativement aux autres types d'acides humiques, augmente avec la profondeur. Quant à H2 et Hx, on peut constater qu'ils représentent des proportions relativement petites des substances humiques, correspondant à moins de 10 % et très souvent équivalent à une proportion inférieure à 5 %. Il peut même arriver que ces acides humiques ne se retrouvent pas dans le sol, comme il est observé sur l'un des profils pour la fraction H2, et généralement, ils n'existent pas à partir d'une certaine profondeur.

En plus cette caractérisation ayant pour base les valeurs obtenues pour les différentes fractions, la matière organique est encore caractérisée en considérant deux constantes définies à partir de ces valeurs—le taux d'humification de la matière organique (pourcentage d'humus par rapport à la matière organique totale) et une autre constante que nous désignons par taux d'évolution de l'humus.

Le taux d'humification (Tableau 4), qui en général diminue graduellement avec la profondeur, indique une matière organique moyennement humifiée. Ce taux varie entre 58,6 et 73,7 % en surface (0-20/23 cm); il varie entre 38,3 et 56,3 % en profondeur, les valeurs supérieures à 50 % étant peu fréquentes. Il existe en réalité une diminution de l'humification dans le profil ce qui est d'ailleurs compréhensible étant donné que l'activité microbienne diminue dans le même sens et que, en profondeur, les conditions sont moins favorables aux réactions chimiques et biochimiques indispensables à la formation de l'humus.

En tenant compte de la différence existant entre les fractions H1, F2 et H1 d'une part (complexes humiques d'une plus grande mobilité et de molécule moins condensée, et de ce fait relativement peu évolués) et les fractions plus évoluées H2, Hx et Hm d'autre part (complexes plus polymérisés et qui dans leurs totalités constituent des complexes argilo-humiques) il semble que l'humus peut être caractérisé par la valeur du pourcentage de H2 + Hx + Hm exprimée par rapport au total des substances humiques—constante que, comme on a dit ci-dessus, nous désignons par taux d'évolution de l'humus. Cette constante présente des valeurs de 33,6 %, 50,8 %.
et 42.7 % à la surface, et varie entre 23,1 et 39,8 % sur le reste du profil (Tableau 4). À l'exception d'un échantillon où les deux ensembles de fractions présentent des proportions égales (indiquant un humus moyennement évolué), les résultats obtenus révèlent que l'humus des sols étudiés est peu évolué, très fréquemment les composés humiques plus polymérisés représentant moins d'un tiers de la totalité des substances humiques. Il semble aussi se discerner une tendance à ce que le taux d’évolution diminue avec la profondeur. Bien qu'une variation uniforme au long du profil n'ait pas été constatée, il arrive cependant que les valeurs observées aux niveaux 0-8/10 cm et 8/10-20/23 cm ne sont jamais atteintes sur quelque niveau inférieur. Ce fait peut être expliqué, en partie, par l'augmentation de l'acidité en profondeur et par la diminution des teneurs en bases dans le même sens, constatées sur ces sols, ces conditions rendant évidemment le moyen moins favorable au processus de polymérisation de la molécule humique.

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Tyurin, I. V. (1940)—Problemy sov. Pochvov. 11, 173-188.

**RÉSUMÉ**

On a étudié la matière organique de trois profils de sols ferrallitiques typiques d'Angola, en suivant la méthode de fractionnement de Tyurin. En

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**Tableau 4**

<table>
<thead>
<tr>
<th>Profils</th>
<th>Profondeur (cm)</th>
<th>Taux d'humification</th>
<th>Taux d'évolution de l'humus</th>
<th>Rapport ac. fulviques/ac. humiques</th>
</tr>
</thead>
<tbody>
<tr>
<td>P99/56</td>
<td>0–10</td>
<td>65·9</td>
<td>33·6</td>
<td>0·6</td>
</tr>
<tr>
<td>(Hb27)</td>
<td>10–20</td>
<td>65·8</td>
<td>29·1</td>
<td>0·8</td>
</tr>
<tr>
<td></td>
<td>20–46</td>
<td>56·3</td>
<td>23·1</td>
<td>2·9</td>
</tr>
<tr>
<td></td>
<td>46–90</td>
<td>49·1</td>
<td>25·0</td>
<td>18·5</td>
</tr>
<tr>
<td></td>
<td>90–145</td>
<td>42·7</td>
<td>28·9</td>
<td>26·0</td>
</tr>
<tr>
<td>P211/56</td>
<td>0–8</td>
<td>65·2</td>
<td>50·8</td>
<td>0·5</td>
</tr>
<tr>
<td>(Hb28)</td>
<td>8–23</td>
<td>58·6</td>
<td>39·8</td>
<td>1·4</td>
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<td></td>
<td>23–38</td>
<td>47·6</td>
<td>32·4</td>
<td>6·7</td>
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<td>38·9</td>
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<td>42·3</td>
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<td>37·0</td>
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<tr>
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<td>0–8</td>
<td>73·7</td>
<td>42·7</td>
<td>0·8</td>
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<td>(Hb33)</td>
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<tr>
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<td>20–50</td>
<td>55·3</td>
<td>24·0</td>
<td>8·3</td>
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<tr>
<td></td>
<td>50–100</td>
<td>47·0</td>
<td>31·0</td>
<td>19·0</td>
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</table>
plus des fractions de Tyurin ($F_1$, $F_2$, $H_1$, $H_2$ et $H_3$) on a déterminé également les humines ($H_m$). La matière organique a été caractérisée non seulement en tenant compte des teneurs des différentes fractions, mais encore par l'utilisation de deux constantes calculées à partir de ces valeurs—le taux d’humification de la matière organique, et une autre constante que nous désignons par taux d’évolution de l’humus.

Les aspects fondamentaux suivants ont été observés :

1. La teneur du sol est réduite dans les différentes fractions, et sur chacune d’elles on a pu constater une diminution avec la profondeur. La variation est d’une manière générale assez rapide, ce qui indique une accumulation des substances humiques aux couches superficielles.

2. Dans le sol superficiel (0-8/10 cm) les acides humiques représentent la fraction la plus importante de l’humus; les acides fulviques prédominent inférieurement, constituant plus de 50 % des substances humiques à n’importe quel niveau au-dessous de 20 cm; les humines, en général, sont la seconde fraction en importance, représentant environ 20-35 % de l’humus. On a constaté, en plus, une augmentation de la proportion d’acides fulviques dans le profil alors que celle des acides humiques diminue; la proportion des humines varie très peu.

3. En ce qui concerne la fraction fulvique, si on excepte le niveau superficiel, ce sont normalement les acides fulviques libres ($F_i$) qui prédominent; ce phénomène devient plus accentué en profondeur.

4. Les acides humiques, en général, sont prédominalement constitués par des acides humiques libres ($H_i$). Les fractions $H_2$ et $H_3$ représentant des proportions relativement petites et parfois même n’existent pas dans le sol.

5. La matière organique se présente sur quelque niveau du profil en général moyennement humifié, le degré d’humification diminuant avec la profondeur.

6. L’humus se présente peu évolué.

**Summary**

The organic matter of three profiles of typical ferrallitic soils from Angola was studied, following Tyurin’s fractionation scheme. Besides Tyurin’s fractions ($F_1$, $F_2$, $H_1$, $H_2$ and $H_3$) the humins ($H_m$) were also determined.

The characterization of organic matter was based on the values of the different fractions and also using two constants derived from those values; the degree of humification and another constant which we designate degree of humus evolution. The following statements can be made.

1. The content of each fraction is low and all the values show a rapid decrease with depth.

2. In the top soil, the humic acids represent the most important humus fraction; the fulvic acids predominate in the remainder of the profile, accounting for more than 50% of the humus in any level
below 20 cm; the humins, in general, are the second most important fraction, representing about 20-35% of the humus. The proportion of fulvic acids increases with depth while that of humic acids diminishes, there being only a slight change in the proportion of humins.

3. Except in the top soil, fraction $F_1$ normally predominates in the fulvic acids.

4. Fraction $H_1$ is generally the predominant fraction in the humic acids. Fractions $H_2$ and $H_3$ are low or even absent.

5. The organic matter generally shows an average degree of humification which decreases with depth.

6. The humus shows a weak development.

**ZUSAMMENFASSUNG**

Die organische Substanz in drei Profilen der typischen ferralitischen Böden (Lateritböden) in Angola ist bei Anwendung der Tyurin Fraktionierungs methode untersucht worden. Ausser den Tyurin-Fraktionen ($F_1$, $F_2$, $H_1$, $H_2$ und $H_3$) wurden auch die Humine ($H_m$) festgestellt. Die organische Substanz wurde nicht nur auf Grund des Gehaltes der verschiedenen Fraktionen bestimmt, sondern auch mittels zweier Konstanten, die von diesen Anfangswerten aus, berechnet waren—der Humifizierungsgrad der organischen Substanz und eine andere Konstante, welche wir als Humusentwicklungsgrad bezeichnen.

Die wesentlichen Erscheinungen waren wie folgt:

1. Der Bodengehalt ist geringer in den verschiedenen Fraktionen und in jeder hat man mit zunehmender Tiefe eine Abnahme festgestellt. Die Veränderung ist gewöhnlich ziemlich rasch, was auf eine Akkumulation der Humussubstanzen in den Oberschichten hinweist.

2. In der Bodenoberfläche (0-8/10 cm) bilden die Huminsäuren die wichtigste Fraktion des Gesamthumus; die Fulvosäuren sind vorherrschend auf grösserer Tiefe, sie stellen mehr als 50% der Humussubstanzen auf jeglicher Tiefe unter 20 cm dar; die Humine sind überhaupt die zweit-wichtigste Fraktion—ungefähr 20-35% des Gesamthumus. Ferner wurde festgestellt, dass der Anteil der Fulvosäuren im Profil stieg, dagegen der Gehalt der Huminsäuren fiel; der Humingehalt veränderte sich sehr wenig.

3. Was die Fulvosfraktion anbetrifft, sind die freien Fulvosäuren, mit Ausnahme der Bodenoberfläche, gewöhnlich vorherrschend; diese Erscheinung ist in der Tiefe mehr ausgesprochen.

4. Gewöhnlich bestehen die Huminsäuren hauptsächlich aus freien Huminsäuren ($H_1$), die Fraktionen $H_2$ und $H_3$ sind in kleineren Mengen und sind zuweilen sogar überhaupt nicht vorhanden.

5. Die organische Substanz befindet sich auf einem gewöhnlich mässig humifizierten Profilniveau, wobei der Humifizierungsgrad mit der Tiefe abnimmt.

6. Der Humus ist wenig entwickelt.
SOME CASES OF PODSOLIZATION UNDER TROPICAL CONDITIONS IN ANGOLA

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Several authors (Vageler, 1930; Baldwin et al., 1938; Costa, 1944; Kellogg, 1947; Jenny, 1948; Mohr, 1954; Sys, 1960; D’Hoore, 1964) have admitted the possibility of occurrence, or have even referred to cases of podsolization under tropical conditions. In the Portuguese overseas province of Angola, soils with similar morphology to those generally referred to as podsolized soils have been reported since 1959. Firstly, their occurrence was considered purely accidental under such unfavourable climatic and vegetational conditions as those in Angola. However, as the soil survey progressed, the area covered by soils of this type was found to be quite considerable. This fact led us to carry out a detailed study.

In the present note five soil profiles were chosen covering a wide field of morphological evidence for podsolization. The general information on these soils is given in Table 1.

Among the morphological descriptions purposely omitted in this paper, we should mention the following points, which are either implicit or can

<table>
<thead>
<tr>
<th>Profiles</th>
<th>Lithology</th>
<th>Vegetation</th>
<th>Moisture index (Thornthwaite)</th>
<th>Mean annual R (mm)</th>
<th>Mean annual T (°C)</th>
<th>Dry season (months)</th>
</tr>
</thead>
<tbody>
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<td>P.32/60</td>
<td>loose sandy sediments</td>
<td>Moist forest</td>
<td>30 (B)</td>
<td>1500</td>
<td>23/24</td>
<td>3 — 4</td>
</tr>
<tr>
<td>P.213c/60</td>
<td>loose sandy sediments</td>
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<td>700</td>
<td>25/26</td>
<td>4 — 5</td>
</tr>
<tr>
<td>P.175/64</td>
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<td>Arboreal savannah</td>
<td>30 (B)</td>
<td>1050</td>
<td>20</td>
<td>± 6</td>
</tr>
<tr>
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<td>loose sandy sediments</td>
<td>Woodland</td>
<td>12 (C)</td>
<td>900</td>
<td>21</td>
<td>6 — 7</td>
</tr>
<tr>
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<td>loose sandy sediments</td>
<td>Herbaceous savannah</td>
<td>50 (B)</td>
<td>1500</td>
<td>22/23</td>
<td>6 — 7</td>
</tr>
</tbody>
</table>

265
<table>
<thead>
<tr>
<th>Profiles</th>
<th>Horizons</th>
<th>Depths (cm)</th>
<th>&quot;Moisture %&quot; at 15 atmospheres</th>
<th>Clay %</th>
<th>&quot;Moisture %&quot; at 15 atmospheres &quot;clay %&quot; ratio</th>
<th>Organic C %</th>
<th>Free FeO3 %</th>
<th>C.E.C.m.e./100g</th>
<th>C.E.C. change on heating %</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.32/60</td>
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<td>170 — 205</td>
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<td>3.7</td>
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<td>5.11</td>
<td>3.71</td>
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<tr>
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<td>17.6</td>
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<td>2.67</td>
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<td>12.90</td>
<td>9.50</td>
</tr>
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<td>B2</td>
<td>80 — 110</td>
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<td>0.9</td>
<td>0.66</td>
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<td>traces</td>
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<td>1.40</td>
</tr>
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<td>40 — 80</td>
<td>1.8</td>
<td>1.3</td>
<td>1.61</td>
<td>0.94</td>
<td>traces</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
be deduced: (1) only one profile showed a surface organic horizon and, even in this case, a very thin one; (2) all the profiles showed a coarse texture within the depth examined; (3) complete absence of structure or very weak structure in the surface level; (4) absence of pans; (5) a very high permeability; (6) very easy drainage throughout the whole depth examined, or in a depth limited by the presence of a water table (P.358c/65); (7) existence in all the cases of a B₂ horizon with a value and chroma clearly lower than those at overlying and underlying levels.

The analytical study was carried out with two different objectives: (1) search for evidence of the presence of amorphous materials in the B₂ horizon, with a view to establish similarities with the spodic horizon of the American authors (Soil Survey Staff, 1960 and 1964); (2) characterization of organic matter and its dynamic study throughout the profile (the main part of this work).

As to the first above mentioned objective, our data showed that in the inorganic colloidal complex the amorphous material did not play any important role. In effect, the X-ray diagrams obtained with and without extraction of the amorphous fraction showed no measurable difference and they indicated dominance of kaolinite either incompletely crystallized or in course of alteration. However the decrease in C.E.C. after heating at 200°C in N₂ showed values between 26 and 36%, and the ratio % water content at 15 atmospheres/% clay was relatively high, being always above 0.4 and greater than 1.0 in some cases (Table 2).

Taken together, these facts suggest that in the soils studied, the B₂ horizon showed certain properties derived from the presence of an amorphous fraction in which the role played by organic matter dominates in a large measure.

This conclusion led us to consider as more important our second way of research. Thus in samples pertaining to horizons A₁ and B₂, and to a layer intermediate between A₁ and B₂, total organic matter was fractionated according to Tyurin's technique (Duchaufour, 1960). The results are presented in Tables 3 and 4 under a different arrangement, and their analysis is of great importance.

The data assembled in Table 3 allow us to characterize the organic matter involved in the genesis of the soils under study more easily than the data presented in Table 4.

The degree of humification of organic matter, considering the humified portion covering the humins besides the fulvic and humic acids, showed values between 56 and 72% in A₁, the values being systematically higher in B₂ (Table 3). The degree of evolution of the humus, i.e. its content in humic acids linked to clay, and in humins (Ricardo, 1967), showed low values which result from a dominance of fulvic acids and forms of humic acids (H₁ fraction) similar to the former (Table 3). In effect, the mobile fractions $F_1 + F_2 + H_1$ make up between about 57 and 98% of the total humus and, within that total mobile portion, fraction $H_1$ reaches values which, excepting those of horizons $A_{22}$ and $B_2$ of P.213c/60, are always very high (Table 3). Although it is implicit in the previous comments, it
### Table 3

**Selected Characterization Data for Organic Matter and Horizons**

<table>
<thead>
<tr>
<th>Profiles</th>
<th>Horizons</th>
<th>Depths (cm)</th>
<th>Total org. mat.</th>
<th>Humus</th>
<th>Humification degree</th>
<th>$C_{%}$</th>
<th>$C_{%}$</th>
<th>C in the $H_1$ fraction</th>
<th>C/N</th>
<th>Base saturation</th>
<th>pH (KCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.32/60</td>
<td>$A_{11}$</td>
<td>0—10</td>
<td>15.97</td>
<td>10.79</td>
<td>68</td>
<td>7.40</td>
<td>3.39</td>
<td>65</td>
<td>16</td>
<td>6.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>$A_{22}$</td>
<td>103—137</td>
<td>2.86</td>
<td>2.64</td>
<td>92</td>
<td>2.20</td>
<td>0.44</td>
<td>53</td>
<td>24</td>
<td>14.3</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>$B_2$</td>
<td>170—205</td>
<td>5.73</td>
<td>5.12</td>
<td>89</td>
<td>4.71</td>
<td>0.41</td>
<td>61</td>
<td>29</td>
<td>10.9</td>
<td>4.7</td>
</tr>
<tr>
<td>P.213c/60</td>
<td>$A_{11}$</td>
<td>0—11</td>
<td>23.91</td>
<td>17.29</td>
<td>72</td>
<td>10.45</td>
<td>6.84</td>
<td>81</td>
<td>15</td>
<td>10.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>$A_{22}$</td>
<td>53—85</td>
<td>1.22</td>
<td>1.22</td>
<td>100</td>
<td>1.01</td>
<td>0.21</td>
<td>22</td>
<td>15</td>
<td>38.5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>$B_2$</td>
<td>127—165</td>
<td>3.24</td>
<td>3.17</td>
<td>98</td>
<td>3.10</td>
<td>0.07</td>
<td>8</td>
<td>19</td>
<td>11.0</td>
<td>4.6</td>
</tr>
<tr>
<td>P.175/64</td>
<td>$A_{11}$</td>
<td>0—20</td>
<td>3.99</td>
<td>2.38</td>
<td>60</td>
<td>1.49</td>
<td>0.89</td>
<td>64</td>
<td>25</td>
<td>18.9</td>
<td>4.1</td>
</tr>
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<td>$A_{22}$</td>
<td>60—110</td>
<td>1.38</td>
<td>1.01</td>
<td>73</td>
<td>0.74</td>
<td>0.27</td>
<td>57</td>
<td>14</td>
<td>23.4</td>
<td>4.4</td>
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<tr>
<td></td>
<td>$B_2$</td>
<td>140—175</td>
<td>26.71</td>
<td>25.82</td>
<td>97</td>
<td>24.16</td>
<td>1.66</td>
<td>96</td>
<td>41</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td>P.271c/64</td>
<td>$A_{11}$</td>
<td>0—14</td>
<td>6.74</td>
<td>3.77</td>
<td>56</td>
<td>2.14</td>
<td>1.63</td>
<td>79</td>
<td>20</td>
<td>17.7</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>$A_{2}$</td>
<td>14—46</td>
<td>1.49</td>
<td>1.44</td>
<td>97</td>
<td>1.11</td>
<td>0.33</td>
<td>70</td>
<td>15</td>
<td>19.5</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>$B_2$</td>
<td>80—110</td>
<td>2.31</td>
<td>1.93</td>
<td>84</td>
<td>1.58</td>
<td>0.35</td>
<td>56</td>
<td>18</td>
<td>10.0</td>
<td>4.4</td>
</tr>
<tr>
<td>P.358c/65</td>
<td>$A_1$</td>
<td>0—9</td>
<td>16.59</td>
<td>10.38</td>
<td>63</td>
<td>6.30</td>
<td>4.08</td>
<td>61</td>
<td>24</td>
<td>16.6</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>$A_2$</td>
<td>9—39</td>
<td>8.19</td>
<td>6.71</td>
<td>82</td>
<td>5.21</td>
<td>1.50</td>
<td>75</td>
<td>18</td>
<td>6.7</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>$B_2$</td>
<td>40—80</td>
<td>9.37</td>
<td>7.03</td>
<td>75</td>
<td>6.09</td>
<td>0.94</td>
<td>69</td>
<td>20</td>
<td>5.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Profiles</td>
<td>Horizons</td>
<td>Depths (cm)</td>
<td>Carbon (in fractions) %&lt;sub&gt;oo&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$F_1$ (a)</td>
<td>(b)</td>
<td>$F_2$ (a)</td>
<td>(b)</td>
<td>$H_1$ (a)</td>
<td>(b)</td>
<td>$H_2$ (a)</td>
<td>(b)</td>
<td>$H_3$ (a)</td>
</tr>
<tr>
<td>P.32/60</td>
<td>$A_{11}$</td>
<td>0—10</td>
<td>0.63</td>
<td>58</td>
<td>1.95</td>
<td>181</td>
<td>4.82</td>
<td>447</td>
<td>0.00</td>
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<td>0.00</td>
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<tr>
<td></td>
<td>$A_{23}$</td>
<td>103—137</td>
<td>0.70</td>
<td>265</td>
<td>0.33</td>
<td>125</td>
<td>1.17</td>
<td>443</td>
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<td>0</td>
<td>0.15</td>
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<tr>
<td></td>
<td>$B_2$</td>
<td>170—205</td>
<td>0.99</td>
<td>193</td>
<td>0.85</td>
<td>166</td>
<td>2.87</td>
<td>561</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>P.213c/60</td>
<td>$A_{11}$</td>
<td>0—11</td>
<td>0.37</td>
<td>21</td>
<td>1.57</td>
<td>91</td>
<td>8.51</td>
<td>492</td>
<td>0.00</td>
<td>0</td>
<td>2.66</td>
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<tr>
<td></td>
<td>$A_{22}$</td>
<td>53—85</td>
<td>0.50</td>
<td>410</td>
<td>0.29</td>
<td>238</td>
<td>0.22</td>
<td>180</td>
<td>0.00</td>
<td>0</td>
<td>0.14</td>
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<tr>
<td></td>
<td>$B_2$</td>
<td>127—165</td>
<td>2.69</td>
<td>849</td>
<td>0.15</td>
<td>47</td>
<td>0.26</td>
<td>82</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>P.175/64</td>
<td>$A_{11}$</td>
<td>0—20</td>
<td>0.09</td>
<td>38</td>
<td>0.44</td>
<td>185</td>
<td>0.96</td>
<td>403</td>
<td>0.00</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>$A_{22}$</td>
<td>60—110</td>
<td>0.15</td>
<td>149</td>
<td>0.17</td>
<td>168</td>
<td>0.42</td>
<td>416</td>
<td>0.00</td>
<td>0</td>
<td>0.07</td>
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<tr>
<td></td>
<td>$B_2$</td>
<td>140—175</td>
<td>0.17</td>
<td>7</td>
<td>0.76</td>
<td>29</td>
<td>23.23</td>
<td>900</td>
<td>0.00</td>
<td>0</td>
<td>0.95</td>
</tr>
<tr>
<td>P.271c/64</td>
<td>$A_{11}$</td>
<td>0—14</td>
<td>0.09</td>
<td>24</td>
<td>0.36</td>
<td>95</td>
<td>1.69</td>
<td>448</td>
<td>0.03</td>
<td>8</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>$A_{2}$</td>
<td>14—46</td>
<td>0.11</td>
<td>76</td>
<td>0.22</td>
<td>153</td>
<td>0.78</td>
<td>542</td>
<td>0.00</td>
<td>0</td>
<td>0.07</td>
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<tr>
<td></td>
<td>$B_2$</td>
<td>80—110</td>
<td>0.40</td>
<td>207</td>
<td>0.29</td>
<td>150</td>
<td>0.89</td>
<td>461</td>
<td>0.00</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>P.358c/65</td>
<td>$A_1$</td>
<td>0—9</td>
<td>0.21</td>
<td>20</td>
<td>2.26</td>
<td>218</td>
<td>3.83</td>
<td>369</td>
<td>0.35</td>
<td>34</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>$A_2$</td>
<td>9—39</td>
<td>0.24</td>
<td>36</td>
<td>1.07</td>
<td>151</td>
<td>3.90</td>
<td>581</td>
<td>0.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>$B_2$</td>
<td>40—80</td>
<td>1.29</td>
<td>183</td>
<td>0.61</td>
<td>87</td>
<td>4.19</td>
<td>596</td>
<td>0.00</td>
<td>0</td>
<td>0.18</td>
</tr>
</tbody>
</table>

(a) in the fine earth;  (b) in the humified fraction.
should be noted that among the humic acids, fraction \( H_1 \) (the mobile form) is always found in high proportion, namely in the \( B_2 \) horizon (Table 3).

It should also be mentioned that the values of the \( C/N \) ratio in \( A_1 \) vary between 15 and 25, and are above 18 (and frequently above 20) in the \( B_2 \); that the base saturation degree in \( A_1 \) shows values always less than 20\%, and the same applies to the \( B_2 \) horizon; and that the \( pH \) (KCl) varies between 3.5 and 4.1 in \( A_1 \), and between 4.0 and 4.7 in \( B_2 \) horizon (Table 3).

On the other hand the data assembled in Table 4 and also some of the constants in Table 3 allow us to follow the dynamics of organic matter throughout the soil profile.

The data referring either to the total organic matter content or to the humus content point to a fairly well defined and sometimes important accumulation of those constituents in the \( B_2 \) horizon (Table 3).

Moreover, the \( F_1 \) fraction showed values consistently higher in \( B_2 \) than in \( A_1 \), indicating an accumulation of the fraction in the former horizon (Table 4). The variation with depth of the values referring to \( F_2 \) fraction was somewhat irregular, showing in \( P.32/60 \) and \( P.271c/64 \) an accumulation in \( B_2 \) relative to \( A_2 \), in \( P.175/64 \) showing an accumulation in \( B_2 \) even when compared to \( A_1 \) and, in \( P.213c/60 \) and \( P.358c/65 \) showing a consistent fall with depth (Table 4). As to \( H_1 \) fraction there was always an accumulation in relation to \( A_2 \) and, in \( P.175/64 \) and \( P.358c/65 \), even in relation to \( A_1 \) (Table 4). Finally analysing the variation of the total mobile fraction with depth we can always observe an accumulation in \( B_2 \) relative to \( A_2 \) and, in \( P.175/64 \), even in relation to \( A_1 \) (Table 3).

Summarizing, we may thus state that the soils under study show: (1) organic matter in \( A_1 \) with a medium or relatively high humification, but with the humus dominated by hydrosoluble compounds weakly polymerized, which migrate in the profile and accumulate in \( B_2 \); (2) low degree of humus evolution, clearly lower in \( B_2 \) than in \( A_1 \); (3) low or relatively low mineralization of the humus; (4) very low base saturation degree in \( A_1 \) and values of \( pH \) (KCl) very low in the entire profile.

From these facts it seems possible to conclude (although only one profile shows an \( O_1 \) horizon) that the organic matter involved in the genesis of the soils studied is similar to oligotrophic "moder", which is the type of humus characterizing the "lessivé podsolique" and the "ocre podsolique" soils (Duchaufour, 1965). In effect the fulvic acids and the humic acids low in nitrogen, which clearly dominate the samples analysed, play an active role in the podsolization process which can thus be initiated and then proceed with a greater or lesser intensity. The genesis of this type of humus should, in the present case, be intimately linked to the extreme poverty of the parent rock in bases and to its high permeability and, sometimes, to the short or even very short dry season.

As to the absence of \( O_1 \) and \( O_2 \) horizons, it should be noted that for example in Australia there have been also descriptions of podsolized soils without any of those horizons (Stephens, 1962).

Taking the values of the ratio between the \( H_1 \) fractions, expressed as a
percentage of their respective mobile global fractions, in $B_2$ and $A_1$ horizons 
$[H_1\% \ (F_1 + F_2 + H_1) \text{ in } B_2/H_1\% \ (F_1 + F_2 + H_1) \text{ in } A_1]$, we attempted 
to assess the five soils studied in relation to the different stages of podsolization 
in which they are found, and to relate the values of that ratio to the 
values of drainage index (Hénin, 1945) and to the position that the respective 
soils may occupy in the American classification.

<table>
<thead>
<tr>
<th>Profiles</th>
<th>Podsolization index (%)</th>
<th>Drainage index (mm)</th>
<th>American classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. 175/64</td>
<td>150</td>
<td>456</td>
<td>Typic Tropohumods</td>
</tr>
<tr>
<td>P. 358c/65</td>
<td>113</td>
<td>871</td>
<td>Typic Tropaquods</td>
</tr>
<tr>
<td>P. 32/60</td>
<td>94</td>
<td>855</td>
<td>Typic Tropohumods</td>
</tr>
<tr>
<td>P. 271c/64</td>
<td>71</td>
<td>314</td>
<td>Spodic Quartzipsamments</td>
</tr>
<tr>
<td>P. 213c/60</td>
<td>10</td>
<td>147</td>
<td>Spodic Quartzipsamments</td>
</tr>
</tbody>
</table>

From the analysis of Table 5 and taking into account the values 
assumed by the so-called podsolization index, the following order may be 
established:

P.175/64 > P.358c/65 > P.32/60 > P.271c/64 > P.213c/60

This ordering of the profiles according to progressively decreasing stages 
of podsolization is in agreement with the ease with which we can distinguish morphologically $B_2$ horizon in the different cases.

The correspondence with the values of the drainage index is relatively 
satisfactory with the exception of P.175/64 which is anomalous.

Finally, as to the correspondence of these soils in the American classification (Soil Survey Staff, 1960 and 1967), it can be shown that the soils with higher values of podsolization index may be included in the Typic Tropohumods and in the Typic Tropaquods whereas those which show lower values of this index may be classified as Spodic Quartzipsamments.

ACKNOWLEDGMENT

The author is indebted to Dr. Iolanda Mateus who carried out the 
majority of the analytical determinations.

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Costa, J. V. Botelho da (1944)—"Apontamentos de Agrologia”. [Lisboa].
The paper deals with podsolized soils occurring in Angola under climatic and vegetational conditions markedly different from those usually associated with podsolization.

Five profiles were chosen for study, with special emphasis on the characterization of organic matter, including the determination of the different humic compounds and their quantitative variation throughout the profile.

The data seemed to indicate that the humus under study was of the oligotrophic "moder" type, whose podsolizing nature is well known.

An index (podsolization index) based on the ratio of $H_1$ (as percentage of the total mobile fractions) in the $B_2$ and $A_1$ horizons was suggested for assessing podsolization. The assumed values of this index agreed fairly well with the morphological evidence of podsolization in the field. On the other hand, also in relation to this index, the soils that in the American system can be classified as Typic Tropohumods and Typic Tropaquods showed higher values, whereas those that can be included in the Spodic Quartzipsamments showed lower values.

There was also found, for the soils studied (strongly permeable materials poor in bases being the dominant factor of podsolization), a fairly regular correspondence between the podsolization index and Hénin's drainage index.
PODSOLIZATION UNDER TROPICAL CONDITIONS

RéSUMÉ

On étudie des sols podzolisés en Angola sous conditions climatiques et végétales très différentes de celles qui sont généralement associées au phénomène de la podzolisation. L'étude a été portée sur cinq profils, particulièrement en ce qui concerne la détermination des différents composés humiques et aussi leur distribution à travers le profil.

D'après les résultats, on a rapproché l'humus étudié au "moder" forestier dont les propriétés podzolisantes sont bien connues.

Un indice basé sur la relation des fractions $H_1$ (exprimées en pourcentage des fractions mobiles totales) en $B_2$ et $A_1$ a été suggéré pour caractériser les phases de podzolisation. On a vérifié que cet indice a pris des valeurs qui sont ordonnées selon l'évidence morphologique de podzolisation. D'autre part les valeurs les plus élevées étaient vérifiées pour les profils classés comme Typic Tropohumods et Typic Tropaquods, et les plus basses pour ceux classés comme Spodic Quartzipsamments (classification Américaine).

Pour les sols étudiés (la pauvreté de la roche-mère en bases étant le principal facteur de la mise en place de la podzolisation) une régulière correspondance a été constatée entre les valeurs de l'indice de podzolisation et celles de l'indice de drainage de Hénin.

ZUSAMMENFASSUNG

Der Bericht behandelt podsolierte Böden, die in Angola vorkommen, unter klimatischen und pflanzlichen Verhältnissen die sich von denen gewöhnlich mit Podsolierung verbundenen Verhältnissen auffallend unterscheiden.

Für die Untersuchung wurden fünf Profile ausgesucht mit besonderer Berücksichtigung auf die Merkmale der organischen Stoffe, einschliesslich der Bestimmung der verschiedenen Humus-Zusammensetzungen und deren quantitativen Verschiedenheiten durch das ganze Profil.

Die Daten schienen anzudeuten, dass der untersuchte Humus zu der oligotrophischen "moder" Type gehörte, dessen Podsolierungsweise wohl bekannt ist.


Man fand auch, dass die untersuchten Böden (stark durchlässig, basenarme Materiale bekannt als die Hauptfaktoren der Podsolierung) eine ziemlich gleichmässige Übereinstimmung zwischen dem Podsolierungs-Verzeichnis und Hénin's Entwässerungs Verzeichnis aufwiesen.
THE NATURE OF BACTERIUM-CLAY INTERACTIONS AND ITS SIGNIFICANCE IN SURVIVAL OF RHIZOBIUM UNDER ARID CONDITIONS

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Although many factors influence the distribution of legumes generally, an important factor in arid and semi-arid areas could be the ability of the appropriate root-nodule bacteria to survive in the soil during hot, dry periods. The majority of legumes found in the more arid regions of Australia are those associated with the so-called slow-growing types of root-nodule bacteria. These legumes include species of Acacia, Swainsona and Psoralea (Beadle, 1964). Plants associated with fast-growing bacteria, namely the indigenous Trigonella suavissima Lindl. and introduced Medicago species, are well established in semi-arid areas but are normally restricted to soils of relatively heavy texture (Hely and Brockwell, 1962; Beadle, 1964). In the present review an attempt is made to assess the relative roles of (a) the heat tolerance of these groups of bacteria, and (b) the interaction between the bacterial cells and clay colloids in influencing the survival of rhizobia and, consequently, the distribution of legumes in these arid and semi-arid areas. The nature of the interaction between bacterial cells and colloidal montmorillonite also is considered.

Heat Susceptibility of Root-Nodule-Bacteria and the Modifying Effects of Clay Colloids

Vandecaveye (1927) presented evidence that cells of Rhizobium leguminosarum were able to survive repeated exposure to hot, dry summers in certain soils. In a study of failure of peas on a sandy soil, Vyas and Prasad (1960) attributed the poor survival of R. leguminosarum to a lower heat tolerance than that of slow-growing bacteria associated with species of Dolichos, Vigna, Cyamopsis and Cicer. They suggested that clay in a heavier textured soil protected the pea bacteria from the effects of high temperatures. Sanderson (1962) reported that R. trifolii naturally present in an air-dry loamy soil survived temperatures in excess of 90°C.

An apparent relationship of nodulation failure in clovers and medics with textural properties of the soils (Marshall et al., 1963) and the success in overcoming the problem by additions of montmorillonite to a sandy soil (Marshall and Roberts, 1963) suggested an investigation of the effects of colloidal materials on the heat susceptibility of root-nodule bacteria in dry soils. Using sterile soil inoculated with R. trifolii strain TA1, Marshall (1964) demonstrated that this organism failed to survive in yellow and
grey sandy soils heated to 70°C, whilst excellent survival was obtained in red sands and soils of heavier texture. The addition of montmorillonite, illite or haematite to a grey sand protected *R. trifolii* from the effects of desiccation and exposure to high temperatures. On the other hand, kaolinite and goethite failed to protect the bacteria. A comparison of the effects of kaolinite, illite and montmorillonite on survival is given in Fig. 1. It is important to note that the protective effects observed were not related to biotic interactions (e.g. adsorption of antibiotics) since sterile soil systems were employed. Another fast-growing species *R. meliloti* also was very susceptible to heating in dry soils. In view of the absence of nodulation

![Fig. 1.—Influence of clays on survival of *R. trifolii* TA1 in a grey sandy soil. S = sand alone; K, M or I = sand + 5% kaolinite, montmorillonite or illite. Soil dried overnight at 30°C following inoculating and then exposed at the temperatures shown for a further 5 hr. Log initial inoculum/g soil = 8.55 (adapted from Marshall, 1964).](image)

problems on lupins grown in the same sandy soils (Marshall *et al.*, 1963), it is significant that the slow-growing species *R. lupini* and *R. japonicum* exhibited a remarkable degree of heat resistance in such soils (Marshall, 1964).

Ecological Implications of the Heat Susceptibility of Root-Nodule-Bacteria in Dry Soils

Based on the observed differences in heat susceptibility of different root-nodule bacteria in dry soils, it is reasonable to postulate that legumes associated with fast-growing bacteria may be limited to relatively cool, moist regions unless suitable protective colloids are present in soils of
hot, dry regions. The slow-growing bacteria and, consequently, their associated legumes may be more adapted to dry, hot conditions where the soils lack even low levels of montmorillonite or illite. These proposals could account for the very restricted distribution of _Trigonella suavissima_ and _Medicago_ species and the more widespread occurrence of legumes such as _Acacia_ and _Psoralea_ in arid and semi-arid regions of Australia (Beadle, 1964).

These views are supported by the findings of Vyas and Prasad (1960). The report by Wilkins (1967) of an ecological adaptation to high temperature resistance by strains of _Rhizobium_ nodulating _Acacia_, _Lotus_ and _Psoralea_ is open to criticism. Although survival in heated soil is related to the inherent resistance of a particular strain of root-nodule bacteria, it is also a function of the initial numbers of the bacteria present in a soil. In comparing survival of rhizobia in different soils, Wilkins has made no attempt to determine initial numbers present in these soils. In addition, the high tolerance to heat of _R. meliloti_ reported by Wilkins may result from protection by clay colloids in the soils employed. Brockwell and Phillips (1965) have noted that peat afforded protection to _R. meliloti_ under hot, dry conditions.

### Rhizobium-Montmorillonite Interaction

In any study of the protective effect of montmorillonite on root-nodule bacteria the nature of the interaction between the bacterial cells and the clay must be considered. A lack of information concerning the surface charge characteristics of the root-nodule bacteria prompted a detailed study of these properties (Marshall, 1967). Based on the electrophoretic mobilities of the bacteria over the pH range from 2.0 to 10.7, evidence was obtained for a simple acidic (carboxyl) surface on slow-growing rhizobia. The fast-growing _R. trifolii_ TA1 exhibited similar surface properties, whilst most of the fast-growing bacteria examined possessed a more complex surface consisting predominantly of carboxyl groups with some basic (amino) groups also present. These amino groups may be distributed either in a diffuse or a

![Diagram](image)

**Fig. 2.** Diagrammatic representation of the dissociated surface ionogenic groups on bacteria possessing either a simple carboxyl or a complex carboxyl-amino surface. For the latter type, alternative arrangements of the amino groups are shown (adapted from Marshall, 1967).
non-diffuse pattern over the bacterial surface. The situation at \( \text{pH} \ 7.0 \) where both the carboxyl and the amino groups are dissociated (Marshall, 1967) is presented in Fig. 2.

A uniform increase in the electrophoretic mobility of 7 cultures of *Rhizobium* spp. following the addition of colloidal montmorillonite and the fact that the mobility values at high clay concentration (100 \( \mu \)g clay/ml) approached that of Na-montmorillonite itself suggests that the clay almost completely envelopes the outer surface of the cells (Marshall, 1968). The amount of clay adsorbed by the different bacteria was determined from electrophoretic mobility data by the limiting mobility method of Nevo *et al.* (1955). No consistent relationship was observed between the amount of clay adsorbed per cell and the relative growth rate, ionogenic surface and surface charge density of the bacteria. There was a definite correlation, however, between the amount of clay adsorbed per unit area of cell surface and the ionogenic properties of the bacterial surface. Bacteria with a simple carboxyl surface (including *R. trifolii* TA1) adsorbed from 0.50 to 0.59 \( \times 10^{-6} \ \mu \)g clay/\( \mu \)m\(^2\), whilst those with a complex carboxyl-amino surface adsorbed from 0.26 to 0.32 \( \times 10^{-6} \ \mu \)g/\( \mu \)m\(^2\). These values are of the same order of magnitude as those found by Lahav (1962) for adsorption of Na-montmorillonite on *Bacillus subtilis*. The higher level of adsorbed clay corresponds to approximately 200 \( \mu \)m\(^2\) of clay surface per \( \mu \)m\(^2\) of bacterial surface. In order to account for these large amounts of clay it is necessary to consider an edge-to-face association between the clay platelets and the bacterial surface rather than a face-to-face association. Spectrophotometric assay at 245 m\( \mu \) of the montmorillonite remaining in suspension at different ionic strengths of \( \text{NaCl} \) indicated that the clay was flocculated at the ionic strength of 0.05 used in these studies (Marshall, 1968).

A diagrammatic interpretation of the observed difference in the amount

![Diagram of clay association](image-url)

*Fig. 3.—Diagrammatic representation of the association between clay platelets and bacterial surfaces. A predominantly edge-to-face arrangement is shown for a simple carboxyl surface and a tendency towards some face-to-face association for complex carboxyl-amino surfaces (adapted from Marshall, 1968).*
of clay adsorbed by cells with carboxyl and carboxyl-amino surfaces is presented in Fig. 3. Electrostatic attraction between the positive charges at the edge of clay platelets and the negatively charged (carboxyl) surface of the bacterial cell could result in an edge-to-face relationship as depicted. The presence of positively charged amino groups at the bacterial surface would tend to repel the edges of the clay platelets and, particularly if the amino groups occur close together, may result in a limited degree of face-to-face interaction.

It is suggested that this montmorillonite envelope might protect the bacteria from exposure to high temperatures by modifying the rate of water loss from the cells.

Preliminary evidence indicates that Na illite increases the apparent electrophoretic mobility of bacterial cells of normally low mobility, but has no influence on cells with mobilities similar to that of the illite itself. Interactions between bacteria and kaolinite, haematite or goethite have not yet been investigated.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. A. V. Blackmore, C.S.I.R.O., Division of Soils, and Dr. J. A. Beattie of this Department for their constructive comments on the interactions between cells and clays.

REFERENCES


Summary

It is proposed that the widespread distribution in arid and semi-arid regions of legumes associated with slow-growing bacteria may be related to the ability of these bacteria to survive exposure to high temperatures in dry soils. The limited distribution of medic bacteria in such areas may be a function of the low heat resistance of these bacteria and the need for the protective effect of certain soil colloids.

Electrophoretic studies of montmorillonite-bacterium interactions suggest an edge-to-face association between clay platelets and the bacterial surface. Cells possessing a simple carboxyl surface adsorbed almost twice as much Na montmorillonite per unit area of cell surface as cells with a complex carboxyl-amino surface.

Résumé

Il est proposé que la répartition étendue dans des régions arides et semi-arides de plantes légumineuses associées à des bactéries à croissance lente, est liée à la capacité de ces bactéries de survivre à l'exposition aux températures élevées dans des sols secs. La répartition limitée des bactéries médiques dans de telles régions est peut-être une fonction de la résistance faible de ces bactéries à la chaleur et du besoin de l'effet protecteur de certains colloïdes dans le sol.

Des études électrophorétiques des interactions du montmorillonite avec les bactéries suggèrent une association de champ-a-face entre des plaquettes argileuses et la surface bactérienne. Des cellules possédant une simple surface de carboxyl adsorbèrent presque deux fois plus de montmorillonite Na par unité de surface que des cellules à surface complexe de carboxyl-amino.

Zusammenfassung

Es wird angenommen, dass die weitverbreitete Verteilung von Leguminosen in Verbindung mit langsam wachsenden Bakterien in ariden und semiariden Regionen, mit der Fähigkeit dieser Bakterien, der Auslieferung an hohe Temperaturen in trockenen Böden zu widerstehen, verbunden ist. Die beschränkte Verteilung medizinischer Bakterien in solchen Gebieten ist vielleicht eine Funktion des geringen Hitzewiderstandes dieser Bakterien und des Bedürfnisses für den schützenden Effekt gewisser Bodenkolloide.

Enzymes play an essential part in the life of the soil as biological catalysts. The biochemical processes of soil formation are carried out by the participation of enzyme systems. Soil enzymes are the by-products of living organisms which inhabit the soil. After secretion by these organisms, some enzymes are inactivated, and the remainder is absorbed by soil particles where it retains its activity for a long time. The extent of enzyme fixation is closely related to soil-formation conditions, the physico-chemical properties and the dispersion of soils (Hofmann 1952, Hoffmann 1959, Galstyan 1963). In this connection it is of considerable interest to elucidate the nature of enzyme fixation by soil particles of various sizes.

Investigations were carried out with heavy-textured surface soil samples from Armenia: a leached chernozem, a non-calcareous brown soil and a reclaimed saline alkali soil.

**Experimental Methods**

Our method can be briefly described as follows: pure enzyme preparations were added to the soils devoid of enzyme activity, and followed by a detailed study on the distribution and fixation of enzymes in the various size fractions. Laboratory experiments on sterilized brown and reclaimed alkaline sodium soils were carried out. The chemical composition of the reclaimed alkaline soil was as follows: water-soluble salts—0.11%; $CO_3^{2-}$—none; $HCO_3^-$—0.06%. The alkaline soil under natural conditions and after reclamation does not possess hydrolytic enzyme activity, and possesses insignificant dehydrogenase activity (0.02 mg TPF). To the sieved (1 mm) soils was added a water-solution of the enzyme preparation of known activity, estimated on 1 g soil per hr: invertase ($\beta$-fructofuranosidase, 3.2.1.26*) —20 mg glucose, amylase ($\alpha$- and $\beta$-amylase 3.2.1.1, 3.2.1.2)—24 mg maltose, urease (3.5.1.5)—16.3 mg ammonium and glucosedehydrogenase (1.1.1.47)—11 mg triphenylformazan (TPF). Interaction between enzymes and soils took place in about 20 days, during which period toluene was added periodically as an antiseptic.

The complete dispersion of the soil was achieved by grinding soil for 30 minutes in a rubber-tipped pestle and mortar, to form a dough. This was followed by transferring the dispersed suspension into an 18-litre glass vessel to separate the soil into the various particle sizes. The velocity

*From index of enzymes, accepted by the Commission on Enzymes of the International Union of Biochemistry, in 1961.
of sedimentation of the soil particles was estimated according to Stokes' formula. The suspension was syphoned off and evaporated to dryness in an oven at 45°C. The air-dried fractions were passed through a sieve (0.25 mm) after crushing the lumps in an agate mortar. This procedure for separating the soil into fractions was chosen to avoid the influence of chemical agents, which might alter the enzyme activity and the properties of soil particles. The separated as well as the unseparated soil samples were analyzed.

Enzyme activity was estimated by methods worked out in our laboratory. Invertase was estimated as follows: 1 g samples of the fractions and soils were placed in 50 ml flasks, to which were added 5 ml of a 5% sucrose solution, 5 ml of an acetate buffer (pH 4.7) and 5 drops of toluene. The flasks were closed by cork stoppers, shaken and placed in an incubator at 30°C for 24 hours; after which the contents were filtered and the reducing sugars in the filtrate were estimated by the Bertrand method. Invertase activity was expressed in mg of glucose per g of sample for a period of 24 hours.

Amylase activity was determined as follows: 1 g samples of the fractions and soils were placed in flasks to which were added 5 ml of 2% starch paste solution, 5 ml acetate buffer (pH 5.9) and 5 drops of toluene. The enzyme reaction took place in an incubator at 30°C for 24 hours, after which the contents were filtered and the reducing sugars in the filtrate were determined by the Bertrand method. Amylase activity was expressed in mg of maltose per g of sample for 24 hours.

Urease activity was determined by the micro-kjeldahl procedure. 1 g samples of the fractions and soils were placed in 50 ml flasks to which were added 5 ml of a 10% urea solution, 5 ml of phosphate buffer (pH 6.7) and 5 drops of toluene. After shaking, the flasks were incubated in an incubator at 30°C for 24 hours. At the end of the incubation period the contents were separated by decantation and 15 ml of 1 M potassium chloride solution was added to the soil samples to completely remove the adsorbed ammonia. The contents were shaken for 5 minutes and filtered. The soil samples were thoroughly washed with distilled water to a final volume of 50 ml. Ammonia was distilled off from the filtrate (10 ml) after adding 5 ml of 2% NaOH solution. Distillation of the ammonia was carried out for 15 minutes. The amount of ammonia produced by the enzyme was estimated by titration. Urease activity was expressed in mg NH₃-N per g of sample for 24 hours.

Dehydrogenase activity was measured by adding 10 mg of CaCO₃ to 1 g of soil sample in a vacuum flask, the contents being thoroughly stirred and moistened by a mixture of 1% glucose solution and 1 ml of 1% 2, 3, 5—triphenyltetrazolium chloride solution (TTC). The air from the flask was pumped out for two minutes (to 10-12 mm Hg). After carefully shaking the contents, the flasks were incubated at 30°C for 24 hours, and then 25 ml of ethyl alcohol was added, the flasks shaken again for 5 minutes and the contents filtered. The optical density of the coloured formazan complex was read at 500-600 nm using 5 mm cuvettes. Dehydro-
<table>
<thead>
<tr>
<th>Soil Condition</th>
<th>Fixation of Enzymes (%)</th>
<th>Percent</th>
<th>Humin</th>
<th>Humus</th>
<th>Total</th>
<th>Percent</th>
<th>Enzymes in Each Fraction %</th>
<th>Enzymes in Each Fraction %</th>
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*Not determined.
Expressed in terms of mg of nitrifier per gram.
Expressed as mg of Humin.
Expressed as mg of humus.
Expressed as mg of enzymes.

**Table 1**

**FIXATION OF ENZYMES BY SOIL FRACTIONS OF VARIOUS PARTICLE SIZES**

<table>
<thead>
<tr>
<th>Soil Condition</th>
<th>Fixation of Enzymes (%)</th>
<th>Percent</th>
<th>Humin</th>
<th>Humus</th>
<th>Total</th>
<th>Percent</th>
<th>Enzymes in Each Fraction %</th>
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genase activity was assayed in terms of triphenylformazan (mg/g soil/24 hr).

Check analyses were included using soil samples and fractions sterilized by dry heat at 180°C for 3 hours, as well as substrate without soil.

**DISCUSSION OF RESULTS**

The results show that enzyme fixation mainly took place on the clay and silt fractions. The data obtained (Table 1) show that the nature of the fixation of the enzymes by soil varied considerably with particle size. Most of the invertase was bound to the fine silt fractions (0.005-0.001 mm) whereas amylase was bound to the fine silt and clay fractions. Amylase was not discovered in particles exceeding 0.01 mm. Urease was associated with clay, fine and medium silt fractions (0.01-0.005 mm). This enzyme, in contrast to the others, was also found with the fine sandy fractions (0.1-0.05 mm). Dehydrogenase was found mainly with the clay fractions and its activity decreased in the coarser particles. Enzyme activity as a whole was absent from sand particles.

A similar pattern of enzyme activity was observed for the textural fractions of natural soils (Table 2). The distribution of enzyme activity in the different soil fractions of the non-calcareous brown soil and the leached chernozem was similar to that which occurred when enzymes were artificially added.

Therefore a correlation exists between the degree of enzyme fixation and the soil particle size. The enzymes are bound in the soil not only by the colloidal particles, but also by the silt fractions (Hoffman 1959). Fine and medium silt fractions, which are the main enzyme fixers, contain definite amounts of micro-aggregates, consisting of organic-matter-binding clay particles. During enzyme fixation by silt and clay particles, the molecular structure of the enzymes is maintained as a whole, especially their tertiary and quaternary patterns, which determine their catalytic properties.

Every genetic soil type has an upper limit of enzyme fixation, which is clearly demonstrated by the experimental data. Although significant amounts of enzymes were added to the reclaimed and sterilized brown soil samples, they were bound in approximately equal amounts, very near to those found in natural brown soils. We can see that each soil is characterized by a definite capacity to fix individual enzymes.

The distribution of enzyme activity according to particle size is closely connected with organic matter. Hydrolase activity was found to be related with the amount of organic matter. Most of the organic matter was present in the silt fractions, which were characterized by large amounts of hydrolytic enzymes—invertase, amylase and urease. Humin substances play an essential part in the fixation of enzymes. They are bound as humates in the silt and clay fractions. Considerable amounts of humic acid are associated with the silt fractions. The humus binding capacity falls sharply as the particle size is increased.

Dehydrogenase activity is correlated with the total amount of microorganisms. The total amount of adsorbed microorganisms and their separate
# Table 2

The distribution of enzyme activities on soil fractions of different particle size

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<th>Fractions, mm</th>
<th>Percent of each fraction</th>
<th>Humus</th>
<th>Total nitrogen, mg/100 g soil</th>
<th>Percent humus in each fraction</th>
<th>Total microorganisms, 10^6 g</th>
<th>Invertase* per gram of particles</th>
<th>Total mg 100 g soil</th>
<th>Amylase* per gram of particles</th>
<th>Total mg 100 g soil</th>
<th>Urease‡ per gram of particles</th>
<th>Total mg 100 g soil</th>
<th>Dehydrogenase§ per gram of particles</th>
<th>Total mg 100 g soil</th>
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Brown non-calcareous

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Lead-contaminated

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<th>Total nitrogen, mg/100 g soil</th>
<th>Percent humus in each fraction</th>
<th>Total microorganisms, 10^6 g</th>
<th>Invertase* per gram of particles</th>
<th>Total mg 100 g soil</th>
<th>Amylase* per gram of particles</th>
<th>Total mg 100 g soil</th>
<th>Urease‡ per gram of particles</th>
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<td>0.005 - 0.001</td>
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</tbody>
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* Expressed as mg of glucose.
† Expressed as mg of maltoose.
‡ Expressed as mg of NH₃.
§ Expressed in terms of mg of triphenylformazan.

Nut determined.
groups (bacteria, fungi, actinomyces, etc.) is relatively higher in the clay fractions than in any other soil particles. An increase in particle size caused a fall in both the degree of microorganism adsorption and the dehydrogenase activity. Although the coarser fractions adsorb a significant amount of microorganisms, enzymes are not adsorbed, because the coarser particles do not possess either organic substances or clay minerals.

Enzyme fixation also depends on mineralogical and chemical composition of the fractions. In the separated fractions the contents of primary and clay minerals were studied. The coarser fractions consisted essentially of feldspars, including plagioclase, containing about 85-95% $\text{SiO}_2$. It is known that $\text{SiO}_2$ possesses insignificant adsorbing capacity. As a result the coarser particles do not bind enzymes. The coarser fractions from the primary minerals also contain considerable amounts of hypersthene, hornblende, augite, magnetite, etc. The content of heavy minerals decreases in the highly dispersed soil particles. The clay and fine silt fractions contain few heavy minerals. Thus they do not participate in the fixation of enzymes.

Clay minerals (montmorillonite, hydromicas and kaolinite) predominate in the finer size fractions, particularly those containing clay and fine silt. Clay minerals contain relatively little $\text{SiO}_2$ and considerable amounts of sesquioxides. The latter act as potent enzyme adsorbents. It is known that secondary minerals have a large adsorbing capacity for enzymes and proteins (McLaren, Peterson and Barshad 1958, Kiss 1958a, 1958b).

Enzymes, which are adsorbed by textural soil fractions, are usually desorbed with great difficulty. Enzymes already combined with such fractions cannot be extracted either by organic solvents or by distilled water. It follows that the bond between the enzyme molecules and soil particles is strong. The fixation of enzymes by these fine soil particles prevents their diffusion.

During enzyme fixation an important role is played by the pH of the medium. When the pH coincides with that of the optimum for enzyme activity they are more strongly bound by soil particles.

The significant activity of the enzymes, observed after their being adsorbed by soil particles shows that the active centre of their molecule remains free. The binding of the enzyme molecules must take place using other functional groups not involved in catalytic reactions. To some extent the selective adsorption of the enzymes implies that the biochemical reactions catalyzed by them are correspondingly localized in the structural elements of the individual fractions. Therefore we may conclude that enzymes which are adsorbed by highly dispersed soil particles do not lose their unique structure or biochemical specificity.

REFERENCES


FIXATION OF ENZYMES

Summary

In an attempt to determine some peculiarities of enzyme fixation by soil fractions of various particle sizes, experiments were carried out on two surface soil-samples: non-calcareous brown soil, from which enzymes were removed by sterilization and on a reclaimed alkaline sodium soil. The alkaline soil under natural conditions does not possess hydrolytic enzyme activity, but contains some dehydrogenase activity.

The results show that enzyme fixation takes place mainly with the clay and silt fractions. Its nature is quite varied. Relatively high invertase activity was observed in the fine silt fractions; amylase activity in the fine silt and clay fractions; urease activity in the above-mentioned fractions and in the medium silt fraction; and dehydrogenase activity in the clay. Enzyme activity as a whole is less in the coarser particle fractions. Enzyme activity is absent from sand particles.

The distribution of enzyme activity is directly correlated with the amount of organic matter, adsorbed microorganisms and the mineralogical contents of the soil fractions.

Enzyme fixation by highly dispersed particles prevents their diffusion from the soil. During enzyme fixation the active centre of their molecule remains free. Thus enzymes do not lose their unique structure or their quite definite biochemical specificity.

RÉSUMÉ

Dans le but de déterminer quelques particularités de la fixation d'enzymes par les fractions du sol dont les particules se groupent suivant leur diamètre, on a fait quelques essais sur deux échantillons de sol superficiel : 1) sol brun non-calcaire dont on a extrait les enzymes par stérilisation, 2) sol alcalin sodique récupéré. Le sol alcalin, à l'état naturel, n'a pas d'activité hydrolytique, mais agit quelque peu comme déshydrogénateur.

Les résultats montrent que la fixation d'enzyme a lieu surtout pour les fractions d'argile et de limon. Le mode de fixation est varié. Une activité invertine relativement grande a été observée dans les fractions fines de limon; une activité amylase a paru dans les petites fractions de limon et d'argile; une activité urée dans les fractions ci-dessus mentionnées et dans le limon moyen et une activité déshydrogénatrice dans l'argile. L'activité enzymatique dans l'ensemble est moindre dans les fractions à particules plus grossières. Il n'y a pas d'activité enzymatique dans les particules de sable.

La distribution de l'activité enzymatique est en rapport direct avec la
quantité de matières organiques, les microorganismes adsorbés et la teneur en minéraux des fractions du sol.

La fixation d’enzyme par les particules grandement dispersées empêche leur diffusion dans le sol. Pendant la fixation d’enzyme, le centre actif de leur molécule reste libre. Ainsi les enzymes gardent leur structure propre ou leur spécificité biochimique définie.

ZUSAMMENFASSUNG

In einem Versuch, einige Besonderheiten der Enzymbindung durch Bodenfraktionen verschiedener Korngrössen zu bestimmen, wurden Experimente an zwei Oberflächenbodenproben durchgeführt: an nicht kalkhaltigem braunen Boden, aus dem Enzyme durch Sterilisation entfernt worden waren, und an einem verbesserten alkalischen Natriumboden. Der alkalische Boden weist im natürlichen Zustand keine hydrolytische Aktivität auf, dagegen aber eine wasserentziehende Aktivität.


Die Verteilung der Enzymaktivität steht in direkter Wechselbeziehung zur Menge des organischen Stoffes, sowie des adsorbierten Mikroorganismus und den mineralogischen Gehalten der Bodenfraktionen.

Die Enzymbindung durch weitherrstreute Teilchen verhindert deren Diffusion aus dem Boden; das aktive Zentrum ihrer Moleküle bleibt während der Bindung frei. Somit verlieren Enzyme nicht ihre eigene Struktur oder ihre ziemlich ausgeprägte biochemische Eigenheit.
INTRODUCTION

In a previous study (Wada and Inoue 1967), a laboratory experiment was done simulating the early reactions that occur when humic substances derived from rotting plant residues are added to soils. Two soils containing allophane and montmorillonite were compared. The differences in retention of the humic substances and their subsequent change on incubation suggested aluminium silicate clay plays an important role in the over-all accumulation of organic matter in soils.

A few studies have been made of the adsorption of soil humic or fulvic acids on clay, as reviewed by Greenland (1965), but these have only limited value, in the opinion of the authors, in investigation of the humus-clay interaction in nature. In soil, the formation of soil organic matter proceeds under the influence of both clays and other components. The formation of the clay-organic matter complex does not necessarily take place between such preformed high-molecular-weight humic or fulvic acids and soil clays. The purpose of the present study is to provide some experimental data on interactions between newly formed humic substances and soil clays.

MATERIALS AND METHODS

(a) Aqueous Extract of Humified Clover

Rotted clover leaves, a product of bacterial lysis of the plant tissue were chosen as a source of the water-soluble, newly formed humic substance. Some data on the chemical nature of similar materials were given by Kononova (1961). The methods of preparation and extraction were the same as those described previously (Wada and Inoue, 1967). The freeze dried extract had 24.6% carbon, 3.8% hydrogen, 3.7% nitrogen and 48.8% ash. Elution chromatography through Sephadex gel indicated the presence of two coloured components differing in “molecular weight”, namely less than 1,500 and 1,500 to 10,000 (Fig. 1). The higher molecular weight component was dark brown in colour, whereas the lower one was bright orange. This is illustrated in the O.D.400/O.D.600 values for the middle fractions of the respective components (Fig. 1; G15 curve). 10.4 and 20.0, where O.D.400 and O.D.600 denote the optical density of the fractionated extracts at 400 and 600 μg. The infrared absorption spectra show fairly strong absorption bands in the region from 1,660 to 1,600 cm⁻¹ and at 1,400 cm⁻¹ suggesting the presence of COO⁻ and CO-NH₂ groups.
Fig. 1.—Elution curves of humified clover extract through Sephadex gel G 50 and G 15 with exclusion limits at 10,000 and 1,500 in "molecular weight", respectively. Column dimensions: 40 x 1 cm. Eluant; water (45 ml). Sample charge: 0.5 ml (2.5 g carbon per liter).
EXTRACT OF HUMIFIED CLOVER ON CLAYS

(b) Clay Samples

TABLE 1
DESCRIPTION OF SAMPLES

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<tr>
<th>Sample</th>
<th>Clay mineral species</th>
<th>Si/Al</th>
<th>References</th>
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<tr>
<td>905</td>
<td>Allophane “Imogolite” (10 : 4)*</td>
<td>1/1.52</td>
<td>Yoshinaga and Aomine (1962a, b)</td>
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<td></td>
<td></td>
<td>1/1.89</td>
<td></td>
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<tr>
<td>1041</td>
<td>Allophane “Imogolite” (10 : 1)</td>
<td>1/1.53</td>
<td>Yoshinaga and Aomine (1962a)</td>
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<td>VA</td>
<td>Allophane</td>
<td>1/1.06</td>
<td>Aomine and Wada (1962)</td>
</tr>
<tr>
<td>VH</td>
<td>Halloysite (fully hydrated)</td>
<td>1/0.94</td>
<td>Aomine and Wada (1962)</td>
</tr>
<tr>
<td>Mt</td>
<td>Montmorillonite</td>
<td>—</td>
<td>API No. 26</td>
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<tr>
<td>W116</td>
<td>Montmorillonite illite and vermiculite</td>
<td>—</td>
<td>Wada and Inoue (1967)</td>
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* Approximate allophane : imogolite concentration ratio.

EXPERIMENTAL RESULTS

Carbon adsorbed (g /100g clay)

Fig. 2.—Adsorption of humified clover extract on Ca saturated soil clays and clays (clay solution ratio; 300 mg/25 ml).
Soil clay and clay samples used are listed in Table 1. The < 0.5 μm fraction was separated by acid or alkali dispersion and centrifugation after repeated $H_2O_2$ treatments and preserved in a dilute $NaCl$ solution. Before the adsorption experiments, aliquots of suspension containing known amounts of clay were converted to the $Ca$ form by washing with 1 N $CaCl_2$ or to the $Al$ form by dialysis against 0.001 N $AlCl_3$.

(c) Adsorption Experiments

The adsorption experiments were carried out in 25 ml of suspension containing 300 mg or 100 mg clay and varying amounts of the humified clover extract. Duplicate suspensions were shaken at 15-20°C for 1 hour, and centrifuged for 15 minutes at 8,000 G. After evaporation, the carbon content in the supernatant was determined by Tyurin’s method (Kononova 1961) while its optical density at 400 and 600 mμ was determined after appropriate dilution.

As Figure 2 indicates, the extent of adsorption of the aqueous extract of the humified clover shows a marked dependence on the main mineral

---

**Fig. 3.**—Effect of exchangeable cation of clay on adsorption of humified clover extract (clay solution ratio: 100 mg/25 ml). Open circles; $Ca$ system. Closed circles; $Al$ system. Figures in brackets; $pH$ of clay-humus system. $pH$ of clay system; 1041, 5.4 ($Ca$) and 4.3 ($Al$); VA, 7.0 ($Ca$) and 5.9 ($Al$); Mt, 7.2 ($Ca$) and 4.2 ($Al$).
species of the clays, although all these give Langmuir type adsorption curves. Adsorption maxima of 14.5 to 16.7 g carbon per 100 g clay are found for the allophanes including "imogolite" with a Si/Al ratio less than 1/1.5, whereas much smaller values, 0.7 to 2.1 g, are found for layer silicates with expandable 2:1 and 1:1 structures. An allophane with a Si/Al ratio of about 1/1 gives an intermediate value.

The change of the main exchangeable cation of the clays, from Ca to Al and the associated change in pH of either the clay or the clay-humus system essentially do not affect the situation (Fig. 3). Since appreciable amounts of K are introduced from the rotted clover, the former Ca and Al systems are actually Ca-K and Al-K systems. The amount of K increases, and the pH of the system rises with increasing concentration of organic matter. The atomic ratio of Ca/K or Al/K in the system varies roughly from 3/1 to 1/10 in the concentration range studied. No difference, however, was found between the Al and Ca systems even at low concentrations of organic matter.

No positive correlation exists between the adsorption maximum and

<table>
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<th>Sample</th>
<th>905</th>
<th>1041</th>
<th>VA</th>
<th>VH</th>
<th>Mt</th>
<th>W116</th>
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<td>Maximum adsorption (C, g/100 g clay)</td>
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<td>14.5</td>
<td>6.1</td>
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<tr>
<td>Extractable iron oxides %</td>
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<td>0.2</td>
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Table 2

The colour change of the clover extract due to adsorption was deter-

| Sample | Percent reduction in carbon concentration | Percent reduction in optical density at 400 mμ | Percent reduction in optical density at 600 mμ | O.D. 400/O.D. 600 
Before adsorption | After adsorption |
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<td>905</td>
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<td>79</td>
<td>90</td>
<td>12.7</td>
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<tr>
<td>Mt</td>
<td>26</td>
<td>30</td>
<td>34</td>
<td>12.7</td>
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</table>
mined for an allophane and a montmorillonite clay. The results (Table 3) demonstrate some preferential adsorption of coloured components, particularly those of darker brown colour. This preference is much more marked for allophane than montmorillonite. As already described in the MATERIALS section, there is a close correlation between "molecular weight" and colouration of the humic components. The result then suggests that the higher molecular weight components (1,500-10,000) are preferentially adsorbed. This supposition was confirmed by comparison of the elution chromato-

A

Organic complex

120 °C

Air dry

H₂O₂ treated

120 °C

Air dry

8 13 18 Å
Fig. 4.—X-ray diffraction patterns of clay-humus complexes and their $H_2O_2$-treatment products. Parallel orientation.

A. Ca—allophane (905)—humified clover extract system.
B. Ca or Al—montmorillonite (Mt)—humified clover extract system.
graphs of the humic extract before and after adsorption. In the case of the allophane, the ratios of the higher to the lower molecular weight components estimated from the sum of the respective O.D.\(_{600}\) and O.D.\(_{400}\) terms, decreased from 5.9 to 1.3 and from 3.0 to 0.7 respectively.

The X-ray diffraction patterns of the allophane-humus complex and its \(H_2O_2\) treatment product are reproduced in Figure 4A. A modification possibly due to the presence of the humus molecule in the “imogolite” structure is seen in the development of an 18.8A peak within the broad 13 to 18A diffraction band upon heating at 120°C. A similar result was obtained in comparison between the clay and clay-humus complex.

In montmorillonite, a partial penetration of the humus molecule between the silicate layers is suggested (Fig. 4B). The appearance of the 12.6A peak is due to the introduction of \(K\) from the rotted clover. The approximate atomic ratios of \(Ca\) to \(K\) and \(Al\) to \(K\) in the system are about 1 to 1. In the air-dry state, the adsorbed humus molecule stabilizes the interlayer spaces against collapse due to the introduction of \(K\). At 110°C, both of the humus-clay complexes collapse to 10.5A, so penetration may occur only at the edges.

**DISCUSSION**

The magnitude of the differences between the allophane and the layer silicates is important in view of the large differences in the organic matter content between the volcanic ash and non-volcanic ash soils, under comparable climate and vegetation. No such large difference was found between allophane and layer silicates in the adsorption of humic acids extracted from soils (Kobo and Fujisawa 1963, 1964).

In the present system, the access of the humus molecule to the surface of allophane is ensured by a neutral to slightly alkaline \(pH\), when allophane has few net electric charges. Simultaneous operation of two adsorption mechanisms could be deduced from the present observation. The first is the interaction between the positive and negative charges of the allophane surface and the humus molecule, respectively. The adsorption curves can be explained by assuming the end-on adsorption of monofunctional humates on the anion-exchange sites, such as \(Al(OH)\_2^+\) groups in allophane. The preferential adsorption of humus molecules with “molecular weights” 1,500 to 10,000 and 50 percent carbon content suggests that the order of anion-exchange capacity 20 to 3 m-equiv. per 100 g clay could account for the observed extent of adsorption, i.e. 15 g carbon per 100 g clay. The blocking of the anion exchange sites of allophane with humus accumulated in nature was observed by Iimura (1961). The increase in adsorption with decreasing \(Si/Al\) ratio may also support the cation-anion interaction.

It is unlikely, however, that such localized interactions alone can stabilize the adsorbed, high-molecular-weight humus molecule. As our previous study (Wada and Inoue 1967) indicated, once formed the allophane-humus complex is very stable against leaching and microbial degradation. This could be expected, as adsorption is due essentially to interactions between organic and inorganic macro-molecules, and where bonding
occurs at a large number of points, largely due to van der Waals interactions. If the humus molecules have somewhat flexible, linear configurations, a hypothetical, porous chain-structure of allophane and "imogolite" (Wada 1967) would favour this type of multiple interaction.

In the layer silicate systems, observed saturation levels are fairly close to those found for K-layer silicates-humic or fulvic acid systems (Evans and Russell 1959). The saturation at a low concentration of organic matter indicates the presence of only limited adsorption sites on the clay and suggest ion-ion attraction. X-ray studies indicate only a partial penetration of the humus molecule, with cations at or near the edge surface probably acting as bridges linking the humus molecules to clay. Since the work of Meyer (1935), a similar "edge-cation" or "edge-oxide" linking mechanism has been suggested for the interaction between humic or fulvic acids and layer silicates. An important difference between the present humified clover extract and the soil humic or fulvic acids is in the effect of exchangeable cations or sesquioxides on adsorption. In the latter systems, the presence of di- or polyvalent cations and sesquioxides often causes a remarkable increase of adsorption (e.g. Evans and Russell 1959, Kobo and Fujisawa 1963, 1964, for review, see Greenland 1965).

REFERENCES
Greenland, D. J. (1965)—Soils Fertil. 28, 521-532.

SUMMARY
Adsorption of newly formed humic substances from aqueous extracts of humified clover by various soil clays and clays was measured. The carbon concentration ranged up to 5 g per liter in the Ca-K system. All systems gave Langmuir type adsorption curves, but there were remarkable differences in the extent of adsorption between the minerals. The adsorption maximum expressed in g carbon per 100 g clay was 15 to 17 for allophanes with a Si/Al ratio less than 1/1·5; 6·1 for allophane with a Si/Al ratio 1/1; 0·7 for halloysite and 1·4 to 2·1 for montmorillonites. Neither the change of the exchangeable cation of the clay from Ca to Al, nor the presence of extractable iron oxides affected the differences in the extent of adsorption between the minerals. A preferential adsorption of darker coloured components with higher molecular weight (1,500 to 10,000) was very marked for allophane.

Adsorption mechanisms were considered in the light of these results and those of the X-ray analyses. The magnitude of the differences between
amorphous and crystalline layer silicates is important in view of the large differences in the accumulation of organic matter between the volcanic ash and non-volcanic ash soils.

RÉSUMÉ

L'adsorption de l'extrait aqueux d'un trèfle humifié sous la forme d'une substance humique nouvellement formée sur différents sols argileux et argiles fut mesurée jusqu'à atteindre une concentration en carbone de 5 g par litre dans le système Ca-K. Elles donnèrent toutes des courbes d'adsorption du type Langmuir, mais il y avait des différences remarquables dans l'étendue d'adsorption entre les minéraux. L'adsorption maximum exprimée en g de carbone par 100 g d'argile fut 15 à 17 pour les allophanes avec le rapport Si/Al de moins de 1/1,5, 6,1 pour l'allophane avec un rapport Si/Al de 1/1, 0,7 pour l'halloysite et 1,4 à 2,1 pour les montmorillonites. Ou, soit le changement du cation échangeable de Ca en Al de l'argile, ou, soit la présence d'oxydes de fer pouvant être extraits, n'affectèrent pas les différences du niveau de l'adsorption entre les minéraux. Une adsorption préférentielle des composés de couleur plus sombre à poids moléculaire plus élevé, 1500 à 10000 fut très marquée pour l'allopaphane.

On examina les mécanismes d'adsorption à la lumière de ces résultats en même temps que des analyses par rayons X. L'ampleur des différences entre les silicates en couche amorphe et cristalline est importante en considération des grandes différences dans l'accumulation de la matière organique entre les sols de cendres volcaniques et ceux de cendres non-volcaniques.

ZUSAMMENFASSUNG

Die Adsorption von einem wässerigen Extraktd humifizierten Klees, als ein neugeformter Humusstoff, auf verschiedenen Boden-Tonen und Tonen wurde bis zu einer Kohlenstoff-konzentration von 5 g per Liter in dem Ca-K System gemessen. Man erhielt in allen Fällen Adsorptions-Kurven des Langmuir Typs, aber es bestanden merkliche Unterschiede im Grad der Adsorption zwischen den Mineralen. Die in g Kohlenstoff pro 100 g Ton ausgedrückte Maximaladsorption betrug 15 bis 17 für Allophan mit dem Si/Al Verhältnisquotient kleiner als 1/1,5, 6,1 für Allophan mit dem Si/Al Verhältnisquotient 1/1, 0,7 für Halloysit und 1,4 bis 2,1 für Montmorillonite. Weder der Wechsel der austauschbaren Kation von Ca auf Al des Tones noch die Anwesenheit ausziehbarer Eisenoxide hatten eine Wirkung auf die Unterschiede im Grad der Adsorption zwischen den Mineralen. Eine bevorzugte Adsorption der dunkler gefärbten Bestandteile mit höherem Molekulargewicht — 1500 bis 10000 — war im Falle von Allophan sehr auffallend.

Die Adsorptions-Mechanismen wurden in Hinblick auf diese Resultate und zusammen mit der Röntgen-Analyse betrachtet. Im Hinblick auf die grossen Unterschiede in der Akkumulation organischer Stoffe zwischen den vulkanischen Asche-und den nicht vulkanischen Ascheböden ist die Grösse der Unterschiede zwischen den amorphen und den kristallin-schichtigen Silikaten wichtig.
ACTIVITIES OF ENZYMES HYDROLYSING SUCROSE AND STARCH IN SOME GRASSLAND SOILS

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INTRODUCTION

Several aspects of enzymes in soils have been recently reviewed by Durand (1965), Kozlov (1966), and Voets and Dedeken (1966). The few systematic examinations of different groups of soils have shown that both qualitative and quantitative differences can occur between enzymes in different soils but that interpretation of results is often difficult because of the many variables involved.

The distribution of enzymes hydrolysing sucrose and starch in different major groups of New Zealand grassland soils and their relationships to climate and other soil factors are considered in this report.

MATERIALS AND METHODS

To standardise the influence of vegetation as much as possible, samples were taken under grazed introduced pastures of grasses and clovers or in tussock grassland adjacent to plants of Festuca novae-zelandiae and Poa colensoi. The tussock-grassland soils were formed on schist parent material on the Old Man Range, Central Otago, and were approximately 45 cm deep.

Topsoils were sampled at a depth of 1 to 7.5 cm, generally as 2.5 cm diameter cores. Samples were also taken from the middle of the A, AB, and B horizons of the Old Man Range soils. Several cores were pooled, sieved through a 2 mm sieve and the fine material stored, without preliminary air-drying, at 4°C; under these storage conditions, enzyme activities remain essentially unchanged (Ross, 1965 b).

Analytical procedures have been described by Ross (1965 a, 1966). One milli-unit (mU) of enzyme is the amount that will catalyse the hydrolysis of carbohydrate to give $1 \times 10^{-3}$ μ moles “glucose” per minute at 37°C or 30°C on incubation in the present systems with toluene, acetate-phosphate buffer (pH 5.5) and substrate; values for the quantity of sucrose or starch hydrolysed were corrected for the activity of a soil on incubation with toluene and buffer without added substrate. Dehydrogenase activities were determined by a modification of Thalmann’s method (1966) carried out at 30°C in a Tris buffer (pH 7.6) containing triphenyltetrazolium chloride.

In most of these studies, data were subjected to analyses of variance and covariance and relationships between properties assessed by the calculation of simple and multiple correlation coefficients.
RESULTS

Influence of vegetation

Initial experiments with three soils showed that enzyme activities could be significantly different under broadleaf forests and under pastures; these differences could not be attributed solely to differences in pH values or contents of moisture or organic C (Ross, 1966). In pastures, enzyme activities could also be influenced considerably by the nature of the different pasture plants. At each of four sites, the ratios of enzymes hydrolysing sucrose to enzymes hydrolysing starch were always significantly higher under single spaced plants of cocksfoot (*Dactylis glomerata*) and prairie grass (*Bromus* spp.) than under plants of red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) (Ross, 1966). Enzyme activities were not significantly different under the tussock grasses *Festuca novae-zelandiae* and *Poa colensoi*, at the same sites on the Old Man Range (Ross and Roberts, 1968).

Influence of period of sampling

Activities of these enzymes generally showed significant differences over a year in eight pasture soils sampled at bi-monthly intervals (Ross, 1965a) but a regular seasonal pattern was not evident. In the four tussock-grassland soils, sampled at three different periods, differences between periods were found in some of the topsoil samples but not generally in pit samples taken at different horizons (Ross and Roberts, 1968).

Influence of soils

At different sites, the activities of these enzymes could differ significantly under the same species of spaced pasture plants (Ross, 1966). Values of enzyme activities could also vary widely in forty-three different pasture soils. Enzymes hydrolysing sucrose ranged from 72 to 348 mU/g soil and enzymes hydrolysing starch from 1.3 to 59.8 mU/g soil. Some patterns were apparent in the values of these activities in different groups of zonal soils, formed from siliceous rocks. In these soils, enzymes hydrolysing starch increased and ratios of enzymes hydrolysing sucrose to enzymes hydrolysing starch decreased from brown-grey earths to yellow-brown earths. Negative and highly significant relationships between annual rainfall or soil moisture and sucrose-hydrolysing enzymes became evident only when their activities were calculated on the basis of soil organic C.

Shallow soils over porous gravels were found to have features more usually associated with soils in drier environments and those with impeded drainage could be correlated with wetter soils.

In general, enzyme values in intrazonal soils were very similar to those found in the zonal soils under similar climatic conditions.

Differences between the four tussock-grassland soils were also evident (Table 1). Starch-hydrolysing enzymes again differed with soil group. In contrast to the pasture soils, enzymes hydrolysing sucrose also differed with soil groups whereas enzyme ratios did not. In the pit samples, enzyme
### Table 1

<table>
<thead>
<tr>
<th>Soil no.</th>
<th>Soil</th>
<th>Mean annual temperature, °C</th>
<th>Annual precipitation, mm</th>
<th>Moisture, %</th>
<th>pH</th>
<th>Organic C%</th>
<th>Clay %</th>
<th>No substrate added</th>
<th>Substrate added Sucrose</th>
<th>Starch</th>
<th>Sucrose Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wharekaka st.l.</td>
<td>12.5</td>
<td>760</td>
<td>15</td>
<td>6.6</td>
<td>1.8</td>
<td>17</td>
<td>2.3</td>
<td>97</td>
<td>10.5</td>
<td>9.4</td>
</tr>
<tr>
<td>2</td>
<td>Pirinoa st.l.</td>
<td>12.5</td>
<td>1,120</td>
<td>22</td>
<td>6.1</td>
<td>3.4</td>
<td>21</td>
<td>2.5</td>
<td>122</td>
<td>14.8</td>
<td>8.5</td>
</tr>
<tr>
<td>3</td>
<td>Ngario st.l.</td>
<td>12.5</td>
<td>1,520</td>
<td>24</td>
<td>5.5</td>
<td>6.4</td>
<td>29</td>
<td>4.5</td>
<td>108</td>
<td>19.5</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>Runanga st.l.</td>
<td>12.5</td>
<td>2,460</td>
<td>57</td>
<td>5.2</td>
<td>10.5</td>
<td>26</td>
<td>7.2</td>
<td>88</td>
<td>25.9</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>Conroy f.s.l.</td>
<td>9.4</td>
<td>360</td>
<td>26</td>
<td>6.2</td>
<td>2.6</td>
<td>15</td>
<td>2.2</td>
<td>145</td>
<td>11.5</td>
<td>15.1</td>
</tr>
<tr>
<td>6</td>
<td>Blackstone f.s.l</td>
<td>5.6</td>
<td>840</td>
<td>25</td>
<td>6.5</td>
<td>2.9</td>
<td>19</td>
<td>1.6</td>
<td>187</td>
<td>13.6</td>
<td>16.5</td>
</tr>
<tr>
<td>7</td>
<td>Carrick s.l.</td>
<td>3.3</td>
<td>1,140</td>
<td>33</td>
<td>5.3</td>
<td>3.2</td>
<td>9</td>
<td>3.4</td>
<td>189</td>
<td>15.3</td>
<td>14.6</td>
</tr>
<tr>
<td>8</td>
<td>Obelisk s.l.</td>
<td>0.6</td>
<td>1,320</td>
<td>42</td>
<td>5.0</td>
<td>4.0</td>
<td>8</td>
<td>4.9</td>
<td>241</td>
<td>17.5</td>
<td>15.9</td>
</tr>
</tbody>
</table>

1. † Data for soils 1-4 were taken from New Zealand Meteorological Service (1966). Date for soils 5-8 are approximate and were calculated from data given by Mark (1962, 1965).
2. ‡ Enzymes in soils 1-4 were determined at 30°C and in soils 5-8 at 37°C.
3. Soils 1-4 occurred under grass-clover pastures and soils 5-8 under tussock grassland.
4. Classification of soils: 1, central yellow-grey earth; 2, central yellow-brown earth to yellow-grey earth intergrade; 3, central yellow-brown earth; 4, southern yellow-brown earth; 5, brown-grey earth; 6, southern yellow-grey earth; 7-8 high country yellow-brown earth.
5. Results are means of five samples of soils 1-4 collected in February 1967, and of twelve samples of soils 5-8 collected in January, March and October 1965.
values usually differed significantly between soils in the A and B horizons and less in the AB horizon. Changes of enzyme activities with depth were generally similar in different soils although some significant differences were found at some periods. Obelisk soil at the highest altitude showed the greatest changes from the A to the AB horizon (Ross and Roberts, 1968).

A study is now being undertaken to examine the possible effect of climatic variables under more defined conditions in (i) a sequence of seven pasture soils under constant mean annual temperature and different rainfalls and (ii) a sequence of five soils under constant rainfall and different mean annual temperatures. Results from the initial sampling of some of the rainfall sequence are given in Table 1 (soils nos. 1-4) and are, in general, very similar to those found in the previous survey of pasture soils. At this stage, it is not possible to detect any major patterns in the temperature sequence of soils.

**Influence of climate and soil factors on enzyme activities**

Correlation coefficients between some different factors and enzyme activities of the topsoils are given in Table 2. The coefficients with soil moisture, organic C, and pH in the tussock-grassland soils (experiment C, Table 2) were computed by removing the variances due to soils, seasons, and interactions between soils and seasons. In all soils, annual rainfall, soil moisture, and organic carbon were generally very significantly correlated with values of enzymes hydrolysing starch, and also with reducing sugars produced on incubating soils without added substrate. Some major differences between pasture and tussock-grassland soils were, however, evident, particularly in the relationships of enzymes hydrolysing sucrose with soil moisture and organic C.

Values of pH were negatively and significantly correlated with enzyme activities without added substrate and with enzymes hydrolysing starch in pasture soils only. pH was not significantly correlated with enzymes hydrolysing sucrose in any of the surveys.

In the fifteen pasture soils for which clay contents were available (Ross, 1966), and in soils of the rainfall sequence (Table 1), enzyme activities without added substrate and enzymes hydrolysing starch, but not sucrose, were positively and significantly correlated with clay contents. In the tussock-grassland soils, in contrast, relationships between enzyme activities and the contents of clays, which were predominantly illite or inter-layered hydrous micas, tended to be negative (Table 1). Although the clay and moisture contents of eight intrazonal soils appeared together to explain more of the variations of starch-hydrolysing activities than a combination of other factors (Ross, 1966), evidence was available that other factors could influence these activities more predominantly in intrazonal soils also. For example, very marked differences in both sucrose- and starch-hydrolysing activities were found in two pasture soils that had almost identical values of pH, organic carbon, and contents of clay (51 and 63 per cent), consisting of nearly pure montmorillonite; these soils occurred, however, under different mean annual temperatures and rainfall (Ross, 1966).
### Table 2

**Correlation Coefficients for Climate and Soil Factors and Enzyme Activities of Topsoils**

<table>
<thead>
<tr>
<th>Experiment no.†</th>
<th>Vegetative cover</th>
<th>Number of different soils</th>
<th>Source of Variation</th>
<th>Enzymes, mU/g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>No added substrate</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Sucrose</strong></td>
</tr>
<tr>
<td>A</td>
<td>Grass-clover pasture</td>
<td>43</td>
<td>41</td>
<td>Mean annual temperature</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41</td>
<td>Annual rainfall</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41</td>
<td>Soil moisture</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41</td>
<td>Soil organic C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41</td>
<td>Soil pH</td>
</tr>
<tr>
<td>B</td>
<td>Grass-clover pasture</td>
<td>7</td>
<td>5</td>
<td>Annual rainfall</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td>Soil moisture</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td>Soil organic C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td>Soil pH</td>
</tr>
<tr>
<td>C</td>
<td>Tussock grassland</td>
<td>4</td>
<td>2</td>
<td>Mean annual temperature</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>Annual rainfall</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>Soil moisture‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>Soil organic C‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>Soil pH‡</td>
</tr>
</tbody>
</table>

1. † Mean annual temperature and annual rainfall relationships: (A) temperature generally increased as rainfall increased; (B) temperature approximately 12.5°C at all sites; rainfall increased from 760 to 2,460 mm; (C) temperature decreased from 9.4°C to 0.6°C and rainfall increased from 360 to 1,320 mm.

2. ‡ Variances due to soils, seasons, and interactions between soils and seasons were removed.

3. *, **, *** = \( P < 0.05, 0.01, 0.001 \), respectively.

Oxygen uptakes were positively, and usually significantly, correlated with amounts of reducing sugars produced by soils without added substrate and with activities of starch-hydrolysing enzymes (Ross, 1965 a). Relationships between oxygen uptakes and activities of sucrose-hydrolysing enzymes were not significant in pasture soils, agreeing with the findings of Drobnik and Saifert (1955) and other workers.
Results from two sampling periods in the current investigation of the sequence of pasture soils under different rainfalls indicate that dehydrogenase activities are not significantly related to the activities of the hydrolytic enzymes studied. This investigation is being continued.

**DISCUSSION**

Measurements of enzyme activities now form part of the general biological study of soils and are considered to give a comparatively stable assessment of specific biochemical processes. Several difficulties are, however, encountered in estimating their activities and in assessing their significance in relationship to various environmental factors. Vegetation has been shown in this and overseas studies to play a major role in influencing enzyme activities. Marked seasonal effects are often found. Depth or texture of soil can also influence results considerably. Because of these, and other influences, variations of the activity of any enzyme within a particular soil can be appreciable. In spite of these difficulties, it has been shown from the study of selected soil sequences in this and other work, Antoniu and Orenski (1960) and Galstyan and Tatevosyan (1964), that at least some enzymes vary broadly with soil groups.

Clays are usually considered to influence the stability of enzymes in soil and their metabolic efficiency (Durand, 1965). Present results do not permit any generalisations about possible relationships between contents of enzymes and clays since relationships were either absent in some enzyme systems or contrasting in other systems in these soils under different grassland covers.

In all these New Zealand soils, enzymes hydrolysing starch and amounts of reducing sugars produced from soils without added substrate were always significantly correlated with organic C, and usually also with rainfall and soil moisture. Because of these associations in properties, a significant difference was generally found between major groups of zonal soils.

The significant differences found in the activities of sucrose-hydrolysing enzymes in the different tussock-grassland soils could again be largely explained by differences in soil organic C. Galstyan and Tatevosyan (1964) have reported that the activities of sucrose-hydrolysing enzymes in Armenian soils were also closely related to soil organic matter and increased with elevation above sea level.

Factors possibly contributing to the different and more complicated picture found with sucrose-hydrolysing enzymes in these New Zealand pasture soils, where activities were not directly related to soil organic C, include (1) different combinations of mean annual temperature and annual rainfall at the pasture and tussock-grassland sites, (2) different proportions of grasses and legumes at the different pasture sites, and (3) different soil microbial populations. The current studies on rainfall and temperature sequences appear to indicate that climatic variables were not solely responsible. Moreover, gross differences in the proportions of grasses and legumes were not regularly observed at the different pasture sites. Differ-
ences in the chemical composition of the pastures are, however, likely to have occurred under the different climatic conditions (Vaadia et al., 1961). Sucrose-hydrolysing activities, which appeared to be related more to the composition than to the amount of organic matter present in pasture soils, could have been influenced by the direct addition of enzymes from pasture plants or, more probably, by microbial changes in soil arising from the availability of different amounts of substrates from plant material (Drobnik, 1955).

Ratios of enzymes hydrolysing sucrose : enzymes hydrolysing starch could be used for differentiating major groups of soils in some, but not all, environments.

Although it is widely recognised that measurements of soil biochemical activities, such as enzymes and respiration, and the enumeration of various groups of organisms provide different aspects of the biological complexity of soils, some interrelationships between such properties could be found, often, probably, because of their mutually significant relationships to soil organic carbon and other factors. Activities of different enzyme systems in soil can, however, obviously differ between themselves and it is clear from the present studies that even enzymes hydrolysing water-soluble carbohydrates, such as sucrose and starch, can be present in considerably different proportions in many soils.

As more data accumulate, the significance of enzyme levels in soils and their role in the economy of general soil processes should become more apparent. Overseas investigations have thrown some light on the origin of enzymes in soil, but their relationship with vegetative cover and different groups of soil organisms, their rates of change with changing environments, their stability in relationship to clays and organic matter, and the activity of free enzymes compared with those in living cells, are all topics requiring further study.

CONCLUSIONS

Enzyme activities could be influenced considerably by vegetative cover, period of sampling, and depth of soil. The influence of clays seemed less than that of other factors.

Enzymes hydrolysing starch were positively correlated with soil moisture and organic carbon in all these grassland soils. Enzymes hydrolysing sucrose were also significantly correlated with these factors in the mountain soils under tussock grassland. In the pasture soils, no single factor could explain much of the variations of sucrose-hydrolysing enzymes, but their greatest proportions appeared to occur in the organic matter of the drier soils. Because of these relationships with particular soil properties, some enzyme activities could differ systematically in different soil groups.

A separate study appears necessary to assess the significance of the activity of any enzyme system in soil since even the two glycoside hydrolases studied here could proportionately differ considerably in different soils.

Positive inter-relationships of enzyme activities with oxygen uptakes
could occur in some situations, due probably to their mutually significant relationships to soil organic carbon and other factors.

ACKNOWLEDGEMENTS

The collaboration of Mr. H. S. Roberts, Applied Mathematics Division, Wellington, the assistance of Soil Bureau pedologists, the analyses of clays by Dr. G. G. C. Claridge, and the analytical assistance of Messrs. I. W. Boyd, I. R. Gibson, and D. E. Harding, are gratefully acknowledged.

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SUMMARY

The possible influence of different soil and environmental factors on activities of these enzymes has been studied in several groups of New Zealand soils under pastures of grasses and clovers and in a mountain soil sequence under tussock grassland.

Enzyme activities could be influenced considerably by vegetative cover, period of sampling, and depth of soil. Clay contents of the soils generally appeared less important in influencing activities.
No single factor could explain much of the variations of activities of enzymes hydrolysing sucrose in the pasture soils but the greatest proportions of these enzymes appeared to occur in the organic matter of the drier soils. In the mountain soils, there was, in contrast, a very marked positive correlation of these enzymes with soil organic carbon.

Enzymes hydrolysing starch were positively correlated with soil moisture and organic carbon in all soils.

These enzyme activities were not always significantly correlated between themselves nor with values of oxygen uptake of the soils, numbers of viable bacteria, nor dehydrogenase activities.

In some cases, enzyme activities could be related to soil groups. In other situations, relationships with soil groups were not found due to the effect of local environmental factors.

The significance of enzyme measurements is discussed.

RÉSUMÉ

L'influence possible de différents facteurs du sol et du milieu sur les activités de ces enzymes a été étudiée sur plusieurs groupes de sols de Nouvelle-Zélande couverts de pâtures d’herbe et de trèfle et sur une série de sols de montagne sous des prés de touffes d’herbe.


On ne peut expliquer par un seul facteur la plupart des variations dans les activités des enzymes qui hydrolysent le sucrose dans les sols de pâturage, mais les plus grandes proportions de ces enzymes semblaient se présenter dans la matière organique des sols plus secs. Dans les sols de montagne, il y avait, par contre, une corrélation positive très marquée de ces enzymes avec le carbone organique du sol.

