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Phytoremediation for phenanthrene and pyrene contaminated soils

GAO Yan-zheng, ZHU Li-zhong*

(Department of Environmental Sciences, Zhejiang University, Hangzhou 310028, China. E-mail: zlz@zju.edu.cn; gaoyanzheng@eyou.com)

Abstract: Phytoremediation of soil contaminated with phenanthrene and pyrene was investigated using twelve plant species. Plant uptake and accumulation of these chemicals were evaluated. At the end of the experiment(45 d), the remaining respective concentrations of soil phenanthrene and pyrene in spiked vegetated soils, with initial phenanthrene of 133.3 mg/kg and pyrene of 171.5 mg/kg, were 8.71—16.4 and 44.9—65.0 mg/kg, generally 4.7%—49.4% and 7.1%—35.9% lower than their concentrations in the non-vegetated soils. The loss of phenanthrene and pyrene in vegetated spiked soils were 88.2%—93.0% and 62.3%—73.8% of the added amounts of these contaminants, respectively. Although plant uptake and accumulation of these compounds were evident, and root concentrations and RCFs (root concentration factors; defined as the ratio of PAH concentrations in roots and in the soils on a dry weight basis) of these compounds significantly positively correlated to root lipid contents, plant uptake and accumulation only accounted for less than 0.01% and 0.23% of the enhanced loss of these chemicals in vegetated versus non-vegetated soils. In contrast, plant-promoted microbial biodegradation was the dominant mechanism of the phytoremediation for soil phenanthrene and pyrene contamination. Results from this study suggested a feasibility of the establishment of phytoremediation for soil PAH contamination.

Keywords: phytoremediation; phenanthrene; pyrene; polycyclic aromatic hydrocarbons(PAHs); soil

Introduction

Soil contamination by polycyclic aromatic hydrocarbons (PAHs) poses a great threat worldwide to the agricultural food quality and human health, and calls for an immediate action to remediate the contaminated sites (Wilson, 1993). Various physiochemical processes have been used to restore the PAH contaminated soils. However, up to the present time there is still scarce of cost-effective and reliable remediation techniques on a large scale (Gao, 2003a).

The prospect of using vegetation to enhance the degradation of organic contaminants in soil systems is an attractive cost-effective alternative to traditional engineering approaches (Schnoor, 1995; Sung, 2002). In the last decades, plant-enhanced remediation of soil PAHs has been reported (Reilley, 1996; Binet, 2000). However, the mechanisms involved are still under well elucidation. Application of the phytoremediation on a large scale as an effective and reliable remediation option requires a reasonable understanding of how plants contribute to the dissipation of PAHs in contaminated soils.

The principle contributions of plants to phytoremediation are either stimulation of soil microbial activity and degradation of contaminants or plant direct uptake and accumulation of the contaminants (Sung, 2001). To the best of our knowledge, most of current studies on phytoremediation for PAH contaminated soils focus on the former contribution of plants (Binet, 2000), and limited information was available on the contribution of plant direct uptake and accumulation on a quantitative scale.

Over the last few years, plant uptake and accumulation of PAHs have been investigated (Jones, 1989; Howsam, 2001; Zhu, 2004). Studies have focused on the foliage uptake and accumulation of PAHs from the atmosphere as a result of the deposition of particle-bound compounds and the retention of vapor phase PAHs on the waxy leaf cuticle (Trapp, 1990; Simonich, 1994; Howsam, 2001). Recently, the root uptake of PAHs from soils has been revealed. Some workers found the direct relationships between the soil and root PAH concentrations, and the root concentrations increased with the octanol-water partition coefficients of the compounds (Kipopoulou, 1999; Chiou, 2001); whilst others have found that no such relationship existed (Wild, 1992). Results reported are not usually identical. Furthermore, information is scant on soil-plant transfer factors and on correlations between plant PAH concentrations and plant compositions. As a consequence, there are difficulties to evaluate the plant contribution to phytoremediation process.

The aim of this paper was to investigate the phytoremediation for PAH contaminated soils using twelve plant species. Based on the observation of plant uptake and accumulation, plant contributions to phytoremediation of soil PAH contamination were evaluated on a quantitative scale. This study may demonstrate the potential for phytoremediation as an effective and low cost strategy for PAH contaminated soils.

