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Enhancement of phenanthrene and pyrene degradation in rhizosphere of tall fescue (*Festuca arundinacea*)

Sardar Alam Cheema[a], Muhammad Imran Khan[a], Xianjin Tang[a], Congkai Zhang[a], Chaofeng Shen[a,*], Zaffar Malik[b], Shafaqat Ali[c], Jianjun Yang[a], Kaili Shen[a], Xincai Chen[a], Yingxu Chen[a]

[a] *Institute of Environmental Science and Technology, Zhejiang University, Hangzhou 310029, PR China*
[b] *MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Environmental and Resources Science, Zhejiang University, Hangzhou 310029, PR China*
[c] *Department of Agronomy, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, PR China*

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ABSTRACT

A greenhouse experiment was conducted with varying concentrations of phenanthrene (11–344 mg kg^{-1}) and pyrene (15–335 mg kg^{-1}) spiked in the soil to evaluate the phytoremediation of PAHs contaminated soil using tall fescue (*Festuca arundinacea*). After 65-day of tall fescue growth, plant biomass, microbial viable counts, dehydrogenase activity, water-soluble phenolic compounds, phenanthrene and pyrene residual concentrations and removal percentages were determined. The results showed that target PAHs (phenanthrene and pyrene) did not affect plant biomass at lower concentrations but a reduced biomass (only 53.5% of shoot and 29.7% of root compared to control) was observed at higher concentrations. Higher biological activities (microbial viable counts, water-soluble phenolic compounds, dehydrogenase activity) and PAHs degradation rates were detected in planted soils than unplanted controls. After harvest, 91.7–97.8% of phenanthrene and 70.8–90.0% of pyrene had been degraded in the planted soils, which were 1.88–3.19% and 8.85–20.69% larger than those in corresponding unplanted soils. This enhanced dissipation of target PAHs in planted soils might be derived from increased biological activity in the rhizosphere. The results of the present study suggest that the presence of tall fescue roots were effective in promoting the phytoremediation of PAHs contaminated soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants [1]. A wide variety of PAHs are found in the environment as a result of the incomplete combustion of organic matter, emission sources, automobile exhausts, stationary matter (e.g. coal-fired, electricity generating power plants), domestic matter (e.g. tobacco smoke and residential wood or coal combustion), area source matter (e.g. forest fires and agricultural burning) and also in food [2]. Many PAHs have toxic, mutagenic and/or carcinogenic properties [3]. Since, PAHs can persist in the environment, various remediation strategies are being developed to restore contaminated sites. Physico-chemical methods can be used for remediation of PAHs contaminated soils and although effective, usually they are expensive and sometimes damaging to the structure and texture of the soil [4].

Phytoremediation has gained acceptance in the last decade as a cost effective, non-invasive technology complementary to engineering-based traditional approaches [5]. Several studies have showed the suitability of phytoremediation for soils polluted by PAHs [4,6–10]. Lee et al. [9] compared PAHs degradation ability of four plant species and reported enhanced phenanthrene and pyrene degradation in the planted soils (i.e. >99 and 77–94% of phenanthrene and pyrene, respectively) compared to unplanted soils (i.e. 99% and 69% phenanthrene and pyrene, respectively). Chen et al. [11] noted 30.4% and 37.7% of ^{14}C-pyrene mineralization in soil planted with switch grass and tall fescue, respectively, while only 4.3% mineralization was observed for the unplanted control. The presence of plants enhanced the dissipation of PAHs as compared to bulk soil. This enhanced degradation might be attributed to the higher densities and greater activities of microorganisms in rhizosphere than bulk soil [12].

It has long been known that plants release a vast range of organic materials through roots into the rhizosphere [13]. These exudates include water-soluble, insoluble, and volatile compounds such as sugars, amino acids, organic acids, nucleotides, flavonones, phenolic compounds and certain enzymes [14]. With the release of these materials, a plant may support growth and metabolic activities of diverse microbial communities in the rhizosphere [15–18]. Some organic compounds (i.e. phenolics, organic acids, alcohols and pro-

* Corresponding author. Tel.: +86 571 8603 6775; fax: +86 571 8697 1898.
E-mail address: ysxzt@zju.edu.cn (C. Shen).