Les enzymes qui hydrolysent l’amidon avaient une corrélation positive avec l’humidité du sol et le carbone organique dans tous les sols.

Ces activités d’enzymes n’avaient pas toujours une corrélation significative entre elles-mêmes, ni avec les valeurs de l’absorption d’oxygène des sols, nombre de bactéries viables, ni avec les activités de déhydrogénase.

Dans certains cas, les activités des enzymes pourraient être reliées aux groupes de sols. Dans d’autres situations, à cause de l’effet de facteurs dans le milieu local, on ne trouva pas de relation avec les groupes de sols.

La signification des mesures d’enzymes est discutée.

ZUSAMMENFASSUNG

Der etwaige Einfluss verschiedener Boden- und Milieufaktoren auf die Tätigkeit der Enzyme ist in mehreren Gruppen der Neuseeländischen Böden untersucht worden, und zwar in Gras- und Kleeweiden sowie in einer Gebirgsbodensequenz unter Buschelgrasland.

Die Tätigkeit der Enzyme kann durch die Pflanzendecke, dem Zeitpunkt

Starke hydrolysierende Enzyme entsprechen der Bodenfeuchtigkeit und dem organischen Kohlenstoff in allen Böden.

Es gab keine bedeutende Korrelation zwischen den Enzymtätigkeiten selbst und dem Sauerstoffaufnahmewert der Böden, der Zahl der lebensefähigen Bakterien oder mit den Tätigkeiten des Wasserstoffentzuges.

In einigen Fällen konnte man Beziehungen zwischen Enzymtätigkeiten und Bodengruppen finden. An anderen Stellen wurden Beziehungen zu Bodengruppen infolge der Milieueinwirkung nicht gefunden.

Die Bedeutung der Enzymmessungen ist diskutiert.
INTERACTIONS BETWEEN SOIL HUMIC ACIDS AND BACTERIA

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INTRODUCTION

In a previous study (Ladd and Brisbane, 1967), it was shown that 30-40% of the acid-hydrolysable amino acid-nitrogen of several soil humic acids was released by the proteolytic enzyme Pronase, thus establishing the presence of protein or peptide components in humic acids. Maximal release of amino acids occurred within 24 hours. This paper shows that under non-sterile conditions, the observed concentrations of released amino acids either (i) decreased markedly or (ii) remained comparatively stable on further incubation. The pattern obtained was related to the humic acid substrate and to its effectiveness as a bactericidal agent.

MATERIALS AND METHODS

Humic acids

Soils were extracted with 0.5N NaOH and the humic acids precipitated with acid as described by Ladd and Butler (1966). The properties of humic acids D, G, I, J and K have been described by Ladd and Brisbane (1967).

Enzymic hydrolysis of humic acids

Humic acids were dissolved in 0.1N NaOH (1 m mole NaOH/100 mg humic acid), titrated to pH 7.0 with 0.1N HCl, and diluted to a final concentration of 4.0 mg/ml. The proteolytic enzyme Pronase-B, prepared from Streptomyces griseus, was obtained from Calbiochem, Los Angeles, California.

Unless otherwise stated the reaction was carried out in tapered, glass centrifuge tubes (capacity, 15 ml) and was started by the addition of freshly prepared solutions of Pronase (0.2 ml of 0.2 mg/ml) to humic acid (0.3 ml of 4 mg/ml) in a final reaction volume of 1.0 ml. After incubation at 37°C the reaction was stopped by the addition of 0.2 ml 2N HCl. When bacterial counts were made, 0.2 ml of each reaction mixture was removed immediately before the addition of acid. The precipitated humic acid was centrifuged (2,000 g for 10 minutes) and a sample (0.7 ml) of the supernatant was evaporated to dryness in vacuo at room temperature, treated with methanolic potassium borate to remove ammonia (Connell et al., 1955), and assayed for amino acids by the procedure of Moore and Stein (1954).

Some experiments were performed under non-sterile conditions; others
in the presence of bacteriostatic concentrations (5%) of ethyl alcohol. In other experiments the components of the reaction mixture were sterilized; humic acids by autoclaving neutral solutions at 120°C for 20 minutes, and Pronase by passage through a 'Millipore' filter (pore size, 0.22 μ). The components of the reaction were transferred aseptically to sterile centrifuge tubes.

**Counting and isolation of bacteria**

Bacterial numbers were estimated from serial ten-fold dilutions of each sample. Yeast extract-peptone agar plates (Sperber and Rovira, 1959) were inoculated with six replicate drops using the Miles and Misra technique (1938), incubated at 37°C for 2 to 3 days, and counts were made of drops containing less than 300 colonies.

Bacteria were isolated from unsterilized reaction mixtures of humic acid K and Pronase, using dilution plates of yeast extract-peptone agar incubated at 37°C. A yellow bacterial isolate was obtained from the reaction mixture at the beginning of the reaction period. After three days' incubation two types of bacteria were obtained, one isolate formed white colonies and the other pink colonies.

**Properties of bacterial isolates**

The tests used for the identification of the isolates followed procedures laid down by the Society of American Bacteriologists (1957), except that the basal peptone medium used for the sugar tests was modified to have the following composition—Peptone 2.0 g, yeast extract 2.0 g, K₂HPO₄ 0.2 g, water 1 litre; the pH was adjusted to 7.0 with 1 N NaOH. The Hugh and Leifson sugar tests followed the procedure of Thornley (1960). Motility was observed by phase contrast microscopy of living cultures, and flagellation by electron microscopy of nickel-shadowed preparations. Sensitivity to antibiotics was tested with 'Multodisks'* 11·14D and 30·1H.

All three isolates were aerobic, Gram-negative rods, not acid-fast, sensitive to 5% ethanol, resistant to penicillin (5 units) and streptomycin (25 μg). Acid production was not marked. Glucose and lactose were oxidized aerobically when tested with the sensitive Hugh and Leifson method; but not when tested by the standard procedures using bromocresol purple as an indicator.

The bacteria which grew as small, white, circular, glistening colonies were identified as *Pseudomonas* sp. This bacterium was a motile rod (0·7 by 1·1 to 2·2 μ) with a single polar flagellum. It was actively proteolytic, hydrolysing nutrient gelatin (crateriform liquefaction) in one week, and producing clearing in a milk agar plate. Although it does not appear to possess an arginine dihydrolase system (Thornley, 1960) there is an alkaline reaction on the aerobic arginine medium within three days. The second isolate, identified as *Flavobacterium* sp., grew as small, yellow, waxy colonies. This motile bacterium (0·8 by 1·0 to 3·0 μ) was not proteolytic when tested on gelatin and casein. The third isolate which grew as small, pink, glistening, circular colonies was not identified and was designated

* Obtained from Oxoid Ltd.
HAP 1. It was a non-motile rod (0.5 by 0.8 to 1.6 μ) which was strongly proteolytic, hydrolysing gelatin (stratiform liquefaction) and producing clearing in a milk agar plate. It did not grow well on peptone medium, unless yeast extract was added.

RESULTS AND DISCUSSION

The effect of ethanol on the release of amino acids from humic acids

Figure 1 shows the amounts of amino acids released from five humic acids by Pronase. None of the reaction components was sterilized, but in one series (Figure 1b) 5% ethanol was present to prevent bacterial growth (Nomoto, Narahashi and Murakami, 1960). Humic acids gave a slight colour with the ninhydrin reagent, and Pronase released amino acids by auto-digestion; therefore, to obtain the net amount of amino acid released by Pronase from humic acid, it was necessary to subtract controls of both humic acid alone and Pronase alone.

Fig. 1.—Release of amino acids from soil humic acids by Pronase, in the presence and absence of alcohol. Amino acid-nitrogen (μg/ml) released by the action of 0.04 mg of Pronase on 1.2 mg of humic acid, reacted at 37°C in the presence and absence of 5% alcohol. Reaction volume, 1.0 ml. Controls of Pronase and humic acid alone, have been subtracted. Control values at 72 hours for treatments with and without alcohol were as follows—Pronase, 1.7, 1.3; humic acid J, 2.1, 1.8; K, 2.4, 1.4; D, 1.4, 1.0; G, 1.1, 1.2; I, 1.6, 1.2.

For a given humic acid, Pronase released within the first 6 hours similar amounts of amino acids both in the presence and absence of alcohol, showing that 5% alcohol did not inhibit pronase activity. However
as the reaction continued, amino acid concentrations decreased in three of the five non-sterile reaction mixtures (Figure 1a), but not in those containing alcohol. In some instances (D, I) the amounts of amino acid-nitrogen in reaction mixtures of humic acid plus Pronase were less than the sum of those measured in the respective humic acid and Pronase controls; thus correction for these controls led to negative values. The decreases in the amino acid concentrations were deduced to be due to bacterial growth which was inhibited by ethanol.

This was confirmed in separate experiments using humic acids I and K and Pronase under sterile and non-sterile conditions. In one experiment, the reaction mixtures of Pronase and humic acids I or K contained initially 3.8 x 10^3 and 3.4 x 10^3 bacteria/ml, respectively. After 72 hours' reaction in the absence of alcohol, the bacterial numbers in mixtures containing K increased markedly to 2.1 x 10^7 bacteria/ml, but in those containing I the numbers were only 3.5 x 10^4 bacteria/ml. When alcohol was present, no bacteria were detected after 72 hours.

### TABLE 1

**RELATIONSHIP BETWEEN BACTERIAL NUMBERS AND AMINO ACID CONCENTRATION**

<table>
<thead>
<tr>
<th>Humic acids</th>
<th>Reaction time (hr.)</th>
<th>Non-sterile</th>
<th>Sterile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amino acid-nitrogen (µg/ml)</td>
<td>Bacterial no. (cells/ml)</td>
<td>Amino acid-nitrogen (µg/ml)</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
<td>2.4</td>
<td>1.2 x 10^4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.9</td>
<td>4.6 x 10^3</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>3.0</td>
<td>7.0 x 10^7</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2.6</td>
<td>1.5 x 10^8</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>2.7</td>
<td>9.1 x 10^3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.9</td>
<td>1.1 x 10^8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.1</td>
<td>5.2 x 10^5</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2.0</td>
<td>1.6 x 10^6</td>
</tr>
</tbody>
</table>

Amino acid-nitrogen (µg/ml) and bacterial numbers (cells/ml) in reaction mixtures of Pronase (0.06 mg) and humic acid (2.0 mg, Na salt at pH 7.0). Reacted at 37°C; total volume 1.5 ml. Data are uncorrected for Pronase or humic acid controls.

In a second experiment, bacterial numbers were related to the recovery of released amino acids (Table 1). The data on amino acid concentrations have not been corrected (as was done in Figure 1) for controls, pronase alone and humic acid alone. Unsterilized reaction mixtures containing Pronase and humic acid K showed a decrease in the amino acid concentrations after 24 hours; the bacterial numbers were then 7.0 x 10^7 cells/ml. A similar relationship between amino acids and bacteria occurred with reaction mixtures containing humic acid I. After 48 hours' reaction, bacterial numbers reached 1.6 x 10^8 cells/ml and there was a decrease in amino acid concentration. In most other experiments with humic acid I (e.g. Figures 1 and 2) the bacteria did not reach sufficiently high numbers to affect the amino acid concentrations. Generally, there was no measurable
change in the amino acid concentration until the bacteria numbered around $10^7$ or $10^8$ cells/ml. When the reactions were carried out under sterile conditions the concentrations of released amino acids remained relatively constant and there was no bacterial growth. In both of these experiments bacteria grew more readily in the presence of humic acid K than in the presence of I.

**Bactericidal activity of humic acids and Pronase**

On the basis of the above experiments, differences in the abilities of bacteria to utilize amino acids released by Pronase from different humic acids (Figure 1a), may be due to one or more of the following factors: (a) chance differences in the number and nature of the bacteria present in the various reaction mixtures; (b) differences in the effectiveness of humic acids to inhibit bacterial growth, i.e. humic acids G and I inhibit bacterial growth but humic acids D, J, and K do not; (c) differences in the effectiveness of humic acids to nullify an inhibitory action of Pronase on bacterial growth. These latter two alternatives were tested using three bacterial isolates obtained from reaction mixtures of humic acid K and Pronase.

Suspensions of each of the isolates were added separately to sterilized solutions of humic acids G, I, J, and K, neutralized to pH 7.0. Within thirty minutes, sterilized Pronase solutions were added and all tubes were incubated at 37°C. Treatments included bacterial suspensions alone, humic acids without Pronase, and Pronase without humic acids. Uninoculated controls were also run. The concentration of humic acids (1-2 mg/ml) and of Pronase (0.04 mg/ml) were identical to those used in the experiment illustrated in Figure 1. At varying intervals after addition of enzyme (0, 6, 24 and 48 hours), 0.2 ml aliquots of each incubation mixture were removed, diluted, and the bacterial numbers estimated.

Uninoculated tubes, with or without humic acids or Pronase remained sterile. The extent of growth in the inoculated tubes depended on the type of humic acid and organism used and on the presence or absence of Pronase (Figure 2).

(a) *Pseudomonas* sp.: In the absence of both Pronase and humic acids (control), the *Pseudomonas* sp. grew within 48 hours from an initial inoculum of $5 \times 10^3$ cells/ml to a population of $3 \times 10^7$ cells/ml (Figure 2b). Both humic acids J and K stimulated growth, the bacterial population rising in their presence to $2.6 \times 10^5$ cells/ml within 48 hours (Figure 2a, 2b). By contrast, humic acids G and I had a marked bactericidal effect on the *Pseudomonas* sp., causing their numbers to fall within 6 hours to an undetectable level.

(b) *Flavobacterium* sp.: In the absence of Pronase and humic acids, the numbers of *Flavobacterium* sp. remained relatively constant throughout the experimental period (Figure 2d). All of the humic acids, in the concentrations used, showed bactericidal activity against the *Flavobacterium* sp. Humic acid K was least effective, whereas humic acids G and I again displayed strong bactericidal activities.
Fig. 2.—Effect of Pronase and humic acids on the viability of three bacterial isolates. Suspensions of bacteria (*Pseudomonas* sp., *Flavobacterium* sp., or HAP 1) were incubated with humic acids (1-2 mg) in the presence and absence of Pronase (0-04 mg) at 37°C for periods up to 48 hours. pH 7-0; reaction volume, 1-0 ml.
In contrast with the *Pseudomonas* sp., the *Flavobacterium* sp. was killed by the Pronase (Figure 2c). From an initial inoculum of $9.6 \times 10^4$ cells/ml, Pronase reduced the bacterial numbers to $1.3 \times 10^2$ cells/ml within 6 hours, and after 24 hours no viable organisms could be detected. The bactericidal activities of Pronase mixed with either humic acid J or K were less than those of Pronase alone.

(c) HAP 1: In the absence of Pronase and humic acids, the numbers of HAP 1 fell within 48 hours from an inoculum level of $2 \times 10^5$ cells/ml to $3.6 \times 10^3$ cells/ml (Figure 2f). Pronase displayed a strong bactericidal activity against HAP 1 (Figure 2e), as was also observed with the *Flavobacterium* sp. Unlike the other organisms considered, HAP 1 was not so susceptible to the bactericidal action of humic acids G and I (Figure 2f). Humic acid I was again the most toxic, but its effect was not obvious until 48 hours' incubation. Humic acids G and J showed slight bactericidal activities, again observed after 48 hours' incubation. Humic acid K caused a relative stimulation of growth of HAP 1.

The bactericidal activities of humic acids incubated with Pronase were less than those of Pronase or humic acids alone. This interaction between Pronase and humic acids may be due to (a) release of amino acid substrates from humic acids, thus stimulating bacterial growth, or (b) reactions between Pronase and humic acids which decrease the bactericidal activity of both of these compounds.

It has been shown that humic acids vary in their toxicity towards individual bacteria, both qualitatively and quantitatively. The nature of this bactericidal action of humic acid is being investigated further. It would therefore be of interest to know whether or not humic compounds have any bactericidal effect in soil. Certainly the toxicity of humic acids may partly explain their resistance to decomposition by bacteria, when studied under laboratory conditions.

Pronase preparations, obtained from *Streptomyces griseus*, are also bactericidal. The possibility that some constituent other than Pronase enzyme itself is the effective bactericidal agent is not eliminated; however none of the organisms tested is sensitive to 25 $\mu$g of streptomycin, the antibiotic produced by *S. griseus*.

Of the three bacterial isolates tested, the *Pseudomonas* sp. was the dominant organism in unsterilized reaction mixtures of humic acid K after incubation with Pronase, and its growth was mainly responsible for the subsequent disappearance of amino acids liberated from the humic acid. The behaviour of the *Pseudomonas* sp. towards Pronase and humic acids G, I, and J was entirely compatible with the observed pattern of release and disappearance of amino acids derived from the proteolysis of the unsterilized humic acids. The remaining two isolates played only minor roles in this regard. The *Flavobacterium* sp. was sensitive to the bactericidal action of both Pronase and humic acid, and could be isolated only at the commencement of incubation. Isolate HAP 1, although less sensitive to the action of the humic acids, did not grow well and utilized amino acids as growth substrates relatively poorly. Despite the bactericidal activities of
some humic acids and of Pronase, it is obviously important to carry out proteolytic studies under sterile conditions or in the presence of a bacteriostatic agent.

ACKNOWLEDGMENTS

The authors are indebted to Dr. R. J. Swaby for gifts of humic acid, and to Mr. M. Amato for skilled technical assistance.

REFERENCES


SUMMARY

The proteolytic enzyme Pronase released amino acids from soil humic acids. In some cases bacterial growth occurred causing a decrease in the amino acid concentration, which became marked when the bacterial population reached $10^7$ to $10^8$ cells/ml. Three bacteria were isolated from humic acid-Pronase mixtures, and studies were made of their growth in the presence of four humic acids (with and without Pronase). Bacteria varied in their susceptibility to a given humic acid and to Pronase. There was also a range in the bactericidal activities of humic acids. However, there was an interaction between the humic acids and Pronase, causing each to become a less effective bactericidal agent.

RÉSUMÉ

L'enzyme protéolytique Pronase libéra les amino-acides des acides humiques du sol. Dans certains cas, il apparut un développement bactérien causant une diminution de la concentration en amino-acide, qui devint importante lorsque la population bactérienne atteignit $10^7$ à $10^8$ cellules/ml. Trois bactéries furent isolées des mélanges acides humiques-Pronase, et on étudia leur croissance en présence de quatre acides humiques (avec ou sans Pronase). Les bactéries répondirent différemment à un acide humique donné et à la Pronase. Il y eut aussi une gamme dans les activités bactericiennes des acides humiques. Cependant, il y eut une interaction entre les acides humiques et la Pronase, ce qui fit que chacun devint un agent bactericide moins efficace.

ZUSAMMENFASSUNG

Die proteolytische enzymatische Pronase entließ Aminosäuren von Boden-Huminsäuren. In einigen Fällen kam es zu Bakterienwachstum, das eine Verminderung in der Aminosäure-Konzentration verursachte, die sich...
bemerken machte, wenn die Bakterienbevölkerung $10^7$ bis $10^8$ Zellen/ml erreichte.

Drei Bakterienarten wurden aus der Huminsäure-Pronase Mischung isoliert und Studien ihres Wachstums wurden in Gegenwart von vier Huminsäuren (mit oder ohne Pronase) vollzogen.

THE SUSCEPTIBILITY OF NITROGENOUS COMPONENTS OF HUMIC ACIDS TO ENZYME ATTACK—INHIBITION OF PRONASE ACTIVITY

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C.S.I.R.O., Division of Soils, Adelaide, Australia

INTRODUCTION

Ladd and Brisbane (1967) have shown that the proteolytic enzyme, pronase, releases amino acids from soil humic acids, thus demonstrating the presence of peptide or protein components in the humic acid preparations. Pronase action on different humic acids released different percentages of their content of acid-hydrolysable, amino acid-nitrogen.

Since pronase has a wide specificity in its activity towards peptide bonds (Nomoto, Narahashi and Murakami, 1960b), variations in the amino acids released by pronase from humic acids may be due to varying degrees of inhibition of pronase activity by the humic acids, and/or variations in the accessibility of the protein moiety of humic acids to attack by pronase.

Inhibition of pronase activity has been investigated using humic acids extracted with solutions of sodium pyrophosphate and dilute alkali from soils of known crop history.

MATERIALS AND METHODS

Humic acids

Samples of red-brown earth from eight experimental sites of known crop history located at Urrbrae, South Australia (permanent rotation trial C1, Waite Agricultural Research Institute) and Deniliquin, N.S.W. (Rixon, 1965) were sources of humic acids. Four replicate samples* of top soil (0-7·6 cm) were taken from each site, air dried at 35°C and sieved (1·0 mm); equal quantities from each replicate were mixed, suspended in water to remove plant debris and stirred with 0·1N HCl for two consecutive periods of 24 hours. Sub-samples of each soil were extracted with either 0·1M sodium pyrophosphate, pH 7·0, or 0·5N NaOH (5 litres extractant/kg of soil) for 24 hours at room temperature. Ex extractions with alkali were carried out under nitrogen. The extraction procedure was repeated once. Soils extracted with pyrophosphate solutions were subsequently extracted with 0·5N NaOH. Humic acids were precipitated

* Samples of Deniliquin soils were kindly provided by Dr. A. J. Rixon, Division of Plant Industry, C.S.I.R.O.
from the extracts with \( HCl \) at \( pH \) 1 to 1.8, their clay contents reduced by successive centrifugation at neutral and acidic \( pHs \) (Posner, 1966), dried at room temperature \( in \ \text{vacuo} \), ground and stored in a desiccator.

**Assay substrates**

Bovine serum albumin was obtained from the Commonwealth Serum Laboratories, Melbourne, Victoria, and \( N \)-carbobenzoxy-glycyl \( L \)-leucine (CBZ-glycyl leucine) from the Cyclo Chemical Corporation, Los Angeles, California.

**Enzyme**

Pronase-B was obtained from Calbiochem, Los Angeles, California.

**Assays of pronase activity**

Albumin (0.3 mg) or CBZ-glycyl leucine (2 \( \mu \) moles) was incubated with pronase (0.02 or 0.08 mg, respectively) in a 2.0 ml reaction volume, \( pH \) 7.0, for 60 minutes at 37°C. The reaction was terminated by the addition of 0.1 ml 5N \( HCl \), centrifuged (2,000 g, 10 minutes), and aliquots of acidified supernatant taken for estimation of amino acid-nitrogen. Under these conditions the release of amino acid-nitrogen was proportional to both time and pronase concentration.

Unless otherwise stated, inhibition of pronase activity by humic acids was assayed by addition of standard substrate to pronase, preincubated with the neutralised humic acids for 60 minutes at 37°C. Corrections were made for the release of amino acid-nitrogen from the humic acid and from the autodigestion of pronase.

**Methylation of humic acids**

Dried, ground humic acids were suspended in ether solutions of diazomethane for 100 hours at approximately 0°C, the diazomethane being replaced successively with fresh solutions until the reaction was complete. The methylated humic acids were centrifuged, washed with ether and dried \( in \ \text{vacuo} \) at 40°C.

Amino acid-nitrogen was determined by the method of Moore and Stein (1954), and carboxyl content by that of Schnitzer and Gupta (1965).

**RESULTS AND DISCUSSION**

(a) **Inhibition of pronase activity by humic acids**

Inhibition of pronase activity by humic acids was assayed using two standard substrates, a protein, albumin, and a derivative of a simple dipeptide, CBZ-glycyl leucine (Figure 1).

(1) **Substrate: CBZ-glycyl leucine**

Pronase activity on CBZ-glycyl leucine was particularly sensitive to inhibition: 50% inhibition was obtained with concentrations as low as 1 to 3 \( \mu \)g/ml. Inhibition curves had similar slopes over a wide range of humic acid concentrations, irrespective of the extractant used in the preparation of the humic acids.

Humic acids from Deniliquin soils were as effective inhibitors of
HUMIC ACIDS AND ENZYME ATTACK

Fig. 1.—The effect of humic acids on pronase activity. Percent inhibitions were calculated from pronase activity without humic acids. In the absence of humic acid, pronase liberated 8.0 μg amino acid-N from CBZ-glycyl leucine and 7.7 μg amino acid-N from albumin. Humic acids designated by source and extractant as follows: 1(a) Pyrophosphate extract of Urrbrae permanent pasture. 1(b) NaOH extract after pyrophosphate treatment of Urrbrae permanent pasture. 2(a) Pyrophosphate extract of Urrbrae wheat-fallow. 2(b) NaOH extract after pyrophosphate treatment of Urrbrae wheat-fallow.

pronase action as those from Urrbrae soils, the inhibition versus concentration curves again having similar slopes.

The most significant differences in inhibition occurred between humic acids removed with different extractants, those extracted with alkali being more effective inhibitors.

(ii) Substrate: albumin

Pronase activity against albumin was about ten times less sensitive to inhibition by humic acid than pronase activity against CBZ-glycyl leucine.

Again, humic acids from Deniliquin soils were as effective as those
from Urrbrae soils. However, the inhibition curves of alkali-extracted humic acids differed in slope from those of humic acids extracted with pyrophosphate. At high concentrations of humic acids from both soils, the alkali-extracted humic acids were more effective inhibitors. At low concentrations, pyrophosphate-extracted humic acids were more inhibitory than alkali-extracted humic acids (observed with humic acids from Deniliquin soils and deduced by extrapolation of inhibition curves of humic acids from Urrbrae soils).

The more effective inhibition of pronase activity against CBZ-glycyl leucine than against albumin is probably due to a lower affinity of the enzyme for the peptide derivative than for the protein. Also, the humic acids may react to some extent with albumin (but not with CBZ-glycyl leucine due to blocking of the terminal amino group), thus reducing the effective concentration of humic acid capable of reacting with the enzyme.

(b) Reversibility of inhibition

(i) Effect of substrate concentration

Increasing substrate concentrations decreased the percentage inhibition of pronase activity, following preincubation of the enzyme with humic acids. Figure 2 shows a Lineweaver-Burk double reciprocal plot of substrate concentration versus reaction velocity, in the presence and absence of various humic acids. The lines intersect at a common point on the vertical axis typical of competitive inhibition.

![Graph](image_url)

**Fig. 2.—** The effect of substrate concentration on the inhibition of pronase activity by humic acids. CBZ-glycyl leucine:—pronase (0.08 mg) was pre-incubated with humic acids (8 μg) for 60 min. at 37°C before addition of substrate; reaction volume 6.0 ml. Albumin:—pronase (0.02 mg) was pre-incubated with humic acids (0.1 mg) for 45 min. at 37°C before addition of substrate; reaction volume 2.0 ml. Humic acids designated as in Figure 1.
It is unlikely that humic acids inhibit by combining with the substrate, CBZ-glycyl leucine. Figure 2 shows that without humic acid a decrease of 8 μ moles in substrate concentration (i.e. from 10 μ moles to 2 μ moles) caused only a 45% inhibition (i.e. a decrease in reaction velocity from 0.67 to 0.37 μ moles amino acid nitrogen/hour). A similar experiment using 24 μg of humic acids and 10 μ moles of CBZ-glycyl leucine showed inhibitions of 57.5, 65.5, 46.0, and 75.0% for humic acids 1a, 1b, 2a and 2b respectively. If the effect of humic acid were to remove substrate, 24 μg of humic acids must remove 8 μ moles (2,600 μg) of CBZ-glycyl leucine. This is considered to be remote, especially since CBZ-glycyl leucine and humic acids can be separated by dialysis, hence any reaction between them must be reversible. Therefore, it may be concluded that humic acids inhibit by combining reversibly with the enzyme, at or near a reactive site (but not necessarily to the exclusion of other sites on the enzyme surface), thus effectively decreasing the affinity of the enzyme for its substrate. Increasing substrate concentrations decreased inhibition by displacing humic acids from the active site of the enzyme. A similar situation applied with albumin as substrate, with the difference that humic acids probably combine with the substrate as well as enzyme. Evidence presented below indicates that such a reaction, if it took place, was also reversible.

(ii) Effect of preincubation of humic acids with substrate or enzyme

When preincubated with enzyme, compounds acting as irreversible inhibitors generally show an increased inhibition with time, eventually causing complete inhibition if the concentration of inhibitor is sufficiently high. Humic acids from Urrbrae soils, pre-incubated for varying times either with substrate or pronase, caused an immediate inhibition of enzyme activity. Evidence presented below indicates that such a reaction, if it took place, was also reversible.

### Table 1

**The Effect of Preincubation of Humic Acids with Substrate or Enzyme on Pronase Activity**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Humic acid concentration (μg/ml)</th>
<th>Pre-incubation time (minutes)</th>
<th>Percentage inhibition by humic acids*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Preincubated with substrate Preincubated with enzyme</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1(a)† 1(b) 2(a) 2(b) 1(a) 1(b) 2(a) 2(b)</td>
</tr>
<tr>
<td>CBZ—glycyl leucine</td>
<td>1.0</td>
<td>0</td>
<td>26 41 16 31 44 21 10 20</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>180</td>
<td>23 41 18 30 47 24 10 20</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0</td>
<td>57 73 46 66 76 54 10 20</td>
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<tr>
<td></td>
<td>120</td>
<td>180</td>
<td>57 72 46 65 76 58 10 20</td>
</tr>
<tr>
<td>Serum albumin</td>
<td></td>
<td></td>
<td>64 80 58 83 70 54 60 82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59 77 52 79 66 72 60 87</td>
</tr>
</tbody>
</table>

* Inhibition calculated from relative pronase activities in the presence and absence of humic acids.
† Humic acids designated as in Figure 1.
activity (Table 1). The extent of the inhibition was independent of time over the three hour pre-incubation period.

(iii) Effect of divalent cations

Table 2 shows that increasing concentrations of divalent cations, Ca$^{2+}$ and Co$^{2+}$, decreased inhibition of pronase activity by humic acids. In the absence of humic acids, both Ca$^{2+}$ and Co$^{2+}$ stimulated pronase activity. The inhibition caused by the humic acids (4 $\mu$g/ml) was almost entirely removed by Co$^{2+}$ but at a concentration (10$^{-8}$M) approximately one hundred times greater than the quantity of cobalt calculated to react with the carboxyl groups of the humic acid. Other divalent cations, e.g. Mg$^{2+}$ and Mn$^{2+}$ also decreased inhibition by humic acids.

In these experiments, CBZ-glycyl leucine was the assay substrate; this permitted humic acids to be used in concentrations to obtain adequate inhibition without precipitating in the presence of Ca$^{2+}$ or other divalent cations. The effect of Ca$^{2+}$ in relieving the inhibition was observed whether or not the humic acids were pre-incubated with enzyme or with Ca$^{2+}$, indicating the reversibility of the inhibition.

(c) Effect of methylation of humic acids on enzyme inhibition

The results have shown that inhibition of pronase activity by humic acids is competitive, reversible and compatible with the hypothesis, that binding of enzyme to inhibitor involves acidic groups of humic acids. This has been further substantiated by showing that methylation of humic acids removed their ability to inhibit pronase. Table 3 shows the activities...
obtained in the presence of equal concentrations of humic acids, based on absorbance at 260 μm. It was assumed that methylation had no appreciable effect on the specific absorbance of the humic acids. Humic acids diluted ten fold still inhibited to a greater extent than the undiluted methylated derivatives.

**TABLE 3**

EFFECT OF METHYLATION OF HUMIC ACIDS ON THEIR INHIBITION OF PRONASE ACTIVITY

<table>
<thead>
<tr>
<th>Humic acid</th>
<th>Pronase activity (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methylated humic acids</td>
</tr>
<tr>
<td>†1(a)</td>
<td>92</td>
</tr>
<tr>
<td>(b)</td>
<td>97</td>
</tr>
<tr>
<td>(c)</td>
<td>98</td>
</tr>
<tr>
<td>2(a)</td>
<td>92</td>
</tr>
<tr>
<td>(b)</td>
<td>94</td>
</tr>
<tr>
<td>(c)</td>
<td>96</td>
</tr>
</tbody>
</table>

* Activities are expressed as percentages of pronase activity in the absence of humic acid. All humic acids present in equal concentrations based on absorbance at 260 μm (absorbance units/ml = 0.5), were preincubated with pronase (0.08 mg) for 60 min. at 37°C before addition of assay substrate (2 μ moles CBZ-glycyl leucine). Reaction volume, 2.3 ml. Reaction medium 9% dimethyl sulfoxide.

†1(a) Pyrophosphate extract of Urrbrae permanent pasture.
(b) NaOH extract after pyrophosphate treatment of Urrbrae permanent pasture.
(c) NaOH extract of Urrbrae permanent pasture.
2(a) Pyrophosphate extract of Urrbrae wheat-fallow.
(b) NaOH extract following pyrophosphate treatment of Urrbrae wheat-fallow.
(c) NaOH extract of Urrbrae wheat-fallow.

The reaction was carried out in a medium of 9% dimethyl sulfoxide in this buffer, pH 7.0. This solvent had no effect either on pronase activity towards CBZ-glycyl leucine, or on the extent of inhibition by the unmethylated humic acids; yet dissolved sufficient quantities of methylated humic acids to permit comparisons to be made.

The results demonstrate that carboxyl and/or acidic phenol groups of humic acids are probably involved in the inhibition of pronase activity. Attempts were made to correlate carboxyl content of the humic acids with their ability to inhibit pronase. The results were inconclusive. No correlation was obtained with albumin as substrate, except for the two humic acids extracted with alkali from Urrbrae loam. With CBZ-glycyl leucine as substrate, inhibition by all humic acids from Urrbrae loam, whether extracted with pyrophosphate or alkali, correlated with carboxyl content. No such correlation was observed for the Deniliquin humic acids, whose carboxyl contents ranged from 3.6 to 4.8 m-equiv./g. From the inhibition versus concentration curves the maximal difference in carboxyl content would produce a difference in inhibition of about 4%, which is similar to experimental error.

Despite the imperfect relationship between inhibition and total carboxyl,
the effect of divalent cations and methylation on the ability of humic acid to inhibit, suggests that some acidic groups are involved in the inhibitory process.

Pronase activity is also stimulated by calcium in the absence of humic acids (Table 2, Nomoto et al. 1960a). If calcium is essential for pronase activity, humic acids may inhibit by combination with calcium. We have shown (unpublished data) a marked inhibition of pronase activity by the complexing agents ethylene diamine tetraacetate and α-phenanthroline.

Future work will involve testing the effect of humic acids on other proteases, such as trypsin, which is also stimulated by calcium, and chymotrypsin, whose activity is independent of divalent cations.

Conclusions

The release of amino acids from humic acids by proteolytic enzymes will be influenced by the extent of the inhibition due to humic acids. However, it has proved difficult to distinguish this inhibition from other possible controlling effects such as protein accessibility.

Acknowledgments

The authors acknowledge with pleasure the very able technical assistance of Mr. M. Amato.

References


Summary

The proteolytic enzyme pronase was inhibited by soil humic acids. Concentrations approximating 2 and 20 μg/ml of humic acid brought about 50% inhibition of pronase action on CBZ-glycyl leucine and albumin respectively. Pre-incubation studies have shown that the inhibition is immediate, and is decreased by divalent cations and by increased substrate concentrations. The inhibition is thus reversible and competitive. Methylated humic acids do not inhibit pronase activity.
Although the correlation between total carboxyl and inhibition is inconclusive, the effects of divalent cations and methylation clearly implicate the acidic groups of humic acids in the inhibition mechanism.

RéSUMÉ

L'enzyme pronase protéolytique fut inhibée par des acides humiques de sol. Des concentrations approximativement de 2 et 20 μg/ml d'acide humique ont produit environ 50 % d'inhibition de l'action pronase sur le leucine CBZ-glycyl et l'albumine respectivement. Des études de pré-incubation ont démontré que l'inhibition est immédiate et qu'elle est diminuée par des cations divalents et par des concentrations substrates augmentées. Donc l'inhibition est reversible et compétitive. Les acides humiques méthylés n'ont pas d'effet inhibiteur sur l'activité du pronase.

Quoique la corrélation entre le carboxyl total et l'inhibition ne tire pas à conclusion, les effets des cations divalents et de la méthylation impliquent clairement les groupes acides des acides humiques dans le mécanisme de l'inhibition.

ZUSAMMENFASSUNG


Obwohl die Wechselbeziehung zwischen totalen Carboxyl und der Hemmung nicht genügend bewiesen ist, bringt die Wirkung von zweiwertigen Kationen und die Denaturierung die Säuregruppen der Humussäure in deutlichen Zusammenhang mit dem Hemmungsvorgang.
EFFECT OF SOIL PROFILE MODIFICATION ON PLANT ROOT DEVELOPMENT

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INTRODUCTION

Houston Black clay and associated soils have been grouped with other deep, dark-coloured clay soils under the general term Grumusol by Oakes and Thorp (1950) and more recently classified as Udic Pellusterts (Vertisols) (Soil Survey Staff, U.S. Department of Agriculture, 1960, 1967). These soils are deep, dense, very slowly permeable montmorillonitic clays. They have been described by Templin et al. (1956) and characterized mineralogically and chemically by Kunze and Templin (1956). They occupy approximately 5 million acres in Texas.

Typically, Houston Black clay is friable at the surface due to its self-mulching characteristics but grades quickly into a very plastic dense clay with low porosity. Consequently, root development of annual crops is restricted to the upper part of the soil profile. Since a period of low precipitation usually occurs during the growing season, crop yields are often reduced by moisture stress even though the subsoil may be quite wet.

Deep tillage investigations have been conducted throughout the United States with varying degrees of success. At times tillage to depths below ordinary plow depth have not resulted in benefits commensurate with the costs involved (Van Doren and Haynes, 1961). However, on soils where a specific problem exists at some profile depth that can be reached with tillage equipment, positive results have been obtained. For example, Fehrenbacher et al. (1958) have shown that where corn root development was restricted in claypan soils in Illinois, mixing and fertilizing of the profile to depths up to 36 inches increased root growth and penetration. Hauser and Taylor (1964) showed that breaking up the dense B horizon of Pullman silty clay loam (Reddish Chestnut) by disc ploughing 24 inches deep increased moisture storage. Other studies where improved water penetration and root development have resulted from deep tillage include the work of Patrick et al. (1959) and Saveson et al. (1961). In most instances, crop yield increases have been associated with deep tillage only when restricting layers in the profile limit water intake and root penetration and where seasonal distribution of rainfall is such that moisture stress can be expected to occur. The dense nature of Houston Black clay together with low summer rainfall in the Texas Blacklands suggested that deep profile mixing of this soil would improve root development and hence improve water use efficiency.
EXPERIMENTAL PROCEDURE

During late summer, 1963, when soil moisture in the upper 2 feet of Houston Black clay was near the wilting point, the following tillage treatments were established: (a) Check treatment (conventional tillage of bedding following crop harvest with subsequent rebedding before spring planting CT); (b) Rototilling 24 in. deep, RT. (Successive 4 in. layers were rototilled and removed from the plot area until the desired depth of 24 in. was reached after which the mixed soil was replaced on the plot area). In the summer of 1964, additional mixing treatments were established as follows: (c) Profile mixed 24 in., PM 2'; and (d) Profile mixed 48 in., PM 4'. These treatments were accomplished with a large ditching machine resulting in complete mixing of the profile to the designated depths.

Grain sorghum (Sorghum vulgare) and cotton (Gossypium hirsutum) were each planted on one-half the experimental area in March and April each year and alternated from year to year. Measurements of plant height and crop yield were made.

Aeration status was determined by direct gas sampling of the soil atmosphere through gas sampling wells at depths of 6, 12, 18, 24, 36, 48, 60, and 72 in. Measurements of soil water content were made periodically during the growing season with a neutron probe. Density was determined by standard gravimetric and nucleonic procedures. Entire root systems were washed from soil monoliths 3-3' wide, 1' thick, and 6' long to evaluate root development.

RESULTS AND DISCUSSION

Oxygen contents of the soil atmosphere by depths in Houston Black clay under grain sorghum at two dates during the 1965 crop season are shown in Figure 1. These data illustrate aeration conditions often found in this soil. During the growing season oxygen deficiencies are present at one time or another at all depths from 6 to 72 in. on soils with conventional tillage.

The effect of profile modification on soil aeration is shown in Figure 1. Two points of importance may be noted. First, only one sample (6 in. depth on April 27) taken from the loosened soil zones had an O₂ content below 10%. On May 26th, low O₂ contents at the 36 in. and deeper depths were from the PM 2' treatment. Thus, these samples were from undisturbed soil. However, many of the samples taken from the undisturbed soils (conventional tillage) on both dates had O₂ contents below 3 or 4%. The second point of interest in Figure 1 is the much greater variability in samples taken from the undisturbed soil. The O₂ curves for the loosened plots are much smoother than those from the undisturbed soils.

These data support the hypothesis that in an undisturbed soil such as Houston Black clay there are isolated areas of good and poor aeration very close to each other. The massive structure and natural cleavage planes found in this soil probably contribute to this characteristic and result in much variation when small gas samples are analyzed from various points in
the soil. Such local variations in gas composition suggest that roots may penetrate some soil layers but may bypass local volumes of soil and not thoroughly utilize the poorly aerated zones. Mechanically disrupting the soil destroyed the massive structure and resulted in improved and more uniform aeration in the loosened portions of the profile.

During the process of washing roots from the soil monoliths, there was visual evidence of differences in soil structure due to profile modification treatment. These differences are illustrated in Figure 2. The soils from Houston Black clay with conventional tillage (Figure 2A) had a massive structure with little change through the profile. The soil from the rototilled treatment (RT) (Figure 2B) still had soil fragments that were unchanged from the time of rototilling in August 1963 until the monoliths were secured in September 1965. The upper 12 in. of soil from the RT treatment is similar to that of the conventional tillage treatment, but the zone from about 18 to 30 in. still had recognizable clods formed in the rototilling process. The striking differences in structure of the soil loosened with the ditching machine are evident in Figure 2C. Large clods formed by the ditching machine appeared to have much greater water stability and did not slake
down as rapidly as the underlying soil from the undisturbed part of the profile or soil at the same depth from conventionally tilled plots.

Mature root systems of grain sorghum grown on Houston Black clay with various profile modification treatments are shown in Figure 3. Some fine roots were lost in the washing process, and some roots were moved from their original position in the soil profile. Root systems extended

Fig. 2.—Soil monoliths of Houston Black clay during root washing process in autumn 1965. A. From conventional tillage treatment. B. From plot rototilled 24 in. deep in August 1963. C. From plot with profile modified to 4 ft. depth with ditching machine in September 1964.

Fig. 3.—Grain sorghum root systems grown in Houston Black clay in 1965. A. From conventional tillage treatment. B. From plot rototilled 24 in. deep in August 1963. C. From plot with profile modified to 4 ft. depth with ditching machine in September 1964.
through the profile, even on soils with the conventional tillage treatment. However, the extent of branching was much less than on soils from the loosened plots.

Figure 3A shows that there were very few sorghum roots below 3 ft. in the conventionally tilled treatment. Note a mass of roots to the left of the picture extending through the entire 5 ft. profile. This was attributed to an improved aeration situation in that zone. It is believed that improved aeration may have existed where a cleavage plane occurred. However, this could not be ascertained since cleavage planes are destroyed in the washing process.

In addition to depth and extent of branching, there were differences in coarseness of the roots. The sorghum root system from the rototilled treatment, Figure 3B, was very fine and fibrous throughout its full depth compared with the root system from the plot with profile modified by the ditching machine, Figure 3C. In this case, the root system was coarse throughout its depth. More roots from this treatment were broken during washing because of the large, heavy clods attached to them. The difference in coarseness of roots was associated with the larger voids created by the ditching machine.

Root systems of cotton also were obtained. Similar differences in morphology of cotton roots due to profile modification were observed. Cotton roots in the check treatment penetrated almost 5 feet of the soil profile but had very little lateral branching compared with other treatments.

Growth differences of the aerial portions of cotton and sorghum due to soil profile modification were not as striking as differences in root development. Cotton growth rate was increased by soil profile modification in all 3 years of the experiment, but the growth rate of grain sorghum was improved only 2 years by the RT treatment and only 1 year by profile loosening with

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1964</th>
<th>1965</th>
<th>1966</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lint Cotton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check — CT</td>
<td>186a*</td>
<td>245a</td>
<td>360a</td>
</tr>
<tr>
<td>Rototilled — RT</td>
<td>498b</td>
<td>335b</td>
<td>565b</td>
</tr>
<tr>
<td>PM 2’</td>
<td>—</td>
<td>330b</td>
<td>560b</td>
</tr>
<tr>
<td>PM 4’</td>
<td>—</td>
<td>450c</td>
<td>585b</td>
</tr>
<tr>
<td><strong>Sorghum grain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check — CT</td>
<td>3640a</td>
<td>3900a</td>
<td>4760a</td>
</tr>
<tr>
<td>Rototilled — RT</td>
<td>4160b</td>
<td>5180b</td>
<td>5700b</td>
</tr>
<tr>
<td>PM 2’</td>
<td>—</td>
<td>3810a</td>
<td>5080b</td>
</tr>
<tr>
<td>PM 4’</td>
<td>—</td>
<td>4520b</td>
<td>5350b</td>
</tr>
</tbody>
</table>

* Means within a year column followed by the same letter are not significantly different at the 5% level by Duncan’s Multiple Range Test.
Fig. 4.—Changes in soil water content with time in Houston Black clay under cotton. Check—conventional tillage of bedding and rebeding. Rototilled—treatment consisted of rototilling and removing successive 4 in. layers of soil to a depth of 24 in. after which the mixed soil was replaced.
the ditching machine. In fact, early season growth of sorghum was delayed by the ditching machine treatment in 2 years of the experiment. This delay was attributed to poor soil-root contact and suggests the importance of a firm seedbed. Even though plant growth rates due to profile modification treatment were not always greater than with conventional tillage, crop yields were increased each year of the experiment. The yield data are presented in Table 1.

Soil water content by depths (1965 crop season) under cotton due to treatment are shown in Figure 4. The data illustrate soil water depletion patterns obtained during the experiment. Even though there is greater root proliferation by both cotton and sorghum in modified soil profiles, there is not always a great difference in water use by plants on modified soils and on conventionally tilled soil. Usually, however, water is used from greater depths earlier in the season on modified profiles, particularly by cotton. The more rapid use of water to greater depths probably accounts for much of the crop yield differences obtained.

Bulk density measurements made in 1967, four years after the roto-tilling treatment and three years after loosening with a ditching machine, showed that density of the loosened soil layers was still lower than similar depth layers from conventionally tilled plots. Observations of the profiles revealed that the surface 8-12 in. had redeveloped a massive structure very much like that of undisturbed soils apparently due to water slaking and compactive forces of tractor tires, but the profile below this depth appeared to have undergone little structural change. This observation, coupled with continued increases in crop yield and root development, suggests that profile modification of heavy clay soils may persist for several years.

REFERENCES


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Soil Survey Staff (1967)—Supplement to soil classification system (7th approximation). 207 pp. (USDA, Soil Conservation Service.)


Summary

Houston Black clay is a deep, dense, impervious, montmorillonitic clay soil. Root development of annual crops is restricted to the upper part of the soil profile and crop yields may be reduced by moisture stress even though the subsoil may be quite wet. Profiles of Houston Black clay were loosened and mixed mechanically to study relations between structural changes and root development, aeration, plant growth, and water use.

Oxygen content of undisturbed soil was extremely low at one time or another during the growing season at all depths from 6 to 72 in. Profile modification resulted in more uniform and improved oxygen concentrations. Greater root development of cotton and sorghum was noted in the modified profiles and resulted in more rapid soil water depletion to greater depths. Differences in plant growth due to profile modification were not as great as root growth differences, but yields of both cotton and grain sorghum were increased by the loosening treatments.

Bulk density measurements and observation of the profiles together with continued increases in crop yield and root development over 3 years indicate that the benefits of modifying the structure of heavy clay soils by mechanical means may persist for several years.

Résumé

L’argile Houston Black est un sol argileux montmorillonoidé profond, dense, imperméable. Le développement des racines de cultures annuelles est restreint à la partie supérieure du profil du sol et les rendements des cultures peuvent être réduits par la force de tension de l’eau bien que le sous-sol soit assez mouillé. Des profils d’argile Houston Black furent ameublis et mélangés mécaniquement pour étudier les relations entre les changements structurels et le développement des racines, l’aération, la croissance des plantes, et la consommation d’eau.

Le contenu d’oxygène du sol laissé intact fut extrêmement bas à un moment ou l’autre pendant la saison de croissance et à toutes profondeurs de 6 à 72 inches. Une modification du profil a produit des concentrations plus uniformes et améliorées d’oxygène. Un plus grand développement de racines de sorgho et de coton fut observé et a produit un épuisement plus rapide de l’eau du sol à de plus grandes profondeurs. Les différences de croissances de plantes dues à une modification du profil ne furent pas aussi grandes que les différences de croissance des racines, mais les rendements de coton et de sorgho furent augmentés par les traitements d’ameublissement.

Des mesures de la densité apparente et l’observation des profils jointes aux augmentations continuelles du rendement de la culture pendant trois ans indiquent que les bénéfices d’une modification de la structure des sols d’argile lourds par des moyens mécaniques peuvent persister indéfiniment.

Zusammenfassung

Der Houston Black Clay ist ein tiefer, dichter, undurchdringlicher montmorillonitischer Tonboden. Die Entwicklung der Wurzeln von einjährigen...


Massendichtemessungen, und Beobachtung der Profile gemeinsam mit einer anhaltenden Erhöhung der Ernteerträge und Entwicklung der Wurzeln, über drei Jahre weisen darauf hin, das die Vorteile, welche durch die Abänderung der Struktur von schweren Tonböden mit mechanischen Mitteln erreicht wurden für eine unbegrenzte Zeit anhalten könnten.
Plant growth, and especially root growth, are affected by many biological, chemical, and physical properties of the soil. The biological properties will not be considered in this paper except to state that species characteristics are important.

Food production is concentrated on soils developed under temperate to tropical climatic conditions. Under these conditions, many soils have developed layers with extreme physical and/or chemical heterogeneity. Particularly noticeable are the fine-textured, compact subhorizons, as contrasted to the coarser-textured and less compact surface horizons. Not readily visible, but easily detectable, is the chemical heterogeneity. The surface layer usually contains more organic matter, more nutrients, and fewer toxic substances, such as soluble aluminium and manganese, than the deeper layers. Root growth is adversely affected by these compact layers, by the deficiency of nutrients, and by the toxic substances.

Efforts to ameliorate certain poor physical conditions have been practised for more than a hundred years. A summary of the results up to 1952 was published by Lutz (1952). With the advent of modern fertilizer technology and modern machinery, many attempts have been made to increase depth of rooting by supplying fertilizer and lime to the deeper layers. Whether the factors limiting root growth were physical or chemical, it has been the principal thesis of the investigators that if root growth were deepened, the plants could obtain water and nutrients from a larger volume of soil. Apparently, very little attention has been given to the availability, or perhaps more correctly the unavailability, of water and nutrients in these adverse layers of the soil. It is the purpose of this paper to discuss this point in light of past and current research.

Many investigators have studied the effects of soil physical and/or chemical properties on root development under field or laboratory conditions. Some of the more recent ones are: Adams and Lund (1966), Adams and Wear (1957), Barley (1963), Barley et al. (1965), de Roo (1957, 1961), Engelbert and Truog (1956), Feibenschneider et al. (1958), Foy et al. (1965a, b), Goedewaagen et al. (1955), Kohnke and Bertrand (1956), Larson et al. (1960), Long (1959), Pearson (1966), and Taylor and Gardner (1963). Many of these investigators have reported increased
depth of rooting resulting from the physical or chemical treatments of the deeper layers. However, in many cases, crop yields were decreased considerably. When increases were obtained they were usually uneconomical; only rarely have the increases been economically profitable.

The question naturally arises as to why treatment of these heterogeneous sublayers usually has not increased crop yields. Is there fallacy in the reasoning that physical or chemical treatment of these sublayers will result in the roots getting more water and nutrients? Let us examine the situation.

The development of the root system of a plant is as much a species characteristic as the development of the above-ground part. Many species produce relatively shallow root systems regardless of the soil strata. Other species that characteristically have a long tap root have a major portion of the total root system relatively close to the soil surface. This has been shown by de Roo (1957, 1961), Goedewaagen et al. (1955), Long (1959), Moore and Rhoades (1966), and by numerous other investigators. Typical of these are the data of Long (1959) shown in Table 1. They show that

<table>
<thead>
<tr>
<th>Crop</th>
<th>Depth (cm)</th>
<th>Total Root Weight (Kg/ha)</th>
<th>Percent of Total Root Weight to Given Depth</th>
<th>Sum of Percent to Depth Shown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>0-15</td>
<td>1087</td>
<td>57.3</td>
<td>79.6</td>
</tr>
<tr>
<td></td>
<td>15-40</td>
<td></td>
<td>22.3</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>0-15</td>
<td>2570</td>
<td>69.0</td>
<td>86.6</td>
</tr>
<tr>
<td></td>
<td>15-38</td>
<td></td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td>0-13</td>
<td>2005</td>
<td>79.1</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>13-30</td>
<td></td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>0-15</td>
<td>13400</td>
<td>47.3</td>
<td>74.7</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td></td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-45</td>
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<td>11.7</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>0-15</td>
<td>5830</td>
<td>78.0</td>
<td>89.9</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td></td>
<td>11.9</td>
<td></td>
</tr>
</tbody>
</table>

more than three-fourths of the total root weight is produced in the upper layers of the soil, with by far the greater percentage being produced in the upper 15 centimeters. The root system of the alfalfa on line 4 extended to a total depth of approximately 200 centimeters and weighed 2.3 times as much as that of the alfalfa on line 5, in which essentially all of the roots were within the top 60 centimeters of soils. In spite of these differences, the three-year average yield was 9 per cent greater where the root system was more shallow. Apparently, some factor other than total weight or total length of roots is more important.
The surface area and length of a unit mass of roots is important. Let us examine the surface area and length of one kilogram of roots (or root hairs) of various diameters (Table 2). These calculations are based on cylindrical roots with a density of 95 g/cm³. It is, of course, obvious that the large roots have a short length, and an extremely small surface area, per unit mass. Expressing root distribution in soils in terms of weight per given depth is less meaningful than giving total root length or surface area per unit volume of soil. This is extremely important in terms of water or nutrient-supplying capacity from deeper soil layers, since usually only the large roots penetrate the deeper layers. Even if there is a great mass of large roots in the deeper layers, they will be relatively ineffective in obtaining water or nutrients. Even though the root mass is large, the surface area is small. If, as in many temperate to tropical humid region soils, the sublayer is fine textured, the hydraulic conductivity will be too slow to supply a significant quantity of water to the limited root surface. This is true regardless of the tremendous difference in potential that might exist between the root surface and a point only a few centimeters distant in the soil as shown by Gardner (1960, 1964, 1966) and Gardner and Ehlig (1962). Mason et al. (1957) reported saturated hydraulic conductivities on thousands of soil profiles from the Southeastern United States. The average value for the B horizon was 0.00064 cm per second, which was less than one-third that of the A horizon. Some values were as low as 0.00008 cm per second. Even if the water in the B horizon of such soils is held at very low tensions, the amount moved will be too small to be of significant value. In other words, a few large roots are essentially useless. The water in the B horizon of such soils can be useful only if the soil is thoroughly permeated with small roots, thus affording a large total root surface area within close proximity to the water. So far, essentially all physical and chemical treatments have failed to produce the desired root proliferation under natural field and climatic conditions.

Gardner (1960, 1966) suggested that under certain conditions considerable water might move from deeper layers to overlying layers. This would...
be true particularly if there were not large differences in texture or in hydraulic conductivity of the different layers, as is true in many dry-land soils. In humid region soils that have dense, compact fine-textured $B$ horizons with low hydraulic conductivity, negligible quantities of water would be expected to move from the $B$ to the coarser-textured $A$ horizons. If horizontal movement within the fine-textured layer is too slow to be of any benefit, then upward movement into a coarser-textured layer would be even less significant.

In humid regions, another factor affecting both water movement and root distribution is the amount and frequency of rainfall. In much of the humid region of the United States, the rainfall during the principal growing season occurs mostly as small showers.

TABLE 3.
SUMMER RAINFALL AMOUNT AND FREQUENCY.
TOTAL FOR 10 YEARS (1951-60), GREENSBORO, N.C., U.S.A.$^a$

<table>
<thead>
<tr>
<th>Rainfall Amount (mm/day)</th>
<th>Total days in 10 years with rainfall amount as indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
</tr>
<tr>
<td>0 - 2.3</td>
<td>19</td>
</tr>
<tr>
<td>2.5 - 6.1</td>
<td>19</td>
</tr>
<tr>
<td>6.4 - 12.5</td>
<td>2.5</td>
</tr>
<tr>
<td>12.7 - 25.2</td>
<td>2</td>
</tr>
<tr>
<td>25.4 - 50.6</td>
<td>1</td>
</tr>
<tr>
<td>50.8 +</td>
<td></td>
</tr>
</tbody>
</table>

Daily Potential ET$^b$ (mm/day) 3.50 4.22 3.84 3.38 3.73

$^a$ Courtesy Mr. A. V. Hardy, U.S. Weather Bureau, Raleigh, N.C.
$^b$ Van Bavel and Verlinden (1956).

Typical of a large segment of the Eastern United States are the data from Greensboro, North Carolina (Table 3). The data are totals for a ten-year period; not averages. Even though at Greensboro, June, July, and August have higher rainfall than any other months in the year, there were only 30 days in ten years when the daily rate exceeded 25 millimeters—an average of only one day per month. In that area, rainfalls of more than 25 mm per day usually come as hard downpours, and runoff is high. Infiltration, therefore, amounts to considerably less than the total rainfall. Table 3 shows that most of the rains occur as light showers, with the total rainfall in many instances being less than the daily potential evapotranspiration.

Even if all the rainfall infiltrates, the soil is rarely wetted below normal plow depth during the principal growing season. In an area with rainfall characteristics and potential evapotranspiration as shown in Table 3, deep wetting during the summer is the exception. Therefore, the fine-textured,
compact subhorizon becomes relatively dry and hard early in the season, before roots have had an opportunity to penetrate it. Under these conditions, root growth is profuse in the uppermost layer that is wetted frequently, but very sparse in the dry sublayer. Roots just do not grow in dry soils. Only on soils with extremely sandy or shallow topsoils, or with very low water-holding capacity, are the sublayers wetted during the growing season. Since the rainfall is sufficient to penetrate to a depth of only 15 to 20 cm, attempts to facilitate water movement below that depth are usually fruitless. This is true whether the attempts are physical, that is, breaking up the compact layers, or chemical, such as the addition of lime and fertilizer. As indicated earlier, under practical field conditions almost all attempts at physical or chemical manipulation of the soil below good plow depth, that is, below 20 to 30 cm, have not given economically profitable increases in crop yields. Typical of these are the data from a Coastal Plain soil at Laurinburg, North Carolina, where the soil was ploughed to depths of 18, 38, and 56 cm (Table 4). Considerable additional fertilizer and lime were worked into the deep-plowed plots. It is obvious from the data that the deeper plowing and the additional fertilizer and lime were of no benefit on cotton, corn, or tobacco.

Data on soil water-holding capacity and on water content show that the deeper plowing increased the 15-bar percentage and decreased the available water content of the topsoil (Figure 1). The increase in the 15-bar percentage at both the 18- and 38-cm depths is explained by mixing of clay from deeper layers with the sandy loam A horizon; and, the increase in the 15-bar percentage at the 56-cm depth is explained by the compaction of the soil at that depth. The 15-bar percentage was determined on core samples.

There was a high percentage of available water present at the 38- and 56-cm depths. Field examination showed that there were large roots at

<table>
<thead>
<tr>
<th>Depth of Plowing (cm)</th>
<th>Yield (Kg/ha)</th>
<th>Seed Cotton</th>
<th>Corn</th>
<th>Tobacco</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1838</td>
<td>7001</td>
<td>2440</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>2006</td>
<td>6340</td>
<td>2432</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>1850</td>
<td>6310</td>
<td>2346</td>
<td></td>
</tr>
</tbody>
</table>

*a Average of 2 years, 6 replications, and 2 fertility levels.
* Average of 2 years, 3 replications, 2 N, and 5 P and K levels.
* Average of 2 years, 4 replications, and 2 fertility levels.
Fig. 1.—Effect of depth of plowing on the 15-bar percentage and on the summer-average soil water content ($P_1$, $P_2$, and $P_3$ = plowing depth of 18, 38, and 56 cm, respectively).

these depths but the root distribution and the total root surface area, coupled with the low hydraulic conductivity of the soil, was such that water was not used in appreciable quantities from the deeper layers. Also, during the one summer in which the moisture determinations were made, topsoil moisture was depleted for only short periods. The following year it was depleted, but yields on the three plow depths followed the same pattern.

REFERENCES

SUMMARY

Availability of soil water to plant roots is a function of both water movement and of root distribution. The thesis is presented that root distribution is more important than water movement. Most agricultural crops have a large percentage of the roots in the top 20 to 30 cm of soil. This may be due to: (a) plant species characteristics; (b) physical and/or chemical heterogeneity of the soil; or (c) insufficient rainfall during the growing season to penetrate deeper. Previous efforts to deepen the root zone have usually not proven economically feasible under practical field conditions. The altered sublayers are usually penetrated by only a few large roots, with a short total length and a very small surface area per unit mass. With insufficient rainfall to penetrate the altered layers, their water content is usually low, and water movement to the few large roots is practically negligible. For water in the deeper layers to be useful, those layers must be permeated by small roots with a large surface area per unit mass. If the top 20 to 30 cm is relatively homogeneous and in good physical and chemical condition for root development, efforts to ameliorate deeper layers are usually not economically beneficial. However, correction of physical or chemical barriers within the top 20 to 30 cm is apt to be profitable.

RÉSUMÉ

La disponibilité de l'eau du sol pour les racines des plantes est fonction à la fois du mouvement d'eau et de la distribution des racines. On propose la thèse que la distribution des racines est plus importante que le mouvement d'eau. Dans la plupart des cultures agricoles, on trouve un grand pourcentage de racines dans les 20 à 30 cm de la partie supérieure du sol. Ceci peut provenir de: (a) caractéristiques d'espèces de plantes; (b) hétérogénéité physique et/ou chimique du sol; (c) précipitations insuffisantes pendant la saison de croissance pour pénétrer plus profondément. Généralement, des efforts antérieurs pour établir plus profondément la zone des racines ne se sont pas montrés économiquement réalisables sous les conditions pratiques du champ. Les sous couches altérées sont pénétrées généralement par quelques grandes racines seulement, avec une longueur totale courte et une très petite surface par unité de masse. Avec des précipitations insuffisantes pour pénétrer dans les couches altérées, leur teneur en eau est généralement basse, et le mouvement de l'eau vers quelques grandes racines est pratiquement négligeable. Pour que l'eau, dans les couches plus profondes, puisse être utile, ces couches doivent être pénétrées par de petites racines avec une grande surface par unité de masse. Si les 20 à 30
cm supérieurs sont relativement homogènes et fournissent de bonnes conditions physiques et chimiques pour le développement des racines, des efforts pour améliorer les couches plus profondes ne sont généralement pas advantageux économiquement. Cependant, une correction des barrières physiques ou chimiques à l’intérieur des 20 à 30 cm supérieurs a tendance à être profitable.

ZUSAMMENFASSUNG

Verfügbarkeit von Bodenwasser für Pflanzenwurzeln ist eine Funktion sowohl der Wasserbewegung als auch der Wurzelanordnung. Die These wird vorgeschlagen, dass die Wurzelanordnung wichtiger ist, als die Wasserbewegung. Die meisten landwirtschaftlichen Saaten haben einen grossen Prozentsatz der Wurzeln in den obersten 20 bis 30 cm des Bodens. Dies kann den folgenden Umständen zugeschrieben werden:
a) Charakteristik der Pflanzentypen
b) Physikalische und/oder chemische Heterogenität des Bodens, oder
c) Ungenügender Regenfall während der Wachstumszeit um tiefer eindringen zu können.

Frühere Versuche die Wurzelzone zu vertiefen, haben sich, unter normalen Feldbedingungen, meistens nicht als ökonomisch ausführbar erwiesen.

SOIL CULTIVATION AS A FACTOR AFFECTING YIELDS

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Soil cultivation is one of the most ancient agricultural practices, but it has the weakest basis as compared to other agrotechnical measures. There is conflicting scientific evidence relating to deep ploughing. Obviously this problem must be considered in accordance with soil conditions and properties of cultivated plants.

We carried out long experiments on krasnozems and subtropical podzolic soils of Western Georgia, aiming to establish an optimal depth of soil tillage prior to planting tea.

Both soil types develop in humid subtropical climate with annual rainfalls ranging from 1500 mm to 2500 mm and a mean annual temperature equal to 14.7°C. Krasnozemic soils occur in the hilly regions with rounded topography, slope gradients approximately 10-25°, and do not occur higher than 200-250 m above sea level. Parent rocks are deep weathering crusts of andesite-basaltic porphyrites and boulder-pebble deposits. The subtropical podzolic soils occur on ancient slightly undulated accumulative terrace and on pebble-boulder deposits as well.

Krasnozems and subtropical podzolic soils differ considerably in their properties: the first having a weakly differentiated profile, rather loose fabric, well-developed structure and high porosity and permeability. Subtropical podzolic soils possess a strongly differentiated profile and a bright very compact illuvial horizon with abundant iron concretions, frequently cemented to form a hardpan. Data on the contents of humus and the main nutritive elements are presented in Table 1. It should be added that both soil types have an acid reaction throughout the profile: $pH_{KCl}$ ranges between 4.0 and 4.5.

Some of the physical properties of these soils are given in Table 2. Krasnozems have better physical properties than the subtropical podzolic soils; the latter require special agrotechnical measures for the improvement of physical properties and for destroying the ortstein (concretionary) horizon (Daraselia, 1947).

Field tests have shown advantages of deep soil tillage (Daraselia, 1948). Deep tillage increased the total porosity and air-retaining capacity of lower soil horizons. More pronounced alterations took place on subtropical podzolic soils where the porosity of illuvial compact horizons increased after tillage by 10%.

The type of cultivation used influences the fabric of the cultivated soil layers. After trenching the highest porosity and moisture-content were observed below, in the humus-rich layer.
## Table 1

CONTENT OF HUMUS, NITROGEN, PHOSPHORUS AND POTASSIUM IN EXPERIMENTAL SOILS

<table>
<thead>
<tr>
<th>Soil</th>
<th>Depth cm</th>
<th>Total content</th>
<th></th>
<th>Hydrolyzed nitrogen</th>
<th>Mobile forms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Humus %</td>
<td>Nitrogen %</td>
<td>$P_2O_5$ %</td>
<td>$K_2O$ %</td>
</tr>
<tr>
<td>Krasnozem on</td>
<td>0—10</td>
<td>7.00</td>
<td>0.35</td>
<td>0.19</td>
<td>0.95</td>
</tr>
<tr>
<td>pebbles</td>
<td>25—35</td>
<td>2.40</td>
<td>0.20</td>
<td>0.17</td>
<td>0.81</td>
</tr>
<tr>
<td>Anaseuli</td>
<td>45—55</td>
<td>0.45</td>
<td>0.10</td>
<td>0.08</td>
<td>0.75</td>
</tr>
<tr>
<td>80—90</td>
<td></td>
<td>0.30</td>
<td>0.08</td>
<td>0.07</td>
<td>0.88</td>
</tr>
<tr>
<td>Subtropical</td>
<td>0—10</td>
<td>5.00</td>
<td>0.28</td>
<td>0.14</td>
<td>1.15</td>
</tr>
<tr>
<td>podzolic,</td>
<td>25—35</td>
<td>0.85</td>
<td>0.16</td>
<td>0.09</td>
<td>0.97</td>
</tr>
<tr>
<td>Zugdidi</td>
<td>50—60</td>
<td>0.50</td>
<td>0.12</td>
<td>0.06</td>
<td>0.98</td>
</tr>
<tr>
<td>70—80</td>
<td></td>
<td>0.20</td>
<td>0.10</td>
<td>0.08</td>
<td>0.65</td>
</tr>
<tr>
<td>Soil and location</td>
<td>Depth cm</td>
<td>Humus %</td>
<td>Bulk density g/cm³</td>
<td>Density g/cm³</td>
<td>Total porosity %</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------</td>
<td>---------</td>
<td>-------------------</td>
<td>---------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Krasnozem on pebbles, virgin land, Anaseuli</td>
<td>5—10</td>
<td>6·8</td>
<td>0·84</td>
<td>2·64</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>30—35</td>
<td>1·6</td>
<td>1·08</td>
<td>2·72</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>60—65</td>
<td>0·9</td>
<td>1·23</td>
<td>2·72</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>100—105</td>
<td>—</td>
<td>1·13</td>
<td>2·71</td>
<td>60</td>
</tr>
<tr>
<td>Subtropical podzolic virgin soil, Zugdidi</td>
<td>0—5</td>
<td>4·5</td>
<td>1·00</td>
<td>2·61</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>10—15</td>
<td>—</td>
<td>1·02</td>
<td>2·66</td>
<td>60</td>
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<tr>
<td></td>
<td>30—35</td>
<td>1·1</td>
<td>1·10</td>
<td>2·76</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>50—55</td>
<td>0·6</td>
<td>1·34</td>
<td>2·72</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>75—80</td>
<td>—</td>
<td>1·42</td>
<td>2·74</td>
<td>48</td>
</tr>
</tbody>
</table>
To find out how long the alterations due to cultivation last in the soil we made a periodic study of some physical properties. The results obtained showed a positive influence of deep tillage over a long period. Table 3 contains results of bulk density determination after 25 years of soil cultivation. With tillage to 54 cm the whole tilled layer maintained a loose fabric which is normally associated with well structured soils.

Depths and methods of soil cultivation determine the character of development and distribution of roots of a tea plant. Experiments showed that with increase of tillage depth the amount of roots increased, especially in the subsoil. This was most marked with trenching, where roots were concentrated in the humus-rich layer. Table 4 illustrates the root distribution of plants grown without any amendments under different cultivation procedures.

Application of fertilizers exerts a strong influence not only on the total root mass, but also affects its distribution in the soil profile. A considerable increase of small roots occurred after the addition of mineral

<table>
<thead>
<tr>
<th>Layer cm</th>
<th>Overdigging to 25 cm %</th>
<th>Overdigging to 45 cm %</th>
<th>Trenching to 45 cm %</th>
<th>Overdigging to 54 cm %</th>
<th>Trenching to 54 cm %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0—10</td>
<td>20.5</td>
<td>15.4</td>
<td>1.5</td>
<td>18.7</td>
<td>2.4</td>
</tr>
<tr>
<td>10—20</td>
<td>33.5</td>
<td>32.4</td>
<td>25.2</td>
<td>28.6</td>
<td>17.5</td>
</tr>
<tr>
<td>20—30</td>
<td>27.1</td>
<td>20.2</td>
<td>32.5</td>
<td>23.5</td>
<td>22.6</td>
</tr>
<tr>
<td>30—40</td>
<td>10.7</td>
<td>20.6</td>
<td>28.3</td>
<td>15.3</td>
<td>33.9</td>
</tr>
<tr>
<td>40—50</td>
<td>6.9</td>
<td>7.4</td>
<td>6.8</td>
<td>7.0</td>
<td>16.8</td>
</tr>
<tr>
<td>50—60</td>
<td>1.3</td>
<td>4.0</td>
<td>5.7</td>
<td>6.9</td>
<td>6.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth cm</th>
<th>Depth cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0—10</td>
<td>10—20</td>
</tr>
<tr>
<td>20—30</td>
<td>30—40</td>
</tr>
<tr>
<td>40—50</td>
<td>0—50</td>
</tr>
<tr>
<td>No fertilizer added</td>
<td>32 31 24 12 6 105</td>
</tr>
<tr>
<td>With N P K</td>
<td>117 94 32 29 17 289</td>
</tr>
</tbody>
</table>
fertilizers (NPK) as shown in Table 5. Phosphorus fertilizers exert a most stimulating influence on the development of root systems. In krasnozems, phosphorus is fixed with iron and aluminium oxides, and unavailable for plants. Experiments carried out demonstrated that phosphate fertilizers strongly increase the total mass of small roots which are especially abundant in the fertilized layer and independent of the depth of fertilizer placement.

Long-term application of mineral fertilizers (NPK) at 10-15 cm favoured the concentration of roots of tea plants in the topsoil with 80% of all the roots in the top 20 cm. Such a concentration resulting from a shallow placement of phosphorus amendments proved to be undesirable. First of all it decreased the water supply to the plant; water reserves in the topsoil fell rapidly owing to physical evaporation and consumption by plant roots, while the subsoil contained much water, not readily available for plants because of root scarcity there and of slow capillary rises. Besides, if root concentration in the topsoil is high, roots are injured during cultivation, and the yield decreases by 10% or even more.

Thus a large application of rational doses of mineral amendments sharply increased the productivity of tea plantations, making it 5-6 times higher in comparison with that of non-fertilized plantations. In the same time, however, due to shallow placement of fertilizer the water supply system of tea plants became worse. In years with relatively low precipitation during the vegetation period tea plants suffer from water deficiency, and yield falls. Consequently, two new problems appeared: investigation

<table>
<thead>
<tr>
<th>Depth of soil cultivation cm</th>
<th>Depth of P application cm</th>
<th>Sample depth cm</th>
<th>At start of experiment % of roots</th>
<th>At the 5th year of experiment % of roots</th>
<th>Tea leaf yield kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-40</td>
<td>0-35</td>
<td>0-10</td>
<td>30.4</td>
<td>37.0</td>
<td>5420</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-20</td>
<td>24.5</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-30</td>
<td>23.5</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-40</td>
<td>14.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>40-50</td>
<td>4.9</td>
<td>1.1</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50-60</td>
<td>1.9</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth of soil cultivation cm</th>
<th>Depth of P application cm</th>
<th>Sample depth cm</th>
<th>At start of experiment % of roots</th>
<th>At the 5th year of experiment % of roots</th>
<th>Tea leaf yield kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0-15</td>
<td>0-10</td>
<td>30.4</td>
<td>53.6</td>
<td>4232</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-20</td>
<td>23.9</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-30</td>
<td>22.3</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-40</td>
<td>16.7</td>
<td>2.7</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>40-50</td>
<td>4.8</td>
<td>2.4</td>
<td></td>
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<td></td>
<td></td>
<td>50-60</td>
<td>1.8</td>
<td>0.2</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth of soil cultivation cm</th>
<th>Depth of P application cm</th>
<th>Sample depth cm</th>
<th>At start of experiment % of roots</th>
<th>At the 5th year of experiment % of roots</th>
<th>Tea leaf yield kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-40</td>
<td>0-15</td>
<td>0-10</td>
<td>26.1</td>
<td>52.4</td>
<td>4600</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-20</td>
<td>37.0</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-30</td>
<td>22.0</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-40</td>
<td>12.1</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>40-50</td>
<td>1.8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-60</td>
<td>1.0</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>
of effects of artificial sprinkler irrigation in the tea plantations and elaboration of methods for deepening the root system.

Tests with sprinkler irrigation gave positive results: yields increased by 25-30% during several years with annual high and stable amounts of tea leaves collected. This practice is used now in many tea plantations in Georgia (Daraselia, Gvasava, 1959). Tests on methods to deepen the root system of the plants demonstrated the advantages of deep placement of phosphates compared with shallow placement.

With deep application of phosphorus the total amount of tea plant roots as well as the proportion in the subsoil, increased considerably (Table 6). The experiment was carried out on a 20-year-old plantation with the following conditions of tillage: (1) 35-40 cm with phosphorus introduced into the layer 0-35 cm; (2) 15 cm with phosphorus introduced into the layer 0-15 cm; (3) 35-40 cm with phosphorus introduced into the layer 0-15 cm. Each plot of the experiment received: superphosphate at 1000 kg P₂O₅ per hectare (being the requirement for 8-10 years); potassium at 150 kg K₂O per hectare; nitrogen in the form of ammonium nitrate was applied annually at 200 kg N per hectare at a depth of 5-6 cm. Table 6 shows that phosphorus placement at the deeper level has increased the tea-leaf yield during several years by 18%. Such an increase may be explained by a deeper and more regular root distribution and, as a consequence, by greater use of moisture and phosphates from the subsoil.

The effect of deep phosphorus application on root development (especially in the lower soil layers) proved to be still greater when this fertilizer was applied in the process of seed-bed preparation before the establishment of the plantation. Peculiarities of root distribution by a young tea plant related to the depth of phosphorus placement are indicated in Figure 1. When phosphorus is applied throughout the cultivated layer (0-45

![Fig. 1.—The percentage distribution of small roots (1 mm size or less) as affected by phosphorus distribution; A—soil cultivated to a depth of 45 cm with P incorporated to the same depth; B—soil cultivated to 45 cm, but P placed only to 15 cm depth.](image-url)
cm), plants develop a greater root-mass with a relatively regular distribution among the soil layers.

A machine has now been constructed for deep phosphate application in existing tea plantations.

When new tea plantations are established phosphorus is introduced during the deep seed-bed preparation at 500-1000 kg $P_2O_5$ per ha, distributed throughout the whole tillage depth.

Summing up, we can state that a system of soil cultivation and fertilizing, developed by the Institute of Tea and Subtropical Crops, provided a sharp rise of tea plantation productivity, bringing an average leaf yield to 4500 kg/ha and on the most productive plantations to 6-8 thousands kg per hectare. Creation of such plantations promoted a continuous increase of soil productivity. The abundant annual leaf drop and the storage of the cut material in rows, equal to 4-5 tons of green mass, favoured an increase in the organic matter content of the soil by 2% humus during 25 years and an increase in the productivity of the soil (Daraselia, 1964).

REFERENCES

SUMMARY
Soil tillage prior to planting is of considerable importance for the establishment of highly productive tea plantations. A deep tillage to 45 cm and 54 cm on krasnozems with their good physical properties and on subtropical podzolic soils with a compact illuvial horizon increased the productivity of the tea plantations. The depth of cultivation was more important than the method of cultivation and a tillage depth of 45 cm proved to be best in practice.

Fertilizing and depth of fertilizer placement was important. Surface application of mineral fertilizers raised tea plantation productivity 5-6 times, but at the same time led to a higher concentration of roots in the topsoil (80% of all the roots in the layer 0-20 cm). Root concentration in the topsoil decreased water supply to a tea plant because the water was lost quickly by evaporation from the soil surface and by root absorption, while deeper layers contained significant amounts of water.

Tests on krasnozems showed the great stimulating influence of phosphorus fertilizers on the development of small roots and showed the necessity for deep application of this fertilizer. Deep introduction of phosphorus (0-35 cm) contributed to a significant increase of the total
root mass, and to stronger root development in deeper layers, thus providing a more complete use of moisture and phosphates in the subsoil and an increase in tea leaf yields by approximately 18%.

**Résumé**

La préparation du sol avant la plantation est d'une importance considérable pour établir des exploitations de thé à grande productivité. La mise en état du sol à une profondeur de 45 cm à 54 cm dans les krasnozems à bonnes propriétés physiques et dans les sols podzoliques subtropicaux à horizon illuvial dense a augmenté la productivité des plantations de thé. La profondeur de la culture importait plus que la méthode et un travail à 45 cm de profondeur s'est avéré le meilleur dans la pratique.

La fumure et la répartition des engrais comptent beaucoup. L'application en surface d'engrais minéraux a quintuplé la productivité mais a provoqué en même temps une plus grande concentration des racines dans la couche de surface (80% des racines dans la couche de 0-20 cm). La concentration des racines en surface a diminué l'apport de l'eau au théier car l'eau s'évaporait rapidement de la surface et était absorbée par les racines alors que des couches plus profondes contenaient des réserves d'eau considérables.

Les essais dans les krasnozems ont mis en évidence l'action stimulante des engrais phosphoriques sur le développement des petites racines et ont amené à appliquer ces engrais en profondeur. L'apport du phosphore en profondeur (0-35 cm) a contribué à une augmentation sensible de l'ensemble des racines et au développement des grosses racines dans les couches profondes, bénéficiant de cette manière de toute l'humidité et des phosphates du sous-sol. Le rendement des feuilles de thé a tout de suite augmenté d'environ 18%.

**Zusammenfassung**

Bodenbearbeitung vor der Anpflanzung ist für die Errichtung von ertragsreichen Teeplantagen von grosser Bedeutung. Auf Krasnozemen mit ihren guten physikalischen Eigenschaften, sowie auf subtropischen podzolischen Böden mit einem festen illuvialen Horizont, wurde die Ertragsfähigkeit der Teeplantagen durch tiefe Bodenbearbeitung von 45 cm und 54 cm erhöht. Die Anbautiefe war wichtiger als die Anbaumethode, und eine Bodenbearbeitungstiefe von 45 cm, erweist sich praktisch am günstigsten.

Düngung und die Tiefe der Düngeanwendung war wichtig. Oberflächenanwendung mineralischer Düngemittel, erhöhten um 5-6 Mal die Ertragsfähigkeit der Teeplantagen, aber führte gleichzeitig zu einer grösseren Wurzelkonzentration in der Ackerkrume, (80% aller Wurzeln in der 0-20 cm Schicht). Durch Wurzelkonzentration in der Ackerkrume nimmt die Wasserzufuhr zu der Teepflanze ab, infolge schnellen Wasserverlustes durch Bodenoberflächenausdünstung, und Wurzelauflnahme, während tiefeere Schichten bedeutende Wassermengen enthielten.
Versuche auf Krasnozemen erweisen den stark anregenden Einfluss der Phosphordünger auf die Entwicklung kleiner Wurzeln, sowie den Bedarf für tiefe Anbringung dieses Düngemittels. Tiefe Phosphoreinführung (0-35 cm) trug zu einer bedeutenden Erhöhung der gesamten Wurzelmasse sowie zu der stärkeren Wurzelentwicklung in den tieferen Schichten bei, demgemäß wurde eine vollkommener Ausnutzung der Feuchtigkeit und Phosphate in den Unterböden, und eine Zunahme von etwa 18% an Teeblättererträgen erreicht.
EFFECT OF DEEP PROFILE MIXING AND AMENDMENT ADDITIONS ON SOIL CHARACTERISTICS AND CROP PRODUCTION OF A SPODOSOL

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Department of Soils
Institute of Food and Agricultural Sciences
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The spodic horizon in Leon fine sand usually is between 10 and 20 cm in thickness and lies within the 30 to 50 cm depth. It is higher in O.M. (organic matter), Al, Fe, and mineral fines than adjacent horizons, and has a high degree of conformity with the watertable. Profiles of Spodosols are strongly acid and very low in fertility. Root proliferation is limited to the profile above the spodic horizon unless the soil is drained. Yields on superficially drained soils vary with the moisture distribution. They are influenced by excess moisture and poor aeration during periods of high rainfall, and by drought during dry periods due to the shallow root system. In the following study, the effect on soil characteristics and crop growth, of profile mixing to a depth of 90 cm, and deep placement of certain soil amendments, is reported.

HISTORY

Robertson, et al. (1957, 1959, 1965) conducted experiments on a wide range of sandy soils in Florida and showed that breaking the hardpan or plough sole increased root penetration, and that placement of fertilizer improved root development in the fertilizer zone. This often was accompanied by increased yield. However, there was very little, if any, residual effect on crop yields. In a later study (Robertson, et al., 1966) it was found that crops grown on soil from the spodic horizons of Leon fine sand responded more to P and Ca additions than did the surface soil, suggesting that lack of nutrients in the spodic horizon might inhibit root proliferation in this horizen even following drainage of Spodosols. Under acid conditions, significant movement of fertilizer P has been observed (Neller, 1951), but this movement is very limited in soils with pH above about 5·5. Calcium moves readily as soluble salts into sandy subsoils. This results in appreciable replacement of titratable acidity in strongly acid soils, but massive movement of calcium bicarbonate from liming materials cannot be appreciable beyond an advancing front more acid than about pH 5·8 (Volk, 1955, 1956).

1 Florida Agricultural Experiment Stations Journal Series No. 2725.
The experiment consisted of five treatments in a Latin square (Table 1). The plots were 14·8 x 16·5 m in size. Before treatment, six lines of 10-

<table>
<thead>
<tr>
<th>No.</th>
<th>Initial Treatment</th>
<th>Subsequent Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Material* Rate</td>
<td>Mixed**</td>
</tr>
<tr>
<td></td>
<td>kg/ha cm</td>
<td>kg/ha cm</td>
</tr>
<tr>
<td>1</td>
<td>L 8063</td>
<td>90 L 1500</td>
</tr>
<tr>
<td>2</td>
<td>L + P 8063 + 7055</td>
<td>90 L 1500</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>90 L 3000</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>None L 3000</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>None L 1500</td>
</tr>
</tbody>
</table>

* L = high calcic lime; P = rock phosphate. FTE503 (balanced fritted microelement source) was broadcast on all plots at 13·5 kg/ha.
** Depth of soil mixing.

cm tile were placed at 90 cm depth between plot tiers. The deep mixing of the soil and simultaneous incorporation of high calcic limetone and rock phosphate in the initial treatment was accomplished by broadcasting the total amount of material on the surface and following with repeated transits with an endless chain bucket type ditching machine making a cut 45 cm wide and 90 cm deep. The soil from the first transit of a plot was piled temporarily on the surface. The soil from subsequent transits was placed in the previously opened trench. There was always a width of 135 cm between each transit so that the walls of the trench would not cave in. The loose soil backfilled into the trench was packed with the treads of a large bulldozer. One series of transits completed a quarter of the plot area. Repeating this operation three times with a 45-cm shift in location each time completed the mixing process. Finally the soil from the first trench was moved into the last trench of the plot with a bulldozer and front-end loader. This process of soil homogenization is more efficient than deep ploughing. After completion of the deep mixing and deep incorporation treatments, the shallow incorporations (Table 1) were broadcast on the surface and the entire area rotavated to 15-cm depth.

One-half the area was planted to Florida 200 corn and one-half to Pensacola bahiagrass (*Paspalum notatum*), using the split plot technique. The experiment was not irrigated as a possible benefit of the treatments would be the development of a deeper root system and greater drought tolerance. An attempt to introduce clover, *Trifolium repens*, in combination with the grass the first year was not successful. Past experience by the authors indicates that changed moisture relations introduced by the drainage did not allow the clover germinated to survive the spring dry season. This factor needs further evaluation in drainage of sandy Spodosols. Grass yields were taken in 1963 and 1964. The grass was destroyed by tillage after the
last harvest in 1964 and in 1965, 1966, and 1967, the plots were planted
to sorghum, corn, and corn, respectively.

The corn and sorghum received 770 kg/ha of 2-27-4-54-6-81 at
planting, followed by approximately 200 kg/ha of N from NH₄NO₃ as a
side dressing 30 days after planting. Grass received the same fertilizer as
corn except that the mixed fertilizer was broadcast in the spring and the
N top dressing added in split applications following each cutting.

Corn ear leaf samples were collected at tasseling time and analysed for
total N, P, K, Ca and Mg. Similar analyses were made on the grass at
each harvest. The sorghum was harvested at ground level and analysed for
nitrogen only.

Soil samples were taken initially to a depth of 15 cm and annually to
a depth of 60 cm in 15 cm increments. Soil pH, O.M., P, K, Ca, and Mg
determinations were made. The pH was determined in water and O.M. by
the Walkley-Black method (Walkley, 1935). The P, K, Ca, and Mg were
extracted with 1N NH₄OAc (pH 4·8). The 1967 samples were frac­
tionated by the method of Chang and Jackson (Chang and Jackson, 1957,
and Hsu and Jackson, 1960) to obtain water soluble-P, Al-P, Fe-P, and acid

<table>
<thead>
<tr>
<th>cm</th>
<th>0-15</th>
<th>15-30</th>
<th>30-45</th>
<th>45-60</th>
<th>Avg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% O.M.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.62</td>
<td>1.51</td>
<td>1.45</td>
<td>1.43</td>
<td>1.50</td>
</tr>
<tr>
<td>2</td>
<td>1.45</td>
<td>1.38</td>
<td>1.28</td>
<td>1.22</td>
<td>1.33</td>
</tr>
<tr>
<td>3</td>
<td>1.51</td>
<td>1.49</td>
<td>1.35</td>
<td>1.47</td>
<td>1.46</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.53</td>
<td>1.46</td>
<td>1.36</td>
<td>1.39</td>
<td>1.43</td>
</tr>
<tr>
<td>4</td>
<td>2.24</td>
<td>1.59</td>
<td>1.43</td>
<td>1.53</td>
<td>1.70</td>
</tr>
<tr>
<td>5</td>
<td>2.08</td>
<td>1.62</td>
<td>1.25</td>
<td>1.33</td>
<td>1.57</td>
</tr>
<tr>
<td>Avg.</td>
<td>2.16</td>
<td>1.60</td>
<td>1.34</td>
<td>1.43</td>
<td>1.64</td>
</tr>
<tr>
<td>1967</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.41</td>
<td>1.47</td>
<td>1.25</td>
<td>1.46</td>
<td>1.40</td>
</tr>
<tr>
<td>2</td>
<td>1.46</td>
<td>1.42</td>
<td>1.05</td>
<td>1.20</td>
<td>1.28</td>
</tr>
<tr>
<td>3</td>
<td>1.38</td>
<td>1.28</td>
<td>0.99</td>
<td>1.16</td>
<td>1.20</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.42</td>
<td>1.39</td>
<td>1.10</td>
<td>1.27</td>
<td>1.29</td>
</tr>
<tr>
<td>4</td>
<td>1.89</td>
<td>1.58</td>
<td>1.53</td>
<td>1.61</td>
<td>1.65</td>
</tr>
<tr>
<td>5</td>
<td>1.87</td>
<td>1.42</td>
<td>1.18</td>
<td>1.62</td>
<td>1.52</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.88</td>
<td>1.50</td>
<td>1.36</td>
<td>1.62</td>
<td>1.59</td>
</tr>
</tbody>
</table>

1 Treatments 1, 2, and 3 were mixed and/or had lime and phosphate placed 90 cm in the
profile in addition to surface treatment. Treatments 4 and 5 had only surface treatment
See Table 1 for details.
soluble-P. Phosphorus was determined on the final residue. Organic P was the difference between P removed by digestion with 5N HCl from soil ignited at 600°C and that removed by similar extraction from non-ignited soil (Bray and Kurtz, 1945).

In 1967, soil cores were taken in 15 cm depth intervals to 60 cm using a tube 3.25 cm in diameter. A composite sample of six cores per plot was taken 3 cm from the base of corn plants. Viable roots were separated out and weighed.

RESULTS AND DISCUSSIONS

The data in Table 2 show that tillage to 90 cm distributed the O.M. in the profile. Initial soil samples were not taken for variability because the area appeared to be relatively uniform. Variability that did exist is suggested by comparison of O.M. found at various depths in treatments 4 versus 5 (Gammon et al., 1953). Similar differences existed between mixed and undisturbed treatments in 1964 and 1967. However, 1967 values were lower than 1964. The decrease amounted to 10% and 3% for the mixed and undisturbed treatments. Progressive changes in organic matter decomposition following initial drainage may have accounted for the general decrease, but the relative decrease in the mixed as compared to the undisturbed subsoils may have been due to improved drainage and aeration introduced by destruction of the spodic horizon.

Lower soil density may have existed and introduced an error in relative O.M. contents, but continued decrease in O.M. between 1964 and 1967

<table>
<thead>
<tr>
<th>Depth sampled (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
</tr>
<tr>
<td>0-15</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>1965</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1360</td>
</tr>
<tr>
<td>2</td>
</tr>
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<td>1352</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>1010</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>1185</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>769</td>
</tr>
<tr>
<td>Extractable Ca (kg/ha)²</td>
</tr>
<tr>
<td>1967</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1340</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>1213</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>1039</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>1174</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>753</td>
</tr>
</tbody>
</table>

1 See Table 1 for details of treatments.
2 Ca extracted with 1N NH₄OAc (pH 4.8).
suggested that differences were valid. The O.M. reduction in the undisturbed treatments was significant only in the top 30 cm.

The distribution of Ca in the profile of the mixed as compared to the undisturbed treatments (Table 3) further indicated the relative effectiveness of the mixing. The higher pH in treatment 3 where the soil was mixed was not consistently associated with higher Ca values as compared to treatment 4 where it was undisturbed.

### Table 4

<table>
<thead>
<tr>
<th>Ca Sources</th>
<th>Extracted by:</th>
<th>Accounted for²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1962 lime</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fertilizer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Original soil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5N HCl</td>
<td>1N NH₄OAc³</td>
</tr>
<tr>
<td></td>
<td>5N HCl</td>
<td>1N NH₄OAc³</td>
</tr>
<tr>
<td>Treatment¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6500</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>880</td>
<td>8380</td>
</tr>
<tr>
<td></td>
<td>6898</td>
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</tr>
<tr>
<td></td>
<td>82</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>1500</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>880</td>
<td>3380</td>
</tr>
<tr>
<td></td>
<td>2094</td>
<td>1742</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>52</td>
</tr>
</tbody>
</table>

¹ See Table 1 for details of treatments.
² Plant removal, which is not included, probably was less than 100 kg/ha during the 5-year period.
³ 1N ammonium acetate, adjusted to pH 4·8.

Data in Table 4 show the distribution of the Ca in the soil five years after start of the experiment. The original soil Ca, plus Ca applied as lime and in the fertilizer was estimated and compared with extractable Ca for treatments 2 and 5. Samples were taken to 90 cm in 1964 and 1965. Unreported data on these samples showed that Ca distribution in the 60 to 90 cm depth was similar to that in the shallower subsoil depths. Although plant removal of Ca was not calculated for Table 4, it was estimated to be less than 100 kg/ha. The higher values for 5N HCl extraction as compared to extraction with 1N NH₄OAc (pH 4·8) may be attributed to the coarse residues of limestone not dissolved by 1N NH₄OAc. Since 5N HCl extraction did not account for all the Ca even when a maximum value for plant removal was assumed, it was evident that some leaching of Ca occurred. The higher percentage loss for treatment 5 may be related to the lower replaceable bases other than Ca in the profile (Volk 1965, 1966).

The data in Table 5 show that a high percentage of P in undisturbed soil was in Al-P form. Added P apparently was primarily in organic and acid soluble fractions. There was some indication that Al-P was increased in the subsoil. The acid soluble P was in the form of Ca-phosphates. The organic-P is distributed in the profile in a manner similar to that of O.M. (Table 2). Higher P values for most fractions in the surface increments may be attributed to residual fertilizer. Apparently there was little leaching of P since most of the applied P was recovered.

