Phytotoxicity assay of selected plants to Pyrene contaminated soil

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Abstract
Three selected plants (Alfalfa, oilseed rape and perennial ryegrass) were tested for their ability to germinate and grow in a soil contaminated with a typical polycyclic aromatic hydrocarbon (PAH), Pyrene, at two concentrations: 50 mg/kg or 100 mg/kg dry soil. When compared to control plants grown in uncontaminated soil, pyrene did not inhibit the germination of the three tested species even at the highest concentration (100 mg/kg), with a germination percentage of alfalfa, oilseed rape and ryegrass of 95, 90 and 86%, respectively. The shoot and root lengths of the three plants were also unaffected by the presence of pyrene at both concentrations. Based on these results, the three tested species can be considered as pyrene tolerant plants, and can be used in phytoremediation experiments. Among the tested species, oilseed rape seemed to be the most suitable plant, probably because of the greater root length. Greenhouse studies are in progress to evaluate the development of these plants in the advanced growth stages, and their effect on pyrene level in soil.

Key Words
PAH, pyrene, phytoremediation, alfalfa, oilseed rape, perennial ryegrass.

Introduction

Recently, the PAHs contribute to the environmental matrix has gained an increased attention by the research community (Henner *et al*. 1997). PAHs are a class of very hazardous pollutants that accumulated increasingly in the environment. They consist of two or more conjugated aromatic rings ranging from a simple two-ring compound to more complex six-ring compounds. The US Environmental Protection Agency (EPA) has indicated 16 PAHs as priority pollutants for carcinogenic risk **(**Agency for Toxic Substances and Disease Registry**,** ATSDR).

In general, the commonly used remediation technologies for PAHs contaminated soils are very expensive, and sometimes can damage the natural soil structure and texture. (Lundstedt *et al.* 2006). On the opposite, the use of plants, their associated microflora and agronomical techniques to lower soil toxicity is considered to be a safe, efficient, eco-friendly and economic means of removing pollutants from contaminated soil, as proved by the numerous phytoremediation studies carried out in the last years (Venkata *et al*. 2006).Several evidences show that the concentration of PAHs can be strongly reduced by the presence of plants, which enhance their removal when compared to bulk soils.

Numerous plant species were tested for their ability to remediate soils contaminated with pyrene. In particular, the PAHs degradation is enhanced in the rhizosphere compartment, where rhizodeposition stimulates the growth of microorganisms, characterized by great densities and activities than those of surrounding soil (Fan *et al*. 2008). For example, the advantage of choosing legumes is due to their ability to fix atmospheric nitrogen, whose availability in PAH-contaminated soils is often limited (Hutchinson *et al.* 2001). Similarly, the benefit of choosing grasses is due to their extensive fibrous root system, which would provide a larger surface for colonization by soil microorganisms than a taproot (White *et al*. 2006). Anyway , an initial phytotoxicity bioassays can be a useful and effective screening tool to eliminate plants which are sensitive to the contaminants found in soil and reduce the number of plants for pot or greenhouse phytoremediation studies (Kirk *et al.* 2002).

Pyrene was selected among the 16 EPA priority PAHs as the model compound since it exhibits intermediate toxicity, hydrophobicity and environmental persistence , and represents the dominant PAH produced by incomplete combustion of oil and oil-byproducts. Pyrene is a four-ringed PAH, colorless, and biodegradable (Kanaly and Harayama 2000). According to the current legislation (D.Lgs.152-2006, All.4), the pyrene level in sites of private and public residential use and of industrial use should not exceed 5 mg/kg and 50 mg/kg, respectively.

Materials and methods
An uncontaminated soil (no previously history of contamination with pyrene) was collected from a farm in Turi, South Italy. It has a clay loam texture, pH 7.81, organic carbon content 25 mg/kg, total nitrogen 2.4 g/kg, available phosphorous 40.7 mg/kg and exchangeable potassium 626 mg/kg. Before use the soil was dried in greenhouse to constant weight.

Three plant species were chosen (Alfalfa (*Medicago sativa* L), Perennial ryegrass (*Lolium perenne L.*) and oilseed rape (*Brassica napus)*) according to their endemic spread (indigenous species) and their nature (legumes and grass). Seeds were purchased by N. Sgaravatti and C. Sementi S.P.A., Italy.