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teins) in root exudates may serve as carbon and nitrogen sources for the growth and long-term survival of microorganisms, which are capable of degrading organic pollutants [19]. Bacterial populations can be as much as 2–4 times higher in the rhizosphere than the populations in the surrounding bulk soils; which may promote the degradation of PAHs in rhizosphere soils [8,19]. This indicates that plants have the potential to increase the degradation of organic pollutants by promoting the growth of soil microorganisms.

This study was conducted to investigate soil PAHs remediation by plants in order to contribute to the technology for field phytoremediation in practice. The experiment was designed to evaluate the ability of tall fescue contribution to PAH removal. Furthermore, the phenanthrene and pyrene removal mechanism was investigated to determine if phytodegradation, especially rhizodegradation, plays a role in PAH removal. In addition, toxicity of target PAHs to tall fescue was assessed to determine the appropriate concentrations for phytoremediation by tall fescue. Tall fescue was selected because of its extensive, fibrous root system providing a large root surface for the growth of microbial populations. Additionally, this species has been previously used for phytoremediation and shown to be more tolerant of PAHs than other tested species [4].

2. Materials and methods

2.1. Chemicals

Phenanthrene and pyrene with a purity of 99.9% were obtained from Sigma–Aldrich Co. Ltd., UK. All the other chemicals used in the study were of analytical purity.

2.2. Soil

An uncontaminated soil with undetectable phenanthrene and pyrene was collected from the upper 15 cm layer of a rice experimental field of Hua Jia Chi campus of Zhejiang University, Hangzhou, China. The soil was air-dried and passed through 2 mm sieve to remove stones and roots. The particle size distribution (50.5% sand, 37% silt, and 12.5% clay) identified the soil as a sandy loam soil. The organic matter content was 2.1% and the pH was 5.95. The cation exchange capacity (CEC) was 7.76 cmol kg^{-1} and electrical conductivity (EC) was 254.5 $\mu s\,cm^{-1}$. The nutrient levels were 1.78% of total N, 9.39 mg kg^{-1} of total P and 0.981% of total K.

2.3. Experimental design

Soils were spiked with different amounts of a mixture of high purity phenanthrene and pyrene dissolved in acetone (10% of the total quantity of soil to be used was spiked for each treatment). When acetone was evaporated off, the spiked soils were mixed with un-polluted soils and sieved through a 2 mm mesh to achieve homogeneity [20]. The soils were then put in plastic bins and placed at room temperature for 6 weeks for aging. After aging, the soils were fertilized with 1.64 g of KH_2PO_2 and 2.28 g of NH_4NO_3 kg^{-1} dry wt of soil and again sieved to obtain homogeneity. Control soils were treated in the same way but without phenanthrene and pyrene. The concentrations of phenanthrene and pyrene in treated soils were measured before transferring to experimental pots. Measured concentrations (mg kg^{-1}) of PAHs (phenanthrene + pyrene) in different treatments were T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5) T4 (200.0 + 199.3), and T5 (344.2 + 335.8). The treated soils were packed into greenhouse pots (500 g DW soil pot^{-1}) lined with gravel, sand and 0.1-mm mesh at the bottom to aid drainage and avoid soil loss [20]. These pots were then transferred to the greenhouse and maintained for 7 days at field moisture before transplanting seedlings.

Tall fescue seeds were germinated on moist perlite and 15 seedlings were transplanted to the greenhouse pots 10–15 days after germination. One week after transplantation, the plants were thinned to 10 per pot. Three replicates of each treatment were prepared in a completely randomized manner. Seedling transplanting date was considered the starting time of experiment. The pots were watered as needed and fertilized every 2 weeks with inorganic salt solution (Hoagland's solution). The position of pots was changed randomly every week. After 65 days of plant growth, the soils and plants were sampled. The planted and unplanted soils were carefully collected, homogenized and divided into two sets, one for chemical analysis and other for biological analysis. Soil samples were stored at 4 °C before analysis.