The means of four-years' data of corn yields (Table 6) show significant differences due to treatments in 2 out of 4 years. Highest yields came from the treatments where the profile was undisturbed. Of the three deep treat-
TABLE 5  
EFFECT OF LIME AND PHOSPHATE MIXED TO 90 CM ON VARIOUS PHOSPHATE FRACTIONS 5 YEARS AFTER START OF THE EXPERIMENT$^1$

<table>
<thead>
<tr>
<th>Fraction $^2$</th>
<th>Depth sampled (cm)</th>
<th>0-15</th>
<th>15-30</th>
<th>30-45</th>
<th>45-60</th>
<th>Total</th>
<th>Grand Total$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lime mixed 90 cm (Treatment 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic P</td>
<td>$^6$Organic P</td>
<td>78</td>
<td>51</td>
<td>55</td>
<td>40</td>
<td>224</td>
<td>533</td>
</tr>
<tr>
<td>Water soluble P</td>
<td>$^6$Water soluble P</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Al-P</td>
<td>$^6$Al-P</td>
<td>61</td>
<td>30</td>
<td>18</td>
<td>28</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>Fe-P</td>
<td>$^6$Fe-P</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Acid soluble P</td>
<td>$^6$Acid soluble P</td>
<td>62</td>
<td>39</td>
<td>30</td>
<td>8</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lime and phosphate mixed 90 cm (Treatment 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3056</td>
</tr>
<tr>
<td>Organic P</td>
<td>$^6$Organic P</td>
<td>613</td>
<td>458</td>
<td>259</td>
<td>369</td>
<td>1699</td>
<td></td>
</tr>
<tr>
<td>Water soluble P</td>
<td>$^6$Water soluble P</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Al-P</td>
<td>$^6$Al-P</td>
<td>69</td>
<td>47</td>
<td>31</td>
<td>35</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>Fe-P</td>
<td>$^6$Fe-P</td>
<td>16</td>
<td>13</td>
<td>9</td>
<td>10</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Acid soluble P</td>
<td>$^6$Acid soluble P</td>
<td>403</td>
<td>308</td>
<td>162</td>
<td>242</td>
<td>1115</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed only to 90 cm (Treatment 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>557</td>
</tr>
<tr>
<td>Organic P</td>
<td>$^6$Organic P</td>
<td>95</td>
<td>45</td>
<td>37</td>
<td>36</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Water soluble P</td>
<td>$^6$Water soluble P</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Al-P</td>
<td>$^6$Al-P</td>
<td>72</td>
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<td>23</td>
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<td>Fe-P</td>
<td>$^6$Fe-P</td>
<td>15</td>
<td>8</td>
<td>4</td>
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<td>31</td>
<td></td>
</tr>
<tr>
<td>Acid soluble P</td>
<td>$^6$Acid soluble P</td>
<td>81</td>
<td>33</td>
<td>18</td>
<td>13</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Undisturbed soil (Treatment 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>429</td>
</tr>
<tr>
<td>Organic P</td>
<td>$^6$Organic P</td>
<td>119</td>
<td>56</td>
<td>26</td>
<td>43</td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>Water soluble P</td>
<td>$^6$Water soluble P</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Al-P</td>
<td>$^6$Al-P</td>
<td>50</td>
<td>24</td>
<td>9</td>
<td>21</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Fe-P</td>
<td>$^6$Fe-P</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Acid soluble P</td>
<td>$^6$Acid soluble P</td>
<td>26</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Initial phosphate application shown in Table 1 plus approximately 175 kg/ha applied in the fertilizer plus native soil P. Phosphate removal in the crops was approximately 60 kg/ha. See Table 1 for details of treatments.

$^2$ Organic P is difference between P removed by 5N HCl from soil ignited at 600°C and that removed by similar extraction from non ignited soil. Water soluble P, Al-P, Fe-P, and acid soluble P were extracted by subsequent extraction with 1 N NH$_4$Cl, 0.5N NH$_4$F, 0.1N NaOH, and 0.5N H$_2$SO$_4$.

$^3$ Does not include "occluded" P, which was extremely low.

ments, the lime-phosphate treatment was the best every year and on the average significantly better than deep placement of lime and soil mixing only. This indicated that under the conditions of the experiment, availability of P limited the production of corn. Mixing the soil without subsoil addition of lime and phosphate (treatment 3) reduced the yield below that of treatment 4 where the profile was undisturbed. Reduction of organic matter in the surface soil, and possibly less drought tolerance as a result of increased rate of drainage following destruction of the spodic horizon, may
be the reasons for lower yields. Corn root penetration was determined in 1967, a year in which rainfall was well distributed. Regardless of mixing or placement of amendments, corn root penetration was largely confined to the surface 30 cm. There was no apparent difference in root distribution between the 0-15 and 15-30 cm depths. In other experiments in which physical barriers were broken with a subsoiler and a long drought period occurred during early growth, corn roots penetrated much deeper into the soil than noted in the current tests (Robertson and Hutton, 1965). Chemical data on ear leaf samples from corn were not consistently affected by treatments.

Bahiagrass and sorghum yields reported in Table 7 were not significantly affected by the various treatments, but the uptake of N was significantly higher for undisturbed than mixed treatments. The lower N content for mixed treatments probably was the result of dispersion of surface O.M.

### Table 6

**CORN EAR YIELDS AS AFFECTED BY LIME AND PHOSPHATE PLACEMENT**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7397</td>
<td>4749a</td>
<td>4127</td>
<td>5041a</td>
<td>5328a</td>
</tr>
<tr>
<td>2</td>
<td>7708</td>
<td>5606b</td>
<td>4672</td>
<td>5609a</td>
<td>5898b</td>
</tr>
<tr>
<td>3</td>
<td>7397</td>
<td>4671a</td>
<td>4204</td>
<td>5061a</td>
<td>5333a</td>
</tr>
<tr>
<td>4</td>
<td>7708</td>
<td>5606b</td>
<td>4905</td>
<td>6774b</td>
<td>6248b</td>
</tr>
<tr>
<td>5</td>
<td>7163</td>
<td>5840b</td>
<td>4594</td>
<td>6618b</td>
<td>6057ab</td>
</tr>
</tbody>
</table>

1 Values followed by the same letter or no letter are not significantly different at the 5% level of probability.
2 See Table 1 for details of treatments.

### Table 7

**GRASS AND SORGHUM YIELDS AND NUTRIENT CONTENT AS AFFECTED BY PLACEMENT OF LIME AND PHOSPHATE**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1963</th>
<th>1964</th>
<th>1965</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Yield</td>
<td>Uptake</td>
<td>Uptake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>4521</td>
<td>7642</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>4927</td>
<td>7287</td>
<td>94</td>
</tr>
<tr>
<td>3</td>
<td>5046</td>
<td>6977</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>5428</td>
<td>7593</td>
<td>101</td>
</tr>
<tr>
<td>5</td>
<td>4736</td>
<td>7894</td>
<td>105</td>
</tr>
</tbody>
</table>

1 See footnote 1 Table 6.
2 In 1963, 1964 and 1965 there were accumulations of 2, 3, and 1 cuttings, respectively.
3 All yields are oven dry weight.
4 See Table 1 for details of treatments.
throughout the profile. The O.M. was both a source of N and a retainer of applied N. Mixing of the profile increased the Ca content of bahiagrass. Deep placement of lime improved the P content of bahiagrass, but uptake was generally better from the undisturbed soil than for the mixed treatments. Higher O.M. in the surface of the undisturbed profile probably supplied a relatively active medium for decomposition of rock phosphate and maintenance of availability of applied readily available phosphate. The grass yields were low in comparison to those from more highly fertilized areas but were realistic for the amount of N applied, as indicated by percentage of N recovered (Blue et al., 1961, Volk, 1966).

**REFERENCES**


Hsu, P. H. and Jackson, M. L. (1960)—Inorganic phosphate transformations by chemical weathering as influenced by pH. *Soil Sci.* 90, 6-24.


**SUMMARY**

Following alteration by drainage, the effects of deep treatments consisting of mixing the profile of a Spodosol (Leon fine sand) with or without limestone and P (rock phosphate) to a depth of 90 cm were compared with surface liming at low and high rates.

Over a 5-year period the O.M. decreased, and the decrease was more rapid for the deep treatments than for the undisturbed soil. Improved drainage and aeration as a result of mixing were probably responsible. After
5 years, \( P \) applied as rock phosphate was primarily in organic and acid soluble forms. No appreciable amount was lost by leaching. Approximately one-half of the total soil \( Ca \) was extractable with \( 1N \) \( NH_4OAc \) (\( pH \) 4–8).

When the soil profile was deep mixed, corn yields were less than those from the undisturbed soil. The reduced yields were probably the result of dilution of the surface soil O.M. Lime addition to the subsoil had no effect on yield, but mixing with lime and phosphate gave yields comparable to the undisturbed treatments. Corn root distribution was not affected by deep treatments. Roots were confined to the top 30 cm. The nutritional value of \( P \) apparently compensated for the detrimental effect of mixing.

Bahiagrass and sorghum yields were not affected by the deep treatments. However, nitrogen content of the grass was always lower, on the deep treatments indicating a possible differential response to soil organic matter status. Phosphorus uptake was reduced and \( Ca \) uptake of the bahiagrass was increased by soil mixing.

**RÉSUMÉ**

À la suite de l’altération par le drainage, des traitements profonds consistant en le mélange d’un profil de Spodosol (sable fin Léon) avec ou sans calcaire avec \( P \) (phosphate de roche) à une profondeur de 90 cm furent comparés au chaulage de la surface à un degré bas ou élevé.

Pendant une période de 5 ans l’O.M. diminua, mais la décroissance fut plus rapide pour les traitements profonds que pour le sol non dérangé. Le drainage amélioré et l’aération par suite du mélange en étaient probablement la cause. Des analyses du sol après 5 ans montrèrent que \( P \) appliqué comme phosphate de roche était principalement sous des formes organiques et solubles dans les acides. Aucune quantité sensible ne fut perdue par lessivage. A peu près la moitié du \( Ca \) total du sol pourrait être extraite avec \( 1N \) \( NH_4OAc \) (\( pH \) 4–8).

L’examen du monolithe de sol 5 ans après le traitement initial montra que la répartition des racines de blé n’était pas affectée par les traitements profonds. Les racines étaient limitées aux 30 cm supérieurs. Quand le profil du sol fut mélangé, les rendements de blé furent moindres que ceux du sol non dérangé. L’addition de calcaire au sous-sol ne fut pas meilleure que le mélange seul. Pourtant un mélange avec du calcaire et du phosphate donna des rendements comparables à ceux provenant des traitements du sol non dérangé. Evidemment la valeur nutritive de \( P \) compense l’effet nuisible de mélanger le sol.

Les rendements d’herbe de bahaia et de sorgho ne furent pas affectés par les traitements profonds. Pourtant, la teneur en azote de l’herbe était toujours plus basse sur les traitements profonds, montrant une réponse différentielle possible à la condition de la matière organique dans le sol. Pour l’herbe de bahaia, l’absorption de phosphore fut réduite tandis que celle de \( Ca \) fut augmentée par le mélange.

L’efficacité du mélange profond du sol au moyen d’un appareil à creuser des fossés en comparaison du labour profond est evidente par l’homogénéité notée par le prélèvement d’échantillons subséquents. Cepen-
Infolge einer Veränderung durch Entwässerung, wurden Tiefebehand- lungen welche in Mischung des Spodosol-Profiles (Leon Feinsand) mit oder ohne Kalkstein und P (Mineralischem Phosphat) bis zu einer Tiefe von 90 cm bestanden, mit Oberflächen Kalkung bei geringen und hohen Zugaben, verglichen.


L'INFLUENCE DE LA ROTATION, DE LA FUMURE ET DE L'ENFOISSEMENT DES PAILLES SUR LA STRUCTURE DU SOL

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Institut d'Agronomie générale et cultures herbacées, Université de Bari, Italie

Malgré le grand nombre de recherches effectuées depuis plusieurs années sur la rotation des cultures et de la fumure (organique ou minérale) l'importance pratique de ces sujets, les vicissitudes économiques qui entraînent des changements continus dans le choix des cultures et surtout les nombreuses possibilités d'interaction entre ces deux facteurs et les conditions écologiques, des données expérimentales supplémentaires dans ce domaine sont nécessaires, surtout dans certaines régions où la culture des céréales est la plus économique.

L'Institut d'Agronomie générale et de culture herbacées de Bari a entrepris, il y a 12 ans, des recherches dans trois localités caractéristiques de la région Méditerranéenne. On donne ici les résultats afférents à la structure du sol et à la vitesse d'infiltration de l'eau.

MATÉRIAUX ET MÉTHODES

Les recherches ont été effectuées dans trois localités: Foggia, Bari et Castellaneta (Tarante), les caractéristiques principales du sol sont données dans le tableau 1. Dans toutes ces régions le climat varie entre semi-aride et humide (454-605-487 mm/ans respectivement à Foggia, Bari et Castel-

<table>
<thead>
<tr>
<th>TABLEAU 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARACTÈRES DES SOLS DES ESSAIS</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>squelette ((\phi &gt; 2) mm); % du total</td>
</tr>
<tr>
<td>sable (2 &gt; (\phi &gt; 0,02) mm); % terre fine</td>
</tr>
<tr>
<td>limon (0,02 &gt; (\phi &gt; 2\mu)); &quot; &quot; &quot;</td>
</tr>
<tr>
<td>argile ((\phi &lt; 2\mu)); &quot; &quot; &quot;</td>
</tr>
<tr>
<td>matière organique; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>calcaire; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>N total; % &quot; &quot; &quot;</td>
</tr>
<tr>
<td>(P_2O_5) total; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>(K_2O) total; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>(P_2O_5) assimil.(Ferrari); p.p.m. &quot; &quot; &quot;</td>
</tr>
<tr>
<td>(K_2O) assimil.(Dirk-Scheffer); &quot; &quot; &quot;</td>
</tr>
</tbody>
</table>

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### Tableau 2 - Traitements appliqués dans les 3 essais

<table>
<thead>
<tr>
<th>Cultures et rotations</th>
<th>Témoins ( (P_2O_5) ) ( (q/ha) ) ( (kg/ha) )</th>
<th>Avec fumier ( (P_2O_5) ) ( (q/ha) ) ( (kg/ha) )</th>
<th>Minérale ( N ) ( P_2O_5 ) ( (kg/ha) )</th>
<th>Organique ( ^{11} ) et minérale fumier ( N ) ( P_2O_5 ) ( (q/ha) ) ( (kg/ha) )</th>
<th>Minérale double ( N ) ( P_2O_5 ) ( (kg/ha) ) ( (kg/ha) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section factorielle (pour les 3 champs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotation de 4 ans:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plante cultivée ( ^{12} ):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blé</td>
<td>(36) 100 (- 36)</td>
<td>66.6 33.3 (36*22)</td>
<td>0</td>
<td>66.6 33.3 (36*22)</td>
<td>133.3 66.6 (36*22)</td>
</tr>
<tr>
<td>Fourrage annuel ( ^{14} ):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blé</td>
<td>(36) 0 (- 36)</td>
<td>66.6 16.6 (36*22)</td>
<td>0</td>
<td>66.6 16.6 (36*22)</td>
<td>133.3 33.3 (36*22)</td>
</tr>
<tr>
<td>Section non factorielle (blé en monocult.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Champ de Foggia et Castellaneta:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Série azote différencié:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*Note: Les chiffres entre parenthèses représentent les doses utilisées en kg/ha.*
<table>
<thead>
<tr>
<th></th>
<th>100 0</th>
<th>150 50 (+ 22)</th>
<th>paille 100 50 (+ 22)</th>
<th>paille 100 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>série phosphate différencié</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>traitements augmenté de 100 kg/ha de $K_2O^{(4)}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>traitements isolés: jachère$^{(6)}$</td>
<td>100 0</td>
<td>150 50 (+ 22)</td>
<td>paille$^{(5)}$ 100 50 (+ 22)</td>
<td></td>
</tr>
</tbody>
</table>

1. Le mot "paille" indique l'enfouissement de toute la paille produite par la parcelle dans l'année précédente. Ce traitement remplace le fumier.

2. Les valeurs entre parenthèses (+ 36) indiquent la dose de $P_2O_5$ appliquée comme base à tout le champ, à l'exception du "témoin absolu" ou des traitements isolés. L'indication (+ 36*22) veut dire que la dose complémentaire de $P_2O_5$ était de 36 kg/ha (lire plus 36 ou 22) dans les champs de Foggia et Castellaneta, et 22 kg/ha à Bari.

3. Betterave à sucre à Foggia; tomate à Castellaneta (Taranto); coton à Bari.

4. Mélange de féverole, vesce etavoine.

5. La fumure potassique a été additionnée à celle d'azote, phosphore et, dans un cas, de paille.

laneta), la distribution annuelle des précipitations est typiquement Méditerranéenne. Le sol du champ de Foggia est argileux, profond et fertile; le sol de Bari est une "terra rossa" d'origine alluviale récente, riche en calcaire; le sol de Castellaneta consiste en sables rouges d'origine pléistocène.

1er essai. Champ de Foggia. Cet essai a été effectué pour comparer trois cultures consécutives: une rotation de 4 ans, une de 3 ans et une monoculture de blé. et 5 régimes de fertilisation par an, à savoir, témoin sans fumure, épandage de fumier, engrais chimiques, engrais organiques et chimiques, engrais chimiques doubles. Pour la monoculture céréalière on a effectué en outre des traitements supplémentaires comprenant une série de trois doses croissantes d'azote, deux parcelles avec enfouissement de toute la paille produite par ces mêmes parcelles l'année précédente, l'une sans azote et l'autre avec engrais azoté. Le champ entier recevait en plus chaque année une fumure de base de 36 kg/ha de $P_2O_5$; un témoin absolu, sans cette fumure de base, a été établi également.

Toutes les phases des rotations comparées ont été effectuées chaque année. En tout on a étudié 46 traitements dont 40 à structure factorielle.

La dose simple des engrais minéraux a été établie de façon à fournir à peu près la même quantité d'éléments fertilisants que le fumier (composition normale).

La quantité d'engrais apportée aux parcelles sous rotations a été calculée de façon à fournir au sol des doses moyennes égales aux doses appliquées en monoculture. Au cours de la rotation les engrais ont été répartis inégalement suivant les critères considérés les meilleurs sur la base de recherches précédentes. L'enfouissement de la paille et du fumier a été effectué pendant le labourage, vers la fin du mois de septembre. Tableau 2 donne les résultats détaillés de ces traitements.

L'essai, répété 3 fois, a été effectué suivant un plan expérimental de parcelles où des traitements différents sont distribués au hasard. Parcelles de $(7.00 \times 7.14)$ m$^2$; surface mesurée 25 m$^2$. Essai commencé en automne 1954.


3ème essai. Champ de Bari. En utilisant des caisses pareilles à celles du 2ème essai et à côté de celui-ci, on a étudié pendant 3 ans l'influence de l'enfouissement de grandes quantités de paille (0-50-200 q/ha) et de
<table>
<thead>
<tr>
<th></th>
<th>témoin</th>
<th>fumier</th>
<th>fertil. minérale</th>
<th>fumier et fertil. minérale</th>
<th>fertil. minérale double</th>
<th>moyennes</th>
</tr>
</thead>
<tbody>
<tr>
<td>rotation de 4 ans</td>
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<tr>
<td>betêche</td>
<td>38.5</td>
<td>38.9</td>
<td>38.4</td>
<td>42.2</td>
<td>35.8</td>
<td>38.7</td>
</tr>
<tr>
<td>blé</td>
<td>34.4</td>
<td>42.8</td>
<td>38.9</td>
<td>35.7</td>
<td>39.0</td>
<td>38.4</td>
</tr>
<tr>
<td>fourrage an.</td>
<td>38.3</td>
<td>39.2</td>
<td>41.1</td>
<td>39.1</td>
<td>38.7</td>
<td>39.5</td>
</tr>
<tr>
<td>blé</td>
<td>43.5</td>
<td>37.7</td>
<td>37.2</td>
<td>39.6</td>
<td>37.6</td>
<td>39.1</td>
</tr>
<tr>
<td>rotation de 3 ans</td>
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<td></td>
</tr>
<tr>
<td>fourrage an.</td>
<td>43.2</td>
<td>39.4</td>
<td>41.3</td>
<td>42.0</td>
<td>43.8</td>
<td>41.9</td>
</tr>
<tr>
<td>blé</td>
<td>39.1</td>
<td>40.8</td>
<td>43.5</td>
<td>42.4</td>
<td>40.3</td>
<td>41.2</td>
</tr>
<tr>
<td>blé</td>
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<td>43.4</td>
<td>40.9</td>
<td>41.9</td>
<td>39.9</td>
<td>41.2</td>
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<tr>
<td>monoculture</td>
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</tr>
<tr>
<td>blé</td>
<td>39.8</td>
<td>46.9</td>
<td>43.1</td>
<td>43.4</td>
<td>39.4</td>
<td>42.5</td>
</tr>
</tbody>
</table>

effets significatifs à P = 0.01: rotation; blé des 3 rotations

effets significatifs à P = 0.05: cultures x fumures
quatre formes d’azote (nitrate de calcium, nitrate d’ammonium, sulfate d’ammonium, urée) à deux niveaux différents (50-20 kg/ha de N et un témoin); le tout factoriellement combiné. Les traitements ont été appliqués pendant la première année seulement. Au cours de la 3ème année l’essai a été complété par l’introduction de la comparaison entre un groupe de parcelles sous jachère et un autre groupe sous culture de blé. Traitements distribués au hasard, répétés 4 fois; dans la 3ème année il y a eu une “confusion” partielle dans l’interaction des formes d’azote x cultures.

4ème essai. Champ de Castellaneta (Tarante). L’essai a été effectué suivant un plan identique à celui de Foggia. La seule différence consistait dans la culture—à Castellaneta la tomate a été choisie comme la plus typique.

Pour tous les essais, le 3ème excepté, l’azote a été fourni sous forme de nitrate d’ammonium (20-21%), le phosphore comme superphosphate (18-20%) et la potasse comme sulfate de potassium (50-52%). La composition chimique du fumier employé pendant les différentes années et dans tous les essais a varié légèrement.

L’examen de l’effet des traitements sur les structures du sol a été effectué à deux occasions en utilisant des méthodes différentes. En juillet 1961, sept ans après le commencement des recherches, deux échantillons de sol ont été prélevés à 5 cm et à 30 cm de profondeur dans chaque caisse des essais de Bari. Après séchage à l’air la stabilité de leur structure a été étudiée par la méthode de Tiulin, modifiée plusieurs fois, (tamisage à l’eau, mailles carrées de 0,25 mm, course 3 cm, fréquence 30 osc/min). Les résultats sont exprimés en pourcentage de terre fine (diamètre > 0,25 mm) qui a résisté au traitement.

D’autres déterminations ont été effectuées en 1957-58 par la même méthode et dans les mêmes caisses, en prélevant des échantillons chaque mois pendant une année solaire.

Dans les essais de Foggia et Castellaneta les échantillons prélevés de chaque parcelle en octobre 1966, 12 ans après le commencement des recherches, ont fourni une moyenne de sol de 5 points de la parcelle et d’une couche de 30 cm. On a introduit cette fois le pré-traitement différentiel des échantillons suivant la méthode de Hénin, c’est-à-dire pré-traitement avec alcool absolu, avec benzène et un témoin sans pré-traitement. Les mailles du tamis ont été réduites à 0,2 mm.

Les déterminations de la vitesse d’infiltration ont été effectuées dans le 3ème essai (Bari) seulement, elles ont été répétées deux fois: en novembre 1957—deux ans après le commencement de l’essai, et en mars 1959—à la fin de l’essai. On a employé l’appareil de Müntze-Musgrave. La faible vitesse relevée dans le premier cas a suggéré l’emploi de la caisse entière comme surface d’infiltration; dans le 2ème cas, par contre, on a employé le cylindre double.

L’analyse statistique des données afférentes à la stabilité de la structure du sol a été effectuée après transformation des données en angles et retransformation inverse des moyennes. Seules les données significativement différentes sont présentées.
L’INFLUENCE SUR LA STRUCTURE DU SOL

DISCUSSION DES RÉSULTATS

Les données des tableaux 3, 4 et 5 démontrent clairement que dans tous ces trois sols la rotation avait une influence sur la stabilité des agrégats. En considérant la moyenne des parcelles soumises à toutes les cultures à chaque rotation, on constate dans tous les champs une stabilité inférieure dans le cas de rotation de 4 ans. La monoculture de blé a donné, presque dans tous les cas, une stabilité plus élevée; à Bari seulement, associée à une forte fertilisation minérale, la stabilité de la structure était réduite en comparaison avec celle des parcelles en rotation.

En étudiant les détails on peut observer que seulement dans le cas du sol sablonneux de Castellaneta les différences entre les trois cultures sont évidentes. La tomate (culture intercalaire) a une structure beaucoup plus instable que les autres cultures. Les différences les plus évidentes sont observées en comparant les résultats obtenus lorsque la culture du blé est incorporée dans les trois rotations. Ces différences sont significatives dans les essais de Foggia et Tarante, et correspondent avec l’effet moyen des rotations.

L’influence négative de la jachère sur la stabilité de la structure est évidente à Bari (17,2 contre 25,8 du blé en monoculture). Cet effet est confirmé par la détermination de la vitesse d’infiltration (Bari. 3ème essai

**TABLEAU 4**

AGRÉGATS RÉSISTANTS (φ > 0,25 MM). CAISSES CÉMENTÉES; BARI. TRAITEMENTS FACTORIELS

<table>
<thead>
<tr>
<th>rotation et culture</th>
<th>fumure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>organique et</td>
</tr>
<tr>
<td></td>
<td>minérale</td>
</tr>
<tr>
<td></td>
<td>minérale</td>
</tr>
<tr>
<td></td>
<td>double</td>
</tr>
<tr>
<td></td>
<td>moyenne</td>
</tr>
<tr>
<td>rotation de 4 ans (moyenne)</td>
<td>25,4</td>
</tr>
<tr>
<td>coton</td>
<td>24,9</td>
</tr>
<tr>
<td>blé</td>
<td>23,1</td>
</tr>
<tr>
<td>fourrage annuel</td>
<td>28,4</td>
</tr>
<tr>
<td>blé</td>
<td>25,5</td>
</tr>
<tr>
<td>moyenne des 2 blés</td>
<td>24,3</td>
</tr>
<tr>
<td>rotation de 3 ans (moyenne)</td>
<td>28,2</td>
</tr>
<tr>
<td>fourrage annuel</td>
<td>32,5</td>
</tr>
<tr>
<td>blé</td>
<td>28,3</td>
</tr>
<tr>
<td>blé</td>
<td>23,8</td>
</tr>
<tr>
<td>moyenne des 2 blés</td>
<td>26,1</td>
</tr>
<tr>
<td>blé en monoculture</td>
<td>31,2</td>
</tr>
<tr>
<td>jachère continuelle</td>
<td>—</td>
</tr>
</tbody>
</table>

effets significatifs à $P = 0,01$: rot. x fum.; cultures x rot. x fum.; fumier/minérale.
**Tableau 5**

**AGRÉGATS RÉSISTANTS (φ > 0,2 MM; MOYENNE DES 3 PRÉ-TRAITEMENTS). CHAMP DE CASTELLANETA. TRAITEMENTS FACTORIELS**

<table>
<thead>
<tr>
<th>Rotation de 4 ans:</th>
<th>Rotation de 3 ans:</th>
<th>Monoculture:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomate</td>
<td>Fourrage an.</td>
<td>Blé</td>
</tr>
<tr>
<td>18,5</td>
<td>23,8</td>
<td>26,7</td>
</tr>
<tr>
<td>Blé</td>
<td>Blé</td>
<td>Blé</td>
</tr>
<tr>
<td>23,4</td>
<td>25,6</td>
<td>26,7</td>
</tr>
<tr>
<td>Fourrage an.</td>
<td>Blé</td>
<td></td>
</tr>
<tr>
<td>21,3</td>
<td>26,2</td>
<td></td>
</tr>
<tr>
<td>Blé</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24,4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21,8</td>
<td>24,5</td>
<td>26,7</td>
</tr>
</tbody>
</table>

Effet moyen de la fumure:
- Témoin: 22,0
- Fumier: 24,8
- Fert. minéral: 23,2
- Fumier et fertilisat. min. paille: 24,8
- Fert. min. double: 23,7

Effets moyens des cultures:
- Tomate: 18,5
- Fourrage an.: 21,3
- Blé: 25,3

Effet du blé dans les différentes rotations:
- Blé en rot. de 4 ans: 23,9
- " " " 3 " : 26,0
- " " monocult. : 26,7

Effets significatifs à $P = 0,01$: rotations, cultures.
" " " $P = 0,05$: fumures, blé en rotations différentes.

**Tableau 6**

**EFFETS DE LA MATIÈRE ORGANIQUE SUR LA STABILITÉ DES AGREGATS, SOUMIS À DIFFÉRENTS TRAITEMENTS. CHAMP DE CASTELLANETA**

<table>
<thead>
<tr>
<th></th>
<th>Sans fumier</th>
<th>Avec fumier</th>
<th>Sans paille</th>
<th>Avec enfouiss. de paille</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sans prétraitement:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sans fertilis. minérale</td>
<td>11,5</td>
<td>14,5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Avec fertilis. minérale</td>
<td>12,8</td>
<td>14,0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moyenne</td>
<td>12,2</td>
<td>14,3</td>
<td>14,2</td>
<td>21,3</td>
</tr>
<tr>
<td>Prétraitement à l'alcool</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sans fertilis. minérale</td>
<td>61,2</td>
<td>63,5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Avec fertilis. minérale</td>
<td>62,7</td>
<td>61,0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moyenne</td>
<td>62,0</td>
<td>62,3</td>
<td>65,0</td>
<td>68,2</td>
</tr>
<tr>
<td>Prétraitement au benzène</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sans fertilis. minérale</td>
<td>4,9</td>
<td>6,2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Avec fertilis. minérale</td>
<td>5,2</td>
<td>5,7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moyenne</td>
<td>5,0</td>
<td>6,0</td>
<td>5,9</td>
<td>10,1</td>
</tr>
</tbody>
</table>

Effets significatifs à $P = 0,01$: fumier et paille, sans prétraitement et benzène.
" " " $P = 0,05$: interaction fumier x minérale, sans prétraitement et alcool.
L'influence sur la structure du sol

5,2 x 10^{-2} cm/sec après 1 an de jachère, contre 6,7 x 10^{-2} cm/sec après blé.

La fumure, comme telle, avait une faible influence sur la stabilité de la structure; à Castellaneta, seulement, les différences enregistrées sont significatives et montrent clairement l'efficacité du fumier. Dans certains cas, toutefois, la fumure a quelque peu influencé l'effet de la rotation (à Bari) ou les cultures (à Foggia), sur la stabilité de structure: à Bari une influence négative de la fumure minérale se manifeste particulièrement sur le blé en monoculture (interaction culture x rotation x fumure). A Foggia, bien que moins clairement, on rencontre des valeurs extrêmes en comparant le blé en rotation de 4 ans sans fumure (34,4), avec le blé en monoculture traité avec fumier (46,9).

L'efficacité de l'épandage de fumier n'est pas évidente à Foggia mais on la trouve à Bari (en moyenne 30,6 avec fumier, contre 19,9 sans fumier) et à Castellaneta. Dans ce dernier champ on peut observer (Tableau 6) que le pré-traitement au benzène et la méthode classique rendent cet effet plus évident, tandis que le pré-traitement à l'alcool éthylique le rend négligeable. Ce résultat confirme la valeur diagnostique des pré-traitements prévus par la méthode de Hénin.

L'influence favorable de l'enfouissement de la paille est significative à Bari (19,8 sans paille, 28,6 avec paille) et à Castellaneta (14,2 contre 21,3); bien que peu importante elle semble exister à Foggia (41,5 contre 44,5).

L'effet favorable de la paille est démontré par les données de vitesse d'infiltration (3ème essai). Deux ans après le traitement, cette vitesse était en moyenne de 8,8 x 10^{-4} cm/sec sans paille, contre 12,4 x 10^{-4} dans les parcelles avec enfouissement de paille.

L'augmentation de l'engrais azoté seulement n'a donné des résultats significatifs qu'à Bari, en montrant une faible réduction de stabilité aux doses les plus basses et ensuite un effet favorable (67,8-64,7-76,0-97,5 en augmentant l'azote de 0 à 150 kg/ha).

La fumure phosphatée n'a pas influencé la stabilité de la structure dans aucun des champs. La fumure potassique a donné un résultat favorable à Bari: (25,3 sans K. contre 33,3 avec K; P = 0,05).

Aucune influence significative des traitements n'a été observée à Bari dans les échantillons prélevées à 30 cm de profondeur.

L'examen des données obtenues a été effectué chaque mois, de décembre à la fin de juin et a montré une amélioration progressive de la stabilité de structure, liée aux facteurs climatiques.

Résumé

On donne ici les résultats les plus importants obtenus à partir de deux essais de 12 ans, d'un essai de 7 ans et d'un de 3 ans, concernant la structure du sol. Les traitements comprennent des comparaisons entre rotations (de 4 et de 3 ans) et la monoculture de blé, et des comparaisons entre fertilisations minérales et organiques, y compris l'enfouissement de la paille.

Les résultats soulignent l'importance de la rotation et des cultures.
Dans tous les essais une forte réduction de la stabilité de la structure est causée par la rotation de 4 ans. La monoculture de blé a donné généralement les résultats les meilleurs; la tomate et la jachère continuelle—les pires.

L'action de la fertilisation est moins évidente. L'épandage de fumier et l'enfouissement des pailles ont déterminé, presque dans tous les cas, un effet remarquable; ce dernier traitement augmente la vitesse d'infiltration de l'eau dans le sol. Seuls les engrais azotés et potassiques ont donné des résultats favorables à la structure du sol dans les champs. L'effet du phosphore a été nul.

La stabilité des agrégats est augmentée en passant de l'hiver à l'été.

ZUSAMMENFASSUNG


Die Ergebnisse zeigen die Wichtigkeit der Fruchtfolge und des Pflanzenanbaus. Alle Versuche haben bewiesen, dass die bedeutendste Abnahme in der Festigkeit der Bodenstruktur bei der 4-jährigen Fruchtfolge stattfindet. Die Weizen-Monokultur hat im allgemeinen die besten Ergebnisse erwiesen; Tomatenanbau und Dauerbrache—die schlechtesten.


Die Stabilität der Aggregate steigt beim Übergang vom Winter zum Sommer.

SUMMARY

The main results obtained from two 12-year trials, one 7-year trial and one 3-year trial are given, concerning soil structure. Treatments include comparisons between rotations (4-year and 3-year) and wheat monoculture, and comparisons between mineral and organic fertilizings, including the burying of straw.

The results show the big importance of rotation and of crops. In all trials, a marked reduction of structural stability is caused by the 4-year rotation. Wheat monoculture generally gave the best results; tomato and continuous fallow the worst.

The effect of fertiliser is less obvious. Manure-dressing and straw-drilling had in nearly all cases a noticeable effect; the latter increased the speed of water infiltration into the soil. The nitrogen fertiliser and potassic fertiliser fields were the only ones showing some results favorable to the soil structure. The effect of phosphorus is nil.

The stability of aggregates increases when passing from winter to summer.
Fertilizers in which the minerals become available to plants over an extended period have been of interest to agronomists for some time. Thus far, only a few such materials such as urea-formaldehyde, C-D urea (2-keto-4 methyl-6 ureidohexahydro-pyrimidine) and IBDU (1, 1 diureido isobutane) are in commercial use and these are used mostly on specialty crops in limited volume because of cost considerations. The use of coatings on granular, soluble fertilizers has been considered by some (Oertli and Lunt 1962, Army 1963) to be a promising technique for achieving controlled nutrient availability. A newly developed technique of the Thiokol Chemical Company for coating fertilizer is not only effective but also promises to be reasonable in cost. Potential advantages include the reduction in frequency of fertilizer application, reduction of injury hazard from large applications, and greater utilization efficiency where leaching losses are normally high. The principal objective of the studies reported here was to examine the efficiency of recovery of coated and uncoated urea by cropping to corn in two soils.

**DESCRIPTION AND PROPERTIES OF THE COATED FERTILIZER**

In the Thiokol coating process, spherodized urea is pretreated to alter its surface characteristics and is then sprayed with a hot mixture of sulphur and a plasticizing agent typically followed by a sealer of soft wax. This fills pin holes and small cracks. Variations in coating components and technical finesse may be brought to bear on the coating process to give various rates of release. A ton (2,240 lb) of urea may be coated with 150 to 300 lb of the modified sulphur and about 20 lb of sealer to control release rates below 1% per day. The cost of coating materials would be a few dollars per ton of urea. The finished product is hard, freeflowing, and free of dust. Figure 1 shows the rate of release of urea to water as a function of time at 23°C. from 3 experimental batches of coated fertilizer labelled “A”, “B” and “C”. It is apparent that by manipulation of the coating technique a considerable range of release rates is obtainable. It has been shown by studying individual granules that the rapid release rate in the first 48 hours is due to some granules being improperly coated.

The completely coated granules vary in coating thickness and thus yield release curves of the shape shown. The release of urea from single granules is generally linear which is consistent with a diffusion mechanism. The membrane is slightly more permeable with increasing acidity. Soil moisture
content in the range which supports plant growth has little effect on the release rate.

Figure 2 shows the response of grass to an application of 6 lb of N per 1000 square feet from the three coated fertilizers shown in Figure 1. The experiment was performed by removing 2.5 x 10 cm deep plugs of soil from established Alta fescue turf, growing in pots, deficient only in nitrogen and growing initially at a rate of about 15-20% of optimum. The appropriate amount of fertilizer was mixed in the soil plugs which were then used to refill the holes. Irrigation rates were adjusted so that leachate was about 10% of applied irrigation water. Under these conditions, unless the grass is supplied with a super-abundance of nitrogen, the nitrogen in the root zone is approximately quantitatively recovered by the roots and the clipping yield reflects fairly well (except at low yields) the nitrogen accumulation by the plant. In the study, a separate liquid fertilizer treatment provided a reference point for maximum growth. At the rate used, material "A" maintained maximum yields (response was parallel to the liquid fertilizer treatment) for about 7 weeks. Material "C" performed much better than would have been expected from the water elution data. This is believed to be due to the adsorption of part of the wax sealer by the soil, which results in a faster release rate. Comparison of water elution and release of urea from coated urea in soil indicates that release in moist soil is never appreciably slower than in water (it is often about the same) and
CONTROLLED NUTRIENT RELEASE

![Graph](image)

**Fig. 2.—** Growth of Alta fescue after the addition of 6 pounds of nitrogen per 1000 square feet into the soil from the three experimental coated urea samples (A, B and C) shown in Figure 1. (Upper curve is optimally growing Alta fescue maintained by liquid fertilization shown for comparison).

sometimes faster. Studies such as the above have shown substantial release of nitrogen for about 80 to 100 days from coated urea in soil. When coated fertilizers are applied to the surface of soil, the release rate may be slower because of intermittent drying.

**EFFICIENCY OF RECOVERY OF NITROGEN FROM COATED UREA**

**Experiment 1.** Golden Bantam corn was planted in Moreno sandy loam (64% F. sand and V.F. sand; 26% silt plus clay) on April 28, 1966. Twenty-five lb of P<sub>2</sub>O<sub>5</sub> from single superphosphate and 75 lb of K<sub>2</sub>O from KCl were disked in prior to planting. Treatment variables involved the source, amount, and timing of the nitrogen fertilizer and are summarized in Table 1. Plots consisted of 4 rows, each 3 feet wide and 8 feet long. Corn was planted at one foot intervals (14,520 plants/acre). Treatments were replicated 4 times and arranged in a randomized block. Two blocks were set up. One was sprinkler irrigated at approximately a normal rate, about 25% in excess of the estimated evapotranspiration; once or twice per week prior to tasselling and twice per week after tasselling. This block received
a total of 22 inches of water from planting to harvest. The other block was irrigated at the same frequency but for twice as long and received a total of 40 inches of water. One inside row from each plot was harvested August 2, dried, ground, quartered, and samples taken for analysis to estimate nitrogen recovery. Data from Sayer (1948) indicates N absorption is about complete one month after tasselling. On August 25, the other inside row was harvested and measured for stover and grain yield. The coated fertilizer used in this study released 1/3 of its nitrogen in the first 5 days and about 0.8% per day of the initial application during the first weeks thereafter.

Experiment 2. A study similar to the above was conducted at a different location on Yolo loam. The treatment variables of nitrogen sources, amounts, and time of application are summarized in Table 2. Plot size and number of replications were the same as above. It was not deemed necessary to add potassium or phosphorus. Only one irrigation rate was used—about 25% in excess of estimated evapotranspiration. Planting was July 8, 1966. The inside rows were harvested for stover and grain yields on September 15, 1966, at which time samples were also obtained for nitrogen analysis. A total of 11 inches of irrigation water was applied by sprinkler during the study. The coated fertilizer used in this study released about 25% of its nitrogen in the first 5 days and in the first weeks thereafter about 0.8% per day.

RESULTS AND DISCUSSION

The results of the study on Moreno sandy loam are summarized in Table 1. Grain yields at the low irrigation rate were comparable when treatments 2, 3, and 4 are compared with 5, 6, and 7. Nitrogen recovery was significantly superior only on treatment 7 as compared to 4. Where single applications of coated and regular urea are compared (treatments 2 and 8) the response to coated urea was much superior. However, the advantage essentially disappeared where two applications were made (compare treatments 3 and 9) of soluble urea. Thus, in an easily leachable soil, coated fertilizer did not improve yields as compared to regular urea when several split applications of fertilizer were made and irrigation was carefully managed.

On the other hand, the single application of coated fertilizer gave comparable yields and, therefore, may represent a labour and convenience advantage. At the high irrigation rates, yields and nitrogen recovery rates were better with coated urea at 50 and 100 lb per acre application rates but the differences in performance narrowed at the 200 lb rate of application. Treatments 2 and 3 were superior to 8 and 9.

On the Yolo loam soil which is inherently higher in fertility than the Moreno soil, yields and nitrogen recovery were comparable between the two sources of nitrogen.

While advantages in yields and efficiency of nitrogen recovery were not obtained as compared to 3 split applications of soluble fertilizer in well-
## TABLE 1
YIELDS AND NITROGEN RECOVERY OF GOLDEN BANTAM CORN ON MORENO SANDY LOAM AS INFLUENCED BY NITROGEN TREATMENTS AND IRRIGATION PRACTICES

<table>
<thead>
<tr>
<th>Treatment***</th>
<th>Yields, lb/acre</th>
<th>% of Applied Nitrogen Recovered****</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stover</td>
<td>Grain</td>
</tr>
<tr>
<td>lb N/acre—Source</td>
<td>L*</td>
<td>H**</td>
</tr>
<tr>
<td>1. No N added</td>
<td>1560</td>
<td>1430</td>
</tr>
<tr>
<td>2. 50-coated urea</td>
<td>3200</td>
<td>2740</td>
</tr>
<tr>
<td>3. 100-coated urea</td>
<td>3570</td>
<td>3020</td>
</tr>
<tr>
<td>4. 200-coated urea</td>
<td>3920</td>
<td>3940</td>
</tr>
<tr>
<td>5. 50-urea</td>
<td>2920</td>
<td>2320</td>
</tr>
<tr>
<td>6. 100-urea</td>
<td>3750</td>
<td>2780</td>
</tr>
<tr>
<td>7. 200-urea</td>
<td>4200</td>
<td>3580</td>
</tr>
<tr>
<td>8. 50-urea</td>
<td>2200</td>
<td>1480</td>
</tr>
<tr>
<td>9. 100-urea</td>
<td>3210</td>
<td>2650</td>
</tr>
<tr>
<td>LSD(05) Treat. Means</td>
<td>360</td>
<td>310</td>
</tr>
</tbody>
</table>

* Low irrigation treatment.
** High irrigation treatment.
*** Timing of fertilizer applications: (Corn was about 12-15 inches high June 12; first tassels appeared July 1).
**** Assumption is made that difference between N recovered and that absorbed by treatment, comes from fertilizer.

Treatments 2, 3 and 4. All applied in a band 2 inches to the sides of the seed and 3 inches below. 28 Apr.
Treatments 5, 6 and 7. 25 lb N in a band on 28 Apr. The remainder in equal portions top dressed 12 June and 5 July.
Treatment 8. 50 lb N in a band on 28 Apr.
Treatment 9. 25 lb N in a band on 28 Apr. and remainder top dressed 12 June.
Note. 1 lb/ac = 1.12 kg/ha.

## TABLE 2
YIELDS AND NITROGEN RECOVERY OF GOLDEN BANTAM CORN ON YOLO LOAM AS INFLUENCED BY NITROGEN TREATMENTS

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Yield, lb/acre</th>
<th>% of Applied Nitrogen Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>lb N/acre—Source</td>
<td>Stover</td>
<td>Grain</td>
</tr>
<tr>
<td>1. No N added</td>
<td>2900</td>
<td>3590</td>
</tr>
<tr>
<td>2. 50-coated urea</td>
<td>3410</td>
<td>4450</td>
</tr>
<tr>
<td>3. 100-coated urea</td>
<td>4280</td>
<td>4830</td>
</tr>
<tr>
<td>4. 200-coated urea</td>
<td>4920</td>
<td>5340</td>
</tr>
<tr>
<td>5. 50-urea</td>
<td>3910</td>
<td>4310</td>
</tr>
<tr>
<td>6. 100-urea</td>
<td>4200</td>
<td>4910</td>
</tr>
<tr>
<td>7. 200-urea</td>
<td>5080</td>
<td>5260</td>
</tr>
<tr>
<td>LSD(05) Treat. Means</td>
<td>430</td>
<td>5-1</td>
</tr>
</tbody>
</table>

* Coated fertilizers were applied in a band 2 inches to the side and 3 inches below seed at time of planting 8 July. Urea was applied at 25 lb in bands at planting. The remainder was applied in equal portions as a top dressing on 18 Aug. and 1 Sept.
First tassels appeared 15 Aug.
Note. 1 lb/acre = 1·12 kg/ha.
managed irrigation schedules, it is noteworthy that yields and efficiency of single applications of coated fertilizer were approximately comparable where cropping was completed in 70 to over 100 days. The potential labour savings may also be significant in specific situations.

Undoubtedly, the most critical nutritional period in the life of many crops is during the seedling stage when the root system is poorly developed. Leaching losses during this period contribute heavily to poor nitrogen recovery of many row crops. This study and other data, not reported here, have shown coated urea is well adapted as a starter fertilizer in highly permeable soils. This is particularly important in some rapidly maturing crops.

While there has been only limited research on the merits of coated fertilizers in crop management (Beaton et al. 1967, Heilman et al. 1966, Lunt and Oertli 1962, Voth et al. 1963) it is probable that low-cost, coated fertilizers will be an important management asset in various situations, especially where root systems of crops are poorly established and where the potentiality of leaching of nutrients is great.

ACKNOWLEDGMENTS

The author wishes to thank the Thiokol Chemical Company for supplying coated urea and for partial financial support of these studies.

REFERENCES


SUMMARY

An inexpensive technique for coating urea with sulphur and a plasticizing agent and sealing with soft wax has been developed by Thiokol Chemical Corporation. After the first few days, the coated fertilizer may have a release rate of less than one percent per day. The magnitude of the release rate can be substantially regulated by the coating technique. Release rates in soils may be higher than elution in water due to adsorption of the sealing wax in soils. Experimental materials on soils have shown substantial release at slow rates for about 80 to 100 days.

Yields and nitrogen recovery by corn have been about as good from a single application of coated fertilizer as from 3 applications of regular urea in a sandy loam and a loam soil when irrigation practices were carefully managed to avoid large leaching losses. Under conditions of high leaching or single applications of urea, yield and nitrogen recovery from comparable applications of coated urea were superior.
Une technique peu coûteuse enrober l'urée de soufre et d'un plastifiant et sceller l'ensemble avec de la cire molle a été développée chez Thiokol Chemical Corporation. Après les quelques premiers jours, l'engrais enrobé peut donner un taux d'écoulement de moins de un pour cent par jour. La magnitude du taux d'écoulement peut être réglée substantiellement par la technique d'enrobage. Les taux d'écoulement dans des sols peuvent être plus élevés qu'une élution dans l'eau en raison de l'adsorption de la cire dans les sols. Des échantillons expérimentaux dans les sols ont montré un écoulement substantiel mais lent pendant 80 à 100 jours.

Les rendements et la récupération d'azote par le maïs ont été à peu près aussi bons avec une seule application d'engrais enrobés qu'avec 3 applications d'urée normale dans un limon sableux et dans un limon quand les irrigations ont été faites soigneusement pour éviter de larges pertes par lessivage. Dans ces conditions de lessivage élevé ou d'une seule application d'urée, le rendement et la récupération d'azote pour des applications comparables d'urée enrobée furent supérieurs.

ZUSAMMENFASSUNG


Wenn die Bewässerung sorgfältig durchgeführt wird, um grosse Auslaugungsverluste zu vermeiden, ist die Ausbeute und die Wiederaufnahme des Stickstoffs durch den Mais, bei einer einzigen Anwendung des umhüllten Dungers mit einer dreimaligen Anwendung des gewöhnlichen Harnstoffs in sandigem Lehm und Lehmboden gleichwertig. Bei höherer Auslaugung oder bei einer einzigen Anwendung des Harnstoffs, waren die Ausbeute und die Wiederaufnahme des Stickstoffs höher, als bei Anwendung des umhüllten Harnstoffs unter vergleichbaren Bedingungen.
DISSOLUTION AND LEACHING OF FERTILIZER GRANULES

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INTRODUCTION

In situations where the net vertical flux of soil water is downward, significant amounts of mobile plant nutrients can be leached beyond the root zone. In the sands characteristic of large areas of recently developed soils in southern Australia, even relatively immobile ions like phosphate are subject to leaching while the fluxes of sulphate and nitrate can result in either temporary or chronic deficiencies during the growing season.

Recent studies have shown that the effectiveness of gypsum as a sulphur fertilizer on these soils depends greatly on the particle size of the material (Powrie, 1967). Fine gypsum is leached so rapidly from the topsoil that it may have little effect on plant yield. On the other hand coarser material persists for a much longer period and has proved to be a highly satisfactory source of sulphur.

The rate of loss of gypsum from the rooting zone is dependent on two processes. Firstly, the sparingly soluble particles must dissolve in the soil water and, secondly, the resulting solution must move downward from the site of application. Factors governing the rates of these processes are examined here.

ANALYSIS AND DISCUSSION

Because fertilizer losses may be limited by processes other than leaching it is of interest to obtain estimates of the rate of dissolution of fertilizer granules in soils. The dissolution process is analogous to mass transfer from a soluble particle or granule suspended in a fluid which may be in motion or at rest. One approach to this kind of problem is that given by Bird, Stewart and Lightfoot (1960) following Sherwood and Pigford (1952). The data presented in Figure 22 of Sherwood and Pigford (loc. cit.) corresponding to the relation

\[ W_m = k_m 4\pi r^2 \frac{x_a - x_s}{1 - x_s} \]

appeared sufficiently encouraging to extend their approach to this particular problem.

In (1) the mass flux \( W_m \) is a function of the transfer coefficient \( k_m \), particle radius \( r \) and a molar ratio gradient term. The concentration, \( x_s \), refers to the concentration of solute at the particle surface and \( x_s \), to the concentration of solute in the fluid upstream from the particle. Mass transfer coefficients \( k_m \) were calculated from the following relation, assuming the particles, in this case gypsum, approximated to spheres.
The transfer coefficient $k_m$ is thus a function of physical properties of the fluid ($C_w$, $\rho_w$, $\mu$), the diffusivity of the solute in the soil ($D$), the velocity of fluid flow ($V_x$) and particle radius ($r$).

The time course of particle dissolution was obtained from

$$t = \int_{r_1}^{r_2} \rho_m 4\pi r^2 W_m^{-1} \, dr$$

which could be simplified to

$$t = n \int_{r_1}^{r_2} \frac{1}{k_m} \, dr$$

where $n$ is a constant.

For the parameters used

$$t = 1080 \, r^2 \text{ day.}$$

In Figure 1 is shown the relation between particle radius and time. The calculated time course of weight change of single particles is shown in Figures 2a and 2b. The ratio of weight of the particle at a given time to its initial weight was adopted to simplify further analysis. In Figure 2a is given also the time course of loss by leaching of a completely dispersed, soluble material with similar physical and chemical properties to those of

![Graph](image-url)
Fig. 2.—Time course of change of weight of gypsum particles and completely dispersed material. (1 day $\equiv 0.42$ cm. rainfall.)
the granules. This relation was calculated from the method proposed by Day and Forsythe (1957) and used by Gardner (1965), that is

\[ C_t = C_i Z_i (4\pi k t)^{-1/2} \exp \left( -\frac{(Z - Vt)^2}{4kt} \right) \]

The proportion of the original mass of material still present in the top 10 cm of soil at time \( t \) was obtained by suitable graphical averaging of \( C_i \) for \( Z_o < Z < 10 \text{ cm} \)

It is clear that the rate of loss of mass by leaching in sands under conditions of intermittent rainfall (see Gardner, 1965) exceeds the rate of dissolution of individual particles for particles sizes greater than about 2.5 mm diameter. The duration of particles extends from a day or two at the fine end of the scale to about 1000 days for particles of 2 cm diameter. This time scale can be interpreted in terms of rainfall for the calculations have been based on 25 cm of rainfall falling over a period of 60 days (i.e. 0.42 cm per day). The latter corresponds roughly to the June and July rainfall in parts of South Australia where sulphur deficiency is acute and where problems in finding a persistent sulphur fertilizer have arisen.

Further calculations were made for \( V_x = 0 \), that is, the conditions in which the fluid about the particle is still. These estimates of rates of particle dissolution were compared with estimates obtained from a solution of the diffusion equation for a spherical source (see Crank, pg. 27, equation 3.8). The estimates of rates of particle dissolution obtained by each method were in good agreement. It is interesting to note that for \( V_x = 1 \text{ cm per day}, t \approx 1080 \text{ r}^2 \text{ day} \) and for \( V_x = 0, t \approx 1190 \text{ r}^2 \text{ day} \). It is clear that although movement of the fluid past the particle increases the rate of dissolution, this increase is small. In essence, the rate of particle dissolution is diffusion controlled or limited and velocities of soil water movement are so small as to have little effect.

In fertilizer application studies it is more usual to consider responses in terms of amount of fertilizer per unit area of land surface. Clearly it is of interest to examine the rate of dissolution and leaching of fertilizer in these same terms as well as in terms of single particle dissolution. For uniform application of fertilizer, that is, the same mass per unit area, the number of particles present is inversely proportional to \( r^3 \). The relations presented in Figure 2 were appropriately adjusted to bring them to a common level of application of fertilizer. Further, the ratio between rate of dissolution of particulate fertilizer and the rate of leaching of a dispersed fertilizer has been calculated. This ratio is shown in relation to particle radius in Figure 3. The comparable curve for individual particles is also shown. Rates have been based in all cases on mass lost in the period from \( t = 0 \) to \( t = t_i \), where \( t_i \) is the time taken for half the initial mass to disappear.

When the rate of dissolution of single particles with respect to rate of leaching is less than 1, there is a relatively slow change in the ratio with increase in particle radius. However when particle number per unit area is taken into account there is a relatively narrow range of desirable particle
sizes. For gypsum, under the conditions here specified, it is not until particle radius exceeds about 0.5 cm that there will be a significant reduction in rate of loss in terms of total mass per unit area per unit of rainfall. Once radius exceeds about 0.7 cm, the rate of loss of fertilizer would be very small compared with the rate of leaching of wholly dispersed materials.

Application to Fertilizer Practice

With sparingly soluble substances like gypsum, fertilizer particle size can be adjusted to take advantage of diffusion limited rate of dissolution of particles. Choice of particle size depends on the solubility of the material, particle shape, the conductivity of the soil to water, the diffusivity of ions in the soil and both the amount and distribution of rainfall and evaporation.

Data showing the relationship between gypsum particle size, extent of leaching and the yield and sulphur uptake of herbage is shown in Figure 4. Experimental details have been described elsewhere (Powrie, 1967). Briefly, crushed rock gypsum of varying particle size was broadcast on an annual pasture growing on a sandy podsol at Mt. Compass, South Australia. Soil sulphate was measured after 19 mm of rain had fallen. Pasture yield and sulphur uptake was determined after a further 38 mm of rainfall.

Increasing particle size resulted in a marked reduction of loss of
R. J. MILLENTOON AND J. K. POWRIE

Fig. 4.—Effect of gypsum particle size on recovery of applied sulphate, sulphur uptake and yield of herbage.

Although the experimentally determined relationship between leaching and particle size was similar in form, it differed in magnitude from the calculated one. Percolation between time of application of gypsum and soil sampling in the field experiment was estimated at 11.9 mm by deducting the mean evaporation for the period from the measured rainfall. On this basis it is clear that the gypsum particles were much more persistent than the predicted rates of leaching would indicate. For example the calculated particle diameters which would have been associated with the measured losses of sulphate for the two largest particle sizes were about three times those actually used. (The diameters were assumed to be those of spheres equal in weight to that of the irregular particles actually used.)

The discrepancy between observed and predicted leaching losses could arise for a number of reasons. Chief among these is the inability to define precisely the actual rate and duration of percolation in the field experiment.

**Diagram:**

- **Yield of Dry Herbage Due to Gypsum**
  - 0
  - 0.5
  - 1.0
  - 1.5
  - 2.0
  - 2.5

- **Mean Gypsum Particle Diameter (mm.)**
  - 0
  - 1.0
  - 2.0
  - 3.0

- **Herbage Sulphur Uptake due to Gypsum (kg./ha.)**
  - 0
  - 1.0
  - 2.0
  - 3.0

- **Soil Sulphur**
  - 0
  - 1.0
  - 2.0
  - 3.0

- **Sulphur Uptake**
  - 0
  - 1.0
  - 2.0
  - 3.0

- **Yield Herbage**
  - 0
  - 1.0
  - 2.0
  - 3.0

**Graph:**

- **Recovery of Applied Sulphur in Soil (kg./ha.)**
  - 0
  - 1.0
  - 2.0
  - 3.0
  - 4.0
  - 5.0
  - 1000
  - 1500
  - 2000
  - 3000

- **Sulphate by leaching. Furthermore, improved retention of sulphate was reflected in both yield of herbage and sulphur uptake.**

**Table:**

<table>
<thead>
<tr>
<th>Mean Gypsum Particle Diameter (mm.)</th>
<th>Yield of Dry Herbage</th>
<th>Herbage Sulphur Uptake due to Gypsum (kg./ha.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2.0</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Note:**

- The discrepancies observed could arise from:
  - The inability to define precisely the actual rate and duration of percolation in the field experiment.
  - A number of reasons, including the inability to define the actual rate and duration of percolation in the field experiment.
In addition diffusivity of gypsum would have varied from time to time since dissolution would have taken place over a range of moisture contents. Also since the gypsum was applied to the soil surface dissolution may well have been retarded by the limited contact of the particles with the soil.

In practice it is likely that optimum particle size of gypsum would need to be determined experimentally for given conditions and materials. However the method of calculating rates of dissolution and leaching described here serves as a useful guide to the form of the leaching process and the order of particle size which should be used to achieve required persistence of fertilizer under prescribed conditions of leaching.

REFERENCES


NOMENCLATURE

\( \text{D} \) = diffusivity of ion in soil when soil is saturated (5 \( x \) \( 10^{-6} \text{ cm}^2 \text{ sec}^{-1} \))

\( \mu \) = viscosity of water, (poises)

\( \rho_w \) = density of water (gm ml \(^{-1} \))

\( \rho_m \) = molar density of particles (gm mole ml \(^{-1} \))

\( \nu_w \) = velocity of water in soil (cm sec \(^{-1} \))

\( \lambda = \frac{q}{\theta} \) and \( \theta \) is capillary conductivity; \( h \) is total "head" in cm (cm sec \(^{-1} \))

\( k_m \) = mass transfer coefficient (gm mole cm \(^{-1} \text{ sec}^{-1} \))

\( r = \text{ particle radius (cm)} \)

\( X_o = \text{ concentration of solute at particle surface (mole mole}^{-1} \))

\( -C_o \)

\( C \)

\( X_T = \text{ concentration of solute distant from particle (mole mole}^{-1} \))

\( W_m = \text{ mass flux (gm mole per spherical granule sec}^{-1} \))

\( C_o = \text{ molar concentration of solute at particle surface (gm mole cm}^{-3} \))

\( C = \text{ molar density of solution} \)

\( = C_o + C_w \) where \( C_o \) molar density of water

\( C_t = \text{ concentration of dispersed fertilizer in soil solution at time } t \) at depth \( Z \) (gm cm \(^{-3} \))

\( C_i = \text{ initial concentration of dispersed fertilizer in soil solution at } t = 0 \) and within the soil layer \( Z = 0 \) to \( Z = Z \), (gm cm \(^{-3} \))

\( \kappa = \text{ dispersion coefficient (cm}^2 \text{ day}^{-1} \))

\( = \frac{q}{\theta} \)

\( \beta = \text{ constant, } 2 \leq \beta \leq 20 \) for conditions examined (cm)

\( \theta = \text{ soil water content, (ml ml}^{-1} \))

\( V = \text{ rainfall (cm) in period } t = 0 \) to \( t = t \)

\( = \theta \nu, t \)

\( \frac{W_t}{W_o} \) = ratio of particle weight at time \( t \) to initial weight.
Factors controlling the dissolution and leaching of fertilizer granules are examined using gypsum as an example. Particular attention is given to the effects of particle diameter because of the practical significance of granule size in controlling the movement of fertilizers.

The calculated course of change with time, of radius and weight of gypsum particles of varying size, is compared with the leaching of completely dispersed calcium sulphate. It is shown that leaching rate exceeds dissolution rate where particle diameter exceeds a critical value (2.5 mm for the postulated conditions). The effect of velocity of water movement is shown to be small and therefore particle dissolution is essentially diffusion controlled.

The effect of particle size on the efficiency of gypsum as a sulphur fertilizer is illustrated using data from a field experiment conducted in an area of South Australia where soil and climatic factors are conducive to the rapid leaching of soluble fertilizers. Under these conditions seasonal correction of sulphur deficiency by means of gypsum could only be achieved when particle diameter exceeded 2.5 mm.

On étudie les facteurs influant sur la dissolution et le lessivage de granules d’engrais, en utilisant le gypse comme exemple. On fait particulièrement attention aux effets dus au diamètre des particules à cause de l’importance pratique de la taille des granules dans le contrôle du mouvement des engrais.

On compare la direction calculée du changement en fonction du temps, du rayon et du poids des particules de gypse de différentes tailles, avec le lessivage du sulfate de calcium entièrement dispersé. On montre que la vitesse de lessivage est supérieure à la vitesse de dissolution lorsque le diamètre des particules est supérieur à une valeur critique (2,5 mm pour les conditions proposées). On montre que l’effet de la vitesse du mouvement de l’eau est faible et que par conséquent la dissolution des particules est essentiellement gouvernée par la diffusion.

L’effet de la taille des particules sur l’efficacité du gypse en tant qu’engrais de soufre est démontré en utilisant les données d’une expérience au champ réalisée dans une région de l’Australie du Sud où le sol et les facteurs climatiques sont propices à un lessivage rapide d’engrais solubles. Dans ces conditions, une correction saisonnière de la carence en soufre à l’aide de gypse pouvait être réalisée seulement lorsque le diamètre des particules était supérieur à 2,5 mm.

RÉSUMÉ

On estudié los factores influenciando la disolución y el leaching de granos de fertilizantes, utilizando el yeso como ejemplo. Se da especial atención a los efectos del diámetro de las partículas debido a la importancia práctica de la granulación en el control del movimiento de los fertilizantes.

Se compara el curso calculado de cambio con el tiempo, del radio y el peso de partículas de yeso de tamaño variable, con el leaching del sulfato de calcio completamente dispersado. Se muestra que el ritmo de leaching supera el ritmo de disolución cuando el diámetro de las partículas supera un valor crítico (2.5 mm para las condiciones hipotéticas). El efecto de la velocidad de movimiento del agua es pequeño y, por lo tanto, la disolución de las partículas es principalmente controlada por difusión.

El efecto del tamaño de partícula sobre la eficiencia del yeso como fertilizante de sulfuro se ilustra usando datos de un experimento de campo realizado en una región de Australia del Sur donde factores del suelo y climáticos son favorables al leaching rápido de fertilizantes solubles. Bajo estas condiciones, una corrección estacional de la deficiencia de sulfuro mediante el uso de yeso sólo podría lograrse cuando el diámetro de las partículas superaba 2.5 mm.

RéSUMÉ

On estudié los facteurs influenciant sur la dissolution et le lessivage de granules d’engrais, en utilisant le gypse comme exemple. On fait particulièrement attention aux effets dus au diamètre des particules à cause de l’importance pratique de la taille des granules dans le contrôle du mouvement des engrais.

On compare la direction calculée du changement en fonction du temps, du rayon et du poids des particules de gypse de différentes tailles, avec le lessivage du sulfate de calcium entièrement dispersé. On montre que la vitesse de lessivage est supérieure à la vitesse de dissolution lorsque le diamètre des particules est supérieur à une valeur critique (2,5 mm pour les conditions proposées). On montre que l’effet de la vitesse du mouvement de l’eau est faible et que par conséquent la dissolution des particules est essentiellement gouvernée par la diffusion.

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ZUSAMMENFASSUNG

Faktoren, die die Auflösung und Auslaugung der Düngerkörnchen kontrollieren, sind untersucht worden wobei Gips als Beispiel genommen wurde. Besondere Aufmerksamkeit wurde dem Einfluss der Teilchendurchmesser geschenkt, wegen der praktischen Bedeutung der Korngrösse in der Kontrolle der Verteilung des Düngemittels.
Die kalkulierte Veränderung des Radius und des Gewichtes der verschiedenen grossen Gipseilchen ist, mit der Zeit, der Auslaugung vollkommen verteilten Kalziumsulfates verglichen worden. Wo der Teilchendurchmesser einen kritischen Wert übersteigt (2·5 Mm für vorausgesetzte Verhältnisse) erweist es sich, dass der Auslaufungsgrad den Auflösungsgrad übertrifft. Der Einfluss der Geschwindigkeit der Wasserbewegung erweist sich als gering und Teibchenauslöschung ist deshalb hauptsächlich durch Diffusion kontrolliert.

Der Einfluss der Korngrösse auf die Wirksamkeit des Gipses als Sulfatdünger wird an Hand von Feldversuchsdaten, welche in einem Bereich Süd-Australiens durchgeführt wurden, wo Boden und klimatische Umstände für das rapide Auslaufen der löslichen Dünger verantwortlich sind, erläutert. Unter diesen Bedingungen konnte eine jahreszeitliche Berichtigung des Sulfatmangels durch Gipsanwendung nur erreicht werden, wenn der Teilchendurchmesser 2·5 Mm überschritt.
AERAGRONOMY—THE KEY TO SOIL CONSERVATION
IN NEW ZEALAND

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INTRODUCTION

Aeragronomy—the hitching of the waggon of agronomy to the star of aeronautics—has given soil technology new dimensions in space and time. It has broken the slope barrier and given wings to several laborious agronomical operations. Necessity became the mother of invention in controlling accelerated land deterioration, soil erosion and flooding on the steep unploughable hill and mountain lands caused by deforestation, burning and overgrazing by sheep and a scourge of rabbits and other noxious animals.

THE PROBLEM

The complex range of agronomic problems debilitating the land—decline in fertility, pasture deterioration, invasion of weeds, reversion to native shrubs and fern, explosion of noxious animal populations, ill-thrift in live stock, decline in carrying capacity and abandonment of the land—accelerated wind, sheet, scree, slip, flow and gully erosion and increased flooding hazards. It was essential to develop ways and means of mobilizing constructive interactions between several factors of the ecosystem in order to attain maximum production consistent with minimizing soil erosion and flooding.

PRELIMINARY INVESTIGATIONS

The first quantitative measurement of several factors governing infiltration, run off and soil loss made with a rainfall simulator indicated the relative effects of intensity and duration of rain with respect to types of vegetation, slope, soil type, soil structure, litter, and roots as well as the positive changes induced by burning, trampling and cultivation. The capacity of the vegetative canopy in intercepting rain and conducting it into the soil via the stems and roots and its protection of the soil against direct impact of raindrops proved it to be a very effective mechanism.

Burning, trampling and cultivation progressively increased run off and soil loss and decreased infiltration according to the severity of application from as high as 4” per hour to less than ½” per hour on certain soils. Subsequent measurements with a North Fork infiltrometer showed that management factors greatly reduced the infiltration rates of many soils as compared with their original condition (Campbell 1945). Further investigations into soil erosion, flooding and restoration of eroded land in Hawkes
Bay helped to clarify the processes operative in land deterioration (Campbell 1951a). The almost cataclysmal changes brought about in "breaking in" land for farming on hill country not only charged the increased volume of run off with its full erosive capacity, but also predisposed the steep erodible soils and soft shattered rocks to the full erosive potential of intense rains, frost, gravity and wind (Campbell 1946). This allowed more precise definition of the problem as one of constructively improving the water controlling and erosion resisting capacity of the induced grassland and soils, sufficiently by management, to take over the functions of the displaced native vegetation (Campbell 1946).

**DEVELOPING SOIL CONSERVATION PRACTICES**

Practical field investigations and trials on 12 typical eroded hill country farms on several soil types revealed that soil conservation practices could be integrated with modified pastoral management to progressively control the movement of water and soil erosion by suitable combinations of practices. These comprised rebuilding fertility with fertilizer, revegetation with grasses and clovers, recuperative grazing with cattle, strategic fencing according to aspect, contouring where possible, tree planting of unstable slopes and gullies, flood control dams and control of fire and pests such as rabbits, deer, goats and opossums (Campbell 1955).

In all cases it was necessary to topdress with phosphate and certain trace elements to establish clovers thus to initiate a fertility-building and structure-improving cycle which could be accelerated by the requisite management. This focused attention on the need to develop a practical method of applying phosphate on unploughable hill country.

**DEVELOPMENT OF AERIAL FARMING**

Since the key to reversing the degenerative trends of management lay in raising fertility with phosphate to introduce clovers, exploratory trials and demonstrations of practical aerial topdressing and seeding techniques were adopted by the Soil Conservation Council in 1947.

The final 1000 acre demonstration of an aerial topdressing system for hill country led to its wholesale adoption by farmers and efficient exploitation by the Aviation Industry (Campbell 1951b, 1951c).

The meteoric growth of aerial farming is best indicated by its performance to date of doing over 16 million man days of work, in 15 million topdressing flights, to distribute over 9 million tons of fertilizer on hill country pastures since its inception in 1950 (see Fig. 1). While it distributed over one million tons last year the Industry has a capacity almost to double this with the full utilization of about 300 aircraft employed, which range in size from the medium sized 12 cwt. capacity aircraft to large 5 ton carrying capacity aircraft. Thousands of tons of lime have also been distributed on deficient areas at reduced, out of season rates, at as low as 4 dollars per ton.

The 13,000 tons of clover and grass seed distributed play a striking role in raising the nitrogen status of soils and assuring fuller utilization of
Fig. 1.—Statistics of Aerial Farming in New Zealand.
the phosphate to facilitate the quick return of dung and urine through the animal cycle.

One of the most spectacular successes has been that of controlling the century old scourge of rabbits by literally bombing them to extinction with over 70,000 tons of poisoned baits. Aerial distribution of 100,000 tons of weedicides and insecticides has given their control a new high level of efficiency on rugged hill country pastures for the first time in history. The dropping of over 13,000 tons of fencing materials, so essential to the full utilization of pastures, has overcome transport problems on remote hill country.

Aircraft have made the poisoning of noxious animals such as opossums, deer and goats practical on the more remote and rugged mountain catchments. A combined operation of helicopter and fixed wing aircraft makes the harvesting of deer meat very effective from mountainous areas where excessive populations ruin natural protective vegetation.

Aerial firefighting equipment and techniques have been developed, making it possible to convert topdressing planes to fire fighters within half an hour, when the need arises, to play their full role in modern forest and rural fire fighting (Campbell 1959).

RESULTS OF AERIAL FARMING ON PROGRESSIVE FARMS

In order to evaluate the contribution being made by aerial farming on typical hill country farms a questionnaire was distributed through operators to their most progressive clients.

Records of treatment and performance, although complicated by several other management factors, do give a picture of the overall results of improving unploughable hill country with aerial farming and prudent management. In the absence, to date, of any critical laboratory information on the build up of fertility with fertilizing on hill country this provides a means of evaluating progress. There is remarkable consistency between the results of such surveys carried out in 1960 and 1967 on different farms where less than 20% of the land has been ploughed.

The results from 23 farms in 19 counties are presented in Table 1, after 13 years of aerial farming, while those from 20 farms in 15 counties after 9 years of operation are given in Table 2.

The order of increases is quite remarkable insofar as this type of country was hitherto regarded as marginal for farming owing to land deterioration.

The average area of the farms is well above the N.Z. average for hill country farms and a much lower order of efficiency could be expected.

Approximately 80% of these farms are commanded by aerial farming despite the precipitous nature of much of such country, but this of course includes 20% which are cultivable.

The phosphate used (15 cwt/acre or approx. 1 cwt/acre per year) is not high when it is appreciated that much greater quantities are used during the initial improvement period and are tapered off to about 1 cwt/acre per year. Experience is proving that up to 10 cwt/acre in the first year is necessary to boost fertility sufficiently to effectively cater for the new higher
AERAGRONOMY AND SOIL CONSERVATION

**TABLE 1**
AREAS, TREATMENT, CARRYING CAPACITY AND PRODUCTION OF 23 TYPICAL HILL COUNTRY FARMS 1967

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area in acres</td>
<td>60,493</td>
<td>2630</td>
</tr>
<tr>
<td>Area cultivable acres</td>
<td>12,523</td>
<td>544</td>
</tr>
<tr>
<td>Area Topdressed acres</td>
<td>48,037</td>
<td>2088</td>
</tr>
<tr>
<td>Period Topdressed</td>
<td>13 years</td>
<td></td>
</tr>
<tr>
<td>Air Strips</td>
<td>21</td>
<td>1 per farm, approx.</td>
</tr>
<tr>
<td>Phosphate used in tons</td>
<td>34,947</td>
<td>1519 or 15-5 cwt/ac</td>
</tr>
<tr>
<td>Lime used in tons</td>
<td>10.798</td>
<td>473 or 4-5 cwt/ac</td>
</tr>
<tr>
<td>Clover and grass seed tons</td>
<td>80.3</td>
<td>3.4 or 3-6 lbs/ac</td>
</tr>
<tr>
<td>Subdivision Fencing in Chains</td>
<td>14,470</td>
<td>629 or 3 chains/ac</td>
</tr>
</tbody>
</table>

Carrying Capacity

<table>
<thead>
<tr>
<th>Sheep—</th>
<th>Before</th>
<th>After</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Average</td>
<td>Total</td>
</tr>
<tr>
<td>Ewes</td>
<td>41,650</td>
<td>1,810</td>
<td>88,569</td>
</tr>
<tr>
<td>Other</td>
<td>11,121</td>
<td>483</td>
<td>46,685</td>
</tr>
<tr>
<td>Cattle—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding Cows</td>
<td>1,288</td>
<td>56</td>
<td>5,381</td>
</tr>
<tr>
<td>Other</td>
<td>1,885</td>
<td>82</td>
<td>6,122</td>
</tr>
<tr>
<td>Production—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wool (Bales)</td>
<td>1,393</td>
<td>60</td>
<td>4,260</td>
</tr>
<tr>
<td>Fat Lambs</td>
<td>1,374</td>
<td>596</td>
<td>43,769</td>
</tr>
<tr>
<td>Fat Cattle</td>
<td>457</td>
<td>19</td>
<td>2,275</td>
</tr>
</tbody>
</table>

**TABLE 2**
AVERAGE AREA TREATMENT, CARRYING CAPACITY AND PRODUCTION OF 20 HILL COUNTRY FARMS 1960

<table>
<thead>
<tr>
<th>Area of Farm</th>
<th>2,400 acres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area Topdressed</td>
<td>1,706 acres</td>
</tr>
<tr>
<td>Phosphate used</td>
<td>875 tons or 10-2 cwt/ac</td>
</tr>
<tr>
<td>Lime used</td>
<td>233 tons or 2-4 cwt/ac</td>
</tr>
<tr>
<td>Clover and grass seed</td>
<td>2.1 tons or 2 lbs/ac</td>
</tr>
<tr>
<td>Subdivision Fencing</td>
<td>237 chains or 14 chains/ac</td>
</tr>
</tbody>
</table>

Carrying Capacity—

<table>
<thead>
<tr>
<th>Sheep—</th>
<th>Before</th>
<th>After</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewes</td>
<td>1,156</td>
<td>2,430</td>
<td>110%</td>
</tr>
<tr>
<td>Others</td>
<td>600</td>
<td>1,660</td>
<td>166%</td>
</tr>
<tr>
<td>Cattle—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
<td>65</td>
<td>162</td>
<td>149%</td>
</tr>
<tr>
<td>Others</td>
<td>356</td>
<td>1,289</td>
<td>262%</td>
</tr>
<tr>
<td>Production—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wool (bales)</td>
<td>42</td>
<td>125</td>
<td>197%</td>
</tr>
<tr>
<td>Fat Lambs</td>
<td>356</td>
<td>1,289</td>
<td>262%</td>
</tr>
<tr>
<td>Fat Cattle</td>
<td>13</td>
<td>70</td>
<td>438%</td>
</tr>
</tbody>
</table>
levels of production possible. The results indicate that there is considerable latitude for improving the fertilizing programmes on many of these farms.

The quantity of lime used is rather surprising as there is not a marked response to lime on the average hill country.

The quantity of seed used is surprisingly low when the beneficial results of more generous seeding on such country are well known, particu-

<table>
<thead>
<tr>
<th>Soil Type, Topdressing and Carrying Capacity (in Sheep Equivalents) and Predicted Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lime cwt/ac</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>1 Otematae</td>
</tr>
<tr>
<td>2 Rodney</td>
</tr>
<tr>
<td>3 Waikato</td>
</tr>
<tr>
<td>4 Ohinemuri</td>
</tr>
<tr>
<td>5 Waipa</td>
</tr>
<tr>
<td>6 Rotorua</td>
</tr>
<tr>
<td>7 Rotorua</td>
</tr>
<tr>
<td>8 Rotorua</td>
</tr>
<tr>
<td>9 Waimana</td>
</tr>
<tr>
<td>10 Hawke Bay Gwacas 74, Oharu 79, Ruahine, 124, Whangaehu 114, Taihape 140</td>
</tr>
<tr>
<td>11 Hawke Bay Otamauri 11H sandy loam</td>
</tr>
<tr>
<td>12 Dannevirke Wanstand 25aH</td>
</tr>
<tr>
<td>13 Masterton Atua 29 and 29H, Taihape 114a, Matakonoha 120, Bluff 113, Kumoreoa 29f and FH</td>
</tr>
<tr>
<td>15 Kaikoura Weld and Medway hill soils</td>
</tr>
<tr>
<td>16 Marlborough Haldon and Hurunui</td>
</tr>
<tr>
<td>17 Waipara Onepunga and Waikari hillside</td>
</tr>
<tr>
<td>18 Waimate Hurunui steep land</td>
</tr>
<tr>
<td>19 Southland Kihiku 60%, Kauoira 40%</td>
</tr>
<tr>
<td>20 Wallace Mangapiri 85%</td>
</tr>
</tbody>
</table>
larly if it is applied after paddocks have been severely grazed with cattle and the seed trampled in afterwards. The paucity of fencing on the average and the great variability of subdivision fencing used (from 0-2 chain/ac to 2 chains/ac) indicates that full utilization of the fertilizer is not being achieved, as approx. 1 ch per acre is required. The above figures are consistent with those of the 1960 survey, but there is a marked trend to increase fencing relative to the other factors, indicating greater emphasis on grazing management.

With regard to carrying capacity the results are quite consistent, but do highlight the marked upward trend in dry sheep and breeding cows, which suggest an economy of labour.

The 1960 figures contrasted strongly with the New Zealand average figures which showed an increase of 50% in the cattle and sheep populations over a comparable period.

The contrast is even more striking between the New Zealand average figures of 120% and 100% increase in sheep and cattle respectively over the same period as the 1967 survey, where, with the exception of ewes, the increases are of the order of 300% in dry sheep and cattle. The marked upward trend in cattle is consistent with improving hill country pastures.

With regard to production, the figures of both surveys are quite consistent and emphasize the increase in wool and fat stock, the latter being a relatively new product of the hitherto "store stock" hill country.

If we use carrying capacity in terms of sheep equivalents as index of soil fertility, the fertility levels of these typical hill country farms have been raised 180%. However if we use production as an index of fertility, its level has been raised by at least 200%.

Response of Hill Country Soils

The results of the 1960 survey were interpreted in terms of soil types and related data, as shown on Table 3.

The most significant feature is that the actual results from development and improved management have exceeded the predictions of potential carrying capacity published in the Soil Survey 1954. The performance of the wide range of soils in terms of carrying capacity is remarkably uniform in the North Island, when the wide range of parent materials and the physical differences between them are considered.

The dominating influence of fertilizing and management are thus emphasized.

Pastoral Responses

The overall transformation of poor, run-down, eroded and weed infested pastures in the North Island to clover rich, well balanced, more protective and productive pastures has exceeded all expectations. On two soil conservation reserves the carrying capacity* has been increased on unploughable

* For the purposes of this comparison, five sheep are considered to be equivalent to one cattle beast.
hills from 1 1/2 ewes/ac to over 5 ewes on one area, to as high as 10 ewes/ac, under mob stocking, on the other (Campbell 1964).

In the South Island clovers and grasses have been successfully introduced into the depleted and eroded native tussock grassland to raise carrying capacity from 1/4 to 1 1/2 sheep/ac (Campbell 1966).

CONTROL OF SOIL EROSION AND FLOODING

One of the most important results has been that of controlling wind, sheet, scree and gully erosion on very severely eroded and depleted South Island high country with a much more highly productive thatch of mixed pasture that promotes infiltration and reduces the flooding hazard.

The deeper rooting protective pastures developed in the North Island play a prominent part in increasing infiltration and decreasing run off, sheet, slip and gully erosion (Campbell 1964, 1965, 1966).

These responses on stable hill country and eroded hill country lay solid foundations for conservation farm plans and catchment control schemes.

To date over 1000 conservation farm plans and 12 catchment control schemes covering over 3 million acres are operative.

DIRECT HARVEST OF INCREASED PRODUCTION

The impact of aerially distributing approximately 300 million dollars worth of fertilizer, seeds and chemicals is however best assessed in terms of increased national carrying capacity and production. During the seventeen years of operation sheep and cattle populations have increased from 33 million and 2 million to 60 million and 4 million respectively. Since the greater proportion of sheep and cattle are carried on hill country and aerial farming is confined to hill country, it is estimated that half these gains can be attributed to aerial farming. Thus an increase of 27 million sheep equivalents worth 4 dollars each can be credited to aerial farming, i.e. 108 million dollars of increased annual production.

Expressed in terms of land improvement on the basis that enough land to carry a sheep is worth 25 dollars the cumulative value of aerial farming and improved management has raised the value of the land resource by 675 million dollars.

CONCLUSION

The marriage of aeronautics and agronomy has made possible the mechanization of hill country farming and revolutionized fertility building and pasture improvement to become the most dynamic agricultural movement of our times.

It has also laid the foundations of a soil conservation farming system urgently needed on unstable slopes, so seriously threatened by soil erosion and flooding.

With its capacity to fertilize, seed, kill weeds and insects, poison noxious animals and deliver fencing materials and supplies on unploughable hill country, aerogronomy enhances man’s capacity to improve soils and focuses on soil science the responsibility of refining the techniques.
Challenging new frontiers awaiting research and development are:—improved spreading of chemicals and seeds, pelleted seed injection, effective aerial introduction of microorganisms where necessary, refined aerial firefighting techniques and aerial cultivation to introduce protective plants on harsh, actively eroding, inaccessible sites.

REFERENCES


SUMMARY

Aeragronomy—the teaming up of two scientific disciplines—has given new dimensions to soil technology in space and time.

With its capacity to rebuild soil fertility and facilitate improved land management by way of fertilizing, seeding, weed and insect killing, poisoning of rabbits, deer, goats and opossums and delivery of fencing materials, aeragronomy is solving a complex of problems stemming from depletion of fertility, land deterioration, soil erosion and flooding in New Zealand.

The integrated soil conservation and prudent grazing management practices developed by investigation and trial on eroded hill country depended upon aerial farming to raise fertility levels to make them economic and practical.

Once aerial farming techniques were developed and demonstrated farmers and aviators responded and helped build a new industry rapidly. The industry has grown and has a capacity of distributing 1 million tons of fertilizer and other materials per annum and so meet the needs of the 20 million acres of improvable hill country pastures.

The results speak for themselves in changing the appearance of the entire hill country pastoral landscapes. On farms surveyed the carrying capacity has been increased 2 fold and production at least 3 fold.

Severely eroded landscapes have been revegetated and the national production raised by 100 million dollars annually.
L'Aéragronomie, l'assemblage de deux disciplines scientifiques, a donné des dimensions nouvelles à la technologie du sol dans l'espace et dans le temps.

Avec sa capacité de reconstruire la fertilité du sol et de faciliter l'amélioration du terrain par la fertilisation, la semelle, l'extermination des mauvaises herbes et des insectes, l'empoisonnement des lapins, des cerfs, des chèvres et des singes et la livraison des matériaux de clôture, l'Aéragronomie est en train de résoudre en N.Z., un complexe de problèmes évolus de l'épuisement de la fertilité, de la détérioration du terrain, de l'érosion du sol et de l'inondation.

Les pratiques de conservation intégrée du sol et de conduite prudente des pâturages, développées par expérimentation et investigation dans les régions de collines érodées, comprenaient sur l'agriculture aérienne pour hausser le niveau de fertilité pour les rendre économiques et pratiques.

Une fois que les techniques de d'agriculture aérienne furent développées et démontrées, les fermiers et les aviateurs répondirent à l'appel et aidèrent à construire rapidement une industrie nouvelle. L'industrie s'est développée tellement qu'elle peut distribuer 1 million de tonnes d'engrais et d'autres matériaux par an et de telle façon répondre aux besoins de 20 million d'acres de pâturages montagneux qui peuvent être améliorés.

Les résultats sont évidents en soi, car ils ont changé l'apparence du terrain pastoral montagneux en entier. Sur des fermes inspectées, la capacité de transport a doublé et la production a au moins triplée.

Les terrains sévèrement érodés ont été révégétés et la production nationale a augmenté de 100 millions de dollars par an.

Die Luftagronomie—das Zusammenkoppeln zweier wissenschaftlicher Disziplinen—hat der Bodentechnologie neue Dimensionen in Raum und Zeit gegeben.


Die integrierte Bodenkonservierung und kluge Beweidungshandhabungen, die sich durch Untersuchungen und Versuche auf erodiertem Hügelland entwickelt haben, hingen von der Landbearbeitung aus der Luft ab, um Fruchtbarkeitsstände zu heben und sie wirtschaftlich und praktisch zu machen.

Sobald die Landwirtschaftstechniken aus der Luft einmal entwickelt und demonstriert waren, reagierten Landwirte und Aviatiker gleichermaßen und halfen rasch eine neue Industrie aufzubauen. Die Industrie ist zu einer
Verteilungskapazität von 1 millionen Tonnen Düngemittel und anderer Materialien im Jahr angewachsen und kommt so den Bedürfnissen der 20 Millionen Morgen verbesserungsfähigen Hügellandweideflächen entgegen.

Die Resultate sprechen für sich selbst durch die Veränderung im Aussehen der gesamten Landschaft des bergigen Weidelandes. Auf Farmen welche untersucht wurden hat sich die Beweidungskapazität verdoppelt und die Produktion mindestens verdreifacht.

Schwer erodierte Landschaften wurden wieder bepflanzt und die Nationale Produktion stieg um 100 Millionen Dollar im Jahre.
A COMPARISON OF PHOSPHATIC AND SULPHUR CONTAINING FERTILIZERS FOR PASTURE PRODUCTION ON RESTIAD PEAT IN THE WAIKATO DISTRICT

F. C. HUPKENS VAN DER ELST
Ruakura Agricultural Research Centre, Hamilton, New Zealand

INTRODUCTION

The high moor or raised peat bog formations found in the Waikato basin lie above flood level and contain on a dry matter basis about 95% organic matter and 5% mineral matter. To convert the native vegetation into pastures, drainage and heavy liming are a first essential; also, high rates of applications of phosphate and potassium are required for some years (Elliott and Thompson 1950) as well as copper and molybdenum (Hupkens van der Elst 1962). On pastures established by these means clover growth is usually excellent for two to three years but deteriorates after this period. The deterioration of the pasture could be partly due to physical changes of the peat such as irreversible drying of the top soil. This point of view is supported by the observation that deterioration is more severe after dry summers than after wet ones. Because of the low mineral content of these peat soils it was considered possible that anions were not held very firmly and that this could lead to mineral deficiency where leaching occurs. In preliminary investigations, phosphate applied as monocalcium phosphate leached through fibrous undecomposed peat but not through partly mineralised peat. As phosphates are more strongly absorbed by the soil than sulphates, possible leaching losses of both elements had to be investigated. For this reason field experiments were conducted to compare water soluble with non water soluble forms of phosphatic fertilisers and secondly to compare gypsum with elemental sulphur on fibrous undecomposed peat and on partly mineralised peat.

EXPERIMENTAL

Two field trials were conducted on virgin peat formed by a variety of plants of which the dominant species are members of the Restianaceae (Campbell, 1964). One trial was on undecomposed fibrous peat which had a loss on ignition of 95%. The other trial was on peat which as a result of drainage was slightly more decomposed and had a loss on ignition of 92%. Because of the acid nature of the peat, 2 tons of lime per acre were applied initially, half of which was worked 9 in. deep into the soil with a rotary hoe, while the other half together with 10 lb of copper sulphate and 1 oz of sodium molybdate per acre was applied to the surface just prior to sowing the seed.
Phosphorus
Superphosphate, serpentine superphosphate (a serpentine reverted superphosphate with 25% serpentine rock), lime reverted superphosphate (50-50 mixture) and basic slag were applied in autumn 1963 at the rate of 32 lb/ac P (36 kg/ha) in the first year, but because of poor pasture growth annual dressings were increased to 64 lb/ac (72 kg/ha) in the following years. The percentage of water soluble, citric soluble and total phosphorus were respectively 9.2, 9.2 and 9.5 for superphosphate; 0, 5.6 and 7.3 for serpentine superphosphate; 0, 3.4 and 4.0 for lime reverted superphosphate, and 0, 6.5 and 8.1 for basic slag.

Sulphur
Gypsum was applied either as a single autumn dressing or at 3 monthly intervals to give the same total application as for the single dressing. The rate of application was equivalent to the sulphur present in superphosphate, namely 31 S lb/ac (35 kg/ha) in the first year and 62 lb/ac (70 kg/ha) thereafter. Plots receiving elemental sulphur were topdressed in autumn with 31 lb/ac (35 kg/ha) of flowers of sulphur.

Potassium
Potassium chloride was applied in autumn at a rate of 50 lbK/ac (56 kg/ha) in the first year, but annual dressings were increased to 100 lb/ac (112 kg/ha) in the following years.

Seed
The seed mixture used in this experiment contained perennial ryegrass (*Lolium perenne* L.), short-rotation ryegrass (*Lolium perenne* L. × *Lolium multiflorum* Lam.), cocksfoot (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.) and lotus major (*Lotus uliginosus*), sown down in autumn at a rate of 30 lb/ac (34 kg/ha).

The lay-out of the trials was a randomised block design with 10 treatments and 4 replications. A mowing and clippings returned technique as described by Lynch (1960) was used to measure pasture production.

RESULTS
Though peat soils are uniformly low in their initial fertility, the pasture growth varies greatly from place to place due to differences in soil elevation brought about by the very uneven subsidence. Consequently only large differences between treatments reach significance and it is difficult to bring out small trends due to the large coefficient of variation.

Phosphate responses
Figure 1 shows the annual production of pasture on undecomposed peat with dressings of superphosphate, serpentine superphosphate, and lime-reverted superphosphate, all treatments receiving a basic KCl dressing. The first cut was taken 5 months after the application of the fertilisers.
ANNUAL PASTURE PRODUCTION on UNDECOMPOSED PEAT
(lb Wet Wt/240 sq. ft Plot)

Fig. 1.—Annual pasture production of plots topdressed with superphosphate, serpentine superphosphate and lime reverted superphosphate.

TABLE 1
ANNUAL PASTURE PRODUCTION (LB/AC DRY MATTER) FROM PEAT TREATED WITH DIFFERENT FORMS OF PHOSPHATE

<table>
<thead>
<tr>
<th>Type of peat</th>
<th>Undecomposed peat</th>
<th>Slightly decomposed peat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>1964/65</td>
<td>1965/66*</td>
</tr>
<tr>
<td>Superphosphate + KCl</td>
<td>6860 bB (7680)**</td>
<td>3950 bB (4420)</td>
</tr>
<tr>
<td>Serpentine superphosphate + KCl</td>
<td>9780 aA (10950)</td>
<td>5920 aA (6630)</td>
</tr>
<tr>
<td>Lime reverted superphosphate + KCl</td>
<td>8570 abAB (9590)</td>
<td>5430 aAB (6080)</td>
</tr>
<tr>
<td>Coeff. of variation per cent.</td>
<td>15·8</td>
<td>14·6</td>
</tr>
</tbody>
</table>

* Winter, Spring and Summer only.
** kg/ha.
Treatments which have a letter in common do not differ significantly: a 5 per cent. level of significance is denoted by small letters, a 1 per cent. level by capitals. (Duncan, 1955).
Table 1 shows the annual pasture production of these three treatments, Duncan’s (1955) multiple range test was used to show significant differences.

For the major part of the two years, pasture production due to superphosphate was inferior to the production due to serpentine superphosphate and to lime-reverted superphosphate on the undecomposed peat. On the slightly decomposed peat there were no significant differences in yield due to forms of phosphatic fertilisers used.

