

# Plant Contribution to the Remediation of PAH-Contaminated Soil of Dagang Oilfield by Fire Phoenix

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## Research Article

**Keywords:** Phytoremediation, PAHs, dissipation, uptake, Fire Phoenix

**Posted Date:** June 22nd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-570890/v1>

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**Version of Record:** A version of this preprint was published at Environmental Science and Pollution Research on January 29th, 2022. See the published version at <https://doi.org/10.1007/s11356-021-18230-7>.

# Abstract

Pot experiments were conducted to evaluate plant contribution during remediation of the polycyclic aromatic hydrocarbons (PAH)-contaminated soil of Dagang Oilfield by Fire Phoenix. The results showed that Fire Phoenix could grow in soil contaminated by high and low concentrations of PAHs. After being planted for 150 days, the total removal rate of six PAHs in the high and low PAH concentrations was 80.36% and 79.79%, significantly higher than the 58.79% and 53.29% of the unplanted control group, respectively. Thus, Fire Phoenix can effectively repair the soil contaminated by different concentrations of PAHs. In high concentrations of PAHs, the results indicated a positive linear relationship between PAH absorption in tissues of Fire Phoenix and the growth time in the early stage. In contrast, the contents of PAHs were just slightly increased in the late period of plant growth. The main factor for the dissipation of PAHs was plant-promoted biodegradation (99.04–99.93%), suggesting a low contribution of PAH uptake and transformation (0.07–0.96%). The results revealed that Fire Phoenix did not remove the PAHs in the soil by accumulation but promoted PAH dissipation in the soil by stimulating the microbial metabolism in the rhizosphere.

## Highlights

- The total removal rate of six PAHs highly concentrated in the soil was 80.36%.
- The main factor for PAH dissipation was plant-promoted biodegradation (99.04%–99.93%).
- PAH dissipation through uptake and transformation by Fire Phoenix was insignificant (0.07%–0.96%).

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) refer to condensed ring compounds in which two or more benzene rings are arranged in linear, angular, or clustered shapes. They have the characteristics of hydrophobicity, low vapor pressure, and high octanol-water partition coefficient (Rey-Salgueiro et al. 2008; Haritash and Kaushik 2009; Venny et al. 2012; Bezza and Chirwa 2017). Owing to the known toxicity (carcinogenicity, teratogenicity, and mutagenicity) (Garcia-Falcon and Simal-Gandara 2005), strong inertness, stable properties, and other characteristics, they have been added to the priority pollutants list of the European Union (EU), Environmental Protection Agency (EPA), Agency of Toxic Substances and Disease Register (ATSDR), and International Agency for Research on Cancer (IARC) (Garciafalcon 2005; Rey-Salgueiro et al. 2008). PAHs are widely found in the soil, sediments, groundwater, and atmosphere (Bamforth and Singleton 2005). As hydrophobic organic pollutants, PAHs are preferentially distributed to non-aqueous systems. They are strongly adsorbent in soil, sediment, and solids in water (Congiu and Ortega-Calvo 2014) and do not degrade easily under natural conditions. Consequently, PAHs usually accumulate in the soil as a long-term environmental gathering place. Not only do PAHs remaining in the soil affect soil natural functions, but PAHs in the soil accumulate in the food chain and directly or indirectly endanger human health. According to reports, the binding force between soil and PAHs varies

from simple adsorption to covalent binding (Haritash and Kaushik 2009; Venny et al. 2012), challenging the development of PAH removal technology in the soil.

Scientists recognized that plants can metabolize toxic compounds decades ago, which led to using plants to treat organic pollutants (Reichenauer and Germida 2008). The degradation rate of organic compounds in planted soil is higher than that in unplanted soil, prompting an increment in researches on phytoremediation of contaminated soils (Ma et al. 2012; Wang et al. 2012). Phytoremediation has now become an effective, non-invasive, and cheap method to repair soil contaminated by organic matter (Wiltse et al. 1998). Plants and their rhizospheric microbial system absorb, degrade, volatilize, and transform pollutants in the environment, which can help to restore contaminated areas (Chaney et al. 1997; Brooks et al. 1998; Zhang et al. 2010).

Anderson et al. (1993) discovered that plants can remediate PAH-contaminated soil through direct or indirect absorption, separation, and degradation. Subsequent studies have shown that plants can promote the degradation of PAHs in the soil by stimulating microbial metabolism in the rhizosphere (Frutos et al. 2012). Sun et al. (2011) recommended intercropping alfalfa and tall fescue to promote the dissipation of PAHs in the soil. It reveals that the planting of two plant species may increase microbial richness in the rhizosphere, which leads to the positive effect on the removal of the soil PAHs. The phytoremediation process is an effective approach to enhance biodegradation by plants, and plant absorption plays a small role in this process (Sun and Zhou 2016). Phytoremediation can also be improved by growing plants that stimulate rhizobacteria. Rhizobacteria can relieve plant environmental pressure and increase the survival rate of plants under undesirable conditions (Boer and Wagelmans 2016). The factors to be considered in evolution of the ability of plants as phytoremediation agents are: i) the type and toxicity of pollutants and the concentration in the soil; ii) the ability of plants to transfer pollutants from the soil to various tissues; iii) the concentration of pollutants in the soil after remediation (Anderson et al. 1993; Ficko et al. 2010).

Soil pollution by petroleum products has attracted attention worldwide. Petroleum and its derivatives are unavoidably released into the environment during petroleum extraction, storage, and transportation. Petroleum hydrocarbons include mixtures of alkanes PAHs, resins, and other organic compounds (Gao et al. 2019). Therefore, the leakage of oil during exploitation and use is one of the critical reasons for PAH pollution of soils. Six PAHs, anthracene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(k)fluoranthene, measured in the soil of Dagang Oilfield in Tianjin, China, were the research object in this study. Fire Phoenix, a combination of *Festuca arundinacea* Schreb., *F. elata* Keng ex E. Alexeev, and *F. gigantea* (L.)Vill, was selected as the test plant by our laboratory after years of experimentation (Liu et al. 2015). The purpose of the present study was: (1) to examine the PAH uptake and transformation in Fire Phoenix plants and (2) to determine the mechanism of PAH phytodegradation. The results of the study are expected to provide some insight into the feasibility of PAH-contaminated soil using plant-enhanced phytoremediation.

