Journal of Hazardous Materials 177 (2010) 384–389

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation

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ARTICLE INFO

Article history:
Received 3 June 2009
Received in revised form 4 December 2009
Accepted 7 December 2009
Available online 16 December 2009

Keywords:
PAHs
Phenanthrene
Pyrene
Phytoremediation
Combined plant cultivation

ABSTRACT

The present study was conducted to investigate the capability of four plant species (tall fescue, ryegrass, alfalfa, and rape seed) grown alone and in combination to the degradation of phenanthrene and pyrene (polycyclic aromatic hydrocarbons, PAHs) in spiked soil. After 65 days of plant growth, plant biomass, dehydrogenase activity, water-soluble phenolic (WSP) compounds, plant uptake and accumulation and residual concentrations of phenanthrene and pyrene were determined. Our results showed that presence of vegetation significantly enhanced the dissipation of phenanthrene and pyrene from contaminated soils. Higher degradation rates of PAHs were observed in the combined plant cultivation (98.3–99.2% phenanthrene and 88.1–95.7% pyrene) compared to the single plant cultivation (97.0–98.0% phenanthrene and 79.8–86.0% pyrene). Contribution of direct plant uptake and accumulation of phenanthrene and pyrene was very low compared to the plant enhanced dissipation. By contrast, plant-promoted biodegradation was the predominant contribution to the remediation enhancement. The correlation analysis indicates a negative relation between biological activities (dehydrogenase activity and WSP compounds) and residual concentrations of phenanthrene and pyrene in planted soils. Our results suggest that phytoremediation could be a feasible choice for PAHs contaminated soil. Moreover, the combined plant cultivation has potential to enhance the process.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) in soil are pollutants of major concern due to their recalcitrance and mutagenic/carcinogenic properties, and there is serious concern about their environmental presence, especially their potential for bioaccumulation in food chains [1]. They are produced during fossil fuel combustion, waste incineration, or as by-products of industrial processes, such as coal gasification and petroleum refining, and often released in large quantities into the environment [2]. Because of their environmental importance, PAHs are listed as priority pollutants, and remediation of soil contaminated with PAHs is of great importance. Due to the fact that engineering-based remedial technologies are expensive and disruptive, there is a growing interest in developing new remediation technologies that are environment friendly and less expensive [3].

Phytoremediation is a promising alternative approach to soil remediation due to its cost effectiveness, convenience and environmental acceptability. Plants may contribute to the dissipation of PAHs through various mechanisms, such as plant uptake and accumulation, increase of microbial activities, improvement of physical and chemical conditions of soils, and adsorption of pollutants in the rhizosphere [4]. However, the impacts of each process have not been clearly elucidated. Laboratory and pot experiments had demonstrated that plants have enhanced dissipation of PAHs when compared to unplanted controls [5–7]. Recently, Fan et al. [8] reported that the degradation of phenanthrene and pyrene can be promoted by four different plant species, including two grasses and two legumes. Liste and Alexander [9] studied the capability of nine plant species to promote the degradation of pyrene in soil and reported higher degradation rates in vegetated soil.

These results were based on single plant species; however, terrestrial ecosystem is a complex ecosystem. In the rhizosphere, processes determining transport and bioavailability of PAHs are more complex than in unvegetated soils. In addition, when plants grown as a multi-species mixture, the interaction of roots modifies the root physiology, root colonization, root surface properties and properties of the rhizosphere [10,11]. Combinations of root types and exudate patterns are assumed to allow greater infiltration of and stimulation of microbial communities, with a net positive

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doi:10.1016/j.jhazmat.2009.12.044

stimulation of microbial catabolic potential [12]. The underlying assumption is that the effects of mixed plant populations will be proportionally cumulative, with the positive benefits of each individual plant species summing to a greater whole. Little information is available on phytoremediation by combined plants cultivation in soils contaminated with PAHs.

The present study was conducted to investigate the effect of single and mix plant cultivation on the PAHs removal from soil. In addition, some mechanisms were investigated to determine the role of plants in enhancing PAHs dissipation. Phenanthrene and pyrene were used as target PAHs. Tall fescue (*Festuca arundinacea*), ryegrass (*Lolium perenne*), alfalfa (*Medicago sativa*), and rape seed (*Brassica napus*) were selected to reflect the typical species found in the region and to cover the range of root physiology and morphology.