2.4. Analytical methods

2.4.1. Plant biomass

After 65 days of growth, the plants were harvested and separated into tall fescue shoots and roots. These were washed separately in tap water followed by distilled water, freeze-dried and weighed.

2.4.2. Microbial numbers

To enumerate the viable microbial population, aqueous extracts of 3 g soil samples were serially diluted and spread on nutrient agar for bacteria and streptomycin-rose bengal agar for fungi. Plates were incubated for 3–5 days at 28 °C prior to counting the numbers of colony forming units (CFU).

2.4.3. Dehydrogenase activity

Soil dehydrogenase activity was measured by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF). Briefly, 5 g soil sample was incubated for 24 h at 37 °C in 5 ml of TTC solution (5 g L^{-1} in 0.2 mol L^{-1} Tris–HCl buffer, pH 7.4). Two drops of concentrated H_2SO_4 were immediately added after incubation to stop the reaction. The sample was then blended with 5 ml of toluene to extract TPF and shaken for 30 min at 250 r/min (25 °C), followed by centrifugation at 5000 × *g* for 5 min, and absorbance of color in the extract was taken at 492 nm. Soil dehydrogenase activity was measured as μg TPF g^{-1} dry soil 24 h^{-1} [21,22].

2.4.4. Water-soluble phenols

Water-soluble phenols were quantified colorimetrically following Carter [23]. Soils were extracted with 25 ml distilled water for 4 h with shaking, followed by centrifugation at 3000 × *g* for 15 min. A 20 ml aliquot of extract or standard was placed in a 200 mm × 25-mm test tube, and then 3 ml of Na_2CO_3 solution was added followed by 1 ml of Folin–Ciocalteau reagent. The solution was mixed well and allowed to stand for 1 h at room temperature. Light absorbance was read at 750 nm. Vanillic acid was used as the standard, and the amount of phenolic compounds is expressed as vanillic acid equivalents (μg vanillic acid g^{-1} soil).

2.4.5. PAH analysis

Two grams of freeze-dried soil sample was mixed with 15 ml of a mixture of dichloromethane:acetone (1:1) in a glass centrifuge tube and extracted three times by ultrasonic treatment for 5 min with an Ultrasonic Disrupter followed by centrifugation at 3000 rpm for 5 min to separate the supernatant from the soil. The supernatant was collected in a 100 ml round bottom flask. The extracted solutions were concentrated to about 1–2 ml in a rotary evaporator, dissolved in 10 ml *n*-hexane and loaded on to a column packed with layers of silica gel (200–300 mesh), neutral aluminum oxide (100–200 mesh), and anhydrous sodium sulphate followed by elution with a 70 ml mixture of hexane and dichloromethane (7:3, v/v). The analyte fraction was reconcentrated in a rotary evaporator to 1 ml and further carefully evaporated to dryness under a

gentle stream of nitrogen. The residue was dissolved in 1.5 ml of a mixture of methanol:water (90:10) and carefully passed through a 0.45-μm Teflon filter to remove particulate matter prior to analysis. The HPLC analyses were performed with an Agilent 1100 Serials liquid chromatograph (equipped with a vacuum degasser, quaternary pump, autosampler, column compartment, diode array and multiple wavelength detectors, and a hypersil reversed-phase ODS-C-18 column made by the Agilent Company, USA) by using methanol–water (90:10) as the mobile phase at a flow rate of 1 ml min^{-1}. Phenanthrene and pyrene were detected by absorbance at 220 and 234 nm, respectively. The solvents used for chromatographic analyses were HPLC grade.

2.5. Statistical analysis

All values presented for the chemical and biological analyses of soil are the means of three replicates. Correlation coefficient (r) between PAHs (phenanthrene and pyrene) residual concentration and other variables were calculated using Pearson correlation analysis in Statistical Package for Social Science (SPSS) (version 16.0 for Windows) and means were compared using least significant differences calculated at a significance level of $P = 0.05$.