## 2. Materials And Methods

## 2.1 Experimental design

The original contaminated soil was collected from Dagang Oilfield in Tianjin City, China. The concentration of PAHs in the soil ranged from 300.42 to 450.68 mg·kg<sup>-1</sup>; the PAHs were anthracene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(k)fluoranthene. Non-polluted natural soil was collected from Zhonger Village, Fushun County, Fushun City, Liaoning Province, and no PAHs were detected in the non-polluted soil samples. According to the pre-experimental results, no plants could grow on the original contaminated soil. Therefore, the original contaminated soil and the non-polluted natural soil were proportioned into two concentrations of PAH-contaminated soil, A and B. Seeds of Fire Phoenix were purchased from the Kelaowu Seeds Company, Beijing, China.

The physicochemical properties of PAHs used in this experiment are shown in Table 1. The physical and chemical characteristics of the experimental soils are shown in Table 2.

Table 1: Physicochemical properties of polycyclic aromatic hydrocarbons (PAHs).

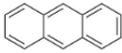
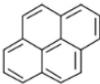
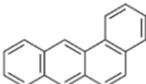
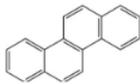
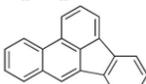
PAHs	Chemical structure	Ring number	Water solubility	Log (Kow)	Vapor pressure(Pa)
Anthracene		3	0.0045	4.54	0.001
Pyrene		4	0.13	5.18	0.0004
Benzo[a]anthracene		4	0.011	5.91	2.80E-05
Chrysene		4	0.006	5.91	5.70E-07
Benzo[b]fluoranthene		5	0.0015	5.80	8.06E-08
Benzo[k]fluoranthene		5	0.0008	6.00	5.20E-08

Table 2: Physicochemical characteristics of the experimental soils.

Contaminated soil	PAHs (mg·kg <sup>-1</sup> )	Physical and chemical properties				
		pH	Carbon content	Phosphorus content	Available P	Available N
			(%)	(%)	(mg·kg <sup>-1</sup> )	(mg·kg <sup>-1</sup> )
A	90.02~113.31	7.64	7.13	0.37	27.95	278.10
B	128.38~149.95	7.23	16.83	0.82	19.80	468.70

The two PAH concentration concentrations in the soil were 90.02~113.31 and 128.38~149.95 mg·kg<sup>-1</sup>. Contaminated soil (1.5 kg) was placed at the bottom of flowerpots, which was evenly covered by 0.1 kg of uncontaminated natural soil. Fire Phoenix seeds (0.5 g) were evenly sprinkled on the natural soil and covered with 0.1 kg of uncontaminated natural soil. A 150-d pot experiment was performed at the Shenyang Ecological Experimental Station at the Chinese Academy of Sciences. This experiment comprised six soil treatments (Table 3), four planting periods (according to the periods of Fire Phoenix planting; 0, 60, 120, and 150 days), and the experiment was repeated three times; a total of 6 × 4 × 3 = 72 pots were planted. The plants were grown in a greenhouse with a day/night temperature of 25/15°C and were watered every two days to keep water content approximately 25%. The greenhouse temperature was preset and adjusted automatically. The experiment ran from April 29, 2020 to September 29, 2020.

Rhizosphere soil and plant samples were collected on the 0, 60, 120, and 150 days. Biomass and the concentration of PAHs were evaluated in the plants, and soil samples were analyzed.

Table 3: Experimental plan for remediation of PAH-contaminated soils.

Treatment	PAHs concentration (mg·kg <sup>-1</sup> )	Plant in the soil
CK-NP	0	No
CK-P	0	Yes
A-NP	90.02~113.31	No
A-P	90.02~113.31	Yes
B-NP	128.38~149.95	No
B-P	128.38~149.95	Yes

CK-P stands for plants growing in natural soil without PAH pollution; A-P stands for plants growing in the contaminated soil A (PAH content: 90.02~113.31 mg·kg<sup>-1</sup>); B-P stands for plants growing in the contaminated soil B (PAH content: 128.38 ~149.95 mg·kg<sup>-1</sup>); NP stands for treatments without plants.

## 2.2 Biomass determination and calculation

Wash the plants with distilled water and measure the height of the plants. Separate the roots and shoots parts and dry them in an oven at 70°C for 48 hours, then the dry weight of the roots and shoots parts were weighed and recorded (Zheng et al. 2021).

To assess the outcome of contaminated soil on plant growth, the equation for calculating the rate of growth inhibition is:

$$\text{Growth inhibition ratios (\%)} = \left(1 - \frac{\text{growth in contaminated soil}}{\text{growth in uncontaminated soil}}\right) \times 100,$$

where growth represents shoots and roots biomass.

### 2.3 PAH extraction and analysis in soils

The PAHs in the soils were extracted by ultrasonic extraction and detected by gas chromatography (6890N Network Gas Chromatograph; Agilent; Santa Clara, CA, USA). Liu et al. (2015) recorded detailed information about the extraction and detection methods. The recovery rate of PAHs was determined by adding a known concentration of a PAH solution mixture (EPA 16 PAHs, 1 mg/kg) to uncontaminated soil. The recoveries were  $89.37 \pm 3.80\%$  for anthracene,  $88.81 \pm 7.06\%$  for pyrene,  $89.00 \pm 7.73\%$  for chrysene,  $89.68 \pm 10.52\%$  for benzo(a)anthracene,  $89.68 \pm 10.52\%$  for benzo(b)fluoranthene, and  $89.07 \pm 12.35\%$  for benzo(k)fluoranthene.

The calculation formula of the degradation rate of PAHs is:

$$DR = \frac{C_0 - C_1}{C_0},$$

where DR is the degradation rate,  $C_0$  is the initial concentration of PAHs in the soil sample ( $\text{mg}\cdot\text{kg}^{-1}$ ), and  $C_1$  is the concentration of PAHs in the soil sample on days 60, 120, and 150 ( $\text{mg}\cdot\text{kg}^{-1}$ ).