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

Soil was spiked with a mixture of high purity phenanthrene and pyrene in acetone (10% of the total quantity of soil to be used was spiked for each treatment). When acetone was evaporated off, the spiked soil was mixed with un-polluted soil and sieved through a 2 mm mesh to achieve homogeneity [13]. The soil was then put in plastic bin and placed at room temperature for 6 weeks for aging. After aging, the soil was fertilized with 1.64 g of KH_2PO_2 and 2.28 g of NH_4NO_3 kg^{-1} dry weight of soil and again sieved to obtain homogeneity. Control soil was treated in the same way but without phenanthrene and pyrene. The concentrations of phenanthrene and pyrene in treated soil were measured before transferring to experimental pots. Measured concentrations of phenanthrene and pyrene in soil were 200.0 and 199.3 mg kg^{-1}, respectively. The treated and untreated soils were packed into greenhouse pots (500 g dry weight soil pot^{-1}) lined with gravel, sand and 0.1-mm mesh at the bottom to aid drainage and avoid soil loss [13]. These pots were then shifted to the greenhouse and maintained for 7 days at field moisture before transplanting seedlings.

Plant species were chosen to reflect the typical species found in the region and to cover the range of physiology and root morphology. Tall fescue (*F. arundinacea*), ryegrass (*L. perenne*), alfalfa (*M. sativa*), and rapeseed (*B. napus*) were chosen as the plant species (as: P0 = no plant, P1 = tall fescue, P2 = ryegrass, P3 = alfalfa, P4 = rapeseed, P5 = P1 + P3, P6 = P1 + P4, P7 = P3 + P4). Seeds of each plant were germinated and grown on moist perlite in growth chamber and seedlings were transplanted to the greenhouse pots 10–15 days after germination. Ten seedlings of tall fescue or ryegrass or alfalfa and six seedlings of rapeseed in single plant cultivation pots were used. Five seedlings of tall fescue or alfalfa and three seedlings of rapeseed were grown in combined cultivation pots. Three replicates of each treatment were prepared in a completely randomized manner. Variable seedling number for the experiment was grown in each pot to produce the equal biomass per pot. Seedling transplanting date was considered the starting time of experiment. The pots were humidified 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 shoots and roots. These were washed separately in tap water followed by distilled water, freeze-dried and weighed.

2.4.2. Water-soluble phenols

Water-soluble phenols were quantified colorimetrically according to Carter [14]. 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).

2.4.3. 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 rpm (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} [13].

2.4.4. PAH analysis

Two grams of freeze-dried soil sample was mixed with 15 ml mixture of dichloromethane and 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 1–2 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.

Plant samples were ground and homogenized, and certain amounts were extracted using the same method as to soils. 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 6 cm 10% $AgNO_3$ silica gel, 10 cm deactivated silica gel, 12 cm 50% H_2SO_4 silica gel and 1 cm of Na_2SO_4 followed by elution with a 70 ml mixture of hexane and dichloromethane (7:3, v/v). The remained part of the procedure was also the same as for soils. 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.

2.5. Statistical analysis

All values presented for the chemical and biological analyses of soil are the means of three replicates. Correlation coefficients (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. Plant biomass

Plant biomass was measured at the end of experiment to explore the ability of plant species to grow in PAHs contaminated soil. The shoot and root biomasses of plants on a dry weight basis grown in the soil contaminated with phenanthrene and pyrene are shown in Table 1. Results show that different plant species displayed different responses to the presence of PAHs in the soil. Root and shoot yields of all plants were significantly lower in PAHs-treated soils than in control soils at the end of experiment. The greatest reduction in biomass was observed in alfalfa, which produced approximately 35% of the biomass of control. Rape seed was the most resistant to the presence of PAHs. Results also reveal that root/shoot ratios of the plant species under investigation decreased as a result of PAHs amendment of the soil. The decrease in the root/shoot ratios of plants can be attributed to the more negative effect of PAHs on the plants root than shoot.

