Germination of *Lepidium sativum* as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are pollutants. They occur as common constituents of petroleum, coal tar and shale oil, but are most frequently formed by incomplete combustion of fossil fuels (Pothuluri and Cerniglia, 1994). Their fate in nature is of great environmental concern due to their toxic, mutagenic and carcinogenic properties (La Flamme and Hite, 1978; Pahlmann and Pelkonen, 1987).

Although GC-MS analysis of PAH has been successfully used to determine the concentration of PAH during the remediation of the pollutants in soil, the methodology remains expensive and requires a high degree of skill in the use of the instruments.

Previously, it was indicated that photomodified PAH had a marked inhibition of root fresh weight of the Canola plant (Ren et al., 1996). The phytotoxicity of these pollutants could, therefore, possibly be used to establish a monitoring method that would indicate the presence of this compound in soil. This suggested that germination efficiency of selected plants, could be used as a bioindicator of pollutants. In this study, our objective was to select and use an appropriate plant with a short germination period and to evaluate it as a potential bioindicator of PAH pollution. In this study, we investigated the germination efficiency *of Lepidium sativum* exposed to different concentrations of PAH in both the presence and absence of a surfactant in soil. Surfactants have been shown to increase the desorption and bioavailability of PAH in soil (Aronstein et al., 1991). We also evaluated the possibility of using germination of *L. sativum* as a bioindicator of PAH removal from contaminated soil.

2. Materials and methods

2.1. Chemicals

All solvents (dichloromethane 99% and trichloromethane 99%), non-ionic surfactant (Triton X-100) and PAH (naphthalene 98%, pyrene 98%, fluorene 98%, phenanthrene 98% and anthracene 98%) were purchased from Sigma-Aldrich, SA.

2.2. Soil

The soils used in the experiments were labelled A, B, C and D. Soil A was a predominantly sandy loam soil taken from the CSIR site (Pretoria, SA), soil B was an industrial soil taken from an industrial oil site (Secunda, South Africa), soil C was also an industrial soil (Kwazulu-Natal, SA) containing ilmenite (6%), rutile (0.4%), zircon (1%), leucoxene (0.3%), magnetite (1%) and kyanite (1%). Soil D was a white playpen sand bought from Lion Bridge SA (Pty) Ltd. Soils C and D were used in the experiment to test the germination of *L. sativum* in different soils that are not contaminated with PAH. The industrial soil (soil B) consisted of heterogeneous soil material from an oil refinery with a contamination of -1:2 g PAH per kg soil. The artificial contamination of soil A with PAH was done as described by Leyval and Binet (1998).

2.3. Plants

The seeds of white buffalo grass *(Panacum maximum)* were purchased from AGRICOL (Pty) Ltd, SA and those of *L. sativum* were purchased from Lion Bridge (Pty) Ltd, SA. The grass seeds were sown in trays (38 cm x 38 cm) containing soil A. The seedlings of P. *maximum* were used in the phytoremediation experiment (vegetated microcosm) while *L. sativum* seeds were used as potential bioindicator ofPAH removal.

2.4. L. sativum bioindicator

The seeds of *L. sativum* were exposed to different concentrations (50, 150, 300, 500 and 1000 ppm) of PAHs in soil A. For each treatment, 75 ml ofdeionised water was added to 375 g soil to bring the soil moisture to 75% field capacity (14% on wet weight). The soil was mixed and then divided into three large polystyrene Petri dishes (150 mm x 25 mm) after

which 50 seeds of *L.sativum* were sown in each Petri dish. Three replica plates were used for each treatment. The plates were placed next to the window allowing sufficient light for photosynthesis. After 3 days, the seedlings were counted. The sensitivity of *L. sativum* germination to PAH was assessed by seedling count as well as by the weight of the seedlings. Germination was defined as a visible cracking of the seed coat with a measurable root or shoot production. Five seedlings from each of the three plates were weighed and the average taken. The above experiment was repeated with the addition of Triton X-100 (a surfactant) at a concentration of 100 µg/g soil. The treatment in which PAHs were absent, as well as the treatment containing Triton X-100 with no PAH served as controls. The germination of *L. sativum* was also evaluated using different soil types. The germination procedure was repeated using the uncontaminated soil A, industrial soils (B and C) as well as soil D. The statistical processing was performed with SPSS for Windows release 7.5.2.