### 2.4 PAH extraction and analysis in plants

After freeze-drying plant shoot and root samples, they were ground separately; 0.5 g of each sample was weighed into a centrifuge tube, and 10 mL of an acetone and n-hexane mixture (1:1; v:v) was added. Ultrasound extraction was performed for 60 minutes, and the ultrasonic temperature was below 32°C. The mixture was, then, centrifuged for 10 min at 4000 rpm, and the supernatant was collected in a round-bottom flask. The collected supernatant was centrifuged again and this procedure was totally repeated three times. The flask was rotated, and the content evaporated to dryness at 40°C. After rinsing the interior of the flask with 5.0 mL of n-hexane and purifying the resulting solution through a silica gel column and rinsing it with 20 mL of eluent (n-hexane and dichloromethane; 1:1; v:v), the eluent was collected. The eluent was, then, evaporated to dryness using an evaporator; chromatographic hexane was used to dilute the content into a 10-mL volumetric flask. The gas-chromatographic (Agilent 6890N) method was the same as that used for PAHs in the soil. In plant samples, the recoveries were  $92.83 \pm$

5.70% for anthracene,  $93.21 \pm 3.68\%$  for pyrene,  $94.21 \pm 5.57\%$  for chrysene,  $92.76 \pm 6.51\%$  for benzo(a)anthracene,  $92.38 \pm 8.96\%$  for benzo(b)fluoranthene, and  $95.46 \pm 8.71\%$  for benzo(k)fluoranthene.

## 2.5 Statistical analysis

Sampling and chemical analyses were repeated three times to reduce experimental errors and improve experimental repeatability. Analysis of variance (ANOVA) was performed on the collected data using SPSS v. 17.0 (IBM, Armonk, NY, USA). When a significant difference occurred, means were compared using the LSD test at  $P < 0.05$  level.

# 3. Results And Discussion

## 3.1 The impact of PAH pollution stress on the growth of Fire Phoenix

According to Fig. 1(a), there was no significant difference in plant height of Fire Phoenix in the three treatment groups after a 60-day culture. After a 120-day culture, the difference between CK-P and A-P treatment groups was not significant. The plant-height inhibition rate of A-P treatment group was 8.65%; the difference between CK-P and B-P treatment groups was significant, and the plant height inhibition rate of B-P treatment group was 19.75%. After a 150-day culture, there were significant differences among CK-P, A-P, and B-P treatment groups. The plant height inhibition rate of the A-P treatment group was 12.20% and that of B-P treatment group was 19.90%. However, the difference between A-P and B-P treatments was not significant. The height inhibition rate of Fire Phoenix plants did not increase and stabilize at approximately 20% with prolonged planting time in the high-concentration PAH soil. This result indicates that the Fire Phoenix plant had specific adaptability in the highly contaminated soil after a 150-day culture. It also indirectly indicates that Fire Phoenix can endure the highly toxic effects of pollutants during growth.

Fig. 1(b) shows changes in shoots and roots biomass in different treatment groups over different periods. The biomass after 120 and 150 days was significantly higher than the biomass after 60 days, which indicates that Fire Phoenix did not show growth reduction nor necrosis under the stress of either concentration of PAHs. After 60, 120, and 150 days of Fire Phoenix planting, the shoot biomass inhibition rates in the A-P treatment group were 38.15%, 20.55%, and 38.17%, respectively. The rate of growth inhibition of the shoots biomass reached the minimum after 120 days, which indicates that the Fire Phoenix plants adapted to the contaminated soil environment at 120 days. Fire Phoenix showed good adaptability to low-concentration PAH pollution. The shoot biomass inhibition rates in the B-P treatment group were 65.18%, 47.74%, and 61.07% at 60, 120, and 150 days, respectively. High-concentration PAH contamination had a more substantial inhibitory effect on shoot biomass of plants than the low-concentration PAH contamination did. After 60, 120, and 150 days, the inhibition rate of root biomass in low-concentration PAH-contaminated soil was 17.68%, 27.18%, and 22.08%, and the inhibition rates of root biomass were 24.89%, 33.95%, and 30.11% in high-concentration PAH-contaminated soil, respectively. With the extension of the cultivation time, the growth inhibition rate of the root biomass

showed a downward trend during the growth process from 120 to 150 days. This result indicates that the roots of Fire Phoenix plants constantly adapted to the soil environment under high concentrations of PAHs, presenting strong tolerance to soils highly polluted by PAHs.

The effect of plant repair on PAH-contaminated soil largely depends on the growth status of the plants (Xu et al. 2006), and the PAH organic pollutants may have toxic effects on plants (Ahammed et al. 2012b), causing growth reduction, chlorosis, and necrosis. Physiologically, PAHs can induce oxidative stress, DNA damage, cell death, change antioxidant enzymes, and inhibit photosynthesis (Alkio et al. 2005; Liu et al. 2009). Ahammed et al. (2012a) studied the growth, photosynthetic machinery, and antioxidant system responses of five vegetable crops under phenanthrene stress; the authors found that PAHs can penetrate plant cells and accumulate in tissues, damaging plant organelles such as chloroplasts. Sverdrup et al. (2003) found that eight polycyclic aromatic compounds with logKow-values ranging from 3.5 to 5.2 inhibited the initial growth of terrestrial plants, corroborating our results, which showed inhibition of the biomass of the Fire Phoenix plants in the early growth period. After 150 days of cultivation, no obvious toxic symptoms were found in either treatment group, indicating that the Fire Phoenix had a good tolerance to PAH pollution stress.

Andreolli et al. (2013) found that the biomass growth, stem length, and root dry weight of poplars after treatment with PAHs were reduced by approximately 65%, 54%, and 60%, respectively, compared with poplars without PAH treatment. Gao and Zhu (2004) observed that a low PAH content had no evident influence on plant biomass, but the growth inhibition effect was noticeable at high concentrations. Cheema et al. (2009) also verified that the roots biomass was only 29.7% of that of the control group when the concentrations of pyrene and phenanthrene in the soil were 344 and 336 mg·kg<sup>-1</sup>, respectively. These conclusions were consistent with our results. Reilley et al. (1996) believed that PAHs might weaken the ability of contaminated soil to supply water and nutrients for plants, which leads to a reduction in biomass. In addition, this study revealed that the inhibitory effect of PAH pollution on the shoots of Fire Phoenix was greater than that of the roots; however, the results obtained in some studies were the opposite of that verified in our study (Cheema et al. 2009). The possible reason for this discrepancy may be the different plants that were used in the experiments. Fire Phoenix plants did not show apparent signs of toxic stress. Moreover, the biomass of the roots of the Fire Phoenix plants showed a downward trend with the extension of the cultivation time, indicating that the roots were constantly adapting to the contaminated soil environment, showing a strong tolerance to soil contaminated with a high concentration of PAHs. In summary, Fire Phoenix plants can grow in soil contaminated by both high and low concentrations of PAHs, so this plant is a viable option for phytoremediation.