The reduction in plant biomass grown in PAHs polluted soil 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 [15]. Reilley et al. [16] 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 under PAHs contamination, the plant species tested did not exhibit apparent signs of stress or toxicity, and it appears that vegetation establishment with these plants in PAH-contaminated soil is feasible.

Table 2
Water-soluble concentrations (μg vanillic acid g^{-1} soil) of phenolic compounds in control (PAHs−) and PAHs-treated (PAHs+) soils planted with different plant treatments after 65 days of plant growth.

Treatment	PAHs−	PAHs+
P0	3.53 ± 0.19BCa	3.71 ± 0.17Da
P1	3.80 ± 0.07ABb	4.82 ± 0.35BCa
P2	3.43 ± 0.54BCDb	4.51 ± 0.23Ca
P3	4.03 ± 0.25Aa	4.60 ± 0.39Ca
P4	3.83 ± 0.13ABb	5.31 ± 0.68ABa
P5	3.06 ± 0.22Db	5.04 ± 0.49ABCa
P6	3.34 ± 0.07CDb	5.63 ± 0.19Aa
P7	3.60 ± 0.30ABCb	5.07 ± 0.27ABCa

Values in each column followed with different capital letters (A–D) indicated significant ($p \leq 0.05$) differences among different plant treatments, and in each row followed with different lowercase letters (a and b) indicated significant difference between control (PAHs−) and PAHs-treated (PAHs+) soils. Values represent means ± standard deviation. Where P0 = no plant; P1 = tall fescue (*F. arundinacea*); P2 = ryegrass (*L. perenne*); P3 = alfalfa (*M. sativa*); P4 = rape seed (*B. napus*); P5 = P1 + P3; P6 = P1 + P4 and P7 = P3 + P4.

3.2. Water-soluble phenolic compounds (WSP compounds)

Water-soluble phenolic (WSP) compounds were monitored to evaluate the influence of plant species alone and in combination on the amount of phenolic compounds to understand the significance of these factors in the bioremediation of PAHs. Plants that release high concentrations of phenol into the rhizosphere may selectively foster the growth of PCB-degrading bacteria [17]. Liste and Alexander [18] suggested that the exudation capacity of phenolic compounds can be used as a screening method for the use of plant species in phytoremediation. At the end of 65 days of experimental period, higher water-soluble phenolic compounds were detected in PAH-treated soils than the uncontaminated soils (Table 2). After 65 days of plants growth, WSP compounds of the PAHs polluted soil were 3.71–5.63 μg vanillic acid g^{-1} of soil against 3.06–4.03 μg vanillic acid g^{-1} soil from uncontaminated soil.

The present results concur with the findings from Lee et al. [19], showing a positive correlation between WSP and content of phenanthrene and pyrene in planted soils. In the present study, the enhanced detection of WSP compounds in the rhizosphere of plants grown in PAHs contaminated soils 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. Among all plant treatments grown in PAHs contaminated soils, more phenolic compounds were observed in the rhizosphere of P6 (5.63) and P4 (5.31) than of the other plant treatments. According to Liste and Alexander [18], these two plant treatments might be useful for the phytoremediation of soils polluted with aromatic compounds. And this has confirmed by our

Table 1
Biomass (g dry weight pot^{-1}) of shoots and roots and root shoot ratio (W_{root}/W_{shoot}) of different plant species grown in control (PAHs−) and PAHs-treated (PAHs+) soils after 65 days of plant growth.

Treatment	Root		Shoot		W_{root}/W_{shoot}	
	PAHs−	PAHs+	PAHs−	PAHs+	PAHs−	PAHs+
P1	1.70 ± 0.22a	0.80 ± 0.07b	9.36 ± 0.34a	6.00 ± 0.65b	0.171	0.133
P2	1.98 ± 0.29a	1.00 ± 0.09b	9.44 ± 0.38a	7.28 ± 1.08b	0.210	0.137
P3	1.56 ± 0.27a	0.48 ± 0.09b	6.39 ± 1.11a	2.60 ± 0.75b	0.243	0.183
P4	1.37 ± 0.26a	0.82 ± 0.19b	7.91 ± 0.90a	5.70 ± 1.28b	0.171	0.140

Values in each column followed with different lowercase letters (a and b) indicated significant ($p \leq 0.05$) difference between plant biomass grown in control (PAHs−) and PAHs-treated (PAHs+) soils. Values represent means ± standard deviation. Where P1 = tall fescue (*F. arundinacea*); P2 = ryegrass (*L. perenne*); P3 = alfalfa (*M. sativa*) and P4 = rape seed (*B. napus*).