Table 1
Treatments used during rhizoremediation

Treatments	Additions/preparations
T_0 (Control)	Soil A + 100 µg/g Triton + grass
T1 (Treatment 1)	Soil A + 100 µg/g Triton + PAH(1000 ppm) + grass
T_2 (Treatment 2)	Soil A + 100 µg/g Triton + PAH(1000 ppm)
T_3 (Treatment 3)	Soil A + PAH (1000 ppm)
T_4 (Treatment 4)	Diluted Soil B + 100 µg/g Triton + grass
T_5 (Treatment 5)	Diluted Soil B + 100 µg/g Triton

2.5. Vegetated and non-vegetated microcosms

The use of *L. sativum* germination as a bioindicator of PAHs removal from contaminated soil was evaluated during phytoremediation of PAH in different soils (Table 1). Treatments 4 and 5 were prepared by mixing 350 g of soil B with 150 g of soil A to make the diluted soil B shown in the table. PAHs were added to the soil as described earlier. Five-hundred grams of each soil preparation (Table 1) were placed in pots with saucers for leachate collection. Two seedlings of the white buffalo grass were planted in each pot and the pots placed in the green house at ambient temperature and natural day-night cycles. Hundred millilitres of water was used to water the plants every 2 days. In instances were leachates were produced, the leachate was used to water the same pots. Each treatment had 10 pots and one pot for each treatment was sacrificed every week and the *L. sativum* germination method carried out as described previously. Three replicates plates were used for each treatment.

2.6. Chemical analysis

Residual PAH in treatments T_1, T_2 and T_3 were quantified after extraction with dichloromethane, using a Varian Saturn 2000 Ion Trap Gas Chromatography/Mass Spectrometer equipped with a Chrompack CP-SIL 8CB-MS (5% phenyl) Fused Silica Capillary Column (30 m x 0:25 mm x 0:25 um). The detector was tuned according to EPA 8270C using DFTPP. Injector temperature was 230°C, oven temperature program: 30°C (6 min), 10°C/min, 300°C (7 min). Chemical analysis was not carried out for T_4 andT_5.

3. Results

3.1. L. sativum bioindicator

The ability of *L. sativum* seeds to germinate in soil contaminated with different concentrations of PAHs was investigated. Germination levels of the seeds decreased with an increase in the concentration of the PAH (Fig. 1a). At higher PAH concentration (1000 ppm), the germination level of *L. sativum* was lower by a factor of three, compared to germination levels at 50 ppm. The presence of Triton X-100 in the soil did not increase the toxicity of the PAH to seed germination (Fig. 1b) as the surfactant by itself had no effect on the seed germination. The level of germination also decreased with increasing concentration of PAH. The effect of PAH on the fresh weight of the seedlings was also investigated in the experiment. The seedling fresh weight of *L. sativum* decreased with an increase in the concentration of PAH (Fig. 2a and b). At higher PAH concentration, the weight of the seedlings was lower by a factor of two compared to the seedlings exposed to lower PAH concentration.

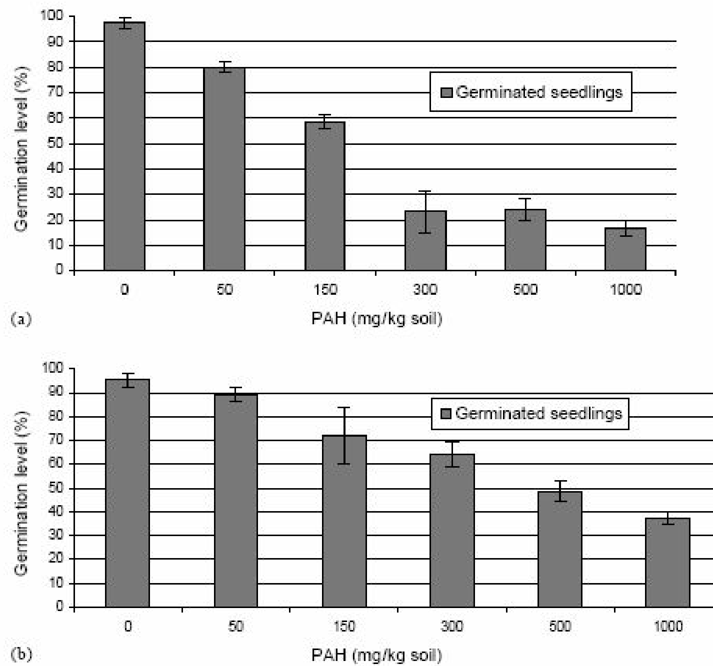

Fig. 1. The level of germination of *L. sativum* in soil contaminated with different concentrations of PAH: (A) germination of *L. sativum* in the absence of the surfactant (Triton X-100) and (B) germination levels in the presence of surfactant (100 μg/g soil). The surfactant was added to make the PAH bioavailable. Error bars represent standard deviations. There was a significant difference between the treatments used ($P < 0.05$).