**Sulphur responses**

Figure 2 shows the relative pasture production from plots receiving basic slag (i) without sulphur, (ii) with sulphur applied in the form of gypsum once per year in the autumn, (iii) with gypsum in split applications at 3 monthly intervals, and (iv) with elemental sulphur. Table 2 gives the annual dry matter production of these treatments. Similar responses to sulphur were obtained on both types of peat.

Gypsum and sulphur increased pasture production by about 400%, but in 1965 (Figure 2) 9 months after application, the pasture on the
plots that had received gypsum in a single dressing yielded less than plots topdressed with elemental sulphur.

Chemical composition of the herbage

Table 3 shows the concentration of phosphorus and sulphur in the dry herbage from plots which had been topdressed 10 months before sampling. At the first sampling in 1965, after all plots had received phosphorus equivalent to 9 cwt/ac (1130 kg/ha) of superphosphate, herbage phosphorus levels were low. A year later, however, after the application of another 6 cwt/ac of superphosphate equivalent, the phosphorus levels in the herbage were very much higher, particularly on the low-producing plots.

Sulphur levels in both years were low with the exception of slightly higher levels from plots which had received split applications of gypsum and from plots topdressed with elemental sulphur. Many values are much below the critical levels suggested by McNaught & Chrisstoffels (1961).

A third trial was laid down to compare spring and autumn topdressing of superphosphate and serpentine superphosphate applied with and without additional sulphur. The rates of application were the same as in the previous trial.

The results set out in Table 4 show again that serpentine superphosphate gave better production than superphosphate when both fertilisers were applied in autumn, but no differences were obtained after an application of additional sulphur to the superphosphate plots. No differences in pasture production were obtained when both superphosphate and serpentine superphosphate were applied in spring; the total annual production from a spring application appeared to be nearly 100% higher than from an autumn application.
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sulphur content as % of dry matter</th>
<th>Phosphorus content as % of dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clovers</td>
<td>Grasses</td>
</tr>
<tr>
<td>Basic slag—no sulphur</td>
<td>-14</td>
<td>-18</td>
</tr>
<tr>
<td>Basic slag gypsum 1 ×</td>
<td>-18</td>
<td>-17</td>
</tr>
</tbody>
</table>

\(u\) = undecomposed fibrous peat, \(pd\) = partly decomposed peat.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of Application</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superphosphate</td>
<td>Autumn</td>
<td>1140 aA</td>
<td>1820 bC</td>
<td>1040 cB</td>
<td>4010 cC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1280)**</td>
<td>(2040)</td>
<td>(1160)</td>
<td>(4490)</td>
</tr>
<tr>
<td>Superphosphate + sulphur</td>
<td>Autumn</td>
<td>1360 aA</td>
<td>2620 cB</td>
<td>1970 aA</td>
<td>5960 bB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1520)</td>
<td>(2210)</td>
<td>(1510)</td>
<td>(6670)</td>
</tr>
<tr>
<td>Serpentine superphosphate</td>
<td>Autumn</td>
<td>1680 aA</td>
<td>2790 bcB</td>
<td>1350 bB</td>
<td>5820 bB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1880)</td>
<td>(1510)</td>
<td>(1510)</td>
<td>(6520)</td>
</tr>
<tr>
<td>Serpentine superphosphate + sulphur</td>
<td>Autumn</td>
<td>1640 aA</td>
<td>3100 bB</td>
<td>1730 aA</td>
<td>6480 bB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1840)</td>
<td>(1940)</td>
<td>(1510)</td>
<td>(7260)</td>
</tr>
<tr>
<td>Superphosphate</td>
<td>Spring</td>
<td>1460 aA</td>
<td>5320 aA</td>
<td>1820 aA</td>
<td>8600 aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1640)</td>
<td>(2040)</td>
<td>(2040)</td>
<td>(9640)</td>
</tr>
<tr>
<td>Superphosphate - sulphur</td>
<td>Spring</td>
<td>1760 aA</td>
<td>5330 aA</td>
<td>1920 aA</td>
<td>9000 aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1970)</td>
<td>(2150)</td>
<td>(2150)</td>
<td>(10090)</td>
</tr>
<tr>
<td>Serpentine superphosphate</td>
<td>Spring</td>
<td>1830 aA</td>
<td>5470 aA</td>
<td>1890 aA</td>
<td>9190 aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2050)</td>
<td>(2120)</td>
<td>(2120)</td>
<td>(10300)</td>
</tr>
<tr>
<td>Serpentine superphosphate + sulphur</td>
<td>Spring</td>
<td>1770 aA</td>
<td>5270 aA</td>
<td>1920 aA</td>
<td>8960 aA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1980)</td>
<td>(2150)</td>
<td>(2150)</td>
<td>(10030)</td>
</tr>
<tr>
<td>C.V.</td>
<td></td>
<td>21.8%</td>
<td>10.1%</td>
<td>13.1%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

* See footnote Table 1.
** kg/ha.
A number of facts needed to be considered before deciding whether leaching of phosphate or sulphate or perhaps both were responsible for the inferior growth on the superphosphate plots (see Table 1) on the undecomposed peat.

In leaching experiments, Hogg (personal communication) showed that by using a laboratory test in which soils were leached with 10 cm of water per week for 4 weeks, phosphate losses on peat occurred from superphosphate, but not from reverted forms of superphosphate. In further investigations Hogg and Cooper (1964) found that leaching losses of phosphates on a podzolised sand amounted to 36%, but that leaching of sulphate under similar conditions was more severe and could amount to 90% of the S applied. These results proved helpful in the interpretation of the field results.

Askew (1942) showed that after ½ hour shaking with water there was more soluble sulphate in superphosphate than in serpentine superphosphate and lime reverted superphosphate. From Table 3 it can be seen that in 1966, phosphate levels in the pasture herbage were high in all plots, but they were particularly high in the herbage from the superphosphate plots. In contrast sulphate levels were low, and the lowest levels were found on the superphosphate plots. Therefore it appears reasonable to assume that sulphur rather than phosphorus was responsible for the inferior growth on the superphosphate plots on the undecomposed peat.

This is borne out by the results in the third trial (Table 4) in which yields on the superphosphate plus elemental sulphur plots were not inferior to those treated with serpentine superphosphate.

When fertilisers were applied in spring instead of autumn, annual production increased by nearly 100% and differences between treatments no longer existed. The high production during the summer period is typical for peat soils, which usually show strong growth in late spring and summer, but poor growth in winter and early spring.

Most response differences can be explained by the loss of nutrients through leaching, as adverse growing conditions during the wet winter cause much of the applied phosphates and sulphates to become unavailable through leaching and fixation. After topdressing in spring, weather conditions are favourable for vigorous pasture growth and the applied fertiliser can be utilised immediately, therefore losses by leaching are likely to be considerably smaller. The sulphur responses presented in Table 2 show a highly significant response to sulphur at the 1% level. They also show that in 1966 a single autumn application of gypsum was inferior to split applications of gypsum and to flowers of sulphur. The irregular responses in the previous year could be explained by the after effect of cultivation which stimulated the breakdown of organic sulphates in the soil. Sulphur concentrations in the herbage (Table 3) show correlation with the pasture dry matter production; they also show that sulphur levels in herbage from plots topdressed with 1 single application of gypsum 9 months previously were little higher than control, indicating that the
pasture had become very sulphur deficient. Responses to elemental sulphur and split applications of gypsum were equal and both were superior to a single application of gypsum. These trials were carried out under conditions of excessive humidity in winter and adequate moisture in summer, in a district with a temperate climate. Under conditions of lower rainfall and a colder climate in the South Island gypsum was shown by Ludecke (1965) to be superior to elemental sulphur. If in future phosphatic fertilisers such as double superphosphate or rock phosphate become more economic to apply than superphosphate, then additional sulphur, preferably in the form of elemental sulphur, will have to be added.

ACKNOWLEDGMENTS

Thanks are due to Mr. K. J. McNaught for the herbage analyses, Mr. D. E. Hogg for the chemical analyses in the leaching experiments and also for their valuable advice; Mr. J. Corby for his assistance in carrying out the field work; and the statistical section who were responsible for the computation of the results.

REFERENCES


SUMMARY

Applications of phosphatic fertilisers have been the main reason for the rapid development of New Zealand’s agriculture since 1900.

In the North Island an autumn application of superphosphate is the most effective form of phosphatic fertiliser to boost pasture growth for the critical winter period on most soils. On undecomposed peat soils results show that superphosphate is inferior to serpentine superphosphate and lime-reverted superphosphate when all are applied in autumn. However, adding elemental sulphur to superphosphate raised pasture production to the same level as that obtained from serpentine superphosphate.

Comparison of production from autumn and spring applications showed that,
(a) pasture production was nearly doubled when superphosphate was applied by spring topdressing, and
(b) additional sulphur had no effect on pasture growth when both superphosphate or serpentine superphosphate were applied in spring.

Dry matter yield, leaching data and plant analyses indicate that leaching of sulphates was the main reason for the inferior production from superphosphate applied in autumn.

The results suggest that, in the development of raw peat, the most economic method is to apply unfortified normal superphosphate in spring rather than superphosphate plus sulphur or a reverted form of superphosphate in either spring or autumn.

RéSUMÉ

Les applications d’engrais phosphatiques ont sans doute été la cause principale du développement rapide de l’agriculture en Nouvelle-Zélande dès les années 1900.

Dans l’île du Nord, on a trouvé qu’une application de superphosphate en automne était la forme d’engrais phosphatique la plus efficace pour encourager la croissance d’un pâturage durant la période critique d’hiver sur la plupart des sols, mais pour les sols de tourbe non-décomposée, le superphosphate s’est avéré inférieur au superphosphate serpentin et au superphosphate transformé par la chaux, quand tous les trois étaient appliqués en automne.

La production de fourrage augmentait de la même façon après l’addition de soufre élémentaire au superphosphate, ou celle de superphosphate serpentin.

En comparant les applications d’automne à celles du printemps, on a trouvé que:
a) La production de fourrage était presque doublée après un épandage en couverture de superphosphate, au printemps.
b) L’addition de soufre n’avait pas d’effet sur la croissance dans les cas où du superphosphate et du superphosphate serpentin étaient appliqués au printemps.

Les données concernant le lessivage de la matière sèche et l’analyse des plantes indiquent que les lessivage des sulphates était la cause principale d’une production inférieure après l’application de superphosphate en automne.

Les résultats obtenus suggèrent que dans le développement de la tourbe brute, la méthode la plus économique est d’appliquer un superphosphate normal non fortifié au printemps, plutôt que du superphosphate + soufre ou une forme transformée de superphosphate soit au printemps, soit en automne.
ZUSAMMENFASSUNG

Der Hauptgrund für die rasche Entwicklung des neusee-ländischen Ackerbaues seit der jahrhundertwende ist ohne Zweifel die Anwendung von Phosphatdüngern.


Der Zusatz von Elementarschwefel zum Superphosphat brachte die Futtererträge auf die selbe Höhe wie Serpentin-Superphosphat.

Beim Vergleich zwischen Düngung im Herbst und im Frühling stellte sich heraus, dass:

a. Futtererträge von Superphosphat allein durch Zugaben im Frühling verdoppelt werden konnten;

b. extra Gaben von Schwefel keinen Einfluss auf den Futterertrag ausübten, wenn Superphosphat und Serpentin-Superphosphat im Frühling gegeben wurden.

Erträge an Trockenmaterial, Auslaugungs- und Pflanzen-analysezahlen weisen darauf hin, dass die Auslaugung von Sulfaten der Hauptgrund für die geringeren Erträge nach Superphosphat-düngung im Herbst waren.

Diese Ergebnisse deuten an, dass beim Entwickeln von rohen Torfböden die Anwendung von unverstärktem Normal-Superphosphat im Frühling wirksam ist, als Superphosphat und Schwefel oder eine "lime reverted" Form Superphosphats im Frühling oder Herbst.
NUTRIENT CYCLE AND SOIL FERTILITY ON RED FERRALLITIC SOILS

E. JONES
Cotton Research Corporation, Cotton Research Station, Namulonge, Uganda

INTRODUCTION

The view that an improved and non-exploitative system of arable farming for the tropics must be developed from shifting cultivation, has often been expressed (Gourou 1961, Nye and Greenland 1960, Kellogg 1963). Shifting cultivation and crop rotation in tropical soils are characterised by a fall in fertility during the arable phase, and a build-up following a period of rest in perennial vegetation. This cycle of fertility has been attributed to changes in soil structure and/or soil nutrient status. A detailed investigation of the effect of three years’ rest in Elephant grass (Pennisetum purpureum) on the structure of the Buganda red ferrallitic soils, showed that the measurable improvement in structure following a rest period disappeared after 12 months of arable cultivation, whereas the improved fertility continued for at least three years (Pereira et al. 1954). The second possibility, the improvement in soil nutrient status, has not been investigated in Uganda and is based on a cycle of nutrients in a profile. During the arable phase, nutrients are leached into the sub-soil beyond the rooting depth of annual crops. The deep roots of the rest phase vegetation take up the leached nutrients and return them to the top-soil. This cycle of nutrients in a profile has been shown to be effective in building up the nutrient status of the soil plus vegetation in a 40 year old forest in Ghana (Nye and Greenland 1960) and in a five to thirty year fallow in Congo Kinshasa (Laudelout and Meyer 1954).

An account is given below of preliminary trials to evaluate the cycle of nutrients in the profile during the course of a rotation.

EXPERIMENTAL

This study was conducted on the farm of the Cotton Research Station situated 17 miles north of Kampala. The arable soils have been described as “ferrallitic sandy clay to sandy clay loam soils with a dominant red colour developed on a basement complex” (Harrop 1962). They are generally deep and free draining. The annual rainfall for the farm varies between 1125-1500 mm and is distributed in two peaks, the first in March-April and May and the second in September, October and November. The natural vegetation of the area has been described as “savanna-like communities derived from forest” (Trapnell and Langdale-Brown 1962).

The simplest approach to the evaluation of the cycle of nutrients in the profile is to measure the change in the nutrient content of the topsoil during
the course of a rotation. The preliminary trials reported here were designed
to give some indication of the magnitude of the nutrient changes, and what
sampling and other problems of technique would have to be overcome. For
the arable phase study, six plots recently cleared from an Elephant grass
rest were compared with six plots cleared from a Rhodes grass (Chloris
gayana) rest. A sequence of crops, cotton, groundnuts, beans, maize and
cotton, was grown on the plots during the 2½ years period of sampling.
Soil samples 0-6 in., 6-12 in. and 12-18 in. were collected at six monthly
intervals and a record of the crop yields was kept to allow calculation of
the nutrients removed in the crops. For the rest phase studies seven resting
treatments replicated twice were planted on land which had completed
three years in arable cultivation. The rest treatments were, Elephant grass
and Rhodes grass undisturbed and self-mulched every six months, Elephant
grass cut and carried every six months to simulate its use for mulching,
natural regeneration and sugar cane up to the first ratoon crop. Soil samples
were collected from 0-6 in., 6-12 in. and 12-18 in. before and after the
rest period. Random strips of the standing vegetation and surface trash
were weighed and sampled for chemical analysis.

Plant materials were analysed for N, P, K, Ca and Mg by standard
methods. Soil analysis carried out on the total soil included: total nitrogen,
macro-Kjeldahl; total phosphorus, perchloric acid digestion; organic carbon,
Walkley and Black; exchangeable calcium, potassium and magnesium,
extracion with 0.1 N hydrochloric acid and pH, 1:5 soil, 0.01 M CaCl₂
suspension.

RESULTS

Soil samples collected from the 0-6 in. horizon showed a consistent
pattern of change in composition following both the rest and arable period.
In contrast the 6-12 in. and 12-18 in. samples gave no consistent change.
It is possible that there was no change in the composition of the soil below
six inches, but a more likely explanation is that the natural variation in the
soils at this depth is greater than the change due to treatment. Using a
fixed sampling pattern it should be possible to test this explanation.

In retrospect it was realized that the soil and vegetation sampling
procedures used in the preliminary studies were inadequate to detect any
differences between the resting treatments, unless these were very large.
No consistent differences between treatments were found, and consequently
the results presented in Table 1 are average values for the seven treatments
in the rest phase and the two treatments in the arable phase.

In order to stress the quantitative aspects of the gains and losses of
nutrients, the results have been expressed on a volumetric basis, assuming
a constant bulk density value. In another study the author has shown that
changes in bulk density do occur during the course of rotation, but their
application would not materially change the pattern of results given in
Table 1. Martin (1944) failed to show consistent changes in bulk density in
a similar rotation in Uganda.

The results given in Table 1 show that there was an increase in all
### Table 1
CHANGES IN THE COMPOSITION OF THE SOIL (0 — 6 IN.) FOLLOWING A PERIOD OF REST AND ARABLE*

<table>
<thead>
<tr>
<th></th>
<th>‘Org.’ C</th>
<th>Total</th>
<th>Exchangeable</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>P</td>
<td>Ca</td>
</tr>
<tr>
<td>Rest phase, average of 14 plots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial soil composition</td>
<td>34,300</td>
<td>2,600</td>
<td>690</td>
<td>3,300</td>
</tr>
<tr>
<td>Change in soil after 2.5 years rest</td>
<td>+5,600</td>
<td>+268</td>
<td>-3</td>
<td>+292</td>
</tr>
<tr>
<td>Nutrients in the vegetation</td>
<td>560</td>
<td>65</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Nett change: soil plus vegetation</td>
<td>+6,200</td>
<td>+333</td>
<td>+12</td>
<td>+321</td>
</tr>
<tr>
<td>Arable phase, average of 12 plots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial soil composition</td>
<td>40,600</td>
<td>2,800</td>
<td>520</td>
<td>2,850</td>
</tr>
<tr>
<td>Estimates of nutrients removed in crops</td>
<td>—</td>
<td>92</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Nett change in soil after 2.5 years arable</td>
<td>-4,800</td>
<td>-520</td>
<td>-34</td>
<td>-364</td>
</tr>
<tr>
<td>S.E. diff between nett change in arable and rest</td>
<td>±1,660</td>
<td>±115</td>
<td>±15</td>
<td>±84</td>
</tr>
</tbody>
</table>

* In lb/ac assuming 1 acre — 6 inches = 2.0 × 10^6 lb.
factors measured following a rest period, and a decrease in the same
factors following a period in arable cultivation. The trials were not designed
to allow a strict comparison of the gains in the rest phase with the loss
in the arable phase but statistical analysis of the pooled data showed no
statistically significant differences between the nett changes following the
two phases. The results indicate that changes in soil composition during an
arable period are corrected by resting the land in perennial vegetation for
an equivalent period of time.

The gains and losses of nutrients are small relative to their total content
in the soil, but if they are considered as their equivalents in inorganic
fertilizers, their importance in the soil fertility cycle associated with
rotations is understandable.

The changes in organic carbon content of the soil probably reflect
changes in soil structure which have been associated with this rotation on
these soils (Martin 1944). Increases and decreases in organic carbon will
also change the cation exchange capacity of these kaolinitic clay soils. The
fact that pH values increase with increasing carbon content and vice-versa,
implies that the rate of gain or loss of cations is more rapid than the gain
and loss of organic carbon. Nye and Greenland (1960) in their review of
soils under shifting cultivation found no evidence of a gain in pH during
the early years of a rest period, and they postulated that gains of organic
carbon and cations would run parallel. Conditions during the rest period
in this study were ideal for the pumping up of cations and other nutrients
from the sub-soil. The topsoil remained dry for long periods in the year
owing to the active growth of the grasses, and under these conditions, the
roots are forced to use sub-soil water and nutrients, the latter being pumped
up into the top-soil through the plant.

Total phosphorus changes in the soil are very small and probably
reflect low requirement of the rest vegetation, low availability of the
nutrient in the sub-soil during the rest phase and the immobile nature of
the nutrient during the arable phase. The changes in the organic carbon
and pH levels in the soil will affect the availability of phosphorus to the
arable crops. Le Mare (1960) showed that phosphorus uptake by cotton
in these soils was positively correlated with organic matter content and pH
values up to 6.4.

The nitrogen gain to the topsoil plus vegetation following the rest
period averaged 333 lb/ac N. Since the rest vegetation contained very few
legumes the origin of the nitrogen is of interest. Using the rainwater analysis
of Visser (1964), it may be calculated that rainfall could contribute 40-50
lb/ac N over the period of rest. Mills (1953) working in Buganda
suggested that nitrate nitrogen leached into the subsoil during the arable
phase was recovered by the deep roots of the rest vegetation and returned
to the surface soil. Simpson (1961) also working in Buganda put forward
the same suggestion to account for a soil gain of 202 lb N per acre follow-
ing two years in grass.

In an attempt to evaluate the subsoil nitrate as a source of the nitrogen
### Table 2

The nitrate nitrogen content* of the soil profile between one and nine ft. in the arable and rest phase

<table>
<thead>
<tr>
<th>Length of rest</th>
<th>2 year rest</th>
<th>1 year rest</th>
<th>1 year rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>adjacent plots</td>
<td>same plot</td>
<td>adjacent plot</td>
</tr>
<tr>
<td>Arable profile</td>
<td>384 511 230</td>
<td>308 395 272</td>
<td>282 482 405 337</td>
</tr>
<tr>
<td>Resting profile</td>
<td>103 110 97</td>
<td>226 264 122</td>
<td>90 116 226 122</td>
</tr>
<tr>
<td>Difference</td>
<td>281 501 133</td>
<td>82 131 150</td>
<td>192 366 179 215</td>
</tr>
</tbody>
</table>

* In lb nitrogen per acre — 8 feet. The profiles were sampled at intervals of one ft. and it was assumed that 1 acre ft. = 4×10^6 lb.
gain following a rest, the nitrate content of 1-9 ft profiles in the rest and arable phase was determined.

The results for ten pairs of soil profiles, either before and after a period of rest, or from adjacent sites in cultivation and rest are presented in Table 2. Without exception there was less nitrate nitrogen in the rest profiles than in the arable phase profiles. The difference varied from 82-501 lb/ac nitrogen. Since little or no leaching occurs during a rest phase it may be concluded that the difference between the rest and arable profiles is due to the uptake of nitrate by the roots of the rest vegetation. The gain of nitrogen to the topsoil from this source would be 200-250 lb/ac.

The gain in soil nitrogen from rainfall and leached nitrates accounts for the bulk of the nitrogen gain following the rest phase. Other possible sources of nitrogen on which there is no information available for Buganda are non-symbiotic nitrogen fixing organisms either in the soil (Moore and Abaelu 1959) or in the plant phyllosphere (Ruinen 1965). The contributions from both these sources are likely to be small.

The results given in Table 2 show that nitrate nitrogen is accumulated in the subsoil during the arable phase. This would account for the loss of 428 lb/ac of nitrogen following an arable period as shown in Table 1. Meiklejohn (1953) stated that losses of nitrogen by denitrification are not important in these soils.

DISCUSSION

A cycle of nutrients in the profile has been shown to occur during the course of a rotation on the red ferrallitic soils. These soils in common with many tropical soils are deeply weathered with little or no reserves of unweathered minerals, particularly in the topsoil (Jones 1963). Any loss of nutrients from the topsoil, by leaching, crop removal and fixation, can only be replaced by uptake from the subsoil by the deep roots of the vegetation. Leaching conditions do occur under arable crops at the two rainfall peaks, and the soils are deep enough to allow the leached nutrients to move beyond the depth of arable crop roots. Under these conditions it is conceivable that the cycle of nutrients in the profile is important in the maintenance of fertility in a rotation. This is supported by the findings of Scott (1961), who showed that the cation content of East African soils under natural vegetation attained a maximum at rainfalls of 900-1000 mm per annum.

The present study was initiated because all attempts at using inorganic fertilizers to maintain fertility under arable cultivation on these soils had failed. The results from many fertilizer trials showed that with the possible exception of nitrogen on maize and phosphorus occasionally, no other nutrient was deficient. The only certain methods of maintaining fertility was to rest the land under perennial vegetation or apply farmyard manure (Stephens, personal communication). The latter is never likely to be practical in an equable climate. Consequently, it was argued that any system of maintaining fertility under arable cultivation would have to
achieve the same effects as a resting period, or be capable of counteracting the effects of arable cultivation.

The results presented show that nutrients are lost during the arable phase and gained during the resting phase. The study did not cover sulphur and the micro-nutrients, but there is no reason to suppose that they are not also gained and lost during a rotation. Associated with the gains and losses of nutrients are changes in the pH values and organic matter content of the soils.

On the basis that farming practice must imitate the rest or counteract the arable, it would appear, that in order to maintain fertility under arable cultivation, inorganic fertilizers supplying all the major nutrients and possibly the micro-nutrients will have to be applied. In addition pH levels would have to be maintained by liming, and farming practice should aim at conserving organic matter. Organic matter in itself is unlikely to be important except where anti-erosion measures are inadequate. This comprehensive approach to the maintenance of soil fertility is in agreement with the use of farmyard manure as a method of maintaining fertility. Similar conclusions have been reached in other areas of old deeply weathered soils (Sene 1966, Roche et al. 1966, Velley et al. 1966, Orchard, personal communication).

Consideration of the mode of formation of the red ferrallitic soils may provide an explanation of why the comprehensive approach is necessary.

The red ferrallitic soils of Buganda are old soils which have undergone a number of weathering and re-sorting cycles (Ollier 1959, Radwanski and Ollier 1959). During the most recent period of their development they have supported a forest type vegetation which would have attained a state of equilibrium with the soil. In short any nutrients in the soil in excess of the requirement of the soil-vegetation cycle would have been removed by leaching or fixed in forms unavailable to plants. Therefore the proportion of different nutrients available in the soil would be correct for supporting the natural vegetation, and would probably be roughly correct for arable crops. Addition of one or two nutrients as inorganic fertilizers could create an imbalance of nutrients unless the arable crop requires a higher proportion of the applied nutrient, e.g. nitrogen on maize. This situation may prevail for a number of years after clearing for cultivation, but since the mechanisms involved in the loss of fertility, leaching and chemical fixation, are not going to affect each nutrient proportionally, deficiencies of individual nutrients can be expected to develop in the long term.

ACKNOWLEDGMENTS

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SUMMARY

Shifting cultivation and crop rotation which includes a period of rest in perennial vegetation are known methods of long term farming in tropical soils. A study of resting under perennial vegetation showed an increase in organic carbon, pH, nitrogen, phosphorus, calcium, potassium and magnesium in the topsoil, whereas losses of the same occurred following a period of arable cultivation. The bulk of nitrogen gained following the rest period is shown to be due to uptake by roots of nitrate leached into the subsoil during the arable period.

The relevance of the results to the problem of long term arable cropping on these soils is discussed.

RÉSUMÉ

Le changement de culture, et l'assolement comprenant une période de friche pour la végétation vivace sont des méthodes connues de l'agriculture à long terme dans les sols des tropiques. Une étude de la terre en friche sous la végétation vivace montra une augmentation de carbone organique, de pH, d'azote, de phosphore, de calcium, de potassium et de magnésium dans la couche arable, tandis qu'il y avait des pertes de ceux-ci suivant une période de culture. La plupart de l'azote gagné à la suite de la période de friche se montre due à l'absorption par les racines, du nitrate lessivé dans le sous-sol pendant la période arable.

Le rapport des résultats avec le problème de la culture arable à long terme sur ces sols est discuté.
NUTRIENT CYCLE ON FERRALLITIC SOILS

ZUSAMMENFASSUNG


Die Anwendbarkeit der Resultate auf das Problem der Dauerbewirtschaftung auf diesen Böden wird diskutiert.
COMPACTION OF PASTURE TOPSOILS UNDER WINTER GRAZING

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INTRODUCTION

The winter climate of the grazing areas of New Zealand is wet and rather mild. Thus pasture growth continues at a rate sufficient to support the outdoor grazing of sheep and cows which the temperature permits. Sears (1953) reported that from 7 to 10 percent of the annual pasture yield in his experimental plots at Palmerston North occurred in the winter months, June to August. At this rate of growth a pasture would probably need to be grazed off at least once in the winter. Topsoils are commonly at, or close to, field capacity for much of this season, with intermittent rain bringing them frequently to higher moisture contents for a day or two. Stock treading on such wet soils can compact them to a degree which would not occur at the same stocking rates at other seasons of the year.

COMPAC TION AND STRUCTURAL BREAKDOWN UNDER TREADING

With the introduction of better pasture species and the removal of soil nutrient deficiencies, stock densities on our pastures have built up substantially in the last thirty years. The treading of great numbers of animals produces a permanent increase in the density of the surface soil. In a trial on a gleyed pumiceous soil (Te Kowhai loam) comprising different stocking rates with sheep, mean soil dry densities and large pore contents of the $\frac{3}{4}$ to $1\frac{1}{4}$ in. depth were as set out in Table 1.

Large pore content is defined as the percentage of soil volume drained by a suction of 50 cm of water. It is seen that soil dry density increased and

<table>
<thead>
<tr>
<th>Stock Rate (dry ewes per acre)</th>
<th>Soil Dry Density (g/cc)</th>
<th>Large Pores (% of soil volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>0.790</td>
<td>13.0</td>
</tr>
<tr>
<td>10.7</td>
<td>0.833</td>
<td>11.5</td>
</tr>
<tr>
<td>16.1</td>
<td>0.861</td>
<td>9.3</td>
</tr>
<tr>
<td>21.5</td>
<td>0.878</td>
<td>7.1</td>
</tr>
</tbody>
</table>

429
large pore content decreased progressively as stocking rate built up. The effect of increased treading appeared to be accentuated by closer grazing, which removed the protective cover of grass from the soil.

Such a year-round increased density of the soil and closing up of its large, freely draining pores under heavy stocking is supplemented by further temporary compaction in the wet winter season. New Zealand soils vary greatly in their liability to this type of compaction, young soils from basalt or andesitic ash being the most resistant, and zonal soils the least resistant. This is illustrated by a laboratory comparison of the compactibility of two extreme soils, the brown loam, Kiripaka silt loam and the podzol, Wharekohe silt loam. Five cores of the topsoil of each were saturated, drained to a tension of 10 cm of water and subjected to a series of impressions of a piston 1·5 in. in diameter, conveying a pressure of 30 lb/sq in. (2·1 kg/cm²) to various portions of their upper surfaces until a complete coverage was obtained. Subsequently the mean content of large pores in the zone just under the surface was found to be 11·6% for the brown loam from basalt and 2·0% for the podzol. It seems that the former soil can be expected to retain a reasonably open structure under the most drastic compaction. The figure for the podzol was not much lower than the value of 3·9% found for this soil under natural forest (New Zealand Soil Bur. 1967). Apparently such a soil is always compact and ill-drained in the A horizon. Between these extremes lie soils which vary in their contents of large pores, from adequate to poor, according to previous management. The results of the grazing trials reported here were derived on such soils.

When soils, softened by high contents of moisture but still containing appreciable air pores, are trodden, the increase in dry density (compaction) occurs concurrently with a decrease in large pore volume. Such a change has been recorded in a winter grazing trial (Gradwell 1960, Table 2). When, however, a soil close to saturation is grazed there can be no direct compaction but the penetrations of animal hooves into the topsoil produce a remoulding action which expresses itself as a loss of large pores. This was well illustrated in the grazing trial reported by Gradwell (1960, Table 3). Here, on a recent soil, Manawatu silt loam, the content of large pores in the surface 1·5 in. of the soil fell from 9·5 to 2·9% from June to the end of August, but soil dry density did not increase at all.

**Effects of Structural Breakdown in Winter**

The immediate effects of these changes are softening of the soil and reduction of its ability to supply oxygen to pasture roots. In the trial just mentioned the moisture content of the soil at a tension of 50 cm of water (which approximated to field capacity) rose from 54·6 to 64·2% (dry weight basis) during the winter. The remoulded soil which resulted, containing more water, would likely be softer and permit deeper hoof penetrations on further grazing. Burke, Galvin and Galvin (1964) compared the structural stability of six Irish soils under grazing by means of the ratio plastic limit : field capacity. The sequence into which the soils fell corresponded with experience in the field. The results of this trial on Manawatu
silt loam show that the field capacity of some surface soils under grazing is not a constant but can vary appreciably with management, reducing the stability of the soil, as estimated by the above index, once partial pugging has occurred.

Deep hoof penetrations into soft, wet soils result in extensive tearing and burying of pasture plants. Reductions in regrowth rates may be expected to follow and can be sought in field trials in which regrowth in winter and spring in puddled plots is compared with unpuddled controls. Edmond (1958, 1963) found that treading rates equivalent to 6 to 9 sheep per acre on Manawatu silt loam at field capacity reduced regrowth by 10 to 30% compared with untrodden controls; treading on saturated soil depressed it a further 20 to 35 percent relative to soil at field capacity. Campbell (1966) found that a day's grazing by cows at 60 or 120 to the acre on Te Kowhai gleyed pumiceous soil at about field capacity reduced regrowth, relative to a mowed control with return of clippings, up to 15% but the differences were not significant.

Such field trials on regrowth do not separate the effects of plant injury during pugging from the effects of subsequent oxygen deficiencies in the soil. The latter have to be estimated from separate pot trials. Very low levels of oxygen are found in puddled pasture topsoils. Gradwell (1960, Table 5) showed that air-pore contents of 5 to 7% of soil volume are common in the field in wet weather on Manawatu silt loam. At such values the diffusion of gaseous oxygen through the greater part of the soil mass is very slow (Gradwell 1961, Fig. 4). The oxygen supplying power of massive or puddled pasture topsoils can be very low (Gradwell 1965, Table 8) as measured by the platinum micro-electrode of Lemon and Erickson (1952). Fortunately, the pasture plants grown in New Zealand appear able to tolerate lower levels of soil oxygen than would be required for the growth of most crop species. Gradwell (1965) found that severe reduction of the growth of seedling ryegrass plants occurred only below a very low level of soil aeration, specified as an oxygen flux of $1 \times 10^{-7}$ g cm$^{-2}$ min$^{-1}$ to the micro-electrode, or an air-pore content of 7 or 8% of soil volume, or a specific gaseous diffusivity of 0.005. This probably means that root growth is slowed below these levels. No total exclusion of roots from soil, even at the lowest levels of soil oxygen, was found, in contrast to the results of Letey et al. (1964) for Newport bluegrass, the roots of which failed to penetrate soil with an oxygen supply rate of less than $2 \times 10^{-7}$ g cm$^{-2}$ min$^{-1}$.

The writer's results do not, therefore, support the explanation of shallow rooting of pasture in terms of oxygen deficiency in the underlying soil, unless such deficiencies, at some depth and under a cover of better aerated soil, are more harmful than when they occur near the surface, as in the above quoted trials (Gradwell 1965). In these trials no possibility of preferential growth in a better aerated zone was available to the grass roots.

Aside from this relative tolerance of low soil oxygen, pasture plants have an advantage over crop plants in that their root systems are well grown before being subjected to shortages of oxygen in winter. These are recognized as being less harmful to mature than to young plants cf. Letey
et al. (1962). The application of this finding to ryegrass has been verified by the writer, who failed to detect depressions of top growth in well-developed plants when the supply of soil oxygen about their roots was lowered below $1 \times 10^{-7}$ g cm$^{-2}$ min$^{-1}$ by continuous flushing of nitrogen gas through the soil. Root growth was somewhat reduced. In experiments with seedling plants both top and root growth were invariably depressed at this level of oxygen supply. Similar experiments with well-developed white clover plants showed in most cases appreciable reductions of top growth at very low levels of soil oxygen, similar to those found harmful to ryegrass.

The overall import of these trials seems to be that soil oxygen deficiencies are not as widely important to pastures as has sometimes been supposed. On massive or badly puddled soils, approaching the blue gleying stage in the surface soil, they may be responsible intermittently for reductions in growth.

**Residual Effects in Spring and Summer**

It has already been mentioned that a soil pre-compacted by stock in the drier seasons of the year will suffer further compaction under the same stocking rate in winter. Even where the damage under winter treading takes the form of remoulding rather than immediate density increase the resulting structureless soil will shrink as it dries out in the spring (Gradwell 1960, Table 3). The indications are that this density increase is rather persistent, leading to a more compact soil for the greater part of the year as a result of a purely seasonal puddling.

**Table 2**

**Long-term Effects of Pugging on Soil Dry Density**

<table>
<thead>
<tr>
<th>Soil:</th>
<th>Manawatu silt loam (g/cc)</th>
<th>Te Kowhai sandy loam (g/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pugged plots</td>
<td>1.390</td>
<td>0.901</td>
</tr>
<tr>
<td>Unpugged plots</td>
<td>1.335</td>
<td>0.788</td>
</tr>
</tbody>
</table>

In Table 2 are set out soil dry densities in the surface $1\frac{1}{2}$ in. of Manawatu silt loam and Te Kowhai sandy loam in summer, six months after winter pugging. Each result is compared with similar determinations on control plots not pugged in the preceding winter but otherwise identically managed. In each case the pugged soil was significantly denser. Four months later, just before the next winter, the density difference on Te Kowhai sandy loam had almost disappeared.

There were signs that on these two, rather compact soils, the density increase due to previous pugging was accompanied by deterioration of those physical growth factors likely to be of importance in the dry season—namely, soil hardness and moisture supply. At the time the above density
determinations were made the Te Kowhai soil was perceptibly harder in the pugged plots. Earlier, in the spring, this soil was observed a day after rain had re-wetted it, following a dry spell. The unpugged plots were visibly more deeply and uniformly wetted, indicating a more absorptive surface and a lesser run-off into cracks and hollows. This property could be important in a dry summer.

The increased density of Manawatu silt loam was accompanied by a reduction of 10% in the storage of available moisture in the top two inches. This effect would not be likely to appear in open soils such as brown loams or yellow-brown pumice soils, where compaction would reduce the air voids first, but in already compact soils, close to saturation at field capacity, any further compaction must cut down the volume of water in the soil.

Whatever the cause, both of these soils showed significantly higher soil moisture tensions in the pugged plots in January (Gradwell, 1966).

**Remedial Management**

The merit of the year-round outdoor grazing traditionally practised in New Zealand was economic as it made for low-cost production. As stocking rates increase, however, the damage to pastures on vulnerable soils in winter is becoming so great that some investment in facilities for more controlled grazing should show a profit. Possible facilities range from a yard, with feeding racks, to which the stock may be removed for a day or two at a time during heavy rain, to complete barns, from which they would emerge only occasionally during the driest spells in winter to consume excess grass in the paddocks. As the soils of the New Zealand grazing districts cover a very wide range in structural stability, the severity of the problem varies greatly from place to place and with it the amount of relief from grazing pressure likely to pay for itself. A rating of soils for liability to treading damage would be useful in selecting from management schemes shown to be profitable on various soils, those most appropriate to any soil on which such farm-scale trials had not been carried out.

Meanwhile, soil physics studies draw attention to two aspects of the problem: (1) the cumulative nature of pasture pugging and (2) the appreciable benefit to be gained by removing stock for the brief periods when soils are between saturation and field capacity. A soil partly pugged early in winter will be softer and wetter thereafter. The hooves of animals will do more damage at subsequent grazings than they would on an undamaged soil. Surface water will lie longer in depressions and the selection of reasonably safe times for grazing will become more difficult. The prevention of the initial damage by careful grazing control on all occasions from the beginning of winter is thus doubly important. On most soils not already pugged, structure will be sufficiently open for the moisture content (on a weight basis) to decline by some few percent by drainage in the first day after heavy rain. With the low rates of evapotranspiration prevailing in winter it may be many days thereafter before a further drying of the surface soil of the same magnitude can occur. Thus a
removal of stock from the pastures merely during and shortly after the heaviest rains will be of considerable benefit where more elaborate grazing control is impracticable.

REFERENCES

SUMMARY
Outdoor grazing in the wet winter months at high stocking rates in New Zealand leads to considerable plant damage by hooves and also to loss of large pores from the surface horizons of the more weakly structured soils. The ryegrass and white clover species commonly grown in the pastures appear to be more tolerant to poor soil aeration than most agricultural plants. Nevertheless, reductions in winter and spring growth under treading have been recorded, due either to this cause or to mechanical plant damage. Recovery of good soil structure after winter puddling is rather slow, surface soils affected having been found to be harder and denser six months later. The ability of compact soils to absorb and store water in their surface horizons in the following summer can also be reduced by winter puddling.

There is a trend to partial relief of grazing pressure in the winter. The economics of this vary with the structural stability of the soil concerned.

RÉSUMÉ
En Nouvelle-Zélande, le pacage en plein air par un bétail nombreux durant les mois humides d’hiver commet des dégâts considérables aux plantes (par les sabots des animaux), et aussi la perte des pores de grande dimension des horizons de surface dans les sols à structure faiblement développée. L’ivraie et le trèfle blanc, qui sont courants dans les pâturages, semblent être les espèces qui tolèrent mieux que la plupart des plantes agricoles, une mauvaise aération du sol. Néanmoins, on a enregistré une diminution de croissance en hiver et au printemps venant soit des dégâts susmentionnés, soit des dégâts mécaniques faits aux plantes. Le rétablissement d’une bonne structure du sol après sa destruction pendant l’hiver est assez lent, les parties affectées du sol étant plus dures et plus denses six mois plus tard. La destruction de la structure d’un sol en hiver peut aussi
diminuer sa capacité d'absorber et de garder l'eau dans son horizon de surface, durant l'été qui suit. 

La tendance actuelle est de relâcher partiellement le pâtage pendant l'hiver. Le rendement de cette méthode varie avec la stabilité de la structure du sol en question.

**ZUSAMMENFASSUNG**


Es besteht eine Neigung zur teilweisen Erleichterung der Weidenbeanspruchung im Winter. Die Wirtschaftlichkeit hiervon variiert mit der strukturellen Stabilität des betroffenen Bodens.
THE INFLUENCE OF SOIL TYPE ON INGESTION OF SOIL BY GRAZING ANIMALS

W. B. HEALY
Soil Bureau, Department of Scientific and Industrial Research, Lower Hutt, New Zealand

The influence of soils on grazing animals is usually considered to be a nutritional one through pasture composition and dry matter production. That soils differ in their ability to supply nutrient elements to animals via pasture is well recognised, and the inadequacy of certain New Zealand soils to supply microelements such as Co, Se, Cu, is well known. However, sheep may ingest considerable quantities of soil during grazing. Differences in the amount ingested on various soils, emphasise the influence of physical properties, such as structure, upon health of sheep through the wear of incisor teeth. Chemical properties of the soil may influence animal health through the amount of microelements and insecticides ingested with the bulk soil. This paper presents data on the effect of soil ingestion upon these aspects of animal health in relation to soil type.

EXPERIMENTAL

Soil ingestion was measured by analysis of uncontaminated faecal samples collected from fields. Oven-dried faecal samples were ashed and acid extracted to determine soil content. Full sampling and analytical details together with methods used to calculate daily soil intakes are given by Healy and Ludwig (1965). Tooth measurements were made as described by Ludwig et al. (1966).

Soils were analysed for macro- and micro-elements to estimate possible amounts of elements absorbed by an animal from ingested soil by leaching 10 g of air-dried soil with 200 ml of 0.1N HCl and determining the amounts of elements extracted by appropriate colorimetric, atomic absorption, and spectrographic techniques.

RESULTS AND DISCUSSION

The fact that grazing sheep can ingest substantial amounts of soil came to notice as a result of investigations into excessive wear of sheep incisor teeth. These studies were aimed at establishing the cause or causes of wear and the period of the year when wear is greatest, at least under New Zealand conditions; although initially only a limited number of soils were involved, the importance of soil type on the quantity of soil ingested, and hence wear, was soon evident (Healy and Ludwig, 1965; Cutress and Healy, 1965; Ludwig, Healy and Cutress, 1966).

The seasonal pattern of soil intake under New Zealand conditions, the
quantities of soil ingested, and their relation to soil type is best seen from data relating to excessive wear of incisor teeth. In Figure 1, differences in soil ingestion over a year as indicated by soil content of faeces, are shown for three farms where high, medium and low rates of wear occur. The period from about July to September is a period of low pasture growth characterised by peak soil intakes, which correlate with the degree of wear indicated by mean incisor lengths for 5 year old ewes. Soil ingestion falls to negligible levels with the growth of pasture in spring.

The soil on the high wear farm (4 ewes per acre) is a wet yellow-grey earth with a weakly developed structure and compacted B horizon. Drainage is impeded in winter, and surface casting by earthworms is prominent. The medium wear farm (4 ewes per acre) is on a stony yellow-brown loam which has moderately to strongly developed structure and is free draining. It is firmer, less prone to puddling, and with fewer earthworm casts. The low wear farm has a much lower stocking rate (1 ewe per acre) and this is partly responsible for low soil intakes. It is, however, on a steepland soil which has a well developed structure; this, combined with an organic mat formed beneath unimproved grasses and practically no surface casting, explains the negligible soil intake. On the farms described a ewe would ingest at peak periods about 250, 80, and 10 g of soil per day respectively on high, medium and low wear farms. Over the winter months when pasture growth is low and animal appetite high, a ewe would take in the bulk of the annual soil intake, which would be approximately 15 kg, 5 kg, and 0.5 kg respectively for the three farms on the soils described. Field and Purves (1964) report soil intakes of 198 g/day for animals in mid-December in Scotland, and Arnold et al. (1966) report daily soil intakes of about 300 g in Australia.
Fig. 2.—Relationship between wear of incisor teeth and ingested soil.

Fig. 3.—Effect of supplementary feeding on soil ingestion.
In Figure 2, soil content of faeces from sheep stocked 7 ewes per acre at the Te Awa hill country research area (Grasslands Division, D.S.I.R.) shows a similar high peak in the July-September period. The soil is a transitional yellow-grey earth with weakly developed structure and impeded drainage, and has abundant surface castings by earthworms in winter. Included in this figure is the rate of incisor tooth wear and it follows closely the pattern of soil ingestion.

That ingestion of soil by sheep on soil types associated with excessive rates of wear can be reduced by management is shown by the effects of supplementary feed (Healy, Cutress and Michie, 1967). It is shown in Figure 3 that by supplying supplementary feed in the July-September period on high wear farms, peak levels of soil in faeces were reduced by half and tooth wear for this period reduced by two-thirds as compared with controls. Wear for the whole year was reduced by about half.

Influence of Soil Type on Soil Ingestion

The effects of soil type on soil ingestion were investigated further by extending the study to a number of soils important in the sheep industry, in each case intensively farmed units being chosen. On all farms, sheep ingested some soil in the winter months but the amounts of soil eaten, and the period over which soil ingestion was appreciable, was related to soil type.

In Table 1 the peak soil levels in faeces samples are presented in relation to soil group and structure, since structural properties of topsoils influence puddling, drainage and earthworm casting. Projection of these data enables a broad prediction to be made in regard to New Zealand soils. It is expected that yellow-brown loams, yellow-brown pumice soils, yellow-brown earths, red and brown loams, and brown granular clays will be associated with low rates of soil ingestion; yellow-grey earths, recent soils from silty alluvium, podzolised yellow-brown earths, and podzols, with high rates of soil ingestion.

It should be emphasised that stocking rates and management affect amounts of soil ingested and are superimposed on basic soil properties. The effects of supplementary feed have already been discussed but the effects of stocking rates can be seen for the transitional yellow-grey earths at Te Awa (Fig. 4). Both peak soil levels in faeces and the duration over which soil ingestion is substantial (say over 15%) vary directly with stocking rate. Doubling the stocking rate from 3 to 6.5 ewes per acre probably increases soil intake per ewe by about 3 times.

Soil types with well developed structural properties can in general carry very high stocking intensities provided soil fertility is kept sufficiently high. On such soils ingestion is usually low but if stock numbers are increased above a certain point it would appear that soil ingestion can increase suddenly to a high level. In Table 2, soil content of faeces for May and June are given for a stocking rate study on a yellow-brown loam at Ruakura Animal Research Centre. This is a soil with a well developed structure usually characterised by low soil ingestion. The sharp break in
<table>
<thead>
<tr>
<th>Soil Group</th>
<th>District</th>
<th>Physical Properties</th>
<th>Peak soil in faeces % D.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow-brown earth</td>
<td>N. Auckland</td>
<td>Strongly developed structure</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Southland</td>
<td>Freely to imperfectly drained</td>
<td>30</td>
</tr>
<tr>
<td>Yellow-brown loam</td>
<td>Waikato</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Wairarapa</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Yellow-brown pumice soil</td>
<td>Taupo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Yellow-grey earth</td>
<td>Wairarapa</td>
<td>Weakly developed structure</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Manawatu</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Canterbury</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Podzol</td>
<td>N. Auckland</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Recent (from silty alluvium)</td>
<td>Manawatu</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Canterbury</td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>
soil ingestion for a comparatively small increase in stocking rate from 14·3 to 16·1 dry ewes per acre is striking. At this point available dry

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Soil content of faeces in relation to stocking rates on a yellow-brown loam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry ewes per ac.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>21·5</td>
<td>18</td>
</tr>
<tr>
<td>17·9</td>
<td>22</td>
</tr>
<tr>
<td>16·1</td>
<td>23</td>
</tr>
<tr>
<td>14·3</td>
<td>0</td>
</tr>
<tr>
<td>12·5</td>
<td>2</td>
</tr>
<tr>
<td>10·7</td>
<td>0</td>
</tr>
<tr>
<td>3·6</td>
<td>0</td>
</tr>
</tbody>
</table>

matter also falls sharply but it is suggested that the sudden rise in soil ingestion may also be the result of a sudden deterioration in structure in the upper part of the profile.
Possible Effects of Soil Ingestion on Animal Health

(i) Microelement intake

At peak periods grazing sheep can take in as much as 300 to 400 g of soil per day, so that perhaps 25 kilograms can be ingested over the winter months when pasture growth is low and animal appetite high. At this time it is possible that animals absorb more of certain elements from soil ingested than from pasture consumed.

On the soils selected for the study of ingestion in relation to soil type, the opportunity was taken to make some estimate of the amounts of various elements that might be absorbed by an animal from ingested soil. This was done by leaching soil with 0.1N HCl. This is a very simple system compared with the varied complexing compounds and hydrogen ion concentrations present in the alimentary system of an animal, so the estimate is necessarily a crude one. It is unlikely that any extraction system in the laboratory can hope to reproduce such conditions, but the simple system used here forms some basis for comparison of soils and their contribution to element uptake by animals. In the second column of Table 3 the ranges of elements extracted from the soils under study are given and it can be seen that amounts extracted vary from 5 fold to 50 fold.

In columns 3 and 4 of Table 3 a comparison is made between the

<table>
<thead>
<tr>
<th>Elements</th>
<th>Range extracted from soils mg per 100 g soil</th>
<th>Possible winter uptake of elements by sheep mg per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From Soil</td>
<td>From Pasture*</td>
</tr>
<tr>
<td>Ca</td>
<td>150 — 500</td>
<td>1500</td>
</tr>
<tr>
<td>Mg</td>
<td>10 — 40</td>
<td>120</td>
</tr>
<tr>
<td>P</td>
<td>5 — 120</td>
<td>360</td>
</tr>
<tr>
<td>Mn</td>
<td>1 — 60</td>
<td>180</td>
</tr>
<tr>
<td>Zn</td>
<td>1 — 5</td>
<td>15</td>
</tr>
<tr>
<td>Fe</td>
<td>3 — 35</td>
<td>105</td>
</tr>
<tr>
<td>Cu</td>
<td>0·2 — 1</td>
<td>3</td>
</tr>
<tr>
<td>Mo</td>
<td>0·001 — 0·1</td>
<td>0·3</td>
</tr>
<tr>
<td>Co</td>
<td>0·01 — 1</td>
<td>3</td>
</tr>
<tr>
<td>Ba</td>
<td>1 — 5</td>
<td>15</td>
</tr>
<tr>
<td>Sr</td>
<td>0·1 — 2·5</td>
<td>7·5</td>
</tr>
<tr>
<td>Cr</td>
<td>0·01 — 1</td>
<td>3</td>
</tr>
<tr>
<td>Ni</td>
<td>0·05 — 0·5</td>
<td>1·5</td>
</tr>
<tr>
<td>Se</td>
<td>n.d.</td>
<td>0·05 (estd.)</td>
</tr>
</tbody>
</table>

* Pasture analysis: Ca 0·7%, Mg 0·3%, P 0·5%

Mn 100 ppm; Zn 40 ppm; Fe 250 ppm; Cu 8 ppm; Mo 1 ppm;
Ba 4·0 ppm; Sr 40 ppm; Ni 1 ppm; Cr 1 ppm; Se 0·1 ppm.
amounts of various elements that might be absorbed from ingested soil and from pasture. It has been assumed that 300 g of soil is ingested by the animal per day and highest extraction values for each element in Table 3 are used. A daily intake of 500 g of pasture dry matter of given composition is assumed, and for calculation purposes it is assumed that all elements in the pasture are completely absorbed by the animal. On this basis Mg and P contributions from soil are small, but Ca contribution approaches 50 per cent of that from pasture. The microelement contribution from soil is however much more substantial and in most cases is comparable to pasture contribution; for Mn and Co it is substantially more.

In regard to ingested soil as a source of microelements it is interesting to look at early work on cobalt deficiency in sheep. Rigg and Askew (1934) used soil drenches to correct this condition, and obtained dramatic results with drenches supplying less than 20 g of soil per week. With daily intakes of 300 g of soil per day a weekly total of 2 kilograms would be reached—over 100 times the amount used by Rigg and Askew.

On soils where mineral deficiencies in animals occur or are suspected, the picture is usually one of varying incidence, with animals on one farm showing symptoms while those on an adjacent farm do not. Variation in amounts of soil ingested due to differences in stocking rate and management may be responsible for this scatter.

(ii) Insecticides intake

In general, precautions in regard to insecticides are concerned with ensuring that animals are not permitted to graze treated pastures in the immediate period following application.

The fact that insecticides can remain concentrated in surface horizons and that sheep can ingest this soil suggests that the winter period could be a period of potential insecticide uptake. It does not seem likely that soil ingestion is a major source of D.D.T. intake for grazing animals although it probably contributes to the low background level usually found in animal fat. The persistence of D.D.T. residues in upper horizons probably means that animals are exposed to the risk of D.D.T. for a much greater period than realised, and this risk may be high at periods of peak soil ingestion. Soil ingestion could also be a factor in the buildup of so-called zero-tolerance insecticides such as dieldrin and aldrin.

REFERENCES


Under New Zealand conditions grazing sheep can ingest substantial amounts of soil over the winter period when pasture growth is low and animal appetite high. Soil type is of basic importance in determining quantities of soil taken in; soils characterised by strong structure are associated with low levels of soil ingestion, while those with weak structure are associated with high levels of soil ingestion. Stocking intensity and management also affect soil ingestion and are superimposed on the effects of soil type.

Seasonal variations in soil ingestion are discussed in relation to excessive wear of sheep incisor teeth and the effects of ingested soil on other aspects of animal health such as microelement intake and possible intake of insecticides are also discussed.
THE EFFECT OF CULTURAL TECHNIQUES ON THE GROWTH OF PINUS ELLIOTTII ENGELM., NEAR JERVIS BAY, NEW SOUTH WALES

H. D. WARING

Forest Research Institute, Canberra, Australia

The Commonwealth of Australia, through the Department of the Interior, is responsible for forestry operations in an area of Commonwealth Territory near Jervis Bay (35° 10' S. 150° 40' E.). Since 1956 it has been active here in the establishment of plantations of introduced pines, and at present 473 acres have been planted.

The average annual rainfall (for the period 1900-1944) is 45.0 in. with 132 wet days per year and with a slight winter maximum. The climate is mild with an average maximum temperature in January of 74.5°F and an average minimum in July of 48.6°F.

A survey carried out by the writer shows that the Territory consists of about 35% Upper Marine Series (Nowra Grit) and about 65% Recent material (sands of oceanic origin). In the past, conditions have been optimum for the transport of sand by wind and vast quantities originally deposited by water have been moved distances up to a mile and piled, usually irregularly but sometimes into regular seif dunes. Currently these dunes are fixed. The topography therefore is hilly, consisting largely of sand dunes of variable depth over a sandstone basement. The sands carry a sclerophyll forest dominated by Eucalyptus pilularis Sm. up to 100 ft high in the protected corridors and 65-70 ft high on the exposed dune crests.

On this dune system the soils consist of Aeolian Sands, Podzols, Groundwater Podzols, and Acid Swamp Soils (Stephens, 1953). In locations where the sand parent material is of the same age, soil changes can be correlated with variation in the soil water regime, which ranges from very dry on high dunes and exposed situations (Aeolian Sands) to very wet swampy areas (Acid Swamp Soils). The best commercial hardwood stands are found on the soils occupying the middle of the range on Groundwater Podzols and the wetter Podzols where the stands are well protected from the winds.

**EXPERIMENTAL**

Investigations into the nutrition of several Pinus species on the deep sands of the Commonwealth Territory commenced in 1956 and are continuing. Results show that growth is limited by—

(i) deficiencies of N, P and K;

(ii) periodic acute shortages of soil moisture in the surface three feet of sand due to the low water retaining capacity and also to the vigorous growth of competing shrubs and herbs (Waring, unpublished).

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In 1959 and 1960 an experiment was established to test the effect of cultivation and competing vegetation on the survival and subsequent growth of *P. elliottii*. Four cultural pre-planting treatments ("cultivated" and "not cultivated", with and without a planting furrow) were combined with three degrees of competition control (to be applied for 2 years subsequent to planting). Two replications were established each year. In addition an aluminium foil mulch was tested on the two "not cultivated" treatments in a $2 \times 3 \times 2$ design. The species used was *P. elliottii* at a spacing of 6 ft x 6 ft. Plot size was 1 chain x 1 chain and 63 trees per plot were measured. To ensure that responses would not be influenced by known mineral deficiencies a basal dressing of John Innes Base fertilizer (5-9-11) was applied at a rate of half a ton per acre per year for the first three years.

The planting furrow was formed by disc ploughing (one run every six ft) and resulted in a furrow 16 to 18 in. wide at the top and 12 in. deep. Cultivated plots were disc ploughed completely and then disc harrowed.

The experiment occupies 7.2 acres (net) in a 12-15 acre area surrounded by blackbutt (*E. pilularis*) forest.

The 1959 establishment is designated Compartment 6 and the 1960 portion is Compartment 10. A typical profile of a site in the middle elevation shows grey surface sand grading to a bleached A$_2$ horizon at 15 in. and a D horizon (2-4 ft) of yellowish-brown sand stained and sometimes weakly cemented by iron and organic matter. Below this, there is light yellow sand parent material to depths of up to 75 ft. The surface soil (0-3 in.) has a pH (1:1) of 4.80, total soil nitrogen 0.05%, $P_2O_5$ (concentrated HCl extract) 47 p.p.m. and consists of 91.5% coarse sand and 2.0% fine sand.

The effect of aluminium foil on growth was not measurable owing to late and incomplete application of the treatment. The degrees of competition control were incompletely applied and should perhaps be considered as "no competition control" and "slight competition control".

During the year 1960-61 the water-table rose in some of the lowest sites killing all trees in seven plots of Compartment 6 and in one plot of Compartment 10.

Height was measured from 1959 to 1962 inclusive and both height and diameter in 1966. In July 1966 the volume of timber produced (under bark) was estimated using a volume line obtained by sectional measurements of 40 sample trees in each of the Compartments. The 80 sample trees were chosen at random from 1 in. diameter classes. The number chosen in each class was proportional to the expected variance in volume in that class weighted to take into account the greater amount of work in measuring the largest trees.

Surface soil (0-3 in.) was sampled from all plots in May 1967 and total nitrogen estimated by standard Kjeldahl methods. Five sub-samples were bulked to obtain each plot sample.

Needles were sampled from all plots of the treatments which showed significant growth responses and total $N$ and $P$ determined on a Kjeldahl digestion (Snowdon, 1967). Needles were sampled from just below the
growing points on four trees. These were analysed separately and a plot mean obtained.

**RESULTS AND DISCUSSION**

Owing to the very high rainfall for several years after establishment, the treatments had no significant effect on survival. These conditions combined with the basal application of fertilizer produced an even, vigorous stand of healthy trees.

Tables 1 and 2 show that:

(i) complete cultivation increased volume production by up to 33%;
(ii) furrow planting had a similar effect on non-cultivated land. This is important because the provision of a planting furrow is much simpler in forestry practice than is complete cultivation;
(iii) a planting furrow in addition to cultivation did not result in any increase in production over cultivation alone;
(iv) limited restraint of weed competition during the first two years after planting increased volume by 11-16%.

In the nutrition experiments reported by Waring (1963, 1967) cultural treatments were held constant and the response measured after adding mineral nutrients. In the experiment reported in this paper a response has been obtained by varying cultural treatments after ensuring

---

**TABLE 1**

VOLUME PRODUCTION UNDER BARK (CU. FT. PER PLOT) AT JULY, 1966

*P. elliottii* Jervis Bay Cultivation Experiment; Compartments 6 planted 1959, Compartments 10 planted 1960

<table>
<thead>
<tr>
<th></th>
<th>No Furrow</th>
<th>Furrow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compt. 6</td>
<td>Compt. 10</td>
</tr>
<tr>
<td>Not Cultivated</td>
<td>33.09</td>
<td>23.68</td>
</tr>
<tr>
<td>Cultivated</td>
<td>43.51*</td>
<td>29.65†</td>
</tr>
</tbody>
</table>

* Increase significant at 5%.
† Increase significant at 1%.

**TABLE 2**

THE EFFECT OF COMPETITION CONTROL ON VOLUME UNDER BARK (CU. FT. PER PLOT) JULY, 1966

*P. elliottii* Jervis Bay Cultivation Experiment

<table>
<thead>
<tr>
<th></th>
<th>Compt. 6</th>
<th>Compt. 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No competition control</td>
<td>37.08</td>
<td>24.02</td>
</tr>
<tr>
<td>Medium competition control</td>
<td>40.96</td>
<td>25.57</td>
</tr>
<tr>
<td>Heavy competition control</td>
<td>41.29*</td>
<td>27.93*</td>
</tr>
</tbody>
</table>

* Increase significant at 5%.
that mineral nutrients were in adequate supply. It was previously reported (Waring, 1967) that growth responses resulting from fertilizer applied at time of planting take place in the early years while the tree is relatively free of competition. There is now evidence (Table 3) that the same is true of a growth response resulting from a cultural treatment. This is an interesting confirmation of early response and indicates that any factor which stimulates growth will have the maximum effect while the tree is free of competition.

| Table 3 |
|-----------------
| **HEIGHT INCREMENTS (IN. PER TREE) 1960-61** |
| **P. elliottii Jervis Bay Cultivation Experiment** |
| | No Furrow | Furrow |
| | Compt. 6 | Compt. 10 | Compt. 6 | Compt. 10 |
| Not Cultivated | 22·55 | 8·15 | 27·40 | 8·35 |
| Cultivated | 27·67 | 8·60 | 30·11 | 10·24 |

Table 3 shows that by the end of the second year after planting the height increment response is very similar in pattern and magnitude to the volume response found five years later (see Table 1).

The results indicate that a planting furrow and complete cultivation create equivalent conditions in the immediate environment of the plant and allow a similar early response. This could be due to—

(i) relief from competition for nutrients and moisture and/or
(ii) sufficient cultivation in both cases to allow an effective fertilizer x cultivation interaction to operate during the early period.

It is considered that the interaction is of primary interest since this has been found to be of great importance by Richards (1961) for P. taeda L. and by the writer for P. radiata D. Don (Waring, unpublished).

Excavation of trees at the end of the second year after planting showed that roots of furrow-planted trees tend to follow along the bottom of the

| Table 4 |
|---------------------
| **TOTAL SOIL N IN 0-3 IN. DEPTH, JERVIS BAY CULTIVATION EXPERIMENT, MAY 1967** |
| Data for all plots and surrounding virgin forest |
| | No Furrow | Furrow |
| Not Cultivated | % | .036 | % | .032 |
| Cultivated | .031 | .039 |

Mean of all plots .033* Cultivated .034.  
Not Cultivated .033.  
Mean of 8 samples from surrounding virgin eucalypt forest .044*.  
* Difference significant at 1%.
<table>
<thead>
<tr>
<th>Competition Control</th>
<th>Not Cultivated</th>
<th>Furrow</th>
<th>Cultivation + Furrow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compt. 6</td>
<td>Compt. 10</td>
<td>Compt. 6</td>
</tr>
<tr>
<td>% N</td>
<td>% P</td>
<td>% N</td>
<td>% P</td>
</tr>
<tr>
<td>Nil</td>
<td>1.05</td>
<td>0.155</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>1.14</td>
<td>0.147</td>
<td>0.96</td>
</tr>
<tr>
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<tr>
<td></td>
<td>1.06</td>
<td>0.175</td>
<td>1.18</td>
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<tr>
<td>Heavy</td>
<td>1.02</td>
<td>0.129</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>1.03</td>
<td>0.163</td>
<td>1.02</td>
</tr>
<tr>
<td>Mean</td>
<td>1.03</td>
<td>0.152</td>
<td>1.04</td>
</tr>
</tbody>
</table>

**Table 5**

furrow, while both "furrow not cultivated" and "complete cultivation" produced a better-developed root system than "not cultivated" treatment.

Although the treatment "competition control" was poorly applied, Table 2 shows that even slight control produced a significant response.

Originally "heavy" was meant to be maintained as a bare surface while "medium" was meant to be cleared once per year. However, both of these treatments received one clearing only and that in some cases during the second year.

No trends of any significance could be shown from the analyses of total soil nitrogen (refer Table 4). All plots within the experiment are low in nitrogen with no difference between "cultivated" and "not cultivated" plots. Eight bulked samples representing the surrounding virgin eucalypt forest are 33% higher in total nitrogen indicating a drain on soil nitrogen reserves similar in magnitude to that reported by Hatch (personal communication) for P. pinaster Ait. on comparable soils in Western Australia.

It is of interest that one complete cultivation before planting has not accelerated this loss as was found by Van Goor (1952).

Table 5 shows no significant trends in needle content of N and P. The N contents are unusually even at an average level, while P contents are consistently high (Walker and Youngberg, 1962; Baur, 1959).

The results show that after adding NPK fertilizer to the deep sands of Jervis Bay, a further substantial production increase can be obtained by two relatively inexpensive establishment techniques. These are—

(i) provision of a planting furrow along each row, and
(ii) control of competition during the first two years after planting.

ACKNOWLEDGMENTS

The establishment and maintenance of the experiment has been a co-operative undertaking with the Forests Section, Lands Branch, Department of the Interior. Chemical analyses were carried out by Mr. P. Snowdon and field work was supervised by Mr. A. G. Eilert.

Mr. G. A. McIntyre, Division of Mathematical Statistics, C.S.I.R.O., advised on the design and analysis of experiments.

REFERENCES


SUMMARY

A brief outline is given of the climate, vegetation, geology and soils of the deep sand areas of the Commonwealth Territory, Jervis Bay, New South Wales.

A factorial field experiment established in 1959-60 testing 4 cultural treatments and 3 levels of competition control showed that by 1966:

(1) Furrow planting on non-cultivated land increased volume production by up to 33%.
(2) Complete cultivation had a similar effect.
(3) A planting furrow in addition to cultivation did not increase the effect due to cultivation alone.
(4) Limited restraint of weed competition during the first two years increased volume by up to 16%.

Height measurements in 1961 foreshadowed the 7 year result and it is suggested that a planting furrow or complete cultivation supplies an environment which provides sufficient of a cultivation x fertilizer interaction to stimulate the tree during the important early period of free growth.

Analyses of total nitrogen in surface soil indicates a 25% reduction as a result of the change from eucalypt to pine forest. There were no significant differences in the effects of the various cultural treatments on this reduction in total nitrogen.

RÉSUMÉ

On présente un aperçu du climat, de la végétation, de la géologie et des sols des zones à sables profonds du territoire du Commonwealth, Jervis Bay, New South Wales, Australie.

Un essai au champ établi en 1959-60, pour éprouver 4 traitements culturels et 3 niveaux de contrôle de compétition a démontré que, en 1966 :

(1) La plantation en sillons a augmenté de 33 % la production de volume.
(2) L'effet de culture complète était le même.
(3) Un sillon de plantation ajouté à la culture n'augmentait pas l'effet résultant de la culture seule.
(4) Une restriction limitée de la concurrence des mauvaises herbes pendant les deux premières années a augmenté le volume jusqu'à 16 %.

Les mesures de hauteur en 1961 anticipèrent le résultat de la 7ème année, et on opine qu'un sillon de plantation ou une culture complète offre un milieu qui fournit une interaction de culture x engrais suffisante pour stimuler l'arbre pendant la première période importante de croissance libre.

Des analyses de l'azote total dans le sol superficiel indiquent une réduction de 25 % à la suite d'un changement de la forêt d'eucalyptus à celle de pins. Il n'y avait pas de différences importantes dans les effets des divers traitements culturels sur cette réduction de l'azote total.
Eine kurze Beschreibung von Klima, Vegetation, Geologie und Böden der tiefen Sand-Gegenden des Commonwealth Territory, Jervis Bay, New South Wales, wird gegeben.

Ein Feldexperiment angelegt in 1959-60 zur Prüfung von 4 Kulturbehandlungen und 3 Stufen der Konkurrenzkontrolle zeigte im Jahr 1966:
1) Furchen-Pflanzung erhöhte die Produktions-Masse, bis zu 33 Prozent.
2) Vollkommene Kultivierung hatte ähnliche Erfolge.
3) Eine zuzügliche Pflanzungsfurche zur Kultivierung erhöhte nicht den Erfolg der Kultivierung.
4) Beschränkte Zurückhaltung der Unkraut-Konkurrenz während der ersten zwei Jahre erhöhte das Volumen bis zu 16 Prozent.

Höhenbemessungen in 1961 sagten das 7 jährige Resultat voraus und es ergibt sich, dass eine Pflanzungsfurche oder vollkommene Kultivierung eine Umgebung erzielt, die genügend dazu beiträgt, eine Kultivierung x Dünger-Wechselwirkung zur Stimulierung des freien Wuchses während der wichtigsten frühen Periode des Baumes zu ermöglichen.

MICROBIAL STIMULATION AND INHIBITION OF PLANT GROWTH

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Forschungsanstalt für Landwirtschaft, Braunschweig—Völkenrode, Germany

I. INTRODUCTION

Principally there are two possible and practicable ways to investigate relations between higher plants and soil micro-organisms: the study of relatively simple models (with increasing complexity), and in situ observations under defined conditions. The first approach yields a rather confusing amount of biological data, which we finally hope to synthesize into what was recently termed “biochemical ecology of soil microorganisms” (Alexander 1964). In the second case natural phenomena are described, the importance of environmental factors is estimated, and we hope to end up with an analytical understanding of complex situations.

In general, it seems to be a rule that the strongest stimuli for the study of soil-microbe relationships did not so much originate from the natural curiosity of biologists as from the search for practical applications of single aspects of microbial activities. The significance of this type of research particularly depends on how precisely working hypotheses have been formulated and how critically conclusions have been drawn from experimental results. Earlier reviews which give a more comprehensive account of the subject but also reflect fairly well the discrepancies in soil microbiology, should be consulted for further information (Krasil’nikov 1958, Clarke 1961, Baker and Snyder 1965, Macura and Vancura 1965, Rovira 1965, Gams 1967).

II. TOPOLOGY OF THE ROOT REGION

(a) Facts and Assumptions

The soil, envisaged as the densely packed environment for all soil micro-organisms, grants survival for a great number of organisms but it is only a small number of species which break into limited stages of activity as soon as organic substrates become available. In the course of substrate decomposition, when shortlived successions of micro-organisms appear according to their nutritional requirements and enzymatic abilities, the multiplication and death rate of species should be of paramount interest. Firstly, because microbial activity is an indispensable prerequisite for any stimulating or inhibiting action, and secondly, because the turnover of generations during a given time interval is a more valuable criterion than numbers of individuals at any given moment.

It must be pointed out that the relatively short periods of microbial activity alternate with intervals of dormancy. The mere fact that soils are
inhabited by highly diversified populations of all sorts of micro-organisms very likely results from the widespread ability to survive, rather than to actively "live" in the soil. In spite of a good proportion of self criticism and constant warnings, progress in ecological soil microbiology is seriously hampered by several deep-rooted misconceptions. Here are just a few examples:

Viability or presence of an organism is taken for activity; biochemical abilities of individuals are transferred to the species level without considering microbial variability; in vitro activities of species have been interpreted as biological soil properties; in many instances even a simple biostatistical treatment of the results has been carefully avoided.

(b) Habitat and Inhabitants

It is a convenient, but not necessarily logical practice to distinguish between different areas in the root region. It must be expected rather that each area is subject to continuous change in space and time by growth and senescence of the root system.

The outer surface of the root consists of a layer of epidermal cells coated with a mucilaginous layer. Soluble organic and inorganic substances are excreted by the root, the composition of which is fairly well known (Rovira 1965). But there is considerable uncertainty as to the extent to which the root loses substances in the absence of any acute stress. Since a concentration gradient exists between vacuoles, cytoplasm, and the ambient medium, exudation is thought to be a diffusion process, which is dependent on environmental factors as well as on the physiological stage of the root.

A gradient of exudates develops from the root surface towards the surrounding soil and creates a zone of increased microbial density, the rhizosphere. This area has received considerable attention, a fact which is mainly based on the assumption that the rhizosphere is also characterized by a manifold increase of microbial activity. However, if we imagine the microflora in a non-amended, undisturbed, root-free soil in a more or less dormant stage, an increase above this ecological "zero" is less drastic than it seems to be at first glance. The common practice to express the "rhizosphere effect" in terms of R/S-ratios (root zone/soil zone) was particularly suited to emphasize "increased activity" instead of "deep dormancy".

III. PHYSIOLOGY OF THE ROOT REGION

(a) Euparasitic Relations

The activities of plant parasitic soil organisms are usually rather neglected by soil microbiologists. I should like to point out that this discipline not only has brought about a valuable amount of clearcut information about pathogens, pathogenesis, etiology etc. during the phase of descriptive plant pathology, but also in some exceptional cases has demonstrated how complex host-microbe relationships can be explored and traced to biochemical interactions at the cellular and finally at the molecular level.
(i) **Phytoalexins.** Substances with nonspecific toxicity that belong to this group are by definition formed by the host under the influence of a microbial stimulus (Cruickshank 1963). Soil fungi and root tissues are involved in two cases:

*Rhizoctonia repens* (and other fungi) induce the formation of orcinol in orchid bulbs. Or cinol has a relatively high fungitoxicity (Nüesch 1963).

*Ceratocystis* (-*Ceratostomella*) *fimbriata* (and other fungi) cause, in sweet potatoes, a disturbance of the metabolism and concurrently induce the production of ipomeamarone as the most interesting among several other substances (Uritani et al. 1960).

(ii) **Toxins.** A selective, host-specific toxin with an extreme effectiveness for susceptible cultivars is formed by *Helminthosporium victoriae*, a pathogen on oats. Chemical characterization indicates a peptide structure for the complete toxin (Pringle and Scheffer 1964).

Another host-specific polypeptide with high activity against susceptible sorghum seedlings is produced by the root pathogen *Periconia circinata*, the causal agent of "Milo disease" (Pringle and Scheffer 1963). In both instances malfunction of the host physiology is the general effect of the toxins. At the present time we have an impressive collection of data based on comparative studies, but we are not yet in a position to relate all these data to each other and to trace a continuous reaction chain from the primary chemical action of the toxin to the final expression of the disease symptoms. Nevertheless there are some excellent opportunities in this field, and work that has just started with chemically defined toxins will enhance research to a great extent.

Some Fusaria produce gibberellic acid, well known for its growth-promoting effects on higher plants. A similar action is ascribed to *Helminthosporium sativum* with helminthosporol as the active substance (Kato et al., 1964). To the extent that these metabolites, reaching the root externally and in excess, cause a disturbance of the normal host growth (cf. "bakanae disease" of rice plants) they should be regarded as toxic agents.

An interesting case of "long-distance-action" of volatile toxins has been established by some soil fungi, which have developed a low degree of parasitism. *Marasmius graminum, M. oreades,* and a low temperature fungus of uncertain position, produce HCN, thereby causing the death of higher plants prior to colonisation (Lebeau and Dickson 1955, Lebeau and Hawn 1963, Ylimäki 1967).

(b) **Hemiparasitic Relations**

The demarcation line between parasites and saprophytes becomes increasingly indistinct with intensified study of host-parasitic relationships. This is particularly typical for hemiparasitic organisms, the negative influences of which consist mainly in the production of nonspecific toxic metabolites.

The following dysfunctions have been ascribed to the action of hemiparasites: inhibition of seed germination; stunted growth; root necrosis.
particularly of root tips; decrease in root length and root weight; suppression of root hair formation and decreased root respiration. Lack of knowledge has led to the introduction of terms like “soil sickness”, “soil fatigue” and “plant incompatibility” to circumscribe symptoms of poorly understood origin. The modes of action are, in general terms, metabolic changes in the host. This can result in direct root injuries or in a predisposition of host tissues to a subsequent parasitic attack.

Some results from related experiments (Domsch and Gams, unpublished) are given in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>FUNGI WITH NEGATIVE INFLUENCE ON THE ROOT DRY WEIGHT OF SEEDLINGS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungus</td>
<td>Wheat (±)</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>Emericellopsis minima</td>
<td>57·5 ± 4·0</td>
</tr>
<tr>
<td>Gliocladium catenulatum</td>
<td>63·9 ± 2·8</td>
</tr>
<tr>
<td>Phoma exigua</td>
<td>74·6 ± 3·1</td>
</tr>
<tr>
<td>Gliomastix guttuliformis</td>
<td>63·0 ± 1·7</td>
</tr>
<tr>
<td>Aureobasidium bolleyi</td>
<td>77·7 ± 2·0</td>
</tr>
<tr>
<td>L.S.D. (P = 0·01)</td>
<td>11·8</td>
</tr>
<tr>
<td>(P = 0·05)</td>
<td>8·4</td>
</tr>
<tr>
<td>(P = 0·1)</td>
<td>7·0</td>
</tr>
</tbody>
</table>

* Data are averages from 5 different isolates of each fungus in 10-fold replications. They are expressed as percentages of non-inoculated controls (with standard deviations).

The biochemical nature of the substance(s) or process involved in the considerable reduction of root growth is not yet clear. It is not unlikely that fungi like Emericellopsis minima and Gliocladium roseum produce substances of the cephalosporin and gliotoxin type, respectively. We know of a number of antibiotic substances that are highly phytotoxic (chloromycetin, gliotoxin, griseofulvin, patulin) and some of them are stable enough to occur in the soil as long as they are continuously produced.

An excellent example of connected and logical work on the soil sickness problem in apple nurseries was given by Börner (1959, 1961, 1964), including critical analysis of the possible role of toxic plant constituents (phlorizin), apple root residues as suitable substrate for the formation of patulin by Penicillium expansum, and determination of the phytotoxic properties of patulin. But it is still beyond any doubt that neither one single producer nor one single product can be responsible for any particular soil sickness phenomenon.

There are some hemiparasitic organisms which are not known to produce physiologically active substances. Other modes of action have to be looked for in these cases. Theoretical considerations include the following possibilities:

Some sugars (deoxygalactose, deoxyglucose, mannose, glucosamine) are known to be toxic to roots (Malca and Endo 1965, Malca, Endo and Long 1967). Galactose is an inhibitor of cell extension; it prevents the synthesis of cell wall polysaccharides (Ferguson et al. 1958). A partial
enrichment in the soil is not unlikely, since most of the soil micro-organisms do not freely utilize these sugars, the presence of which has been partially demonstrated in soil extracts.

In this context it should be mentioned that the normal course of protein synthesis can be severely disturbed by amino acids (L-methionine, DL-valine, L-norleucine, hydroxy-L-proline and others) after external application to the root (Street et al. 1960). Amino acids can occur in considerable quantities in the rhizosphere and they have been shown to interfere in several root-parasite complexes (van Andel 1966).

(c) Symbiotic Relations

While relatively primitive relations of the necrobiosis-type have dominated the foregoing, we find a highly developed symbiosis between roots and micro-organisms in mycorrhiza and in the Rhizobium associations of legumes.

(i) Mycorrhiza. The three different types of mycorrhiza are characterized by the relatively long duration and stability of the association and in most cases by the specificity of the organisms involved. Some microbial partners belong to commonly euparasitic genera (Rhizoctonia, Pythium, Armillaria); for unknown reasons they lose their pathogenicity in the course of mycorrhiza formation.

The basic effects of the ectotrophic mycorrhiza are mediated by the pseudoparenchymatic fungal layer between root and soil. This layer increases the total absorbing surface and controls uptake, storage and gradual utilization of ions (alkali metals, phosphates). Experiments with \(^{14}\text{CO}_2\) and labelled sugars proved that the fungus is supplied by the root with carbohydrates (Melin 1963, Harley 1965).

(ii) Rhizobial symbiosis. According to Nutman (1965) and others the following phases can be distinguished. The Rhizobium population in the rhizosphere increases to a density high above the numbers necessary for a successful infection. The first visible symptom is a curling reaction of the root hairs with circumstantial evidence that IAA plus a co-factor are involved. Bacterial polysaccharides induce the formation of polygalacturonase by the host root; the latter event might possibly be one mechanism on which host specificity is based.

(d) Probiotic Relations

Besides the organisms participating in well described and defined associations, some free living bacteria, actinomycetes and fungi are claimed to stimulate plant growth. But it might be worthwhile to consider the following points before entering the field:—

A distinction has to be made between experiments in soil and in artificial culture, also between stimulation of seedlings and measurable effects at the time of harvest.

Experimental proof has to be presented that either the presumptively stimulating growth substances are produced in insufficient amounts by the roots themselves, or that other essential growth processes are involved.

An evaluation of the experimental procedures and the consideration
of possible effects of growth substances lead to the following conclusions:

Most of the stimulations have been demonstrated in model systems. Plants in the seedling stage have been used in most experiments. It is known that seedlings make extensive use of the reserve materials of the endosperm during the first 1 or 2 weeks of growth. The activation and de novo synthesis of aleuronal enzymes is one of the characteristic features of gibberellin (Paleg 1965), which is produced by several fungi. It should be expected that gibberellin would cause an increase in shoot weight, provided it is one of the triggering substances and acting in the seedling stage. Stimulation of the shoot is indeed a widely observed effect (Domsch and Gams, unpublished) exerted by potentially stimulating fungi.

Table 2

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Wheat</th>
<th>Peas</th>
<th>Rape</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sporormia aemulans</em></td>
<td>111.6 ± 3.1</td>
<td>111.8 ± 4.2</td>
<td>102.3 ± 0.8</td>
</tr>
<tr>
<td><em>Verticillium lateritium</em></td>
<td>103.7 ± 1.0</td>
<td>120.8 ± 9.7</td>
<td>101.6 ± 0.3</td>
</tr>
<tr>
<td><em>Cylindrocarpon olidum</em></td>
<td>194.8 ± 2.0</td>
<td>106.9 ± 6.9</td>
<td>108.7 ± 3.1</td>
</tr>
<tr>
<td><em>Plectosphaerella cucumeris</em></td>
<td>94.2 ± 3.3</td>
<td>102.0 ± 4.8</td>
<td>107.0 ± 5.2</td>
</tr>
<tr>
<td><em>Trichoderma apicuum</em></td>
<td>92.4 ± 0.7</td>
<td>105.0 ± 7.0</td>
<td>103.7 ± 0.3</td>
</tr>
<tr>
<td>L.S.D. (P ≤ 0.01)</td>
<td>9.8</td>
<td>19.5</td>
<td>8.4</td>
</tr>
<tr>
<td>(P ≤ 0.05)</td>
<td>7.4</td>
<td>14.8</td>
<td>6.4</td>
</tr>
<tr>
<td>(P ≤ 0.1)</td>
<td>6.2</td>
<td>12.4</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*Data are averages from 5 different isolates of each fungus in 10-fold replications. They are expressed as percentages of non-inoculated controls (with standard deviations).

The data presented in Table 2 are the result of tests with about 300 different species of soil fungi. By comparison with Table 1 it becomes obvious that the recognition of stimulatory effects is much more difficult than the proof of gross inhibition. This is particularly true if calculations are made on a dry weight basis. In our experience underestimations of the control data are among the main errors leading to an incorrect indication of stimulation.

If we consider the main function of growth substances (and co-factors) in the root to be the initiation of root primordia and lateral roots, there seems to be no evidence that roots suffer from a suboptimal level of growth substances and that they have to rely on the microbial activity in the ambient medium for additional supply. It also is a widely accepted fact that the gibberellin effects are not of long duration and have no significant influence on the crop yield (Stuart and Cathey 1961).

Another hypothetical aspect of direct beneficial activities of soil microbes is the production of antibiotics, which are believed to exert a systemic influence on plant pathogens. Again, we know that separate processes of a whole chain are operating (production, uptake, translocation, fungitoxicity, stability), but how they work under natural
conditions remains to be demonstrated. Any attempt made towards an enrichment of the soil with antibiotic-producing micro-organisms should recognize the fact that overproduction of physiologically active substances can reverse the beneficial effects.

IV. COMPLEX ACTIONS IN THE ROOT REGION

The discussion of the interactions between plant roots and soil micro-organisms so far has concentrated on direct relations. It should be mentioned that a number of ecological reaction chains might have considerably more influence on the development of higher plants than the more spectacular direct effects.

Mycelial growth of soil fungi (Griffiths 1965) and polysaccharide production of soil bacteria bind together soil particles, thereby increasing the number of water-stable soil aggregates and facilitating gas exchange of arable soils.

During the growth of pigmented soil fungi numerous phenolic substances are produced and subsequently oxidized to coloured polymers with humus-like properties (Haider and Schetters 1967). More frequently discussed is the incomplete microbial decomposition of organic materials as one of the main sources of humus formation.

The metabolic activity of the first colonizers on fresh substrates can result in the production of organic acids as intermediate metabolites, which hold a key position insofar as they facilitate the solution and transport of minerals. On the other hand they may act as powerful chelating agents rendering essential elements unavailable for immediate use.

Further, the activity of chemoautotrophic microbes results in a net increase in certain elements (carbon, sulphur) which are fixed from gaseous compounds. N-binding organisms are merely mentioned in this context for the sake of completeness, without further discussion.

The excretion of substances of high nutritional value by the root leads to an enrichment of organisms with a high competitive saprophytic ability (Garrett 1956). Consequently organisms (like most soil pathogens) which are lacking this ability are excluded from this region. Here we might very well have a mechanism by which the rhizosphere acts as a “shield of protection” against pathogens.

Within certain limits the soil biophase can be manipulated by amendments, green manure, foliar application of various substances, or by seed or soil inoculation. Each one of these practices causes more or less pronounced changes in germ densities in the microbial habitat. The stability of the newly induced situation determines the degree to which the higher plant will finally be influenced.

It seems that the relations between higher plants and soil micro-organisms have been studied more intensely during the last ten years than ever before. One also gets the impression that the rate of progress and the rate of discouragement are still of the same magnitude as in previous decades. But there is a remarkable tendency to replace the merely
descriptive approach to soil biological phenomena by more basic research in defined systems.

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Uritani, I., Uritani, M., and Yamada, H. (1960)—*Phytopathology* 50, 30-34.

SUMMARY

The influences of soil micro-organisms on the development of higher plants are reviewed under plant physiological, phytopathological, and microbiological aspects.

The root region is described as an area of intensive ecological and...
biochemical interrelationships and as the particular habitat of soil microbes.

Some of the basic concepts of direct microbial stimulations and inhibitions are listed with emphasis on euparasitic, hemiparasitic, symbiotic and probiotic relations.

Indirect microbial influences, representing the effects on soil structure, humus formation, nutritional status of the soil, and the biophase are briefly discussed.

RéSUMÉ

Les influences des microbes du sol sur le développement du végétal supérieur sont présentées sous les aspects physiologique, pathologique et microbiologique.

La région radiculaire est caractérisée comme l'endroit d'échanges écologiques et biochimiques intensifiés, et l'habitat particulier des microbes des sols.

Les bases matérielles des stimulations et inhibitions directes des microbes sont discutées et illustrées par des exemples de relations euparasitiques, hémiparasitiques, symbiotiques et probiotiques.

Parmi les influences indirectes microbiennes les actions sur la structure du sol, formation d'humus, conditions nutritives et biophase sont brièvement mentionnées.

ZUSAMMENFASSUNG

Die Einflüsse von Bodenmikroorganismen auf die Entwicklung höherer Pflanzen werden unter pflanzenphysiologischen, pflanzenpathologischen und mikrobiologischen Gesichtspunkten dargestellt.

Die Wurzelregion wird als Ort intensiver ökologischer und biochemischer Wechselbeziehungen und besonderer mikrobieller Habitate gekennzeichnet.

Mit Beispielen aus euparasitischen, hemiparasitischen, symbiotischen und probiotischen Beziehungen werden die stofflichen Grundlagen direkter mikrobieller Förder- und Hemmwirkungen diskutiert.

Aus der Gruppe der indirekten mikrobiellen Einflüsse werden die Wirkungen auf Bodenstruktur, Humusbildung, Nährstoffzustand des Bodens und Biophase kurz besprochen.
ACTINOMYCETE FLORA OF JAPANESE SOILS

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National Institute of Agricultural Sciences, Nishigahara, Tokyo, Japan

I. INTRODUCTION

Actinomyces have long been known as one of the major groups of the soil population together with bacteria and fungi. But it seems since 1940 that special attention has been paid to this group of microorganisms not only by soil but also by other field microbiologists. This is because it was proved that the production of antibiotics against other organisms including pathogens is a common phenomenon amongst actinomyces (Waksman 1941). Since then it is only natural that many studies have been made on this group of microorganisms in soil, especially relating to their antagonistic property.

In the authors' laboratory, a series of studies was started several years ago of the microbiological characterization of Japanese soils using both the composition of microflora as well as the microbial activities. Some results already obtained have been published (Ishizawa and Toyoda 1964); they proved that volcanic soils, which are especially important for agriculture in Japan, have a characteristic microbial population.

As a part of this study a comparison was made of the composition of the actinomycete flora in volcanic and non-volcanic soil and in cultivated and virgin soil.

II. EXPERIMENTAL

(a) Soil samples used

Soil samples were collected throughout Japan in autumn. Samples were taken from plough-soil in cultivated soils and from the surface layer, after removal of litter, in virgin soils; they were usually used about fourteen days after sampling. Special precautions such as keeping under refrigeration were not taken. A total of 35 volcanic soils (17 virgin, 18 cultivated) and 30 non-volcanic soils (16 virgin, 14 cultivated) was sampled.

(b) Isolation of actinomycetes

Each sample was plated out on albumin agar. After two weeks' incubation at 28 °C, fifty isolates were obtained by random selection from each soil sample.

(c) Morphological, cultural and biochemical characters

(i) Morphology and cultural characters

Each isolate was transferred onto a glucose asparagine agar slope. After three weeks' incubation at 28 °C, extent of growth, aerial and
vegetative mycelium, and soluble pigment were examined. Sporophores were observed under low magnifications.

(ii) Gelatin liquefaction and chromogenesis
Observations were made after one and two weeks' incubation at 28 °C. Gelatin liquefaction was determined by refrigerating cultures on a medium consisting of:
- gelatine—150.0 g, peptone—5.0 g, glucose—20.0 g, distilled water—1000 ml, pH 7.0.

(iii) Utilization of starch and cellulose
After three weeks' incubation at 28 °C, growth was observed and the results were described as amylase or cellulase activity.
Starch agar: soluble starch—10.0 g, NaNO₃—1.0 g, MgCO₃—1.0 g, NaCl—0.5 g, K₂HPO₄—0.3 g, agar—15.0 g, distilled water—1000 ml; pH 7.2 to 7.4.
Cellulose medium: NaNO₃—2.0 g, K₂HPO₄—1.0 g, MgSO₄·7H₂O—0.5 g, KCl—0.5 g, FeSO₄·7H₂O—0.01 g, distilled water—1000 ml; pH 7.0-7.3; a piece of filter paper was immersed in each tube.

(d) Antibiotic spectrum
An indication of the antagonistic properties of the isolates was obtained by using an agar cylinder 9 mm in diameter cut from five day old plate cultures at 28 °C on medium A.
Medium A: 0.5% of each of glucose, glycerin, starch, meat extract, peptone, NaCl, soybean meal, and dried yeast, respectively; Agar 2%; pH 7.0.

Composition of media used to grow each test organism is as follows:
For *Fusarium oxysporum* f. *cucumerinum* Owen, and *Pellicularia filamentosa* Pi-63:
Potato extract—1000 ml, sucrose—20.0 g, agar—18.0 g; pH 7.0. 200 g of potato were gently boiled with 1000 ml of water for one hour and then the extract was made up to 1000 ml.
For *Candida albicans* IAM 4888: peptone—5.0 g, yeast extract—3.0 g, meat extract—3.0 g, glucose—10.0 g, agar—18.0 g, distilled water—1000 ml; pH 7.2.
Observations were made after 24 hours' incubation for bacteria and *Candida*, two days for *Fusarium*, and three days for *Pellicularia* at 28 °C.

III. RESULTS AND DISCUSSION
The average count of actinomycetes in volcanic soil was 10.8 x 10⁶ for cultivated and 4.59 x 10⁶ for virgin soil; that of non-volcanic soil was 8.47 x 10⁶ for cultivated and 4.04 x 10⁶ for virgin soil per g of dry
sample. The number of isolates was 1750 for volcanic and 1500 for non-volcanic soils. A summary of results is presented in the following Tables.

(a) Volcanic soil: non-volcanic soil

As shown in Table 1, the actinomycete florae of each soil were not distinguishable from each other on the basis of morphology (spiral formation of sporophores), although morphology was expected to be one of the important criteria for differentiation especially because the characteristic structure of a volcanic soil allows freer air and liquid movement compared with a non-volcanic soil (Misono 1964). In this respect the size of pores filled with air, rather than the total volume of pore space, might be more important.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Volcanic soil (n = 35)</th>
<th>Non-volcanic soil (n = 30)</th>
<th>Difference between soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiral formation in sporophore</td>
<td>12·3 8·7</td>
<td>10·2 9·2</td>
<td>2·1</td>
</tr>
<tr>
<td>Chromogenesis</td>
<td>20·4 12·8</td>
<td>11·2 11·8</td>
<td>9·2****</td>
</tr>
<tr>
<td>Protease (Gelatin liquefaction)</td>
<td>44·9 18·4</td>
<td>44·3 14·4</td>
<td>0·6</td>
</tr>
<tr>
<td>Amylase</td>
<td>42·8 24·2</td>
<td>48·7 24·7</td>
<td>5·9</td>
</tr>
<tr>
<td>Cellulase</td>
<td>16·5 14·9</td>
<td>20·1 15·9</td>
<td>3·6</td>
</tr>
</tbody>
</table>

**** Significant at 0·5% level.

Although amylase, cellulase, and protease activity could not serve as useful bases for differentiation, chromogenesis did. In other words, the actinomycete flora of volcanic soil is characterised by its higher proportion of chromogenic species compared with non-volcanic soil. In volcanic soil chromogenic species such as *Streptomyces scabies*, *S. lavendulae*, *S. erythrochromogenes*, *S. viridochromogenes*, *S. cinnameus*, and *S. reticuli* were found, whereas in non-volcanic soil non-chromogenic species including *S. albus*, *S. cinereus*, *S. flavus*, *S. ruber*, *S. viridis*, *S. violaceoruber*, *S. fradiae*, *S. griseus*, and *S. hygroscopicus* were noticed. Volcanic soil is usually rich in humus, and black-colored. It is not known whether these characters are related to the abundance of chromogenic types in volcanic soil.