Table 3
Dehydrogenase activities (μg TPF g^{-1} soil) in control (PAHs−) and PAHs-treated (PAHs+) soils planted with different plant treatments after 65 days of plant growth.

Treatment	PAHs−	PAHs+
P0	1.8 ± 0.2Eb	2.5 ± 0.2Ea
P1	47.4 ± 5.7BCb	72.5 ± 14.3BCa
P2	42.2 ± 8.9Ca	57.3 ± 11.2Ca
P3	68.7 ± 16.3Aa	36.6 ± 10.0Db
P4	48.9 ± 4.8BCb	70.3 ± 7.1BCa
P5	24.8 ± 12.5Db	57.6 ± 10.3Ca
P6	43.5 ± 11.8Cb	107.1 ± 10.4Aa
P7	60.9 ± 8.4ABb	85.9 ± 5.7Ba

Values in each column followed with different capital letters (A–E) indicated significant ($p \leq 0.05$) differences among different plant treatments, and in each row followed with different lowercase letters (a and b) indicated significant difference between control (PAHs−) and PAHs-treated (PAHs+) soils. Values represent means ± standard deviation. Where P0 = no plant; P1 = tall fescue (*F. arundinacea*); P2 = ryegrass (*L. perenne*); P3 = alfalfa (*M. sativa*); P4 = rape seed (*B. napus*); P5 = P1 + P3; P6 = P1 + P4 and P7 = P3 + P4.

study with the enhanced PAHs degradation in the rhizosphere of P4 and P6.

3.3. The response of dehydrogenase activity

Microbial extra-cellular soil enzyme activity was monitored at the end of the phytoremediation experiment to determine how different plant species alone and in combination affect microbial activity under PAHs contamination. The activities of dehydrogenase were measured by the reduction of TTC. Table 3 shows that the dehydrogenase activities were much higher in planted soils compared to unplanted controls irrespective to the presence of PAHs in soil. After 65 days of plant growth, the dehydrogenase activities were 1.8–68.7 and 2.5–107.1 μg TPF g^{-1} dry soil in uncontaminated and contaminated soils, respectively. Highest dehydrogenase activity was observed in the rhizosphere of P6 (mix plantation of tall fescue and rape seed) whereas lowest values were observed in the rhizosphere of alfalfa. This matches well with the PAHs degradation data.

Dehydrogenase activity assays in soil have often been used to obtain correlative information on the biological activity of microbial populations in soil, i.e., as an index of total microbial activity [20]. Strong correlations between hydrocarbon removal and dehydrogenase activity are frequently observed [21]. The results also show that presence of PAHs stimulated the dehydrogenase activity in the rhizosphere of all plant treatments except alfalfa. Lee et al. [19] 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 [22]. The higher dehydrogenase activity in the PAHs contaminated soil might be attributed to the increased microbial activity as a result of enhanced root exudation as a result of PAHs toxicity to plants. Walton et al. [23] speculated that when chemical stress occurs in soil, a plant may respond by increasing or changing its exudation to the rhizosphere, which then modifies the microfloral composition or activity of the rhizosphere.

3.4. Plant uptake and accumulation of phenanthrene and pyrene

Concentration of phenanthrene and pyrene in plant roots and shoots and their concentration factors are shown in Tables 4 and 5. Concentrations of phenanthrene and pyrene in roots grown in unspiked control soil were not detectable, where as shoot accumulation was obvious, which should only derive from shoot uptake and accumulation from atmosphere probably through the retention of vapor phase of PAHs on the waxy leaf cuticle [24]. It indicates that the shoot uptake of PAHs from the ambient air, possibly originally volatized from the soils, was an important pathway for these PAHs intake by above-ground parts.