Germination of *L. sativum* in different soils shows that, where PAHs are absent, the level of germination exceeds 95% while in the case where the concentration of PAH are high (1.2 g/kg soil as in soil B), no germination of *L.sativum* occurs (Fig. 3). The composition of soil C did not appear to have any inhibition on the germination of *L. sativum* seeds, as germination levels were comparable to the levels in soils with no PAH (A and D).

3.2. Germination over time in vegetated and non-vegetated soils

The potential of *L. sativum* germination as a bioindicator of PAH removal was investigated during phytoremediation of soil contaminated with PAH. Soil A was artificially contaminated with PAH mixtures of naphthalene, anthracene, phenanthrene, fluorine and pyrene and the grass seedlings of *P. maximum* were planted in treatments T_0, T_1 and T_4. The level of germination in the soil of vegetated treatment (T_4) was significantly higher compared to germination levels in the non-vegetated treatment T_5 (Fig. 4). However, there was no significant difference in the germination level of both the vegetated treatment (T_1) as well as the non-vegetated treatments (T_2 and T_3) containing the artificially contaminated soil A. The data suggest that *P. maximum* may have a positive effect on the removal and or detoxification of the PAH from the soil. The extent of PAH disappearance in vegetated soil is significantly greater than in unvegetated soil (Aprill and Sims, 1990). Initial germination levels were below 15% but increased with the time of the experiment, possibly due to the reduction in the concentration of PAH in the soil (Table 2).

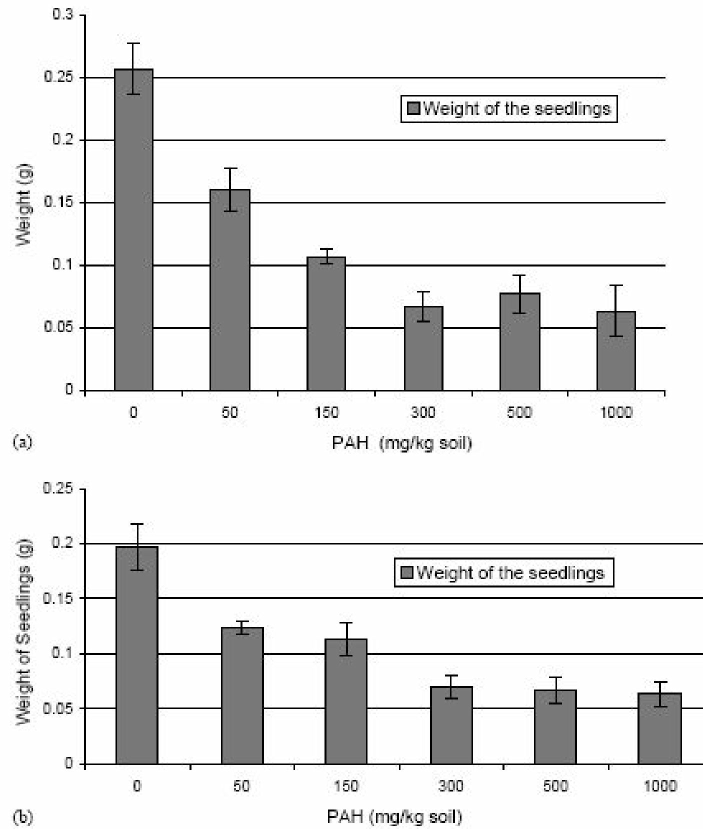

Fig. 2. The fresh weight (wet) of *L. sativum* seedlings in the presence of different concentrations of PAH in soil A: (a) the seedlings fresh weight of *L. sativum* germinated in soil with no Triton X-100 and (b) seedling fresh weight of *L. sativum* in soil with Triton X-100.