Only the results of antibiotic production against the test organism, *Bacillus megaterium*, were found to be usable as a distinguishing criterion (Table 2). Although Valyi-Nagy et al. (1961) reported that the percentage incidence of antagonistic *Streptomyces* isolates was higher in soils rich in organic matter than in poorer soils, volcanic soils rich in humus compared with non-volcanic soils poorer in humus did not show a higher percentage
of isolates with antagonistic activity except in the case of activity against Bacillus megaterium. Although the reason for the discrepancy is not known, the variation in the content of organic matter within the same soil group might be important, as may be seen later in the comparison between virgin and cultivated soil.

### Table 2

PERCENTAGE OF ISOLATES ANTAGONISTIC AGAINST EACH TEST ORGANISM IN VOLCANIC AND NON-VOLCANIC SOIL

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Volcanic soil (n = 35)</th>
<th>Non-volcanic soil (n = 30)</th>
<th>Difference between soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Standard deviation</td>
<td>Average</td>
</tr>
<tr>
<td>Pellicularia filamentosa</td>
<td>32.1</td>
<td>16.4</td>
<td>32.6</td>
</tr>
<tr>
<td>Bacillus cereus var. mycoides IAM 1190</td>
<td>21.3</td>
<td>11.4</td>
<td>16.3</td>
</tr>
<tr>
<td>Bacillus megaterium IAM 1030</td>
<td>26.3</td>
<td>11.9</td>
<td>19.1</td>
</tr>
<tr>
<td>Bacillus subtilis IAM 1069 (ATCC 6633)</td>
<td>27.7</td>
<td>14.7</td>
<td>25.1</td>
</tr>
<tr>
<td>Sarcina lutea IAM 1099</td>
<td>26.9</td>
<td>11.9</td>
<td>22.6</td>
</tr>
</tbody>
</table>

** Significant at 2.5% level.

The results shown in Tables 1 and 2 were from a comparison between volcanic and non-volcanic soils without subdivision into cultivated and virgin soil groups. When comparisons were made between volcanic and non-volcanic cultivated soils or between volcanic and non-volcanic virgin soils the results were similar but the difference between cultivated soils was larger than between virgin soils.

(b) Cultivated soil: virgin soil

As shown in Table 3, no differences were found in actinomycete florae between cultivated and virgin soils on the basis of spiral formation, chromogenesis and protease activity whereas large differences were observed in amylase and cellulase activity. These results are interesting in view of the wide distribution of amylase among Streptomyces (Waksman 1954). Properties such as morphology of sporophore and chromogenesis are said to be fairly stable ones, whereas the hydrolytic activity on starch or cellulose is liable to be effected by environmental conditions (Okami 1961). The results appear to suggest that the actinomycete flora of virgin soil is liable to acquire starch or cellulose hydrolysis activity without a great change in species composition when brought under cultivation, although it is uncertain how much time must elapse for the acquisition of their hydrolytic activity. According to Din Tszyan (1960), the dominant species differ between virgin and cultivated soil. Whether this result
applies to the authors' data will have to be determined after further detailed examination but this has not yet been made. The result that the percentage of cellulose-decomposing actinomycetes seems higher than Din Tszyan's result may be ascribed to the difference in the extent of cellulase activity adopted as the basis for determination.

### Table 3

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Cultivated soil (n = 32)</th>
<th>Virgin soil (n = 33)</th>
<th>Difference between soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Standard deviation</td>
<td>Average</td>
</tr>
<tr>
<td>Spiral formation in sporophores</td>
<td>12.6</td>
<td>8.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Chromogenesis</td>
<td>16.6</td>
<td>12.5</td>
<td>15.8</td>
</tr>
<tr>
<td>Protease (Gelatin liquefaction)</td>
<td>45.6</td>
<td>20.9</td>
<td>43.6</td>
</tr>
<tr>
<td>Amylase</td>
<td>57.2</td>
<td>21.7</td>
<td>34.1</td>
</tr>
<tr>
<td>Cellulase</td>
<td>28.9</td>
<td>13.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

**** Significant at 0.1% level.

The reason for such great differences found between virgin and cultivated soil is not known at present. The establishment of a high proportion of actinomycetes with amylase or cellulase in cultivated soil could be ascribed to an abundant supply of carbonaceous material in cultivated soil, but the general trend is that the content of available organic matter is higher in virgin than in cultivated soil, and therefore the difference in the actinomycete flora on the basis of amylase or cellulase activity seems hardly ascribable to the difference in the supply of the substrate. Concerning this point it may be added that the enzyme activity of the soil as a whole is usually higher in virgin than in cultivated soil (unpublished data). Hofmann and Hoffmann (1955) have shown that cultivated soils have much less amylase activity than grassland. Galstyan (1959) has reported that the amylase activity of soils is closely related to the type of microorganisms present, the pH, and the amount of humus. On the other hand, since the percentage of amylase-active actinomycetes is changed by manural treatment (Ishizawa and Araragi 1963) and by cropping.—Abraham and Herr (1964) have reported a rhizosphere effect—the relationship between the enzyme activity of the soil and its actinomycete flora remains to be solved.

Comparison on the basis of antibiotic production is shown in Table 4. There were four cases where differences were evident and in every case the proportion of isolates antagonistic to the test organisms was higher in
virgin than in cultivated soil. The difference in the case of *Bacillus subtilis* was highly significant. As stated above, since a virgin soil is usually richer in available organic matter, these results seem to agree with those of Valyi-Nagy *et al.* (1961). On the basis of Gram-negative bacteria, a significant difference was not observed.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Cultivated soil (n = 32)</th>
<th>Virgin soil (n = 33)</th>
<th>Difference between soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Standard deviation</td>
<td>Average</td>
</tr>
<tr>
<td><em>Pellicularia filamentosa</em> Pi-63</td>
<td>27·8</td>
<td>14·0</td>
<td>36·7</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> var. <em>mycoides</em> IAM 1190</td>
<td>15·9</td>
<td>7·1</td>
<td>22·1</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em> IAM 1030</td>
<td>21·0</td>
<td>8·2</td>
<td>25·2</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> IAM 1069 (ATCC 6633)</td>
<td>21·4</td>
<td>11·2</td>
<td>31·5</td>
</tr>
<tr>
<td><em>Sarcina lutea</em> IAM 1099</td>
<td>22·1</td>
<td>8·0</td>
<td>27·8</td>
</tr>
</tbody>
</table>

*, ** and **** Significant at 5%, 2·5% and 0·5% level, respectively.

In comparing cultivated and virgin soils, reports that the percentage of antagonistic actinomycetes is not greatly changed by the kind of crop (Ehle 1966, Rehm 1960, Kublanovskaya 1962) may be remembered.

The difference presented in the above Tables was distinct also when comparison was made by the statistical sign test (Miura and Asaka 1953).

At present the authors can assign no reliable reason for the differences found in the actinomycete flora between volcanic and non-volcanic soils or between virgin and cultivated soils. Comparisons of various aspects of the actinomycete flora between each soil will be possible using the data in hand. These will be published elsewhere in the near future.

**IV. ACKNOWLEDGMENT**

The authors wish to express their sincere thanks to the Institute of Applied Microbiology, The University of Tokyo, for the supply of organisms for testing the antagonistic property of isolates.

**V. REFERENCES**


ACTINOMYCETES IN JAPANESE SOILS

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Tsyan Din (1960)—Mikrobiologiya. 29, 104-108.

SUMMARY

As part of a study on microbiological characterization of Japanese soils, actinomycete flora were compared between volcanic and non-volcanic soils and between cultivated and virgin soils using about three thousand isolates obtained by fifty random selections from albumin agar plates per each soil sample.

In comparing volcanic and non-volcanic soils, the proportions of chromogenic actinomycetes and of antibiotic-producing actinomycetes (against Bacillus megaterium) were found to be significantly higher in the former soils.

In comparing virgin soils with cultivated ones, the latter were characterized by significantly higher proportions of actinomycetes with starch or cellulose hydrolytic activity and by lower proportions of actinomycetes antagonistic against Pellicularia filamentosa, Bacillus cereus, Sarcina lutea, and Bacillus subtilis.

RÉSUMÉ

En étudiant le caractère microbiologique des sols Japonais, on a comparé les actinomycètes flora des sols volcaniques et non-volcaniques et/ou des sols cultivés et des sols vierges, en utilisant environ 3.000 "isolats" obtenus de 50 sélections, prises au hasard, des plaques d’albumine —agar, pour chaque échantillon de sol.

En comparant les sols volcaniques et non-volcaniques, on a trouvé que la proportion d’actinomycètes chromogéniques et celle des actinomycètes produisant des antibiotiques contre le Bacille megaterium, était beaucoup plus importante dans le premier genre de sol que dans le deuxième.

Dans une comparaison entre les sols vierges et cultivés, ces derniers sont caractérisés par un nombre plus grand d’actinomycètes ayant une activité hydrolysante de l’amidon ou de la cellulose et par une proportion plus petite d’actinomycètes antagoniques envers les Pellicularia filamentososa, Bacillus cereus, Sarcina lutea et Bacillus subtilis.
ZUSAMMENFASSUNG

Bei Untersuchungen von mikrobiologischen Kennzeichen der Japanischen Böden, wurden Vergleiche betreffend der Aktinomyzeten флора durchgeführt, und zwar zwischen vulkanischen und nicht vulkanischen Böden und/oder zwischen kultivierten und unbebauten Böden. Es wurden etwa 3.000 Isolate gebraucht, die durch 50 Stichproben von Albumin-Agar-Platten pro Bodenprobe erlangt wurden.

Beim Vergleich zwischen vulkanischen und nicht vulkanischen Böden wurde festgestellt, dass das Ausmass von chromogenen Aktinomyzeten und von Antibiotikum erzeugenden Aktinomyzeten (gegen Bacillus megaterium) in den ersterwähnten Böden bedeutend höher war.

Beim Vergleich von unbebauten und kultivierten Böden hatten die letzteren ein bedeutend höheres Ausmass von Aktinomyzeten mit Stärke oder Zellulose hydrolitischen Aktivität und ein geringeres Ausmass von Aktinomyzeten antagonistisch zu Pellicularia filamentosa, Bacillus cereus, Sarcina lutea und Bacillus subtilis.
MICROBIAL INOCULATION OF WHEAT

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Renewed interest in the microbial inoculation of crop plants has followed reports on Soviet experiments (Cooper, 1959; Swaby, pers. comm.), a realistic evaluation of Soviet trials (Mishustin and Naumova, 1962) and reports of field trial results at Rothamsted (Brown et al., 1964). Following the observation in glasshouse trials that inoculation of wheat seed with *Azotobacter chroococcum*, *Bacillus polymyxa* and *Clostridium pasteurianum* induced earlier flowering and increased vegetative growth in wheat (Rovira, 1963, 1965), a series of extensive field trials has been conducted in Southern Australia inoculating these organisms onto wheat seed. Results from these field trials and glasshouse trials on the effects of inoculation upon heading and grain yield, and on the spread of inoculum from seed to roots are presented.

I. MATERIALS AND METHODS

(a) Micro-organisms

*Azotobacter chroococcum*, *Bacillus polymyxa* and *Clostridium pasteurianum* isolated from wheat roots (Rovira, 1963) have been used in field trials. Ten other organisms (8 bacteria, 1 actinomycete and 1 fungus) isolated from wheat roots and known to colonize roots were used in glasshouse trials.

(b) Wheat varieties

The varieties of *Triticum vulgare* used in most of the trials were Gabo and Insignia, with varieties Warigo, Heron and Insignia-49 used less extensively.

(c) Inoculation and planting methods

Three methods of inoculation were used: (a) aqueous suspension of cells grown on agar medium (1963 field trials and glasshouse trials), (b) mixture of radiation-sterilized peat and cells grown on agar medium (1964 field trials), (c) suspension of peat-grown cells in aqueous gum arabic (glasshouse trials). The respective control seeds were treated as follows: (a) with distilled water, (b) with a suspension of sterile peat in distilled water, (c) sterile peat in sterile medium with aqueous gum arabic. Incorporation of peat into the inoculum increased the survival of *Azotobacter* on the seed, permitting inoculation well before seeding. Apart from 3 farm scale trials, superphosphate was applied separately from the seed.
(d) Field trials—1963 and 1964

These were conducted at six sites in South Australia to cover several soil types and annual average rainfall from 32 to 60 cm.

(e) Glasshouse trials

The effects of several organisms on the onset of heading were investigated in Parafield red brown earth. Fifteen seeds, inoculated with aqueous suspension of the culture, were planted into each 13 cm diameter pot containing 2-5 kg soil wet to 60% field capacity. The plants were thinned to 10 per pot and grown in a glasshouse with average temperatures of 25°C max. and 18°C min. and 15 to 16 hour day. There were duplicate pots for each inoculation treatment at two fertility levels, (a) no added plant nutrients and (b) 400 ml plant nutrient solution (Hoagland and Arnon, 1938) per kg soil; 120 ml initially to wet the soil and 280 ml over 5 weeks.

(f) Survival and spread of Azotobacter from seed to roots

These studies were conducted in pots in controlled environment cabinets and in the glasshouse, with and without the addition of superphosphate near the seeds at a rate equivalent to 110 kg/ha. Temperatures were 15-5°C (night) and 20-5°C (day) under 12 hour day conditions. The soils used were: Monarto—sandy Terra Rossa (pH 6.6), and Clinton—loamy Terra Rossa (pH 7.3). Seed inoculated from a 5-day agar culture (liquid inoculum) or from a 21-day peat-grown culture was planted 2-4 cm deep (approx.) into soil wet to 60% field capacity. In the superphosphate experiments overhead watering at 4 days after planting was arranged to simulate a rain of 1.0-1.5 cm in 3 to 4 hours, with another overhead watering at 8 days. In other experiments watering took place from below via a side tube. Samples of seeds and roots, with attached soil, were carefully taken to reduce contamination between the 3 zones, viz. (a) seed with about 0.3 cm of upper roots attached (to reduce cross-contamination between seed and upper root); (b) upper roots, 0.3 to 2.3 cm from the seed; (c) mid-roots, 2.3 to 4.3 cm from the seed; and (d) lower roots, more than 4.3 cm from the seed. The samples, each consisting of 3 seeds or 3 root sections, were shaken for 15 minutes in distilled water and 10-fold dilutions made. Azotobacter numbers were estimated by the most probable number method with 10 drops of 0.02 ml from each dilution per sector of Petri dish containing nitrogen-free agar medium similar to the M9 medium of Parker (1955).

II. Results

(a) Field trials

The grain yields for the uninoculated control plots ("Nil") and the response to inoculation relative to a control value of 100 at each location for 1963 and 1964 are presented in Table 1. There was a much greater tendency for inoculation to increase grain yield than to decrease it. If we accept ±5% as a minimum meaningful yield difference (though not necessarily statistically significant) then, of the 71 comparisons, there were 28 increases and 4 decreases following inoculation.
### EFFECT OF SEED INOCULATION ON GRAIN YIELDS OF WHEAT IN FIELD TRIALS

<table>
<thead>
<tr>
<th>Variety</th>
<th>Year</th>
<th>Inoculum</th>
<th>Site A</th>
<th>Site B</th>
<th>Site C</th>
<th>Site D</th>
<th>Site E</th>
<th>Site F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabo</td>
<td>1963</td>
<td>Nil</td>
<td>100(111)$\phi$</td>
<td>100(452)</td>
<td>100(297)</td>
<td>100(363)</td>
<td>100(311)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Az</td>
<td>138***</td>
<td>116***</td>
<td>90</td>
<td>107</td>
<td>109**</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bs</td>
<td>134***</td>
<td>115***</td>
<td>94</td>
<td>105</td>
<td>103</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl</td>
<td>121***</td>
<td>112**</td>
<td>92</td>
<td>106</td>
<td>102</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mix</td>
<td>137***</td>
<td>121***</td>
<td>81</td>
<td>95</td>
<td>112*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>Nil</td>
<td>100(506)</td>
<td>100(5363)$\theta$</td>
<td>100(303)</td>
<td>—</td>
<td>100(273)</td>
<td>100(381)$\ddagger$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Az</td>
<td>105</td>
<td>102</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bs</td>
<td>104</td>
<td>103</td>
<td>99</td>
<td>—</td>
<td>97</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl</td>
<td>112</td>
<td>101</td>
<td>99</td>
<td>—</td>
<td>104</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mix</td>
<td>112</td>
<td>112*</td>
<td>101</td>
<td>—</td>
<td>105</td>
<td>108</td>
</tr>
<tr>
<td>Insignia</td>
<td>1963</td>
<td>Nil</td>
<td>100(175)</td>
<td>100(533)</td>
<td>100(199)</td>
<td>100(388)</td>
<td>100(332)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Az</td>
<td>98</td>
<td>104</td>
<td>104</td>
<td>95</td>
<td>111*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bs</td>
<td>95</td>
<td>102</td>
<td>104</td>
<td>104</td>
<td>110*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl</td>
<td>99</td>
<td>103</td>
<td>106</td>
<td>94</td>
<td>109*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mix</td>
<td>97</td>
<td>96</td>
<td>99</td>
<td>104</td>
<td>111*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>Nil</td>
<td>100(508)</td>
<td>100(6200)$\theta$</td>
<td>100(228)</td>
<td>—</td>
<td>100(290)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Az</td>
<td>108</td>
<td>113</td>
<td>105</td>
<td>—</td>
<td>106*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bs</td>
<td>112</td>
<td>—</td>
<td>102</td>
<td>—</td>
<td>106*$\phi$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cl</td>
<td>106</td>
<td>—</td>
<td>103</td>
<td>—</td>
<td>103</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mix</td>
<td>101</td>
<td>104</td>
<td>104</td>
<td>—</td>
<td>111*$\phi$</td>
<td>—</td>
</tr>
</tbody>
</table>

**Sites:** A, Waite Institute (red brown earth); B, Parafield (red brown earth); C, Saddleworth (black soil); D, Clinton (loamy Terra Rossa); E, Monarto (sandy Terra Rossa); F, Meningie (deep sand).

**Inoculum:** Az, *Azotobacter*; Bs, *Bacillus*; Cl, *Clostridium*; Mix, *Azotobacter*, *Bacillus* and *Clostridium*.

*$\phi$* Yields in g/plot in parenthesis. Other figures represent yields relative to 100 for the uninoculated control.

**Statistical Significance:** *,$\phi$, **,$\phi$,$\phi$, ***,$\phi$,$\phi$, significant at 5, 1 and 0·1% levels, respectively.

**Plot Size and Replication:** All plots 2·5 x 0·7 m (10 to 18 replicates) except those marked $\theta$, 20 x 1·5 m (4 to 6 replicates) and $\ddagger$, 2 x 3·5 m (7 replicates).
A problem with field trials is that, despite careful selection of each area on a farm for uniformity and considerable replication of each treatment, yield increases greater than 10% are generally required for statistical significance at the 5% level. Nevertheless, increases in yield significant at the 5% level or better occurred in 18 of these inoculation trials.

Despite the range of soils and climates covered by the field trials no relationship was found between inoculation response, soil and climatic conditions.

In 1966, trials were conducted on a large scale on 2 farms in South Australia to assess the practical value of seed inoculated with peat-grown Azotobacter. On one farm inoculation of “Prior” barley increased yield by 5.5% and of “Heron” wheat by 6.8%; on the second farm inoculated “Noyep” barley yielded only 1.8% more grain than did the control.

(b) Glasshouse trials

The most consistent visible result of seed inoculation upon field-grown wheat is the slight advancement in the emergence of the head from the flag leaf. In glasshouse trials the date of emergence of the head rather than grain yield was used to assess inoculation response. Az. chroococcum and 10 organisms isolated from wheat roots were tested using Parafield soil at 2 fertility levels. The date upon which each head emerged fully from the flag leaf was recorded. Table 2 shows the cumulative totals of the number of heads which had emerged on each day from the appearance of the first head for 3 organisms. If the dates upon which 50% of the plants had heads fully emerged are compared, then Table 2 shows that inoculation advanced head emergence by one day in amended soil but had no effect in unamended soil. Several cultures gave slightly taller plants in the unamended soil although no differences in heading time were detected.

<table>
<thead>
<tr>
<th>Days from emergence of first head</th>
<th>Total number of heads fully emerged from 20 plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No fertilizer</td>
</tr>
<tr>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
</tr>
</tbody>
</table>

* Az, Azotobacter chroococcum; 20, an actinomycete; 64, a pseudomonad.
<table>
<thead>
<tr>
<th>Type of inoculum</th>
<th>Days from sowing</th>
<th>Clinton soil Azotobacter per sample</th>
<th>Monarto soil Azotobacter per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>H</td>
</tr>
<tr>
<td>Liquid</td>
<td>0</td>
<td>2.8 x 10⁴</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.4 x 10⁴</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.7 x 10⁴</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.6 x 10⁴</td>
<td>3500</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.4 x 10⁴</td>
<td>2800</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>7.6 x 10⁴</td>
<td>360</td>
</tr>
<tr>
<td>Peat</td>
<td>0</td>
<td>6.0 x 10⁴</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.1 x 10⁴</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.9 x 10⁴</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.0 x 10⁴</td>
<td>2.4 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4.6 x 10⁴</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4.4 x 10⁴</td>
<td>200</td>
</tr>
</tbody>
</table>

* In neither soil were naturally-occurring Azotobacter detected.

Coding of Samples: A — Seed plus 0-3 cm upper roots with attached soil.
B — Upper roots (0.3-2.3 cm from seed) with attached soil.
C — Mid roots (2.3-4.3 cm from seed) with attached soil.
D — Lower roots (4.3 cm to apex, with total root lengths up to about 14 cm).
E — Total roots (B + C + D) with attached soil.

Each count, in general, the mean of 3 assays, each consisting of 2 to 4 individual seeds or root sections.
The long day length of 15 to 16 hours accelerated growth and the plants came into head between 6 and 7 weeks after seeding which reduced any effect of inoculation on heading time. The results are consistent with earlier studies with \textit{Azotobacter}, \textit{Bacillus} and \textit{Clostridium} under short day conditions (Rovira, 1965).

None of the inoculation treatments affected grain yield per pot, although amendment with plant nutrient solution raised grain yield from 2.77 g/pot to 4.15 g/pot due to nitrogen. Crowding of the plants with 10 per pot was deliberate to produce only one head per plant, as it was our aim to study the effects of inoculation upon development of the primary tiller.

(c) \textit{Spread of Azotobacter from seed to roots}

(i) \textit{Comparison of liquid and peat inocula}

Despite the large inoculum of \textit{Azotobacter} ($10^4$ to $10^5$ per seed), comparatively few had been found in the root region of peat-inoculated plants growing in the field. Pot experiments with two soils were conducted to assess the spread of organisms from seeds with liquid (agar-grown) and peat-grown inocula. Although there was wide variability between counts of replicate samples, we concluded from Table 3 that there was generally: (a) no marked difference in root populations with the two inoculation methods, (b) a relatively low number of \textit{Azotobacter} in the rhizosphere even 0.3-2.3 cm from the seed, (c) a 5 to 10-fold rise in numbers on the seed in the first day after seeding, and (d) a decline of \textit{Azotobacter} numbers on the seed after 7 days. In this experiment the numbers of \textit{Azotobacter} on the seed and in the rhizosphere were not affected by soil, but in other experiments there was a lower survival in the sandy Monarto soil.

<table>
<thead>
<tr>
<th>Sample (with attached soil)</th>
<th>Clinton soil (Initial inoculum = $2.7 \times 10^4$ per seed)</th>
<th>Monarto soil (Initial inoculum = $1.8 \times 10^4$ per seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed plus 0.3 cm upper roots attached</td>
<td>1.0 $\times 10^3$</td>
<td>1.7 $\times 10^3$</td>
</tr>
<tr>
<td>Upper roots (0.3 - 2.3 cm from seed)</td>
<td>980</td>
<td>4900</td>
</tr>
<tr>
<td>Lower roots (2.3 cm to apex, total root lengths being up to about 12 cm)</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

Each count is mean of 3 assays, each assay covering 3 different seeds or root sections. Clinton samples assayed after 11 days, Monarto samples after 12 days.
(ii) Effect of superphosphate on *Azotobacter* inoculum

Superphosphate close to the seed under conditions simulating heavy rain following seeding was not detrimental to the survival of *Azotobacter* on the seed nor to its spread to the roots (Table 4). In this experiment mortality of the peat-grown *Azotobacter* on the seed was higher in Monarto soil than in Clinton soil.

**III. DISCUSSION AND CONCLUSIONS**

These investigations have demonstrated the difficulties in establishing that seed inoculation of wheat is an economic proposition. In the 1963 and 1964 field trials an average for all sites of the percent increase or decrease in grain yield over the control following inoculation for each variety and each year shows that the overall increase in yield with inoculation was:

- *Gabo*: +9.5% (1963), +3.8% (1964);
- *Insignia*: +2.0% (1963), +6.0% (1964).

The technical problems of inoculum preparation and survival on seed when planted together with fertilizer have been overcome, but despite extensive field trials no explanation can be offered for increased yields. Under Australian conditions where moisture supply is often limiting after wheat has flowered, earlier flowering induced by seed inoculation should generally increase grain yield. There will be exceptional growing seasons where the reverse may occur.

The difficulty of studying this problem in the field has led us to trials in the glasshouse and controlled environments, and in these trials improved seedling growth or advancement of heading has been used to assess the effects of inoculation. Several organisms which produced greater effects than did *Azotobacter* are being tested further. Some of these organisms are superior root colonizers to *Azotobacter* and hence they may be stronger competitors with the natural soil-borne root colonizers.

The results of experiments in controlled environment and glasshouse trials to be reported elsewhere have shown that inoculation responses with wheat are increased (a) on seedlings of low vigour (Paltridge, pers. comm.), (b) under short day conditions and (c) in soil enriched with inorganic nutrients. These interactions further highlight the complexities of the problem.

Throughout our experiments in both field and glasshouse we have found relatively few *Azotobacter* in the rhizosphere compared with the numbers placed on the seed, whereas Brown *et al.* (1962) found that *Azotobacter* colonized the whole root system “indicating continuous multiplication of the cells”. However, the soils used by Brown *et al.* contained naturally-occurring *Azotobacter*, whereas we did not detect any in our soils, hence the restricted spread we found may be due to less favourable soil environments. We found the *Azotobacter* restricted to the rhizosphere and only occasionally on the root surface, as was reported by Brown *et al.* (1962).

Survival on the seed and spread to roots were not affected by superphosphate but did vary with different soils. Immunofluorescence techniques introduced for fungi in soil by Schmidt and Bankole (1962) are being applied to our investigations of the spread of inoculum from seed to root.
The mechanisms by which these organisms affect plant growth have not been clearly elucidated despite the findings of Vancura (1961) and Jackson et al. (1964) that the response of plants to *Azotobacter* is similar to the response to gibberellins. The fact that so many organisms affect plant growth (Mishustin and Naumova, 1962; Macura and Vancura, 1965) may indicate that gibberellins are not solely responsible for the response of wheat to inoculation.

Until the mechanisms by which these organisms affect plant growth are explained, there is little prospect of placing the large-scale inoculation of seed for practical agriculture upon a sound scientific basis.

IV. ACKNOWLEDGMENTS

We thank Messrs. R. Hart, H. van Dijk and N. Sutherland for assistance in laboratory and field work and the Waite Institute of the University of Adelaide and individual farmers for the planting and harvesting of field trials. The Australian Wheat Research Council has provided financial support for investigations.

V. REFERENCES


SUMMARY

*Azotobacter* and other micro-organisms were used to inoculate seed wheat in pot and field experiments. Advancement of heading noted in the field after *Azotobacter* inoculation was reproduced in glasshouse trials with *Azotobacter* and two other organisms. If ±5% is accepted as a meaningful yield difference (though not always statistically significant) 71 field comparisons of grain yield between inoculated seed and uninoculated controls showed 28 increases, 4 decreases, and 39 with no difference. In pots there was no difference in the survival and spread of *Azotobacter* in sandy or loamy soils, whether seed was inoculated with liquid or peat cultures. However, in the soils used, which lacked naturally-occurring *Azotobacter*, this spread was largely restricted to the upper 2-3 cm of
the root. Pot experiments with seed inoculated from peat-grown *Azotobacter* showed no adverse effect from superphosphate placed near the seed even under conditions simulating rainfall soon after seeding.

**Résumé**

On a utilisé l’*Azotobacter* et d’autres micro-organismes pour inoculer au blé de semence pour des expériences en vases de végétation et au champ. L’augmentation des épis notée dans le champ après inoculation d’*Azotobacter*, s’est reproduite dans des essais en serre avec *Azotobacter* et deux autres organismes. Si on agréée ±5% comme différence significative de récolte (bien que pas toujours statistiquement significative), 71 comparaisons de la récolte de grains du champs, entre de la semence inoculée et des échantillons témoins pas inoculés, montrèrent 28 augmentations, 4 diminutions, et 39 cas n’ayant pas de différence. En vases de végétation il n’y avait aucune différence de survivance ni d’étendue d’*Azotobacter* dans les sols sableux ou limoneux, soit que la semence fut inoculée de cultures liquides ou tourbeuses. Pourtant, dans les sols utilisés, qui manquaient d’*Azotobacter* naturel, cette étendue était limitée aux 2-3 centimètres supérieurs de la racine. Les expériences en vases de végétation avec de la semence inoculée d’*Azotobacter* cultivé dans la tourbe ne montrèrent pas d’effet adverse provenant du superphosphate placé près des graines, même sous des conditions simulant la pluie peu après les semaines.

**Zusammenfassung**

Phytate (myoinositol hexaphosphate) frequently occurs in plants (Cosgrove 1966) and it has been shown that an important fraction of the soil organic phosphorus is in the form of phytates (myo-, scyllo-, DL and neoinositol hexaphosphates) (Bower 1945, Anderson 1956, Cosgrove 1963, Cosgrove and Tate 1963). Caldwell and Black (1958) found that soils under forests had a higher percentage of the total organic phosphorus in the form of inositol hexaphosphate than soils developed under grasslands. They demonstrated that myoinositol hexaphosphate and an assumed isomer of this compound was formed by micro-organisms in soils amended with organic and inorganic nutrients.

Soil and litter phytate may be a potential source of phosphate for plant use, either directly or following decomposition by micro-organisms. Phytase activity is common among soil and rhizosphere micro-organisms (Casida 1959, Greaves and Webley 1965). It has been shown that phytate can be utilized by some plants e.g. maize and tomato (Rogers, Pearson and Pierre 1940), radish and kale (Szemberg 1960) in the absence of microbial phytase activity. As far as it could be ascertained there is no information on the use of phytates by either Pinus radiata or mycorrhizal fungi.

In the present studies the presence of inositol phosphates in Pinus radiata D.Don needles and the phytase activity of pure cultures of mycorrhizal fungi were investigated. The possibility of phytate being used as a carbon source for mycorrhizal fungi was also examined.

1. Materials and Methods

(a) Inositol phosphates in pine needles

(i) Extraction procedure

Mature needles from the lower branches of trees in a 27 year old Pinus radiata D.Don stand in the S.E. of South Australia were dried at 105°C. Two hundred grams were ground and extracted with 1000 ml of 5% (w/v) perchloric acid at room temperature. EDTA was added and the extract was neutralised initially with 5N KOH and then with 1N KOH to pH 7.5 (Turner and Turner 1961) and concentrated to one-fifth volume at 30°C in vacuo and an equal volume of 10% barium acetate was added. The crude barium phytate precipitate was washed with distilled water twice, dried in an oven at 80°C and weighed.
(ii) Paper electrophoresis of inositol phosphates from needles

The precipitate obtained from the pine needles was dissolved in 1N acetic acid and Dowex AG-50W resin (H\(^+\) form, -400 mesh) was added to remove Ba\(^{++}\). The components were separated by electrophoresis on Whatman No. 3MM chromatography paper at 1600V in sodium oxalate, oxalic acid buffer \(pH\ 1.5\) (Seiffert and Agranoff 1965). Reference solutions containing myoinositol hexaphosphate, three inositol pentaphosphate isomers, inositol tetraphosphate, inositol triphosphate, inositol monophosphate and orthophosphate were used for comparison. The phosphorus compounds were detected by the Harrap (1960) phosphomolybdate procedure.

(b) Phytase activity of mycorrhizal fungi

(i) Plate method

Plates of Melin-Norkrans medium, \(pH\ 5.2\) (Melin 1959), containing 5 g per litre of commercial calcium phytate or iron phytate were inoculated with *Rhizopogon luteolus*, *Boletus luteus*, and *Cenococcum graniforme*. Four plates were inoculated with each fungus. A clear zone around the fungus after 12 days' incubation at 25°C indicated solubilization of the phytate.

(ii) Liquid cultures

Thirty ml of sterile Melin-Norkrans liquid medium, \(pH\ 5.2\), in which the \(KH_2PO_4\) was replaced by 0.15 g/30 ml of calcium or ferric phytate was inoculated with 6 discs, 3 mm in diameter, from actively growing cultures of *Rhizopogon luteolus*, *Boletus luteus*, and *Cenococcum graniforme*. The calcium phytate used contained 13% of its total phosphorus in the form of phosphate esters other than the hexaphosphate. The ferric phytate was pure. Two uninoculated control treatments, one buffered with 0.05M potassium phthalate at \(pH\ 4.6\) (the approximate \(pH\) to which the medium drops after fungal growth) and the other at \(pH\ 5.2\), were used for determining the inorganic phosphorus released by mild acid hydrolysis. Four replicates were used. After inoculation the cultures were incubated in shallow solution layers for eleven days in the case of calcium phytate and for one month in the case of ferric phytate at 25°C and then the medium was filtered and the \(pH\) and the inorganic phosphorus in the medium determined by the method of Mehta *et al.* (1954). The mycelium was washed well with distilled water and any residual calcium phytate or ferric phytate on the outside of mycelium was dissolved by washing with 1N HCl or 1N NaOH respectively for 10 min. The mycelium was then washed with distilled water, dried at 80°C and digested with a perchloric, sulphuric, nitric acids mixture (Piper 1944) and the solution was analysed for phosphorus.

The loss of phosphorus from the mycelium on washing with 1N HCl was estimated by growing the fungi in \(^{32}P - KH_2PO_4\) and washing the mycelium with distilled water to get rid of any free phosphorus and subsequently with 1N HCl for 10 min. It was found that the loss of phosphorus
was $2.3\%$ of the phosphorus in the mycelium for *Rhizopogon luteolus*, $18.3\%$ for *Boletus luteus*, and $22.4\%$ for *Cenococcum graniforme*. This shows that the amount of phosphorus in the mycelium given in Table 2 is underestimated.

(c) *Use of phytate as a carbon source*

Phosphate in Melin-Norkrans medium was replaced by sodium phytate (pure inositol hexaphosphate—Sigma Chemical Company, Type V) and

![Electrophoresis of Barium Inositol Phosphates Extracted from Pine Needles.](image-url)

*Fig. 1.*—Electrophoresis of Barium Inositol Phosphates Extracted from Pine Needles.

(a) Needle extract  (b) Reference
1. Inositol hexaphosphate; 2. Inositol pentaphosphate (3 isomers); 3. Inositol tetraphosphate; 4. Inositol triphosphate; 5. Inositol monophosphate; and 6. Orthophosphate.
potassium requirements were met by addition of potassium nitrate. In one half of the experiment phytate was the only major carbon source and in the other, glucose at a suboptimal level for growth (Theodorou, unpublished) was included. Thirty ml of medium was autoclaved at 120°C for 15 min., inoculated with *Rhizopogon luteolus*, *Boletus granulatus*, *B. luteus* and *Cenococcum graniforme* and incubated at 25°C for one month. The mycelium was washed with distilled water, dried at 80°C for 5 hours and weighed. The fungus and solutions from this experiment were also analysed for inorganic phosphorus.

II. RESULTS

(a) *Inositol phosphates in pine needles*

The electrophoretogram of the barium inositol phosphates showed (Figure 1) the presence of inositol hexaphosphate, three isomers of inositol pentaphosphate, inositol tetraphosphate, inositol triphosphate and orthophosphate. The amount of phosphorus in the form of inositol phosphates in this sample of needles was found to be 2% of the total phosphorus.

(b) *Phytase activity of mycorrhizal fungi*

In plates containing calcium phytate the fungal colonies produced a clear zone indicating the solubilization of the phytate. The clear zone for the fungi studied was from 4 mm to 7 mm ahead of the colony edge (Table 1). The same fungi did not produce a clear zone when they were incubated on ferric phytate plates.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Diameter of fungal colony* mm</th>
<th>Margin of clear zone from colony edge* mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizopogon luteolus</em></td>
<td>74</td>
<td>4</td>
</tr>
<tr>
<td><em>Boletus luteus</em></td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td><em>Cenococcum graniforme</em></td>
<td>14</td>
<td>7</td>
</tr>
</tbody>
</table>

* Mean of 4 plates.

The clear zone could have been caused by either chelation, acid and/or enzymic hydrolysis of the phytate. To differentiate between the acid and enzymic hydrolysis the fungi were grown in liquid cultures and acid added to controls. Table 2 gives the amount of phosphorus released from calcium phytate, sodium phytate and ferric phytate. All fungi released significantly greater amounts of phosphorus than the uninoculated controls from calcium and sodium phytates. In the case of ferric phytate only *B. luteus* released significantly greater amounts of phosphorus than the acid treatments.
<table>
<thead>
<tr>
<th>Type of inoculum</th>
<th>Rhizopogon luteolus</th>
<th>Boletus granulatus</th>
<th>Boletus luteus</th>
<th>Cenococcum graniforme</th>
<th>Uninoculated 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4.6</td>
<td>pH 5.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Calcium phytate 0.15 g/30 ml medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P in medium (mg)</td>
<td>1.74</td>
<td>1.68</td>
<td>1.36</td>
<td>1.59</td>
<td>0.48</td>
</tr>
<tr>
<td>P in fungus (mg)</td>
<td>0.73</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total P released (mg)</td>
<td>2.81**</td>
<td>2.41*</td>
<td>1.90***</td>
<td>1.59</td>
<td>0.48</td>
</tr>
<tr>
<td>Final pH of medium</td>
<td>4.6</td>
<td>4.6</td>
<td>5.2</td>
<td>4.6</td>
<td>5.2</td>
</tr>
<tr>
<td>LSD</td>
<td>0.74 P = 0.1, 0.9 P = 0.05, 1.24 P = 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Sodium phytate 0.3 g/30 ml of medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P in medium (mg)</td>
<td>3.89</td>
<td>4.3</td>
<td>8.35</td>
<td>-</td>
<td>2.09</td>
</tr>
<tr>
<td>P in fungus (mg)</td>
<td>1.67</td>
<td>1.04</td>
<td>1.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total P released (mg)</td>
<td>5.56***</td>
<td>5.93***</td>
<td>5.08***</td>
<td>9.50***</td>
<td>-</td>
</tr>
<tr>
<td>LSD</td>
<td>2.86 P = 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Ferric phytate 0.15 g/30 ml of medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P in medium (mg)</td>
<td>1.19</td>
<td>1.19</td>
<td>1.93</td>
<td>0.37</td>
<td>1.19</td>
</tr>
<tr>
<td>P in fungus (mg)</td>
<td>0.35</td>
<td>0.38</td>
<td>0.47</td>
<td>0.87</td>
<td>-</td>
</tr>
<tr>
<td>Total P released (mg)</td>
<td>1.54</td>
<td>1.57</td>
<td>2.40***</td>
<td>1.24</td>
<td>1.19</td>
</tr>
<tr>
<td>LSD</td>
<td>0.37 P = 0.05, 0.53 P = 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Means of 4 replicates.
2 Growth for 11 days at 25 °C. Maximum possible phosphorus released from impurities (13%) have been deducted.
3 Growth for 31 days at 25 °C.
4 Difference between fungus and control at same final pH significant at 10% level*,
   5% level**
   1% level***.
(c) Use of phytate as a carbon source

Table 3 shows the growth of mycorrhizal fungi with phytate partially or completely replacing the carbon and phosphorus source. The best fungal growth was in the presence of glucose. When phytate was added to glucose the dry weight of the fungi was somewhat lower. When phytate was the only energy source the fungus did not grow, the dry weight of the fungus after one month’s incubation being equal to the inoculum weight. In the absence of both glucose and phytate the fungi grew very little.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Dry weight of fungus (mg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal medium + 2·5 g glucose, 1·0 g KH$_2$PO$_4$</td>
<td>Rhizopogon luteolus</td>
</tr>
<tr>
<td>Basal medium + glucose, 0·3 g sodium phytate 30 ml · KNO$_3$</td>
<td></td>
</tr>
<tr>
<td>Basal medium - KH$_2$PO$_4$</td>
<td></td>
</tr>
<tr>
<td>Basal medium - sodium phytate - KNO$_3$</td>
<td></td>
</tr>
</tbody>
</table>

* Mean of 4 replicates.

III. DISCUSSION

It was demonstrated that phytate was present in fresh mature pine needles. It is not certain, however, whether the lower inositol phosphates (pentaphosphate, tetraphosphate, and triphosphate) found in the extract existed as such in the needles or whether they were formed from the hexaphosphate during the extraction procedure. Acid hydrolysis of the inositol hexaphosphate at about pH 4·0 can produce a more complex mixture of lower phosphates than can enzymic hydrolysis (Cosgrove 1966). The electrophoretic pattern in the present study was similar to the bands obtained by Seiffert and Agranoff (1965) in an acid hydrolysate (pH 5·2). The complexity of the bands in the present study suggests that the lower inositol phosphates were an extraction artifact rather than the result of phytase activity in the needles although phytase activity in the needles giving rise to one inositol pentaphosphate has been observed (Theodorou, unpublished).

The clear zone produced by the fungus on the plates with calcium phytate indicated solubilization of the phytate but did not indicate whether this was due to chelation, increased acidity or to phytase activity. Although no direct extraction and assay of phytase from the mycorrhizal fungi was
ACTIVITY OF MYCORRHIZAL FUNGI

made, the release of significantly greater amounts of phosphorus from calcium phytate (after accounting for complete hydrolysis of impurities in the sample) and sodium phytate in the medium with the fungus than in the controls at pH 4.6 and 5.2 indicated enzymic activity rather than acid hydrolysis. The amount of inorganic phosphorus found in the medium suggests that the enzyme may be extra-cellular. Ferric phytate was not hydrolysed readily by mycorrhizal fungi probably because of its insolubility (Cosgrove 1966). Casida (1959) has, however, demonstrated a small phytase activity by an Aspergillus niger strain on iron phytate and there was a suggestion of activity from B. itaeus in the present experiment.

The difference in the activity of fungal phytase on calcium phytate which gave clear zones and ferric phytate which gave none indicates that there is a minimum solubility requirement for enzymic activity. Phytase enzymes act on phytate ions which are in solution but they do not attack solid phytate. Hill and Tyler (1954) have shown that when Ca⁺⁺ is added to a solution of phytate, phytase activity is diminished, because of the formation of insoluble calcium phytate precipitate.

Requirements for inositol are rare in fungi (Cochrane 1958). Inositol is used as a vitamin by Rhizopogon luteolus (Harley 1959). Beadle (1944) found that an inositol requiring mutant of Neurospora crassa could not use phytin for the supply of inositol.

Although the proportion of phytate phosphorus in the sample of needles analysed was low (2%), Caldwell and Black (1958) demonstrated that inositol hexaphosphate in forest soils constituted 24% of the total organic phosphorus and Anderson (1956) showed that one-third of soil organic phosphorus was in the form of inositol hexaphosphate. This important fraction of soil organic phosphorus can be of potential importance for the phosphorus nutrition of pine since as indicated here mycorrhizal fungi may obtain phosphorus from phytate in litter and soil and thus help the nutrition of the host tree.

IV. ACKNOWLEDGMENTS

The author wishes to thank Dr. M. E. Tate, Dr. R. J. Swaby and Mr. G. D. Bowen for valuable advice. This study has been supported by a grant from a number of Australian Forest Organizations.

V. REFERENCES

Harrap, F. E. G. (1960)—Analyst. 85, 452.
SUMMARY

Inositol hexaphosphate was present in needles of Pinus radiata D.Don. Lower inositol phosphates (pentaphosphates, tetraphosphate and triphosphate) were also observed but may have been caused by the method of extraction. The inositol phosphates constituted 2% of the total needle phosphorus. The mycorrhizal fungi Rhizopogon luteolus, Boletus luteus, B. granulatus and Cenococcum graniforme in pure culture showed phytase activity towards calcium and sodium phytate but little towards ferric phytate. Phytate was not used as a carbon source for these mycorrhizal fungi. The importance of the presence of phytates in litter and soil and the phytase activity of mycorrhizal fungi is discussed.

RéSUMÉ

On a trouvé de l'hexaphosphate d'inositol dans les aiguilles de Pinus radiata D. Don. On a remarqué aussi des phosphates inférieurs d'inositol (pentaphosphates, tetraphosphate et triphosphate) mais il se peut qu'ils ont été engendrés par la méthode d'extraction. Les phosphates d'inositol ont constitués 2% du phosphore total des aiguilles. Les mycètes de mycorhize, Rhizopogon luteolus, Boletus luteus, B. granulatus et Cenococcum graniforme, dans une culture pure ont montré de l'activité de phytase vers les phytates de calcium et natrium, mais peu vers le phytate ferrique. On n'a pas utilisé le phytate en tant que source de carbone pour ces mycètes de mycorhize. On discute l'importance de la présence des phytates dans la litière et le sol, et l'activité de phytase des mycètes de mycorhize.

ZUSAMMENFASSUNG


SOIL MICROORGANISMS AND MOLYBDENUM CONCENTRATION IN PLANTS

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Microbiology Department, Medical School, University of Otago, Dunedin, N.Z.

Although many factors such as pH, soil type and nature of soil molybdenum (Barshad 1951a,b, Davies 1956) have been shown to influence the uptake of molybdenum by plants, microorganisms have not yet been implicated. There are however three papers at least from which it might be inferred that microorganisms affect molybdenum availability to plants.

Mosse (1957) showed that mycorrhizal apple seedlings contained a higher concentration of molybdenum than non-mycorrhizal plants, and Donald, Passey and Swaby (1952) showed that in deficient acid-washed quartz crystals microorganisms could fix various major and minor elements including molybdenum. In addition, Davies, Holmes and Lynch (1951) found that molybdenum added as fertiliser remained in the top inch of soil for a considerable time. Because the top three inches of soil contains the greatest number of microorganisms (Alexander 1961) and is also a region of high root density, it seems reasonable to speculate that interactions between microorganisms and plant roots may lead to variation in molybdenum concentration in plants.

Investigation into such possible interactions arose from a dental health survey.

Healy, Ludwig and Losee (1961) in the course of a dental investigation in the Hawke Bay area of New Zealand found that vegetables grown in Napier soils contained higher concentrations of aluminium, molybdenum and titanium and lower concentrations of barium, copper, manganese and strontium than vegetables grown in Hastings soils. The differences in molybdenum concentration were particularly marked and the suggestion was made that molybdenum might influence the incidence of dental caries in these areas. Further, the differences in the concentrations of the various elements in the vegetables were thought to be due to soil differences. The Napier and Hastings soils are derived from similar parent materials and contain comparable amounts of most elements including molybdenum (Healy et al. 1961) but there is a difference between them since Napier soils have a recent marine history. A major earthquake occurred in the Hawke Bay area in 1931 and a land mass 60 miles by 10 miles was raised about nine feet at Napier (New Zealand DSIR Bulletin 1933). This area which was originally a lagoon is now used for market gardens and residential land (New Zealand DSIR Bulletin 1939).
This paper presents evidence to support the idea that microorganisms may affect molybdenum concentration in plants.

**MATERIALS AND METHODS**

A description of the area and chemical composition of the soils is given by Healy *et al.* (1961). The Napier market garden soil is described as an Ahuriri silt loam (pH 7.2) and that from Hastings as a Twyford sandy loam (pH 6.2) (New Zealand DSIR Bulletin 1954). The methods of soil sampling and preparation are given by Loutit and Loutit (1966). Where sterile soils were used, air-dried soils were sterilised by gamma irradiation (2.5 megarads).

All experiments were carried out under aseptic conditions and all water used was double-distilled, the second distillation being made in a glass still. The radish *Raphanus sativus* L. variety “White Icicle” was used as the experimental plant. Sound seed was surface sterilised and after germination on moistened glasswool was transferred to test-tubes (32 x 200 mm) containing 35 g of sieved air-dried soil. All experiments were carried out with the soil at 60% water holding capacity (WHC) and an aluminium cap on each tube ensured that water was not lost through evaporation. The tubes, in wooden racks, were transferred to a glass-house where temperatures were within the range 10°C to 35°C.

Chemicals added to the soil were reagent grade only (British Drug Houses Ltd., Analar Grade) and the following additives were prepared:

(a) Sodium molybdate (Na$_2$MoO$_4$·2H$_2$O) solution to give 10$^{-3}$ Mo/ml.

(b) A solution of carbon, phosphorus, nitrogen and sulphur (C, P, N and S) to give in each 35 g of soil, C (2% glucose), P (0.04% KH$_2$PO$_4$ adjusted to pH 6.95 with KOH), N (0.08% NH$_4$NO$_3$) and S (1.6 x 10$^{-3}$% MgSO$_4$·7H$_2$O). These proportions were suggested by the work of Stotzky and Norman (1961a, b, 1964).

(c) Where Mo was added with C, P, N and S, sodium molybdate was incorporated in the above mixture to give a Mo concentration of 10$^{-3}$ Mo/ml.

In all experiments plants were grown for four weeks and harvested by cutting the stem at the base. The tops were washed in sterile water to remove any soil particles, blotted and placed in weighed Petri dishes, dried at 80°C and weighed when cool. The material was ashed at 540°C and the molybdenum determined by the method of Healy (1964) using dithiol.

Estimates of numbers of bacteria and fungi in rhizosphere and soil away from plant roots were made by the dilution-plate count technique. Soil extract agar was used as the plating medium for bacteria and acidified Czapek-Dox agar for fungi.
MICROORGANISMS AND MOLYBDENUM

EXPERIMENTAL

The radish was chosen as an experimental plant as the smooth-coated seeds were easily sterilised and germinated rapidly. Seeds were sterilised to prevent the seed coat microflora from influencing the rhizosphere population.

One batch of seed was used for all experiments since Buxton (1957 a, b) showed that strains of the same plant species produced different effects on the rhizosphere and Rao (1962) showed that under identical growth conditions the rhizospheres of different varieties of peanuts were significantly different.

Environmental conditions were controlled as far as possible because cultural conditions affect both the organisms in the bulk of the soil and the rhizosphere. Taylor (1936) demonstrated that environmental factors influenced soil organisms and Rovira (1959) showed that cultural conditions affected root exudates which would of course influence the rhizosphere population. More recently Rouatt and Katznelson (1960), Rouatt, Peterson, Katznelson and Henderson (1963) and Peterson, Rouatt and Katznelson (1965) have shown that light, temperature and soil moisture affect rhizosphere organisms.

Radishes were grown in sterilised and unsterilised soil from Napier and Hastings. Sodium molybdate was added to some tubes and water where necessary, to bring the soils to 60% WHC. In addition the soil microbial population was stimulated in some tubes by adding carbon, phosphorus, nitrogen and sulphur to see if this would affect the molybdenum (Mo) concentration in the plants.

In these experiments routine examination of soil pH at the end of the experimental period showed that addition of C, P, N and S to unsterilised Hastings soil resulted in a rise in pH from pH 6.2 to between 6.5 and 6.7. No such increase occurred in sterile soils and there was little change in Napier soil. The effect of pH on molybdenum concentration in plants was therefore investigated. The pH of the Hastings soil was adjusted from pH 6.2 to that of Napier (pH 7.2) by the addition of a pre-determined amount of sterile calcium carbonate to sterilised and unsterilised Hastings soil.

RESULTS AND DISCUSSION

Results in Table 1 indicate that the same variety of plant grown in unsterilised Napier and Hastings soils under comparable conditions had a higher concentration of Mo when grown in Napier soil. This repeated in the laboratory the findings of Healy et al. (1961). Addition of Mo as sodium molybdate solution emphasised this difference.

Of interest was the result that changes in Mo concentration in the plants were not accompanied by any changes in dry weights (P > 0.05). Molybdenum was not limiting in Hastings soil so as to cause deficiency symptoms in the plants. Thus we have a system for investigating the effect
<table>
<thead>
<tr>
<th>Place</th>
<th>Treatment</th>
<th>Napier</th>
<th></th>
<th>Hastings</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mo¹ added</td>
<td>No Mo added</td>
<td>CPNS added</td>
<td>No CPNS added</td>
<td>CPNS added</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo² added</td>
<td>No Mo added</td>
<td>CPNS added</td>
<td>No CPNS added</td>
<td>CPNS added</td>
</tr>
<tr>
<td>Unsterilised soil</td>
<td>CPNS added</td>
<td>201²</td>
<td>10</td>
<td>11</td>
<td>545</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>No CPNS added</td>
<td>369</td>
<td>12</td>
<td>11</td>
<td>595</td>
<td>260</td>
</tr>
<tr>
<td>Sterilised soil</td>
<td>CPNS added</td>
<td>255</td>
<td>8</td>
<td>12</td>
<td>43</td>
<td>678</td>
</tr>
<tr>
<td></td>
<td>No CPNS added</td>
<td>89</td>
<td>18</td>
<td>14</td>
<td>42</td>
<td>172</td>
</tr>
</tbody>
</table>

¹ Ten ml. of sodium molybdate solution (10 μg Mo/ml).
² Figures are the mean of two estimations on pooled samples of 3 plants.
³ Sterilised by gamma radiation.
of microorganisms on $Mo$ concentration in plants without necessarily producing changes in the plant metabolism and so introducing other variables.

The most convincing way to demonstrate that differences in $Mo$ concentration in plants were due to microorganism activity seemed to be to grow the plants in sterilised and unsterilised soil. Further if the microbial population could be stimulated by adding $C$, $P$, $N$ and $S$ to the soils one might expect changes in plant $Mo$ concentration. Experiments in which $C$, $P$, $N$ and $S$ were added to soils showed that microbial numbers increased both in the rhizosphere and non-rhizosphere soil, with the greatest increase occurring in Hasting soil treated with $C$, $P$, $N$, $S$ and $Mo$. Results in Table 1 indicate that in fact microorganisms influenced $Mo$ concentration in plants but that the results differed for plants grown in Napier and Hastings soils.

The addition of $C$, $P$, $N$ and $S$ to unsterilised Napier soil did not alter the concentration of $Mo$ in the plants. If $Mo$ was added as well as $C$, $P$, $N$ and $S$ the concentration of $Mo$ in the plants was less than if $Mo$ was added alone. Similar trends were obtained for sterile soil but apparently microorganisms had some influence on the $Mo$ concentration in plants as the results differed for sterilised and unsterilised soil. Increase in numbers of organisms following addition of $C$, $P$, $N$, and $S$ however was of no great importance as the effect of the addition was similar in sterilised and unsterilised soils. The reduction of $Mo$ concentration when $Mo$ was added with $C$, $P$, $N$ and $S$ was probably due to factors other than microorganisms.

Examination of dry weights of plants grown in unsterilised and sterilised Napier soil showed that the addition of $C$, $P$, $N$ and $S$ produced different effects. No increase in dry weights ($P > 0.05$) occurred if $C$, $P$, $N$ and $S$ were added to unsterilised soil with and without the addition of $Mo$. If $C$, $P$, $N$ and $S$ were added to sterilised soil an increase in dry weights ($P < 0.001$) occurred independently of the addition of $Mo$. Presumably in unsterilised soil the microorganisms used the added substances before they could be used by the plant, and this has been shown in subsequent experiments. In Napier soil then, it appeared that microorganisms affect the concentration of $Mo$ in plants but an increase in the microbial population following the addition of $C$, $P$, $N$ and $S$ did not affect the $Mo$ concentration in the plants and microbial factors were not of importance in affecting the concentration of $Mo$ in the plants.

The addition of $C$, $P$, $N$ and $S$ to Hastings soil gave very different results. Following the addition of $C$, $P$, $N$ and $S$ to unsterilised soil an increase in $Mo$ concentration occurred in plants whether $Mo$ was added or not (Table 1), although the effect was more marked if extra $Mo$ was provided. If, however, plants were grown in sterile soil to which $C$, $P$, $N$, $S$ and $Mo$ had been added the results were quite different. Instead of an increase in $Mo$ concentration the concentration was less than if $Mo$ was added alone. This was a similar result to that obtained for sterilised and unsterilised Napier soil. The obvious differences in results for sterilised and
unsterilised Hastings soils indicated that microorganisms influenced the $Mo$ concentration in plants but that in sterilised soil other factors influenced $Mo$ uptake.

Examination of dry weights of plants grown in Hastings soil proved interesting. No increase in dry weights of plants ($P > 0.05$) occurred in unsterilised soils if $C, P, N$, and $S$ were added with and without $Mo$ indicating that $Mo$ was in no way limiting. In sterile soil however the addition of $C, P, N$, and $S$ together with $Mo$ caused an increase in dry weight ($P < 0.001$). If $C, P, N$, and $S$ were added without $Mo$ the dry weights fell ($P < 0.01$). The results indicated that in sterile soil, in the absence of added $Mo$ but with $C, P, N$, and $S$ present, some non-microbial factors caused a decrease in dry weight.

There appear to be two ways at least in which microorganisms may affect $Mo$ concentration in plants if grown in Hastings soil. Microorganisms apparently prevent some $Mo$ entering the plant but an increase in microbial numbers following addition of $C, P, N$, and $S$ ensures that more $Mo$ accumulates in the plant rather than less. That this is a microbiological effect is deduced from the fact that no such result is obtained in sterilised soil. It is probable that microorganisms also freed some $Mo$ held in the soil, because addition of $C, P, N$, and $S$ resulted in increased $Mo$ concentration in plants grown in unsterilised soil but not in those grown in sterilised soil. A possible explanation is that the increase in $pH$ from 6.2 to 6.5-6.7 which occurred in unsterilised soil to which $C, P, N$, and $S$ had been added may have affected $Mo$ availability. Microorganisms may have produced substances which resulted in an increase in $pH$. No such effect was noted in sterilised soil.

From experiments in which the $pH$ of the Hastings soil was adjusted to that of Napier soil (Table 2) it became obvious that $pH$ adjustment was not the only factor regulating $Mo$ concentration in plants, as the results were not comparable in sterilised $pH$ adjusted Hastings and Napier soil. The results indicate that differences in soil $pH$ are important in explaining why Napier vegetables contain a higher concentration of $Mo$ than those from Hastings. The results do establish however that microbiological factors influence $Mo$ concentration in plants and under the conditions of the Hastings soil microbiological factors are extremely important.

The results are of significance because, although adjustment of $pH$ by liming may increase the availability of $Mo$ to plants, the balance of other trace elements in soil may be upset by such a practice (Schütte 1964). If $Mo$ concentration in plants can be affected by factors other than $pH$ then it is important to study these factors. The role of microorganisms in affecting $Mo$ concentration in plants may explain in part the observation that addition of sodium molybdate to an acid soil does not always result in the $Mo$ becoming unavailable. Cunningham and Hogan (1949) found a persistent and high increase in $Mo$ concentration in plants grown in an acid soil to which sodium molybdate had been added. Mitchell (1955)
### Table 2

MOLYBDENUM CONCENTRATION (ppm DRY WEIGHT) AND DRY WEIGHT (g PER PLANT) OF RADISHES GROWN IN NAPIER AND HASTINGS SOIL (WITH AND WITHOUT ADJUSTMENT OF pH)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Sterilised(^1)</th>
<th>Unsterilised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mo(^a) added</td>
<td>No Mo added</td>
</tr>
<tr>
<td>Napien (pH 7.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo Conc.</td>
<td>1201(^a)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1205</td>
<td>17</td>
</tr>
<tr>
<td>D.W.</td>
<td>0.0168(^a)</td>
<td>0.0100</td>
</tr>
<tr>
<td></td>
<td>0.0118</td>
<td>0.0122</td>
</tr>
<tr>
<td>Hastings (pH adjusted to 7.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo Conc.</td>
<td>857</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>747</td>
<td>7</td>
</tr>
<tr>
<td>D.W.</td>
<td>0.0097</td>
<td>0.0091</td>
</tr>
<tr>
<td></td>
<td>0.0122</td>
<td>0.0119</td>
</tr>
<tr>
<td>Hastings (Unadjusted pH 6.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo Conc.</td>
<td>266</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>445</td>
<td>2</td>
</tr>
<tr>
<td>D.W.</td>
<td>0.0118</td>
<td>0.0073</td>
</tr>
<tr>
<td></td>
<td>0.0107</td>
<td>0.0170</td>
</tr>
</tbody>
</table>

\(^1\) Sterilised by gamma radiation.
\(^\) Ten ml. of sodium molybdate (10y Mo/ml).
\(^3\) Figures are the mean of 2 estimations on pooled samples of 3 plants.
\(^4\) Figures are the mean weight of 3 plants.

in discussing the high Mo content of plants grown in an acid soil suggested that Mo might form an organic complex in the soil preventing the fixation of Mo in an unavailable form. Part of the organic complex could be Mo in microbial cells. Microbiological factors may also explain in part why addition of Mo to a pasture results in only some pasture species showing an increase in Mo concentration.

Any information that can be obtained about factors influencing Mo concentration in plants must surely be important if variation in Mo content is likely to affect not only dental health as has been suggested (Healy et al. 1961) but animal health.

**ACKNOWLEDGMENTS**

This work was supported, in part, by a grant from the New Zealand Medical Research Council to the Dental Research Unit. I am indebted to Dr. W. B. Healy of the Soil Bureau, D.S.I.R., and to Dr. R. S. Malthus of the Nutrition Research Unit, Medical School, University of Otago, for assistance with molybdenum estimations.
REFERENCES

Healy, W. B. (1964)—Determination of molybdenum in biological materials such as teeth, bone, liver, urine and water using dithiol. New Zealand, D.S.I.R. Soil Bureau Rep. 3.
Schütte, K. H. (1964)—“The biology of the trace elements, their role in nutrition”. [Crosby Lockwood, London].
An experimental system has been established using the radish *Raphanus sativus* L. variety “White Icicle”, which allows the investigation *in vitro* of the effect of soil microorganisms on the availability of molybdenum to plants.

The system has been used to study the reason why vegetables grown in two soils, derived from similar parent materials and containing comparable amounts of total molybdenum, contain different concentrations of molybdenum. In one soil microorganisms have a marked effect on the molybdenum concentration in the plants, while in the other soil non-microbial factors are of greater importance in influencing molybdenum concentration.

**Résumé**

Un système expérimental a été mis au point en employant la variété de radis “White Icicle” (*Raphanus sativus* L.), qui permet l’investigation *in vitro* de l’effet des microorganismes du sol sur la disponibilité du molybdène pour les plantes.

On a utilisé ce système pour étudier les raisons pour lesquelles les légumes cultivés sur deux sols dérivés de matériaux originaires semblables et contenant des quantités comparables de molybdène total, contiennent des concentrations de molybdène différentes. Dans l’un des sols, les microorganismes ont un effet prononcé sur la concentration du molybdène dans les plantes, tandis que dans l’autre sols, les facteurs non-microbiens exercent une plus grande influence sur la concentration du molybdène.

**Zusammenfassung**


THE BACTERIA OF DECOMPOSING CEREAL STRAW, AND THEIR EFFECTS ON PLANT GROWTH

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INTRODUCTION

Straw toxicity is frequently recorded when cereal crops are seeded into soils containing recently incorporated stubble or where heavy mulches remain partly-decomposed on the surface. The causes have been attributed to microbial activities, either by tie-up of nitrogen or sulphur, by production of phytotoxic antibiotics, or by release of polyphenols and organic acids from straw. But relatively little is known about the ability of specific organisms to induce the toxic effects.

Although deleterious effects associated with the growth of grasses, particularly upon horticultural crops, were described many years ago, Collison (1925) was one of the first to report inhibitory substances in wheat straw. Nevertheless nitrogen tie-up was regarded as the major component of these effects upon growth until there was a clear-cut demonstration by Benedict (1941) that dead roots of brome-grass were a source of chemical inhibitors. The identity of phenolic phytotoxins from cereals came from the identification of ferulic, p-coumaric, vanillic, syringic and p-hydroxybenzoic acids in aqueous and ethanolic extracts of straw, plant parts and cereal soils. Phenolcarboxylic acids have now been demonstrated to occur in other plant residues and to persist in soils in amounts sufficient to induce phytotoxicity. But whilst these toxic compounds occur in straw, the conditions under which decomposition proceeds are important. Mishustin and Erofeev (1966) stress that it is only under anaerobic conditions that the phenolcarboxylic acids themselves are released and not further decomposed.

A different sort of toxic compound is that synthesised by microorganisms, and here most attention has been paid to the antibiotics formed by fungi, with emphasis upon gliotoxin, patulin and citrinin. There may be many others formed in soils, but difficulties with extraction procedures may have nullified their demonstration (Soulides 1964).

The third sort of growth-inhibiting substance which is formed by microorganisms is the suite of organic acids produced under anaerobic conditions. Studies on the physiological “Akiochi” disease showed that butyric and acetic acids formed from decomposition of organic materials and green manure crops inhibited root growth of rice seedlings; organic acids have also been shown to inhibit the growth of sugar cane. Samtsevich and Borisova (1963) have also drawn attention to production of volatile sub-
stances by soil microorganisms which inhibit root elongation of winter wheat.

The activities of microorganisms on buried cereal straw may thus result in phytotoxicity in a variety of ways, apart from considerations of immobilization of plant nutrients during the decomposition process. The study of wheat straw decomposition in a buried layer under South Australian conditions has shown that phytotoxicity develops rapidly and is acute within three or four days (Harris—unpublished data). During this time, there is intensive activity of a rapidly-developing bacterial flora, and the source of toxicity has been sought as products of bacterial metabolism.

**THE EFFECTS OF STRAW ON PLANTS**

The decomposition of a layer of wheat straw buried in soil proceeds as it does because there is a characteristic ecological environment. Although cereal straws have high cellulose and low nitrogen contents, these do not influence the course of decomposition as much as their content of readily-available soluble substrates which support the rapid bacterial attack on them and give rise to the proliferation of a population of large size.

Experimentally, straw decomposition was studied by incorporating layers of amounts equivalent to nil, 550, 1100, 1650 and 2200 lb/ac in a coarse river-sand, watering up with plant nutrient solution, then seeding with oats, lucerne and subterranean clover as test plants. Decomposition commenced when the pots were watered up with plant nutrient solution to an estimated field capacity. The pots sown to cereal received a complete plant nutrient solution containing 10 m-equiv/1 nitrate-nitrogen; the pots with legumes received nitrogen-free solution. The cultivars used were

| Equivalent straw level | Lucerne | | Clover | | Oats | |
|------------------------|---------|----------------|---------|----------------|---------|
|                        |         |                |         |                |         |
| Nil                    | 3700    | 4566           | 6169    | 2295           | 4955    |
| 550 lb./ac             | 2287    | 2279           | 2161    | 785            | 1852    |
|                        | (38-3%) | (50-1%)        | (65-0%) | (65-8%)        | (63-2%) |
| 1100 lb./ac            | 1245    | 1401           | 1746    | 644            | 1505    |
|                        | (66-4%) | (69-3%)        | (71-7%) | (72-0%)        | (69-6%) |
| 1650 lb./ac            | 719     | 739            | 1252    | 524            | 1282    |
|                        | (80-6%) | (83-8%)        | (79-7%) | (77-2%)        | (74-1%) |
| 2200 lb./ac            | 418     | 631            | 779     | 342            | 1006    |
|                        | (88-7%) | (86-2%)        | (87-4%) | (85-1%)        | (79-7%) |
|                        |         |                | (58-2%) |                |         |

**Table 1**

REDUCTION IN DRY MATTER PRODUCTION OF LUCERNE, SUBTERRANEAN CLOVER AND OATS CAUSED BY LAYERS OF WHEAT STRAW AT DIFFERENT RATES

Mean weights in mg per pot and percentage reduction
BACTERIA OF DECOMPOSING STRAW

"Early Burt" oats, "Hunter River" lucerne and "Yarkoop" subterranean clover. The legume seeds received rhizobial inoculation with an appropriate mixture of three effective strains of *Rhizobium meliloti* or of *Rhizobium trifolii*.

Toxicity produced during decomposition of the straw layer had severe effects upon the growth of all three test plants. Both legumes show particularly severe effects, and their sensitivity to toxicity is much greater than that of the cereal (oats). The data of Table 1 for plants harvested after ten weeks show that a severe percentage reduction in both tops and roots occurs even at the lowest straw level (550 lb/ac) for the legumes, whereas higher levels were necessary to produce a similar order of reduction in oat roots. Nevertheless, the effect on oat tops is large and is severe enough to be of serious consequence. It was noted that roots of the two legumes would not penetrate the straw layer, whereas some degree of penetration did take place with oat roots, although roots in the straw were discoloured and showed considerable numbers of necrotic lesions, as have been reported by Carley and Watson (1967).

MICROBIAL ACTIVITY IN STRAW

In parallel series of pots, the straw environment was monitored by inserted electrodes to give readings of reaction (*pH*) and oxidation-reduction potential (*Eh*) on a Jones *pH* meter, and oxygen tension on a Beckman oxygen analyser with 24 hour chart recorder. The pots were set up dry with the electrodes set vertically in the straw layer; the readings commenced with watering-up. When the wheat straw was layered in sand, a complete plant nutrient solution was used; when the straw layer was in a red-brown earth soil (typical of many cereal soils in South Australia) watering-up was carried out with distilled water.

The first noticeable effects begin about 6 hours (at 25°C) after watering up, when the oxygen tension begins to decrease. By 20 hours it has fallen gradually from $pO_2 = 160$ mm to about 100 mm, but from 24 to 30 hours the fall is precipitous to $pO_2 = 20$ mm and beyond. During this period the *pH* remains in the 6-2-6-7 range; the oxidation-reduction potential moves from +190 mV to +80 mV; temperature rise does not usually exceed 0.5°C. Between 30 and 48 hours there is an onset of anaerobic conditions when $pO_2$ stabilizes at less than 2 mm (and is no longer accurately recorded on this equipment), the reaction becomes more neutral, usually stabilizing near *pH* 7.2, but may rise temporarily to *pH* 7.7 during peak carbon dioxide production; the oxidation-reduction potential may temporarily become as low as -120 mV, but usually stabilizes close to 60 mV. Anaerobic to microaerophilic conditions will persist for many days (it has been followed for up to 42 days) as long as the straw layer remains moist. A rise above $pO_2 = 10$mm is indicative of lack of moisture. Gas samples from the straw layer removed via a capillary and injected into a mass spectrometer often showed no oxygen peak at all, but high levels of carbon dioxide often exceeded 12%. Similar order values are obtained from soils as light as coarse river sands where oxygen
diffusion rates would be high, or as heavy as clay loams of poor structure. It would seem that the straw environment characterizes these data rather than soil in which the layer is held. The straw is usually holding about 60% moisture content, and is surprisingly impervious to water movement through it.

The early stages of straw decomposition are seen to be carried out by a microflora which is facultatively anaerobic and dominated by aerogenic fermenters. In this way the initially aerobic flora consumes oxygen at a rate greater than that replenishable by diffusion, and the vigorous evolution of carbon dioxide assists in flushing away incoming gases. The rapid consumption of the gaseous oxygen enforces further decomposition processes to be carried out via an essentially anaerobic route. Thus the conditions of toxicity postulated by Mishutin and Erofeev (1966) usually apply to straw decomposed between soil layers.

**CHARACTERIZATION OF THE MICROORGANISMS**

Decomposing straw sampled 7 days after the onset of microbial action supported a bacterial population in excess of $10^9$ organisms per g net weight. Straw was diluted with sterile filtered tap-water in a hundred-fold dilution series to at least $10^{-5}$, then two-fold to $10^{-12}$ in MacConkey broth and a glucose-wheat straw-nutrient gelatin medium in tubes. Where growth occurred, the higher dilution positive tubes were plated out on nutrient agar, potato-dextrose agar and medium B of King, Ward and Raney (1954). A range of selected colonies were replated and purified for examination by standard morphological, physiological and biochemical test procedures in order to identify them. Representative strains were studied for their abilities to produce antibiotics and to produce inhibitory effects upon plant growth and seed germination.

The early stages of decomposition are dominated by representatives of three bacterial families, the Enterobacteriaceae, Achromobacteriaceae and Bacillaceae. The aerogenic fermenters dominate early stages, and bacteria resembling *Enterobacter (Aerobacter) cloacae* are responsible for vigorous gas production. They may also be important in lowering the oxidation-reduction potential (Fulde and Fabian, 1955). The other common representative of the Enterobacteriaceae is the anaerogenic yellow-pigmented *Erwinia (Xanthomonas) herbicola*, a group of strains similar to those described by Graham and Hodgkiss (1967) from plants. The *cereus-megaterium* group of the genus *Bacillus* are the spore-forming representatives of the carbohydrate-attacking bacteria. All these organisms strongly reduce nitrate to nitrite. Two groups of the Achromobacteriaceae which do not give strong oxidizing reactions on sugars may be referred to the genera *Alcaligenes* with white to creamy colonies and *Flavobacterium* with yellow colonies. In addition to these five main groups, a number of others were commonly encountered, viz. members of *Micrococcus, Sarcina, Bifidobacterium, Pseudomonas* and *Streptomyces*, but numbers were never large. Conspicuous absentees were anaerobic clostridia.
Fig. 1.—Inhibition of germination of lucerne and subterranean clover seeds by culture fluids of bacterial isolates from straw growing on a straw infusion medium.
ANTIBIOTIC ACTIVITY OF ISOLATES

A range of isolated bacteria were tested for activity against five test organisms using filter-paper discs saturated with culture filtrate of organisms grown on straw infusion. The test organisms were: *Sporosarcina ureae, Serratia marcescens, Pseudomonas fluorescens, Bacillus brevis* and *Sarcina flava*; zones of antibiotic activity were not found for any of the tested isolates. It is presumed that production of antibiotics by bacteria does not account for straw toxicity under our conditions.

INHIBITION OF SEED GERMINATION

Twenty-three isolated bacteria were inoculated into wheat straw infusion in deep culture tubes. Seeds of lucerne or subterranean clover were set to germinate on filter paper saturated with culture filtrate. The results (after 5 days at 22°C) have been expressed as percentage inhibition of germination in Figure 1, from which it can be seen that most strains of bacteria were markedly inhibitory. The effects on subterranean clover (cultivar “Mt. Barker”) were extremely severe where almost all organisms completely suppressed germination. This was over-sensitive for bioassay purposes, but effects on lucerne (cultivar “Hunter River”) were also acute and general, with 30-40% modal inhibition.

INHIBITION OF ROOT ELONGATION

The closed dish assay developed by Parker (1964, 1966) and used by Horowitz (1966) and Eshel and Warren (1967) for bioassaying residual herbicide toxicity in soils provides the basis for a sensitive and elegant assay procedure for phytotoxicity. We used large Petri dishes (120 mm diam. x 15 mm depth) filled completely with 250 g coarse, washed river-sand, and dry-sterilized at 165°C for 2 hours. The “Hunter River” cultivar of lucerne used as test plant was surface sterilized (alcohol-peroxide) then set to germinate in Petri dishes on filter-paper moistened with sterile tap-water. Uniform germinated seeds with radicals 1 cm long were selected for testing.

Four bacteria were selected for testing for toxin production on three bacteriological media, viz. nutrient broth, 10% wheat straw infusion and 10% milled wheat straw infusion. Duplicate cultures of each organism were incubated for 7 days at 28°C in 24 x 150 mm culture tubes containing 30 ml medium. The supernatants (25 ml) were pipetted onto the sterile sand in large dishes to provide a soil culture of each test organism. Approximately three-quarters way across each dish, germinated lucerne seeds were set in line as a chord from which the radicals pointed to the far side of the dish. The seeds were gently pressed into the moist sand by applying the lid and securing it in place with packaging tape. The chord was ruled on the outside of the dish lid with a marking crayon, and the taped dish inverted to be held at an acute angle to the vertical with the chord horizontal; in this position the dishes were transferred to a darkened incubator (24°C). The length of root produced by each seedling was marked on the glass lid at 24-hour intervals for 5 days. Controls
Fig. 2.—Growth-response curves for lucerne roots in sand culture obtained by closed dish assay of four bacteria isolated from straw and showing the influence of culture medium on toxicity. SI = straw infusion; MW = milled wheat straw infusion; NB = nutrient broth.
were established using (i) sterile distilled water and (ii) sterile culture media. As far as possible, aseptic precautions were maintained throughout. Growth-response curves were plotted for controls and test organisms.

By use of this bioassay technique it was found that choice of the bacteriological medium was important, in that ordinary nutrient broth was quite toxic to lucerne roots, inducing more than 60% inhibition. Although the growth of test organisms in it increased inhibition to 90% (usually), care must be taken in interpreting data when there is such a high background toxicity. Sterile wheat straw infusion showed 20 to 25% inhibition; and when the straw was milled first, this could increase by another 10%, showing that even such simple media were not without toxic backgrounds, although this is not surprising in view of phenoliccarboxylic acid contents. But the growth of four test organisms in these media increased root inhibition up to more than 70%. Figure 2 shows that this was least for Bacillus megaterium and greatest for a Flavobacterium isolate. The type of growth-response curve for four organisms is shown in Figure 2, where the influence of the medium is clearly evident. Similar responses could be found for a range of bacteria isolated from straw and Figure 3 illustrates the effects of ten of them on lucerne roots. Subsequent experiments were carried out in straw infusion only.

Experiments with a wide variety of isolates from wheat straw showed that almost all of them were capable of a fair degree of root inhibition of lucerne. This is in keeping with the observations of the pot experiments, where lucerne roots did not penetrate the layer of rotting straw. Some

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**Fig. 3.**—Inhibition of root growth of lucerne by ten bacteria isolated from straw and grown in two culture media. The isolates are coded F = Flavobacterium spp.; P = Pseudomonas spp.; G = Gram positive bacteria (7 = Bacillus megaterium, 8 = Micrococcus luteus, 9 = Sarcina flava); E = Erwinia herbicola.
mixtures of organisms may also be more inhibitory than the individuals alone. This can be illustrated by Figure 4, where a mixed culture of *Bacillus megaterium* and *Flavobacterium* sp. is more inhibitory (54%) than either singly (34% and 40% respectively). Thus, straw toxicity appears to be due largely to selective environmental conditions favouring groups of facultatively anaerobic bacteria in the families Enterobacteriaceae, Achromobacteriaceae and Bacillaceae; and these tend to be toxicogenic to plants by causing inhibition of root growth.

**REFERENCES**

Summary

When cereal straw is incorporated into soil, toxic effects upon plant growth frequently follow. Although this has been attributed to a number of causes—especially to tie-up of plant nutrients, antibiotic production by fungi, as well as release of toxic phenol-carboxylic acids and organic acids—pot experiments under South Australian conditions have shown that phytotoxicity is associated with an early flush of bacterial growth supported by soluble constituents of the straw which were readily attacked. The decomposition of a straw layer in soil proceeds as an aerogenic fermentation supporting a suite of facultatively anaerobic bacteria dominated by members of the families Enterobacteriaceae, Achromobacteriaceae and Bacillaceae. The characteristics of typical isolates of Enterobacter cloacae, Erwinia herbicola, Alcaligenes denitrificans, Flavobacterium sp. and Bacillus megaterium are given. Most strains of these organisms were found to be toxigenic when grown on a simple straw infusion medium and shown to inhibit seed germination of lucerne and subterranean clover. Effects upon root growth of lucerne were demonstrated using a close dish assay procedure.

Résumé

Lorsqu'on incorpore de la paille de céréale dans le sol, souvent il s'ensuit des effets toxiques sur la croissance de la plante. Bien qu'on en ait tenu responsables diverses causes—surtout la non-disponibilité des substances nutritives de la plante, la production antibiotique par les champignons, ainsi que la libération d'acides toxiques phénolcarboxyliques et d'acides organiques—des expériences en pot sous les conditions de l'Australie Méridionale ont montré que la phytotoxicité est associée à une abondance, au départ, de croissance bactérienne soutenue par les constituants solubles de la paille qui avaient déjà été attaqués. La décomposition d'une couche de paille dans le sol se réalise sous la forme d'une fermentation aérogénique et soutient une série de bactéries facultativement anaérobiques dominées par des membres des familles Enterobacteriaceae, Achromobacteriaceae et Bacillaceae. On donne les caractéristiques d'isolées typiques de Enterobacter cloacae, Erwinia herbicola, Alcaligenes denitrificans, Flavobacterium sp. et Bacillus megaterium. On a trouvé que la plupart des espèces de ces organismes étaient toxigènes, lorsqu'on les cultivait sur un simple environnement de paille infusée et elles ont montré qu'elles inhibent la germination des graines de lucerne et du trèfle sotterrane. On a démontré les effets sur la croissance des racines de lucerne en se servant d'un plateau fermé comme procédure analytique.

Zusammenfassung

Wenn Getreidestroh im Boden aufgenommen worden ist, folgen häufig giftige Effekte auf das Pflanzenwachstum. Obwohl dies einer Reihe von Gründen zugeschrieben wurde—besonders der Verbindung von Pflanzenährstoffen, der antibiotischen Produktion durch Pilze, sowie der Frei-
lassung giftiger, phenolkarbozylnhaltiger- und organischer Säuren haben Gefäßversuche unter südaustralischen Bedingungen gezeigt, dass Phyto-
toxizität mit einer frühen Fülle bakteriellen Wachstums verbunden ist, das durch lösliche, leicht angriffbare Strohbestandteile unterstützt wird. Die Zersetzung einer Strohschicht im Boden wirkt wie eine aerogene Fer-
tmentation, welche ein Gefolge anpassungsfähiger anaerobe Bakterien, beherrscht von Mitgliedern der Familien Enterobacteriaceae, Achromo-
bacteriaceae und Bacillaceae, unterhält. Die Merkmale der typischen Isolate von Enterobacter cloacae, Erwinia herbicola, Alcaligenes denitrifi-
cans, Flavobacterium sp. und Bacillus megaterium werden gegeben. Die meisten Stämme dieser Organismen waren toxigenisch, wenn sie auf einem einfachen Strohinfusionsmedium gezogen worden waren und hemmten die Saatkeimung von Luzerne und Untergrundklee. Wirkungen auf das Wurzel-
wachstum von Luzerne wurden mit einem geschlossenen Gefäßprobever-
fahren demonstriert.
AUTOMATIC COLORIMETRIC METHODS FOR THE DETERMINATION OF NITROGEN IN DIGESTS AND EXTRACTS OF SOILS

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I. INTRODUCTION

This paper describes methods for determining ammonium-$N$ in Kjeldahl digests of soils, and for ammonium-$N$, nitrate-$N$, and nitrite-$N$ in soil extracts, using the Technicon AutoAnalyzer (Technicon Instruments Corp., Chauncey, N.Y.).

II. DETERMINATION OF AMMONIUM-$N$ IN SOIL DIGESTS

Soil samples are digested by the conventional macro-Kjeldahl method (Bremner 1965a) using either the reduced iron or the salicylic acid modifications to include nitrate-$N$. Copper must not be used as a catalyst (see below).

(a) The Method

Dialysis is used to remove hydroxides of iron and manganese that precipitate when excess sodium hydroxide is added to soil digests. A modification of Logsdon's procedure (Logsdon, 1960) is then used to

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Fig. 1.—Flow scheme and manifold for the determination of ammonium-$N$ in Kjeldahl digests. The symbols $\bigcirc$, $\bullet$ refer, respectively, to the upper and lower positions in the double end-blocks of the proportioning pump.
determine ammonium-N. The flow scheme and manifold are summarized in Figure 1.

Reagents—
Analytical grade materials were used, except for the sodium hypo­
chlorite.
Sodium hydroxide solution, 2.5N.
Sodium phenate solution—250 g of phenol were dissolved in 1 litre of 5N
sodium hydroxide solution and made up to 4 litres with distilled water.
Ethanol solution, 25% v/v, aqueous, containing 2 drops of Tween 20
wetting agent per litre.
Alkaline hypochlorite solution—This was prepared by mixing equal volumes
of a commercial preparation of sodium hypochlorite containing 9-10% w/v free chlorine, and 5N sodium hydroxide solution.
Standard ammonium sulphate solution—4.7168 g of ammonium sulphate
dissolved in 1 litre of 4N sulphuric acid gave a solution containing
1000 ppm of ammonium-N, from which the calibrating solutions were
prepared by dilution.

The purpose of the ethanol reagent is to increase sensitivity (Crowther
and Large, 1956). Sodium hydroxide is included in the hypochlorite
reagent to raise the alkalinity to the optimum for colour development
(pH 13.1). The sensitivity of the reaction is increased by using a higher
concentration of sodium hypochlorite (see also Namiki, Kakita, and Gotô,
1964) than that used by Logsdon (1960). We observed a marked drift
in the calibration curve when the alkaline hypochlorite solution was too
dilute. We also confirmed that the sensitivity could be increased further by
using a higher concentration of sodium phenate (Varley, 1966).

The concentration of alkali in the sample stream after mixing the
sodium hydroxide solution with the diluted digest is important. If the stream
is too alkaline (above about 2N sodium hydroxide) the dialyser membrane
is rapidly destroyed; if it is acid, hydroxides precipitate when the recipient
stream is reacted with alkaline sodium phenate and hypochlorite. The
concentration of acid in the diluted digests and standard solutions should be
between 2N and 4N. It was found that absorbance increased when the acid
concentration was below 2N and decreased markedly as the acid concen­
tration was increased from 4N to 7N. Sulphuric acid (2-4N) should be
pumped into the sample stream at all times to avoid passing concentrated
sodium hydroxide solution through the dialyser. For the same reason the
interval between pumping from successive tubes on the turntable should be
as short as possible. Solution is pumped from each tube for 2.3 minutes,
with 0.2 minutes between tubes. Thus about 24 solutions can be deter­
mined per hour.

A reproducible proportion (about 30%) of the ammonium-N in the
sample stream passes to the recipient stream. The rate of dialysis tends
to fall slowly during the first ten hours of operation of a new membrane;
thereafter it is stable. An average figure for the life of a membrane is 60
hours of running time.
Possible interferences were studied using a mixing device that provides a solution of ammonium-N in which the concentration of an interfering substance increases linearly with time (Lindquist, 1968). The threshold concentrations of interfering substances can thus be determined. No interference was detected from potassium sulphate, sodium sulphate, selenium, or iron and manganese sulphates, at the concentrations found in digests prepared by the salicylic acid or reduced iron methods. Copper sulphate reduces the permeability of the dialysis membrane and interferes with colour development (at about 12 ppm of copper in the recipient stream).

The volume of the diluted digests must be corrected because of insoluble soil residues and precipitated salts. For example, with a sandy loam soil, where the whole digest including sand was transferred to a 200 ml volumetric flask, the mean volume of supernatant was found to be 195.8 ml (coefficient of variation 0.13%). With another sandy loam soil, this time leaving the coarse sand in the digestion flask, the volume of supernatant was 198.8 ml (coefficient of variation 0.17%).

The timing system is set to give wide, flat-topped peaks on the recorder chart. This is done so that short-term fluctuations in the trace can be readily observed and disregarded in reading the peak height. The other advantage in working with a wide, flat-topped peak is that the effect of carry-over from a previous sample can be detected and ignored in reading the peak height. Neither of these requirements are satisfied by rounded or pointed peaks.

Normally, two calibration curves are constructed for each batch. The unknowns are read from a curve covering the range 0-160 ppm of N; the optimum range for reading from the curve (Ayres, 1949) is 40-160 ppm. The digestion blanks are read from a second calibration curve covering the range 0-5 ppm.

(b) Results

The method has been used over a two-year period in a study of soil-N changes under pastures. The samples were run on the AutoAnalyzer in batches of 36 solutions. Each batch contained 22 samples, 2 digestion blanks, 9 calibrating solutions (ammonium sulphate), 1 standard digest, plus two other ammonium sulphate solutions placed at the beginning and end of each run to measure drift. The remaining solutions, including the calibrating solutions, were placed in random order on the turntable. The standard digest was taken from a bulk sample of soil digests prepared at the beginning of the two-year period. Its function was to measure long-term time trends in the procedure, but no significant trends were detected. However, there were random errors. During the two-year period the standard digest was run on 90 different occasions and the coefficient of variation per unit was 0.6% on a mean of 88.5 ppm of ammonium-N. Duplicate readings on a random sample of unknowns, repeated in different batches, also had a coefficient of variation of 0.6%. For comparison, duplicate digestions on sub-samples of the same soil, run at least one day apart, had a coefficient of variation of 2.4%. This variation includes sub-
sampling error (10 g sub-samples from 200 g of soil plus roots ground < 2 mm) and digestion error.

These errors are not likely to be important in analysing soil samples from field experiments. The figures in Table 1 were calculated using data from a sandy loam soil under pasture in Central Queensland, which contained 1680 kg N/ha in the top 15 cm (Vallis, unpublished results). The coefficient of variation between field samples, each composed of 8 cores, 5 cm in diameter, to a depth of 15 cm, was 9.0%. The coefficient of variation between analyses was 2.4%.

For this kind of experiment there is little point in doing more than one analysis per field sample, or in trying to improve precision in the laboratory (which has the same effect on confidence limits).

### III. Determination of Mineral-N in Soil Extracts

Procedures have also been developed for the determination of ammonium-N, nitrate-N, and nitrite-N in soil extracts. Hitherto it has not been possible to determine all three forms of nitrogen on a single extract, except by distillation (Bremner and Keeney, 1966). The methods described below have been used mostly for extracts prepared with 0.5N K₂SO₄ solution, but they can also be used for extracts made with N K₂SO₄ and N KCl. Filtration is unnecessary if the suspensions are allowed to settle before transferring the supernatants to the sample tubes.

(a) **Ammonium-N**

The flow sheet is a modified version of the one shown in Figure 1. Dialysis is not required for the soil extracts that we have used and the sequence of adding the reagents is different (this latter change was made chiefly so that the manifold could also be used for acid extracts). A segmented stream of 2.5N sodium hydroxide receives the sample solution (pumped at 1.2 ml a minute), ethanol reagent, and sodium phenate, and is mixed before the sodium hypochlorite is introduced. To prevent precipitation of hydroxides in alkaline solution, sodium citrate (1% w/v, analytical reagent grade) and tartaric acid (2% w/v, analytical reagent grade) are included in the sodium hydroxide reagent (Dabin, 1966). These reagents also increase the colour intensity.
DETERMINATION OF NITROGEN

The calibration curve is determined with solutions containing 0.25 ppm of ammonium-N; the optimum range for reading is 5-25 ppm. The possibility of interferences was studied by adding ammonium-N to soil extracts (0.5N K₂SO₄). A mean recovery of 99.4% was measured, with a coefficient of variation between duplicate readings on the same extract, run in the same batch, of 0.3%. Comparison of results obtained by this procedure and by distillation (Bremner and Keeney, 1966) on a batch of 16 soil extracts showed a slightly higher mean value for the AutoAnalyzer procedure (3.13 ppm ammonium-N versus 2.95 ppm, the difference being significant at \( P < 0.01 \)).

(b) Nitrate-N

In this method, nitrate is reduced to nitrite and the nitrite is determined colorimetrically by the Griess-Ilosvay procedure. An outline of the flow scheme is shown in Figure 2. It is similar to those described by Britt (1962), Henricksen (1965) and Terrey (1966), but differs in the use of dialysis and in the use of disodium pyrophosphate to reduce interference by calcium and magnesium (Lindquist, unpublished results). The use of dialysis for determining nitrate in blood has been reported by Litchfield (1967).

Reagents—
Analytical-grade materials were used.
Sodium hydroxide solution, 0.1N, containing 0.5% w/v disodium pyrophosphate.
Copper sulphate solution—0.05 g dissolved in a litre of distilled water.
Hydrazine sulphate solution—0.6 g dissolved in a litre of distilled water.
The acetone, sulphanilamide, and \( N-(1\text{-naphthyl}) \) ethylene diamine reagents were prepared as described by Terrey (1966).
Use of the gradient mixing device showed that interference becomes significant (> 1% fall in transmittance) at about the following concentrations: calcium 2000 ppm, magnesium 380 ppm, copper 20 ppm. Nitrite interferes and must first be destroyed with sulphanic acid, as described by Bremner and Keeney (1966). Urea may also interfere, since it reduces nitrite to \( N_2 \) (Bremner, 1965b).

Each solution is pumped for 2-8 minutes with 0-2 minutes delay between samples. Solutions of potassium nitrate in extracting solution (e.g. 0-5N \( K_2SO_4 \)) are used to establish the calibration curve (1-12-5 ppm N) which is read most precisely in the range 2-12-5 ppm. The recovery of nitrate-N added to extracts of various soils (not kraznozems) averaged 98-7%, with a coefficient of variation between duplicate readings on the same extract, run in the same batch, of 0-5%. Two kraznozem soils gave only 95% recovery of nitrate-N. A comparison of the Auto-Analyzer method with distillation (Bremner and Keeney, 1966) on 16 soil extracts showed mean values of 16-43 and 15-62 ppm nitrate-N respectively. The difference was significant (\( P < 0.01 \)).

(c) Nitrite-N

The flow scheme for determination of nitrite-N is as shown in Figure 2, except that the hydrazine sulphate and acetone reagents are replaced with distilled water. Nitrate at five times the concentration of nitrite does not interfere significantly. The sensitivity of the method is about twice that for nitrate-N, and the calibration curve is read most precisely in the range 1-6 ppm nitrite-N. The average recovery of nitrite-N added to soil extracts was 101-0%.