### **3.2 PAH degradation**

Fig. 2 shows the removal rate of PAHs after 60 days of remediation. The removal rate of six PAHs from treatment group A-P ranged from 38.41% to 53.91%, which was 9.64%–28.56% higher than that of the control group. Growing plants had a better repair effect on the 4-ring pyrene, benzo(a)anthracene, and

chrysene, and the removal rate was approximately 25% compared with that of the control group. The removal rate of the six PAHs from the B-P treatment group was 21.12%–56.17%, which was 9.27%–48.30% higher than that of the control group. As shown in Fig. 3, after 120 days of restoration, the removal rate of PAHs in the soil was further improved. In the treatment group A-P and B-P, the removal rate of six PAHs generally reached approximately 65% and 60%, respectively. Compared with the unplanted control group, the removal rate was improved.

As shown in Fig. 4, 150 days after Fire Phoenix was planted, the total removal rate of the six PAHs in the A-P and B-P treatment groups reached 80.36% and 79.79%, which were significantly higher than that of the unplanted control group. This result indicates that the plants presented good PAH recovery in both concentrations. The removal rate of 4-ring benzo(a)anthracene in the A-P treatment group reached 84.18%, which was 19.04% higher than that of the unplanted soil; the removal rate of 5-ring benzo(b)fluoranthene and benzene(k)fluoranthene reached 81.35% and 80.14%, which was 15.82% and 30.34% higher than those of the unplanted soil, respectively. The removal rate of 4-ring chrysene and 5-ring benzo(b)fluoranthene in the B-P treatment group reached 88.42% and 81.63%, the removal rate was increased by 31.90% and 31.63%, respectively, compared with that of the unplanted soil.

From Fig. 2 to 4, it can be seen that planting Fire Phoenix can significantly improve the degradation rate of PAHs in the soil. Phytoremediation has always been regarded as a cost-effective way to remove organic pollutants from the soil, and plants can improve soil structure (Andreolli et al. 2013). The degradation rate of organic pollutants was higher in the rhizosphere than in bulk soil (He et al. 2005). Kosnar et al. (2020) conducted a three-year experiment to investigate the function of willow on the phytoremediation of PAHs contaminated soil produced by straw combustion. The authors verified that the total degradation rate of PAHs in the soil with willow growth was 50.9%, and PAHs in naturally attenuated soil (unplanted soil) were only reduced by 9.9%. The mechanism of phytoremediation included biophysical and biochemical processes, such as adsorption, translocation, and transport, and the mineralization and transformation of plant enzymes (Gao et al. 2010; Perelo 2010). Moreover, the positive function of roots on improved PAH degradation due to the interactions between root exudates, microbes, and contaminants that stimulate microbial activity, enzyme-catalyzed processes, or co-metabolic processes have been observed (Joner and Leyval 2003; Gao et al. 2010).

The removal rate of various pollutants in the rhizosphere soil of Fire Phoenix was significantly affected by the phytoremediation time. When planting time reached 150 days, the removal rate of the pollutants was greatly improved. Alves et al. found that *M. sativa* L. 'Crioula' showed great potential and could be a phytoremediation tool to treat soils contaminated by pyrene, anthracene, and phenanthrene. With the extension of planting time, the degradation rate of PAHs in the soil increased by 10% within 20 days (Alves et al. 2018), which was consistent with our results. With the increasing planting time, the remediation effect of Fire Phoenix on PAH-contaminated soil increased, which may have been due to the continuous enrichment of the biodiversity of the soil bacterial community (Dai et al. 2020). In previous studies, it was verified that changes in active bacterial communities associated with atmospheric roots were a crucial factor in the success of phytoremediation (Agarry et al. 2013; Hou et al. 2015). Xu et al.

(2014) investigated the soil where ryegrass had been grown; the results indicated that the biodiversity of the soil bacterial community improved with the increase in growth time. And Guo et al. (2018) found that tall fescue significantly improved the ability to remove PAHs from contaminated soil. This elimination was related to the change in the structure of the bacterial community over time.

### 3.3 Migration of PAHs in plants

Table 4: Polycyclic aromatic hydrocarbon concentration in the different tissues of Fire Phoenix in the A-P treatment (PAH content: 90.02~113.31 mg·kg<sup>-1</sup>).

Compound	60 days		120 days		150 days	
	Shoot	Root	Shoot	Root	Shoot	Root
Ant	1.46 ± 0.56	3.22 ± 0.46	2.07 ± 0.694	2.44 ± 0.47	2.37 ± 0.71	3.42 ± 0.69
Pyr	1.25 ± 0.08	1.18 ± 0.15	4.90 ± 0.29	5.16 ± 0.36	ND	0.58 ± 0.03
BaA	0.72 ± 0.04	1.49 ± 0.31	0.99 ± 0.34	1.78 ± 0.29	ND	0.82 ± 0.18
Chr	ND	3.28 ± 0.67	2.89 ± 0.62	3.35 ± 0.54	0.37 ± 0.02	0.89 ± 0.23
BbF	ND	1.60 ± 0.06	1.62 ± 0.48	2.09 ± 0.39	ND	ND
BkF	ND	2.63 ± 0.218	2.17 ± 0.43	2.74 ± 0.34	ND	2.32 ± 0.016
Σ	3.43 ± 0.68	13.4 ± 1.868	14.64 ± 2.854	17.56 ± 2.39	2.74 ± 0.73	8.03 ± 1.146

Ant, anthracene; Pyr, pyrene; BaA, benzo(a)anthracene; Chr, chrysene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; ND, not detected.

Table 5: Polycyclic aromatic hydrocarbons concentration in the different tissues of Fire Phoenix in the B-P treatment (PAH content: 128.38 ~149.95 mg·kg<sup>-1</sup>).