1 Materials and methods

1.1 Chemicals

Phenanthrene and pyrene were provided by Aldrich Chemical Co. with a purity >98%. The respective molecular weights of phenanthrene and pyrene are 178.23 and 202.26 g/mol; $\log K_{ow}$ (K_{ow} is the octanol-water partition

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coefficient) are 4.46 and 4.88(Yaws, 1999).

1.2 Contaminated soils

A vadose zone soil collected from Hangzhou, China was air-dried and sieved through a 3 mm sieve. The soil has a pH of 5.05 and organic matter of 1.45%. This PAH-free soil was then spiked with various concentrations of phenanthrene and pyrene dissolved in acetone. The acetone was evaporated under hood, and the spiked soils with various phenanthrene and pyrene concentrations were mixed with a bulk of unpolluted soil and homogenized. To ensure the homogeneity, soils were then sieved through 3 mm mesh. The final PAH concentrations in treated soils were measured, and soil phenanthrene and pyrene concentrations on a dry weight basis were 133.3 and 171.5 mg/kg. These PAH treated soils were then packed into greenhouse pots, and equilibrated in a greenhouse for four days after the addition of water to maintain soil moisture at 50% of the field capacity.

1.3 Experimental design

Plant species used in this study included three-colored amaranth (*Amaranthus tricolor* Linn.), flowering Chinese cabbage (*Brassica parachinensis* Bailey), radish (*Raphnus sativus* L.), water spinach(*Lpomoea aquatica* Forsk), green soybean (*glycine max* Merr.), kidney bean (*Phaseolus vulgaris* L.), pakchoi (*Brassica chinensis* L.), broccoli (*Brassica oleracea* L.), sinage (*Spinacea oleracea* L.), capsicum (*Capsicum annuum* L.), eggplant (*Solanium melongena* L.), and ryegrass(*Lolium multiflorum* Lam). All plants were grown directly in the treated soils. The seedlings were then thinned 7—10 d after emergence to leave eight plants per pot for ryegrass and three plants per pot for other species, giving (1) non-vegetated pots with spiked soil, (2) microbe-inhibited non-vegetated pots with spiked soil(0.5% $HgCl_2$ was used to inhibit the microbial activity), and (3) vegetated pots with spiked soil. Each treatment was established with three replicates. The treated pots were randomly placed in a small greenhouse and exchanged their positions every two days. Soils were carefully watered as needed and fertilized every 2 weeks with an inorganic salt solution(Reilley, 1996; Liste, 2000). Plants were harvested after 45 d of growth after sowing. Soils and plants were destructively sampled from the pots. The plant shoot and root tissues were washed with distilled water to remove any soil particles. The moisture contents of soil and plant samples, and the lipid contents of the whole plant and plant parts were measured as described previously by Simonich and Hites (Simonich, 1994).

1.4 PAH analysis for soil and plant samples

The soil samples from vegetated pots were collected, homogenized and passed through a 20 mesh standard sieve. Soil samples were mixed with anhydrous Na_2SO_4 and extracted by ultrasonication into dichloromethane. The soil extracts were filtrated through a silica gel column with elution of 1∶1 hexane and dichloromethane. The solvents were evaporated using a rotary evaporator, and exchanged to methanol with a final volume of 2 ml. The final soil extracts were then analyzed by high performance liquid chromatograph (HPLC) after filtration through 0.22 μm syringe filters. The recoveries of phenanthrene and pyrene through the complete analytical process were 91.3%—95.6% with the relative standard deviation(RSD) less than 3.1%.

Plant samples were ground, homogenized, and extracted by ultrasonication with 1∶1(v/v) solvent mixture of acetone and hexane. The solvent was decanted and collected. The plant samples were re-extracted with the replenished fresh solvent and sonicated. This process was repeated for three times. The solvent fractions were combined and passed through an anhydrous Na_2SO_4 column with elution of 1∶1(v/v) solution of acetone and hexane. The solvents were then evaporated using a rotary evaporator and exchanged to 2 ml hexane, followed by filtration through 2 g silica gel column with elution of 11 ml of 1∶1 (v/v) hexane and dichloromethane. The samples were then evaporated and exchanged to methanol with a final volume of 2 ml. After filtration through 0.22 μm syringe filters, the samples were analyzed by HPLC (Simonich, 1994; Kipopoulou, 1999; Gao, 2004). Recoveries of known amounts of phenanthrene and pyrene through the entire procedure averaged 103.1% (n = 5, *RSD* 3.34%) and 89.4% (n = 5, *RSD* 4.05%), respectively. The employed system of HPLC contained a UV detector and a ϕ4.6 × 150 mm reverse phase C_{18} column. The mobile phase was methanol-water(83∶17) with a flow rate of 1 ml/min. Phenanthrene and pyrene was detected at 245 and 234 nm, and their limits of detection were 44.1 and 50.2 pg, respectively. Concentrations of phenanthrene and pyrene in soil or plant samples were contrasted using $p < 0.05$ unless indicated otherwise based on 3 replicates for each treatment. All data were processed by the software package of SPSS.