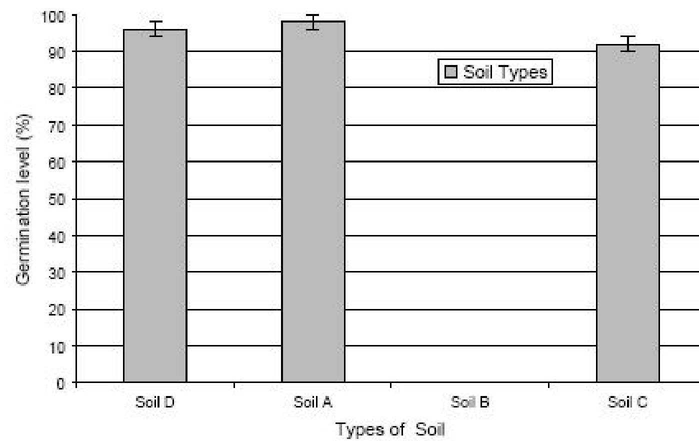

Fig. 3. Germination of *L. sativum* in different soil types. Soil A (sandy loam soil not contaminated with PAH), soil B (historically PAH polluted industrial soil), soil C (soil contaminated with heavy minerals but with no PAH), soil D (white playpen sand free of PAH). Error bars represent standard deviations. There was no significant difference between the treatments in which germination occurred ($P < 0.01$).

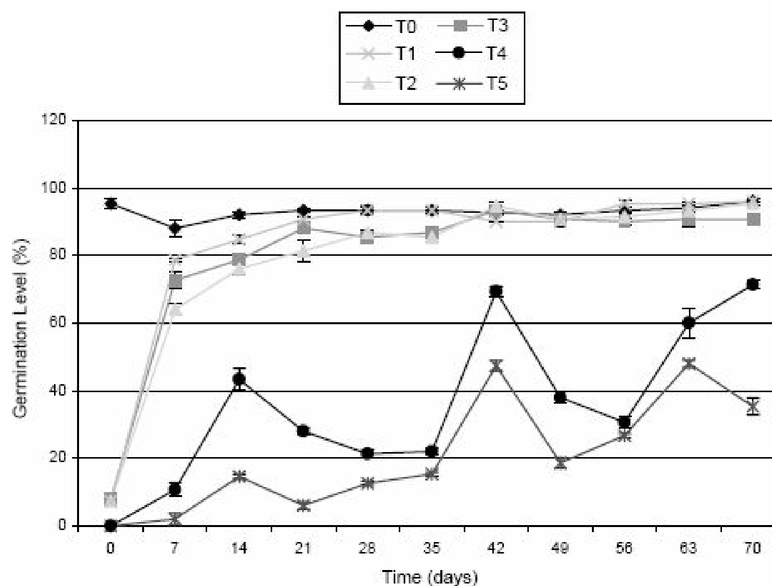

Fig. 4. *L. sativum* germination during phytoremediation of contaminated industrial soil as well as soil artificially contaminated with PAH. Error bars represent standard deviations. T_1 was not significantly different from T_2 and T_3. However, T_4 was significantly different from T_5 ($P < 0.05$).

Table 2
PAH concentrations (mg/kg soil) in planted and non-planted soil using EPA analytical method 8270C

Days of incubation	Naphthalene			Fluorene			Phenathrene			Anthracene			Pyrene		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
7	143	106	197	433	536	572	679	757	711	737	696	703	720	824	696
42	2.46	3.81	20.4	82.7	146	183	296	510	502	571	437	512	335	402	397
70	0.82	0.52	1.7	17.1	20.3	37.4	50.4	56.5	63.4	182	135	193	184	205	211

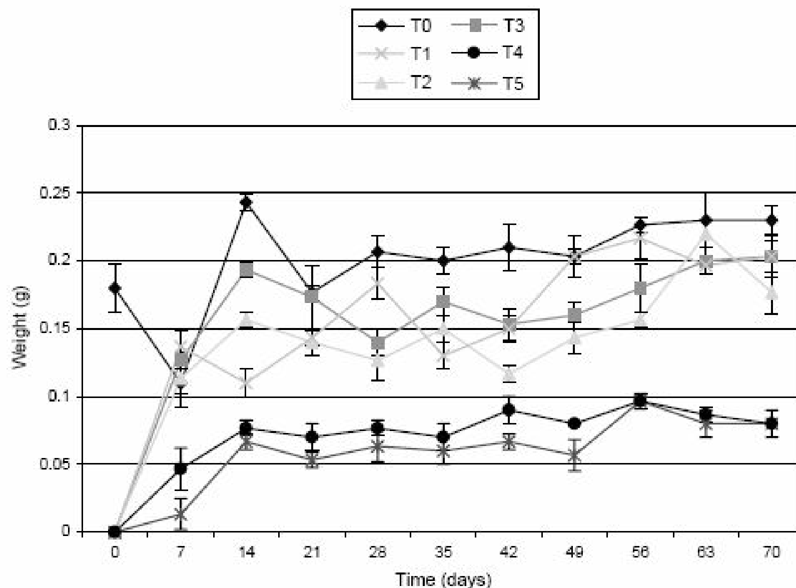

Fig. 5. The average weight (wet) of *L. sativum* seedlings during phytoremediation of PAH in contaminated soil.