Compound	60 days		120 days		150 days	
	Shoot	Root	Shoot	Root	Shoot	Root
Ant	1.71 ± 0.20	2.51 ± 0.599	1.20 ± 0.49	1.74 ± 0.15	2.93 ± 0.66	4.07 ± 0.71
Pyr	ND	0.71 ± 0.31	0.37 ± 0.03	3.39 ± 0.59	1.67 ± 0.13	2.14 ± 0.025
BaA	ND	0.88 ± 0.33	0.52 ± 0.32	1.51 ± 0.25	0.97 ± 0.24	2.75 ± 0.18
Chr	ND	1.64 ± 0.422	3.69 ± 0.87	4.06 ± 0.66	1.68 ± 0.44	6.17 ± 0.87
BbF	ND	ND	1.40 ± 0.59	2.03 ± 0.91	1.67 ± 0.14	1.28 ± 0.23
BkF	ND	1.50 ± 0.509	1.75 ± 0.59	2.98 ± 0.98	1.78 ± 0.14	3.13 ± 0.38
Σ	1.71 ± 0.20	7.24 ± 2.17	8.93 ± 2.89	15.71 ± 3.54	10.70 ± 1.75	19.54 ± 2.395

Ant, anthracene; Pyr, pyrene; BaA, benzo(a)anthracene; Chr, chrysene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; ND, not detected.

Table 4 shows that under the stress of low-concentration PAH pollution, six PAHs were found in the roots of the Fire Phoenix after 60 days of planting. Only the 3-ring and 4-ring compounds anthracene, pyrene, and benzo(a)anthracene were detected in the shoots of the plants; the 5-ring compounds benzo(b)fluoranthene and benzo(k)fluoranthene were not detected. After 120 days of planting, the absorption of PAHs by Fire Phoenix reached the maximum, and the content of PAHs in the roots and shoots reached 17.56 and 14.64 mg·kg<sup>-1</sup>, respectively. Table 5 shows that under the stress of high-concentration PAH pollution. After 60 days of planting, in addition to the five-ring benzo(b)fluoranthene, the other five PAHs were detected in plant roots; only anthracene was discovered in the shoots of the plants. After 120 days of planting, the accumulation of PAHs by the roots and shoots of Fire Phoenix reached 15.71 and 8.93 mg·kg<sup>-1</sup>, respectively. After 150 days of planting, the accumulation of PAHs by the roots and shoots reached 19.54 and 10.70 mg·kg<sup>-1</sup>, respectively. The PAH levels in the plants after 150 days and 120 days were not significantly different.

A large number of studies have shown that lipophilic organic pollutants depended on Kow entering plant roots from the soil (Gao et al. 2008). And literature showed that the bioavailability of PAHs from plant roots was positively correlated with Kow (Su and Zhu 2008). Studies have shown that most of the water-soluble organic matter with lgKow < 4 can be absorbed by plant roots directly, while hydrophobic organic pollutants with lgKow > 4 were strongly adsorbed on the epidermis of plant roots or soil particles and were not easily absorbed and translocated by plants. A previous study has shown that most of the accumulation of PAHs in the shoots was transmitted via the roots, and a small part of the PAHs from the

atmosphere remained on the surface of waxy leaves (Sun and Zhou 2016). In our experiment, under the stress of 128.38~149.95 mg·g<sup>-1</sup> PAH pollution, the concentration of PAHs in the plant after 120 and 150 days was not significantly different. In the later stage of Fire Phoenix growth, the content of PAHs did not increase significantly with the extension of growth time. However, in the examination of the uptake and translocation of benzo[a]pyrene by two kinds of ornamental plants, Sun and Zhou (2016) found that the PAH accumulation in the two plants increased with the extension of growth time, which may be a different phenomenon due to the use of different plant species. PAHs were found in the roots and shoots of Fire Phoenix, which indicates that PAHs have been actively transferred from the roots to shoots. Researchers studied the accumulation and distribution of PAHs in rice and found that significant differences between adjacent rice tissues (e.g., roots and stem), and the results indicated that the transport of PAHs was difficult to occur (Tao et al. 2006). It was understood that PAHs were proactively transmitted into cells through H<sup>+</sup>-coupled symporters, and transporters in various plants had different appetencies for PAHs (Zhang et al. 2012; Yin et al. 2014).

### 3.4 Plant contribution to plant-enhanced remediation of soil PAHs

Table 6: Contribution of plant to the removal of polycyclic aromatic hydrocarbons in soil.

Growth time	Treatments (mg kg <sup>-1</sup> )	T <sub>d</sub> (mg pot <sup>-1</sup> )	P <sub>ac</sub> (μg kg <sup>-1</sup> )	P <sub>ac</sub> / T <sub>d</sub> (%)
60 days	90.02~113.31	32.82	96.58	0.29
	128.38~149.95	43.85	29.23	0.07
120 days	90.02~113.31	37.23	358.83	0.96
	128.38~149.95	49.88	221.30	0.44
150 days	90.02~113.31	28.91	120.24	0.42
	128.38~149.95	56.03	274.58	0.49

The dissipation mechanism of PAHs in rhizospheric soil involves leaching, abiotic dissipation (surface adsorption, photooxidation, and volatilization), biodegradation, plant uptake, and accumulation (Gao and Zhu 2004; Sun and Zhou 2016). The removal of PAHs in non-rhizospheric soil is leaching, abiotic dissipation, and biodegradation. Therefore, the loss of PAHs in planted soil and non-planted soil can be described as

$$T_p = T_l + T_a + T_b + P_{ac} \quad (1)$$

$$T_{unp} = T_l + T_a + T_b^* \quad (2),$$

where  $T_p$  and  $T_{unp}$  represent the loss of PAHs (mg pot<sup>-1</sup>) in planted soils and unplanted soils,  $T_l$  represents the dissipation of leaching, and  $T_a$  stands for abiotic dissipation.  $T_b$  and  $T_b^*$  are the

dissipation of biodegradation in soils with and without plants, respectively.  $P_{ac}$  is the uptake and accumulation of PAHs in Fire Phoenix plants. Researchers verified that 4- and 5-ring PAHs in leachate were undetectable from soils with or without plants (Sun and Zhou 2016). And Reilley et al. found that the various abiotic dissipation of PAHs in rhizospheric soil and non-rhizospheric soil was negligible (Reilley et al. 1996). Therefore, the dissipation enhancement ( $T_d$ ) of PAHs in rhizospheric soil versus non-rhizosphere soil is

$$T_d = T_p - T_{unp} = P_{ac} + T_b - T_b^* \quad (3)$$

$$T_{bp} = T_b - T_b^* \quad (4)$$

In Eq. (4),  $T_{bp}$  represents the dissipation of PAHs by plant-promoted biodegradation.