3. Results and discussion

3.1. The biomass of tall fescue

Tall fescue biomass was measured to study the toxic effects of PAHs on plants to determine the appropriate concentration of PAHs for phytoremediation. The shoot and root biomasses of tall fescue on a dry weight basis grown in the soil contaminated with different concentrations of phenanthrene and pyrene are shown in Fig. 1. At harvest, the shoot biomass in unspiked soil (T0) was 9.95 g pot^{-1}. No significant difference was shown among the shoot biomasses in pots with lightly spiked soil (T1–T3) and those with control (T0). When phenanthrene and pyrene concentrations exceeded 200 mg kg^{-1} soil, shoot biomass decreased significantly with the increase of phenanthrene and pyrene concentrations. When the phenanthrene and pyrene concentration was 344 and 336 mg kg^{-1} soil, respectively, shoot biomass was only 53.5% of the control.

According to Fig. 1, the inhibitory effect of PAHs on root dry biomass was more pronounced than that on shoot dry biomass. Even at low concentration of phenanthrene (70 mg kg^{-1} soil) and pyrene (72 mg kg^{-1} soil), the reduction in the root biomass was significant. The decreasing trend was consistently observed with the increase in initial PAHs concentration and root biomass was only 29.7% of the control when the phenanthrene and pyrene concentrations were 344 and 336 mg kg^{-1} in the soil, respectively.

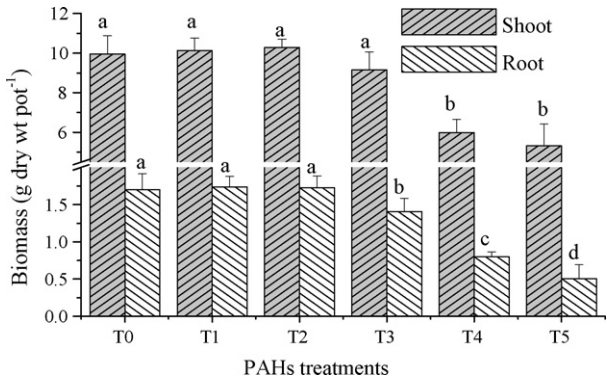

Fig. 1. Root and shoot biomass (g dry wt pot^{-1}) of the tall fescue growing in soil with different concentrations of phenanthrene and pyrene after 65-day of growth. Error bars represent the standard deviation of three sampled pots. Columns denoted by different letters indicated significant ($P \leq 0.05$) differences among different treatments. Where T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5), T4 (200.0 + 199.3) and T5 (344.2 + 335.8) are different concentration levels (mg kg^{-1}) of PAHs (phenanthrene + pyrene).

The present study clearly demonstrates that PAHs concentration and total biomass production have a direct relationship with each other. The lower PAHs concentration proved to be less toxic regarding biomass production. Similar to our present findings, Gao and Zhu [24] observed no significant effect of lower PAHs on plant biomass but growth inhibition was obvious at higher concentrations. The reduction in plant biomass that was observed on higher PAHs concentration might result from the inherent toxicity of PAHs. Plants, are sensitive to low-molecular-weight volatile hydrocarbons, which are soluble in hydrophobic plant materials and can penetrate cell membranes [25,26]. Reilley et al. [7] suggested indirect adverse effects of PAHs; PAHs might reduce the ability of contaminated soil to provide water and nutrients to plants, leading to a decline in biomass production. Although biomass was reduced on higher concentrations of PAHs, tall fescue could grow normally within certain concentration ranges of phenanthrene and pyrene. Additionally, it was noted that plants did not exhibit apparent signs of toxicity stress, suggesting that this species can be grown in PAHs contaminated soils and hence is a feasible choice for phytoremediation.

3.2. Microbial number

After 65-day growth of tall fescue in the soil polluted with phenanthrene and pyrene, the total number of bacteria and fungi were counted and results from microbial plate counts are given in Table 1. The tabulated data revealed that bacterial numbers of the soils planted with tall fescue were $6.33 \pm 1.20 \times 10^7$ to $11.85 \pm 1.79 \times 10^7$ CFU g^{-1} dry soil, which were significantly higher than in unplanted soil ($3.01 \pm 0.98 \times 10^7$ to $5.69 \pm 0.80 \times 10^7$ CFU g^{-1} dry soil). The numbers of fungi in planted soils were 1.5–2.0 times more than bulk soils. The results also indicated that the PAHs have a stimulatory effect on microbial count both in planted and unplanted soils.