IV. ACKNOWLEDGMENTS

This work was financed by the Australian Meat Research Committee. The authors also acknowledge the advice and assistance received from Dr. M. P. Hegarty, Dr. A. E. Martin, and Mr. M. F. Robins, who suggested several features used in the manifold for ammonium-N.

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DETERMINATION OF NITROGEN

SUMMARY

The paper describes methods for the determination of nitrogen in soil digests and soil extracts, using the Technicon AutoAnalyzer.

Digests: Diluted Kjeldahl digests (containing 0-160 ppm N) are reacted with sodium hydroxide and dialysed against water. Ammonium-N in the recipient water stream is determined colorimetrically. About 24 solutions can be determined per hour of running time.

In routine use over a two-year period the coefficient of variation between duplicate determinations on the same digest was 0·6%, and between duplicate digestions on the same soil sample 2·4%. Results are quoted which show that the limits of detection of soil-N changes in field experiments are set by the variation between field samples, not in the analytical techniques.

Extracts: Methods are described for the determination of mineral-N in unfiltered soil extracts. The method for ammonium-N is similar to that for digests, except that sodium citrate and tartaric acid are used instead of dialysis to prevent interference by hydroxides during colour development. In the method for nitrate, nitrate is reduced to nitrite (after dialysis) and the nitrite is determined by the Griess-Ilosvay procedure. Nitrite-N can be determined separately on the nitrate manifold simply by replacing two reagents with distilled water.

RÉSUMÉ

L'étude décrit les méthodes de détermination directe de l'azote dans les digestes et les extraits de sol, utilisant l'autoanalyseur Technicon.


Au cours d'une période de deux ans d'emploi routinier, le coefficient de variation entre deux déterminations faites du même digeste était de 0·6%, et entre des digestions doubles faites sur le même échantillon du sol, 2·4%. On cite les résultats montrant que les limites de détection des changements de l'azote du sol dans les expériences au champ sont fixées par la variation des échantillons prélevés et non celle des techniques analytiques.

Extraits: On décrit les méthodes de détermination de l'azote minéral dans les extraits de sol non filtrés. La méthode employée pour l'ammonium-N est semblable à celle dont on se sert pour les digestes, sauf que l'on utilise le citrate de sodium et l'acide tartarique au lieu d'une dialyse pour prévenir l'interférence d'hydroxydes pendant le développement de la couleur.

Dans la méthode relative au nitrate, on réduit le nitrate en nitrite (après dialyse) et le nitrite est déterminé par le procédé de Griess-Ilosvay. Le nitrite-N peut être déterminé séparément des milieux nitrates, en remplaçant simplement deux réactifs par de l'eau distillée.
ZUSAMMENFASSUNG

Der Artikel beschreibt Methoden zur direkten Feststellung von Stickstoff in Bodenausscheidungen und Bodenextraktionen mit dem Technicon AutoAnalysator.


Im Routine Gebrauch der sich über eine Periode von 2 Jahren erstreckte war der Koeffizient der Variation einer Doppelbestimmung an derselben Ausscheidung 0-6% und an einer Doppelausscheidung an derselben Bodenprobe 2-4%.

Resultate sind angeführt, welche zeigen, dass im Feldversuch die Grenzen der Bestimmung der Boden-N-Änderungen auf Unterschieden der Feldproben beruhen und nicht auf den analytischen Methoden.


In der für Nitrate verwendeten Methode wird das Nitrat nach der Dialyse zum Nitrit reduziert und das Nitrit wird durch das Griess-Ilosvay Verfahren bestimmt. Nitrit Stickstoff kann separat in der Nitratsammelleitung einfach durch Ersetzen von zwei Reagenten durch destilliertes Wasser festgesetzt werden.
SIGNIFICANCE OF ERRORS IN $^{15}\text{N}$ MEASUREMENTS IN SOIL: PLANT RESEARCH

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I. INTRODUCTION

Tracer experiments using $^{15}\text{N}$ have been widely employed, yet it is surprising to discover that most published analytical procedures for the mass spectrometer have not, in any important detail, altered much since Rittenberg's pioneering work nearly 30 years ago (Rittenberg et al. 1939). We contend that these procedures have become too stereotyped to enable the inherent versatility of $^{15}\text{N}$ data to be properly exploited.

Studies of $\text{N}$ metabolism in the soil or the plant embrace many spheres of interest in which $^{15}\text{N}$ can be employed to great advantage, ranging from the level of the plant or microbial cell to that of the field experiment. Each of these situations requires a different approach in technique, since the amounts of $\text{N}_2$ gas available and its enrichment will vary greatly, as also will the precision demanded of the measurements. Each procedure adopted will usually be a compromise between opposing factors and each will possess its own sources of error. It is important that the overall errors of measurement should be revealed during the analytical program. It is neither necessary nor desirable to describe a procedure in detail for each situation. So much depends on the number of samples to be analyzed, on the technical competence and experience of the mass spectrometer operator, and on the experimental aims. In this paper we shall review briefly some of the well-documented sources of error in $^{15}\text{N}$ measurements, and discuss their importance in typical situations in soil: plant research; in this way we hope to clarify what could be a complex operation for inexperienced users.

II. INVESTIGATION OF ERRORS

Although the conversion of total $\text{N}$ to ammonium $\text{N}$ is subject to error (Martin and Skyring 1962, Bremner, Cheng and Edwards 1963, Bremner 1965) in this paper it is assumed to be quantitative. If only a portion of the total $\text{N}$ is required (e.g. that from amino-acid) the conversion must also be specific. It is worth noting that commercially available sources of $^{16}\text{N}$ should be analyzed accurately for total $\text{N}$ and $^{15}\text{N}$; many articles do not discuss this important operation.

Tracer experiments are usually designed to measure the content of isotope in a component of a system. For radioactive isotopes, if $C$ is the counting rate per unit weight and $w$ is the weight of component, then the total tracer content is $Cw$, whereas for $^{15}\text{N}$, it is $N\omega a$, where $N$ is the nitrogen content and $\omega$ is the $^{15}\text{N}$ abundance. Hence $^{15}\text{N}$ contents are subject
to more possibilities of error than contents of radioactive tracer, since they are the products of three parameters rather than two.

In practice, variations in $a$ can occur such that $a$ and $N$ covary in opposite directions when the same amounts of $^{15}N$ are added to replicates varying in total $N$ content. The between-replicate error in the amounts of $^{15}N$ would then be lower than that of either $N$ or $a$. This cancelling out of errors is partly responsible for the high precision of $^{15}N$ data.

(a) The "Black Box" Approach

The need to obtain estimates of error has been stressed in the previous section. Modifications to an existing technique often reveal unsuspected sources of error. The experimenter may not be able to perform the actual analyses himself, and may employ an assistant or send the samples to another laboratory. For these reasons it is essential to check the procedure by submitting standard samples of known enrichment under conditions identical to those of the unknown samples, and to maintain this check at frequent intervals throughout the analytical program. By thus regarding the whole procedure as a "black box", the necessary estimate of error for any particular system of analysis can be obtained.

(b) Preparation of Standards

It is easy to measure very accurately the $^{15}N$ abundance in samples highly enriched in $^{15}N$ (compounds with over 99 at. % $^{15}N$ are commercially available). It is also easy to measure accurately the $^{15}N$ abundance in unlabelled samples by comparing them with atmospheric $N_2$, which has a constant and accurately known $^{15}N$ abundance (Junk and Svec 1958). Standards of $^{15}N$ abundance accurate to within a fraction of 1% can thus be made quite simply by mixing solutions of the two in known proportion. Standard solutions thus prepared are introduced at random into successive batches of samples and analyzed with the unknowns.

III. SOURCES OF ERROR

(a) Instrumental Errors

Instrumental errors may be random (from ion beam fluctuations) or systematic (isotopic discrimination, non-linearity of amplifiers, defects in optics, etc.). Systematic errors can be allowed for by calibration with $^{15}N$ standards of known composition; they rarely interfere with $^{15}N$ measurements. Random instrumental errors are best determined by analyzing successive samples from a standard gas sample. No background corrections need be made, and the observations are used directly to calculate instrumental variation. Normally these errors are very small; typical values reported in the literature are $< 0.02\%$ (Junk and Svec 1958), $0.03-0.04\%$ (Martin et al. 1963) and $< 0.02\%$ (Hüser et al. 1960). It is generally accepted that more precise ion current ratios are obtained with double-collector systems than with a single ion beam collector. This is true, but quite satisfactory precision can be attained with the latter if sufficient replicate readings are taken.
ERRORS IN $^{15}$N MEASUREMENTS

(b) Air Contamination

The effect of air contamination depends on the volume of the gas sample and its $^{15}$N enrichment (Hüser et al. 1960). At abundances over 10 at. %, the ion current ratio usually measured is $(i_{28} + i_{29})/i_{30}$, which is relatively insensitive to the presence of air. Data for samples containing $>$ 70 at. % $^{15}$N are best calculated from replicated measurements of $i_{29}$ and $i_{30}$ on a single collector. These suffice to give the required precision and no air correction is necessary.

The literature shows that some prefer to use the $^{40}$Ar peak ($i_{40}$) to correct for air (Holt and Hughes 1955; Capindale and Tomlin 1957; Hüser et al. 1960; Newman and Oliver 1966) while others (Rittenberg 1948; Yemm and Willis 1956; Sims and Cocking 1958) prefer the $^{16}$O$_2$ ($i_{32}$) peak. Two assumptions are made in the calculation: (1) That the $^{14}$N/$^{15}$N ratio in the contaminating air is equal to that of ordinary air. This assumption is almost certainly true, yet Bremner (1965) perpetuates an error by Rittenberg in neglecting to correct for the peak at m/e 29. (2) That the $i_{28}/i_{16}$ or the $i_{28}/i_{32}$ ratio is the same as for air. This is certainly not true (Hüser et al. 1960; Martin et al. 1963), since dissolved air (representing some or all of the contamination) is richer in O$_2$ and Ar than ordinary air, and using $i_{28}/i_{16}$ or $i_{28}/i_{32}$ ratios for ordinary air would overestimate the contamination. We have observed that the $i_{28}/i_{16}$ ratio is constant under our conditions, giving a value of 24 (cf. 55 for ordinary air), whereas the $i_{28}/i_{32}$ ratio is too variable for reliable correction.

In attempts to design a technique for rapid analysis we have been able to keep air contamination at low levels using the following procedure: each ammonium sulphate solution is evaporated to dryness in a small glass tube (approx. 6 x 1.5 cm) which can be closed with a tightly-fitting screw cap. The contents can thus be stored dry indefinitely. For analysis, the uncapped vessel is fixed to an assembly using a neoprene O-ring and reacted with NaOBr solution that has been vacuum de-gassed and stored in a glass reservoir under He at a pressure of a few cm of mercury. The need for de-gassing each sample solution and separate portions of NaOBr reagent are thus avoided. Trials have shown (Ross, unpublished) that residual air is less than 0.25% under routine conditions in which only 1 min is allowed for evacuating the sample tube and attachments.

(c) Background Spectrum

Most mass spectrometers show background peaks at masses 28, 29 and 30. The $i_{28}/i_{29}$ background ratio is commonly greater than that for atmospheric N$_2$, due to the relatively larger hydrocarbon contribution at mass 29 and the higher natural abundance of carbon isotopes which contribute to peaks arising from $^{12}$CO$^+$ and $^{13}$CO$^+$. Our observations show that the heights of the background peaks are not seriously affected by sample pressure in the ion source, and that they are relatively stable. Hence a simple correction can be applied to gas samples of normal pressure.
There are other sources of contamination that should be avoided.

(i) **Substances other than** N\textsubscript{2} **at the** N **isotope peaks.**

Bremner et al. (1963) list some of the contaminant ions but primary alcohols should be included (see Beynon 1960, p. 349). Ethanol contained in indicator solutions employed in the titration step is a potential hazard if dry ice is used as refrigerant, unless the titrated distillate is evaporated completely to dryness before conversion with hypobromite. Traces of ethanol persist in the vacuum system of the mass spectrometer and can contaminate a long sequence of \textsuperscript{15}N determinations (Martin et al. 1963). Because of the need to exclude organic substances from each distillate, we prefer to titrate ammonium borate solutions electrometrically.

Methylamine is still mentioned in the literature (Hüser et al. 1960, Bremner et al. 1963, Bremner 1965) as a potential contaminant, a legacy from Rittenberg’s (1948) original observation of an unknown peak at mass 29. Tests by the authors showed that, although the methylamine peaks at masses 29 and 30 decreased slowly after shutting off the inlet valve, there was no special problem in pumping this compound from the analyzer tube. Further trials showed that methylamine does not survive Kjeldahl digestion (even using the low ratio of \textsubscript{2}K\textsubscript{2}SO\textsubscript{4} : \textsubscript{5}H\textsubscript{2}SO\textsubscript{4} of 0.1) for more than 30 minutes. It is time this ghost was finally laid.

Some other contaminants should be briefly mentioned. Nitrous oxide is always produced in small quantities in the reaction between NaOBr and ammonium salts, and can interfere in the measurement of \textsubscript{15}N in samples of high enrichment. Although \textsubscript{2}N\textsubscript{2}O can be frozen out with liquid nitrogen, this process is slow; enough time should be allowed to complete its condensation, unless a very efficient trap is used.

When dry ice is used instead of liquid nitrogen in the gas-handling system, enough CO\textsubscript{2} can be produced from the alkali in the NaOBr solution to interfere with the N isotope peaks if the sample contains excess acid. It is safer to adjust the pH of the sample to about 3 with dilute sulphuric acid before evaporation.

Water vapour should never be allowed into the mass spectrometer analyzer tube. It reacts with tungsten filaments to form CO\textsuperscript{2+}, and is very difficult to remove.

(ii) **Memory effects due to hold-up in the distillation step or in the mass spectrometer inlet system.**

Memory effects result from isotopic exchange or adsorption processes during certain stages of the analysis, causing cross-contamination of samples. Newman (1966) showed a hold-up of about 1% of \textsubscript{15}N from 350 \mu g N in a borosilicate glass condenser; tests in this laboratory showed a mean hold-up of 0.5% on a 500 \mu g sample of N. Newman suggests that a chemi-sorbed layer of ammonia on the glass surface is responsible for the hold-up, and shows that a silver condenser virtually eliminates the memory. We have found a stainless steel condenser, attached to a silver-plated spray trap, also satisfactory; hold-up being reduced to < 0.1% provided the assembly is steamed for 4 minutes between distillations. Bremner (1965)
ERRORS IN $^{15}$N MEASUREMENTS recommends the removal of contamination by distilling ethanol between distillations of ammonium, but this involves the risk of contamination mentioned above.

Memory can also occur in the liquid nitrogen trap used to purify the gas sample. In one trial, run in duplicate, a sample of $N_2$ containing 50 at. % excess $^{15}N$ was passed through a perfectly dry trap immersed in liquid nitrogen, followed by a sample of unlabelled $N_2$. Duplicate values of the enrichment of the latter were both $-0.0013$ at. % excess, whereas when the 50 at. % sample was passed through the trap which contained traces of water, the following "unlabelled" $N_2$ sample acquired an enrichment of $+0.0450$ at. %. If the trap was immersed in dry ice-acetone, no such memory effects were observed. It was concluded that memory can occur by sorption of $N_2$ on ice at liquid nitrogen temperatures. Since a liquid nitrogen trap is desirable to remove contaminants, it should be as small as practicable and should be pumped out at room temperature between determinations to remove water and the previous $N_2$ sample. A spiral of 1.5-2 mm diameter stainless steel tube, wound three or four times into a helix about 3 cm diameter, provides a satisfactory trap.

(iii) Other potential sources of contamination.

Ammonium chloride is volatile. Tests showed that prolonged heating of ammonium chloride residues on a waterbath resulted in extensive losses; even during the evaporation stage itself, 8% of the $N$ was lost. It is therefore possible that cross contamination could occur between adjacent vessels on a waterbath. Ammonium sulphate is non-volatile provided the evaporating solution is below $pH 3$, so it is best to titrate ammonium distillates with sulphuric acid and then adjust the $pH$ to this value with more acid before evaporation.

IV. PRECAUTIONS TO MINIMISE ERROR

Analytical procedures cannot be expected to yield precise results under all conditions. In soil fertility studies it is frequently necessary to analyze samples of very high enrichment, samples very near the natural abundance of $^{15}N$, or very small samples of gas. Each of these restrictions requires special attention in different ways.

(a) Samples of Normal Size but High Enrichment

These include very highly enriched salts used as source material, e.g. fertilizers, or samples of gas for $N$ fixation experiments. The $^{15}N$ balance data could be vitiated by inaccuracies in the analysis of the source of $^{15}N$, and if the mass spectrometer has a double collector there may be limits beyond which a ratio of ion currents cannot be determined.

Errors in measurement of separate peaks for enrichments $>70\%$ are not serious if enough replicate readings are obtained for each sample. It is possible to dilute the highly enriched salt with unlabelled carrier (of known enrichment) so that double-collector readings can be obtained, provided both sources are pure and their proportions known with sufficient accuracy, but this complicates the procedure and is unnecessary.
Memory effects from the distillation of $^{14}N$ in a previous sample should be eliminated by pre-distilling a small aliquot of the test solution.

(b) **Samples of Normal Size but Low Enrichment**

Soil samples containing $N$ of near natural abundance are common in field experiments on $N$ balance; accurate data are necessary because tracer content is calculated from the differences between two abundances of about the same magnitude, one of which is the natural abundance of the soil. There is evidence that many surface soils are slightly enriched in $^{15}N$ (Cheng et al. 1964) which must be allowed for. During crucial analyses the recorder techniques described by McKinney et al. (1950) and by Galimov et al. (1965) are satisfactory, since these minimise the effects of instrumental error discussed previously.

In these samples, air contamination is not as important in natural abundance measurements as for higher enrichments, but memory effects must be avoided; the logical procedure is to avoid processing highly enriched samples at the time that samples of low tracer content are being analyzed. It is also important to use the same standard throughout the experiment. Enrichment data are sensitive to small changes in the comparison standard.

(c) **Small Samples**

Soil extracts usually contain small amounts of ammonium and nitrate and it is usually impracticable to distil more than 50 ml, which may yield ammonium samples containing 25 $\mu g\ N$ or less. There is no theoretical difficulty in analyzing samples of this size, as normal ion source pressures can be achieved by using smaller expansion vessels in the inlet system. Practical difficulties occur because of the likelihood of air contamination during the handling of small samples. Sorption of ammonia from the laboratory atmosphere can also be important either during evaporation or storage. As mentioned above, storage is no problem if the ammonium salt is dry, and contamination during evaporation can be avoided if concentration is achieved by a hot stream of ammonia-free air (Bremner and Edwards 1965).

V. **Precision of $^{15}N$ Enrichment**

Although high precision can be obtained with mass spectrometers using special techniques, under routine operation the variability of the results is greater. Nömmik (1957) found a coefficient of variation of 0.3% in an analysis of highly enriched nitrate (31.7 at. % excess) measurements of the $f_{am}/f_{no}$ ratio from nitric oxide. Lower enrichments show coefficients of variation between 1-3% (Martin et al. 1963, Owens 1960, Guebler et al. 1962) and Smith et al. (1963) show a variation of 1.1% in measurements of the natural abundance of soil $N$. At very low enrichments the coefficient of variation tends to be very high. Table 1 gives results of a "black box" type of study, in which samples containing 500 $\mu g\ N$ of both a labelled and an unlabelled ammonium sulphate solution were randomly introduced into
an analytical program over a period of 10 weeks. About half the samples were distilled and titrated in the usual way, and the rest were analyzed directly to study any effect of distillation.

### Table 1

**ERRORS IN $^{15}$N ENRICHMENT IN TWO AMMONIUM SULPHATE STANDARDS DURING ROUTINE ANALYSIS**

<table>
<thead>
<tr>
<th>Source</th>
<th>Treatments</th>
<th>No. of samples</th>
<th>Mean enrichment at. $^{15}$N* % excess</th>
<th>S.D.</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled $(NH_4)_2SO_4$</td>
<td>Distilled</td>
<td>14</td>
<td>0.8842</td>
<td>0.0141</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Undistilled</td>
<td>10</td>
<td>0.8942</td>
<td>0.0101</td>
<td>1.1</td>
</tr>
<tr>
<td>Unlabelled $(NH_4)_2SO_4$</td>
<td>Distilled</td>
<td>15</td>
<td>0.0008</td>
<td>0.0024</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Undistilled</td>
<td>10</td>
<td>-0.0004</td>
<td>0.0008</td>
<td>—</td>
</tr>
</tbody>
</table>

* Corrected for background and “air”.

Although distillation seemed to alter the enrichments of samples and caused more variability in the measurements, the changes in enrichment were statistically non-significant ($P < 0.05$). The results show that, under the operating conditions that are probably typical of the analysis of large numbers of labelled samples, variability can be limited to 1.2%.

**VI. ACKNOWLEDGMENTS**

We thank Dr. E. F. Henzell for access to some of his data, and Mrs. K. Newton for the mass spectrometric measurements.

**REFERENCES**


A. E. MARTIN AND P. J. ROSS

Many procedures for $^{15}$N analysis have been published but most of them lack the adaptability required for tracer studies in soil:plant research. Different kinds of tracer experiments may require different approaches in mass-spectrometric technique, and each technique has its own complement of potential errors. The main sources of error (e.g. instrumental, contamination by air and other substances, and memory effects) are reviewed, and discussed in relation to three examples commonly encountered in this field of study: highly enriched samples, samples with a $^{15}$N abundance close to the natural value, and small samples such as those obtained from soil extracts. The importance of providing a satisfactory estimate of error is emphasized; this can only be achieved by submitting $^{15}$N standards at random for isotopic analysis during the course of analyzing a large batch of experimental samples. It is shown that, under routine operating conditions, a coefficient of variation of 1-2% can be expected for samples containing about 1 at. % excess $^{15}$N.

SUMMARY

Many procedures for $^{15}$N analysis have been published but most of them lack the adaptability required for tracer studies in soil:plant research. Different kinds of tracer experiments may require different approaches in mass-spectrometric technique, and each technique has its own complement of potential errors. The main sources of error (e.g. instrumental, contamination by air and other substances, and memory effects) are reviewed, and discussed in relation to three examples commonly encountered in this field of study: highly enriched samples, samples with a $^{15}$N abundance close to the natural value, and small samples such as those obtained from soil extracts. The importance of providing a satisfactory estimate of error is emphasized; this can only be achieved by submitting $^{15}$N standards at random for isotopic analysis during the course of analyzing a large batch of experimental samples. It is shown that, under routine operating conditions, a coefficient of variation of 1-2% can be expected for samples containing about 1 at. % excess $^{15}$N.

RÉSUMÉ

Beaucoup de procédés pour l'analyse du $^{15}$N ont été déjà publiés, mais pour la plupart ils manquent l'adaptabilité requise pour l'étude des traceurs dans les recherches sol: plante. Les genres différents d'expériences portant sur les traceurs peuvent demander différentes méthodes dans la technique spectrométrique pour mesurer les masses, et chaque technique comporte des possibilités d'erreurs potentielles. On considère donc les causes principales de ces erreurs, (par exemple, défauts d'appareil, contamination par l'air et autres substances, effets de la mémoire); ces causes sont étudiées par rapport à trois exemples que l'on relève fréquemment dans ce domaine d'études: les échantillons fortement enrichis, les échantillons munis d'une abondance $^{15}$N avoisinant la valeur naturelle, et les petits échantillons, par exemple, ceux qu'on obtient des extraits de sol. On souligne l'importance qu'il y a à fournir une évaluation suffisante des erreurs, ce qu'on peut réaliser uniquement en soumettant les valeurs $^{15}$N au hasard à l'analyse isotopique au cours de l'analyse d'un grand lot d'échantillons d'essai. On démontre que, dans des conditions d'opérer
normales, on peut s’attendre à un co-efficient de variation de 1-2% pour les échantillons qui contiennent à peu près 1 au % supérieur a $^{15}$N.

ZUSAMMENFASSUNG

Viele Verfahren für $^{15}$N-Analysen sind veröffentlicht worden, aber die meisten vermissen die Anpassungsfähigkeit, für die Spuren (Tracer) Studien im Boden: Pflanzen-Forschung. Verschiedene Arten der Spuren-Experimente mögen verschiedene Schritte in der mass-spektrometrischen Technik benötigen, und jede Technik hat ihren eigenen Anteil an Potentialfehlern. Die Hauptsachen dieser Fehler (z.B. durch Instrumente, Verunreinigung durch Luft und andere Ursachen, und Gedächtnisvermögen) werden in Betracht gezogen und werden in Beziehung zu drei Beispielen diskutiert, die gewöhnlich in diesem Felde der Studien vorkommen: stark angereicherte Proben, Proben mit einem $^{15}$N Überfluss nahe des natürlichen Wertes, und kleine Proben, z.B. die von Boden-Extrakten erhalten sind. Die Wichtigkeit, eine richtige Bewertung der Fehler zu ermöglichen, muss betont werden; kann nur dadurch ausgeführt werden, indem man, während der Analysendurchführung einer grossen Anzahl experimentaler Proben, $^{15}$N-Masstäbe, wie der Zufall sie bringt, einer isotopischen Analyse unterwirft. Es wird ersichtlich, dass unter normalen Bedingungen, ein Variations-Koeffizient von 1-2% für Proben, die ungefähr 1 at. % Überfluss $^{15}$N enthalten, angenommen werden kann.
THE EXTRACTION AND PARTIAL CHARACTERIZATION OF NON-HYDROLYSABLE NITROGEN IN SOIL

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Although it is well known that most of the nitrogen in arable soils is organic in nature, only part of it has been chemically identified. Studies using boiling acids and alkalis have shown that amino-acids and amino-sugars are present in soil organic matter, and account for 20-40, and 5-10, percent of the total soil nitrogen, respectively (Bremner 1965a, b).

However, Bremner (1949) found that only 68-87 percent of the soil nitrogen was dissolved by boiling acid or alkali, and apart from an electrophoretic study by Johnston (1959) little work has been published on the nature of the material not dissolved by these treatments.

It has been suggested that the acid-insoluble fraction may be composed of nitrogenous complexes formed by interaction between oxidised lignins and ammonia (Mattson and Koutler-Andersson 1943), melanin type compounds (Kono nova 1961), or mineral-organic nitrogen complexes (Kono nova 1961).

The aim of the present work was to study the nature of the nitrogenous compounds in soil which are not released by boiling with 6N acid. Particular attention has been devoted to the suggestion that organic nitrogen is protected from acid attack by clay minerals.

MATERIALS AND METHODS

(a) Soils

A partial description of the soils used in this study is given in Table 1. The 0-4" layer of surface soil was collected, air-dried, and ground to pass a 2 mm mesh sieve before analysis. These soils were chosen because they are known to contain some 2:1 type clay minerals.

(b) Hydrolysis of soils

(i) In an autoclave

The soil (10 g) was weighed into a 4 oz Macartney bottle; 80 ml of 6N sulphuric acid was added, the bottle sealed, placed in an autoclave, and heated at 120°C for 4 hours. The residue was separated from the acid extract by filtration* and washed with 6N sulphuric acid (Treatment 1).

* In all the experiments reported in this paper, the acid extract was separated from the soil residue by filtration through a Whatman No. 42 paper and washed with the appropriate strength of acid. The combined filtrate and washings were diluted to 150 ml.

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<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Location</th>
<th>Soil Group</th>
<th>Parent Material</th>
<th>Texture</th>
<th>pH</th>
<th>Total N</th>
<th>Inorganic N (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Berridale</td>
<td>Black earth</td>
<td>Basalt</td>
<td>Medium clay</td>
<td>6.8</td>
<td>2420</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>N.S.W.</td>
<td>Black earth</td>
<td>Basalt</td>
<td>Clay loam</td>
<td>6.7</td>
<td>3300</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Crookwell</td>
<td>Reddish chocolate</td>
<td>Basalt</td>
<td>Clay loam</td>
<td>6.5</td>
<td>2520</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>N.S.W.</td>
<td>Yellow podzolic</td>
<td>Granodiorite</td>
<td>Sandy loam</td>
<td>5.2</td>
<td>2700</td>
<td>16</td>
</tr>
</tbody>
</table>
The washed residue was air dried and subjected to, (A) a second boiling with sulphuric acid (Treatment 9), or as described below, (B) treatment with 2N hydrofluoric acid (Treatment 7), (C) treatment with 5N hydrofluoric acid (Treatment 8), or (D) extraction with sodium hydroxide (Treatment 10).

(ii) Under reflux
To a 10 g soil sample in a 250 ml round bottom flask, 100 ml of 6N hydrochloric acid (Treatment 2) or 6N sulphuric acid (Treatment 3) was added. The flask was attached to a water jacketed condenser, placed on an electric heater, and the contents boiled for 24 hours. The residue from the 6N sulphuric acid treatment was subjected to a second (Treatment 4) and third extraction (Treatment 5) with boiling 6N sulphuric acid under reflux.

(iii) Under reflux after dilute sulphuric acid pretreatment (Treatment 6)
The soil (10 g) was treated with 100 ml of 0.55N sulphuric acid for 1 hour at 20°C in vacuo. The mixture was then filtered. The soil residue was boiled with 100 ml of 0.55N sulphuric acid under reflux for 3 hours, and the residue from this treatment boiled with 100 ml of 6N sulphuric acid for 24 hours under reflux. The final residue was again separated from the acid extract by filtration.

(iv) Hydrofluoric acid treatment of residues
Washed residues from the autoclaved treatment (Treatment 1) were shaken with 100 ml of 2N HF:2N HCl solution (Treatment 7) or 100 ml of 5N HF:1N HCl:0.6N H₂SO₄ (Treatment 8) in polyethylene bottles for 16 hours at 20°C. The bulk of the acids was removed by evaporation in vacuo at 40°C, and the residues subjected to a second treatment with 6N sulphuric acid in the autoclave as described under (b) (i). Acid extract and residue were separated by filtration.

(c) Sodium hydroxide extraction of acid-treated soil (Treatment 10)

(i) Extraction
The washed residue from treatment 1 was shaken with 50 ml of 0.5N sodium hydroxide solution for 1 hour, at 20°C, in a reciprocating shaker. The extract and residue were separated by centrifugation at 3000 r.p.m. and the residue subjected to six similar extractions with sodium hydroxide solution.

Aliquots were taken from each extract for nitrogen analysis and the remainder was bulked for further study.

(ii) Fractionation of extract
An aliquot of the sodium hydroxide extract was adjusted to pH 1 by the addition of concentrated hydrochloric acid. The acid-soluble fraction was separated from the acid-insoluble fraction by filtration.

A second aliquot was dialysed against distilled water in cellulose dialysis tubing. The non-dialysable fraction was subjected to hydrolysis with 6N hydrochloric acid under reflux for 24 hours, and the soluble and insoluble fractions separated by filtration.

A third aliquot of the sodium hydroxide extract was made 6N with
respect to hydrochloric acid and then boiled for 24 hours under reflux. Insoluble material was removed by filtration.

(d) Total nitrogen determination

Total nitrogen was determined on soils and aliquots of extracts by a semi-micro Kjeldahl method using selenium as a catalyst.

(e) Statistical analysis

All treatments were replicated and the results (in ppm N) were subjected to an analysis of variance. However, for the readers' convenience, only the means of the replicates, expressed as a percentage of the total soil nitrogen are given in the tables.

RESULTS AND DISCUSSION

(a) 6N Acid treatments

The total amounts of soluble nitrogen determined by treatments 1-10 are given in Table 2. These results show that autoclaving the soil with

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Soil 1</th>
<th>Soil 2</th>
<th>Soil 3</th>
<th>Soil 4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>75-1</td>
<td>75-1</td>
<td>78-8</td>
<td>81-5</td>
<td>77-6</td>
</tr>
<tr>
<td>2.</td>
<td>81-1</td>
<td>82-3</td>
<td>86-1</td>
<td>75-8</td>
<td>81-3</td>
</tr>
<tr>
<td>3.</td>
<td>80-3</td>
<td>82-5</td>
<td>84-8</td>
<td>82-9</td>
<td>82-6</td>
</tr>
<tr>
<td>4.</td>
<td>83-3</td>
<td>85-1</td>
<td>87-4</td>
<td>84-7</td>
<td>85-1</td>
</tr>
<tr>
<td>5.</td>
<td>86-2</td>
<td>87-6</td>
<td>88-5</td>
<td>85-4</td>
<td>86-9</td>
</tr>
<tr>
<td>6.</td>
<td>84-2</td>
<td>87-2</td>
<td>88-1</td>
<td>84-9</td>
<td>86-1</td>
</tr>
<tr>
<td>7.</td>
<td>82-8</td>
<td>83-2</td>
<td>84-7</td>
<td>85-1</td>
<td>84-0</td>
</tr>
<tr>
<td>8.</td>
<td>83-9</td>
<td>84-0</td>
<td>86-3</td>
<td>85-8</td>
<td>85-0</td>
</tr>
<tr>
<td>9.</td>
<td>82-3</td>
<td>81-2</td>
<td>83-7</td>
<td>84-7</td>
<td>83-0</td>
</tr>
<tr>
<td>10.</td>
<td>95-5</td>
<td>90-4</td>
<td>92-6</td>
<td>95-9</td>
<td>93-6</td>
</tr>
</tbody>
</table>

* 1. Soil heated with 6N sulphuric acid at 120°C for 4 hr.
2. Soil boiled with 6N hydrochloric acid under reflux for 24 hr.
3. Soil boiled with 6N sulphuric acid under reflux for 24 hr.
4. Treatment 3 followed by a second treatment 3 on residue from 3.
5. Treatment 4 followed by a third treatment 3 on residue from 4.
6. Soil soaked in 0·55N sulphuric acid for 1 hr at 20°C in vacuo; the residue was then boiled with 0·55N sulphuric acid for 3 hr under reflux; the residue from this treatment was then boiled with 6N sulphuric acid under reflux for 24 hr.
7. Treatment 1, followed by treatment of residue from 1 with 2N HF:2N HCl for 16 hr then 2nd treatment 1 on residue.
8. Treatment 1, followed by treatment of residue from 1 with 5N HF:1N HCl:0·6N H2SO4 for 16 hr and 2nd treatment 1 on residue.
9. Treatment 1, followed by a second treatment 1 on residue from 1.
10. Treatment 1, followed by seven extractions of the residue from 1 with 50 ml of 0·5N sodium hydroxide solution.

acid (Treatment 1) gave lower yields of nitrogen (mean 77·6 percent) than the other methods of hydrolysis (significant at P < 5% for soils 1, 2 and 3). This method was tested because it is convenient for handling
large numbers of samples and because it yields maximum recovery of amino-sugar nitrogen in four hours (Stevenson 1957).

Bremner (1949) showed that 68-87 percent of the organic nitrogen was rapidly dissolved when soils were boiled under reflux with 6N hydrochloric acid, and that the amount dissolved was not significantly increased by extending the hydrolysis time beyond 12 hours. Bremner's results suggest that either "hydrolysable" nitrogen is a specific fraction or that the "non-hydrolysable" nitrogen is protected in some way from acid attack. These two alternative explanations have been examined.

Apart from soil 4, there was no significant (P < 5%) difference in the results obtained by boiling the soils under reflux for 24 hours with 6N hydrochloric acid or 6N sulphuric acid (compare means of 81.3 and 82.6 percent respectively, Table 2). However, boiling the soil with 6N sulphuric acid under reflux for a second (Treatment 4), and third (Treatment 5) 24 hour period significantly (P < 5%) increased the amount of nitrogen hydrolysed (to means of 85.1 and 86.9 percent of the total soil nitrogen, respectively). The acid was changed at the end of the 24 and 48 hour period to displace any equilibrium reactions which may have been formed, and to compensate for any decrease in acid strength due to reaction with the soil.

Persson (private communication) found that by using a dilute acid pretreatment similar to that published by Waksman and Stevens (1930), he could extract a large amount of organic matter from soil. He suggested that this higher result may be caused by the removal of sugars and organic substances of low molecular weight which could condense with nitrogenous compounds, during boiling with 6N acids, to form insoluble complexes.

More nitrogen was dissolved by this procedure (Treatment 6), than was dissolved by one or two treatments with boiling sulphuric acid (compare mean of 86.1 percent for treatment 6, with means of 82.6 and 85.1 percent for treatments 3 and 4, or 77.6 and 83.0 percent for treatments 1 and 9).

(b) Hydrofluoric acid treatment

Reaction of the acid treated residues from treatment 1 with hydrofluoric acid, followed by a second acid extraction (Treatments 7 and 8), released more soluble nitrogen (means 84.0 and 85.0 percent) than either one (mean 77.6 percent) or two acid extractions in the autoclave (mean 83.0 percent; Treatment 9). (Differences significant at, P < 5%.)

Even though the increase due to hydrofluoric acid is small (difference between treatments 7 and 9 or 8 and 9), it suggests that inorganic material in the soil is protecting part of the nitrogen from acid attack. Clay minerals do not completely protect organic nitrogen complexed with them from 6N acid attack (Miller and Freney, unpublished) but they slow down markedly the rate of this reaction, and one would expect to have nitrogen released from similar complexes in the soil by imposing successive 6N acid treatments, e.g. treatments 4, 5, 9, thus reducing any effect due to release by hydrofluoric acid.
Further evidence for the protection of soluble nitrogen by inorganic minerals was obtained from the results of repeated alkaline extraction of acid treated residues. Up to 95.9 percent of the total soil nitrogen (mean 93.6) could be extracted by this technique (Treatment 10). The seventh alkaline extraction removed little nitrogen from the residues (mean 0.15 percent of total soil nitrogen).

The nitrogen extracted by alkali could be separated into a number of fractions (see Tables 3 and 4). Not all of this alkali-soluble material was high molecular weight material since it could be separated into an acid-soluble fraction, "fulvic acid", and an acid-insoluble fraction, "humic acid". (Table 3), and a large portion of the alkali extracted material passed through a dialysis tubing (Table 4).

In addition, most of the alkali-soluble nitrogen (which represents 16 percent of the total soil N) was hydrolysed by boiling in 6N hydrochloric acid for 24 hours (mean 12.3 percent of total soil N). These results increased the total acid-soluble nitrogen to a mean of 90.0 percent of the total soil nitrogen.

The results show that an appreciable fraction of the alkali-soluble material was also acid-soluble, or that it could be hydrolysed with boiling acid, after it had been extracted from the soil, yet it was not possible to dissolve this material in the presence of soil by the 6N acid treatment.

It is of course possible that there was some decomposition of the organic nitrogen during the alkaline extraction which may account for the low molecular weight material in the alkaline extract. However the

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Soil 1</th>
<th>Soil 2</th>
<th>Soil 3</th>
<th>Soil 4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen extracted</td>
<td>20.4</td>
<td>13.3</td>
<td>14.4</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Acid soluble N*</td>
<td>8.4</td>
<td>6.0</td>
<td>7.3</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>Acid insoluble N*</td>
<td>12.0</td>
<td>9.3</td>
<td>4.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Acid hydrolysable N**</td>
<td>15.4</td>
<td>12.9</td>
<td>11.7</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>Non-hydrolysable N**</td>
<td>5.0</td>
<td>2.4</td>
<td>2.1</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Total acid hydrolysable N***</td>
<td>90.6</td>
<td>88.0</td>
<td>90.5</td>
<td>90.0</td>
<td></td>
</tr>
</tbody>
</table>

* Obtained by adjusting the pH of the sodium hydroxide extracts to 1, and separation of the two fractions by filtration.
** Sodium hydroxide extract made 6N with respect to hydrochloric acid, boiled under reflux for 24 hours and filtered.
*** Sum of nitrogen dissolved from soil with 6N sulphuric acid (Treatment 1), plus acid hydrolysable nitrogen** in sodium hydroxide extract.
results for acid-soluble nitrogen (Table 3) and non-dialysable nitrogen (Table 4) show that there was still some high molecular weight material in the extract and that a large part of this could be dissolved in boiling acid.

### Table 4
Fractionation of the Nitrogen in Dialysed Sodium Hydroxide Extracts of Acid Treated Soils (Treatment 1)

<table>
<thead>
<tr>
<th></th>
<th>Soil 1</th>
<th>Soil 2</th>
<th>Soil 3</th>
<th>Soil 4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysable N*</td>
<td>10.9</td>
<td>6.1</td>
<td>7.7</td>
<td>9.6</td>
<td>8.6</td>
</tr>
<tr>
<td>Non-dialysable N*</td>
<td>9.5</td>
<td>9.2</td>
<td>6.1</td>
<td>4.8</td>
<td>7.4</td>
</tr>
<tr>
<td>Hydrolysable, Non-dialysable N**</td>
<td>4.3</td>
<td>5.1</td>
<td>3.7</td>
<td>2.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Non-hydrolysable, Non-dialysable N**</td>
<td>5.2</td>
<td>4.1</td>
<td>2.4</td>
<td>2.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Dialysable plus hydrolysable, non-dialysable N</td>
<td>15.2</td>
<td>11.2</td>
<td>11.4</td>
<td>12.2</td>
<td>12.5</td>
</tr>
</tbody>
</table>

---

* Sodium hydroxide extract dialysed, against distilled water, in cellulose dialysis tubing obtained from Visking Company, Chicago, Illinois.

** The non-dialysable material was boiled for 24 hours with 6N hydrochloric acid and filtered.

This acid-soluble material may have been protected from acid attack in the soil by inorganic minerals, or it may have been converted to acid-soluble material by degradation and repolymerisation during the alkaline extraction.

A large amount of silica was extracted by the sodium hydroxide treatment, and this formed a gel as soon as acid was added to the system. As it was thought possible that additional nitrogen may have been protected from hydrolysis by entrapment in the precipitated silica gel, the alkaline extract was dialysed to remove sodium silicate and the non-dialysed material was boiled with 6N hydrochloric acid. The results of this treatment, given in Table 4, show that there was still some nitrogen not dissolved by the acid treatment (mean 3.5 percent) and this was not greatly different from the result obtained by direct hydrolysis of the alkaline extract (Table 3, mean 3.7 percent). In addition the amount of acid-hydrolysable nitrogen in the undialysed sodium hydroxide extract (Table 3, mean 12.3 percent) was similar to the sum of the dialysable and hydrolysable non-dialysable nitrogen (Table 4, mean 12.5 percent).

While it is appreciated that the dialysable nitrogen may not all be acid-soluble, it is difficult to test this because of the precipitation of silica when the dialysate is acidified.

The results presented above for alkali extracted material suggest that part of the soil nitrogen is protected from acid attack by some fraction of the soil. The large amount of silica extracted along with this material, in
addition to the results from the hydrofluoric acid study suggests that the protection is by silicates. That all of this acid-soluble nitrogen was not released by the hydrofluoric acid treatments may mean that the acid used was not strong enough to dissolve all the silicates. The strength of the hydrofluoric acid was not increased because of the possibility of breakdown of organic nitrogen compounds.

Bremner (1955) showed that a considerable fraction of the nitrogen (20-60 percent) in humic acid preparations, obtained by extraction of untreated soils with sodium hydroxide and sodium pyrophosphate, was not dissolved by boiling acid. As the ash content of these preparations was low (<1 percent) one cannot suggest protection from acid hydrolysis by inorganic materials. It is possible, as suggested by Persson, that some condensation occurred during the extraction, precipitation or hydrolysis of Bremner's humic acid preparations. Similar formation of non-hydrolysable material may have been avoided in the present work because of the preliminary acid treatment before the alkaline extraction.

REFERENCES


SUMMARY

The amount of nitrogen extracted from soil by boiling with 6N acid was not fixed. The quantity dissolved could be increased by pre-treating the soil with dilute sulphuric acid, and by a hydrofluoric acid treatment.

After the soil had been extracted with 6N sulphuric acid, additional nitrogen was extracted from the residue by dilute sodium hydroxide solution. Most of this alkali-extracted nitrogen was dissolved by boiling in 6N acid, thus increasing the acid-soluble nitrogen in the soils studied to a mean of 90 percent of the total soil nitrogen.

The alkali-soluble nitrogen was readily fractionated into an acid-soluble fraction and an acid-insoluble fraction by adjustment of the extract pH to 1. In addition, a fraction of the alkali-soluble nitrogen passed through a cellulose dialysis membrane suggesting that it was low-molecular weight material.

The results are consistent with the hypothesis that some soil nitrogen is protected from acid hydrolysis by soil minerals.
La quantité d’azote extraite du sol par cuisson avec de l’acide 6N n’est pas fixée. La quantité dissoute peut être augmentée en traitant le sol au préalable avec une dilution d’acide sulphurique, suivi par untraitement avec l’acide fluorhydrique.

Après avoir extrait le sol à l’aide du 6N acide sulphurique, un supplément d’azote fut extrait du résidu par une solution d’hydroxyde de sodium diluée. La plus grande partie de l’azote extraite à l’aide d’alcalis était dissoute par cuisson dans l’acide 6N, augmentant ainsi l’azote soluble dans les acides des sols examinés jusqu’à une moyenne de 90% de la totalité d’azote présent dans le sol.

L’azote soluble dans l’alcali était rapidement transformé en fractions solubles et insolubles dans l’acide, en ajustant le pH à 1. En plus, une fraction de l’azote soluble dans l’alcali passa à travers une membrane de dialyse cellulosique, ce qui montre qu’il s’agit d’un matériel d’un poids moléculaire bas.

Les résultats étaient compatibles avec l’hypothèse qu’une partie de l’azote du sol est protégée de l’hydrolyse acide par les minéraux des sols.

**ZUSAMMENFASSUNG**

Die Stickstoffmenge, die dem Boden durch Kochen mit 6N Säure entzogen wurde, ist nicht festgesetzt. Die aufgelöste Menge kann durch eine Vorbehandlung des Bodens mit verdünnter Schwefelsäure, und ebenso durch eine Behandlung mit Fluorwasserstoffsäure erhöht werden.


Die Resultate sind mit der Hypothese vereinbar, dass etwas Bodenstickstoff durch Bodenminerale von Säurehydrolyse beschützt wird.
A PROPOSED TERMINOLOGY FOR SOME CHEMICAL PROPERTIES OF SOILS

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C.S.I.R.O., Division of Soils, Adelaide, Australia

Soil physicists have found it expedient to try to define the terms they use when describing the physical properties of a soil. It was realised (I.S.S.S., 1963) that some terms could be defined in a useful, precise and generally acceptable manner. Others, e.g. field capacity, while useful, are incapable of precise definition. A concerted attempt by soil chemists to define their terms has not been made for a long time. For example the last official attempt to define the conditions for measuring soil pH was in 1930 (I.S.S.S., 1930).

NEED FOR DEFINITIONS

The primary purpose of this paper is to raise the question whether terms used in soil chemistry ought to be defined. When comparing results obtained by different workers it is often possible to make allowance for variations in the definitions and techniques used but difficulties may arise where the conditions of measurement are not given or, in the case of novel techniques, where no comparison with customary procedures is made. The adoption of standard definitions and procedures may help to eliminate these difficulties.

Very often a term can only be defined in terms of some standard technique. In this paper some methods have been outlined and these are believed to be suitable for a wide range of soils. These methods, however, are to be taken as no more than examples of the techniques which might be used and of the considerations which would lead to the adoption of standard procedures.

TERMINOLOGY

Here the terminology and conditions of measurement for only three common chemical properties of soils are discussed, namely soil pH, soluble salts, and ion exchange. A much larger number of terms could perhaps be usefully defined. The measurements of ‘potential’ which are increasingly used in research on nutrient availability and the methods of presentations of ‘capacity/intensity’ functions might well benefit from standardisation.

Some new definitions and terms are suggested. These are acidic, neutral and carbonate soils, net negative charge, exchangeable acidic and basic cations, and acidity.
CHOICE OF REAGENTS

In many chemical methods there are several alternative reagents which could be used. A choice has to be made deliberately, weighing the advantages and disadvantages of each possibility. For measuring the ion-exchange properties of soil samples it is suggested that calcium chloride be used. The chloride ion is chosen because it is commonly present in soils, is easily analysed and does not enhance the solubility of non-exchangeable cations. Further it is not specifically adsorbed. The calcium ion is favoured for three reasons. It is normally the predominant exchangeable cation. It is an efficient displacer of other cations yet does not readily displace them from interlayer sites. It is easily analysed by a variety of methods. It has the disadvantage that exchangeable calcium in the sample has to be separately extracted by some other salt.

SOIL REACTION OR pH

The pH of a soil sample could be defined as the pH of a 1:2.5 suspension of the soil in 0.1M CaCl₂, the liquid junction of the reference electrode being made in the supernatant liquid. A particular advantage of this method is that it gives a lower limit to the pH of a soil containing calcite in equilibrium with a standard atmospheric concentration of CO₂ (300 p.p.m.). Using the second approximation to the Debye-Hückel equation to calculate the activity of the Ca²⁺ ions, the value of this pH at 25°C is 7.4. The choice of a 1:2.5 suspension conforms to the I.S.S.S. recommendation of 1930. The use of 0.1M CaCl₂ has two advantages. The concentration is high enough to minimise the variations in soil pH for differing soluble salt contents in the samples. The same solution is recommended later for the measurement of net negative charge. Olsen and Watanabe (1959) showed that soils may contain more soluble forms of CaCO₃ than calcite. These would increase the pH by 0.1 units at the recommended ratio of soil to solution. For soils containing carbonate, the pH of the soil in the field is so dependent on the partial pressure of CO₂ and on the water content, that such small differences would not be significant.

Soil samples may be classified on the basis of pH. Carbonate soil samples have a pH of 7.4 or over. These samples may contain calcium, magnesium or sodium carbonates. Acidic soil samples have a pH below 6.0. This choice of pH 6.0 as the upper limit is arbitrary. It should be the limiting pH at which most agricultural soils respond to the addition of lime. Neutral soil samples have a pH between 6.0 and 7.4.

TOTAL SOLUBLE SALTS

The conductivity of the extract from a saturated paste (U.S. Salinity Laboratory, 1954) is recommended for assessing the total soluble salts present in a soil sample. It is preferred to methods based on the conductivity of a 1:5 soil and water suspension (Piper, 1942) for two reasons. The amount of soluble salts is not overestimated when sparingly-soluble salts like gypsum are present. An estimate of the percentage exchangeable
sodium in a soil sample can be made from the ratio $\frac{Na}{\sqrt{Ca+Mg}}$ in the extract even when the soil contains gypsum.

If it is necessary to convert conductivity to concentration then it is suggested that the units be milliequivalents per 100g of sample. There is less error in assuming a linear relation between conductivity and salt content if m.e. per 100g is used instead of percentage by weight.

**Net Negative Charge or NNC**

For acidic and neutral samples the NNC would be measured as the difference in milliequivalents of $Ca^{2+}$ and $Cl^-$ held by the soil in equilibrium with 0.1M $CaCl_2$. This measurement would normally be made in the same unbuffered solution as used for the measurement of soil reaction (NNC$_s$). The calcium and chloride ions would be displaced by a solution of a suitable salt, e.g. $NH_4NO_3$. For acidic soils another measurement could be made at a standard pH of 7.4 (NNC$_{7.4}$). For carbonate soil samples without gypsum the NNC$_{S}$ would be measured with unbuffered 0.1M $CaCl_2$ as for non-carbonate soils. The only difference is that the calcium and chloride ions would have to be displaced by a solution which has a minimum solubility for calcium carbonate, e.g. $m\,NH_4NO_3$ (not chloride) in 60% ethanol-water adjusted to pH 8.5 with ammonia (Tucker, 1954) or $m\,NaCOOCH_3$ adjusted to pH 8.2 (Bower et al., 1952). It can be shown that the error in NNC$_S$ caused by neglecting the presence of bicarbonate ions would be much less than 1%. For soils containing gypsum, a correction for the sulphate dissolved by the displacing solution would have to be made.

**Cation Exchange Capacity or CEC**

Most methods in current use require the removal of the excess measuring cation by washing (Rich, 1962). This may cause hydrolysis of the cations from the sample (for kaolinite, Greene-Kelly, 1955; for soils, Sumner, 1963; and Okazaki et al., 1963). It may also affect the retention of cations by soil organic matter (Peech, 1965). In any case the final equilibrium concentration of the measuring cation is undefined. A more satisfactory method is to calculate the measuring salt held by the wet sample at equilibrium. This involves making some assumption about the volume of solvent adsorbed by the soil (Schofield, 1949; Tucker, 1960). At present, therefore, the CEC of a sample cannot be determined exactly. Usually the number of positive charges present in a soil sample at pH 7.4 will be small, although Sumner and Reeve (1966) have shown that iron oxides may retain chloride ions at this pH. Generally NNC$_{7.4}$ for acidic and neutral soils and NNC$_{S}$ for carbonate soils will be a good approximation to the CEC at pH 7.4, and may be preferred as standard measurements.

**Exchangeable Cations**

In order to separate soluble from exchangeable cations, the samples are usually given a preliminary washing. This may cause a loss of exchangeable cations, especially of sodium. The following methods may be the best
available at the present time. For acidic and neutral soils, the sample is washed with 60% ethanol. Since most acidic soils are low in soluble salts little washing is required and the loss of exchangeable cations will be small. The sample is then extracted with an unbuffered salt solution. Suitable salts would be $\text{CaCl}_2$ for $K$ and $\text{Na}$ and $\text{KCl}$ for $\text{Ca}, \text{Mg}$ and $\text{Al}$ ions. Carbonate soil samples are washed with 60% ethanol and the exchangeable cations are then extracted with $\text{m NH}_4\text{Cl}$ in 60% ethanol adjusted to $\text{pH} 8.5$ with $\text{NH}_4\text{Cl}$ (Tucker, 1954). For samples containing gypsum there are no really satisfactory methods. The exchangeable cations may be correlated with the ratio $\text{Na}/\sqrt{\text{Ca+Mg}}$ in the extract from a saturated soil paste (U.S. Salinity Laboratory, 1954).

**Definitions of New Terms**

*Exchangeable basic cations* is the sum of the exchangeable $\text{Na}$, $\text{K}$, $\text{Ca}$, and $\text{Mg}$. The name is intended to give continuity with the old term 'exchangeable bases'. The term 'base' should be dropped because the cations are not bases in any accepted chemical meaning (Leeper, 1948). The term 'exchangeable metal cations' (Piper, 1942) is also unsuitable because it may be understood to include aluminium. *Exchangeable acidic cations* in acidic soils is the sum of exchangeable $\text{H}$ and $\text{Al}$. In the absence of direct measurement this sum could be expressed as the difference between the CEC and the exchangeable basic cations. Its value will depend on the $\text{pH}$ at which the CEC is measured.

*Acidity* is defined as the difference between the net negative charges at $\text{pH} 7.4$ and at the soil $\text{pH}$, the concentration of the measuring salt, $\text{CaCl}_2$, being the same at both $\text{pH}$ values. It is similar in concept to a lime requirement.

It is hoped the proposed terms and methods of measurement given in this paper will lead to an appraisal of current procedures and concepts. This in turn may lead to some agreement on suitable definitions for the chemical properties of soils. Then it is likely that, in experiments with soils or clays, these defined properties would be stated. The results of the experiments would then be more easily appreciated and be of more general application.

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TERMINOLOGY FOR CHEMICAL PROPERTIES

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SUMMARY

A number of terms in the subjects of soil pH, soluble salts, and ion exchange and some possible standard procedures for their measurement are discussed. This is intended to lead to discussion on whether standardisation of method and terminology is required in soil chemistry. The procedures which are outlined are meant to illustrate the considerations which are necessary before the adoption of suitable methods as standards rather than to be firm recommendations.

Some new terms, which should prove useful, are proposed. They include exchangeable basic cations (Na, K, Ca and Mg) and exchangeable acidic cations (H and Al). Calcium chloride solution is suggested as a suitable medium for the measurement of soil pH and net negative charge. It is also proposed that the more easily measured net negative charge could replace cation exchange capacity as the normal exchange measurement for most soils.

RÉSUMÉ

On discute un certain nombre des termes utilisés pour décrire le pH des sols, les sols solubles et l'échange des ions, et, dans le but de les mesurer, on considère quelques possibilités pour des procédés de normalisation. Notre intention est de conduire à une discussion pour savoir si la normalisation des méthodes et de la terminologie dans la chimie du sol est nécessaire. L'on indique des procédés qui ont pour but d'illustrer les considérations qui seraient nécessaires avant l'adoption des méthodes convenables comme normes, ces indications n'étant toutefois pas des recommandations indispensables.

On propose quelques termes nouveaux, qui pourraient être utiles. On cite, par exemple, les cations échangeables basiques (Na, K, Ca et Mg) et les cations échangeables acides (H et Al). On propose la solution du chlorure de calcium comme moyen convenable pour mesurer le pH du sol et la charge négative nette. On propose en outre que la charge négative nette, étant plus facilement mesurée, pourrait bien remplacer la capacité pour l'échange des cations comme mesure normale d'échange pour la plupart des sols.

Die hier umrissenen Verfahren sollen nicht eine feste Empfehlung sein, sondern sollen die Überlegungen beschreiben, die vor der Annahme von angemessenen Standardmethoden nötig sind.


Ferner wird vorgeschlagen, dass die normalerweise als Austauschwert für Böden verwendete Kationenaustausch-Kapazität durch die leichter zu messende netto negative Ladung ersetzt werden könnte.
STUDIES ON SOIL ORGANIC PHOSPHORUS

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Phosphorus is one of the major plant nutrients but many soils, though containing sufficiently abundant amounts, are unable to supply enough phosphorus to satisfy plant requirements. In consequence, the chemical nature of soil phosphorus has received a great deal of attention. The major portion of the research in the past has focussed on the inorganic phosphorus component and considerable knowledge of this fraction has been accumulated through application of various chemical procedures (Chang and Jackson, 1957; Williams, 1960; Hanotiaux, 1966) and methods using isotopic dilution (Spinks and Barber, 1947; Larsen, 1952).

However, in the surface layers of most soils, a substantial portion of the total phosphorus exists in various organic forms. In recent years considerable progress has been made in identifying the substances present and in measuring their quantity.

An investigation has recently been made on a number of contrasting Canadian soils which has provided new information about analytical methods of determining organic phosphorus and also about the nature of some of the compounds and their relationship to other soil properties.

DETERMINATION OF TOTAL ORGANIC PHOSPHORUS

One of the major difficulties in studies on soil organic phosphorus has been the development of reliable methods for its determination. Numerous methods have been proposed, although most are variations of two basic procedures. In one the organic phosphorus is extracted and measured in the extract, and in the other it is mineralized by ignition of the soil and measured by the difference between the amounts of inorganic phosphorus extractable from ignited and unignited samples. Examples of each of these in wide use at the present time are the extraction method of Mehta et al. (1954) and the ignition method of Saunders and Williams (1955). The most probable source of error in extraction methods is thought to be partial mineralization of organic phosphorus during extraction, giving low values, whereas in ignition methods the ignition is believed to increase the solubility of inorganic constituents, giving high values. If both methods are used to analyze soils, it is often found that extraction values are in fact considerably lower than ignition values, but it has not been possible to assess the extent of the errors in each case.

The methods were compared using some Canadian soil profiles and it was observed that ignition gave higher values in nearly all cases and

1 Contribution No. R20 of the Saskatchewan Institute of Pedology.
measured appreciable organic phosphorus even in some C horizons where extraction values were zero. Since there was virtually no organic matter in the C horizons, it was assumed that the ignition values in these cases measured an increase in solubility of inorganic phosphates after ignition. Although the comparative mineral composition of the surface and subsoil horizons may vary appreciably, it was felt that the C horizon ignition values might give an approximate measure of the ignition error throughout the solum. Accordingly, the ignition values for the upper horizons were corrected by subtraction of the C horizon values, and in most cases the results were very close to those obtained by the extraction method (McKercher and Anderson, 1968a). It seems likely that in such soils the extraction method of Mehta et al. (1954) causes no appreciable mineralization of organic phosphorus and gives an accurate measure of this fraction.

Various reports (Williams, Williams and Scott, 1960; Barrow, 1961; Williams, 1966) show that although carbon, nitrogen, and sulphur ratios usually fall within broad limits, the organic phosphorus ratio with respect to any one or all of these elements has a much greater variability. These findings and similar evidence from the Canadian soils (McKercher, 1966) that the organic phosphorus is not closely related to either nitrogen or carbon, suggest that the organic phosphorus accumulation may be largely independent of soil organic matter buildup.

**Fractionations of the Organic Phosphorus**

(a) The identified compounds

Positive identification and quantitative measurement of the individual organic phosphorus constituents in soil have proved difficult but considerable progress has been made as detailed in reviews by Ulrich and Benzler (1955), Anderson (1966, 1967) and Cosgrove (1967).

Three major groups of organic phosphorus compounds have been identified in the soil, namely the phospholipids, the nucleic acids and the inositol phosphates. There is evidence that both phosphoproteins and a sugar phosphate, glucose-1-phosphate, may also exist (Anderson, 1967) but the relative content of the latter must be very low. Phospholipids account for about 1% of the total organic phosphorus (Hance and Anderson, 1963) and nucleic acid phosphorus not more than 5 to 10% (Adams, Bartholomew and Clark, 1954; Anderson, 1958, 1961). The inositol phosphates account for as much as 60% of the total organic phosphorus, but a wide range of values have been reported (Anderson, 1967) and some soils have undetectable amounts (Cosgrove, 1966b).

(b) The inositol phosphates

Soil contains a number of stereoisomeric forms of the inositol phosphates which are found nowhere else in nature (Cosgrove, 1966a). Inositol itself (hexahydroxycyclohexane) appears to be ubiquitous with life and no adequately examined tissue has been found free of the substance (Angyal
SOIL ORGANIC PHOSPHORUS

Combinations occurring in soil include polyphosphates of five of a possible nine stereoisomers, the myo-, scyllo-, neo- and racemic dl- forms. Naturally occurring neoinositol has been identified only from soil sources, and apart from within the soil, the only phosphorylated inositol which appears to occur naturally is myoinositol.

Although the search for the presence in the soil of inositol phosphates, particularly the hexaphosphate, was in progress over half a century ago (Shorey, 1913), it was not until 1940 that inositol and materials having the properties of ferric inositol hexaphosphate were isolated (Dyer, Wrenshall and Smith, 1940; Yoshida, 1940). A further period of years elapsed before techniques were available for a more thorough characterization of the ester fraction (Smith and Clark, 1951; Anderson, 1956; Cosgrove, 1962).

Several stereoisomeric forms of inositol penta- and hexa-phosphates have been identified in a number of soils (Cosgrove, 1963; Cosgrove and Tate, 1963; Martin and Wicken, 1966; McKercher and Anderson, 1968c) but most of the inositol phosphate in soil exists as myoinositol hexaphosphate. Other myoinositol polyphosphates and lower esters of the other isomers of inositol have been detected in very small amounts (Smith and Clark, 1951; Anderson, 1956; Wild and Oke, 1966).

To extract inositol phosphates, the soil is leached with acid to remove calcium, and then extracted with alkali. After a preliminary chemical fractionation of the extract, the esters are finally separated by chromatography on anion resins (Cosgrove, 1963; Anderson, 1964; Martin, 1964a). Both neutral salt solutions and acids have been used to elute the esters from the resins. The most effective separations of myoinositol penta- and hexa-phosphates and the stereoisomers are achieved by a gradient elution with acid (Cosgrove, 1963) but a stepwise increase in concentration (Smith and Clark, 1951) or a combination of both (Schormüller and Bressau, 1960) have also been used. At some stage before the resin fractionations, a treatment must be given to break up the inositol complexes which are known to form with polysaccharides and proteins (Anderson, Peck and Creighton, 1940; Anderson and Hance, 1963; Cosgrove, 1963; Martin, 1964b). This separation of inositol phosphates from other organic materials may be effected by prolonged alkaline hydrolysis (Anderson, 1964) or by oxidation and acid hydrolysis (Cosgrove, 1963).

Procedures have been described by Anderson (1964) and by McKercher and Anderson (1968b) in which the soil inositol phosphates are fractionated by anion exchange chromatography in a formate system. If a stepwise elution with neutral ammonium formate is carried out, then preliminary chemical fractionation of the soil extract can be kept to a minimum, and a fraction obtained which contains the penta- and hexaphosphates of the various isomeric inositols. If a detailed characterization of this fraction is desired, the esters can be readily recovered and chromatographed on an exchange column by eluting with a gradient of hydrochloric acid.

In the Canadian soils examined, the combined inositol penta- and hexa-
phosphates account for up to 71 p.p.m. $P$ in the surface soils and up to 43 p.p.m. in the subsoils representing, on the average, 6% of the total phosphorus and 17% of the organic phosphorus in the soil. The amounts are positively correlated with total phosphorus, total organic phosphorus, and orthophosphate retention capacity. Within the same broad geographical areas, there is a tendency for soils developed under grassland to have somewhat lower inositol penta- and hexa-phosphate contents than those developed under forest. Calcareous soils usually have a lower content than adjacent acid or neutral soils (McKercher, 1966). The amounts found are much lower than have been found in soils in Britain (Anderson, 1964).

Characterization of the individual esters in a number of Canadian and British soils has confirmed the presence of penta- and hexa-phosphates, but their relative amounts are quite variable. In one instance the pentaphosphates exceed the hexaphosphates to a slight extent but in most cases the ratio of hexa- to penta-phosphate is between 2:1 and 4:1. Whether these ratios are reflections of the relative stabilities of the esters in soil is open to question but Arnold (1955) has found that the other lower esters are in turn less stable than the penta- hexaphosphate fraction. Myoinositol phosphates are present in greatest amount, but appreciable amounts of scylloinositol phosphates also occur, supporting the view that the soil inositol phosphates are derived primarily from micro-organisms (Cosgrove, 1966b). The ability of some micro-organisms to synthesize certain inositol phosphates has been established (Caldwell and Black, 1958a; Cosgrove, 1964; Piña and Tatum, 1967).

Amounts of inositol phosphates which have been reported in soils from various parts of the world are listed in Table 1. Some of the values must be regarded as approximate since the investigations in which they were determined were concerned with the nature of the esters and the methods used were not intended to give quantitative values.

(c) Unidentified phosphate ester in a Saskatchewan soil

One Saskatchewan soil has yielded a considerable quantity (Table 2) of an organic phosphorus fraction which is not adsorbed by ion exchange resins (McKercher, 1966). This material has been briefly commented on elsewhere (McKercher and Anderson, 1968c). Its constitution has not been fully resolved but some further characterization has been carried out.

Its composition by weight includes 25% $C$, 0.75% $N$ and 1.4% $P$. The anthrone sugars composed 25% and the uronic acids by carbazole 4%. The phosphorus was recovered in similar quantity by analysis for total $P$ following ignition and also after the acid hydrolysis for sugar depolymerization. The sugars liberated after hydrolysis at 100° for 5 hr in $n H_2SO_4$ included galactose, glucose, mannose, arabinose, xylose, fucose, rhamnose, and three methyl sugars. These are similar to those obtained from the usual soil polysaccharide fractionations (Duff, 1952; Parsons and Tinsley, 1961; Sowden and Ivarson, 1962). An infrared absorption spec-
### Table 1

**Data from the literature concerning content and relationships of the inositol penta- and hexa-phosphates***

<table>
<thead>
<tr>
<th>Reference</th>
<th>Soil Location</th>
<th>Penta- and Hexa-phosphates</th>
<th>Isomer Ratio Myo + dl : Scylo</th>
<th>Ester Ratio Hexa : Penta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson (1964)</td>
<td>Scotland</td>
<td>56 to 460</td>
<td>24 to 58</td>
<td>—</td>
</tr>
<tr>
<td>Cosgrove (1963)</td>
<td>Australia</td>
<td>85 to 300</td>
<td>15 to 20</td>
<td>4 or 5 : 1</td>
</tr>
<tr>
<td>Cosgrove (1966b)</td>
<td>Scotland</td>
<td>—</td>
<td>—</td>
<td>5 : 1</td>
</tr>
<tr>
<td></td>
<td>California</td>
<td>—</td>
<td>—</td>
<td>5 : 1</td>
</tr>
<tr>
<td>Caldwell and Black (1958b)</td>
<td>Iowa</td>
<td>30 to 80</td>
<td>15 to 25</td>
<td>1 : 2 : 1</td>
</tr>
<tr>
<td></td>
<td>Minnesota</td>
<td>35 to 60</td>
<td>10 to 20</td>
<td>0.9 to 1.4 : 1</td>
</tr>
<tr>
<td></td>
<td>Michigan</td>
<td>35 to 40</td>
<td>20 to 25</td>
<td>2 to 3 : 1</td>
</tr>
<tr>
<td>McKercher and Anderson (1968b, c)</td>
<td>Scotland</td>
<td>90 to 460</td>
<td>35 to 50</td>
<td>1.8 to 4.6 : 1</td>
</tr>
<tr>
<td></td>
<td>Saskatchewan</td>
<td>20 to 60</td>
<td>10 to 30</td>
<td>1.4 to 2.7 : 1</td>
</tr>
<tr>
<td></td>
<td>Quebec</td>
<td>60 to 70</td>
<td>18 to 24</td>
<td>1 : 1</td>
</tr>
<tr>
<td>Martin (1964)</td>
<td>New Zealand</td>
<td>55 to 165</td>
<td>12 to 46</td>
<td>8 : 1</td>
</tr>
<tr>
<td>Martin and Wicken (1966)</td>
<td>New Zealand</td>
<td>22 to 340</td>
<td>5 to 26</td>
<td>4 : 1</td>
</tr>
<tr>
<td>Pederson (1953)</td>
<td>Denmark</td>
<td>163</td>
<td>46</td>
<td>—</td>
</tr>
<tr>
<td>Smith and Clark (1951)</td>
<td>Iowa, grassland</td>
<td>15 to 25</td>
<td>7 to 16</td>
<td>4 : 1</td>
</tr>
<tr>
<td></td>
<td>Iowa, forested</td>
<td>90</td>
<td>34</td>
<td>4 : 1</td>
</tr>
<tr>
<td>Thomas and Lynch (1960)</td>
<td>Alberta</td>
<td>10 to 150</td>
<td>10 to 20</td>
<td>0.8 to 2 : 1</td>
</tr>
</tbody>
</table>

* Some values are interpretive approximations of data or graphs and do not necessarily reflect opinions of the authors quoted.
trum indicated a high content of structural hydroxyl and carboxyl groups.

Until more is determined about this fraction, it would be premature to speculate on its manner of phosphate binding, but Martin (1964b) has suggested the possible existence of polymeric phosphate containing compounds, such as phosphorylated polysaccharides, in the soil. Dephosphorylation of the fraction did not occur, however, with alkaline phosphatase.

### Table 2

| COMPARISON OF ORGANIC PHOSPHORUS FRACTIONS IN A REGO DARK BROWN CHERNOZEM FROM SASKATCHEWAN |
|-----------------------------------------------|-----------------------------------------------|
| Total Organic P (method of Mehta et al. 1954) | 200 p.p.m. of soil |
| Inositol Penta- and Hexa-phosphate P           | 31 p.p.m., 15.5% of Organic P                  |
| (McKercher and Anderson, 1968b)               |                                               |
| Unadsorbed Organic P                           | 56 p.p.m., 28% of Organic P                   |

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SOIL ORGANIC PHOSPHORUS


SUMMARY

Methods of analyses for total organic phosphorus are briefly considered. Discussion of some individual chemical groups or components of the organic phosphorus fraction in the soil follows. Particular attention is directed to the inositol phosphates and the fractionation carried out on various soils. Mention is made of a fraction containing more organically bound phosphorus than the inositol phosphate fraction, which occurred in a Canadian Rego Dark Brown Chernozemic soil.

RéSUMÉ

Les diverses méthodes visant à l'analyse de tous les composés organiques contenant du phosphore ont été brièvement repassées. Ceci est suivi en considérant certains groupes de composés organiques contenant le phosphore que l'on retrouve dans le sol. Une attention toute spéciale a été portée sur les esters phosphoriques de l'inositol ainsi que sur les résultats d'analyses plus spécifiques faites sur ce groupe dans différents sols. L'attention est portée à un groupe de composés organiques contenant plus de phosphore que les esters phosphoriques de l'inositol. Un tel groupe a été retracé dans un sol Brun foncé Rego-chernozémique (Rego Dark Brown Chernozemic) canadien.

ZUSAMMENFASSUNG

A COMPARATIVE STUDY ON THE ACCURACY OF THE RADIO-ACTIVE METHOD FOR SOIL MOISTURE DETERMINATION USING THERMAL AND EPITHERMAL NEUTRONS

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The evaluation of a method is generally based on its accuracy and precision. The accuracy of a measurement gives the degree of conformity to the true value, and the precision is the possibility of obtaining each time the same magnitude of a given value by repeated measurements.

Lack of precision may be due to changes in the source-detector-moderator geometry, to decrease in source activity, to changes in the characteristics of the electronic circuit or to inexact reading of the calibration curve. This has already been discussed extensively by a number of authors (Van Bavel 1963, Wack 1964, De Boodt 1967) and will not be treated here. The accuracy of the method poses a greater problem since, up to now, no satisfactory solution has been found for the exact measurement of water content using neutron scattering (Wack 1967). The reasons are, from the theoretical point of view, the following:

1. the influence of the chemical composition of the soil,
2. the change in resolution as a function of the water content, and
3. the dependence of the measurements on the density of the soil.

In the opinion of some workers, e.g. Wack (1967), these effects are so important that the measurement of water content by neutron scattering should be abandoned and replaced by neutron transmission measurements. Such measurements are however at present only feasible for water content determinations near the surface. Other research workers think that the neutron scattering method can be improved through the use of epithermal neutrons.

The Use of Epithermal Neutrons

The scattering of neutrons in the soil is based on two phenomena:

1. Fast neutrons emitted by a radio-active source are slowed down to thermal neutrons through collision with the nuclei of the environment after covering an average slowing down length, \( L_s \), where \( L_s^2 = \frac{1}{8} AB^2 \) (see Figure 1).

2. The slowed down neutrons travel a certain distance called the diffusion length, \( L \), where \( L^2 = \frac{1}{6} BC^2 \) until they are captured by the nuclei of the surrounding medium.

Both processes are involved when thermal neutrons are counted as a measure of the water content, whereas only the first phenomenon is involved.
when using epithermal neutrons. As there is a fundamental difference between scattering and diffusion, it is to be expected that there will be a difference in accuracy when one or the other kind of neutron is detected to measure the same quantity of water.

The Measurement of Epithermal Neutrons

A cadmium foil, 0.065 mm thick, placed around the lithium glass scintillation counter on a neutron probe (see Figure 2), prohibits all thermal neutrons from reaching the detector. However, epithermal neutrons are only slowed down so that they are detected as thermal neutrons once behind the cadmium foil. The neutron flux detected however is strongly diminished. In order to increase the neutron flux the 100 mC Am-Be source is surrounded by an auxiliary moderator, a nylon cylinder of 7 mm wall thickness (probe Nr. 2). For the same reason the lead block separating source and detector is reduced to a height of 15 mm. This distance has proved to be optimal since any further reduction increases the background noise ($\gamma$ rays) more than the neutron flux. Another possible method of measuring epithermal neutrons is to surround a $^3$He counter (10-16 cm active length, gas pressure 10 atm) with cadmium foil. This detector is superior to the BF$_3$ counter (11.5 cm active length) also surrounded with cadmium foil. For both counters with and without cadmium foil the integrated spectrum in water was determined. For the $^3$He counter the plateaus were situated respectively at 310 and 2900 IPS and with the BF$_3$ counter the values were 251 and 550 IPS.

When using a scintillation crystal with (Figure 2, probe Nr. 2) and without (Figure 2, probe Nr. 1) a cadmium foil the values obtained in
the same circumstances were respectively 695 and 1055 IPS. The drop in efficiency (count rate per unit output of fast neutrons) is smallest when the geometry of probe Nr. 2 is used.

Fig. 2.—Two different geometries of a source-detector assembly:—probe Nr. 1: measuring thermal neutrons, probe Nr. 2: measuring epithermal neutrons.

The Influence of the Chemical Nature of the Elements

The probability of a collision between a neutron and an atom in the moderating substance depends on a value called the cross-section. This is not a simple function of the diameter of the atom (Glasstone and Edlund 1953). On collision the neutrons are slowed down. This means a loss of
### Table 1

**Characteristics of the most common elements in the soil**

<table>
<thead>
<tr>
<th>Elements</th>
<th>In the first 45 cm of the soil-layer</th>
<th>In the first 16 km of the earth crust</th>
<th>Scattering fast neutrons (5 MeV)</th>
<th>Scattering slow neutrons</th>
<th>Capture slow neutrons</th>
<th>Atomic weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen (O)</td>
<td>51-0 (1)</td>
<td>46-71 (1)</td>
<td>1.5</td>
<td>4.2</td>
<td>20-10</td>
<td>16.00</td>
</tr>
<tr>
<td>Silicon (Si)</td>
<td>32-3 (2)</td>
<td>27-69 (2)</td>
<td>2.0</td>
<td>1.7</td>
<td>0.16</td>
<td>28.09</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>6-3 (3)</td>
<td>8-07 (3)</td>
<td>2.5</td>
<td>1.4</td>
<td>0.241</td>
<td>26.98</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>2-6 (4)</td>
<td>5-05 (4)</td>
<td>3.7</td>
<td>11.0</td>
<td>2.62</td>
<td>55.82</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>1-9 (5)</td>
<td>3-65 (5)</td>
<td>2.2</td>
<td>3.0</td>
<td>0.44</td>
<td>40.08</td>
</tr>
<tr>
<td>Carbon (C)</td>
<td>1-3 (6)</td>
<td>traces</td>
<td>1-0</td>
<td>4-8</td>
<td>3.73 x 10^-3</td>
<td>12.01</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>1-4 (7)</td>
<td>2-58 (7)</td>
<td>3.5</td>
<td>1.5</td>
<td>7.07</td>
<td>39.10</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>0-8 (8)</td>
<td>2-75 (6)</td>
<td>2-5</td>
<td>4-0</td>
<td>0.525</td>
<td>22.99</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0-7 (9)</td>
<td>0-09 (12)</td>
<td>3-6</td>
<td>2-3</td>
<td>13-2</td>
<td>54.94</td>
</tr>
<tr>
<td>Hydrogen (H)</td>
<td>0-5 (10)</td>
<td>0-14 (10)</td>
<td>1-8</td>
<td>38-0</td>
<td>0.332</td>
<td>1.00</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>0-5 (11)</td>
<td>2-08 (8)</td>
<td>2-0</td>
<td>3-6</td>
<td>0.069</td>
<td>24.32</td>
</tr>
<tr>
<td>Titanium (Ti)</td>
<td>0-4 (12)</td>
<td>0-62 (9)</td>
<td>3-4</td>
<td>4-0</td>
<td>5-8</td>
<td>47.90</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>0-1 (13)</td>
<td>traces (-)</td>
<td>1-5</td>
<td>10-0</td>
<td>1-88</td>
<td>14.01</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>0-05 (14)</td>
<td>0-13 (11)</td>
<td>2-5</td>
<td>5-0</td>
<td>0-20</td>
<td>30.98</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>traces</td>
<td>traces</td>
<td>1-5</td>
<td>4-0</td>
<td>795</td>
<td>10.82</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>traces</td>
<td>traces</td>
<td>4-0</td>
<td>7-0</td>
<td>2450</td>
<td>112.41</td>
</tr>
</tbody>
</table>
energy, which is a function of the mass of the nucleus with which the neutron collides. With heavy nuclei the neutrons will have an inelastic collision and consequently will not lose too much energy. The lighter the nucleus, the more it approaches a mass equal to one. This means a more elastic collision and a greater loss of energy. A collision with a nucleus of the same mass as the neutron (e.g. hydrogen) means a loss of 50% of the energy for the fast neutron.

From Table 1 it can be seen that in this respect the place of the H atom is unique. This is one reason why neutron scattering can be used to determine the amount of water present in the soil. The other reason lies in the chance of interaction with the different nuclei. In this respect thermal and epithermal neutrons differ considerably as can be seen from their cross-sections listed in Table 1.

The cross-section of the scattering fast neutrons when they change from fast neutrons (5 MeV) to slow neutrons (0.025 eV) has the greatest increase in relative as well as in absolute value for hydrogen. This makes it evident that this property contributes much to make the scattering of neutrons appropriate for determining the amount of H atoms present in the soil. Epithermal neutron determination essentially makes use of this property to determine the water content of the soil.

When taking the number of thermal neutrons as a measure of the water content the cross-section of the atoms for capturing slow neutrons is also involved. Here again hydrogen occupies a selected place in the sense that this atom has an extremely low chance of capturing slow neutrons while other elements, which occur commonly in the soil such as C, Mg, Cl, K, Fe, have very high chances of capturing slow neutrons. Consequently the measurement of thermal neutrons gives too low a value and this will be more pronounced the more elements are present with a high cross-section for capturing slow neutrons. This does not happen in the case of epithermal neutrons. A practical result can be seen in Figure 3.

A sandy profile was first scanned at 5 cm intervals with an ordinary neutron moisture probe (Berthold) in which the number of thermal neutrons was counted. The resulting moisture profile (curve A) is characterised by smooth changes from one layer to another with an abnormally low number of counts at a depth of 100 cm. The same profile was scanned a second time with an adapted Berthold probe measuring only the number of epithermal neutrons (curve B).

When comparing curve A with curve B it is obvious that the latter shows more distinct changes in the number of counts (see small arrows). This is explained on the basis of better resolution which is discussed later. Besides, curve B at a depth of 100 cm indicates a much smaller relative number of counts than curve A. This is due to the presence of a B_{iron} horizon at that depth. The profile scanned is a covered podzol under sand. The accuracy of the method is much better when absorbers are present in the soil profile and when use is made of epithermal neutrons. Curves A and B were obtained with the two probes Nr. 1 and Nr. 2 respectively.
Resolution as a Function of Water Content

The resolution of a sub-surface probe is the ability of the probe to resolve changes in water content with depth. The depth of the effective measuring point of the probe below the surface of a homogeneous soil, at which the count rate is a stated fraction (95%) of the count rate in an effectively infinite volume of the same soil, is a practical way to compare the depth resolution for different probes (I.A.E.A. Consultants Meeting, Vienna, 1966).

From the above definition it is evident that when epithermal neutrons are measured the resolution will be smaller as the diffusion length of the neutrons is decreased in the measurement. This has been already verified experimentally (De Boodt 1964). With the two probes, measuring respectively thermal and epithermal neutrons, the data in Table 2 were obtained.

The accuracy is involved in the resolution since all hydrogen present in the soil scanned by the neutrons does not contribute to the same extent in slowing down the neutrons. The hydrogen atoms closest to the source contribute the most (McHenry 1963). In that sense moisture measure-
NEUTRON MOISTURE METER TECHNIQUE

Table 2
MAXIMAL IPS AND RESOLUTION WHEN USING THERMAL OR EPITHERMAL NEUTRONS IN SOIL MOISTURE MEASUREMENTS

<table>
<thead>
<tr>
<th>Moisture content in vol %</th>
<th>10.0</th>
<th>19.5</th>
<th>29.7</th>
<th>41.2</th>
<th>50.8</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe Nr. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thermal neutrons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPS max. minus background</td>
<td>220</td>
<td>400</td>
<td>545</td>
<td>670</td>
<td>730</td>
<td>1055</td>
</tr>
<tr>
<td>Resolution (cm)</td>
<td>40.2</td>
<td>32.2</td>
<td>30.8</td>
<td>27.4</td>
<td>23.4</td>
<td>19.4</td>
</tr>
<tr>
<td>Probe Nr. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epithermal neutrons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPS max. minus background</td>
<td>180</td>
<td>250</td>
<td>320</td>
<td>405</td>
<td>455</td>
<td>695</td>
</tr>
<tr>
<td>Resolution (cm)</td>
<td>32.8</td>
<td>29.8</td>
<td>23.0</td>
<td>26.0</td>
<td>14.4</td>
<td>10.0</td>
</tr>
</tbody>
</table>

ments through neutron scattering is a weighted observation (Wack 1964), so that the accuracy can only be perfect when the soil is homogeneous over the depth of resolution.

As this is not always the case some workers (Wack 1967) believe more in transmission measurements to obtain good accuracy. It is our opinion that when the resolution can be reduced to the size of a horizon where a homogeneous moisture distribution or moisture gradient is expected sub-surface measurements can be made accurate. Thus an increase in accuracy accompanies improvement in resolution. In this respect real gains can be achieved using epithermal neutrons as shown in Table 2.

The Sensitivity of the Method to the Density of the Soil

From Table 1 it is obvious that atoms other than hydrogen present in the medium play a non-negligible part in neutron attenuation. Hence the greater the density the more neutrons are slowed down. Thus sensitivity to the density of the soil can be put equal to virtual amounts of water (De Boodt 1964, Holmes 1966). Increase in density does not only mean more atoms slowing down the neutrons, but also the presence of more absorbers, the absorbers having the opposite effect to the atoms present. Indeed absorbers capture an amount of slow neutrons originating through the presence of atoms other than hydrogen. This means a reduction in the virtual amount of water. Hence measurements based on the number of epithermal neutrons are less accurate than measurements counting thermal neutrons, the latter being subjected to the capturing just mentioned.

This has been proved experimentally. The two probes shown in Figure 2 were again used to carry out measurements with thermal and epithermal neutrons. Two 55-gallon drums were filled with sand-alum mixtures at densities of 1.38 and 1.28 g/cm³. Both had a volume water content of 29.7% in the form of crystal water.
With each probe and on each drum a wetted front experiment was carried out. The results are presented in Figure 4.

Making use of thermal neutrons the maximal IPS were found to be 517 and 495 for densities of 1.38 and 1.28 g/cm$^3$ respectively. Thus a difference of 0.1 g/cm$^3$ density corresponds to a difference of 22 counts per second.

With epithermal neutrons the maximal IPS was 251 and 237 for densities of 1.38 and 1.28 g/cm$^3$ respectively. Thus a difference of 0.1 g/cm$^3$ density corresponds to 14 counts per second. Attention is drawn to the fact that the impulses were measured with a digital scaler during three minutes and recalculated in seconds. The differences observed are highly significant. According to the calibration curves 22 IPS and 14 IPS correspond to differences in humidity of 1.3% and 1.8% respectively.

These figures are of the same order of magnitude as can be obtained theoretically namely a virtual water content of 1.9% with thermal neutrons and 2% with epithermal neutrons (Jensen and Somer 1967).

**ACKNOWLEDGMENTS**

This study has been carried out with the aid of the Joint Division FAO-IAEA (Vienna) for the equipment concerned. Euratom (Brussels) provided the funds to engage an assistant being the junior author.


SUMMARY

The accuracy of the radio-active method for soil moisture determination when epithermal instead of thermal neutrons are used is influenced as follows:

(1) The influence of the chemical composition of the soil is diminished.
(2) The resolution is improved.
(3) The sensitivity to the density of the soil becomes worse.

The two first points are considered to be positive while the third is negative. The theory is given and practical data are reported to support the above conclusions.

RÉSUMÉ

La précision de la méthode lorsqu'on emploie des neutrons épithermiques plutôt que thermiques est influencée comme suit:

1) une diminution de l'influence de la composition chimique du sol.
2) une amélioration du pouvoir résolvant.
3) une augmentation de l'influence de la densité.

Les deux premiers points sont considérés comme étant positifs et le troisième comme négatif. La théorie ainsi que des données résultant des expériences pour appuyer les conclusions sont également présentées.
ZUSAMMENFASSUNG

Bei Verwendung von epithermischen statt thermischen Neutronen wird die Präzision der Methode in mancher Hinsicht geändert:
1) Einfluss der chemischen Zusammensetzung des Bodens verringert.
2) Besseres Auflösungsvermögen.
3) Einfluss der Dichte nimmt zu.

Die beiden ersten Auswirkungen sind als positiv zu betrachten, während der dritte Punkt als negativ angesehen werden muss.

Ergebnisse, die zu den obengenannten Folgerungen Anlass gegeben haben, sind ebenfalls dargestellt worden.
SERIENMÄSSIGE CHARAKTERISIERUNG DES WASSERHAUSHALTES VON BEWÄSSERUNGSBÖDEN IN ARIDEN UND SEMIARIDEN GEBIETEN

G. Husz

I. CHARAKTERISIERUNG DES BODENWASSERHAUSHALTES DURCH DIE SAUGSPANNUNGSKURVE


II. BESTIMMUNG DER SAUGSPANNUNG DES BODENS (KAPILLARPOTENTIAL)


Es erscheint daher die Möglichkeit interessant, für große Serien aus einfach und rasch ermittelbaren bodenkundlichen Daten die pF-Kurve zu berechnen, wie dies bereits De Leenheer et al. (1960) und A. Combou und P. Quantin (1963) getan haben.


Hier bietet sich als erstes die Körnung, also die Textur an. Voraussetzung für ein wirklich rasches Arbeiten ist allerdings eine Bestimmungsmethode für den Rohlton- und Schluffgehalt, die bei ausreichender Genauigkeit auch von angelerntem Personal rasch durchgeführt werden kann. Es kommen hier vor allem Hydrometer-Methoden in Betracht. Wenn man in die Berechnung nicht den Rohlton, also die Korngröße ≤ 2 μ eingehen läßt, sondern eine
Korngröße von \( \leq 5 \mu \) und eine andere von 5 bis 50 \( \mu \), dann können auf diese Weise von einem angelernten Mann mit einem Helfer leicht etwa 60 Proben pro Tag aufgegearbeitet werden.

Um einen ersten Überblick zu bekommen, haben wir uns im Chicamatal Nordperus, das ist in der Küstenwüste, dieser Möglichkeit bedient und haben folgende multiplen Regressionen erhalten (Vergl. G. Husz 1967):

**Tabelle 1**

Gleichungen der multiplen Regression aus Textur und Wassergehalt für verschiedene Saugspannungen

<table>
<thead>
<tr>
<th>( pF )</th>
<th>atm</th>
<th>Gleichungen der multiplen Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-5</td>
<td>0-33</td>
<td>( y_j = 14·8737 + 0·2843x_{1j} + 0·10839x_{2j} )</td>
</tr>
<tr>
<td>3-0</td>
<td>1-00</td>
<td>( y_j = 14·7971 + 0·2861x_{1j} + 0·02294x_{2j} )</td>
</tr>
<tr>
<td>3-9</td>
<td>7-50</td>
<td>( y_j = 8·8005 + 0·3367x_{1j} - 0·03557x_{2j} )</td>
</tr>
<tr>
<td>4-2</td>
<td>15-00</td>
<td>( y_j = 10·2255 + 0·0247x_{1j} - 0·06337x_{2j} )</td>
</tr>
</tbody>
</table>

J = 1, 2, 3, \ldots 161, 162.  
\( y \) = V\% \( H_2O \) (abhängige Variable).  
\( x_1 \) = Kornanteil = 5 \( \mu \) (unabhängige Variable).  
\( x_2 \) = Kornanteil 5 — 50 \( \mu \) (unabhängige Variable).

Eine mathematische Prüfung hat gezeigt, daß das Zahlenmaterial, bzw. die Fehlerverteilung der Gauszschen Fehlerverteilungskurve folgt, was bedeutet, daß die üblichen Gesetzmäßigkeiten und Methoden der Wahrscheinlichkeitsrechnung und Statistik angewandt werden können. Während die varianzanalytische Prüfung für die erhaltene Beziehung im Zustand der Feldkapazität hochsignifikant sowohl für \( x_1 \) als auch für \( x_2 \) ist, ergeben sich für die höheren Druckbereiche Signifikanz von 99\% nur noch für \( x_1 \) (Korngröße \( \leq 5 \mu \)).

Eine andere sehr einfache Möglichkeit wäre die Berechnung der \( pF \)-Kurve aus der Bindigkeitszahl nach Arany (\( K_a \)), eines Wertes, welcher der "Saturationspercentage" der nordamerikanischen Literatur gleichzusetzen ist und etwa der oberen Plastizitätsgrenze nach Atterberg entspricht. Dieser \( K_a \)-Wert wird in Osteuropa, besonders Ungarn, seit langem für die physikalische Charakterisierung von Boden verwendet und es können leicht 100 Proben pro Tag von einer angelernten Person bestimmt werden.

Wir haben an unserem Institut auch aus dieser Zahl und der \( pF \)-Kurve eine Beziehung hergestellt und sie mit einer statistischen Sicherheit von 99\% bestätigt gefunden; leider garantiert eine hochsignifikante Beziehung zwischen zwei Werten, wie im vorliegenden Fall, nicht auch die gewünschte Genauigkeit der zu ermittelnden Werte bei Anwendung dieser Beziehung, das heißt bei Anwendung der Regression.

Dieses Berechnungsergebnis im einzelnen vorzutragen erscheint deswegen berechtigt, weil bei empirisch statistischen Methoden allzuoft die Gefahr einer unerlaubten Verallgemeinerung besteht und die Grenzen der Möglichkeiten bekannt sein sollten: Wir sehen aus der Zusammenstellung in Tabelle 2, daß zum Beispiel mit einer einzigen Bestimmung des \( K_a \)-Wertes eine \( pF \)-Kurve nicht auf befriedigende Weise berechnet werden kann. Selbst bei fünf Proben wird für genaueres Arbeiten in der Agrohydrologie der mögliche
Fehler von 3 $V\%$ Wasser nicht akzeptiert werden können. Macht man aber für eine zu untersuchende Flächeneinheit 20 Bestimmungen, so kann der auftretende Fehler schon eher toleriert werden. Die minimale Anzahl der Proben wird natürlich von der Fragestellung abhängen.

Von solchen einfachen Regressionen ist auch keine höhere Leistung zu erwarten, da, wie wir berechnet haben, nur etwa 30 bis 35% aller Faktoren, die auf die $pF$-Kurve Einfluss ausüben, erfasst wurden. Ebenso selbstverständlich ist, dass durch Einführen von einer Reihe von zusätzlichen Werten, wie Humusgehalt, $CaCO_3$-Gehalt, Strukturkennwerten, Ionenbelag, Art der Tonminerale etc. in multiple Regressionen $pF$-Kurven berechnet werden können, die den direkt in der Druckapparatur ermittelten sehr nahe kommen.