In Table 6, in the soil contaminated with a high concentration of PAHs, the uptake and accumulation of PAHs by plants and the plant-promoted biodegradation had a significant increase in the dissipation of PAHs as the growth time increased. This result confirmed that the degradation rate of PAHs in the rhizospheric soil was significantly affected by the phytoremediation time. The planting time reached 150 days, and the degradation rate of each pollutant was greatly improved. The main factor for the dissipation of PAHs was the plant-promoted biodegradation that accounted for 99.04%–99.93% of PAH dissipation, suggesting the low contribution rate for PAH dissipation through uptake and transformation in Fire Phoenix was only approximately 0.07%–0.96%. It showed that plant accumulation was not the primary mode of PAH removal in soil. Plants promote the degradation of PAHs in the soil by stimulating the metabolism of microorganisms in the rhizosphere (Frutos et al. 2012; Kong et al. 2018). Thus, the rhizosphere was crucial in the phytoremediation of PAHs. The participation of rhizosphere-related microorganisms in the bioremediation of soil PAHs has been investigated (Khan et al. 2013; Li et al. 2019), and the rhizosphere of plants can improve the dissipation of PAHs (Cheema et al. 2010; Yu et al. 2011). The accelerated removal of PAH is mainly associated with increased bacterial activity and diversity. Additionally, The bioavailability of PAHs in rhizospheric soil increased due to the improved soil aeration and permeability, as well as the decomposition of soil aggregates (Hamdi et al. 2007). In addition, the compounds released from the roots (i.e., root exudates) may represent high carbon infiltration into the rhizosphere, and some exudates act as surfactants, increasing the solubility of PAHs. This indicates that the root system can stimulate microbial degradation in the rhizosphere (Li et al. 2019).

## 4. Conclusion

The present study showed that Fire Phoenix plants could grow in soil contaminated by high and low PAH concentrations. The total removal rates of the six PAHs in the high and low PAH concentrations were 80.36% and 79.79%, significantly higher than the 58.79% and 53.29% of the unplanted control group, respectively. Therefore, the removal rate of PAHs in the soil can significantly be increased by planting Fire Phoenix. The main factor in the dissipation of PAHs in this study was plant-promoted biodegradation, which accounted for 99.04–99.93% of the PAH dissipation, suggesting the low contribution uptake and

transformation in the Fire Phoenix is as low as approximately 0.07–0.96%. This result suggests that Fire Phoenix does not remove the PAHs in the soil by accumulation but promotes their dissipation by stimulating the microbial metabolism in the rhizosphere of the plant. The planting of Fire Phoenix can be used to remediate the soil contaminated by PAHs in Dagang Oilfield. Thus, this study provides a new approach to remediate contaminated soils from crude oil-contaminated areas, demonstrating a high practical application value of Fire Phoenix.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analysed during this study are included in this published article.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This work was funded by the National Natural Science Foundation of China (31770545).

### Authors' contributions

Xiaomei Wang: Formal analysis, Writing - original draft, Visualization. Jianping Sun: Writing - original draft, Visualization. Rui Liu: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. Tingyu Zheng: Investigation, Validation. YingNan Tang: Investigation, Validation.

### Acknowledgements

We thank the National Field Research Station of Shenyang Agroecosystems for providing use of their experimental site. This work was funded by the National Natural Science Foundation of China (31770545).