It can be concluded from the results that the growth of microorganisms was stimulated by the presence of plant roots. The roots are known to release organic compounds, such as amino acids, organic acids, sugars, enzymes and complex carbohydrates, providing carbon source and energy for the growth of rhizosphere microorganisms [27–29]. Parrish et al. [30] reported that after 12 months of plant growth, the PAH degrading microbial populations in vegetated treatments were more than 100 times greater than those in unvegetated controls. Corgie et al. [31] found that number of PAHs degrading bacteria as well as phenanthrene degradation declined with distance from plant roots. Therefore, differences between rhizosphere soils and non-rhizosphere soils could be explained by the rhizosphere effect [32].

3.3. Water-soluble phenolic compounds (WSP compounds)

Water-soluble phenolic compounds in the soil were monitored to evaluate the influence of PAHs on the root exudation of tall fescue. Phenolic compounds are an important class of compounds exuded by plant roots and microorganisms capable of using phenolic compounds as a carbon source often have enzymes that can co-metabolise pollutants with similar structures [33]. After tall fescue growth for 65 days, WSP compounds of the planted soil were 3.80–5.08 μg vanillic acid g^{-1}, which were 1.08–1.24 times higher than in unplanted soil (i.e. 3.53–4.08 μg vanillic acid g^{-1} soil). A significant difference in WSP contents between planted and unplanted soil could be seen when phenanthrene and pyrene treatments exceeded 70 and 72 mg kg^{-1}, respectively (Table 2).

Table 1
Bacteria and fungus plate counts in the planted and unplanted soils after 65 days of tall fescue growth.

Treatment	Bacterial count CFU ($\times 10^7$ g^{-1} dry soil)		Fungal count CFU ($\times 10^4$ g^{-1} dry soil)	
	Unplanted	Planted	Unplanted	Planted
T0	3.64 ± 0.80Bb	6.82 ± 1.18Ca	5.74 ± 0.84Bb	8.91 ± 0.50Ca
T1	3.01 ± 0.98Bb	6.33 ± 1.20Ca	5.95 ± 1.13Bb	10.32 ± 1.69BCa
T2	3.47 ± 0.99Bb	7.74 ± 1.32BCa	5.42 ± 1.17Bb	10.86 ± 1.48BCa
T3	3.75 ± 0.59Bb	9.92 ± 0.92ABa	6.41 ± 1.39ABb	12.89 ± 1.38ABa
T4	5.29 ± 0.17Ab	9.89 ± 0.87ABa	7.51 ± 1.35ABb	12.78 ± 1.67ABa
T5	5.69 ± 0.80Ab	11.85 ± 1.79Aa	8.51 ± 1.42Ab	15.19 ± 1.61Aa

Values in each column followed with different capital letters (A–C) indicated significant ($P \leq 0.05$) differences among different treatments, and in each row followed with different lowercase letters (a and b) indicated significant difference between planted and unplanted soils. Values represent means ± standard deviation. Where T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5), T4 (200.0 + 199.3) and T5 (344.2 + 335.8) are different concentration levels (mg kg^{-1}) of PAHs (phenanthrene + pyrene).

The results also indicated that WSP compounds were enhanced with the increase of PAHs levels in planted and unplanted soils. WSP compounds were highest at the highest level of PAHs in soil.

Our present findings are in line of those Lee et al. [9], who found a positive correlation between WSP and content of phenanthrene and pyrene in planted soils. In the present study, the enhanced detection of WSP compounds at higher PAHs concentrations might be because of the increased root exudation and/or root death and decay as a result of PAHs toxicity and/or production of higher quantity of PAHs degradative intermediates as a result of PAHs degradation in planted soil. Siqueira et al. [34] concluded that the majority of phenolic compounds were products of shikimate and acetate pathways in plants and synthesis/accumulation tended to be enhanced by environmental stress. Cerniglia [35] and Kraus et al. [36] also reported that a large portion of phenolic compounds found in PAH-contaminated soils was the result of aromatic ring cleavage of PAHs. This was also confirmed by the detection of significantly higher levels of water-soluble phenols found in the unplanted soil with higher concentration of PAHs in the present study. Phenolic compounds released to soil might result in selective growth and long-term survival of certain soil microbes, a situation conducive to the enhancement of rhizosphere-facilitated degradation of recalcitrant pollutants such as PAHs.