### Tabelle 2

<table>
<thead>
<tr>
<th>atm</th>
<th>$pF$</th>
<th>Regressionsgleichung</th>
<th>Sign.</th>
<th>Anzahl der Werte, aus denen ein Mittelwert gebildet wurde</th>
<th>Fehlergrenzen $V% H_2O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-33</td>
<td>2-5</td>
<td>$y_j = 13-80096 + 0-3707x_j$</td>
<td>99</td>
<td>1</td>
<td>6-17 ± 0-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>5</td>
<td>3-12 ± 0-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>20</td>
<td>1-77 ± 0-21</td>
</tr>
<tr>
<td>1-00</td>
<td>3-0</td>
<td>$y_j = 8-309762 + 0-4084x_j$</td>
<td>99</td>
<td>1</td>
<td>6-51 ± 0-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>5</td>
<td>3-02 ± 0-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>20</td>
<td>1-71 ± 0-19</td>
</tr>
<tr>
<td>7-50</td>
<td>3-9</td>
<td>$y_j = 2-860910 + 0-4179x_j$</td>
<td>99</td>
<td>1</td>
<td>7-05 ± 0-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>5</td>
<td>3-28 ± 0-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>20</td>
<td>1-87 ± 0-19</td>
</tr>
<tr>
<td>15-00</td>
<td>4-2</td>
<td>$y_j = 3-792653 + 0-3262x_j$</td>
<td>99</td>
<td>1</td>
<td>7-43 ± 0-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>5</td>
<td>3-47 ± 0-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99</td>
<td>20</td>
<td>1-95 ± 0-21</td>
</tr>
</tbody>
</table>

$x = K_A$ (Bindigkeitszahl nach Arany = Saturation percentage)  
y = $V\% H_2O$  
j = 1, 2, 3, ..., 161, 162.

### III. Bestimmung des osmotischen Potentialis bei Böden mit erhöhtem Elektrolytgehalt

In ariden und semiariden Gebieten ist der Elektrolytgehalt oft so weit erhöht, dass die dadurch auftretenden osmotischen Werte in der Bodenlösung nicht mehr vernachlässigt werden können, weil sie von der Pflanze bei ihrem Bestreben, Wasser aufzunehmen, zusätzlich überwunden werden müssen.

Nach einem Vorschlag eines Komitees der Internationalen Bodenkundlichen Gesellschaft (1963) vereinfachen wir für praktische Anwendungsziecke in der Form, daß wir das Adsorptionspotential und das Kapillarpotential zum Kapillarpotential zusammenfassen, wodurch das Gesamtpotential


Es hätte nun wenig Sinn, nach dem eingangs gemachten Vorschlag, das Kapillarpotential bei serienmäßiger Bestimmung auf schnellem Wege zu ermitteln, wenn nicht gleichzeitig auch das osmotische Potential ebenso rasch erhalten werden könnte. Die klassischen Methoden der Gefrierpunktserniedrigung, Siedepunktserhöhung, Messung des Dampfdruckes etc. dürften sich für die serienmäßige Routine vorläufig wohl kaum eignen.

Es bietet sich nun die in der nordamerikanischen Literatur stark verbreitete Möglichkeit an, den osmotischen Druck mit Hilfe der elektrischen Leitfähigkeit eines Bodenextraktes zu schätzen (Handbook No. 60—United States Department of Agriculture, 1954); Leider ist diese Methode nur für ganz leichte Sandböden brauchbar: Es wird nämlich die elektrische Leitfähigkeit in der Lösung gemessen, die aus einem Boden im Zustand des “saturation percentage”, oder besser, im Zustand der oberen Plastizitätsgrenze nach Atterberg, bzw. Bindigkeitszahl nach Arany \((K_a)\) extrahiert wurde.

Aber in Nichtsandböden, ist ein linearer Schluß von diesen ermittelten Werten auf andere Bodenfeuchtezustände nicht zulässig. Es ist nämlich unrichtig, anzunehmen, daß die im Saturationsextrakt gelöste Teilchenzahl oder Konzentration (Aktivität) der Ionen bei etwa halber vorhandener Wasser- menge im Vergleich zur “Saturationspercentage” doppelt so groß ist und der osmotische Wert sich dementsprechend nun auch verdoppeln müßte. Die Änderung der Konzentration (Aktivität) der Ionen der Bodenlösung und damit auch die Änderung des osmotischen Druckes folgt vielmehr einer Kurve, die vom Austauscher des Bodens (Tongehalt, Tonart, Humus) und wahrscheinlich auch vom \(pH\)-Wert abhängig ist.


\[ \text{Abb. 1} \]

\[ \text{Abb. 1.} \text{— Konzentration verschiedener wasserlöslicher Salze bei geändertem Wassergehalt.} \]
Verhältnis von 1:1, 1:0-5, 1:K_A, 1:0-25 gewonnen und analysiert. Es zeigte sich, daß die erhaltene Elektrolytkonzentration stark von obigem Bo: Wa—Verhältnis abhängig ist (Vgl. Abb. 1.a, 1.b, 1.c, sowie 2).

Abb. 2

Abb. 2.—Konzentration der wasserlöslichen Salze der Böden des Chicamatales (Peru) in Abhängigkeit vom Wassergehalt.

Eine derartige Kurve wurde schließlich auch für einen typischen Boden des Chicamatales (N-Peru) erstellt und mit ihrer Hilfe konnte nun für jedes beliebige Verhältnis Bo: Wa (Feuchtigkeit des Bodens in Gew. %) die Konzentration der löslichen Salze ermittelt werden. Wir waren also nicht mehr gezwungen, vom osmotischen Wert des Saturationsextraktes auf den von anderen Feuchtigkeitsgehalten zu schließen, sondern es eröffnete sich die Möglichkeit, aus der nun tatsächlich bekannten Konzentration den osmotischen Druck zu ermitteln.

Aus Tabelle 3 geht übrigens hervor, daß nicht nur die Ionenkonzentration eine bestimmte Änderung erfährt, sondern, daß sich auch die Ionenverteilung ändert.

Diese hier auf möglichst einfache Weise aufgezeigten Zusammenhänge haben ihre Erklärung in Überlegungen, die in der Theorie über das Verhalten von Elementarladungen bzw. Ionen in der Helmholtzschen Doppelschicht.
## Tabelle 3
**Analyse von wäfigen Bodenextrakten, gewonnen bei 15 atm Saugspannung**

<table>
<thead>
<tr>
<th>Boden:</th>
<th>pH des Extraktes</th>
<th>Leitfähigkeit des Extraktes</th>
<th>( \Sigma )</th>
<th>( \Sigma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td></td>
<td>( \mu \text{S cm}^{-1} ) (25 °C)</td>
<td>mmol l</td>
<td>mg %</td>
</tr>
<tr>
<td>0.18</td>
<td>7.30</td>
<td>14769</td>
<td>283.89</td>
<td>229.9</td>
</tr>
<tr>
<td>0.25</td>
<td>7.35</td>
<td>13364</td>
<td>249.60</td>
<td>286.5</td>
</tr>
<tr>
<td>0.50</td>
<td>7.10</td>
<td>10461</td>
<td>190.70</td>
<td>457.4</td>
</tr>
<tr>
<td>1.00</td>
<td>6.72</td>
<td>80001</td>
<td>147.80</td>
<td>715.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ca^++</th>
<th>Mg^++</th>
<th>K^+</th>
<th>Na^-</th>
<th>Cl^-</th>
<th>CO3^-</th>
<th>HCO3^-</th>
<th>SO4^-</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
</tr>
<tr>
<td>0.18</td>
<td>6.0</td>
<td>0.30</td>
<td>20.2</td>
<td>1.66</td>
<td>2.7</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>0.25</td>
<td>6.6</td>
<td>0.33</td>
<td>21.9</td>
<td>1.80</td>
<td>5.1</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>0.50</td>
<td>12.4</td>
<td>0.62</td>
<td>31.5</td>
<td>2.59</td>
<td>13.2</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>1.00</td>
<td>30.9</td>
<td>1.54</td>
<td>49.2</td>
<td>4.03</td>
<td>18.0</td>
<td>0.46</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Millimol pro 1 l des Extraktes

<table>
<thead>
<tr>
<th>Ca^++</th>
<th>Mg^++</th>
<th>K^+</th>
<th>Na^-</th>
<th>Cl^-</th>
<th>CO3^-</th>
<th>HCO3^-</th>
<th>SO4^-</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
</tr>
<tr>
<td>0.18</td>
<td>8.33</td>
<td>46.11</td>
<td>3.89</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.25</td>
<td>6.60</td>
<td>36.00</td>
<td>5.20</td>
<td>0.00</td>
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<tr>
<td>0.50</td>
<td>6.20</td>
<td>25.90</td>
<td>6.80</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1.00</td>
<td>7.70</td>
<td>20.25</td>
<td>4.60</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ca^++</th>
<th>Mg^++</th>
<th>K^+</th>
<th>Na^-</th>
<th>Cl^-</th>
<th>CO3^-</th>
<th>HCO3^-</th>
<th>SO4^-</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
<td>mg %</td>
<td>me %</td>
</tr>
<tr>
<td>0.18</td>
<td>106.67</td>
<td>42.78</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>33.33</td>
<td>72.78</td>
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<td>93.20</td>
<td>37.20</td>
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<td>0.00</td>
<td>0.00</td>
<td>5.60</td>
<td>65.80</td>
</tr>
<tr>
<td>0.50</td>
<td>69.20</td>
<td>23.80</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>2.20</td>
<td>56.60</td>
</tr>
<tr>
<td>1.00</td>
<td>54.70</td>
<td>14.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.40</td>
<td>45.13</td>
</tr>
</tbody>
</table>
zusammengefaßt werden können. Seit diese Theorie als Modell für das Verhalten von Ionen in unmittelbarer Nähe von Bodenaustauschern allgemein anerkannt und das Diffusionsverhalten der Ionen in der diffusen Doppelschicht beschrieben wurde (Gouy, Chapman, Stern), waren es in letzter Zeit vor allem auch Bolt et al. (1965), die die Energieverhältnisse in der Doppelschicht auch in Abhängigkeit zum Beispiel von der Konzentration bearbeitet haben.

Es ist nicht möglich, hier auf die in obigen zitierten Arbeiten gewonnenen Erkenntnisse genauer einzugehen; es soll aber auf die wichtigsten Ergebnisse hingewiesen werden:

1. Das Diffusionsbestreben der Ionen bzw. das Potential der Doppelschicht nimmt mit steigender Konzentration der Bodenaussenlösung ab.


Es zeigt sich also, dass Veränderungen der Konzentration der Bodenlösung oder Veränderungen des Feuchtezustandes bei gegebenem Elektrolytgehalt in der Bodenaussenlösung auch Änderungen im Gleichgewicht zwischen Innen- und Aussenlösung mit sich bringen müssen. Selbstverständlich sind diese Gesetzmäßigkeiten auch noch abhängig von Austauscher selbst.

Die Bestimmung des osmotischen Potentiales im pflanzenverfügbaren Wasser muss also derart erfolgen, daß für jeden Feuchtezustand der im eigenen osmotischen Wert unter Berücksichtigung der bei diesem Zustand vorhandenen Ionenkonzentration (Aktivität) und Ionenverteilung ermittelt wird.

Nach Van’t Hoff kann die allgemeine Zustandsgleichung idealer Gase für die Berechnung des osmotischen Druckes in idealen Lösungen angewandt werden:

\[ P_o = \frac{N \cdot R \cdot T}{V} \text{ atm} \]

\( T = \text{Absolute Temperatur} \)
\( R = \text{Allgemeine Gaskonstante (0,082)} \)
\( V = \text{Volumen (1)} \)
\( N = \text{Konzentration der aktiven Teilchen im Volumen } V, \text{ ausgedrückt in mol/l.} \)

Für \( N \) ergibt sich die Schwierigkeit, daß die durch Analyse bestimmte Konzentration nicht eingesetzt werden kann: Für das Zustandekommen und die Größe des osmotischen Wertes ist nämlich bekanntlich die Teilchenzahl und ihre Energie verantwortlich und die oben angeführte Formel gilt außerdem nur für unendlich verdünnte Lösungen.

Der Gesamtanzahl der analysierten Teilchen pro Liter (mol/l) kommt nun eine bestimmte Aktivität zu, die durch die im Vergleich zur unendlichen Verdünnung stark konzentrierten Lösung verringert ist. Diese Verringerung kommt dadurch zustande, daß bei realen Lösungen in einem gegebenen
Zeitpunkt eine bestimmte Anzahl von Teilchen durch Zusammenstoß sich von zwei oder mehreren zu einem Teilchen vereinigt haben. Dieser Vorgang spielt sich in einem dynamischen Gleichgewicht ab und kann durch die Verminderung der elektrischen Leitfähigkeit charakterisiert werden. Wenn die theoretische Leitfähigkeit $L_i$ ist und diejenige der realen Lösung $L_r$, dann ergibt $\frac{L_r}{L_i} = a$, ein Maß für die Verringerung der elektrisch geladenen Teilchen in einer realen Lösung, bzw. ein Maß für die "Aktivität" der analysierten Ionen. Die theoretische Leitfähigkeit $L_i$ einer Lösung kann aus der Ionenleitfähigkeit berechnet werden oder man verdünnt die zu untersuchende Lösung so stark, daß sie als ideal gelten kann, mißt die Leitfähigkeit und multipliziert den erhaltenen Wert nach vorherigem Abzug der Eigenleitfähigkeit des Wassers mit dem Verdünnungsfaktor.

Mit Hilfe einer entsprechenden Graphik (Siehe Abbildung 3) kann für jede beliebige Konzentration in der realen Lösung $L_i$ abgelesen und damit $a$ berechnet werden. Wenn $n$ die Anzahl der analysierten Ionen in mol/l ist, dann ist $\text{n.a.}$ die Anzahl der elektrisch geladenen Teilchen. Der verbleibende Rest aber ergibt sich somit aus der Differenz aus $n - \text{n.a.} = n(1-a)$. Dieser Ausdruck gibt aber nur an, wieviel von den gesamtausgelösten Teilchen ihre Ladung durch Zusammenstoß verloren bzw. neutralisiert, haben, nicht aber, zu wievielen Partikeln sie sich durch diese vorübergehende Vereinigung vermindert haben.

Da wir es im allgemeinen nur mit ein- und zweiwertigen Ionen zu tun haben, gibt es qualitativ gesehen nur die Möglichkeit, daß sich zwei oder drei Teilchen zu einem elektroneutralen Teilchen vereinigen. Im Falle einer NaCl-Lösung zum Beispiel, wären die $n(1-a)$ Teilchen mit dem Faktor $f = 0,5$, im Falle von Na$_2$SO$_4$ mit dem Faktor $f = 0,33$ zu multiplizieren. Üblicherweise liegen Mischsalze vor, sodaß der Faktor $f$ zwischen 0,33 und 0,50 liegt, je nach Art der Ionenkombination. Aus dem Gesagten ist also folgende Formel für $N$ abzuleiten:

$$N = \text{n.a.} + n(1-a)$$

und die Gesamtformel lautet somit:

$$P_o = \frac{RT}{V} n [a \cdot f(1-a)] \text{ atm.}$$

$n$ = Anzahl der analysierten Ionen (mol/l)

$a = \frac{L_r}{L_i}$ = ein Mass für die Aktivität der analysierten Gesamtionen

$f$ = siehe Text.

IV. BESCHNÄMMUNGEN DES GESAMTPOTENTIALS PFLANZENVERFÜGBAREN WASSERS

Das Kapillarpotential für die entsprechenden Punkte der Saugspannungskurve kann mit Hilfe von vorher aufgestellten Regressionsgleichungen, der jeweils zugehörige osmotische Wert nach der Formel: $P_o = \frac{RT}{V} n [a \cdot f(1-a)]$ berechnet werden.
Für die Routine benützt man entweder ein Computerprogramm oder eine Graphik. In Abb. 3 ist eine solche für die chemischen Verhältnisse der Bewässerungsböden des Chicamatales (Nord-Peru) dargestellt.

### Tabelle 4

**Analyse eines Salzbodens im Chicama-Tal (Peru)**

| Probenzahl | Tabelle 4
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Probenzahl</td>
<td>25</td>
</tr>
<tr>
<td>Tiefe (KCl)</td>
<td>0–60 cm</td>
</tr>
<tr>
<td>pH (KCl)</td>
<td>7,85</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>7,30</td>
</tr>
<tr>
<td>Bindigkeitszahl (Kₐ)</td>
<td>48,19</td>
</tr>
<tr>
<td>(= &quot;Satur. percent-age&quot;)</td>
<td></td>
</tr>
<tr>
<td>El. Leitfähigkeit des &quot;Satur. Extrakt&quot;</td>
<td>11,9 μS·cm⁻¹(25°C)</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>9,55%</td>
</tr>
<tr>
<td>Organ. Subst.</td>
<td>1,59%</td>
</tr>
<tr>
<td>Humus</td>
<td>1,34%</td>
</tr>
<tr>
<td>C</td>
<td>0,92%</td>
</tr>
<tr>
<td>N</td>
<td>0,098%</td>
</tr>
<tr>
<td>C/N</td>
<td>9,38%</td>
</tr>
</tbody>
</table>

**Wasserlöslich (Sat.Extr.)**

<table>
<thead>
<tr>
<th>Anzahl am Sorptionskomplex</th>
<th>Wasserlöslich (Sat.Extr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El. Leitfähigkeit des &quot;Satur. Extrakt&quot;</td>
<td>11,9 μS·cm⁻¹(25°C)</td>
</tr>
<tr>
<td>Ca⁺⁺⁺</td>
<td>340,2 mg %</td>
</tr>
<tr>
<td>Mg⁺⁺</td>
<td>151,0 mg %</td>
</tr>
<tr>
<td>K⁺⁺</td>
<td>39,0 mg %</td>
</tr>
<tr>
<td>Na⁺⁺</td>
<td>129,0 mg %</td>
</tr>
<tr>
<td>NH₄⁺⁺</td>
<td>0,9 mg %</td>
</tr>
<tr>
<td>NO₃⁻⁻</td>
<td>4,2 mg %</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>13,8 mg %</td>
</tr>
</tbody>
</table>

**Scheinbare Dichte**

<table>
<thead>
<tr>
<th>Flachenverfügbar</th>
<th>28,42 me %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umtausch—</td>
<td>28,42 me %</td>
</tr>
<tr>
<td>Umtauschkapazität</td>
<td>1,42</td>
</tr>
<tr>
<td>Textur</td>
<td>36,69%</td>
</tr>
<tr>
<td>VON G. HUGZ</td>
<td>15,60%</td>
</tr>
<tr>
<td>Umtauschbar</td>
<td>5,20 me %</td>
</tr>
<tr>
<td>Equivalente Dichte</td>
<td>36,69%</td>
</tr>
<tr>
<td>Ca⁺⁺⁺⁺⁺</td>
<td>340,2 mg %</td>
</tr>
<tr>
<td>Mg⁺⁺⁺⁺⁺</td>
<td>151,0 mg %</td>
</tr>
<tr>
<td>K⁺⁺⁺⁺⁺</td>
<td>39,0 mg %</td>
</tr>
<tr>
<td>Na⁺⁺⁺⁺⁺</td>
<td>129,0 mg %</td>
</tr>
<tr>
<td>NH₄⁺⁺⁺⁺⁺</td>
<td>0,9 mg %</td>
</tr>
<tr>
<td>NO₃⁻⁻⁻⁻⁻</td>
<td>4,2 mg %</td>
</tr>
<tr>
<td>PO₄³⁻⁻⁻⁻⁻</td>
<td>13,8 mg %</td>
</tr>
<tr>
<td>Scheinbare Dichte</td>
<td>28,42 me %</td>
</tr>
<tr>
<td>Umtausch—</td>
<td>28,42 me %</td>
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<tr>
<td>Umtauschkapazität</td>
<td>1,42</td>
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<tr>
<td>Textur</td>
<td>36,69%</td>
</tr>
<tr>
<td>VON G. HUGZ</td>
<td>15,60%</td>
</tr>
<tr>
<td>Umtauschbar</td>
<td>5,20 me %</td>
</tr>
</tbody>
</table>
SERIENMÄSSIGE CHARAKTERISIERUNG

Bei serienmäßigen Bestimmungen von Saugspannungskurven für die physikalische Charakterisierung des Bodens ergibt sich immer wieder die Schwierigkeit, daß die übliche Methodik für große Serien nicht geeignet ist.

Es wird daher vorgeschlagen, das matrische Potential mit Hilfe von Regressionsen, das osmotische Potential aber aus den chemischen Routinedaten mit Hilfe folgender Formel zu berechnen:

**LITERATUR**


Leenheer, L. De et al. (1961) — Monografie der zeepolders Repertorium van de Bodemkundige Eigenschappen der belangrijken Bodemtypen in de Belgische zeepolders, Rijkslandbouwhogeschool, Bodemkundig Laboratorium, Gent.


**ZUSAMMENFASSUNG**

Desorptionskurve eines Salzbodens unter Berücksichtigung des osmotischen Potential des Bodenlösungen bei verschiedenen Wassergehalte.
\[ P_0 = -\frac{RT}{V} n [a + f(1-a)] \]

\( T \) = Absolute Temperatur
\( R \) = Allgemeine Gaskonstante
\( n \) = Anzahl der Analysierten Ionen (mol/l)
\( V \) = Volumen (liter)
\( a \) = Maß für die Aktivität der Teilchen
\( f \) = 0.33 — 0.50 (Siehe Text)

\( P_0 \) kann auch direkt graphisch ermittelt werden.

Somit ergibt sich eine Möglichkeit einer serienmäßigen Bestimmung der Gesamtsaugspannung bzw. der Kurve des Gesamtpotentials.

**SUMMARY**

Routine determinations of desorption curves for physical soil characterisation are generally difficult to carry out because the usual methods are not suitable for large series.

It is proposed to calculate the matrix potential by regressions and the osmotic potential from chemical routine data using the following formula:

\[ P_0 = -\frac{RT}{V} n [a + f(1-a)] \]

\( T \) = Absolute Temperature
\( R \) = Gas Constant
\( V \) = Volume (litres)
\( n \) = Ion-concentration (mol/l)
\( a \) = Factor, indicating Particle activity
\( f \) = Factor varying from 0.33 — 0.50 (see text)

It is possible to read \( P_0 \) directly from the graphs, thus it will be possible to calculate very quickly the total potential for a large number of samples.

**RÉSUMÉ**

Les déterminations routinières de la courbe de désorption pour caractériser les sols présentent souvent des difficultés parce que les méthodes habituelles ne conviennent pas pour les grandes séries.

On propose donc de calculer le potentiel matrice au moyen de régressions et le potentiel osmotique à l'aide de données routinières chimiques, en utilisant la formule suivante:

\[ P_0 = -\frac{RT}{V} n [a + f(1-a), ] \]

\( T \) = la température absolue
\( R \) = la constante de gaz
\( V \) = le volume (litres)
\( n \) = concentration des ions (mol/l)
\( a \) = facteur indiquant l'activité des particules
\( f \) = facteur variant de 0.33 à 0.50

\( P_0 \) peut être déterminé graphiquement, il est donc possible de calculer rapidement le potentiel total pour toute une série d'échantillons.
ALTERSBESTIMMUNG VON BÖDEN DURCH DIE RADIOKOHLENSTOFFDATIERUNGSMETHODE

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1. EINLEITUNG


2. METHODE


Dieses angereicherte Material wird durch Säurebehandlung karbonatfrei gemacht, im Sauerstoffstrom verbrannt und das CO₂ wird gemäß folgenden Reaktionen in Benzol umgewandelt:

<table>
<thead>
<tr>
<th>Probe (C-baltig)</th>
<th>Humus + O₂ → → CO₂ +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karbonat + HClO₄</td>
<td>→ C₂H₄ + 2 LiClO₄</td>
</tr>
<tr>
<td>2CO₂ + 10Li</td>
<td>→ Li₂C₂ + 4 Li₂O</td>
</tr>
<tr>
<td>Li₂C₂ + 2 H₂O</td>
<td>→ C₂H₄ + 2 LiOH</td>
</tr>
<tr>
<td>3 C₂H₂</td>
<td>→ C₂H₄</td>
</tr>
</tbody>
</table>

Katalysator

Das aus der organischen Bodensubstanz hergestellte Benzol wird 1 : 3 mit altem Toluol + Szintillator vermisch und auf seine Aktivität hin gemessen.

Das Bodenalter berechnet sich aus der Aktivitätsmessung wie folgt (ohne Berücksichtigung der wichtigen Fehlerrechnung):

\[ T_{\text{Probealter}} = \frac{\ln 0,95 \times \text{Aktivität d. Kontemporärstandards}}{\text{Aktivität der Probe}} = 8030 \text{ J.} \]

\[
\left( 8030 = \frac{1}{\lambda} = \frac{T/2}{\ln 2} = \frac{5568}{0,69} \right)
\]

3. VERTIKALE VERJÜNGUNG


<table>
<thead>
<tr>
<th>Schichttiefe cm</th>
<th>(^{14}\text{C}-\text{Datum Alter Vor 1950})</th>
<th>Extremer vertikaler Kontaminationsfehler Jahre</th>
<th>Normaler vertikaler Kontaminationsfehler Jahre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>1 000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-30</td>
<td>2 000</td>
<td>400</td>
<td>200</td>
</tr>
<tr>
<td>30-45</td>
<td>3 000</td>
<td>850</td>
<td>400</td>
</tr>
<tr>
<td>45-60</td>
<td>4 000</td>
<td>1 200</td>
<td>600</td>
</tr>
<tr>
<td>60-75</td>
<td>5 000</td>
<td>1 400</td>
<td>700</td>
</tr>
<tr>
<td>75-90</td>
<td>6 000</td>
<td>1 500</td>
<td>800</td>
</tr>
</tbody>
</table>

Extremer Fehler = Einwanderung aus den höher liegenden Schichten: 40%, 20%, 10%, 5%, 2%, 1%.
Fehler in normaler Größenordnung = Einwanderung aus den höher liegenden Schichten: 20%, 10%, 5%, 2%, 1%.

4. BISHIERGE ANWENDUNG DER RADIOKOHLENSTOFFMETHODE ZUR BODENDATIERUNG


Es wird deutlich, daß die Moorhorizonte bis 2 m Tiefenlage eine gewisse Hauptbildungsperiode im älteren Subatlantikum, Subboreal und Atlantikum
Übersichtsplan der Radiokohlenstoff-Datierung des Bodens

<table>
<thead>
<tr>
<th>Alter</th>
<th>Bodentyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1000</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td></td>
</tr>
<tr>
<td>15000</td>
<td></td>
</tr>
<tr>
<td>20000</td>
<td></td>
</tr>
<tr>
<td>25000</td>
<td></td>
</tr>
</tbody>
</table>

Vorhandene Radiokohlenstoffdaten von Bodenmaterial aufgetragen gegen Zeit, postglaziale Klimaphasen und Bodentypen.

Abbildung 1: Vorhandene Radiokohlenstoffdaten von Bodenmaterial aufgetragen gegen Zeit, postglaziale Klimaphasen und Bodentypen.
besitzen, diejenigen aus 2-10 m Tiefe in älterem Atlantikum, Boreal, Präboreal, jüngerer Dryas sowie im Alleröd, und daß die durch mehr als 10 m mächtige Deckschichten begrabenen Moorhorizonte zumeist zwischen Präboreal und den Phasen des Altwürm entstanden sind. Unter den subhydrischen Böden wurden bisher die Gyttjen am stärksten datiert. Abb. 1 zeigt, daß die oberflächennahen (< 2 m tief) besonders häufig im Subboreal und Atlantikum entstanden, die zwischen 2 und 10 m Tiefe anstehenden vornehmlich im Boreal, Präboreal und Jungwürm (Jüngere Dryas, Alleröd, Ältere Dryas, Bölling, Älteste Dryas).


5. EIGENE BODENDATIERUNGEN


5.1 Schwarzerden


1. Tschernozem und Pseudogley-Tschernozem auf Würmloß des Braunschweiger Verbreitungsgebietes (Söllingen, Jerxheim), Maximalalter 5550 ± 80 B.P.
2. Pseudogley-Tschernozem auf Würmloß über Kreideton der Hildesheimer Region (Adlum), Maximalalter 4000 ± 80 B.P.
4. Dunkelbrauner Steppenboden in Würmloß über kalkreichem jungdiluvialem Feinsand bei Wallertheim in Rheinhessen 2560 ± 60 B.P.
5. Prairieboden-artige Schwarzerde auf würmzeitlichem Geschiebemergel, Großenbrode/Ostholstein, Maximalalter $1850 \pm 70$ B.P.

6. Pararendzina-Schwarzerdevorstufe unter Laacher Bims (Alleröd), Michelsberg nördl. Ochtendung, Rheinland-Pfalz $9130 \pm 100$ B.P.

Die schichtweise ermittelten Daten verschiedener Schwarzerdevorkommen zeigen in Abb. 2:

1. Die Vorstellung, daß in Schwarzerden durch die stark entwickelte Bodenfauna eine besonders intensive Materialdurchmischung stattfände, trifft nicht zu. Alle datierten Profile besitzen innerhalb der 10 cm starken Unterteilungsschichten einen deutlichen vertikalen Altersgradienten. Diese Erkenntnis schließt gleichzeitig die mancherorts vertretene Hypothese aus, daβ der Mullhorizont der Schwarzerde sich im Laufe der Zeit nicht nach oben sondern nach unten hin ins kalkreiche Lößmaterial fortgepflanzt habe.

2. Die Alterskurven der Böden in Trockenlagen (Söllingen I, Söllingen II, Adlum II) zeigen einen starken Altersanstieg bis in die C-Material-Grenzschicht, was auf eine langausgedehnte Pararendzinaentstehungsphase im trockenen Kalksteppenmilieu hindeutet. Demgegenüber

**Abb. 2.**—Schichtweise datierte Tschernozeme, Auftragung: Lagerungstiefe (Mitte des Schichtpakets) gegen Alter.
zeigen die Alterskurven in flachen, Stauwasser-beeinflußten Senken (Söllingen III, Söllingen IV, Adlum I) in den untersten 30 cm des A-Horizontes kaum einen Altersanstieg, was auf eine kurze Entstehungsphase mit schneller organischer Substanz-Akkumulation im feuchten bis anmorrigen Milieu hinweist. Es scheint, daß man aus der Gestalt der Alterskurve eines schichtweise datierten Tschernozem-A-Horizontes Rückschlüsse auf die Umweltbedingungen in der Entstehungsphase ziehen kann.


Ein Datum aus vergrabenem Schwarzerdrelikt unter Laacher Trachyttuff bei Michelsberg, Rheinland/Pfalz ergab 9130±100 B.P., mit Korrektur also ein Alter bis zu 11000 Jahren.

Man könnte daher annehmen, daß es sich bei den jüngeren Vorkommen in den Hauptverbreitungsgebieten (s. Abb. 2) um spätere Erosionsformen handelt. Ältere Voralleröd-Tschernozeme sind jedoch in den typischen Verbreitungsgebieten bisher noch nicht gefunden worden.

5.2 Plaggenböden


Eigene Datierungsarbeiten an Plaggenböden:
1. Graue Plaggenböden im Raum Greven/Westfalen auf sandigem Material der Emsniederung, Maximalalter 1300 ± 80 B.P.
2. Brauner Plaggenboden südlich der Stadt Rheine/Westfalen auf sandig-kiesigem Material der Emsniederung, Maximalalter 1260 ± 60 B.P.
3. Grauer Plaggenboden auf altpleistozämem Sand südlich Lengerich/ Westfalen, Maximalalter 1190 ± 70 B.P.

5.3 Podsole


Eigene Datierungen von Podsol-\(A_h\) und \(B_s\)-Horizonten:

1. Podsol \(B_h\)-Horizont, 60-70 cm tief, Sennesand nahe Autobahnüberquerung des Teutoburger Waldes, 930 ± 80 B.P.
2. Podsol \(B_s\)-Horizont, 70-85 cm tief, in Liassand bei Irrel, nahe Echternach, 810 ± 50 B.P.
3. Podsol \(B_h\)-Horizont, 80-95 cm tief, in diluvialem Sand, Darlaten westlich Stolzenau, Niedersachsen, 1220 ± 60 B.P.
4. Darlaten \(B_s\)-Horizont, 95-110 cm tief, in diluvialem Sand, westlich Stolzenau, Niedersachsen, 1165 ± 60 B.P.
5. Podsol \(A_h\)-Horizont, 10-25 cm tief, in Gemisch von diluvialem- und Kreidesand über Maasterrasse, Scherpenseel bei Geilenkirchen, Niederrhein, 2960 ± 70 B.P.
6. Podsol \(B_h\)-Horizont, 65-85 cm tief, sonst wie unter 5., Scherpenseel bei Geilenkirchen, 2570 ± 70 B.P.
7. Podsol $B_h$-Horizont, 60-70 cm tief, in diluvialem Moränensand und -schotter, Wilsede, Lüneburger Heide, 1140 ± 60 B.P.

8. Podsol $B_h$-Horizont, 55-70 cm tief, in diluvialem Moränensand und -schotter, Oberhaverbeck, Lüneburger Heide, 940 ± 50 B.P.


10. Podsol $B_h$-Horizont, obere $B_a$-Schicht, 80-95 cm tief, sonst wie 9., Flaesheim bei Haltern, 2220 ± 90 B.P.

11. Podsol $B_h$-Horizont, untere $B_a$-Schicht 135-170 cm tief, sonst wie 9., Flaesheim bei Haltern, 2420 ± 80 B.P.

Diese Podsoldaten von $A_h$- und $B_h$-Horizonten zeigen:

1. Die Hauptentstehungsphase der Podsole liegt 800-3000 Jahre zurück, also im Subatlantikum und Subboreal.

2. Bei dem zweistöckigem $B_h$-Horizont im Profil Flaesheim besteht nur ein geringer Altersunterschied zwischen den beiden durch vertikale

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**Abb. 4.** — Schichtweise datierte Parabraunerde, Auftragung: Lagerungstiefe (Mitte des Schichtpakets) gegen Alter.
Brücken zusammenhängenden Schichtpaketen. Das deutet daraufhin, daß die eigentliche Ausbildungsphase des mächtigen $B_h$-Horizontes abrupt einsetzt und kurzfristig verläuft.


5.4 Parabraunerde

Radiokohlenstoffdaten von rezenten Parabraunerden sind den Autoren nicht bekannt.

Eigene Daten liegen vor vom Parabraunerdeprofil Frimmersdorf, 35 km

![Graph](image-url)
nordwestlich Köln auf Würm- und wahrscheinlich Rißlöß. Sein Maximalalter ist 1880 ± 80 B.P.

In Abb. 4 sind die Daten von 4 je 25 cm mächtigen Schichten bis 1 m Tiefe aufgetragen. Das Alter von 1880 Jahren stellt die Entstehung der Lößparabraunerde ins Subatlantikum. Für eine endgültige Stellungnahme bedarf es weiterer Datierungen dieses wichtigen Bodentyps.

5.5 *Flaches Niedermoor*

Gemäß Abb. 1 gibt es bereits eine große Anzahl datierter Anmoore und Niedermoore.

Eigene Daten liegen vor von:

1. Begrabenes Anmoor unter lehmig-sandiger Rambla im Alluvium der kleinen Donau, nahe Preßburg, Slowakei, 2460 ± 60 B.P.
2. Flaches Niedermoor auf schwerem alluvialem Lehm über tertiärem Ton (Kalkarer Moor), nahe Billig, südlich Euskirchen/Nordrheinland, Maximalalter 7790 ± 110 B.P.

Die Daten von 20 cm tiefen Schichten in zwei Profilien (Rand und Mitte des Moorgebietes) sind in Abb. 5 dargestellt.


5.6 *Seemarsch*

Ein datiertes Seemarschprofil auf der Insel Nordstrand, Holstein, nahe Süderhafen ergab ein Maximalalter von nahezu 3000 Jahren. Marschdaten benötigen Kontrollmessungen des $^{13}$C/$^{12}$C-Verhältnisses, da der Humus-$C$ hier sowohl aus atmosphärischem- wie auch aus altem Meeres-Karbonatkohlenstoff herrühren kann. Die Subhorizonte zeigen folgende $^{14}$C-Alter:

<table>
<thead>
<tr>
<th>Tiefe</th>
<th>Alter</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-10 cm</td>
<td>modern</td>
</tr>
<tr>
<td>10-20 cm</td>
<td>110 ± 60</td>
</tr>
<tr>
<td>20-30 cm</td>
<td>240 ± 60</td>
</tr>
<tr>
<td>30-40 cm</td>
<td>1310 ± 50</td>
</tr>
<tr>
<td>40-50 cm</td>
<td>1420 ± 70</td>
</tr>
<tr>
<td>50-60 cm</td>
<td>2950 ± 70</td>
</tr>
</tbody>
</table>

5.7 *Rendzinen der Alpen*

Ein besonders mächtiges Tangelrendzinaprofil auf dem Kramer (eingereicht von Dr. T. Dietz und Dr. W. Kerpen) wurde datiert und zeigte ein Maximalalter von 4172 Jahren. Nach Korrektur für vertikale Verjüngung
ALTERSBESTIMMUNG VON BÖDEN

und Unterschied zu dendrochronologischen Ergebnissen (Damon 1966) darf man das wirkliche Alter mit etwa 6000 Jahren beziffern.

Die Subhorizonte zeigen folgende $^{14}$C-Alter:

<table>
<thead>
<tr>
<th>Subhorizonte</th>
<th>Tiefe (cm)</th>
<th>$^{14}$C-Alter</th>
</tr>
</thead>
<tbody>
<tr>
<td>OF</td>
<td>10-15</td>
<td>530 ± 60</td>
</tr>
<tr>
<td>$OH_{11}$</td>
<td>20-35</td>
<td>820 ± 60</td>
</tr>
<tr>
<td>$OH_{12}$</td>
<td>45-55</td>
<td>2050 ± 50</td>
</tr>
<tr>
<td>$OH_{21}$</td>
<td>60-70</td>
<td>3740 ± 90</td>
</tr>
<tr>
<td>$OH_{22}$</td>
<td>75-80</td>
<td>4172 ± 70</td>
</tr>
</tbody>
</table>

5.8 Daten von Fraktionen der organischen Substanz des Bodens


Eigene Gesamt-C- und Huminsäure-C-Daten einer Söllinger Tschernozemprobe zeigen gute Übereinstimmung (Ges.C 2100 ± 80; Hum.S. C 2240 ± 80).

Weitere Untersuchungen konzentrieren sich auf die Altersbestimmung von Fulvosäure-, Braun- und Grauhuminsäure-Fraktionen in Tschernozem $A/C$- und Podsol $B_h$-Horizonten.

Fraulein Elisabeth Kruse vom Institut für Bodenkunde der Universität Bonn sei für ihre sehr wertvolle technische Hilfe herzlich gedankt!

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LITERATUR


ZUSAMMENFASSUNG


In Abb. I sind alle erreichbaren, bisher weltweit gewonnenen, Bodendaten zusammengefaßt. Aus dem vorhandenen Datenmaterial, einschließlich der beschriebenen eigenen Bodendaten, vermag man die Hauptbildungsphasen der Moorböden, Schwarzerden, Plaggenböden und Podsole zu erkennen.


SUMMARY

Briefly the required practical measures for radiocarbon dating of soils, in particular for sample preparation and age calculation, are outlined.

Enrichment of C-content as well as elimination of young roots and cell debris are brought about by a combined sedimentations-sieving-centrifugation pretreatment.

Age supplements, to compensate for rejuvenation of the soil samples due to percolation or soil translocation by the soil-fauna, are assessed by model estimates for average as well as extreme conditions.

In Fig. 1 all obtainable existing radiocarbon dates on soils are listed. These, together with our own dates described subsequently, allow to distinguish the principal time-phases of development for bog soils, chernozems, plaggen soils, and podzols.

Our own radiocarbon dates on low moor, chernozem, para-brown-earth, plaggensoils, podzols, rendzinas and soil-carbon fractions are discussed. Age assessment of all our great soil groups will require a great deal of additional dating effort.
Les mesures pratiques pour datage du sol par la méthode de carbone-14 sont expliquées en détail, particulièrement en ce qui concerne la préparation des échantillons et le calcul de l'âge.

Un enrichissement du taux de C, et une élimination des racines jeunes, aussi bien que des tissus végétaux, sont achevés par une méthode combinée de sédimentation, tamisage et de centrifugation.

Des suppléments en âge, pour compenser le rajeunissement des échantillons de sol au cours de la percolation ou de la translocation par la faune de sol, estimés pour des conditions normales et extrêmes, sont indiqués.

Le Figure 1 contient toutes les dates de sol, par la méthode de radio-carbone, existantes et connues.

Avec les dates propres, figurant dans ce travail, elles permettent de distinguer les phases principales du temps de développement des sols tourbeux, des chernozems, des plaggensols et des podzols.

Les dates propres des sols tourbeux, de chernozem, de sol brun lessivé, de plaggensol, de podsol, de rendzine et de fractionnement de la matière humique, sont discutées. L'estimation de l'âge de tous nos sol-groupes demande encore beaucoup d'effort de datage additionnel.
Einleitung
Um das Porenvolumen eines Bodens zu bestimmen, werden meistens Proben mit Stechzylindern entnommen, an denen die Messungen im Labor durchgeführt werden. Wenn die Veränderung des Porenvolumens in Abhängigkeit von der Zeit verfolgt werden soll, dann müssen bei dieser Arbeitsweise jedesmal neue Proben entnommen werden. Dabei muss jede nachfolgende Entnahme in einem gewissen Abstand von der vorigen Entnahmestelle erfolgen, um zu vermeiden, dass bei der vorigen Entnahme gestörte Bodenbereiche mit erfasst werden. Der räumliche Abstand jeder erneuten Probenentnahme von der vorhergehenden wird oft um so größer sein, je tiefer und damit größer die jeweils auszuhebende Profilgrube ist.
Infolgedessen müssen Unterschiede im Porenvolumen, die im Verlauf der Zeit festgestellt werden, als Ergebnisse des Zusammenwirkens von Veränderungen des Bodens nicht nur in Abhängigkeit von der Zeit (z.B. Rid, 1961), sondern auch in Abhängigkeit vom Raum angesehen werden, sie müssen also nicht unbedingt auf Quellung und Schrumpfung beruhen. In der vorliegenden Arbeit wird versucht, den relativen Anteil von Raum und Zeit, d.h. den Anteil der Heterogenität der Bodenstruktur und den von Quellung und Schrumpfung an den beobachteten Veränderungen zu bestimmen.

Methoden und Material
An 7 Bodenprofilen wurden je zweimal von 0 bis ~ 100 cm Tiefe Stechzylinderproben entnommen, an denen das Gesamtporenvolumen (GPV) und die Wasserspannungskurve bestimmt wurden. Aus der Wasserspannungskurve wurde der Anteil des bei 0,06 und 0,3 at entfernten sowie der des bei 15 at im Boden verbleibenden Wassers und daraus der Anteil an Poren mit Äquivalentdurchmessern von > 50 (sP), 10-0,2 (mP) und < 0,2 μ (fP) berechnet. Die Bestimmungen des GPV wurden mit je 3, die der Wasserspannung mit 4 Parallelen je Horizont durchgeführt. Die Probenentnahme erfolgte mit einem zeitlichen Abstand von 1 Tag bis zu 1 Jahr und einem räumlichen Abstand von 2 m bis zu 100 m.

In 4 von diesen Profilen sowie in 5 weiteren wurden 2 mm starke Eisenplatten (10 x 20 cm) waagerecht in eine ungestörte Profilwand geschlagen und an ihren Enden Stangen festgeschraubt, die nach dem Zuschütten der Grube aus dem Boden ragten. Je Profil wurden 3 Platten in etwa 30, 60 bzw. 100 cm Tiefe angebracht. Die Veränderung der Lage der Platten wurde durch Messen der Veränderung der Abstämde der
TABELLE 1
HERKUNFT UND EIGENSCHAFTEN DER UNTERSUCHTEN BÖDEN

<table>
<thead>
<tr>
<th>Profil</th>
<th>Bodentyp und Ausgangsmaterial</th>
<th>Org. S. (%), Ton (%), Schluff (%)</th>
<th>CaCO₃ (%)</th>
<th>pH</th>
<th>GPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ap/Ah</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Braunerde fluv. glac. S.</td>
<td>2 6 3/10¹</td>
<td>30 4/30¹</td>
<td>0 0</td>
<td>6-8 4-5</td>
</tr>
<tr>
<td>Ame</td>
<td>Vega Auenton</td>
<td>5 27 50</td>
<td>46 32</td>
<td>0 0</td>
<td>6-0 6-3</td>
</tr>
<tr>
<td>Old</td>
<td>Parabraunerde Geschiebelhm</td>
<td>10 7 20</td>
<td>25 24</td>
<td>0 0</td>
<td>3-4 4-0</td>
</tr>
<tr>
<td>IR*</td>
<td>Pseudogley Löß</td>
<td>4 16 10</td>
<td>74 72</td>
<td>0 4</td>
<td>4-3 7-6</td>
</tr>
<tr>
<td>Ho*</td>
<td>Podsol Dünensand</td>
<td>38 1 7</td>
<td>9 1</td>
<td>0 0</td>
<td>2-9 4-2</td>
</tr>
<tr>
<td>BoH*</td>
<td>Pseudogley Geschiebelhm</td>
<td>2 13 15/53¹</td>
<td>25 22/40¹</td>
<td>0 6/30¹</td>
<td>3-7 7-3</td>
</tr>
<tr>
<td>Be*</td>
<td>Pseudogley Geschiebelhm</td>
<td>2 5 22</td>
<td>25 27</td>
<td>0 0</td>
<td>6-2 5-4</td>
</tr>
<tr>
<td>LH**</td>
<td>Pseudogley Löß</td>
<td>6 13 13</td>
<td>74 74</td>
<td>0 0</td>
<td>3-4 5-5</td>
</tr>
<tr>
<td>Ru**</td>
<td>Braunerde Löß</td>
<td>2 9 17</td>
<td>74 77</td>
<td>0 0</td>
<td>6-9 6-8</td>
</tr>
<tr>
<td>Mw**</td>
<td>Vega Auenlehlm</td>
<td>4 29 14</td>
<td>51 64</td>
<td>1 0</td>
<td>7-1 6-5</td>
</tr>
<tr>
<td>Mi**</td>
<td>Braunerde Geschiebesand</td>
<td>4 5 1</td>
<td>10 2</td>
<td>0 0</td>
<td>6-8 4-4</td>
</tr>
<tr>
<td>Gret**</td>
<td>Pseudogley Lias-Ton</td>
<td>2 33 65</td>
<td>40 35</td>
<td>1 0</td>
<td>7-3 6-7</td>
</tr>
</tbody>
</table>

* Böden, an denen außer Stechzylinderuntersuchungen auch Sackungsmessungen durchgeführt wurden.
** Böden, an denen nur Sackungsmessungen durchgeführt wurden.
¹ Unterschiede zwischen 2 Probeentnahmen infolge unterschiedlicher Erfassung von C- und D-Horizonten.

**ERGEBNISSE**

1. Räumlicher und zeitlicher Abstand:

Ein Vergleich der Ergebnisse der Bestimmungen innerhalb der Parallelengruppen der einzelnen Profile zeigt, dass bei der Untersuchung der in Tab. 1 beschriebenen Profile eine deutliche Tendenz der Standardabweichungen für verschiedene Bodentiefen und Porengrößenbereiche auftrat (Abb. 1a). Wie ein Vergleich mit den früher bestimmten Standardabweichungen der gleichen Arbeitsweisen (Hartge, 1965) zeigt, wurden deren Werte nur bei den sgP und beim GPV in 0-20 cm Tiefe deutlich überschritten.

Vergleicht man die Verhältnisse bei einem räumlich wie auch zeitlich größeren Abstand der Probenahmen, so erhält man ein weniger eindeutiges Bild (Abb. 1b). Im Vergleich zu Abb. 1a ist hier die Tendenz der Streuungsabnahme bis in die Schicht 60-80 cm etwa die gleiche, doch sind die Unterschiede zwischen den verschiedenen Porenbereichen nicht so deutlich zu erkennen. Trotzdem ist auch hier die deutliche Abnahme der Streuungen mit zunehmender Tiefe erkennbar, und zwar beim GPV und bei den sgP etwa gleich stark. Der Unterschied der Streuungen dieser beiden Messgrössen zu den anderen ist kleiner als beim Vergleich der Werte innerhalb der gleichen Grube (Abb. 1a). Vollständig abweichend sind die Ergebnisse in der Tiefe 80-100 cm bei den sgP und den fP, weil mit zunehmender Entfernung von der Bodenoberfläche die Wahrschein-
lichkeit zunimmt, bei der Probeentnahme in eine Schicht anderer geologischer Herkunft zu geraten.

Eine solche Schicht (D-Horizont) steht in 5 der 7 untersuchten Profile in ~ 100 cm Tiefe an. Da ihre Oberfläche nicht eben ist, wird sie von der tiefsten hier durchgeführten Probeentnahme gelegentlich in unterschiedlichem Ausmass mit erfasst. Dies führt, wie Abb. 1 zeigt, in erster Linie zu starker Erhöhung der Streuungen bei den sgP. Da die Gruppe der fp stark vom Tongehalt abhängig ist, führt das stellenweise Miterfassen eines D-Horizontes aus tonreichem Material auch bei ihnen zu starken Streuungen.

2. Zeitlicher Abstand allein:

Abb. 2 lässt erkennen, wie stark sich der Abstand der Platten und damit das GPV ändert, wenn es an genau der gleichen Stelle im Verlauf längerer Zeit beobachtet wird. Im 1. Beispiel, einem Sandboden (Mi), treten so gut wie keine Unterschiede auf. Es ist zu erkennen, dass die Platten trotz sorgfältigen Einbaues ihr Niveau im Boden im Verlauf des ersten Beobachtungsjahres noch änderten, um dann nur noch Verschiebungen von ≤ 3 mm für eine 29 cm mächtige Bodenschicht zu zeigen. In 66-93 cm Tiefe sind die Unterschiede noch geringer (Tab. 2). Das 2. Beispiel zeigt bei einem Geschiebelehm Verschiebungen bis zu 6 mm, wobei eine Tendenz zur Schrumpfung in den beiden trockenen Jahren und eine Tendenz zur Quellung in den feuchten Jahren angedeutet ist. Diese Tendenz ist in 60-105 cm Tiefe nicht mehr erkennbar. Die Veränderungen sind hier auch absolut schwächer (Tab. 2). Das 3. Beispiel zeigt die sehr viel stärkeren Verschiebungen in dem Pseudogley aus Lias-Ton. Auch
TABELLE 2
VARIATIONSBREITE DER VERÄNDERUNG DER SCHICHTMÄCHTIGKEIT UND DES GPV 1962-1966

<table>
<thead>
<tr>
<th>Profil</th>
<th>Bodentyp und Ausgangsmat.</th>
<th>Tiefe (cm)</th>
<th>Variationsbreite</th>
<th>( \text{Ablesung (mm)} )</th>
<th>( \text{GPV (%)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi</td>
<td>Braunerde fluv. glac. S.</td>
<td>37-66</td>
<td>3</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>66-93</td>
<td>2.5</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>LH±</td>
<td>Pseudogley</td>
<td>27-62</td>
<td>4</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LÖβ</td>
<td>62-95</td>
<td>3</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>Pseudogley</td>
<td>36-60</td>
<td>5,5</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LÖβ</td>
<td>60-102</td>
<td>4</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Ru††</td>
<td>Parabraunerde LÖβ</td>
<td>44-115</td>
<td>4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Mw††</td>
<td>Vega</td>
<td>40-70</td>
<td>7</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Auenlehms</td>
<td>70-105</td>
<td>4</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>BoH</td>
<td>Pseudogley</td>
<td>40-60</td>
<td>6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Geschiebelehms</td>
<td>60-105</td>
<td>3</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Ho††</td>
<td>Podsol</td>
<td>35-66</td>
<td>5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>66-104</td>
<td>4,5</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Gret</td>
<td>Pseudogley</td>
<td>0-36</td>
<td>22</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lias-Ton</td>
<td>36-68</td>
<td>11</td>
<td>3,5</td>
<td></td>
</tr>
</tbody>
</table>

† erst 1963 begonnen bzw. 1964 beendet.
†† Meßreihe durch Zerstörung unterbrochen.


DISKUSSION UND SCHLUSSFOLGERUNGEN

Vergleicht man die Ergebnisse der Hebungs-Sackungs-Messungen mit denen der Probeentnahmen mit Stechzylindern, so ergibt sich, dass die Standardabweichungen, die bei späteren Wiederholungen einer Probeentnahme neben dem ursprünglichen Entnahmeort auftreten, etwa in der gleichen Größenordnung liegen wie die Variationsbreite der Hebungs-Sackungs-Messungen. Das bedeutet, dass die zeitlichen Veränderungen, die nur durch Quellung und Schrumpfung bedingt wurden, etwa 1/6 des in Abb. 1b dargestellten Fehlers ausmachen.

Der jahreszeitliche und witterungsbedingte Gang der Kurven in Abb. 2 lässt erkennen, dass die beobachteten Veränderungen nicht zufallsbedingt, sondern durch Quellung und Schrumpfung hervorgerufen sind, die teilweise auf jahreszeitlich bedingte Wassergehaltsänderungen zurückzuführen sind.
teilweise aber auch auf solche infolge der allgemeinen Wetterlage oder der Topographie des Standortes. Daher wurde auch in diesem Fall keine Standardabweichung berechnet.


**Literatur**


**Zusammenfassung**

An 7 Bodenprofilen wurden die Veränderungen des Gesamtporenvolumens und verschiedener Porengrössenbereiche in Abhängigkeit von Raum und Zeit durch 2-fache Probeentnahme zu verschiedenen Terminen und in Abständen von 2-100 m untersucht. Die Veränderung in Abhängigkeit von der Zeit allein wurde durch Sackungs- und Hebungsmessungen an 9 Bodenprofilen an ins Profil eingesetzten Platten bestimmt. Die unter-
HETEROGENITÄT DES BODENS


RéSUMÉ

Les variations dans le volume total des pores et les divers domaines des pores, relatifs aux facteurs de temps et d’espace ont été étudiées dans 7 profils du sol par prélèvement d’échantillons, deux par profil, aux intervalles différents et à des distances entre 2 et 100 m. Les variations basées sur le facteur de temps seulement ont été déterminées en mesurant l’action de gonflement et de tassement dans 9 profils à l’aide de plaques enfouies dans les profils. Les sols examinés étaient les suivants: alluvion glaciaire sablonneuse et limoneuse; loess; limon et argile alluviaux, et un argile mésozoïque (lias). Les résultats ont montré que les variations dans le volume des pores, dûes au gonflement et tassement du sol, étaient de l’ordre de 1/6 des variations constatées dans les échantillons prélevés à des intervalles de temps ainsi qu’à des distances différentes. Le changement dans les déviations par rapport à la profondeur du profil, la distance entre les sites de prélèvement (à l’intérieur de la fosse et dans les deux fosse contiguës) et les domaines des pores indique que l’hétérogénéité du sol et surtout la structure du sol étaient la cause principale des variations.

On a trouvé que l’hétérogénéité était plus importante avec des pores plus grands et près de la surface du sol. Il a été conclu, par conséquent, que la structure du sol est plus hétérogène que la texture du sol.

Summary

In 7 soils variations of total pore space and of several ranges of pore diameters as found within one and between two profile pits were investigated and compared with those found by measuring the swelling and shrinking of 9 soils as measured by plates buried in undisturbed soils. The soils investigated were sandy and loamy glacial till, loess, alluvial loam and clay and a mesozoic clay (lias). Results showed that most of the differences found between two adjacent pits dug at different times were due to heterogeneity of soils and to a small extent only (~ 1/6) to lifting-settling action of the soils. Heterogeneity was found to be greatest with big pores and near to the soil surface. So it was concluded that soil structure is more heterogeneous than soil texture.
MONOLITH LYSIMETERS—CONSTRUCTION.
FEATURES AND PRELIMINARY
HYDROLOGICAL RESULTS

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I. INTRODUCTION

Knowledge of soil-water-plant relationships is of great importance in agriculture, allowing the agronomist to get the best performance from many cropping-practices, not only those that conserve moisture or keep soil erosion controlled, but also those that increase crop yields.

It is also necessary to know the water movement over the soil surface, into and through the entire soil profile and the water use by the crop, in order to achieve better control.

The data presented here were obtained from monolith lysimeters, which are located at the experiment station of the Instituto Agronomico in Campinas, Sao Paulo, Brazil. The blocks have the three main soil types of the State of Sao Paulo. Three profile depths have been used under different soil cover treatments. The lysimeter and evaporimeter set-up measures the water and soil nutrient losses by percolation through the soil profile. This can be further related to the surface runoff losses.

II. REVIEW OF LITERATURE

An early bibliographical review on lysimeter studies has been made by Kohnke et al. (1940) which covers about 250 years of lysimeter research. The authors show that most lysimeter studies have emphasized water balance, soil fertility and lysimeter management, very few of them being concerned with planning.

Wallihan (1940) and Colman (1946) refer to lysimeter layout. The lysimeters built at Campinas have been based on the Musgrave type (1935). Among the research works on the soil hydrologic balance are those presented by Martin and Rich (1948) and Colman and Hamilton (1947). Fertility problems have been studied by many others who have covered percolate composition (Roller and Bowen 1944, Plice 1945). Harrold and Dreibelbis (1951) present data on soil nutrient losses for different soil types and soil uses as well as for maximum percolation rate.

Lysimeter studies in Africa have been reported by Haouet (1946). Many works from European countries relate crop cover and soil nutrient status to the percolation loss (Geering 1943, Odelein 1945). Also in Latin
America, Suarez de Castro (1958) refers to soil nutrient and percolation loss; Grohmann et al. (1951) present a reduced-scale monolith lysimeter for laboratory studies which features low construction costs, with lateral movement of water reduced to a minimum. Bertoni and Barreto (1966) obtained results from monolith lysimeters as an approach to the study of the hydrologic cycle.

III. MATERIAL AND METHODS

(a) Description of lysimeters

A set of lysimeter and evaporimeter monolith blocks, of undisturbed soil profiles, was installed at the Instituto Agronomico’s Campinas Experiment Station. It consists of a group of 60 lysimeter blocks, 18 dry-soil evaporimeter blocks, 8 saturated-soil evaporimeter blocks, 3 free-surface water evaporimeter pans, 1 wind recorder, 1 temperature recorder, 2 rain gauges and 5 soil thermometers.

Figure 1 shows a plan and elevation view of the installation. The blocks were located along a tunnel. 30 blocks to each side in a 10-block row arrangement. In the tunnel are kept all containers for surface runoff and percolation water. Each three-block row along the tunnel has one of the three main soil types, podzolic soil on calcareous sandstones, ortho red-yellow podzolic soil and latosolic B terra roxa.
In each block row the first two units are 0-45 m deep, and the six centre ones 0-9 m deep, and the last two are 1-8 m deep and all have the same (0-75 m²) surface area. The lysimeters were made of No. 16 galvanized iron sheets. A ring-like metal sheet baffle was welded to the cylinder wall 0-20 m from its top end, to minimize lateral drainage.

The block units of dry-soil evaporimeters are placed in two 9-unit rows alongside the tunnel stairway. Each row is made up of 3 evaporimeters for each soil type. The evaporimeters are weighed twice a week by means of a balance hanging from a winch hook. The winch is bolted to a steel chassis that can be displaced by hand on rails over the blocks.

The 8 block units of saturated-soil evaporimeters are located on the other side of the tunnel stairway. They are in a single-row arrangement which has 2 evaporimeters for each soil type and 2 more soil blocks with muck soil. Each evaporimeter has a water supply tank placed at a higher level. This tank is connected by a plastic hose to a float-valve box clamped to vertical iron rod thus providing an up-and-down displacement along the evaporimeter’s profile length. The float-valve tank is also connected to the bottom of the evaporimeter to make a liquid system in communicating vessels. Observations can be made on the effect of water table depths inside the evaporimeters. The method permits also the measurement of excess water due to rainfall.

The three free-water evaporimeter pans are placed beside those of saturated-soil. The evaporating pans are 0-33 m deep, with 0-1875, 0-75 and 3-00 m² surface area. Observations are made with vernier hook gauges.

(b) Experimental design

The experimental plan is designed to investigate basic hydrologic and edaphic principles related to soil and water conservation. The basic design is a factorial experiment to study the correlation among all the factors involved.

(ii) Lysimeters

Two combined experiments are included in two factorial designs as follows:

(1) Soil type × profile depth × irrigation (3 × 3 × 2)

The 18 treatment combinations are:

Soil type = 3 variables with 6 replications each: (1) yellow podzolized soils on calcareous sandstones, (2) ortho red-yellow podzolic soils, (3) latosolic B terra roxa.

Profile depth = 3 depth levels with 6 replications each: (1) 0-45 m, (2) 0-90 m, (3) 1-80 m.

Irrigation = 2 soil moisture conditions having 3 replications each: (1) with irrigation, (2) without irrigation.

Soil cover is incorporated with the following crop rotations: cotton, soybeans, corn, meadow, meadow.
(2) **Soil type × soil cover** (3 × 4)

The 12 treatment combinations are:

Soil type = 3 variables with 4 replications each: (1) yellow podzolized soils on calcareous sandstones, (2) ortho red-yellow podzolic soils, (3) latosolic B terra roxa.

Soil cover = 4 variables with three replications each: (1) no cover, bare soil, (2) straw mulch, (3) perennial plant cover (coffee tree), (4) perennial plant cover + straw mulch.

(ii) **Soil Evaporimeters**

Two combined experiments are included in two factorial designs, as follows:

(1) **Soil type × soil use, in dry soil evacipersimeters** (3 × 6)

The 18 treatment combinations are:

Soil type = 3 variables with 6 replications each: (1) yellow podzolized soil on calcareous sandstones, (2) ortho red-yellow podzolic soils, (3) latosolic B terra roxa.

Soil use = 6 variables with 3 replications each: (1) no cover, bare soil, (2) straw mulch, (3) perennial plant cover (coffee tree) + straw mulch, (4) perennial plant cover (coffee tree), (5) perennial plant cover (coffee tree) + dust mulch, (6) perennial plant cover (coffee tree) + irrigation.

(2) **Soil type × soil cover, in saturated soil evaparimeters** (4 × 2)

The 8 treatment combinations are distributed as follows:

Soil type = 4 variables with 2 replications each: (1) yellow podzolized soils on calcareous sandstones, (2) ortho red-yellow podzolic soils, (3) latosolic B terra roxa, (4) alluvial muck soil.

Soil cover = 2 variables with 4 replications each: (1) no cover, bare soil, (2) meadow cover (*Melinis minutiflora*).

(iii) **Free Water Surface Evaporimeter Pans**

There are 3 variables, without replications, having the following evaporating surface areas: 0.1875, 0.75 and 3.00 m².

IV. **RESULTS**

In Table 1 are presented the percolation water data (mm) from the three different soils, the three profile depths, and the several soil covers.

The 5-year annual averages show that the percolation water decreases, according to soil, as follows: (1) yellow podzolized soils on calcareous sandstones, (2) ortho red-yellow podzolic soils, (3) latosolic B terra roxa.

The amount of percolation water, as shown in the table, was not inversely proportional to soil profile depth as might be expected. The smallest profile depths had the greatest percolation, but the greatest profile depths did not have the smallest percolation.
TABLE 1

PERCOLATION WATER (mm) FROM LYSIMETERS

(1) average of 20 lysimeters with the same soil type, (2) average of 12 lysimeters at 0.45m depth, 12 at 0.90m depth, and 12 at 1.80m depth, with the same cover crops, (3) average of 36 lysimeters with annual crops in rotation, and the others average from 6 lysimeters each. Monthly averages from July 1960 to June 1965.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall</th>
<th>Added water</th>
<th>(1) Soil type</th>
<th>(2) Soil depth</th>
<th>(3) Soil cover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>yellow podzol.</td>
<td>ortho red yellow podz.</td>
<td>latos. B terra roxa</td>
</tr>
<tr>
<td>Jul</td>
<td>19.3</td>
<td>94.5</td>
<td>8.15</td>
<td>7.56</td>
<td>6.23</td>
</tr>
<tr>
<td>Aug</td>
<td>25.9</td>
<td>102.4</td>
<td>8.94</td>
<td>7.75</td>
<td>5.69</td>
</tr>
<tr>
<td>Sep</td>
<td>25.1</td>
<td>123.0</td>
<td>4.84</td>
<td>6.80</td>
<td>4.63</td>
</tr>
<tr>
<td>Oct</td>
<td>169.6</td>
<td>63.7</td>
<td>40.64</td>
<td>45.35</td>
<td>29.48</td>
</tr>
<tr>
<td>Nov</td>
<td>115.7</td>
<td>25.3</td>
<td>41.17</td>
<td>35.00</td>
<td>18.18</td>
</tr>
<tr>
<td>Dec</td>
<td>299.9</td>
<td>29.3</td>
<td>107.95</td>
<td>99.26</td>
<td>53.94</td>
</tr>
<tr>
<td>Jan</td>
<td>229.7</td>
<td>30.6</td>
<td>101.30</td>
<td>99.73</td>
<td>57.53</td>
</tr>
<tr>
<td>Feb</td>
<td>284.1</td>
<td>8.0</td>
<td>121.41</td>
<td>117.66</td>
<td>72.80</td>
</tr>
<tr>
<td>Mar</td>
<td>129.8</td>
<td>31.9</td>
<td>60.48</td>
<td>43.03</td>
<td>30.49</td>
</tr>
<tr>
<td>Apr</td>
<td>47.2</td>
<td>163.6</td>
<td>11.24</td>
<td>9.62</td>
<td>6.34</td>
</tr>
<tr>
<td>May</td>
<td>37.4</td>
<td>98.4</td>
<td>16.84</td>
<td>15.90</td>
<td>9.32</td>
</tr>
<tr>
<td>Jun</td>
<td>21.4</td>
<td>82.5</td>
<td>8.94</td>
<td>8.18</td>
<td>6.76</td>
</tr>
</tbody>
</table>

Total 1405.1 853.2 531.90 495.84 301.39 401.20 339.35 377.15 545.81 372.53 212.36 154.47 1284.20
### Table 2

**RUNOFF AND PERCOLATION (mm) IN THREE SOILS PLANTED WITH COFFEE TREES. MONTHLY AVERAGE FROM JULY 1960 TO JUNE 1965**

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall</th>
<th>Added water</th>
<th>Yellow podzolic on calcareous sandstones</th>
<th>Ortho-red yellow podzolic</th>
<th>Latosolic B terra roxa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>runoff</td>
<td>percolation</td>
<td>runoff</td>
</tr>
<tr>
<td>Jul</td>
<td>19.3</td>
<td>94.5</td>
<td>0.18</td>
<td>9.72</td>
<td>0.00</td>
</tr>
<tr>
<td>Aug</td>
<td>25.9</td>
<td>102.4</td>
<td>0.38</td>
<td>8.32</td>
<td>0.00</td>
</tr>
<tr>
<td>Sep</td>
<td>25.1</td>
<td>123.0</td>
<td>0.55</td>
<td>0.86</td>
<td>0.00</td>
</tr>
<tr>
<td>Oct</td>
<td>169.6</td>
<td>63.7</td>
<td>5.50</td>
<td>12.41</td>
<td>2.89</td>
</tr>
<tr>
<td>Dec</td>
<td>299.9</td>
<td>29.3</td>
<td>10.61</td>
<td>75.19</td>
<td>12.72</td>
</tr>
<tr>
<td>Jan</td>
<td>229.7</td>
<td>30.6</td>
<td>21.76</td>
<td>51.03</td>
<td>20.99</td>
</tr>
<tr>
<td>Feb</td>
<td>284.1</td>
<td>8.0</td>
<td>31.40</td>
<td>69.09</td>
<td>43.50</td>
</tr>
<tr>
<td>Apr</td>
<td>47.2</td>
<td>163.6</td>
<td>0.08</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>May</td>
<td>37.4</td>
<td>98.4</td>
<td>0.38</td>
<td>0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Jun</td>
<td>21.4</td>
<td>82.5</td>
<td>0.12</td>
<td>7.23</td>
<td>0.03</td>
</tr>
<tr>
<td>Total</td>
<td>1405.1</td>
<td>853.2</td>
<td>85.84</td>
<td>280.70</td>
<td>106.59</td>
</tr>
</tbody>
</table>
The amount of percolation water decreases with soil cover as follows: (1) straw mulch, (2) bare soil, (3) annual plant cover, (4) perennial plant cover, (5) perennial plant cover + straw mulch.

Table 2 presents data for runoff and percolation water under a coffee crop for the three main soils of the State. The data represent 5-year averages, on a monthly basis.

Evaporation data and the losses of the chemical constituents of the soil through percolation were determined but are not presented in this paper.

V. CONCLUSIONS

On the basis of the data, the following conclusions were drawn:

a. There is a close relation between the amount of rainfall and the percolation.

b. The percolation water was greater in bare soil compared with soil under cover crops. The amount of percolated water under cover crops is smaller than under the straw mulch cover.

c. The percolation water for the three soils studied, averaging all treatments, was greatest in the case of the sandy soil, lowest for the terra roxa, and intermediate for the clayey soil. However, when the soil cover is coffee tree, the amount of percolation water decreases with the type of soil as follows; clay, sand and terra roxa.

d. When the soil cover is coffee tree, the relationships between total rainfall, percolation and runoff water, for the three kinds of soil, are

\[
\begin{align*}
\text{sandy soil} & = 100 : 12.4 : 3.8 \\
\text{clay soil} & = 100 : 12.5 : 4.7 \\
\text{terra roxa} & = 100 : 3.3 : 2.3
\end{align*}
\]

e. The amount of percolation for bare soil is 1.5 times the percolation under annual crops (rotation: cotton, soybeans, corn, meadow, meadow).

f. The 2-year meadow pasture areas were clipped to correspond to grazing periods. Since actual grazing of the lysimeters was impractical, the effect of stock trampling on the soil surface could not be obtained.

VI. REFERENCES


SUMMARY

In this paper the authors present the features of the installation, experimental design and some hydrologic results on precipitation, percolation and runoff from a lysimeter investigation at the Experiment Station of the Instituto Agronomico in Campinas, in the State of Sao Paulo, Brazil.

The lysimeter design comprises sixty monolith block units with undisturbed soil profiles, for three different soil depths, from the main soil types of the State of Sao Paulo. Eighteen dry-soil evaporimeter and eight saturated-soil evaporimeter units integrate the lysimeter design.

The lysimeter investigations at Campinas were planned to measure the effect of various factors on the hydrologic cycle. Different vegetation, soil-type and soil depth conditions are included.

RÉSUMÉ

Dans ce travail les auteurs présentent les caractéristiques d’installation d’une batterie de lysimètres, le plan expérimental et quelques résultats hydrologiques déjà obtenus: précipitation, percolation et ruissellement. Cette installation se trouve à la Station Expérimentale de l’Institut Agronomique, à Campinas, São Paulo, Brésil.

Les installations lysimétriques comprennent soixante blocs monolithes de trois tailles différentes, avec des profils de sols à structure naturelle, représentant les trois principaux types de sol de l’État de São Paulo; elles sont complétées par une batterie de dix-huit éparomètres de sol sec et huit de sol saturé.

Les lysimètres sont constitués par des cylindres métalliques, à l’intérieur desquels se trouvent les profils monolithes de sol; ces cylindres ont été introduits dans le terrain sous pression et un plateau a été placé en-dessous ce qui permet de recueillir les eaux de percolation.

Les recherches avec les lysimètres étaient conduites de façon à pouvoir examiner les différentes phases du cycle hydrologique sous des conditions bien différentes de végétation, sol et épaisseur de profils.
ZUSAMMENFASSUNG

In der vorliegenden Arbeit erörtern die Autoren das Aussehen der Installationen, ihre experimentelle Ausführung und einige hydrologische Ergebnisse.

Die Lysimetersversuche werden auf der Versuchsstation des Instituto Agronomico in Campinas im Staate São Paulo, Brasilien, ausgeführt.

Der Lysimeterentwurf umfasst sechzig Monolithblockeinheiten mit deren ungestörten Bodenprofilen, die aus drei verschiedenen Bodentiefen entnommen wurden, in denen die drei Hauptbodentypen des Staates São Paulo vorkommen. Achtzehn Trockenbodenevaporimeter und acht gesättigte Bodenevaporimeterreinheiten gehören ebenfalls zu dem Lysimeterentwurf.

CORRELATIONS BETWEEN FIELD CAPACITY AND CLAY CONTENT: A GENERALIZED SEMI-EMPIRICAL EQUATION

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Research Institute for Land Reclamation and Soil Science, Bucharest, Romania

For soils with non-impeded internal drainage, moisture content corresponding to a certain suction, $1/3$ atmosphere for example, is a good approximation of the field capacity. As far as, in this range of values, suction is determined by the surface activity of the soil matrix (and not by its structure), a good correlation may be expected between field capacity and clay content. Experimental data to support such a relationship (Shishkov, 1957; Carlson, 1959) are not abundant. Many factors, some of them resulting from the analysis of De Leenheer and Van Ruymbeke (1960), hide the direct effect of clay content. This led Rode (1952) to pessimistic conclusions on the possibility of getting such correlations.

In earlier papers we presented correlations between field capacity and clay content for zonal medium-textured soils (Canarache, 1964; Motoc, Canarache and Dumitriu, 1964) and for sandy soils (Canarache, Dumitriu and Motoc, 1966). Within each of these textural ranges the regressions are linear, but the different slopes of the two regression lines suggest a curvilinear regression for a larger clay content range. The data of Deasunettes (1964) also suggest a curvilinear relationship.

The above mentioned experimental data and conclusions referred to soils with good internal drainage, showing a relative textural homogeneity of the profile and/or a deep ground water table. Such limitations are included in the strict concept of field capacity. From a more practical point of view, related to the actual storing ability of the soil, water retention capacity may be a more useful concept. We understand by water retention capacity the amount of water held by the soil after the rate of downward movement of the excess water has materially decreased, whether the internal drainage is good or impeded. Some indications on this matter we obtained earlier, using experimental data for alluvial soils (Canarache, 1967).

This paper, in which the whole experimental data of our earlier work were used, aims at presenting the complex relationships between water retention capacity, clay content, textural non-homogeneity of the profile and depth to the water table.
MATERIALS AND METHODS

Experimental data used refer to some 400 soil profiles, representative for Romanian soils.

Water retention capacity (equal, for soils with good internal drainage, to field capacity) was measured in the field, sampling the soil 36-60 hours after a 2 sq. m. plot was excessively watered and determining its moisture content; to calculate the water retention capacity as depth of water (mm), bulk density was determined using soil cores of 100 cm³. Soil texture was determined with a pipette, the Katchinski method for the pretreatment of the samples being used. The standard deviation of a series of 10 figures, each of them representing the clay content of 10 successive soil layers 10 cm deep, was used as a numerical index of the textural non-homogeneity of the profile (Canarache, 1967). The depth of the ground water table was measured using special bore-holes in the very vicinity of the plots, when not greater than 4-5 m, or in the nearest well when exceeding this depth.

Water retention capacity, bulk density and texture were determined on soil samples from each genetical horizon, and then calculated for the 0-100 cm depth of the soil profile.*

Statistical calculations were performed using the Romanian made computer CIFA-102.**

RESULTS AND DISCUSSION

In the first stage of the analysis about 260 profiles with good internal drainage, that is fulfilling the limitations of the field capacity concept, were used. The regression of field capacity on clay content was calculated, best fitting being a third degree curve:

\[ FC = -10 + 26.3C - 0.778C^2 + 0.00853C^3 \]  \hspace{1cm} (1)

Within clay content ranges of 0-25 percent and 25-50 percent two linear regressions are a good approximation of this curve.

In the second stage of the analysis about 100 profiles with impeded internal drainage were used. Differences were calculated between the water retention capacity as determined in the field and the field capacity as calculated with Equation (1). These differences were correlated with the index of textural non-homogeneity (Figure 1) and with the depth to the water table (Figure 2). Notwithstanding the wide scatter of the points on the graphs, the general trend is quite clear; the correlation coefficients are significant.

* Symbols used throughout this paper are: FC—field capacity (mm/m); WRC—water retention capacity (mm/m); ΔFC—difference between water retention capacity as determined in the field and field capacity of a soil with the same texture but with non-impeded internal drainage, as calculated with Equation (1) (mm/m); C—clay content (under 0.002 mm diameter) (percent); ITN—index of the textural non-homogeneity; WT—depth to the water table (m).

** The author extends his deep gratitude to Mr. V. Perlea and Mrs. S. Morgenstern for programming and for supervision of the computer operation.
The relationship of the differences between water retention capacity and field capacity to the index of textural non-homogeneity (Figure 1) may be considered as linear, showing a noticeable increase of the water retention capacity in soils with non-homogeneous textural profiles. This may be explained by the well-known effects occurring at points of contact of different-textured soil layers; water is usually held by the soil in such
Fig. 2.—Relationships of the difference between water retention capacity and field capacity to the water table depth.

I: Soils with less than 15 percent of clay, (x),
\[ \Delta FC = 161 - 167 \log WT \quad (r = -0.439^*) \]

II: Soils with more than 15 percent of clay, (●),
\[ \Delta FC = 92 - 120 \log WT \quad (r = -0.544^{**}) \]

instances at suctions lower than 1/3 atmosphere (capillary-suspended water).

An increase of the water retention capacity with higher water tables
FIELD CAPACITY AND CLAY CONTENT

is shown by Figure 2. It may be easily explained by the low values of the suction in such instances (supported water). This decrease is not a linear one: the curve is steeper when the water table is higher than 2 m (soils with depth to the water table less than 0.8-1 m were not used in this analysis) and it shows no further variation of the water retention capacity when the water table is deeper than 3-4 m. A logarithmic equation was used to describe this relationship. An interesting point is that this relationship has to be differentiated according to soil texture, the slope of the curve being steeper for soils with less than 15 percent clay. This may be related to the larger water-free porosity of the sandy soils.

In the third stage of the analysis the experimental data for all soil profiles were used, and multiple regression equations were calculated.

Simple correlation coefficients, as well as partial correlation coefficients (Table 1), seemed to warrant inclusion of all three independent variables in the calculations above mentioned.

<table>
<thead>
<tr>
<th>Variables included in the correlation calculations</th>
<th>Simple correlation coefficient</th>
<th>First order partial correlation coefficient, the variable of which the effect was excluded being C</th>
<th>ITN</th>
<th>log WT</th>
<th>Second order partial correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRC — C</td>
<td>—0.765**</td>
<td>+0.772</td>
<td>+0.806</td>
<td>+0.781</td>
<td></td>
</tr>
<tr>
<td>WRC — ITN</td>
<td>+0.502</td>
<td>+0.523</td>
<td>+0.465</td>
<td>+0.479</td>
<td></td>
</tr>
<tr>
<td>WRC — log WT</td>
<td>—0.252</td>
<td>—0.325</td>
<td>—0.139</td>
<td>—0.225</td>
<td></td>
</tr>
</tbody>
</table>

**All correlation coefficients in Table 1 significant to the one percent level.

In the multiple regression equation, the effect of clay content was expressed including the terms of its first three powers; the results of the first stage of the analysis, reported earlier, led to this conclusion. Earlier analysis (second stage) also led to inclusion of a linear term of the textural non-homogeneity index as well as of a logarithmic term of the water table depth in the multiple regression equation. Terms representing the products of clay content (raised at each of its three first powers) to the logarithm of the water table depth were included as to outline the differentiation according to texture of the water table depth effect.

A multiple regression equation with 9 terms (including the free one) was developed according to the above analysis (Table 2, equation (8)).

To justify from the statistical point of view all the terms of this regression equation, an analysis of variance and partial correlation calculations were performed. The last two columns of Table 2 show that such calculations led to positive conclusions. Nonsignificant F tests or non-
<table>
<thead>
<tr>
<th>No.</th>
<th>New included independent variable</th>
<th>Regression equation</th>
<th>Multiple correlation calculations</th>
<th>Partial correlation coefficient, the effect of all independent variables except the new included one being excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>WRC = 179 + 4.5 C</td>
<td>Simple and multiple correlation coefficient: + 0.765**</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean square for the new included independent variable: 1400712*</td>
<td>80023*</td>
</tr>
<tr>
<td>2</td>
<td>C²</td>
<td>WRC = 140 + 7.7 C + 0.053 C²</td>
<td>0.786*</td>
<td>0.308**</td>
</tr>
<tr>
<td>3</td>
<td>C³</td>
<td>WRC = 110 + 12.0 C + 0.198 C³ + 0.00136 C³</td>
<td>0.795*</td>
<td>0.197*</td>
</tr>
<tr>
<td>4</td>
<td>ITN</td>
<td>WRC = 93 + 11.9 C + 0.203 C² + 0.00153 C³ + 0.0703 ITN</td>
<td>0.814*</td>
<td>0.108**</td>
</tr>
<tr>
<td>5</td>
<td>log WT</td>
<td>WRC = 131 + 12.3 C + 0.208 C² + 0.00137 C³ + 5.49 ITN — 58 log WT</td>
<td>0.822*</td>
<td>0.123**</td>
</tr>
<tr>
<td>6</td>
<td>C. log WT</td>
<td>WRC = 145 + (0.7 log WT + 12.1) C — 0.216 C² + 0.00146 C³ + 5.40 ITN — 82 log WT</td>
<td>0.824*</td>
<td>0.229*</td>
</tr>
<tr>
<td>7</td>
<td>C². log WT</td>
<td>WRC = 212 + (7.2 log WT + 6.6) C — (0.098 log WT + 0.104) C² + 0.00092 C³ + 5.58 ITN — 178 log WT</td>
<td>0.826*</td>
<td>0.219*</td>
</tr>
<tr>
<td>8</td>
<td>C³. log WT</td>
<td>WRC = 308 + (27.3 log WT — 3.7) C — (0.750 log WT — 0.204) C² + (0.00627 log WT — 0.00180) C³ + 5.60 ITN — 348 log WT</td>
<td>0.833*</td>
<td>0.194*</td>
</tr>
</tbody>
</table>

* Significant to the five percent level.
** Significant to the one percent level.
significant partial correlation coefficients were obtained only for some of
the lower powers of clay content, but not for its third power.

Therefore, Equation (8) in Table 2 seems to be warranted from the
statistical point of view as well as from the principal considerations reached
during earlier analyses of the separate effects of the various independent
variables and from their physical interpretation. A semi-empirical character
may thus be attributed to this equation.

Figure 3 shows a more suggestive representation of Equation (8) in

![Graph of water retention capacity (WRC) vs. clay content (C)](image)

**Fig. 3.**—Relationships between water retention capacity and clay content
(calculated with Equation (8) in Table 2), for selected values of the index
of textural non-homogeneity and of the water table depth.

I: ITN = 10, WT = 1 m
II: ITN = 5, WT = 2 m
III: ITN = 2, WT = 5 m
IV: ITN = 0, WT = 10 m

Table 2. Selected figures of the index of textural non-homogeneity and of
the water table depth were used, and the corresponding curves of the water
retention capacity as depending on clay content were plotted. Curves I and
II represent soils with impeded internal drainage, severely affected by the
textural non-homogeneity of the profile and by the water table level, while
curves III and IV represent soils with non-impeded internal drainage, where
the limitations of the field capacity concept are mostly fulfilled.
CONCLUSIONS

1. Some 70 percent of the variations of water retention capacity may be accounted for by the combined effects of clay content, textural non-homogeneity of the profile and depth to the water table. A multiple regression equation, holding a semi-empirical character, was developed to express these effects.

2. Clay content effect may be put under the form of a third degree curve.

3. The effect of the textural non-homogeneity of the profile may be expressed as a linear one, using a numerical index representing the standard deviation of clay content on 10 successive soil layers, each 10 cm deep.

4. The effect of the water table depth may be expressed by a logarithmic equation. It increases with decreasing clay content of the soil.

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Rode, A. A. (1952)—“Potchvennaia vlaga” (Academy of Sciences Publishing House, Moscow).

SUMMARY

Analytical data on about 400 soil profiles were used for statistical calculations. Field capacity (FC) and water retention capacity (WRC) were used as dependent variables, and clay content (C), a numerical index of the textural non-homogeneity of the profile (ITN) and water table depth (WT) as independent variables.

For soils with a good internal drainage the relationship of field capacity to clay content may be described by a third degree curve (Equation (1)).

For soils with a non-homogeneous textural profile and/or a shallow water table, water retention capacity is linearly affected by the index of textural non-homogeneity (Figure 1) and by the logarithm of the water table depth (Figure 2). A multiple regression equation (Equation (8) in Table 2, Figure 3) including all these effects accounted for some 70 percent of the variation of the water retention capacity.

RÉSUMÉ

On a effectué des calculs statistiques pour des données analytiques concernant environ 400 profils de sol. La capacité au champ (FC) et la capacité de rétention pour l’eau (WRC) ont été utilisées en tant que variables dépendantes, tandis que la teneur en argile (C), un indice
The numerical data on non-homogeneous textural profile (ITN) and phreatic water table depth (WT) were used as independent variables.

For soils with good internal drainage, the effect of clay content on field capacity can be expressed by a third-order equation (equation no. 1).

For soils with non-homogeneous textural profile and/or shallow phreatic water table, linear effects of the index of textural differentiation (figure 1) and logarithm of phreatic water table depth (figure 2) on water retention capacity were evidenced. A multiple regression equation (equation no. 8 in Table 2, figure 3) that considers all these effects explains approximately 70% of the variation of water retention capacity.
INTRODUCTION

Numerous data have been gathered during early and present-day investigations in soil biology and biochemistry showing that the soil is not only a suitable environment for microorganisms but it is also a product of their activity. The interactions between microorganisms and soil environment are so close that the soil as a whole would lose its unique character if one of the components was separated from the other. No wonder, that in the past as at present it has been emphasized that the microbial activity should be studied actually in the soil (Winogradsky 1924), and that more direct measurements of the composite activity of microflora in the system “microflora-soil-plant” are needed (Allison 1961).

The studies on the activity of soil biological populations are mainly concerned with the nature, rate and extent of biochemical processes brought about by microorganisms, with the interrelation between basic processes, biochemical pathways and metabolic sequences, and with the regulatory mechanisms governing the growth and enzymatic reaction of an individual cell, as well as that of a microbial community, to environmental factors; and all this in relation to the broad range of the more or less specific soil conditions. Even if the approach is more biochemical, investigations of this kind are not without an ecological basis, as the ecological behaviour of microorganisms, i.e. their interactions with their environment, is inherent in their physiological and biochemical potentialities.

With respect to the complexity and heterogeneity of the soil as a whole, as well as the quantitative and qualitative diversity of the soil population, the investigator is often forced to disregard the soil as a natural habitat for microorganisms and to deal with isolated organisms or pure cultures grown in liquid media, with elective or enrichment methods, with model systems and so on. He has to isolate the organisms from the soil to prove the biological nature of a certain reaction or process. Further he must occasionally observe the degradation processes or metabolic sequences under controlled conditions, even if the complete evaluation of the microbial activity in the complex system “microflora-soil-plant” remains the aim of his work.

It is obvious that the activity or metabolic responses observed under laboratory conditions might differ from those occurring in the soil owing
to the distinct differences between the environmental conditions prevailing in liquid media and those in the natural environment. The most striking dissimilarities can be summarized as follows: (1) In the laboratory mostly pure cultures are investigated whereas, in the soil, mixed microbial populations or communities occur; and intricate interrelations occur between the components of a natural population conditioned by metabolic interactions in the competition for limited sources of nutrition and energy and dependent on environmental factors. (2) The cells are usually homogeneously dispersed in the laboratory media, or may occur in the form of colonies; in the soil, on the other hand, the organisms occur in microenvironments and are “adsorbed” on solid particles. Their activity as well as the activity of enzymes may be affected by sorption phenomena. (3) The substrate used in laboratory investigations is likely to be a chemically defined compound, usually in a high concentration; in the soil, however, composite substrates are available heterogeneous both in composition and in structure and present in amounts that limit growth. (4) The substrate and mineral nutrients are dissolved and homogeneously dispersed in liquid media, but in the soil their distribution is discontinuous and their availability can be affected by sorption, exchange, and surface phenomena.

Therefore, the choice of a method of approach and the techniques to be applied to the study of the complex system, soil-microflora, affects decisively not only the relevance of the answer to a given question but also the reliability and significance of measurements which provide a basis for the evaluation of the activity of biological populations in the soil environment and its function and importance in soil processes.

Qualitative Aspects

The choice of techniques for studying the activity of the soil biological population is, without doubt, conditioned by the purpose of the investigation. To be able to bring about a certain kind of biological activity in the soil we have to answer two questions: does a certain reaction or process take place in the soil, and what is the rate and extent of the particular process? In the first case qualitative estimations and in the latter one quantitative measurements are involved. The qualitative aspects in studying the activity of a soil biological population often lead to the application of those techniques which enable the investigator to ignore the soil. These are mainly the elective and enrichment methods providing an environment which, under laboratory conditions, enables the microorganisms possessing certain properties to dominate the culture. These techniques played an important role at the time of classical discoveries in soil microbiology in the days of Winogradsky and Beijerinck. Certainly their importance has not been lost even today and they have been employed successfully for the isolation of microorganisms which destroy herbicides and various synthetic chemicals (Audus 1964). These methods facilitate the isolation of the selected organism in a pure culture after its accumulation in the medium. This approach is often unavoidable for obtaining evidence for the biological nature of a certain process. On the other hand, it is obvious that the data
obtained by these methods cannot elucidate sufficiently the pathway of a particular process in the soil, in view of the differences between the laboratory media and the soil as environments and conditions of growth for the microflora. They do show, however, that organisms capable of performing a certain reaction or process are present in the soil.

**Quantitative Aspects**

Only the measurements of biological activity carried out in the soil can present a picture characterizing the rate and extent of a particular process and the degree to which a certain kind of biological activity is influenced by physical, chemical, and biological properties of the environment, and what influence the biological activity exerts upon soil properties. For these effects it is necessary to apply quantitative methods.

*Total metabolism*—On studying the biological activity in the soil, most attention is paid to the determination of the so-called total or aggregated biological activity of the soil. This effort is understandable because it would be ideal to comprehend all processes which take place in the soil. Besides, many soil scientists assume that it would be possible in this manner to characterize the soil and its properties from an agronomic point of view. Unfortunately, a short look at textbooks on soil microbiology will show that such an effort is in vain. Despite the frequent discussions on the total biological activity of the soil based on the determination of oxygen uptake and/or carbon dioxide evolution, these measurements do not integrate all the kinds of biological activity occurring in the soil. Both methods, the determination of the oxygen uptake and the carbon dioxide evolution, are suitable mainly in such cases when the oxidation of a substrate added to the soil is to be investigated, usually under aerobic conditions. However, the reactions in the soil in which oxygen is taken up or carbon dioxide evolved are not necessarily always biological (Drobniková and Drobník 1965; Scharpenseel and Beckmann 1964).

The exchange of oxygen and carbon dioxide in the soil is often called the soil respiration, and different methods have been developed for its measurement (Domsch 1962). Principally the oxygen uptake may be estimated by manometric methods using Warburg vessels (Rovira 1953, Chase and Gray 1957, Drobník 1960, Drobniková and Drobník 1965), or by volumetric measurements (Lees 1949). Some disadvantages of manometric measurements, as for example the production of other gases, are eliminated in procedures where the oxygen consumed is supplied electrolytically (Swaby and Passey 1953, Birch and Friend 1956, Wieringa 1958, McGarity, Gilmour and Bollen 1958, Greenwood and Lees 1959). Carbon dioxide is determined by titration, or by gravimetric or conductometric methods after being absorbed in a hydroxide solution. The conductometric method is suitable for continuous or very frequent measurements (Freytag and Igel 1964). An infrared analyser has also been applied to the determination of CO$_2$ (Zöttl 1960). Stotzky (1960) has designed an apparatus for the simultaneous measurement of oxygen uptake and carbon dioxide evolution and for determination of R.O.
Curves representing the rate of oxygen uptake or carbon dioxide evolution during the decomposition of organic material added to the soil have a characteristic form. They have been reported in detail by Drobnik (1960), and can be expressed mathematically (Lees and Porteous 1950, Chase and Gray 1957, Drobnik 1960, Drobnikova and Drobnik 1965). Serial measurements give a picture of the kinetics of the oxidation processes or of the extent of oxidation of an added substrate, i.e. the amount of the material oxidized to carbon dioxide, and the residue in the soil either in the form of a cellular substance, storage material or humified material. The kinetic measurements show the time needed for the development of a population able to utilize the added substrate and, from the exponential part of the curve, it is possible to evaluate the rate of oxidation and the formation of specific enzymes (Drobnikova and Drobnik 1965). Under certain conditions the laboratory measurements of biological activity in the soil can be related to those in field soils (Greenwood 1965). Naturally, great caution is necessary in drawing conclusions since the pathways of the processes can be considerably affected when taking samples by mechanical disruption of aggregates (Rovira and Greacen 1957), and by drying (Birch 1958, Stevenson 1956). Also the volume, the rate of addition, the aeration, and a priming effect (Clark 1965) can influence the results.

Biochemical patterns and metabolic sequences—The measurements of microbial activity on the basis of oxygen uptake or carbon dioxide evolution include a series of reactions occurring in the decomposition process. However, to comprehend the fate of the substance being decomposed and its effect upon other processes and soil properties, the patterns of biochemical transformation in the soil must be known well. The study of metabolic pathways is difficult because the intermediates and the metabolic products occur in minute quantities. It is also necessary to distinguish between products arising from the added material and those originating in the decomposition of the native soil organic matter. The application of specifically labelled materials in combination with sensitive analytical methods, such as spectral analysis and various kinds of chromatography, have proved to be suitable.

In the soil amended with uniformly labelled glucose, intermediates and products of glucose metabolism have been determined by means of partition chromatography (Vancura, Macura and Szolnoki 1964). Different ratios of the amount of carbon oxidized to the amount of carbon retained or assimilated in the soil were found at different levels of the elements of mineral nutrition by Macura et al. (1965). The use of simple compounds (Ivarson and Stevenson 1964, Jansson 1960, Macura et al. 1965) and plant materials labelled with $^{14}$C (Szolnoki and Vágó 1959, Mayaudon and Simonart 1958, 1959, Sörensen 1963) appears to be an appropriate technique for studying the decomposition processes in the soil and the formation of soil organic matter; the incorporation of $^{14}$C into different fractions of humus and soil polysaccharide has been described by Keefer and Mortensen (1963).
Besides the chemical methods, microbiological methods employing the simultaneous adaptation technique can be used for a study of metabolic sequences. If sequential enzyme induction occurs in pure cultures then in the soil studies the sequential induction (Alexander 1964) of populations which are capable of oxidizing the intermediates without a lag phase, takes place. This approach was used in studying the sequences of cysteine oxidation in the soil (Freney 1960), and also the degradation of some pesticides (Alexander 1965). In the author’s laboratory it has facilitated the determination of the metabolic pathways for the oxidation of aromatic compounds in the soil.