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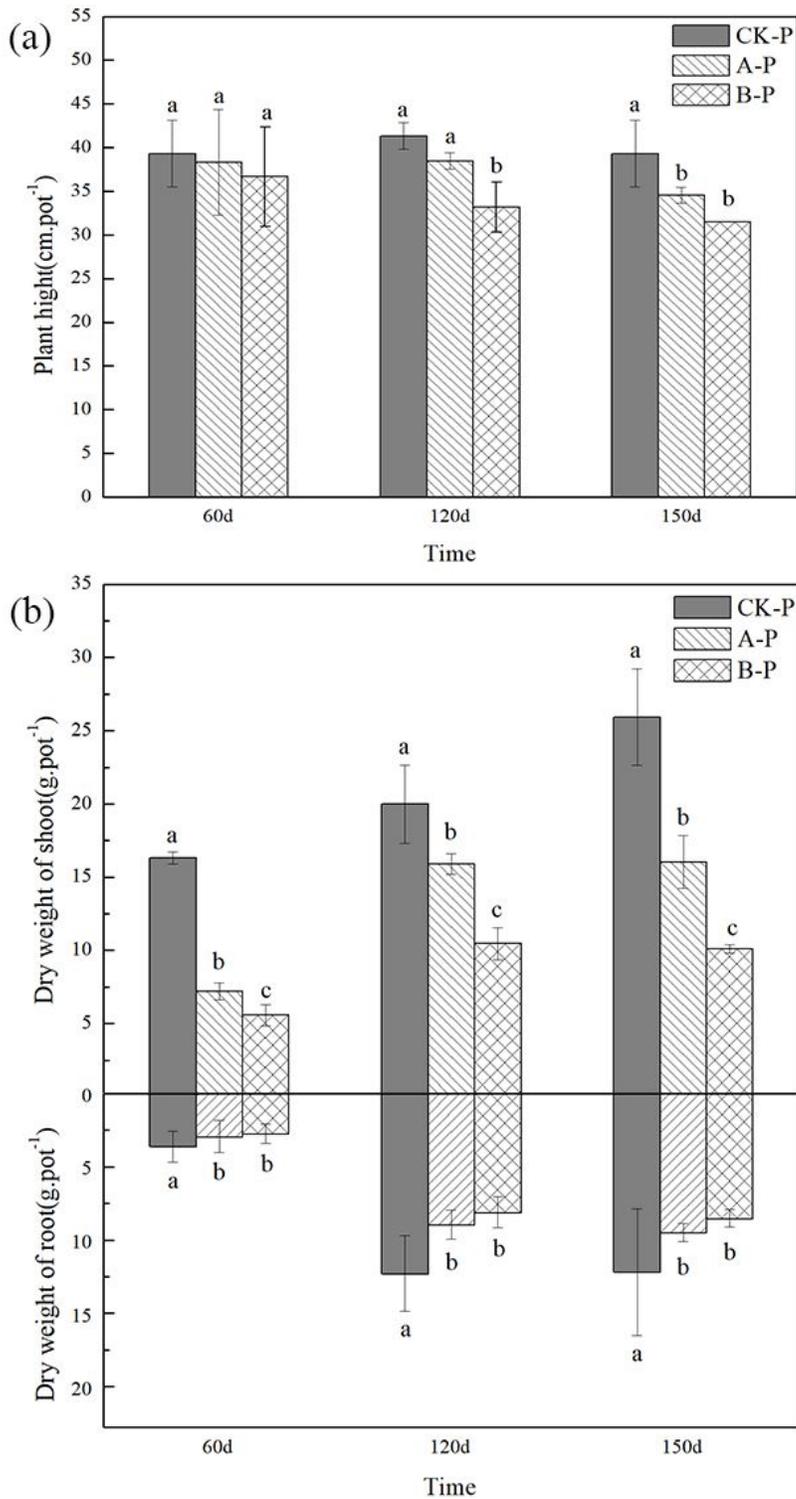
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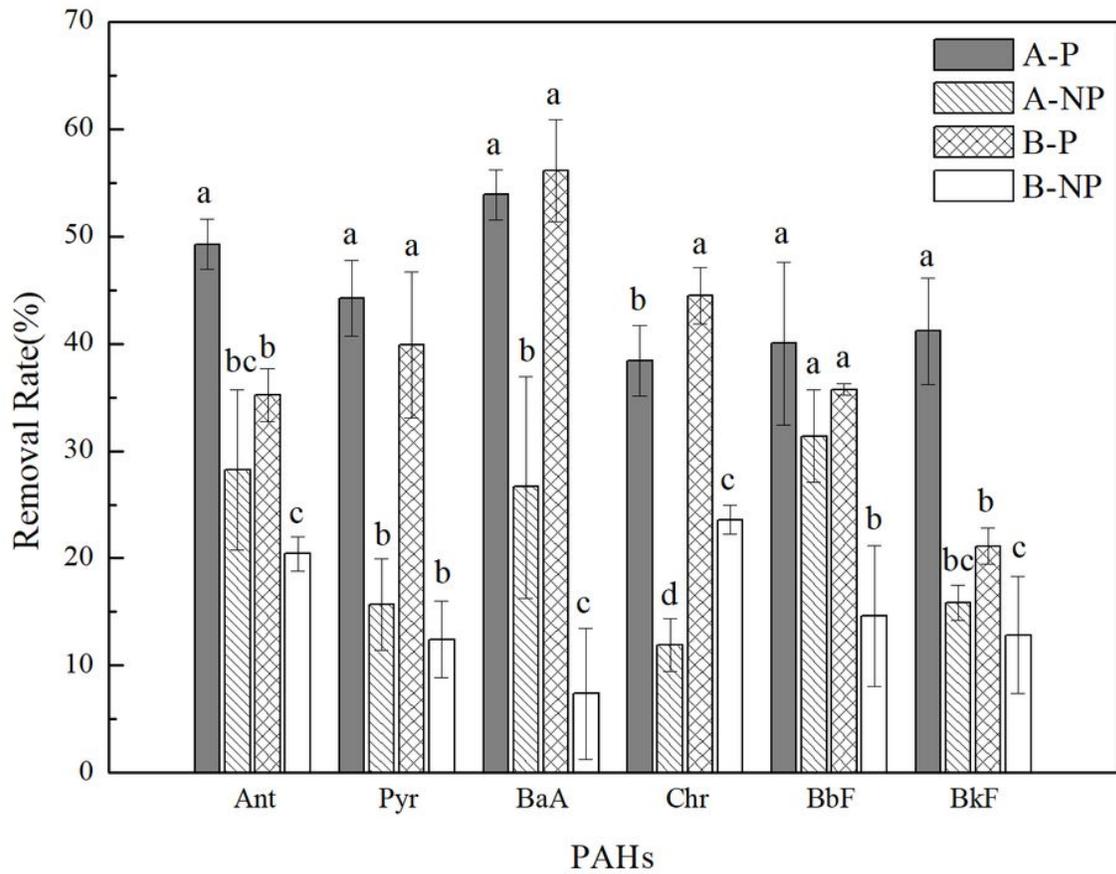
## Figures



**Figure 1**

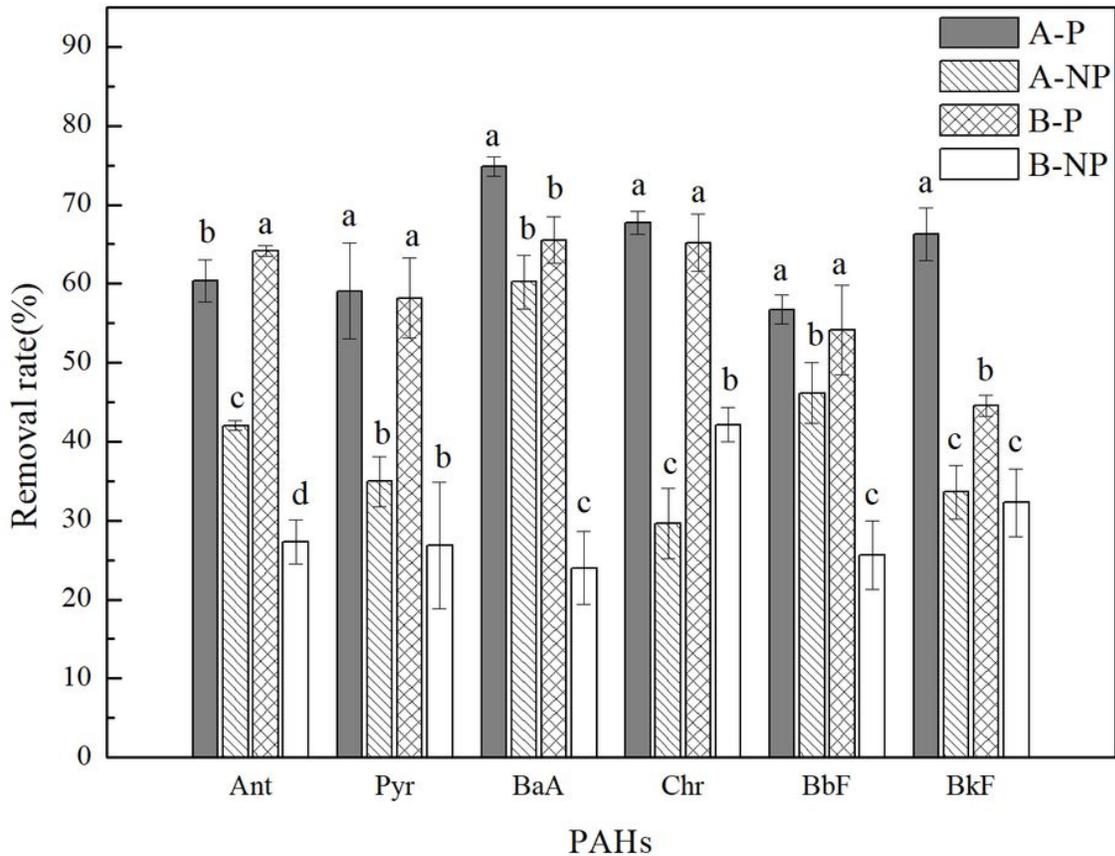
Growth of Fire Phoenix treated with polycyclic aromatic hydrocarbons (PAHs) at 60, 120, and 150 days. (a) Plant height, and (b) shoot and root biomass. CK-P stands for plants growing in natural soil without PAH pollution; A-P stands for plants growing in the contaminated soil A (PAH content: 90.02~113.31 mg·kg<sup>-1</sup>); B-P stands for plants growing in the contaminated soil B (PAH content: 128.38 ~149.95 mg·kg<sup>-1</sup>).