3.4. The response of dehydrogenase activity

The dehydrogenase activities in planted and unplanted soils are shown in Table 3. Since dehydrogenase exists in all microorganisms, it may give a measure of total viable microbial cells [37]. The activities of dehydrogenase were measured by the reduction of TTC. After 65 days of tall fescue growth, the dehydrogenase activity was 2.2–4.2 and 77.0–174.2 μg TPF g^{-1} dry soil in unplanted and planted soils, respectively. The dehydrogenase activities of the planted soils were 33–42 times higher than those of the bulk soils, indicating that the abundance and activities of the microbial communities in the planted soils were enhanced by the presence of the tall fescue roots. Lower concentrations of PAHs did not affect the dehydrogenase activity in planted and unplanted controls but it increased significantly, when phenanthrene and pyrene concentrations were higher than 70 and 72 mg kg^{-1}, respectively. The dehydrogenase activity was the highest at the highest level of PAHs. Lee et al. [9] found that there was a negative correlation between dehydrogenase activity and content of phenanthrene and pyrene in soil. However, a positive correlation between dehydrogenase activity and the content of PAHs has also been observed [38]. In the present study, the stimulation of dehydrogenase activity matches well with the enhanced microbial and fungal counts and higher WSP in soil at higher PAHs levels in planted soil. In unplanted soils at higher PAHs levels, the PAHs and their intermediates might serve as a carbon source for microbial growth and result in stimulation of microbial growth that leads to enhanced dehydrogenase activity.

3.5. The residual concentrations and the degradation rate of PAHs

Concentrations of phenanthrene and pyrene remaining in variously treated soils after 65 days are shown in Fig. 2. The residual concentrations and residual percentage of PAHs in the planted soils were lower than those in the corresponding unplanted soils for all treatments. For example, the residual percentages for phenanthrene and pyrene were 2.2–8.3% and 13.1–29.2% in planted soil whereas in unplanted control soils it was 4.1–10.1%, and 25.4–49.1%, respectively. The dissipation ratios of phenanthrene in planted soils with initial phenanthrene concentrations of 11.5–344.2 mg kg^{-1} dw were 91.7–97.8%, which was 1.88–3.19% larger than those in corresponding unplanted soils. Dissipation ratios of pyrene in variously spiked soils (T1–T5) with tall fescue were 70.8–90.0%, which was 8.9–20.7% higher than those with no tall fescue (Table 4).

Although the soil used in this experiment was paddy soil without any known contamination history, the indigenous microorganisms

Table 2
Water-soluble concentrations of phenolic compounds in the planted and unplanted soils after 65 days of tall fescue growth (μg vanillic acid g^{-1} soil).

Treatment	Unplanted	Planted
T0	3.53 ± 0.19Ba	3.80 ± 0.07Ca
T1	3.70 ± 0.17Ba	3.96 ± 0.32Ca
T2	3.60 ± 0.20Ba	3.87 ± 0.40Ca
T3	3.66 ± 0.21Bb	4.28 ± 0.04BCa
T4	3.78 ± 0.10ABb	4.82 ± 0.35Ba
T5	4.08 ± 0.31Ab	5.08 ± 0.39Aa

Values in each column followed with different capital letters (A–C) indicated significant ($P \leq 0.05$) differences among different treatments, and in each row followed with different lowercase letters (a and b) indicated significant difference between planted and unplanted soils. Values represent means ± standard deviation. Where T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5), T4 (200.0 + 199.3) and T5 (344.2 + 335.8) are different concentration levels (mg kg^{-1}) of PAHs (phenanthrene + pyrene).

Table 3
Dehydrogenase activities in the planted and unplanted soils after 65 days of tall fescue growth (TPF μg g^{-1} dry soil).