The Closed and Open Systems

The methods of soil incubation applied until now are principally batch processes. In these circumstances the soil-microflora system is studied as a closed system. Under natural conditions, however, the soil as a whole is enriched with plant residues and fertilizers which supply the energy and nutrients. Mineral nutrient elements are absorbed by plant roots, and soluble substances can be translocated by mass-flow, diffusion, or leached out. A part of the soil, the rhizosphere, can be more or less continually enriched with energy sources in the form of root exudates or dead plant tissues. It can be assumed that the soil as an environment for microorganisms can provide a varying range of conditions, from a batch process or closed system to a continuous or open system.

Unlike the batch culture where the composition of the medium changes without control and as a result the population changes quantitatively and qualitatively, in a continuous culture the population may be cultivated in a steady state where both the population and medium remain theoretically constant. Naturally, the system used for continuous cultivation differs considerably from the soil as the cells and substrates are homogeneously dispersed in the medium. This, of course, does not occur in the soil, where the cells are adsorbed on a solid carrier—the structural aggregates.

Nevertheless, some aspects of the continuous cultivation technique appear to qualify this approach for studying the activity of soil microbial population. It is a fact, as mentioned above, that under certain conditions (in the rhizosphere) the soil can be enriched continuously with energy sources. Further, it is possible to study the competition of microorganisms under low substrate concentrations, and to investigate other questions connected with the nutrition and growth of microorganisms in the soil, and the regulatory mechanisms governing the development of microbial populations.

The apparatus and procedures for the study of microbiological processes in the soil were reviewed recently (Macura and Malek 1958, Macura 1961, 1966). The results obtained so far show that the method can be applied to the quantitative measurement of the metabolic activity of soil microflora, and also to reproduce some phenomena, particularly those related to the competition and nutrition of microorganisms.
Conclusion

The various techniques used for studying the activity of soil biological populations cover a wide range. The aim of many soil scientists from the very beginning of soil microbiology was to elaborate suitable methods and many of these methods are still valid. In the last decades a great number of techniques developed in microbiology, biochemistry, physics, and chemistry have been adapted for soil biological investigations. They allow a more exact characterization of the soil environment and a more intimate view of the biological processes occurring in the soil. Nevertheless, many questions remain unresolved and their solution very often necessitates a methodical approach and suffers from a lack of suitable techniques. One may expect that scientific developments will provide soil biologists with techniques which will enable them to reveal many hitherto unknown manifestations of biological activity in the soil. However, since the activity of biological populations and communities in the soil as a natural habitat has specific features determined by the special nature of the soil-microorganism-plant system, the approach to the elucidation of basic problems of this biological whole has to be developed by those who have chosen it as the subject of their interest, regardless of whether it be from an academic or from an agronomic point of view.

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Summary

Methods are needed to study the composite activities of microflora in the ecosystem, microflora-soil-plant, and not merely the activities of individuals isolated from this system. They should be applicable to mixed cultures, heterogeneously dispersed through media containing complex food-stuffs, partly soluble, partly sorbed and partly insoluble. Elective and enrichment methods are only qualitative at best, since they create artificial conditions quite unlike those in soil. Their main use is in isolating organisms and not in elucidating those metabolic pathways that actually occur in soil.

Amongst the quantitative methods applicable to total soil metabolism, not even oxygen uptake or carbon dioxide output integrate all types of biological activity and they may also include some non-biological reactions. Respiration methods are most useful where a substrate is added to aerobic soil and the kinetics of its disappearance compared with the synthesis of specific enzymes. However, studies on intermediary metabolism by analysis of intermediates are almost impossible, since only traces survive. This requires the most sensitive analytical methods, involving the use of radioactive substances, spectral analyses and chromatography. The technique of simultaneous adaptation can be used to elucidate pathways in both pure and mixed cultures.

Since the soil complex involves both open and closed systems, neither continuous culture nor batch culture methods are strictly applicable, except for studying separate parts of the whole.

Résumé

Il nous faut des méthodes pour étudier les activités composées des microflores dans l’écosystème microflore-sol-plante, et non point seulement les activités isolées d’individus dans ce système. Les méthodes devraient pouvoir s’appliquer aux cultures mixtes, dispersées de manière hétérogène à travers des milieux contenant des substances nutritives complexes, en partie solubles, en partie adsorbées et en partie insolubles. Les méthodes électives et celles d’enrichissement ne sont que quantitatives au mieux, puisqu’elles créent des conditions artificielles fort différentes de celles dans le sol. Elles sont valables surtout pour isoler les organismes et non point pour éclairer ces sentiers métaboliques qui se présentent dans le sol.

Parmi les méthodes quantitatives capables d’application au métabolisme total du sol, ni le prélèvement d’oxygène ni la production du bioxyde de carbone n’intègre tous les types d’activité biologique, et elles pourraient aussi embrasser quelques réactions non-biologiques. Les méthodes respira-
toires sont les plus utiles là où on ajoute un substratum au sol aérobie et où on compare la cinétique de sa disparition avec la synthèse d’enzymes spécifique. Pourtant, les études sur le métabolisme intermédiaire par l’analyse des intermédiaires est presque impossible, puisque des traces seulement survivent. Ceci exige les méthodes analytiques les plus sensibles, et implique l’utilisation de substances radioactives, d’analyses spectrales et de chromatographie. La technique de l’adaptation simultanée peut être utilisée pour éclaircir les sentiers dans les cultures pure et dans les cultures mixtes.

Puisque le complexe du sol comprend le système ouvert ainsi que le système fermé, ni les méthodes de culture continue ni celles de culture en groupes ne sont proprement applicables, sauf pour l’étude de parties individuelles du tout.

ZUSAMMENFASSUNG

Methoden zur Untersuchung der Kompositen Tätigkeit der Mikroflora im Oecosystem sind erforderlich, Mikroflora-Boden-Pflanze, und nicht nur die Tätigkeiten der Einzelnen, vom System abgesonderten. Sie sollten bei gemischten Kulturen, heterogen verbreitet durch Media mit kompliziertem Nährstoffgehalt, teils löslich, teils adsorbiert und teilweise unlöslich anwendbar sein. Gewählte und bereichende Methoden sind nur qualitativ zweckmässig, weil sie künstliche Bedingungen ganz unterschiedlich von denen im Boden erzeugen. Der Hauptzweck ist die Isolierung der Organismen, und nicht die Klarstellung der im Boden tatsächlich vorgehenden metabolischen Bahn.


Weil der Bodenkomplex offene sowie geschlossene Systeme enthält, sind weder ununterbrochene- noch stellenweise Kultur Methoden genau zutreffend, nur zum Zweck der Untersuchungen einzelner Teile des Ganzen.
MODERN METHODS FOR ESTIMATING THE SOIL MICROPOPULATION AND ITS ACTIVITY

E. N. Mishustin, D. I. Nikitin and I. S. Vostrov

As far as their fundamental features are concerned, microbiological methods of soil investigation are quite diverse, each of the particular techniques providing an answer to some special question. It is evident that, although the set of analytical techniques will vary with the objective set out by the investigator, it is neither necessary nor possible to employ a wide range of methodologies in each individual case.

In this paper an attempt is made to present an analysis of concrete methodological approaches to the elucidation of tasks of scientific and economic character. And it is from this standpoint that the available and projected possibilities are assessed. It is of course impossible to discuss all the diverse methodologies, and we will just dwell on those that seem to us more interesting.

First of all, there arises the question of quantitative indices of soil micropopulation. Methods for direct microscopic studies of the soil are apparently the most objective ones. In the first place mention should be made of Winogradsky's methodology (1952) and its variants, which involve the use of an optical microscope for soil examination. In a number of cases it is advisable to perform direct microscopy of the soil and rhizosphere in conjunction with the use of fluorochromes (Strugger 1944, Zvyagintsev 1964, Hahnel 1964).

For purposes of direct counting of organisms in the soil, it is very convenient to use the capillary chambers of Perfiliyev and Gabe (1961). These investigators have developed a technique for obtaining multiple capillaries 30 to 40μ deep and having a width not exceeding the field of vision of a microscope. This permits counting of the number of organisms in the capillary without any difficulty, after which it is easy to estimate their numbers in a gram of soil. It should be noted that the counting capillaries make it possible to discover many new and highly interesting forms of organisms in the soil.

Recently Nikitin and co-workers (1966) have employed electron microscopy for direct investigation of the soil, which enabled them to detect specimens of many hitherto unknown groups of soil organisms. Attempts are being made at the present time to isolate pure cultures of these organisms. Many of them grow poorly or fail to develop on ordinary nutrient media.

It is well known that to count organisms, extensive use is made of the method of inoculating soil suspensions into various solid and liquid nutrient media.
Of great interest is the approximate ratio between the numbers of organisms obtained for one and the same soil by different methods. Relevant information for soddy podzolic soils taken near Moscow is presented in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Method</th>
<th>Organism numbers in soil, in thousands per gram soil</th>
<th>Ratio between indices in individual analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation into various solid media</td>
<td>1 — 3 ( \times ) 10^4</td>
<td>1</td>
</tr>
<tr>
<td>Direct count using optical microscope</td>
<td>2 — 5 ( \times ) 10^9</td>
<td>150 — 1500</td>
</tr>
<tr>
<td>Direct count using electron microscope</td>
<td>20 — 25 ( \times ) 10^9</td>
<td>up to 15,000</td>
</tr>
</tbody>
</table>

The differences are due to several causes. For instance, many organisms fail to grow on ordinary nutrient media. (In this connection it should be noted that the problem of the so-called "autochthonous" microsphere of the soil has not been solved. Work on this problem is now under way in our laboratory.) Then, direct methods take into account not only living, but also dead organisms, and so on.

It should be mentioned that the vast numbers obtained by means of electron microscopy do not attest to the exceedingly great absolute mass of microbes in the soil, since the greater part of the organisms so revealed are of very small size. Tentative calculations lead to the conclusion that the mass of microbes in the soil does not exceed several tenths of a per cent of soil weight.

Which methods of estimating organism numbers should then be selected by the microbiologist? It is quite evident that direct methods provide more correct data and their application to obtain general biogenic characteristics of the soil is undoubtedly useful. Considering, however, the methodological implications, it seems that today their application on a large scale presents certain difficulties. For that reason, extensive use is made of methods of inoculating organisms into solid media and of methods of titration. These methods have often met with disapproval, which should be considered quite justified. For example, mycelial organisms, such as fungi and actinomycetes, are arbitrarily fractionated into any number of particles. Or, it is not possible to differentiate between the spores and mycelia of these microbes, etc.

Nevertheless, by standardizing the procedures and considering as reliable only statistically significant differences in the samples being compared, and also bearing in mind the inadequacies of the methods used, it is possible to get a correct idea of life in the soil.

Referring to our personal experiences, we may note that such an analysis has enabled us to evolve a concept regarding the differences in the biogenic
nature of different kinds of soil, as well as to solve successfully some problems of agrotechnology (Mishustin 1960, 1966).

Experience shows that it is advisable to employ methods of inoculation of organisms into nutrient media, bearing, however, in mind that in this way only a part of the soil micropopulation can be brought to light. Nevertheless this method does reflect the actual state of soil coenosis. Research should be extended to include the study of unknown groups of organisms, but one should not disregard the results already achieved and the foundations on which the science of soil life has developed.

In quantitative analysis of soil micropopulations, we give much attention to the use of poor nutrient media with different fractions of humus compounds, since many more organisms develop on such media than on commonly used substrates (beef-extract agar, wort agar, etc.).

When using ordinary nutrient media, we prefer agarized substrates because this makes it possible not only to take into account individual groups of soil organisms (bacteria, actinomycetes, fungi, etc.), but also to make an approach to the analysis of the species composition of the soil microflora, and this cannot be done by the titration method. With few exceptions (for instance, in the case of nitrifiers) the physiological groups of micro-organisms are very numerous. The species composition of the soil micropopulation can change within a wide range depending on the environmental conditions. At the same time, it often happens that the environment changes the number of physiological groups very little.

It is clear that it is impossible to analyze the soil composition down to a species, or even to ordinary saprophytes, for that matter. This is why one should try to identify those organisms that are representative of certain conditions and observe their behaviour in the soil. To date, the list of such representative organisms is not very long, but it will increase as soil microbiology advances.

We have widely employed the principle of representative organisms in analyzing microbe communities in various soils. The results of this work have been published (Mishustin 1966).

Perfiliyev's and Gabe's capillary method (1961) opens up new prospects in the field of microbial ecology studies. These investigators have designed a "pedoscope"—a capillary device intended for studying the group composition of the soil and ground organisms. This device consists of a set of capillary cells with five to six rectangular channels, the cells being held fast in the slots of a wide glass holder.

Aristovskaya (1965), who has much experience with pedoscopes, recommends filling the capillaries with a semiliquid agar medium containing humus substances (fulvic acids) as an organic substrate. This creates conditions similar to the soil environment. Agar is used at 0.1% concentration.

After exposure in the soil (generally, for 6 to 8 weeks), the pedoscopes are examined with a microscope. This method permits identification of typical microbe communities of various soils and many interesting forms of organisms.
Other techniques can also give an idea of the density and, partly, of the character of the microbe population of the soil. Worth mentioning is, for example, the method of disseminating fine earth over the surface of solid nutrient media (Novogrudsky 1947, Warcup 1960). These methods can be recommended for comparative studies, but the resultant figures cannot, however, be compared with the data obtained by the conventional methods of inoculation.

Of ancillary importance may be such techniques as analysis of soil sections (Burges and Nicholas 1961, Jones and Griffiths 1964), the methods of Cholodny (1935) and Ziemiecka (1935) and their variants (Rybalkina and Kononenko 1953) as well as some other methods.

We deem it necessary to note once more that individual techniques should not appear as opposing one another, and that practical purposes of investigation can best be served by some or other combinations of them.

In many cases researchers do not confine themselves to eliciting data of a floristic nature, for there is the need to determine the total effect of the activity of the soil micropopulation in chemical terms. This makes it possible to evaluate the potentialities of biological processes and, not infrequently, to draw conclusions of great practical value.

These techniques include, first of all, those making use of the nitrifying capacity of the soil, which provides an indication of the mobilization of nitrogen reserves of the soil, thus supplying valuable information for establishing the dosage of nitrogen fertilizers and for appraising the effect of various factors on the soil.

The nitrification test can be made either by the old technique of Waksman (1923) or by the percolation method (Stevenson and Chase 1953, Robinson 1963). This latter method is now applied on an increasing scale to solve a number of problems of soil microbiology (Macura and Kunc 1965, Macura et al. 1967).

It seems advisable to make similar tests to determine the mobilization of phosphoric acid in the soil. In some soils up to 60% of the total reserves of phosphorus are present in organic form, and it is possible to ascertain what part of it could be easily mobilized. Unfortunately, this problem has not yet been fully solved.

In studying soil biodynamics wide use is made of the phenomenon of $CO_2$ evolution by the soil ("respiration"), and many methods have been devised for this purpose (Lees 1949, Makarov 1957, Vostrov 1961, Vostrov and Petrova 1961). The value of "soil respiration" for the analysis of the effects of various factors on the soil is well known.

In the study of the effect of soil cultivation on microbiological processes, we have employed the method of "applications", i.e. placing strips of linen cloth into the soil and observing their disintegration. The cloth is attached to a glass plate 10 x 50 cm which is inserted in the soil in an upright position. Observations are made for up to six weeks. This method provides a clear picture of the zone of activity of soil organisms.

At present we are making use of a technique similar to that just described, but a more rapid one (Mishustin and Petrova 1963). The cloth
SOIL MICROPOPULATION

is kept in the soil seven to ten days—depending on the weather—and, after being removed from the soil, is dried, cleaned of soil particles and treated with ninhydrin or bromophenol blue; zones of intensive microbial activity become tinted, due to accumulation of metabolites (proteins, amino acids, etc.).

The rate of disintegration of the cellulose is in the main determined by the presence in the soil of soluble forms of nitrogen available to microorganisms. For this reason the above test permits an assessment of the mobilization processes since foci are clearly shown on the linen. The test is very simple to perform and provides valuable indications which may be useful in solving a number of agrotechnical and reclamation problems. Other techniques of investigation do not permit the determination of the microzonal distribution of soil processes with such precision.

To assess soil activity, enzymic criteria may be utilized (Kuprevich 1951, Kozlov 1962, Hofmann 1962, Galstyan 1965, Durand 1965, Kuprevich and Shcherbakova 1966). A considerable body of evidence has been accumulated to show that there is a relationship between the activity of soil enzymes and certain properties of the soil. For example, Galstyan believes that there is an almost direct correlation between the activity of invertase and the rate of soil respiration, while the activity of oxidases depends on the dynamics of the nitrates.

The numerous data available suggest that all measures that improve microbiological processes also intensify enzymatic processes in the soil. This is supported by the finding that enzymes are more active in the root zone containing products of root exosmosis and where vast numbers of organisms accumulate.

All this justifies our paying much attention to enzymatic properties of soil and operating with some new sets of techniques in studying soil properties, and this is to be one of the fundamental methodological tasks of the immediate future.

At the same time it must be stressed that it would be incorrect to expect direct relationships between the number of soil organisms and the activity of soil enzymes, for these relationships are determined by a number of factors, and above all by the fact that soil enzymes are produced not only by micro-organisms, but also by root systems of plants and by soil animals; also, enzymes are not inactivated at an equal rate under different soil and climatic conditions, etc. Table 2 gives a comparison between the activity of individual enzymes in different soils (according to data of Galstyan) and the biogenic nature of soil (according to data of Mishustin).

It seems to us that the representative value of individual enzymes for characterization of soil types and the nature of action of various factors on the soil should be expressed in more precise terms.

The use of chemicals in agriculture sets a number of tasks for microbiology, including that of determining the requirement for fertilizers; and for this purpose microbes (fungi, algae, bacteria) may be used as test organisms. These methods permit one to ascertain how the soil is provided with both micro-organisms and trace elements (Niklas 1931, Butkevich
### Table 2

<table>
<thead>
<tr>
<th>Soil</th>
<th>Humus, %</th>
<th>Activity</th>
<th>Number of organisms per g organic matter, in thousands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invertase</td>
<td>Amylase</td>
<td>Dehydrogenase</td>
</tr>
<tr>
<td>Dark brown</td>
<td>1.7</td>
<td>9.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Chestnut</td>
<td>3.0</td>
<td>25.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Leached chernozem</td>
<td>7.2</td>
<td>45.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Brown</td>
<td>7.8</td>
<td>29.7</td>
<td>10.2</td>
</tr>
<tr>
<td>Meadow-steppe</td>
<td>18.1</td>
<td>53.4</td>
<td>10.2</td>
</tr>
<tr>
<td>Brown mountain-meadow</td>
<td>15.3</td>
<td>66.8</td>
<td>12.1</td>
</tr>
<tr>
<td>Mountain-meadow-peat</td>
<td>21.6</td>
<td>87.2</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Note: (1) Micro-organism numbers were counted following inoculation on starch-ammonia agar.

(2) The activity of invertase is expressed in mg glucose per 1 g soil (during 24 hrs); of amylase, in mg maltose per 1 g soil (during 24 hrs); of dehydrogenase, in mg triphenylformasan per 10 g soil (during 28 hrs); of polyphenoloxidase, in 6 mg purpurogalin per 100 g soil (during 30 min); and the activity of catalase, in O₂ (in cc) per 1 g soil (during 1 min).

1932, Uspensky and co-workers 1935, Hirai 1957, Tchan 1959, Domnier 1964). It should be stressed that, unfortunately, microbiological methods for determining the nutrient requirements of soils for higher plants are used too little in agricultural practice. There is no doubt that these methods could also be applied to ascertain the rate of consumption of herbicides, fungicides and insecticides in the soil.

The brief survey presented here leads to the conclusion that current microbiological methods permit not only the disclosure of many aspects of soil life, but also the resolving of a number of practical tasks. It is unquestionable that their further development should continue.

### References


Domnier, A. (1964)—Roczniki glebozn. 14 (sup), 131-142.


Galstyan, A. Ch. (1965)—Pochvoovedenie (2), 68-74.


Hähnel, W. (1964)—Albrecht-Thaer-Arch. 8(1-7), 139-152.
In recent years numbers of new approaches have been made to the
analysis of the quantitative and qualitative composition of soil microflora,
and to the evaluation of the energy of soil biodynamics. These methods may
provide a valuable addition to current concepts regarding the microscopic
population of the soil and should, therefore, constitute an integral part of
the set of analytical tools used by the investigator. We, however, by no
means imply that the new methods of investigation should replace the
existing ones.

A wide range of methodologies is at the disposal of microbiologists.
Depending on the goals of an investigation, specific complexes of analytic
procedures may be recommended, which would suffice to resolve a given
task.

In principle, the following instances of using microbiological analysis
may be envisaged:
(a) problems of a general biological nature and problems of soil science;
(b) agricultural technology problems (especially related to soil cultivation);
(c) problems of agricultural chemistry, especially those related to the dosage of fertilizers.

When many research projects are carried out in the field of soil science and when practical tasks are to be solved, microbiological criteria can supply valuable data which would supplement those obtained by other techniques of soil investigation or illustrate soil processes not detected by the methods of chemical or agrochemical analysis.

ZUSAMMENFASSUNG

In den vergangenen Jahren wurde eine Anzahl neuer Annäherungen an die Analyse der quantitativen- und qualitativen Komposition der Bodenmikroflora und die Auswertung der Energie der Bodendynamik gemacht.
Diese Methoden können eine wertvolle Bereicherung der gegenwärtigen Begriffe der mikroskopischen Population des Bodens darstellen, und sollten daher einen integralen Teil der von Forschern gebrauchten Satz analytischer Werkzeuge ausmachen. Wir wollen jedoch in keiner Weise andeuten, dass die neuen Forschungsmethoden die bestehenden ersetzen sollten.

Ein weites Bereich von Methodologien steht den Mikrobiologen zur Verfügung. Je nach den Zielen einer Forschung können spezifische Komplexe analytischer Verfahren empfohlen werden, die geeignet wären, die gegebene Aufgabe zu lösen.

Im Prinzip können die folgenden Vorschläge zur Anwendung der mikrobiologischen Analyse ins Auge gefasst werden:

(a) Probleme allgemeiner biologischer Natur und Probleme der Bodenkunde;
(b) landwirtschaftlich-technologische Probleme (besonders solche, die mit der Bodenbearbeitung zu tun haben);
(c) Probleme der landwirtschaftlichen Chemie, besonders solche, die mit der Dosierung von Düngemitteln zusammenhängen.

Wenn viele Forschungsprojekte im Feld der Bodenkunde durchgeführt werden und praktische Aufgaben zu lösen sind, können mikrobiologische Kriterien wertvolle Angaben liefern, welche solche, durch andere Bodenforschungs-Techniken erhaltenen, ergänzen würden. Sie können auch Bodenprozesse illustrieren, die von den Methoden der chemischen- oder agrochemischen Analyse nicht entdeckt wurden.
THE EFFECT OF CARBON DIOXIDE AND OXYGEN MIXTURES IN SOIL ON ROOT-COLONISING FUNGI

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INTRODUCTION

An investigation of the fungi colonising roots of rye grass (Lolium perenne L.) and of white clover (Trifolium repens L.) in several soils under sheep-grazed pasture in New Zealand showed differences in the incidence of certain fungi e.g. Fusarium oxysporum, between warm, moist soils in the north, and cooler moist soils in the south (Thornton 1965).

Moisture and temperature are likely to be the more important environmental factors determining the relative occurrence of fungi (Taylor 1964). However, in addition to any direct effects on specific fungi, moisture and temperature will influence the aeration of soils. The composition of soil air, in addition to any direct effects on specific fungi, may influence root growth and physiology thereby possibly changing the quantity and composition of organic additions to soil from roots and conditioning microbial responses in the root region.

This paper describes the technique developed to investigate the effects of mixtures of carbon dioxide and oxygen on the fungi about the roots of several plants, together with some experimental results.

Apical 2-5 cm portions of roots were chosen for study because any effects are likely to be shown first in this region and events here may well condition later metabolic and pathological aspects of plant growth.

MATERIALS AND METHODS

The soil used for this investigation is classed as Sunnyside loam, a well drained red-yellow podzolic soil developed on reddish brown sandy coastal plain materials (information from U.S. Soil Conservation Service) and was obtained from under a 4- to 5-year old pasture of white clover and cocksfoot (Dactylis glomerata L.).

It was air-dried, screened through a 3/8 in. mesh to remove stones, and thoroughly mixed before storage.

This soil had the following properties: pH 6.1; organic carbon 1.15%; base saturation 66%; cation-exchange capacity 7.6 m-equiv/l00g.

The soil was moistened to levels corresponding to 30% and 50% of the water-holding capacity.

Seeds of spring wheat (Triticum vulgare Host.) var. "Federation 41", red clover (Trifolium pratense L.) and white clover, were surface sterilised...
with 0·17% AgNO₃ (Mead 1935), and germinated for 1 to 4 days on sterile water agar. Vigorously growing seedlings, free of microbial contamination, were transferred to plant culture vessels.

Aseptic seedlings were placed in sterilised glass planting tubes, 10 mm x 40-50 mm constricted at the base to an opening 5 mm wide, filled with “Perlite” (10-30 mesh). When plants had become established a further layer of sterile “Perlite” was added at the top of the tube and the plants were sealed about the stem with a layer each of petrolatum and a silicone rubber compound (Silastic RTV 501 (now 588), Dow Corning Corp.) as described by Stotzky et al. (1962).

Three planting tubes, together with a gas outlet tube, were inserted in the upper stopper of a stoppered glass tube (48 mm OD x 300 mm), filled with moist soil compacted to a density of one g per cc supported on a thin layer of glass wool over quartz sand resting on stone chips. The base of the planting tubes was placed near the soil surface.

Plants were grown under artificial light, 1,000 ft-c (10,800 lux), for 18 hours per day with the root system of the developing plants protected from light by aluminium foil covers.

All experiments were carried out at 16°C, to approximate the mean daily temperatures at 10 cm depth recorded for the growing seasons in the cool, moist, southern New Zealand soils (N.Z. Meteorological Service 1962).

Wheat plants were grown for 14 days and clover plants for 21 days.

The gas mixtures used were: (1) 0·5% carbon dioxide-enriched air (“soil air”), (2) 10% or 20% carbon dioxide-enriched air, and (3) 2% oxygen, 0·5% carbon dioxide and 97·5% nitrogen. These levels have been found in natural soil (Russell 1952; Miller 1960), and are not likely to prevent plant growth (Cannon 1925; Seifriz 1942).

Gas mixtures were moistened and continuously perfused through the soil columns via a tube in the bottom stopper, the rate of flow monitored by allowing the effluent gas to bubble through a flask of water. A gas flow rate of 150 to 200 ml per hour was maintained throughout the experiments.

Roots were treated and sampled for fungi by the general methods described by Thornton (1965).

Six soil tubes were assembled for each treatment: a total of 18 plants per treatment. The two-sample t-test based on range (Moore 1957) was applied to examine significance of data.

**RESULTS**

Although the treatment atmospheres, which were oxygen-low and carbon dioxide-enriched relative to “soil air” caused a reduction in growth in all three kinds of plants, the plants grew sufficiently well for the investigation. Reduction in root length occurred in both treatment atmospheres, and was most marked under low oxygen.

874 of the 3,011 cultured root segments (approx. 29%) produced mycelia. The percentage occurrence of the common fungi isolated from all treatments is given in Table 1.
Table 1
PERCENTAGE OCCURRENCE OF COMMON FUNGI ISOLATED FROM APICAL ROOT SEGMENTS

<table>
<thead>
<tr>
<th>Fungus</th>
<th>White Clover</th>
<th>Red Clover</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium oxysporum</td>
<td>33</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>Non-sporing mycelia</td>
<td>19</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>13</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>4</td>
<td>&lt;1</td>
<td>10</td>
</tr>
<tr>
<td>Absidia spp.</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Gliocladium spp.</td>
<td>6</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sporotrichum spp.</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Humicola sp.</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>2</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>5</td>
</tr>
</tbody>
</table>

No. segments examined: White Clover 862, Red Clover 1112, Wheat 1037
No. segments with mycelia: White Clover 252, Red Clover 353, Wheat 267

These root fungal patterns are dominated by *Fusarium oxysporum* and are broadly similar between the three plants and although similar to those from earlier studies (Thornton 1965) are noteworthy for the unexpected absence of *Cylindrocarpon radicicola* and of *Fusarium culmorum*.

Whereas wheat roots appeared to favour the growth of *Mucor* spp. and of *Trichoderma viride*, clover roots favoured Fusaria and *Gliocladium* spp. (mainly *G. roseum*).

Gas mixtures appeared to affect certain fungi; *Absidia* spp. were isolated mainly from roots in the “soil air” series; *Fusarium solani* was found principally on red clover roots grown in the moister (50% WHC) soil under low oxygen levels; *Rhizoctonia solani* was virtually absent from roots exposed to carbon dioxide-enriched atmospheres.

Perfusion of the soil and roots with atmospheres of different gas mixtures caused little change in the incidence of mycelia on roots in soil moistened to 30% WHC, except on wheat roots where low oxygen conditions caused a significant difference relative to “soil air” (i.e. $M = 0.135, P_{0.01} = 0.130$).

When the soil moisture was increased to 50% WHC, relative to the lower moisture series the incidence of fungi on wheat roots decreased under “soil air” and 10% carbon dioxide-enrichment (i.e. $M = 0.163, P_{0.01} = 0.121$ and $M = 0.100, P_{0.05} = 0.101$ respectively).

At the higher soil moisture the gas mixture treatments, relative to “soil air”, caused no significant differences in the incidence of fungi on wheat roots. With clover roots 10% carbon dioxide-enrichment of soil air caused a significant difference in fungal incidence (i.e. for white clover $M = 0.263, P_{0.01} = 0.147$, and for red clover $M = 0.209, P_{0.05} = 0.176$). The low oxygen treatment had no significant effect on the incidence of fungi on roots. (See Table 2.)
TABLE 2
MEAN NUMBER OF ROOT SEGMENTS WITH MYCELIA FROM PLANTS GROWN IN SOIL AT DIFFERENT MOISTURE LEVELS AND EXPOSED TO SOIL ATMOSPHERES OF DIFFERENT GAS MIXTURES

<table>
<thead>
<tr>
<th>Soil moisture % WHC</th>
<th>Plants</th>
<th>Soil Atmosphere</th>
<th>CO₂—enriched</th>
<th>Low O₁</th>
<th>2% O₂, 0-5% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White clover</td>
<td>&quot;Soil air&quot;</td>
<td>(10%)</td>
<td>(20%)</td>
<td>10% O₂</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td>2-9</td>
<td>2-4</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>Red clover</td>
<td></td>
<td>3-6</td>
<td>2-7</td>
<td>2-6</td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td></td>
<td>3-8</td>
<td>3-5</td>
<td>1-9</td>
</tr>
<tr>
<td>50</td>
<td>White clover</td>
<td></td>
<td>4-5</td>
<td>1-6</td>
<td>3-2</td>
</tr>
<tr>
<td></td>
<td>Red clover</td>
<td></td>
<td>3-9</td>
<td>1-6</td>
<td>4-4</td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td></td>
<td>2-0</td>
<td>1-8</td>
<td>2-2</td>
</tr>
</tbody>
</table>

Approximately 120 — 180 root segments were examined for each kind of plant in each treatment.

From Table 3 it is apparent that the decreased incidence of fungi on clover roots in the more moist soil under 10% carbon dioxide-enriched air is due largely to the relatively unfavourable influence of these conditions on F. oxysporum. The incidence of this fungus on clover roots in the carbon dioxide-enriched air series is significantly different relative to the control "soil air" series: (white clover, $M = 0.23, P_{0.01} = 0.147$; red clover, $M = 0.30, P_{0.01} = 0.244$).

In the less moist soil (30% WHC) 10% carbon dioxide-enriched air appeared to have little effect on the relative incidence of F. oxysporum on root segments.

No reason can be advanced for the low incidence of F. oxysporum on wheat roots grown in "soil air" at the higher moisture level.

DISCUSSION

Studies in vitro elsewhere have shown that fungi respond differently to atmospheres of varying concentrations of carbon dioxide and oxygen (Durbin 1955) and that a greater tolerance to carbon dioxide by certain fungi is thought to account for their growth at depth in soil (Burges and Fenton 1953). The high tolerance to increased levels of carbon dioxide by Fusarium oxysporum (Stotzky and Goos 1965) has facilitated its isolation from soil by a selective plating procedure (Bouhot, Bullit and Louvet 1964).

It was surprising, therefore, to find in the present investigation that 10% carbon dioxide-enrichment of soil air under the more moist conditions caused a sharp decrease in the incidence of F. oxysporum on clover roots. This decrease was unlikely to be related to the inhibitory effect of carbon dioxide on chlamydospore formation in Fusaria (Bourret, Gold and Snyder 1965), which was considered to have significance for the long term survival of chlamydospores in soil.
Table 3

RELATIVE INCIDENCE PER CENT OF FUSARIUM OXYSPORUM ON FUNGUS INFECTED APICAL ROOT SEGMENTS FROM PLANTS GROWN IN SOIL AT DIFFERENT MOISTURE LEVELS AND EXPOSED TO SOIL ATMOSPHERES OF DIFFERENT GAS MIXTURES

<table>
<thead>
<tr>
<th>Soil moisture % WHC</th>
<th>Plants</th>
<th>&quot;Soil air&quot;</th>
<th>CO₂—enriched (10%)</th>
<th>CO₂—enriched (20%)</th>
<th>Low O₂ (2% O₂, 0-5% CO₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>White clover</td>
<td>39</td>
<td>36</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red clover</td>
<td>17</td>
<td>23</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>18</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>White clover</td>
<td>33</td>
<td>8</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red clover</td>
<td>37</td>
<td>0</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>6</td>
<td>13</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

The 16°C temperature used in these investigations may have resulted in an increased solution of carbon dioxide in soil water, which Brown (1922) found to retard fungal growth. The pH changes recorded here were small, i.e. 0·1 unit, and unlikely to be responsible for the inhibitory effect recorded. On the other hand enhanced levels of bicarbonate salts can inhibit the cytochrome oxidase system (Miller and Evans 1956).

The environmental conditions employed in this investigation in addition to any direct influence upon root-colonising fungi, are likely also to affect the physiology of plants (Stolwijk and Thimann 1957, Cline and Erickson 1959, Siegel, Rosen and Renwick 1962) which may possibly influence the quantity and composition of organic materials added by plant roots to soil in the form of root exudates and cells (Rovira 1965). Ayers and Thornton (1968) grew peas under the gas mixture treatments used here and noted changes in the pattern of amino acids released by roots.

A change in soil moisture and aeration levels, and in the nature and quantity of organic substances released from roots, are likely to affect other micro-organisms in the root region (Peterson, Rouatt and Katznelson 1965) and any changes in lytic and antibiotic activities could have important effects on the distribution of fungi about plant roots (Ayers and Papavizas 1963). No examination of this aspect was carried out in the present investigation.

The technique used in the present investigation has shown that, in the presence of plants, the composition of the soil atmosphere can have an important effect on root-colonising fungi. Under moisture levels such that pores would be largely gas filled, a low oxygen atmosphere affected wheat-root-colonising fungi. In soils at a higher moisture level where pores would be largely water filled, a carbon dioxide-enriched atmosphere appeared to have a major effect on clover-root-colonising fungi, particularly Fusarium oxysporum. These results suggest that carbon dioxide levels may be equally important as oxygen levels (Griffen 1966) in influencing fungal activity about plant roots.
ACKNOWLEDGEMENTS

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Soil analyses were done by the U.S. Soil Conservation Service.

REFERENCES

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Moore, P. G. (1957)—Biometrika. 44, 482-489.

SUMMARY

Aseptically reared seedlings of white and red clover and wheat were placed in “planting tubes” containing sterile Perlite, and sealed around the stem with a layer each of petrolatum and a silicone rubber compound (Silastic RTV 588 (previously 501) Dow Corning Corp.). These planting tubes were inserted in the top of soil-containing glass cylinders continuously perfused with one or other of the following gas mixtures: (i) 0-5% carbon dioxide-enriched air (‘soil air’); (ii) 10 or 20% carbon dioxide-enriched air; (iii) 2% oxygen, 0-5% carbon dioxide, 97-5% nitrogen.

Plants were grown for 14 or 21 days at 16°C in soil moistened to 30% and 50% of the water-holding capacity.

Except for wheat grown at the lower soil moisture level low oxygen conditions did not affect root-colonising fungi to any marked degree.

Air enriched with 10% carbon dioxide when perfused into soil moistened to 30% water holding capacity did not cause any marked
changes but when perfused into soil at 50% water holding capacity caused a decrease in the incidence of fungi isolated from clover roots. This decrease was largely due to the unfavourable effect these conditions had on *Fusarium oxysporum*, the species most commonly isolated from roots.

The mechanism for this effect was not determined.

RéSUMÉ

Des plantules de blé et de trèfles blancs et rouges cultivées aseptiquement furent placées dans "des tubes de plantation" contenant du Perlite stérile, et scellées autour de la tige par une couche de petrolatum ainsi qu’une couche d’un composé de silicone caoutchoucé (Silastic RTV 588 (auparavant 501) Dow Corning Corp.). Ces tubes de plantation furent insérés en haut de cylindres de verre contenant du sol perfusé sans arrêt avec l’un ou l’autre des mélanges gazeux suivants: (i) 0,5% d’oxide de carbone — air enrichi ("air du sol"); (ii) 10 (ou 20%) d’oxide de carbone — air enrichi; (iii) 2% oxygène, 0,5% d’oxide de carbone, 97,5% d’azote.

Les plantes furent cultivées pendant 14 ou 21 jours à 16°C dans un sol humidifié à 30% et à 50% du pouvoir de rétention en eau.

Sauf dans le cas du blé cultivé au niveau d’humidité du sol inférieur, les conditions de faible oxygénation ne réagissaient pas sur les champignons colonisateurs de racines de façon particulière.

Lorsqu’il fut perfusé dans un sol humidifié à 30% du pouvoir de rétention en eau, l’air enrichi de 10% d’oxide de carbone ne causa aucun changement prononcé, mais perfusé dans un sol à 50% du pouvoir de rétention en eau, il causa une diminution de l’avènement des champignons isolés des racines de trèfles. Cette diminution eut pour principale cause l’effet défavorable que ces conditions eurent sur le *Fusarium oxysporum*, l’espèce la plus souvent isolée des racines dans la plupart des autres conditions.

Le mécanisme ne fut pas déterminé pour cet effet.

ZUSAMMENFASSUNG

Aseptisch gezogene Sämlinge von weissem und rotem Klee und von Weizen wurden in "Pflanzröhren gesetzt", die mit sterilem Perlit gefüllt waren, die Rohre wurden um die Stengel mit je einer Lage Petrolatum und einer Silizi um-Gummi-Zusammensetzung (Silastic RTV 588 (früher 501) Dow Corning Corp.) abgedichtet. Sie wurden in das obere Ende von Erde enthaltenden Glaszyllindern eingeführt und fortlaufend mit der einen oder anderen der folgenden Gasmischungen behandelt: (i) 0,5% Kohlendioxyd- angereicherte Luft ("Bodenluft"); (ii) 10 (oder 20%) Kohlendioxyd- angereicherte Luft; (iii) 2% Sauerstoff, 0,5% Kohlendioxyd, 97,5% Stickstoff.

Die Pflanzen wurden für 14 oder 21 Tage bei 16°C in einem Boden gezogen, der bis zu 30% und 50% seiner Wasserhaltungskapazität durchfeuchtet war.
Mit der Ausnahme von Weizen, der auf dem niedrigeren Bodenfeuchtigkeitsniveau gezogen worden war, hatten niedrige Sauerstoffbedingungen keinen merkbaren Einfluss auf die in den Wurzeln siedelnden Pilze.

Mit 10% Kohlendioxyd angereicherte Luft, eingeführt in einen bis zu 30% Wasserhaltungs-Kapazität durchfeuchteten Boden, verursachte keine merklichen Veränderungen. Dagegen führte der gleiche Vorgang in einem Boden bei 50% Wasserhaltungskapazität zu einer Verminderung der von Kleewurzeln isolierten Pilze. Diese Verminderung war größtenteils auf die ungünstige Wirkung dieser Bedingungen auf *Fusarium Oxysporum* zurückzuführen, die Spezies, die man im allgemeinen von den Wurzeln isoliert.

Die Ursachen für dieses Verhalten wurden nicht festgestellt.
THE EXUDATION OF $^{14}$C-LABELLED SUBSTANCES FROM ROOTS OF WHEAT SEEDLINGS

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I. INTRODUCTION

In soil under crops or pastures a large proportion of the soil microbial population occurs in the immediate vicinity of the roots of living plants, i.e. in the rhizosphere (Rovira and McDougall, 1967). Proliferation of microorganisms in the rhizosphere is due in part to the utilization of organic substances which exude from roots of living plants. A study of exudates from living roots is therefore basic to an understanding of the maintenance and fluctuation of the rhizosphere population. In the work reported in this paper, exudates from roots of wheat seedlings have been labelled with radioactive carbon, by supplying $^{14}$CO$_2$ to plants during photosynthesis (McDougall and Rovira, 1965).

II. MATERIALS AND METHODS

(a) Culture of Wheat Seedlings Under Aseptic Conditions

Wheat seed (Triticum vulgare c.v., Gabo) was surface sterilized for 30 minutes with the supernatant of a 7% suspension of calcium hypochlorite, washed with sterile distilled water and germinated at 25°C in darkness on seed germinating agar (Rovira, 1959). Two germinated seeds were transferred to each cotton-plugged test tube (3.5 x 25 cm) containing sterile plant nutrient solution, which was modified Hoagland and Arnon solution (Hoagland and Arnon, 1938) made by dissolving the salts (except ferric citrate), sterilizing by steam at 115°C for 20 minutes, adding ferric citrate solution sterilized by the same method, and adjusting the pH to 6.5 with NaOH before dispensing into sterile cotton-plugged tubes. The seeds were supported over the solution on glass fibre mesh held in an open-ended cylinder of stainless steel. The plants were grown for 5-6 days in a cabinet under mercury vapour and incandescent lamps (2,000 foot candles) with a 12 hour day at 21°C and 12 hour night at 15·5°C.

(b) Administration of $^{14}$CO$_2$ to Plants

After several hours in light, air (10 cc) was withdrawn from the tube and replaced with air enriched with $^{14}$CO$_2$ (50-60 μc, generated by lactic acid from Na$^{14}$CO$_3$ of specific activity 1·16 mc per mmole) through an injection port in a rubber stopper. The plants were exposed to light in the controlled environment cabinet, the stoppers removed and the photosynthesis continued in non-radioactive air.
(c) Collection of Exudates

Two methods were used to collect exudates from roots of labelled plants.

(i) Collection of exudates into solutions around the roots

The plants were removed from the tubes by sliding out the steel support containing the plants and each support was suspended from the top of a beaker so the roots were immersed in sterile nutrient solution aerated with sterile air.

(ii) Collection of exudates into damp paper

To determine the sites of exudation the root system of an intact plant was laid on a strip of Whatman No. 1 chromatography paper previously sprayed with 0.5 mM CaSO₄ and 1 root was isolated from the rest by a strip of polyethylene. Another strip of damp paper was placed on this root and the papers were held firmly, but without crushing, against the roots by foam plastic backed by board. The whole assembly including the plant top was placed in a humid atmosphere in a polyethylene bag so that evaporation did not induce movement of exudates in the paper. After 2 hours the position of the isolated root was marked on the adjacent paper strip, the paper strip was removed, the root was cut from the top and root and paper strip were dried at room temperature. Since the paper dried in approximately 10 minutes there was little movement of substances by diffusion. Both paper strip and root were examined with a radiochromatogram strip scanner (see below for method for roots).

(d) Distribution of Carbon-14 Along Roots

The technique of Bowen and Rovira (1967) was modified to count ¹⁴C by mounting dried roots between adhesive-coated chromatography paper strip (4 cm wide) and thin polyethylene (1 mg per cm²; Handiwrap, Dow Chemical Co., Michigan, U.S.A.). Radiation reaching the detectors was decreased about 50% by this covering.

(e) Extraction of Soluble Fraction from Roots

Root tissue was extracted with 95% ethanol at 75°C for 30 minutes. The supernatant was removed and after 2 washings with 95% ethanol, the tissue was extracted with 20% ethanol in the same manner. The extracts and washings were pooled.

(f) Liquid Scintillation Counting of Carbon-14

Since the experiments reported in this paper were not designed to study volatile compounds, ¹⁴CO₂ was removed from solution by acidification with 0.1 volumes of 0.1 M HCl and bubbling air through the solutions for 30 minutes. The solutions were then counted without neutralization.

Samples of extracts of exudates were counted in 10 ml of Bray's solution (Bray, 1960) in Ekco or Packard Tri-carb liquid scintillation counters and the counts were corrected for quenching.
EXUDATION OF ROOT SUBSTANCES

(g) Paper Chromatography

Descending chromatography was used with Whatman No. 1 papers with:

Butanol-acetic acid-water: n-butanol 120 ml, glacial acetic acid 30 ml, water 50 ml.
Phenol-water: phenol 80 g, water to 100 ml.

Radioactive spots in 2-dimensional chromatograms were detected by autoradiography. The radiochromatogram strip scanner was used for chromatogram strips run in 1 dimension. Ninhydrin, 0.1% in ethanol, was used to detect amino acid spots and p-aminophenol (Dawson, Elliott, Elliott and Jones, 1959) was used for sugars. Invertase concentrate (B.D.H.) at room temperature without buffer was used to hydrolyse sucrose in extracts.

(h) Ion Exchange Fractionation of Exudate

Plant nutrient solutions containing exudate were applied to AG 50-H+ (Bio-Rad processed Dowex 50W-X8, 50-100 mesh) followed by 2 resin volumes of water. The effluent from the column was applied to AG 1-acetate (Bio-Rad processed Dowex 1-X4, 50-100 mesh, prepared from chloride form by washing with 2N sodium acetate), and then the column was washed with water.

The AG 50-H+ column was eluted with 3 resin volumes and the AG 1-acetate with 4 resin volumes of 10N acetic acid.

Effluent and eluates from columns were evaporated at 40-50 °C under vacuum to a small volume or to dryness and re-dissolved in water. Aliquots were counted in the liquid scintillation counter. The eluate from AG 50-H+ was chromatographed in 2 dimensions.

III. RESULTS

(a) Translocation of Carbon-14 to Roots

The experiments reported here were mainly concerned with the time required for \(^{14}C\) fixed by photosynthesis to be translocated to and label the

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Radioactivity in root segments (cp 100 sec)</th>
<th>Distance down root from base (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(0-1)</td>
<td>(1-3)</td>
</tr>
<tr>
<td>0.75</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5,320</td>
<td>5,070</td>
</tr>
<tr>
<td>1.25</td>
<td>15,200</td>
<td>4,150*</td>
</tr>
<tr>
<td>1.5</td>
<td>8,680</td>
<td>18,800</td>
</tr>
</tbody>
</table>

* Length = 1.5 cm.
† Basal segment was 6.8 cm from apex.

Plants were allowed to photosynthesize in 50 µg of \(^{14}CO_2\) for 15 minutes and transferred to beakers containing sterile plant nutrient solution. At the above times a plant was removed, a root was cut into sections and each section was extracted with aqueous ethanol.
whole length of the roots. Radioactivity of ethanol extracts of sections of roots harvested at varying intervals is shown in Table 1. No $^{14}$C was detected in the roots before 0.75 hours; after 1.5 hours radioactivity had reached the terminal section of the root which was 5.7 cm from the base. In another experiment the distance reached by $^{14}$C in replicate roots was 5 cm ± 1 cm after 1.5 hours. The rate of translocation seems therefore to be consistent; however, the total amount of $^{14}$C varied widely between roots. Details of distribution of $^{14}$C along roots, obtained with a radiochromatogram scanner, showed that the pattern of distribution differed from root to root (Fig. 1). This difference is probably due to accumulation of $^{14}$C into growing tips of laterals which had reached different stages of development in roots grown under the same conditions. Autoradiographs showed that $^{14}$C did accumulate into the growing tips of primary and secondary roots of wheat seedlings.

(b) Distribution of Carbon-14 in Soluble Fraction of Root Tissue

In extracts prepared from roots 2 hours after $^{14}CO_2$ was applied to tops almost all the $^{14}$C was incorporated into sucrose. This was concluded from the findings that the radioactivity passed through ion exchange resins
(Dowex 50–H+ and Dowex 1-acetate), moved with unlabelled sucrose during co-chromatography in 2 dimensions and was equally distributed in glucose and fructose after extract was incubated with invertase (Fig. 2).

![Achromatogram of ethanol extracts of roots two hours after administration of $^{14}CO_2$.](image)

**Fig. 2.**—Scans of chromatograms of ethanol extracts of roots two hours after administration of $^{14}CO_2$.

After 10-12 hours photosynthesis in non-radioactive air following $^{14}CO_2$ treatment sucrose still contained approximately 50% of the total $^{14}C$ in soluble extracts. Most of non-sucrose $^{14}C$ appeared to be incorporated into raffinose, glucose, fructose, glutamine, aspartic and glutamic acids.

(c) *Exudation of Carbon-14 from Roots*

Radioactive exudates from attached roots into liquid media were detected 3-4 hours after $^{14}CO_2$ was first supplied to the tops of these plants.
The amount of radioactivity in exudates collected for 12 hours after applying $^{14}CO_2$ represented approximately 1% of the amount of $^{14}C$ in the soluble fraction of the same roots extracted at the end of this period.

Exudates collected for 12 hours after exposure to $^{14}CO_2$ were fractionated on ion exchange resins and the recovery of counts was about 33%. Loss of counts is probably due to irreversible adsorption which reached large proportions because of the small amounts of radioactive compounds involved. The neutral fraction obtained after passage of effluent from Dowex 50—H+ through Dowex 1-acetate contained 46% of the recovered counts but the components have not yet been identified. The

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**Fig. 3.**—Comparison of exudation and distribution of $^{14}C$ in soluble fraction along roots.
**EXUDATION OF ROOT SUBSTANCES**

\[ \text{NH}_4\text{OH} \] eluate from Dowex 50-\(H^+\) contained 3 main radioactive spots which coincided with glutamine, glutamic and aspartic acids and which contained approximately equal amounts of \( ^{14}\text{C} \). Several other spots of unknown identity contained less radioactivity.

(d) **Sites of Exudation from Roots**

Results with plants which had been labelled with \( ^{14}\text{C} \) 24 hours previously showed that radioactive substances exuded mainly from the basal regions of roots (Fig. 3). Radioactivity in aqueous ethanol extracts of the same roots are also graphed in Figure 3. Carbon-14 accumulated in larger amounts in the apical section; the amount of \(^{14}\text{C}\) per cm along the rest of the root was much less and approximately equal in 1 cm sections. On the other hand there was relatively little or no exudation of \( ^{14}\text{C}\)-compounds from the root tip. About 25% of roots tested did not exude sufficient \( ^{14}\text{C} \) to be detected by collection into paper. Exudation of ninhydrin positive substances from these roots into paper could not be detected.

**IV. CONCLUSION**

When 8-day wheat seedlings were pulse labelled by photosynthesis in \( ^{14}\text{CO}_2 \), periods of 1-5-2 hours elapsed before the whole lengths of roots became labelled. Exudation of carbon newly incorporated by photosynthesis, commences shortly after this carbon is translocated to the roots. Photosynthetically fixed carbon appears to be translocated as sucrose in wheat since in roots this was the first compound which incorporated almost 100% of \( ^{14}\text{C} \). After reaching the root \( ^{14}\text{C} \) seems to enter a pool of unlabelled sucrose since, after a further 10 hours’ photosynthesis in normal air, approximately 50% of \( ^{14}\text{C} \) in the soluble fraction was still present as sucrose. The remainder was incorporated into glucose, fructose, raffinose, glutamine, glutamic acid, aspartic acid and several unidentified substances.

In an examination of the distribution of \( ^{14}\text{C} \) in exudate almost half the \( ^{14}\text{C} \) recovered from the fractionation procedure was in the neutral fraction when the collection period was 1-12 hours after \( ^{14}\text{CO}_2 \) administration. Glutamine, glutamic and aspartic acids were detected in the cationic fraction. When completed, a comparison between \( ^{14}\text{C}\)-compounds in exudates and soluble fraction may help to elucidate the mechanism of exudation.

Most exudation of \( ^{14}\text{C} \) from these roots occurred from the basal region, even though the amount of \( ^{14}\text{C} \) in the soluble fraction is approximately the same per unit length of root. Exudation from the basal region could not be correlated with emerging of lateral roots; root hairs occurred only near the base of these roots and could perhaps be related to exudation. Pearson and Parkinson (1961) using a similar technique with bean seedlings reported that ninhydrin positive substances exuded from the tip region of roots. The difference between these results and the present findings may be due to species variation and to differences in stages of development at which the plants were examined. In the present study on the sites of exudation the particular compounds have not yet been identified; different
compounds may be exuded at different regions of the root. For example, Frenzel (1960) reported that threonine and asparagine came from the root tip of sunflower roots while leucine, valine, glutamic acid and phenylalanine were exuded in greater amounts from the root hair zone. Also, in pulse labelled plants all components of the soluble fraction may not contain $^14$C. Although pulse labelled plants have advantages in certain experiments with exudates, experiments of the type discussed above would be more conclusive if performed with plants in which the soluble fraction is uniformly labelled.

When wheat roots were deliberately crushed or cut through at any point, considerable exudation onto paper occurred at the affected region showing, as expected, that damage to roots increased exudation. Root damage under field conditions would thus encourage proliferation of microorganisms and perhaps subsequent invasion of the root by pathogens.

V. ACKNOWLEDGMENTS

It is a pleasure to acknowledge the helpful advice given by Dr. A. D. Rovira and the technical assistance of Mrs. R. Fahlbusch. The controlled environment cabinet and radiochromatogram strip scanner were purchased with a Rockefeller Grant.

VI. REFERENCES


SUMMARY

Radioactive techniques have been applied to studies of exudates from wheat seedlings. The seedlings were grown under aseptic conditions and then pulse labelled with $^14$C by allowing the plants to photosynthesize in the presence of $^{14}$CO$_2$. After approximately 1 hour $^14$C appeared in roots, where the label was incorporated into sucrose; 12 hours later sucrose still contained approximately 50% of the total $^14$C in the soluble fraction, while the remainder was distributed into raffinose, glucose, fructose, glutamine, glutamic and aspartic acids and some unidentified compounds. Exudation of $^{14}$C-compounds was detected 3-4 hours after the start of photosynthesis in $^{14}$CO$_2$. Neutral substances were the main fraction of exudates collected 12 hours after labelling and glutamine, glutamic and aspartic acids were the main $^{14}$C-compounds in the cationic fraction.

The sites of exudation from $^{14}$C-labelled roots were detected by collecting exudate on damp paper and locating the position of radioactive sub-
Exudation occurred mainly from the basal portions of roots, even though the soluble fraction of the root tip contained much more $^{14}$C. Increased exudation occurred at places of root damage.

Résumé

Des techniques radioactives ont été appliquées aux études des matières exténuées des plantes de blé. Les plants ont été cultivés sous des conditions aseptiques et ensuite actives avec $^{14}$C, en leur permettant de photosynthétiser en présence de $^{14}$CO$_2$. Après une heure environ, $^{14}$C devint visible dans les racines où il avait été incorporé en sucrose; après 12 heures la sucrose contenait encore à peu près 50% du $^{14}$C total de la fraction soluble, tandis que le reste était réparti en raffinose, glucose, fructose, glutamine, acide glutamique et acide aspartique, et en quelques composés non identifiés. L'exsudation des composés de $^{14}$C fut aperçue 3-4 heures après le commencement de la photosynthèse dans $^{14}$CO$_2$. Des substances neutres composaient la fraction principale des matières exsudées recueillies 12 heures après le traitement des plantes, tandis que la glutamine, l'acide glutamique et l'acide aspartique étaient les composés principaux de $^{14}$C de la fraction des cations.

Les endroits de l'exsudation des racines activées avec du $^{14}$C étaient découverts en recueillant les matières exsudées sur du papier humide et en localisant la position des substances radioactives. L'exsudation avait lieu surtout aux parties fondamentales de la racine, bien que la fraction soluble de l'extrémité de la racine contenait bien plus de $^{14}$C. Il y avait de l'exsudation augmentée aux endroits où les racines étaient endommagées.

Zusammenfassung


Der Ausschwitzungsart an den $^{14}$C markierten Wurzeln wurde festgestellt, indem die Ausschwitzung auf feuchtes Papier gesammelt wurde und die Position der radioaktiven Substanz lokalisiert wurde. Die Ausschwitzung fand hauptsächlich an dem untersten Teil der Wurzeln statt, obwohl der lösliche Teil der Wurzelspitze viel mehr $^{14}$C enthielt. Eine erhöhte Ausschwitzung fand an Stellen statt, wo die Wurzel beschädigt war.
TECHNIQUES FOR THE STUDY OF LOCALIZED MICROBIAL ACTIVITY IN SOIL

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Techniques involving the burying of insoluble organic materials such as insect wings, pieces of lettuce, cellulose, chitin (Okafor, 1966; Tribe, 1963; Tribe, 1957; Okafor, 1966) are well known for their value in studying the ecology of soil micro-organisms during the decomposition of organic matter in soil. No comparable technique was available for soluble organic substrates until Webley and Duff (1962) proposed the use of pellets made of a mixture of the organic substance with kaolin as an inert base. Other workers (Lehr, Brown and Brown, 1959) had already buried pellets in soil to follow the chemical changes which occur during the conversion of monocalcium phosphate to insoluble forms. Later Chandra, Beaton and Reed (1962) similarly used pellets made from inorganic constituents to study the effect of fertilizers on the bacterial population of soil.

The present paper describes methods for the preparation of pellets and aggregates from kaolin and various soluble and insoluble organic materials. These are incubated in or on soils and subsequently investigated by a wide range of techniques. The results obtained at the Macaulay Institute in the study of the localized microbial development and biochemical changes taking place are presented.

PREPARATION OF PELLETS AND AGGREGATES

Pellets. These are made in a hardened steel mould similar to that described and illustrated by Ford and Wilkinson (1954), but without the outer case, since evacuation was unnecessary. The press used was obtained commercially (Epco Ltd., Leeds, England) and modified by the manufacturers to our specifications. It consists of the press frame, hydraulic ram, pusher head, pump and hose supplied with a pressure gauge. For ease of removal of the pellets after release of the press load (5 000 lbs) the die at the base of the mould is first removed and the barrel of the mould with plunger then placed on a device (pellet ejector) made by our instrument workshop (Fig. 1). By gradually turning the screw the plunger of the mould is gently depressed until the pellet is ejected from the base of the mould into the perspex container. The pellet is then ready for use. Kaolin as originally used by Webley and Duff (1962) still continues to be the most suitable base for making the pellets because of its good binding properties. Further, for dilution plate counts, pellets made with it disperse readily when shaken in the initial dilution flasks either on a Microid shaker (Griffin and
Fig. 1.—Diagram of pellet ejector (not drawn to scale). a, screw; b, steel frame; c, plunger; d, mould barrel; e, pellet; f, perspex drawer to receive pellet.

Tatlock Ltd., England) or Whirlimixer (Scientific Industries International Inc. (U.K.) Ltd.). Pellets have been principally used for soluble organic substrates, the ingredients being mixed in a dry state in perspex containers in a Mickle disintegrator. The amount of added organic substrate can be varied as desired. One or two plastic beads are included in these containers to ensure effective mixing before aliquots of the kaolin and the added organic material are moulded in the press. The total weight of each pellet is usually 200 mg and the diameter 9.5 mm. Control pellets of the same weight and size are prepared from kaolin only.

Aggregates. These have been used mainly for insoluble substrates which are available in limited amounts and which it is desired to keep moist during preparation, e.g. fungal walls (D. Jones and D. M. Webley, unpublished). Aqueous suspensions of the walls are thoroughly mixed with kaolin to form a paste of the desired concentration. This paste is forced from a hypodermic syringe (needle detached) on to a tile. The lengths of paste are allowed to dry out slightly at room temperature, cut into 2 mm portions and further dried at room temperature before use. Control aggregates from kaolin alone are prepared in the same way. This method for making aggregates is in essence that used by Griffiths and Jones (1965) for preparing aggregates from a mixture of soil and various organic materials.
Both pellets and aggregates can be sterilized by autoclaving but care is necessary to avoid them being permeated by too much moisture. Recently γ-radiation has been found to be a more satisfactory way of sterilization. For this purpose the pellets and/or aggregates were sealed, using an Audion Sealboy (Stainsby Road, London), into separate compartments in nylon tubing. Each compartment was separated by two seals 1 cm apart. The nylon strips containing the pellets and/or aggregates were sent to the U.K. Atomic Energy Authorities' Radiation Branch, Wantage, England, where they were subjected to 2-5 Megarads, the dose normally used for sterilization of medical goods and soils with moderate numbers of microorganisms. When a pellet or aggregate is required the strip is cut between the two seals with a scissors, leaving the remainder unaffected.

**Incubation of Pellets and Aggregates in and on Soils**

In early work, where pellets were principally used, they were buried half way down in soil (50 g) by adding half the amount of soil to the plastic pot, then the pellet, followed by the remaining soil. At required intervals a pot was removed and the pellet recovered for examination and analysis. One disadvantage of this method is that one cannot make direct microscopic observations on the organisms which are colonizing the pellets. Recently we have placed the pellets and aggregates on the surface of soil contained in small polystyrene weighing bottles with snap-on lids. From time to time these are examined under a stereoscopic microscope and the types of organisms developing noted. By using glass micro-needles it is possible to isolate directly from the pellets or aggregates any unusual or predominant organism (particularly fungi and actinomycetes) which develop.

**Results of Investigations Using Pellets and/or Aggregates**

*Enrichment and Isolation of Micro-organisms which Attack Specific Substrates*

Henderson (1965) working at this Institute used the pellet technique as originally described by Webley and Duff (1962) for the enrichment and isolation from soil of fungi which were capable of degrading aromatic compounds such as α-conidendrin, α-conidendrol, vanillin, syringic and vanillic acids. She buried in soil, pellets composed of a mixture of equal quantities of substrate and kaolin. After incubation, the fungal population was estimated by the dilution plate technique, and the disappearance of the substrates from the pellets during incubation was followed by paper chromatography. Some of the fungi isolated e.g. *Cylindrocarpon, Monocillium, Volutella* etc. had not been found by techniques previously used for such studies.

More recently Jones and Farmer (1967) expanded this work at the Macaulay Institute, and included in their study a lignin preparation extracted from Phragmites, by incubating the pellets on the surface of the soil and examining them 'in situ' using a stereoscopic microscope with illumination from above. They were able to observe directly the fungal
colonists, the spores and hyphae of which could be transferred by means of sterile glass needles directly to agar plates. They found that the range of fungi capable of metabolizing vanillic acid in pure culture could be extended to include *Stilbum* spp., *Humicola* sp. and two unidentified species. In addition, although it was unable to utilize vanillic acid, a new species of an unusual fungus, *Acremoniella velata*, was isolated (D. Jones, unpublished).

**Detection of Metabolic Products and the Rate of Localized Breakdown of Added Substrates**

The underlying intention for the original development of the pellet technique by Webley and Duff (1962) was to test for the production of organic acids in a localized soil environment. Louw and Webley (1959) and Duff and Webley (1959) had already shown that organisms producing 2-ketogluconic acid, present in soil, were particularly active in dissolving, in pure culture, difficulty soluble phosphate fertilizers. Because of their occurrence in such small amounts, some workers (Schwartz and Martin 1955) have concluded that these organic acids have no significant effect on phosphate availability in soils. However, when pellets consisting of kaolin and glucose were incubated in soil, 2-ketogluconic acid could be extracted from the soil in the vicinity of the pellets and detected by paper chromatography. Also, amongst the increased bacterial population that occurred near the pellets, a high proportion of pseudomonads were found, many of which were shown to be capable of producing 2-ketogluconic acid in pure culture. As Whitehead (1963) points out such acids would not be distributed uniformly throughout the soil but would occur in localized zones near particles of decomposing organic matter.

Jones and Farmer (1967) used U.V. absorption spectrometry to detect the loss of lignin from pellets and showed that after 5 months incubation up to 40% of the lignin present had been utilized. They also found, by infrared analysis, that the residual lignin in the pellets was little altered.

Recently a study has been made of the rate of breakdown of the cell walls of pigmented and non-pigmented fungi. For this investigation an unidentified soil fungus (No. 21), in which the degree of wall pigmentation could be varied, was chosen and grown in batch culture under controlled conditions of 

\[ pH \]

, temperature, aeration and agitation. Mycelium harvested after 30 hr was free of pigment but after 70 hr was deeply pigmented—being almost black. Walls were prepared from both the pigmented and non-pigmented mycelium using the method of Crook and Johnson (1962). These were incorporated into aggregates as described previously, and incubated on soil. Two aggregates were removed at periodic intervals, dried, and the total carbon estimated by the combustion method of Van Slyke and Folch (1940). After 4 weeks incubation the non-pigmented walls had lost 65% of their carbon content compared with 20% loss in the pigmented walls. In this connection it is interesting to note the suggestion of Potgieter and Alexander (1966) that melanin-like pigments in hyphal walls may protect them from lysis.
Localized Production of Induced Enzymes

Many measurements of enzyme activities in soil have been carried out in the past (see Volls and Dedeken, 1966). However, no study has been made of the production of induced enzymes when organic substrates are in contact with soil. We have been able to employ aggregates for investigating the induced enzymes associated with the lysis of fungal cell walls. In this study cell walls of *Fusarium culmorum* (W. G. Smith) Sacc., *Mucor ramannianus* Möller and the sterile fungus, previously referred to, were incorporated into aggregates and incubated on soil in the usual manner. It was necessary to preheat the wall suspensions to inactivate their own enzymes before incorporation into aggregates. After suitable periods of incubation one aggregate of each type (including a control) was removed from its respective soil container, dispersed in buffer, and incubated with laminarin in the presence of toluene as described by Jones and Webley (1967). Increase in reducing sugar was estimated in a Technicon auto-analysing after centrifugation. Whereas an active laminarinase was present in the aggregates containing walls of *F. culmorum* or the sterile fungus No. 21 (both pigmented and non-pigmented), negligible activity was obtained in the control aggregates and those containing the *Mucor* walls.

Further evidence for the presence of a glucanase was the detection by paper chromatography of glucose when an extract from fusarium-cell-wall-aggregates was incubated with preheated walls of bakers yeast *Saccharomyces cerevisiae* which is known to contain a β-(1-3) glucan. Examination with optical and electron microscopes showed that substantial lysis of these walls had occurred, but not of those incubated with similar extracts from the kaolin control aggregates.

Direct microscopic examination of the aggregates during their incubation on soil revealed profuse growth of *Streptomyces* spp. Representative isolates when grown in liquid culture produced extracellular enzymes capable of lysing the corresponding cell walls. As was expected from the observations on aggregates, such culture fluids contained an active glucanase where *Fusarium* cell walls were concerned but none with *Mucor*. This difference can be correlated with what is known of the chemical composition of the walls (Skujins, Potgieter and Alexander, 1965; Bartnicki-Garcia and Nickerson, 1962).

The presence of lytic enzymes induced in aggregates incubated on soil will be of special interest to workers concerned with the lysis of microbial tissue in soil. Recently Bumbieris and Lloyd (1966) were unable to obtain soil extracts capable of lysing fungal hyphae. In view of our results with aggregates containing fungal walls, it seems probable that lytic enzymes are produced in localized zones near moribund fungal material which is being colonized by lytic organisms such as *Streptomyces* spp. Thus we may have an analogous situation to that postulated by Whitehead (1963) for the localized production of organic acids in soil.
CONCLUSIONS

The following are considered to be the main advantages of incorporating soluble and insoluble organic substrates into kaolin for the study of the localized activities of the soil microflora.

(a). The materials are uniformly distributed in the pellets and agglomerates and these units are easily reproducible and can be sterilized.

(b). The basic equipment required for making the pellets and agglomerates is relatively simple and inexpensive.

(c). The amount of incorporated organic material can be varied and this is particularly useful where material is available only in small quantities.

(d). Direct microscopic observations can be made of the colonizing organisms which can then be isolated by direct means or by dilution plate methods.

(e). Chemical and biochemical techniques can be applied to the pellets and agglomerates and the surrounding soil, into which the soluble organic materials or their metabolic products may diffuse.

In his excellent review on the biochemical ecology of soil microorganisms, Alexander (1964) echoes the feelings of many soil microbiologists in making a plea for further studies of the micro-environment in order to understand the factors affecting the role and activities of soil microorganisms 'in situ'. He goes on to point out, "Yet, because of the inherent technical difficulties in biochemical experimentation at the microscopic level, progress in understanding the micro-environment has been painfully slow." It is hoped that the techniques presented in this paper may help to stimulate progress in this direction.

ACKNOWLEDGMENTS

We are indebted to the staff of the Institute's instrument workshop for making the pellet ejector and to the staff of the department of Biochemistry (under Dr. J. S. D. Bacon) who carried out the carbon determinations and glucanase assays.

REFERENCES

STUDY OF MICROBIAL ACTIVITY


**SUMMARY**

Techniques for making pellets and aggregates from kaolin as a base in which can be incorporated soluble or insoluble organic constituents are described. The pellets and aggregates which are incubated in or on the surface of soils have been used in a number of investigations to study the localized activity of the soil microflora. Direct microscopic observations of the organisms colonizing the pellets and aggregates can be made and their isolation in pure culture can be effected by glass micro-needles or by plate dilution methods. The rate of decomposition of organic substances incorporated in the pellets and aggregates can also be determined as well as the detection of metabolic products produced in them or the surrounding soil. For this purpose paper chromatography, U.V. absorption, infrared spectrometry and carbon determinations have been employed.

Aggregates have also been employed to study certain aspects of the lysis of microbial cell walls. The induced lytic enzymes and rate of carbon loss have been followed in aggregates, containing preheated fungal cell walls, incubated on the soil surface.

It is suggested that the techniques described are of value in the study of the soil microflora and its activities 'in situ'.

**RéSUMÉ**

Cet article décrit les techniques utilisées pour confectionner des pastilles et des agrégats de kaolin servant de bases auxquelles on peut incorporer des constituants organiques solubles ou insolubles. Les pastilles et agrégats qui ont été incubés dans ou à la surface des sols ont servi dans bon nombre de recherches à étudier l'activité localisée de la microflore du sol. Ceci permet l'observation microscopique directe des organismes colonisant les pastilles et agrégats et leur isolement en culture pure au moyen de microaiguilles de verre ou par dilution de plaque. Le taux de décomposition des substances organiques incorporées aux pastilles et agrégats ainsi que les poduits de métabolisme formés en leur sein ou dans le sol environnant peuvent être déterminés. A cet effet la chromatographie sur papier, l'absorption U.V., la spectrométrie infra rouge et les déterminations de carbone ont été employées.

Les agrégats ont aussi été utilisés pour étudier certains aspects de la lyse des parois cellulaires des micro-organismes. L'induction d'enzymes lysants et le taux de perte en carbone ont été suivis sur des agrégats
contenant des parois cellulaires fongiques préalablement chauffées, incubées à la surface du sol.

On pense que les techniques décrites peuvent être utiles pour l'étude de la microflore du sol et de ses activités "in situ".

**ZUSAMMENFASSUNG**


Aggregate hat man auch benützt, um gewisse Erscheinungen der Mikrobenzellewandanalyse zu erforschen. Die induzierten lytischen Enzyme und das Ausmass der Kohlenstoffverluste wurden in den Aggregaten, die vorgewärmte Pilzzellwände enthielten und auf der Bodenoberfläche inkubiert waren, weiterhin untersucht.

Es wird darauf hingewiesen, dass die beschriebenen Methoden für die Erforschung der Boden mikroflora und deren Aktivität "in situ" sehr wertvoll sind.
TECHNIQUES FOR ASSESSING THE NET EFFECT OF MICROBIAL ACTIVITIES IN SOIL

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I. INTRODUCTION

Over the years there have been many attempts to equate the biological activities of a soil with its fertility. In some cases, specific processes show trends which can be correlated with plant response, but an index of overall biological activity in the soil is not easy to define and the net effect of the numerous processes remains tantalizingly elusive. Microbiologists have had only limited success in equating total or selective counts of microorganisms with overall biological activity. The extreme variability of results, even where the sampling and counting procedures are carried out at short intervals apart and with extensive replications, has deterred the use of this sort of approach. As a result, the tendency has been to turn to a biochemical approach which measures the rate of change of soil constituents.

Three soil processes have received particular attention. They are carbon dioxide evolution, cellulose decomposition and ammonification-nitrification assessments of potential nitrogen supply; and in some cases the processes have been integrated with advantage. Irrespective of whether an individual process or several integrated processes are to be assessed, where there is a measurable rate of reaction it must be assumed that three basic conditions hold. These are that:

(i) There is a population of microorganisms available in the soil to carry out the process(es).
(ii) There is a supply of substrate(s) accessible to the organisms to meet their energy and growth requirements.
(iii) The soil environment is equitable and not inimical to the process(es) being carried out by the indigenous microorganisms.

In this paper several bioassay procedures will be described which aim to determine which of the three assumed conditions is subject to limitation. In infertile soils a process will be abnormal when the population of microorganisms is relatively inactive, when substrates are lacking or when conditions are unsuitable.

II. THE RATIONALE OF THE BIOASSAYS

(a) SLAMCOE Bioassay

The bioassay aims to evaluate effects of Substrate Limitation on the Activity of Microorganisms using Carbon diOxide Estimation (SLAMCOE) of respiratory activity of the natural soil microflora following the wetting of air-dried soil samples by water and by a simple substrate solution.
The re-wetting of a dry soil sample is followed briefly by a flush of enhanced respiration. The level of respiratory activity gives an indication of the turn around rate in the free pools of the cycle. In fertile soil this rate is high, and respiration following re-wetting is likewise high; but in infertile soil where lack of substrates limits microbial activities, the level is low. As lack of substrate is not the only factor which can limit biological activity, it is necessary to gain some estimate of potential respiratory activity in soil when substrate is not limiting. This is done using the bioassay in a form which compares respiration rates on wetting with water and on wetting with a substrate under comparable conditions. Respiration is measured during a confined measuring period, viz. twenty-four hours following wetting.

In fertile soils there is little difference in amounts of carbon dioxide respired during the first twenty-four hours on wetting with water or on wetting with substrate. In infertile soils where lack of substrate limits activity, the difference becomes large. The difference between the two levels of respiratory activity has been found to be of greater significance than either level separately. The most convenient way of expressing the result has been in the form of the ratio

$$K = \frac{(A - B)^2}{A + B}$$

where A is $CO_2$ respired on wetting with substrate, and B that on wetting with water. For respiration measured over eighteen hours (of the initial twenty-four) values of K are usually well less than 100 for fertile soils, and tend to considerably exceed 100 for infertile soils with limiting substrates.

(b) OURMESE Bioassay

The bioassay is a microrespirometric procedure in which the existing soil microflora is swamped by introduction of a massive resting suspension of test organisms whilst existing substrate differences are similarly swamped by supplying a bacteriological medium capable of sustaining vigorous respiration of the test suspension. In a fertile soil which is not inimical to biological activity, the respiration rate of the buffer-substrate-cell suspension will be high, but if conditions are unfavourable, the rate will be low. The bioassay thus utilizes Oxygen Uptake Rate as a Measure of a Equitable Soil Environment (OURMESE). In order to compare the respiration rate in soil with its potential in the absence of soil, a "No Soil" control series consisting of the aerated suspension alone is incubated under the same conditions (time and temperature). Results of the assay are expressed as a ratio (R) of oxygen consumed (as microlitres gas at N.T.P.) by the test suspension in soil to that of the "No Soil" control. Most soils are somewhat inimical to microbial activities, and values of R rarely exceed 1-0 except for soils carrying mature pastures, despite the demonstration by Ellinger and Quastel (1948) that suspensions of microorganisms spread in thin films over soil crumbs can be adequately aerated so that oxygen diffusion rate does not limit respiration. In arable soils the value of R is usually found between 0·60 and 0·85; in soils which are clearly
infertile and in which soil toxicity may be encountered, values are usually less than 0.50 and may be as low as 0.20 where there is acute respiratory poisoning. The OURMERE bioassay thus measures a challenge to the third assumption.

(c) Dehydrogenase Activity

Biological transformations in soils are catalyzed by enzymes, which can be found outside of the cells of living soil microorganisms. The range of enzymes isolated from soils is very large, and various workers have attached different degrees of significance to their occurrence. One of the most interesting of these soil reactions has been the quantitative dehydrogenase test developed by Lenhard (1956, 1966) and variously modified by Stevenson (1959, 1962), Casida, Klein and Santoro (1964), Galstyan (1964), and Kozlov and Mikhaylova (1965). A direct relationship exists between dehydrogenase activity and respiration in the presence of decomposable substrates; but during early stages of decomposition of plant residues, part of the enhanced respiratory activity is due to increased bacterial numbers, and part is due to selective stimulation of certain zymogenous organisms (Stevenson 1959, 1962). Casida, Klein and Santoro (1964) found that dehydrogenase activity paralleled microbial activity in unamended soils, but on addition of substrates it more closely paralleled a selective increase in Gram-positive bacteria. It thus would seem that dehydrogenase activity can be used to estimate endogenous activity where soils have not recently received fresh substrate. It may be a better yardstick to judge the endogenous level of microbial activity than a viable count procedure.

III. Experimental Methods

(a) SLAMCOE Bioassay

An 8 g sample of air-dry soil, spread as a layer on the floor of the 50 ml conical flask of a modified Cavett (Kent-Jones and Taylor 1954) blood test apparatus (Quickfit and Quartz Ltd.) is carefully wetted by pipetting on 2 ml 0.01 M Sørensen phosphate buffer pH 7.0. A similar soil sample is wetted with 2 ml substrate solution. Each flask is capped with a loose-fitting polythene cover, then placed in a 30°C incubator for 5-6 hours to equilibrate. At the end of this period, the Cavett stopper assembly replaces the polythene cover. A filter paper wick 5 x 2 cm is placed in the stopper-well, and 1 ml approx. 0.3 N barium hydroxide solution added. The stopper is held firmly in place on the flask by two springs, and the assembly returned to the incubator for a further 18 hours. Next morning, the contents of the stopper, including the wick, are washed into a small beaker with a jet of distilled water, and contents back-titrated to pH 8.3 with standardized (approx.) 0.01 N hydrochloric acid. Three replicate flasks are run for each soil under test and for each wetting solution. A soil-free blank is used as control. The difference in titration values between blank and treatment is equivalent to the alkali neutralized by respired carbon dioxide, and may be converted to a gas volume by multiplying by a factor (usually 130 to 140).
A 4 g sample of air-dry soil (screened through a 2 mm sieve), spread as a layer on the floor of a Warburg flask is wetted by pipetting on 1 ml buffer-substrate-cell suspension. The cells of the test bacteria are grown on shaken liquid cultures of medium CDSC (Casitone-dextrose-sucrose-citrate), harvested (after 3 days growth at 28°C) by centrifugation, resuspended in 0.05 M Sorensen phosphate buffer pH 7.0 (1 ml buffer per 25 ml culture) and refrigerated for a period not exceeding 21 days. Conventional Warburg microrespirometric procedures are used to measure the oxygen consumed over a four-hour incubation period at 30°C. A preliminary equilibration period of not less than thirty minutes precedes the test period; readings are made at thirty-minute intervals. The "No Soil" control flasks receive 1 ml buffer-substrate-cell suspension plus 1 ml phosphate buffer, and are shaken continuously in the apparatus.

Substrate is supplied from a bacteriological medium CDSC containing: dextrose 5 g; sucrose 5 g; sodium citrate 1 g; Casitone (Difco) 5 g; magnesium glycerophosphate 1 g; sauerkraut juice 20 ml; distilled water to 1 litre. Adjust reaction (if required) to range pH 6.8-7.0. May be dispensed single-strength or double-strength.

Buffer-Substrate-Cell Suspension

9 ml 0.05 M phosphate buffer pH 7.0; 15 ml double-strength medium CDSC; harvested cell suspension—1.5 ml where Rhizobium trifolii is test organism or 4.5 ml for Nocardia rubra; distilled water to 45 ml. For SLAMCOE bioassay replace the harvested cell suspension with distilled water.

IV. APPLICATION OF THE ASSAYS TO AGRICULTURAL SOILS

The establishment of sown pastures on deep, podsolized sands in many parts of Australia is hazardous because legumes are difficult to establish on soils where there is natural toxicity. The nitrogen status is too low to maintain pasture grasses beyond the first year, and the toxicity-sensitive legumes frequently fail before they can contribute by fixation. There are many factors which must be taken into account in solving these problems agronomically, but the microbiological picture reveals why the level of fertility is difficult to raise.

The SLAMCOE bioassay of three sands from Mt. Compass and Moon Hills in South Australia and from Carnamah in Western Australia shows that there is acute substrate limitation depressing microbial activities. Table 1 shows that the carbon dioxide respired on wetting with buffer is usually less than half that found on provision of substrate. The index K has values of the order of 500 in cleared scrub outside of the plots or under poor clover plants. In sites where it is possible to establish good plants in the first year or under second-year plants (which may still be relatively unthrifty), the value of K declines to near 200. At Mt. Compass it does this
Table 1
SLAMCOE BIOASSAY OF SUBSTRATE AVAILABILITY IN THREE SANDY SOILS ON WHICH IT IS DIFFICULT TO ESTABLISH SOWN PASTURES

<table>
<thead>
<tr>
<th>Soil sample and pasture cover</th>
<th>Microlitres respired CO₂</th>
<th>$K = \frac{(A - B)^2}{A + B}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wetting with substrate (A)</td>
<td>Wetting with buffer only (B)</td>
</tr>
<tr>
<td>(a) Moon Hills, S.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>under poor plants in first-year pasture</td>
<td>1947</td>
<td>808</td>
</tr>
<tr>
<td>under good plants in first-year pasture</td>
<td>1947</td>
<td>988</td>
</tr>
<tr>
<td>under moderate plants in second-year pasture</td>
<td>2015</td>
<td>983</td>
</tr>
<tr>
<td>under established plants in older pasture</td>
<td>1799</td>
<td>1032</td>
</tr>
<tr>
<td>(b) Mt. Compass, S.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>outside of lucerne plots</td>
<td>2072</td>
<td>843</td>
</tr>
<tr>
<td>under second-year lucerne plants</td>
<td>1822</td>
<td>1059</td>
</tr>
<tr>
<td>outside of subterranean clover plots</td>
<td>2072</td>
<td>847</td>
</tr>
<tr>
<td>under second-year clover plants</td>
<td>1949</td>
<td>970</td>
</tr>
<tr>
<td>(c) Carnamah, W.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>under first-year subterranean clover</td>
<td>1664</td>
<td>547</td>
</tr>
<tr>
<td>under second-year subterranean clover</td>
<td>1771</td>
<td>687</td>
</tr>
<tr>
<td>under established subterranean clover</td>
<td>1806</td>
<td>806</td>
</tr>
</tbody>
</table>
TABLE 2
OURMESE BIOASSAY WITH TEST ORGANISM NODCARDIA RUBRA IN THREE SANDY SOILS ON WHICH IT IS DIFFICULT TO ESTABLISH SOWN PASTURES

<table>
<thead>
<tr>
<th>Soil sample and pasture cover</th>
<th>Mean oxygen consumption in microlitres over 4 hours at 27°C</th>
<th>R (Ratio to &quot;No Soil&quot; control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Moon Hills, S.A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>under poor plants in first-year pasture</td>
<td>121</td>
<td>0.126</td>
</tr>
<tr>
<td>under good plants in first-year pasture</td>
<td>204</td>
<td>0.212</td>
</tr>
<tr>
<td>under moderate plants in second-year pasture</td>
<td>253</td>
<td>0.264</td>
</tr>
<tr>
<td>under established plants in older pasture</td>
<td>346</td>
<td>0.361</td>
</tr>
<tr>
<td>(b) Mt. Compass, S.A.</td>
<td></td>
<td></td>
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<tr>
<td>outside of lucerne plots</td>
<td>159</td>
<td>0.165</td>
</tr>
<tr>
<td>under second-year lucerne plants</td>
<td>231</td>
<td>0.240</td>
</tr>
<tr>
<td>outside of subterranean clover plots</td>
<td>179</td>
<td>0.187</td>
</tr>
<tr>
<td>under second-year clover plants</td>
<td>274</td>
<td>0.285</td>
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<tr>
<td>(c) Carnamah, W.A.</td>
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<td></td>
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<tr>
<td>under first-year subterranean clover</td>
<td>93</td>
<td>0.282</td>
</tr>
<tr>
<td>under second-year subterranean clover</td>
<td>95</td>
<td>0.288</td>
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<tr>
<td>under established subterranean clover</td>
<td>137</td>
<td>0.414</td>
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"No Soil" control series a and b | 961 |

"No Soil" control series c | 330 |
TABLE 3
A COMPARISON OF DATA FROM SLAMCOE AND OURMESE BIOASSAYS OF A SANDY SOIL UNDER PASTURE DEVELOPMENT FROM BUNDANOON, N.S.W.

<table>
<thead>
<tr>
<th>Soil sample No.</th>
<th>Soil reaction</th>
<th>SLAMCOE bioassay (A) with (B) with substrate</th>
<th>OURMESE bioassay by (a) Rhizobium trifolii (b) Nocardia rubra</th>
<th>ages of experimental pasture:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH (1:5)</td>
<td>(A) with buffer K - (A - B)2 Oxygen consumed</td>
<td>R Oxygen consumed</td>
<td>first-year pasture, heavily fertilized</td>
</tr>
<tr>
<td>5</td>
<td>5.35</td>
<td>1382</td>
<td>13.0 1077 0.893</td>
<td>R 4.65 1205 1.0 897 1.0</td>
</tr>
<tr>
<td>6</td>
<td>5.30</td>
<td>1318</td>
<td>24.2 1073 0.890</td>
<td>R 4.90 1554 1.0 920 1.0</td>
</tr>
<tr>
<td>2</td>
<td>5.30</td>
<td>1356</td>
<td>1.4 1032 0.857</td>
<td>R 4.75 1544 1.0 920 1.0</td>
</tr>
<tr>
<td>10</td>
<td>5.05</td>
<td>943</td>
<td>107 871* 0.799</td>
<td>R 4.90 1554 1.0 920 1.0</td>
</tr>
<tr>
<td>9</td>
<td>4.90</td>
<td>1019</td>
<td>111 666* 0.611</td>
<td>R 4.90 1506 1.0 920 1.0</td>
</tr>
<tr>
<td>4</td>
<td>4.75</td>
<td>1108</td>
<td>75.6 501 0.416</td>
<td>R 4.70 1544 1.0 920 1.0</td>
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<tr>
<td>3</td>
<td>4.70</td>
<td>981</td>
<td>126 406 0.337</td>
<td>R 4.90 1506 1.0 920 1.0</td>
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<tr>
<td>8</td>
<td>4.90</td>
<td>1022</td>
<td>92.7 369 0.306</td>
<td>R 4.90 1506 1.0 920 1.0</td>
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<tr>
<td>7</td>
<td>4.85</td>
<td>920</td>
<td>111 325 0.269</td>
<td>R 4.85 1432 1.0 920 1.0</td>
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<tr>
<td>1</td>
<td>4.55</td>
<td>1151</td>
<td>14.9 255 0.211</td>
<td>R 4.85 1432 1.0 920 1.0</td>
</tr>
</tbody>
</table>

"No Soil" control for samples 1-8 1205 1.0 897 1.0
"No Soil" control for samples 9-10 1090 1.0 926 1.0

For the pastures developed:
- Virgin land: Sample nos. 9 and 10
- Pasture one year old: 1, 3, 4, 5 and 6
- First-year pasture, heavily fertilized: 2
- Pasture three years old: 7
- Pasture five years old: 8

NFT EFFECT OF MICROBIAL ACTIVITIES
more readily under lucerne than under subterranean clover. The highly
toxic Carnamah soil is even more recalcitrant to amelioration.

The acute toxicity of these soils is shown by the OURMESE bioassay
data of Table 2 where values of R less than 0.2 indicate catastrophic
respiratory poisoning. These values of R in this table closely parallel the
values of K from Table 1, with a similar trend of slow amelioration under
successful plant establishment. The severity of initial inhibition of respira­
tion, and the relatively slight gains made in the first few years of a struggling
pasture are evident from the OURMESE bioassay data. *Nocardia rubra*
has been chosen because it is a typical soil actinomycete; *Rhizobium
trifolii* because it is a plant associate. Despite the contrasted ecological
preferences of the two organisms, they are similarly affected by the
toxicity of this soil, and it is not unreasonable to assume that the overall
microbial population will show reduced activity where the soil environment
is far from equitable.

In the soils examined, the OURMESE and the SLAMCOE bioassays
have shown parallelism, and this is usually the case for most soils. In an
acid, sandy soil from Bundanoon, New South Wales, where again there is
low natural fertility and marked decline and unthriftiness in subterranean
clover in the year of establishment a contrasting situation was encountered.
The OURMESE bioassay data of Table 3 show that *Rhizobium trifolii*
is more sensitive than *Nocardia rubra*; the lowest values of R are 0.40
for the latter organism, but the former shows four plots to be well below
this and acutely toxic. The SLAMCOE bioassay data show 126 as the
largest K value and 1.4 as the smallest, these values are low enough to
indicate that there is no lack of decomposable substrate in these soils. The
most interesting contrast is for soil no. 1 where R (for *Rhizobium trifolii*)
is 0.21 yet K has the low value of 14.9. If biological activity is reduced in
these soils it must therefore be due to an inimical environment. The *pH*
data are of interest because they show that the downward trend in the
OURMESE bioassay data can be correlated with increasing acid reaction
of the soil. The assay organism itself is not inhibited by this reaction in its
buffered system, but the soil environment becomes increasingly unfavour­
able under these conditions, and biological activity reflects this course.

V. DISCUSSION

The level of microbial activity has important inter-relationships with
soil fertility. The diversity of biochemical reactions proceeding simul­
taneously in soil requires that some net effect of biological activity be used
to assess the bearing upon mineralization-immobilization processes. Because
biological activity depends upon (i) an adequate population of micro­
organisms, (ii) an adequate substrate, and (iii) an equitable soil environ­
ment, it is necessary that these assumed conditions be tested. Samples
of soils carrying pastures have been bioassayed by SLAMCOE and
OURMESE techniques, which demonstrate where biological activity is
substrate-limited and where soil environment is not equitable. The tech­
niques are applicable to soils both highly and poorly fertile. We are left
with the problem of testing the remaining premise of an adequate microbial population. The difficulties associated with counting procedures are well-known. A biochemical procedure such as dehydrogenase activity has much to recommend it, but its use does not remove certain areas of uncertainty. In experiments on red-brown earth cereal soils for which data were not presented in this paper, the most vigorous dehydrogenase activity was associated with poorly-structured soils of a wheat-fallow rotation shortly after rains had broken the season. This is a period in which the OURMESE bioassay tends to give low values of R, and is associated with activity of anaerobic organisms. In other cases light sands have given high dehydrogenase activity (data not presented). We are thus confronted with an inability to distinguish between the outcome of aerobic and anaerobic activities and their respective significance. The same problem confronts workers attempting to use respiration as an index of biological activity; this has led to stress being placed upon respiratory quotients (e.g. Stotzky 1960). Whilst R.Qs. may indicate that anaerobiosis occurs, it does not provide a solution to our problem. Again, counting procedures usually ignore anaerobes. Soil dehydrogenase activity is assayed under anaerobic conditions, but the enzymes may originate from either anaerobes or aerobes. We now need more information to evaluate the association between microbial growth in soil under various oxygen tensions and the relation of actual dehydrogenase activity to Lenhard's (1966) potential dehydrogenase activity. We may then be able to test the remaining premise. This would permit the assessment of net microbial activity, and enable its relation to soil fertility to be examined.

VI. REFERENCES


SUMMARY

In attempting to relate the overall level of microbial activity to soil fertility there are difficulties in choosing a biochemical process to measure or a counting procedure which is valid. Some of these difficulties stem from assumptions we make in assessing microbial activity as a rate. It becomes necessary to test the factors which limit the rate, and three bioassay techniques are suggested and described. The SLAMCOE bioassay is a respirometric measure of substrate limitation in the biological activity of the natural soil flora. The OURMESE bioassay is a microrespirometric measure of an equitable environment occurring in soil for the dissimilation of added substrate by an introduced population of test bacteria. Soil
dehydrogenase activity is a measure of current metabolic activity of the microbial population. Examples are given of the application of the bioassays to agricultural soils by examining the reasons for limited microbial activity in problem soils for the establishment of sown pasture. The bioassay data show how microbial activities can be related to the trends in soil fertility.

RéSUMÉ

Lorsqu’on essaie de faire un rapport entre le niveau général de l’activité microbienne et la fertilité du sol, quelques difficultés se présentent dans le choix d’un processus biochimique pour prendre des mesures ou d’une procédure d’évaluation qui soit valable. Quelques-unes de ces difficultés ont pour origine des suppositions que nous faisons lorsque l’on considère l’activité microbienne en tant que régime. Il devient nécessaire de tester les facteurs qui limitent le régime, aussi suggère-t-on trois techniques de bioanalyse qu’on décrit. La bioanalyse SLAMCOE est une mesure de respirométrie des limites des substrats dans l’activité microbienne de la flore naturelle du sol. La bioanalyse OURMESE est une mesure microrespirométrique d’un milieu convenable présent dans le sol pour la dissimilation de substrats ajoutés par une population de bactérie témoin qui est introduite. L’activité de déhydrogénèse du sol est une mesure de l’activité métabolique normale de la population microbienne. On donne des exemples de l’application des bioanalyses aux sols agricoles en examinant les raisons pour la limitation de l’activité microbienne dans les sols qui posent des problèmes pour l’établissement de pâture semée. Les données de la bioanalyse montrent comment les activités microbienne peuvent être reliées aux tendances dans la fertilité du sol.

ZUSAMMENFASSUNG

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