All plants accumulated phenanthrene and pyrene in the plant parts when grown in the spiked soils. Great variations of root and shoot phenanthrene and pyrene concentrations were observed among different plant species.

Alfalfa exhibited the highest root concentrations of phenanthrene (5.25 mg kg^{-1}) and pyrene (40.90 mg kg^{-1}), while tall fescue and ryegrass contained the lowest portion of these compounds. The concentrations of PAHs in shoots were far lower than in roots. Highest accumulation of phenanthrene (1.46 mg kg^{-1}) and pyrene (0.39 mg kg^{-1}) was observed in the shoot of rape seed whereas lowest accumulation was recorded in the leaves of ryegrass, i.e., 0.2 mg kg^{-1} of phenanthrene (5.25) and 0.09 mg kg^{-1} of pyrene. The

Table 4
Root concentrations (mg kg^{-1}) and concentration factors (RCFs) of phenanthrene and pyrene of plants grown in PAHs-treated (PAHs+) soils after 65 days of plant growth.

Treatment	Phenanthrene		Pyrene	
	Roots	RCFs	Roots	RCFs
P1	1.60 ± 0.21C	0.38 ± 0.09B	25.62 ± 6.29C	0.82 ± 0.07B
P2	2.30 ± 1.06C	0.58 ± 0.28B	33.75 ± 13.83C	1.09 ± 0.23B
P3	8.17 ± 0.89A	1.37 ± 0.18A	105.82 ± 24.97A	2.60 ± 0.37A
P4	5.33 ± 1.53B	1.37 ± 0.40A	70.48 ± 11.26B	2.62 ± 0.81A

Values in each column followed with different capital letters (A–C) indicated significant ($p \leq 0.05$) differences among different plant treatments. Values represent means ± standard deviation. Where P1 = tall fescue (*F. arundinacea*); P2 = ryegrass (*L. perenne*); P3 = alfalfa (*M. sativa*) and P4 = rape seed (*B. napus*).

Table 5
Shoot concentrations (mg kg^{-1}) and concentration factors (SCFs) of phenanthrene and pyrene of plants grown in control (PAHs−) and PAHs-treated (PAHs+) soils after 65 days of plant growth.

Treatment	PAHs−		PAHs+			
	Phenanthrene	Pyrene	Phenanthrene		Pyrene	
	Shoot	Shoot	Shoot	SCFs	Shoot	SCFs
P1	0.12 ± 0.05B	0.09 ± 0.03B	0.72 ± 0.18B	0.16 ± 0.02C	1.26 ± 0.21B	0.042 ± 0.01B
P2	0.11 ± 0.05B	0.07 ± 0.03B	0.45 ± 0.16B	0.12 ± 0.07C	0.87 ± 0.14B	0.031 ± 0.01B
P3	0.28 ± 0.03A	0.17 ± 0.04A	1.81 ± 0.25A	0.30 ± 0.07B	3.94 ± 0.72A	0.097 ± 0.01A
P4	0.35 ± 0.07A	0.21 ± 0.03A	1.92 ± 0.29A	0.49 ± 0.10A	3.55 ± 0.41A	0.132 ± 0.03A

Values in each column followed with different capital letters (A–C) indicated significant ($p \leq 0.05$) differences among different plant treatments. Values represent means ± standard deviation. Where P1 = tall fescue (*F. arundinacea*); P2 = ryegrass (*L. perenne*); P3 = alfalfa (*M. sativa*) and P4 = rape seed (*B. napus*).

growth conditions of all plant species grown were identical. Thus the disparity of root and shoot uptake of these PAHs would come from plant properties. Several studies have suggested that the root uptake of lipophilic organic compounds could be in correlation with root compositions such as lipid contents [25,26].