The effect of PAH on the weight of the seedlings of *L. sativum* was also investigated over an experimental period of 70 days (Fig. 5). As with the germination of the seedlings, the weight of the seedlings germinated in the respective soils of the vegetated treatments gave similar results. The weight of the seedlings in the industrial soil (soil B) remained low throughout the experiment, possibly because of toxicity due to the presence of recalcitrant PAH.

4. Discussion

PAH phytotoxicity has been described as a physiological toxicity (Bossert and Bartha, 1985; Huang et al, 1996; Ren et al, 1996), and an indirect effect on the ability of the contaminated soil to provide water and nutrients to the plants (Reilley et al, 1996). In this study, *L. sativum* showed sensitivity to PAH as the level of germination decreased with an increase in the concentration of the PAH. The level of germination in soil with no surfactant (Triton X-100) was significantly lower compared to the level of germination in soil with Triton X-100. As light dramatically enhances the toxicity of PAH (Ren et al., 1994), the presence of the surfactant in the soil might have interfered with the absorption of radiation by the PAH and thereby rendering the PAH less toxic. This was contrary to the findings that bioavailability/desorption of PAH in treatments with surfactant tends to be higher at certain surfactant concentration compared to treatments with no surfactant (Aronstein et al., 1991). According to Guha et al. (1998), for a given surfactant concentration, the bioavailability appears to be higher for the lower molecular PAH and there is very little difference in the bioavailability of the same compound as single solute or in different binary or ternary mixtures. The findings may be explained thus, if the PAH were of low molecular weight.

The fresh weight of *L. sativum* seedlings also decreased with an increase in the concentration of PAH in the soil. At a concentration of 300 ppm, the weight of the seedlings was lower compared to the control (0 ppm). There were no noticeable differences in the seedling weight of *L. sativum* in both the absence and the presence of Triton X-100. PAH have been suggested to cause germinated seeds to produce fewer roots (Ren et al, 1996). At the early stages of plant development, root growth is due primarily to cell expansion and not cell division (Taiz and Zeiger, 1991). Cell expansion is probably being impeded, which could for example, be by inhibition of hormone action (auxin) or interference with cellular metabolism (e.g. mitochondrial function).

The data on the germination of *L. sativum* in different soils shows that where PAHs are absent, the level of germination exceeds 95% while in the case of soil B, no germination of *L. sativum* occurs. The composition of soil C did not appear to have any inhibition on the germination of *L. sativum* seeds as germination levels were comparable to the levels in soils with no PAH (soils A and D). The data suggest that *L. sativum* germination is a potential PAH bioindicator.

The data of phytoremediation showed that plants may be playing a significant role in the removal and or detoxification of PAHs in the soil. Germination level of *L. sativum* in soils of vegetated treatment (T_4) was significantly higher compared to the germination level in treatment T_5 while there was no significant difference between the vegetated treatment (T1) and the non-vegetated treatments containing artificially contaminated soil A. The data suggest that *P. maximum* enhanced the detoxification and or dissipation of the PAH in the soil. Vegetated treatments enhance pollutant removal in soil compared to non-vegetated treatments possibly due to the increased microbial activity as well as the deposition of root exudates in the rhizosphere (Siciliano and Germida, 1999).

The germination level of *L. sativum* seeds, increased with the reduction of the PAHs in the soil artificially contaminated with the PAHs. The level of germination in the artificially contaminated soil was nearly the same as the germination level in the control treatment after 3 weeks of the experiment, possibly due to the dissipation of PAH in the soil.

The methodology based on the sensitivity of *L. sativum* (that has a short germination period) to PAH can be used as a monitoring tool in remediation treatments of soil contaminated with PAH. The methodology should be further developed to gain more knowledge on aspects of bioavailability of PAH in both the aged as well as the freshly spiked soil. Also critical is the sensitivity of the seeds to other pollutants (e.g. heavy metals), which are most likely to occur in the presence of the PAHs.

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