2 Results and discussion

2.1 Plant biomass

Root and shoot biomass of tested plant species with the spiked and unspiked soils are shown in Table 1. Although all plant species grown in the spiked soils with initial phenanthrene of 133.3 mg/kg dw and pyrene of 171.5 mg/kg dw showed no outward signs of phytotoxity, there was evident effects of PAHs on plant dry weights. Shoot and root biomass were generally significantly much lower with the contaminated than uncontaminated soils. Plants always formed a relatively denser root system in unspiked soils. It was notable that despite reduced biomass of these plant species with the spiked soils, all tested plants did not exhibit apparent signs of stress and toxicity, suggesting that establishment of vegetation in these soils should be feasible.

Table 1 Shoot and root biomass of test plant species with spiked or unspiked soils after 45 d treatment(g dw/pot)

Plant No.	Plant species	Unspiked soil		Spiked soil	
		Root	Shoot	Root	Shoot
P1	Three-colored amaranth	0.22	0.79	0.10	0.42
P2	Flowering Chinese cabbage	0.64	1.62*	0.32	1.54*
P3	Ryegrass	0.73#	1.48*	0.61#	1.41*
P4	Radish	3.65	5.19	1.59	4.19
P5	Water spinach	0.51	1.93	0.27	1.36
P6	Green soybean	0.74	4.79	0.41	2.80
P7	Kidney bean	0.93	7.32	0.53	4.90
P8	Pakchoi	0.66	4.28	0.46	3.25
P9	Broccoli	0.75	4.07	0.52	3.37
P10	Sinage	0.16	2.09	0.12	1.07
P11	Capsicum	0.31	3.25	0.21	2.68
P12	Eggplant	0.32	3.43	0.21	2.80

Notes: * Means no significant difference between shoot biomass of plants grown in unspiked and spiked soils; # means no significant difference between root biomass of plants grown in unspiked and spiked soils

2.2 Plant-enhanced remediation of soil phenanthrene and pyrene

High-molecular-weight PAHs including phenanthrene and pyrene constitute a group of pollutants whose degradation in contaminated soils often has not been successful(Wilson, 1993). The PAHs are characteristically persistent in soils (Liste, 2000). Accelerating the degradation of these compounds is thus a major challenge.

In this instance, the concentrations of phenanthrene and pyrene in spiked soils declined markedly after 45 d. A more marked rate of disappearance was evident if plants were present. The remaining respective concentrations of soil phenanthrene and pyrene in vegetated soils were 8.71—16.4 and 44.9—65.0 mg/kg dw, generally 4.7%—49.4% and 7.1%—35.9% lower than their concentrations in non-vegetated soils(phenanthrene of 17.2 mg/kg and pyrene of 70.0 mg/kg) (Fig.1). This indicated that the tested plants enhanced the remediation of soil phenanthrene and pyrene. The loss of phenanthrene and pyrene in vegetated spiked soils after 45 d was 88.2%—93.0% and 62.3%—73.8% of the total added amounts of these contaminants, respectively. It is noteworthy that the percentage of the lost pyrene to the total added amounts in spiked soils was always much lower than that of phenanthrene for the same treatment, implying that pyrene was more recalcitrant than phenanthrene in soils.