Root concentration factors (RCFs), and shoot concentration factors (SCFs) were calculated as the ratio of the PAH concentrations in roots/shoots and in soils on a dry weight basis. RCFs of different plants are higher than the SCFs, suggesting that the transfer of tested PAHs from roots to shoots was considerably restricted. The results also show that RCFs of phenanthrene (0.25–0.88) were much lower than those of pyrene (0.49–1.07) for the same soil–plant treatment. It might be explained by the higher K_{ow} (octanol–water partition coefficient) value of pyrene than phenanthrene. Studies have shown that most lipophilic organic compounds (K_{ow} greater than approximately 10^4) partition to the epidermis of the roots [27] and the extent to which a lipophilic organic compound enters in plant roots from contaminated soil depends on the K_{ow}. Generally, the more lipophilicity results in higher root concentrations [24].

3.5. Dissipation of phenanthrene and pyrene in soil

Residual concentrations of phenanthrene and pyrene remaining in soil at the end of phytoremediation experiment are shown in Fig. 1. After 65 days of experiment, initial pyrene (199.3 mg kg^{-1}) and phenanthrene (200 mg kg^{-1}) concentrations significantly decreased in the planted soils as well as in unplanted control, but a more marked rate of disappearance was evident when plants were present. The residual percentages for phenanthrene and pyrene were 0.8–2.9% and 4.2–20.2%, respectively in planted soils, which were significantly lower compared to unplanted control soils (4.1% for phenanthrene and 26.6% for pyrene). The overall extent of PAHs loss was clearly compound-dependent; pyrene degradation ratio was much lower than phenanthrene in all treatments, 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 [19,26,28].

Different planted treatments displayed different degradation rates. Generally, higher PAHs disappearance was found in the combined plant treatments compared to single plantation. Among single plant treatments, rape seed displayed the highest PAHs degradation rate (98% of phenanthrene and 86% of pyrene) followed by ryegrass and tall fescue whereas alfalfa showed the lowest degradation rate: 97% of phenanthrene and 79.8% of pyrene. Among mix plantation the combination of tall fescue and rape seed had the highest removal rate of PAHs: 99.1% of phenanthrene and 95.7% of pyrene.

The major finding in this present study was the presence of plant increasing significantly phenanthrene and pyrene degradations in soil. Obviously, the enhanced dissipation of phenanthrene and pyrene in planted versus unplanted soil would overwhelmingly derive from plant direct uptake and accumulation and promoted biodegradation. However, the amount of PAHs directly accumulated in plants only reached an average of 0.68% of dissipation enhancement for phenanthrene and 0.81% for pyrene in the presence of vegetation. By contrast, plant-promoted biodegradation of phenanthrene and pyrene was the dominant contribution, and about 99% dissipation enhancement of these compounds in planted versus unplanted soil came from plant-promoted biodegradation. Our results concur with the findings of Gao and Zhu [26], who concluded that enhanced dissipation of PAHs in planted soil was mainly because of plant-promoted biodegradation and the contribution of plant uptake and accumulation to the dissipation enhancement of PAHs was negligible. Plants may contribute to the biodegradation of organic compounds by an increase in microbial number [16,29], a promotion in microbial activity [30,31] and a modification in microbial community in rhizosphere [11,31], as results of the massive input of easily degradable organic substances, the improvement of physical and chemical soil conditions, and increased humification and adsorption of pollutants in the rhizosphere. The plant-promoted biodegradation of phenanthrene and pyrene in this study should be results of the sum of above factors.

The enhanced degradation of PAHs in mixed plantation treatments compared to single plantation might be the result of different roots interaction constituents. This may have two possible explanations: (1) roots interaction modified root physiology (enzyme activity, exudation, longevity) in a manner that stimulates PAH degradation, either by root derived enzymes or by rhizosphere organisms, (2) interaction roots colonization affected root surface properties or rhizosphere soil properties that act on PAH availability through adsorption, and improved physical structure of the soil allowing more rapid and deeper penetration of water, nutrients, and microbes [11]. On the other hand, the root systems of plants in combined cultivar with a large surface area and intensive soil penetration also caused more PAH degradation than in individual plants, and combined cultivar plants provided the desired result of increasing the effective depth of remediation. These features were favorable to establish a rhizosphere throughout the soil column.