The general phytotoxicity assay described by Henner *et al*. (1999) and Kirk *et al*. (2002) was followed, with few modifications. For each experiment, 40 g of dried soil were added to a plastic Petri dish in 4 replicates. A stock solution of pyrene (98% purity, SIGMA-ALDRICH) in acetone was added to soil to reach two concentrations, 50 and 100 mg/kg. The spiked soil was carefully mixed and air-dried under fume hood for more than 24 hour, until the smell of acetone had disappeared. Simultaneously, a blank was performed by adding to the soil the same quantity of acetone in order to observe the possible solvent effect on tested plants, and a control with an equivalent amount of water instead of the acetone.

The seeds of Alfalfa, Perennial ryegrass and oilseed rape were immersed in water for 1 hour and then sown in the pyrene spiked soil using 7 seeds per Petri dish. The dishes were arranged in completely randomized design at room temperature and under natural sunlight. To make up for the water loss, each plate has been added with 10–20 ml of water daily. After 14 days, the number of seeds germinated for each treatment was counted and plants were removed to measure shoot and root lengths. Data were statistically analysed using the one-way analysis of variance (ANOVA), and comparisons of means were carried out using the Duncan's test.

Results
The germination data indicate that pyrene did not inhibit the germination of the tested plants (Figure 1). In particular, the germination of the three tested plant species is not significantly affected by the presence of pyrene at both concentrations. These results agree with those of Smith *et al.* (2006), who reported that germination of seven plants (grasses and legumes) is not affected by PAH contamination in soil, whereas dry foliage yield was significantly reduced. The same conclusion was reached by Sverdrup *et al.* (2007), who showed that PAHs have no influence on seed germination of *L. perenna T. pretense* and *Brassica alba*, whereas the growth of these plants, in terms of plant dry weight, is reduced. Huang *et al.* (1996) reported that pyrene induced chlorosis in *Brassica napus* L., but this symptom was not found in this study.

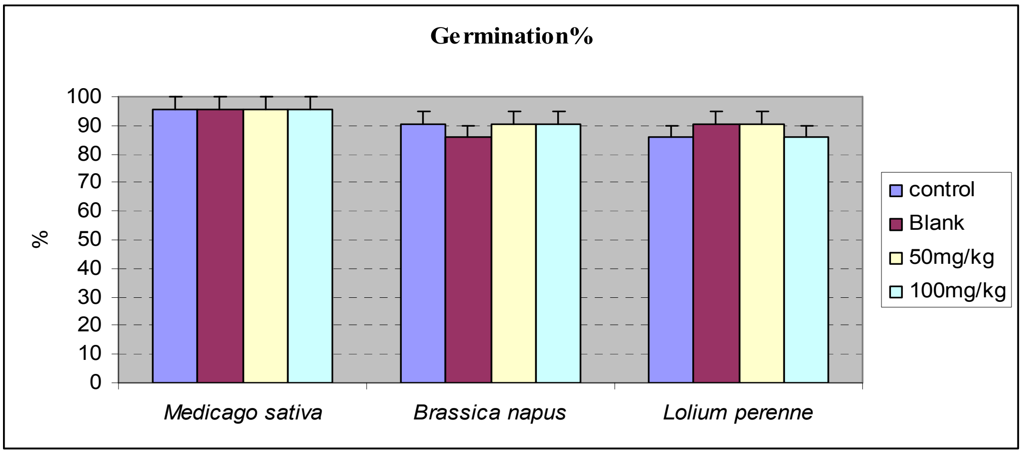

Figure 1. Germination percentage of the three tested plant species.

Similarly, the shoot and root lengths of control alfalfa, oilseed rape and ryegrass plants grown in the presence of pyrene, even at the highest concentration, were not significantly different from those grown in the absence of pyrene (Figures. 2 and 3).