Treatment	Unplanted	Planted
T0	2.32 ± 0.41Db	77.6 ± 16.4Da
T1	2.22 ± 0.33Db	77.0 ± 17.9Da
T2	2.51 ± 0.45CDb	85.5 ± 14.0CDa
T3	3.18 ± 0.38BCb	110.0 ± 13.0Ca
T4	3.40 ± 0.49Bb	142.9 ± 18.9Ba
T5	4.27 ± 0.36Ab	174.2 ± 22.8Aa

Values in each column followed with different capital letters (A–D) indicated significant ($P \leq 0.05$) differences among different treatments, and in each row followed with different lowercase letters (a and b) indicated significant difference between planted and unplanted soils. Values represent means ± standard deviation. Where T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5), T4 (200.0 + 199.3) and T5 (344.2 + 335.8) are different concentration levels (mg kg^{-1}) of PAHs (phenanthrene + pyrene).

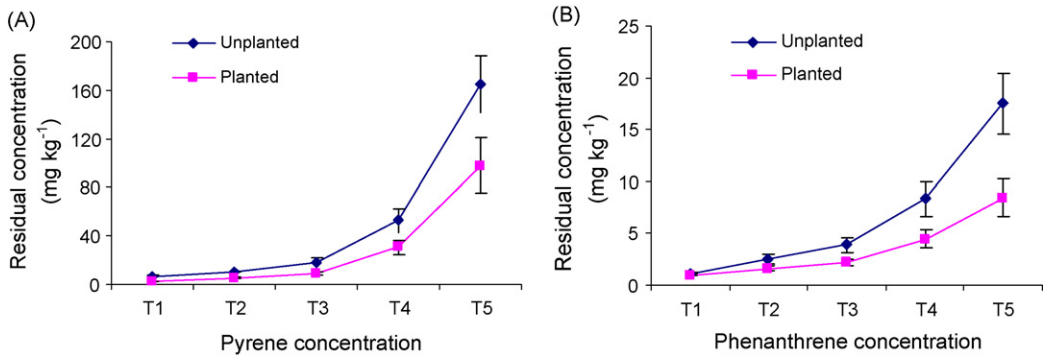

Fig. 2. Residual concentrations of phenanthrene and pyrene in planted and unplanted soils after 65-day of tall fescue growth. Error bars represent the standard deviation of three sampled pots. Where T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5), T4 (200.0 + 199.3) and T5 (344.2 + 335.8) are different concentration levels (mg kg^{-1}) of PAHs (phenanthrene + pyrene).

exhibited a remarkable capacity to degrade 3–4-ring PAHs. The observed results indicated that the dissipation of phenanthrene and pyrene in all treatments was promoted by tall fescue after 65-day of growth based on the residual concentrations and dissipation ratios of tested PAHs in planted and unplanted soils. It was also noted that the overall extent of PAHs loss from all treated soils was clearly compound-dependent; pyrene degradation ratio was much lower than phenanthrene in all treatments (Table 4), which is in agreement with many other studies, suggesting that high-molecular-weight PAHs are more resistant to microbial attack than low-molecular-weight PAHs [9,35,39]. It is also noteworthy that the effects of tall fescue on pyrene degradation were more obvious than on phenanthrene degradation.

The loss of PAHs from soil could be because of biotransformation, biodegradation, plant uptake, or abiotic dissipation, including leaching and volatilization [6,7,40]. Many studies reported that the loss of PAHs from soil by plant uptake/accumulation can be assumed to be negligible [7,26,41]. Dietz and Schnoor [42] demonstrated that hydrophobic compounds with $\log K_{ow} > 4$ are not readily taken up by plants through transpiration due to their hydrophobicity; $\log K_{ow}$ for phenanthrene and pyrene is 4.17 and 5.13, respectively [43]. Phenanthrene and pyrene have low vapor pressures of $10^{-1.00}$ and $10^{-2.05}$ L at mmol^{-1}, respectively [43]. Therefore, the loss of these compounds via volatilization from soil is also unlikely to occur [7]. Abiotic losses by leaching were not measured in the present experiment but are assumed to be insignificant because the water content of the soil was maintained at about 70% of water holding capacity; thus, minimizing leaching, if any leachate was produced, it was re-applied to same pot. The non-ionic, non-polar structure of PAH compounds leads to their partitioning out of the polar water phase and onto hydrophobic surfaces in the soil matrix. Lipophilic soil organic matter acts as an adsorbent and immobilizes hydrophobic PAHs [44]. Our results suggest that the enhanced dissipation of PAHs might be caused by increased rhizosphere microbial density and activity compared to unplanted soil, since the root exudates and plant litter could enhance the bioavailability of the contaminant, provide more substrate for co-metabolic degradation, and modify the soil environment to be more suitable for microbial transformation [45].