The biological parameters measured after 65 days of plant growth were significantly correlated with PAHs concentrations (Table 6). The dehydrogenase activity and water-soluble phenol

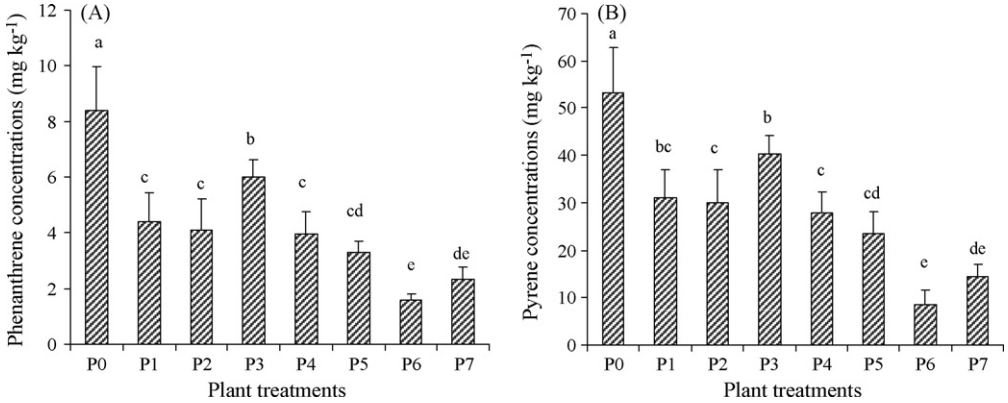

Fig. 1. Residual concentrations (mg kg^{-1}) of phenanthrene and pyrene in soils planted with different plant treatments after 65 days of plant growth. Error bars represent the standard deviation of three sampled pots. Columns denoted by different letters (a–e) indicated significant ($p \leq 0.05$) differences among different treatments. Where P0 = no plant; P1 = tall fescue (*F. arundinacea*); P2 = ryegrass (*L. perenne*); P3 = alfalfa (*M. sativa*); P4 = rape seed (*B. napus*); P5 = P1 + P3; P6 = P1 + P4 and P7 = P3 + P4.

Table 6
Correlation between PAHs residual concentrations and different biological parameters in soils planted with different plant treatments after 65 days of plant growth.

Parameter	Correlation coefficients		
	Pyrene	Water-soluble phenols	Dehydrogenase
Phenanthrene	0.991**	−0.908**	−0.955**
Pyrene		−0.901**	−0.955**
Water-soluble phenols			0.920**

** Significance at $p \leq 0.01$.

content were negatively correlated with phenanthrene and pyrene residual concentrations in planted soils ($p < 0.05$).

4. Conclusions

We investigated the phytoremediation capability of four plant species grown alone and in combination for the PAHs contaminated soil. The presence of vegetation significantly increased the dissipation of phenanthrene and pyrene in the soil environment. Enhanced dissipation in planted versus unplanted soil was 1.18–3.37 mg for phenanthrene and 6.48–22.42 mg for pyrene. As compared to the single plant cultivation, combined plants cultivation significantly enhanced the dissipation rate of PAHs. The selection of different combinations might be especially useful for phytoremediation of soils contaminated with PAHs. All plant species did take up the PAHs in plant parts but contributions of plant off-take of these chemicals to the total remediation enhancement in the presence of vegetation were negligible. By contrast, plant-promoted dissipation was the predominant contribution to the remediation enhancement for soil phenanthrene and pyrene in the presence of vegetation.

Our results suggest that the enhancement of phenanthrene and pyrene disappearance is caused by an increase in the rhizosphere biological activity compared to root free soil. Moreover, combined plant cultivation should be considered while evaluating remedial approaches for contaminated soils. Further research work is required to elucidate the complex processes at the interface of soil, microorganisms and roots which determine the fate of organic pollutants.

Acknowledgements

This work was supported by the Program for Changjiang Scholars and Innovative Research Team in University (IRT0536), National Natural Science Foundation of China (20607019 and 40590392) and Zhejiang Province Bureau of Science and Technology (2007C23037). The authors would like to specially acknowledge Mr. Liu Lei and other members in our group for their great assistance and cooperation during the research.

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