As seen from Fig.1, the seedlings of flowering Chinese cabbage, radish, and green soybean brought about the highest promotion of phenanthrene degradation in vegetated versus non-vegetated soils after 45 d, while the seedlings of green soybean, ryegrass and eggplant did the greatest promotion of pyrene disappearance. Comparing with Table 1, it could be viewed that no relationship was evident between the mass of roots or shoots and the ability of the plants to bring about phenanthrene and pyrene degradation. This is consistent with the observed results that correlations between plant root or shoot biomass and the disappearance of pyrene

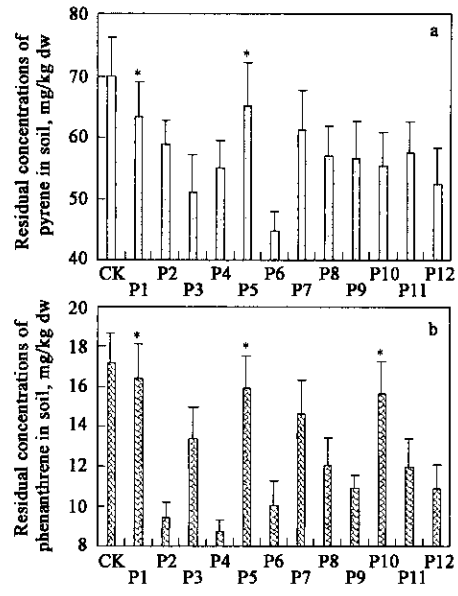

Fig.1 Residual concentrations of phenanthrene and pyrene in spiked vegetated and non-vegetated soils. The initial concentrations of phenanthrene and pyrene in all cases were 133.3 and 171.5 mg/kg dw, respectively (a) pyrene, (b) phenanthrene. * means no significant difference between the residual concentrations of phenanthrene or pyrene in vegetated and non-vegetated soils(CK). P1—P12 are plant No. as denoted in Table 1. Error bars represent ± 1 *SD*

and petroleum hydrocarbons were poor(Wiltse, 1998).

For PAHs are considered to be seriously health risk at very low concentrations, even a small remediation enhancement in the presence of plants is potentially important. The high rate of the added phenanthrene and pyrene disappearance in spiked vegetated soils, the evident promotion of phenanthrene and pyrene degradation in vegetated versus non-vegetated soils, and the healthy plant growth (as illustrated previously) suggested encouraging opportunities to enhance the remediation of soil phenanthrene and pyrene contaminants by vegetation.

2.3 Plant accumulation of phenanthrene and pyrene

It has been revealed that plant uptake and accumulation are the predominant mechanism of the phytoremediation for soil heavy metal contamination(Gao, 2003b). However, the mechanism of phytoremediation for soil PAH contamination is still under well elucidation, because the plant uptake and accumulation of these compounds are not efficiently defined.

Root and shoot concentrations of phenanthrene and pyrene after 45 d were displayed in Table 2. Contents of phenanthrene and pyrene in shoots of experimented plant species with spiked soils varied in range of 0.095—1.955 and 0.231—7.372 mg/kg dw, and those in roots were 0.603—6.715 and 13.10—198.9 mg/kg dw, respectively. Root concentrations of phenanthrene or pyrene were generally much higher than shoot concentrations for a given soil-plant treatment, implying that the translocation of these compounds from roots to shoots was restricted. Plant concentration factors

(PCFs), defined as the ratio of PAH concentrations in plant or plant part and in the soils on a dry weight basis, were obtained. Root concentration factors(RCFs) of phenanthrene and pyrene by these twelve plant species were 0.050—0.670 and 0.276—4.435. Shoot concentration factors (SCFs) of these compounds were 0.006—0.119 and 0.004—0.116, generally much lower than RCFs by the same plant.

Table 2 Root and shoot concentrations and concentration factors of phenanthrene and pyrene with spiked soils after 45 d treatment

Plant No.	Root				Shoot			
	C_{ph}, mg/kg dw	C_{py}, mg/kg dw	RCF_{ph}	RCF_{py}	C_{ph}, mg/kg dw	C_{py}, mg/kg dw	SCF_{ph}	SCF_{py}
P1	1.950	45.11	0.119	0.713	1.955	7.372	0.119	0.116
P2	4.235	111.9	0.450	1.903	0.295	1.282	0.031	0.022
P3	1.849	19.17	0.138	0.375	0.862	1.240	0.064	0.024
P4	2.356	82.92	0.270	1.508	0.388	1.070	0.044	0.019
P5	0.851	38.57	0.053	0.593	0.301	0.498	0.019	0.008
P6	6.715	198.9	0.670	4.435	0.557	2.094	0.056	0.047
P7	2.530	48.50	0.173	0.793	0.349	1.526	0.024	0.025
P8	2.380	74.80	0.197	1.313	0.778	1.935	0.064	0.034
P9	0.805	16.10	0.074	0.285	0.757	0.895	0.069	0.016
P10	2.030	20.45	0.129	0.310	0.095	0.379	0.006	0.007
P11	0.603	13.10	0.050	0.228	0.421	0.325	0.035	0.006
P12	0.849	14.45	0.078	0.276	0.226	0.231	0.021	0.004