Table 4
Removal percentages of PAHs in tall fescue planted and unplanted soils after 65 days of tall fescue growth (%).

Treatment	Pyrene		Phenanthrene	
	Unplanted	Planted	Unplanted	Planted
T1	59.7 ± 6.5Bb	80.4 ± 1.2Aa	89.8 ± 1.2Ba	91.7 ± 0.2Ca
T2	71.2 ± 2.4Ab	83.6 ± 2.6Aa	91.6 ± 1.6Bb	94.8 ± 0.8Ba
T3	74.6 ± 4.8Ab	86.9 ± 1.8Aa	94.4 ± 1.0Ab	96.8 ± 0.4Aa
T4	73.3 ± 4.8Ab	84.4 ± 2.9Aa	95.8 ± 0.8Ab	97.8 ± 0.4Aa
T5	50.8 ± 7.0Bb	70.8 ± 6.6Ba	94.9 ± 0.8Ab	97.5 ± 0.5Aa

Values in each column followed with different capital letters (A–C) indicated significant ($P \leq 0.05$) differences among different treatments, and in each row followed with different lowercase letters (a and b) indicated significant difference between planted and unplanted soils. Values represent means ± standard deviation. Where T0 (0 + 0), T1 (11.5 + 15.6), T2 (30.7 + 35.0), T3 (70.2 + 72.5), T4 (200.0 + 199.3) and T5 (344.2 + 335.8) are different concentration levels (mg kg^{-1}) of PAHs (phenanthrene + pyrene).

Table 5
Correlation between PAH residual concentrations and different biological parameters in planted and unplanted soils after 65 days of tall fescue growth.

Parameters	Phenanthrene	Pyrene
Unplanted soil		
Bacteria	0.911*	0.866*
Fungi	0.951**	0.925**
Dehydrogenase	0.959**	0.919**
WSP compounds	0.962**	0.965**
Planted soil		
Bacteria	0.904*	0.834*
Fungi	0.930**	0.853*
Dehydrogenase	0.972**	0.919**
WSP compounds	0.948**	0.881*
Root	−0.951**	−0.900*
Shoot	−0.923**	−0.869*

* Significance at $P < 0.05$.
** Significance at $P < 0.01$.

The biological parameters measured in unplanted and planted soil at the end of this experiment were significantly correlated with PAH concentration (Table 5). The number of bacteria and fungi, dehydrogenase activity and water-soluble phenol content were positively correlated with PAH concentration both in unplanted and planted soils ($P < 0.05$), whereas PAHs concentrations were negatively correlated to the root and shoot biomass ($P < 0.05$).

4. Conclusions

In conclusion, tall fescue was more or less tolerant to all concentrations of phenanthrene and pyrene in this experiment. The biological activities were enhanced in soils planted with tall fescue relative to unplanted control soils at all PAH levels. In addition, PAHs had stimulatory effect on the soil microbial population, WSP compounds and dehydrogenase activity in both planted and unplanted soils. The presence of tall fescue promoted the dissipation of phenanthrene and pyrene in soils irrespective of the variation of initial concentrations of these chemicals in the experimental soil. Our results suggest that the enhancement of PAH disappearance is caused by an increase in the rhizosphere biologi-

cal activity compared with that of unplanted soil. Results from this greenhouse experiment support the notion that phytoremediation is an effective and environment friendly approach for remediation of PAH-contaminated soils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2008.12.027.

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