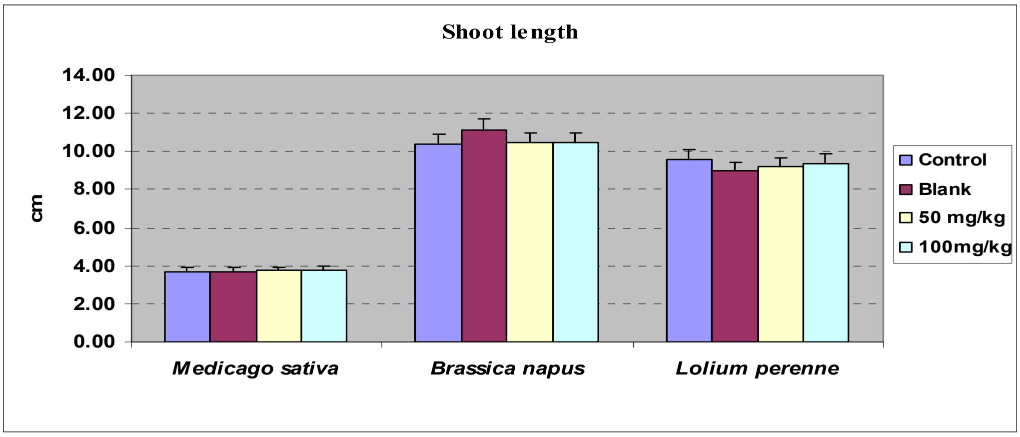

Figure 2. Shoot length of the three tested plant species.

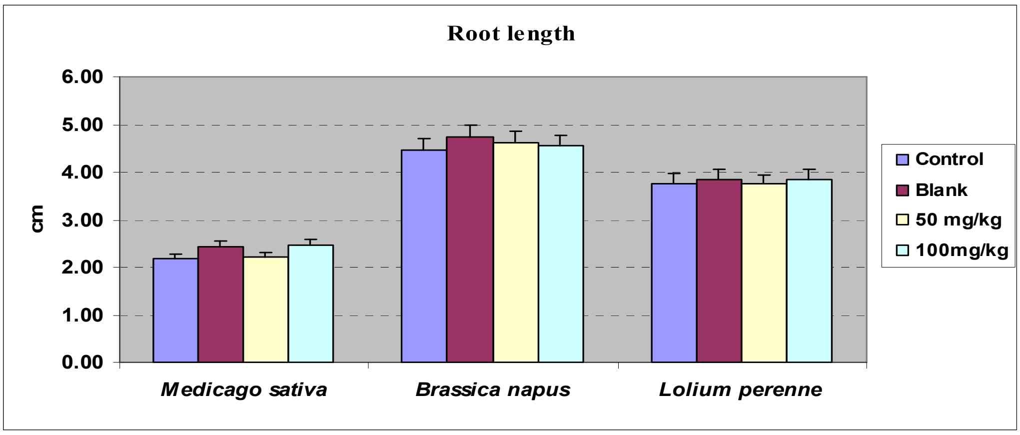

Figure 3. Root length of the three tested plant species.

In this study, we evaluated the extent of seed germination and seedling growth over a relatively short period of time (14 day). Obviously, seed germination represents the first important step to perform an effective phytoremediation. In fact, young seedlings may be particularly susceptible to PAH contaminants, since early periods of seedling growth are extremely important for root development and, consequently, for phytoremediation. Thus, the assessment of a good seedlings growth in PAH-contaminated soils may provide an important indication of the potential value of these plants in phytoremediation.

On the other hand, it is well known that for an effective phytoremediation, it is desirable to have tolerant plants with a root apparatus well distributed in soil, being the root length a parameter to assess the phytoremediation ability of different plant species. Longer roots may increase the rhizosphere area and thereby enhancing the ability to support soil microorganisms as compared to shorter ones (Harvey *et al.* 2002). Based on these considerations, the three tested species can be considered potentially as pyrene tolerant plants. Considering that longer root system is preferred for plants selected for use in phytoremediation, *Brassica napus* should appear to be the most promising plant.

Conclusion

The three plant species tested in this study germinate and grow well in pyrene contaminated soil. They show a good germination pattern, and no negative impact of pyrene has been observed in their shoot and root development. The general response of all tested plant species to pyrene is similar in all cases, both as germination percentage and as shoot and root growth, and no negative effects have been induced by pyrene, also at the highest concentration.

Brassica napus with the longest root seems to be the most suitable plant when grown in pyrene contaminated soil. Although roots of *Medicago sativa* seemed to be shorter than those of *Brassica napus*, the value of nitrogen fixing bacteria in stimulating pyrene remediation in roots of *Medicago sativa* should not be ignored.

PAHs generally do not kill plants, but would slow down or inhibit growth by decreasing plant biomass or elongation. PAHs are also known to induce plant genetic mutation, retard growth, and increase the sensitivity of the plant to other stresses (Maliszewska-Kordybach and Smreczak, 2000). Depending on these considerations, further greenhouse studies are in progress to evaluate the growth and the development of the three plant species in successive advanced stages by observing the effect of pyrene on their foliage yield, plant biomass and dry weight, in addition to their phytoremediation ability to decrease the pyrene level in soils.

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