Notes: C_{ph} and C_{py} were plant concentrations of phenanthrene and pyrene after 45 d; RCF_{ph} and RCF_{py} were root concentration factors of phenanthrene and pyrene; SCF_{ph} and SCF_{py} were shoot concentration factors of phenanthrene and pyrene, respectively. P1—P12 are the plant No. as denoted in Table 1

It was interesting, as manifested in Table 2, that root concentrations and concentration factors of pyrene were always much higher than those of phenanthrene. Studies have revealed that most lipophilic organic compounds partition to the epidermis of the roots, and the extent to which a lipophilic chemical partitions into a plant's roots from soils depends on the K_{ow}. The more lipophilicity of chemicals results in the stronger root accumulation (Trapp, 1990; Chiou, 2001). Although information on root uptake and accumulation of PAHs up to date is scant, the observed results of this study revealed consistently the stronger root accumulation of pyrene than phenanthrene, which should be a result of the higher K_{ow} of pyrene than phenanthrene. It was noteworthy that root concentrations or *RCFs* of phenanthrene and pyrene were significantly positively correlated to root lipid contents at $p < 0.01$ confidence level (Fig. 2 and Fig. 3), suggesting that lipid content should be a good predictor of phenanthrene and pyrene accumulation in plant roots.

The concentrations and distribution of phenanthrene and pyrene in plants are essential in predicting the effectiveness of a phytoremediation operation. As seen from Table 2 and Fig. 1, it was impossible to select the high-efficient-remediation vegetation based on the plant uptake and accumulation of these PAHs. For instance, concentrations and off-take(plant concentrations of target compounds × plant biomass on a dry weight basis) of phenanthrene and pyrene with eggplant were generally much lower than other plant species. In contrast, eggplant manifested a relatively higher performance on the remediation of these compounds.

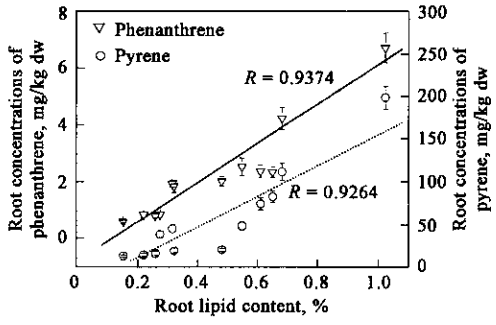

Fig. 2 Correlations of root concentrations with root lipid contents. Error bars represent ± 1 *SD*

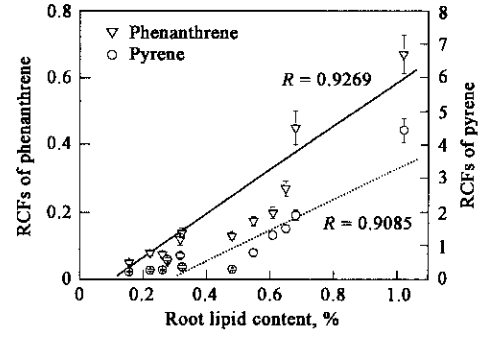

Fig. 3 Correlations of *RCF*s with root lipid contents. Error bars represent ± 1 *SD*

2.4 Contributions of vegetation to phytoremediation

The loss of phenanthrene and pyrene in vegetated soils approximately included leaching, abiotic dissipation(such as sorption, photo-oxidation and volatilization), microbial biodegradation, and plant uptake and accumulation. In contrast, the loss of these PAHs in non-vegetated soils was the sum of leaching, abiotic dissipation and microbial biodegradation. The loss of these compounds in microbe-inhibited non-vegetated soils accounted for the abiotic dissipation. At the end of the experiment(45 d), the loss of phenanthrene and pyrene in the microbe-inhibited non-vegetated soils was 7.68% and 3.76% of the added these chemicals, indicating abiotic dissipation was a minor pathway of the loss of these PAHs from soils. It has been reported that anthracene and pyrene were undetectable in leachate from soils with or without plants(Reilley, 1996), and no leachate was found through our whole experiments. Since the phytovolatilization of PAHs was negligible, and plant metabolism is not significant(Trapp, 1990), and variation of abiotic dissipation of PAHs between planted and unplanted soils was negligible(Reilley, 1996). Thus, obviously, the enhanced loss of tested PAHs in vegetated versus non-vegetated soils would predominantly come from plant uptake and accumulation and plant-promoted microbial biodegradation.

Results above revealed that both plant shoots and roots did accumulate the soil phenanthrene and pyrene, so there is the potential for human exposure to these chemicals from crop

plants. However, plant uptake and accumulation made an insignificant contribution to the enhancement of the loss of these chemicals in spiked vegetated versus non-vegetated soils (Table 3). The enhanced loss of phenanthrene and pyrene from spiked soils in the presence of vegetation were 0.36—4.22 and 2.50—12.5 mg/pot, respectively. By contrast, the total off-take of phenanthrene and pyrene by the accumulation of tested twelve plant species was less than 5.36 and 86.7 μg/pot, which could only account for less than 0.01% and 0.23% of the total enhanced loss of these chemicals from vegetated versus non-vegetated soils, respectively (Table 3). As such, plant-promoted microbial biodegradation of these chemicals in planted soils would be the dominant mechanism of the remediation enhancement in the presence of vegetation.

Table 3 Plant contributions to the remediation enhancement of phenanthrene and pyrene in vegetated versus non-vegetated spiked soils with initial phenanthrene concentration of 133.3 mg/kg and pyrene of 171.5 mg/kg after 45 d

Plant No.	T_d, mg/pot		P_a, μg/pot		A, ‰	
	Pyrene	Phenanthrene	Pyrene	Phenanthrene	Pyrene	Phenanthrene
P1	3.35	0.36	7.67	1.02	0.14	0.02
P2	5.60	3.87	37.9	1.81	0.67	0.03
P3	9.43	1.87	13.5	2.36	0.22	0.04
P4	7.50	4.22	136	5.36	2.33	0.09
P5	2.50	0.62	11.2	0.64	0.21	0.01
P6	12.5	3.57	86.7	4.29	1.37	0.07
P7	4.43	1.25	33.2	3.05	0.60	0.05
P8	6.51	2.53	40.6	3.62	0.71	0.06
P9	6.76	3.11	11.4	2.97	0.20	0.05
P10	7.36	0.74	2.77	0.34	0.05	0.06
P11	6.27	2.56	3.63	1.25	0.06	0.02
P12	8.78	3.11	3.72	0.81	0.06	0.01

Notes: T_d— the enhanced loss of phenanthrene or pyrene in vegetated versus non-vegetated spiked soils; P_a— off-take of phenanthrene and pyrene by plant accumulation with vegetated spiked soils. A— the percentage of phenanthrene and pyrene accumulated by plants to the enhanced loss (T_d) of these compounds in vegetated versus non-vegetated spiked soils

3 Conclusions

The presence of vegetation evidently enhanced the remediation of phenanthrene and pyrene in soil environment. Within 45 d, the remaining respective concentrations of soil phenanthrene and pyrene in spiked vegetated soils were 8.71—16.4 and 44.9—65.0 mg/kg dw, generally 4.7%—49.4% and 7.1%—35.9% lower than their concentrations in non-vegetated soils. The loss of phenanthrene and pyrene in vegetated spiked soils were 88.2%—93.0% and 62.3%—73.8% of the total added amounts of these contaminants, respectively. The seedlings of flowering Chinese cabbage, radish, and green soybean brought about the greatest promotion of phenanthrene degradation in these spiked vegetated versus non-vegetated soils, while the seedlings of green soybean, ryegrass and eggplant did the highest promotion of pyrene disappearance.

Phytoremediation is potentially associated with plant accumulation. Root concentrations and RCFs of these compounds displayed significantly positive correlations with root lipid contents. However, plant uptake and accumulation only accounted for less than 0.01% and 0.23% of the enhanced loss of these chemicals in vegetated versus non-vegetated soils. In contrast, plant-promoted microbial biodegradation was the dominant mechanism of the phytoremediation for soil phenanthrene and pyrene contaminants.

The high loss rate of phenanthene and pyrene from vegetated spiked soils, the significant promotion of the loss in vegetated versus non-vegetated soils, and the healthy plant growth suggested a feasibility of the establishment of phytoremediation for soil PAH contamination.

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