1); NP stands for treatments without plants. Values are the mean  $\pm$  SD of three replicates. Means with different letters are significantly different ( $P < 0.05$ ; LSD test).



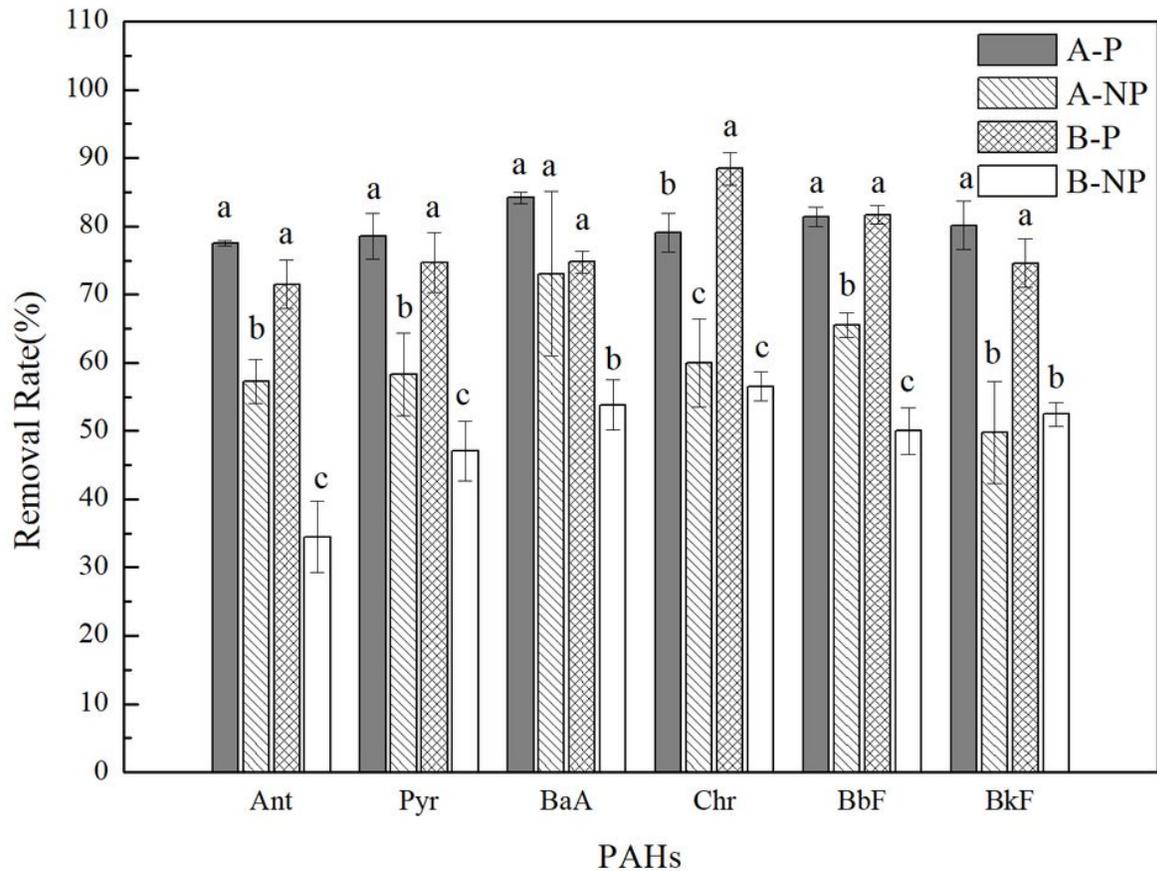
**Figure 2**

The removal rate of polycyclic aromatic hydrocarbons (PAHs) on day 60. A-P stands for plants growing in the contaminated soil A (PAH content: 90.02~113.31 mg·kg<sup>-1</sup>); B-P stands for plants growing in the contaminated soil B (PAH content: 128.38 ~149.95 mg·kg<sup>-1</sup>); NP stands for treatments without plants. Letters indicate statistical difference ( $P > 0.05$ ; LSD test). Ant, anthracene; Pyr, pyrene; BaA, benzo(a)anthracene; Chr, chrysene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene.



**Figure 3**

The removal rate of polycyclic aromatic hydrocarbons (PAHs) on day 120. A-P stands for plants growing in the contaminated soil A (PAH content: 90.02~113.31 mg·kg<sup>-1</sup>); B-P stands for plants growing in the contaminated soil B (PAH content: 128.38 ~149.95 mg·kg<sup>-1</sup>); NP stands for treatments without plants. Letters indicate statistical difference at significances ( $P > 0.05$ ; LSD test). Ant, anthracene; Pyr, pyrene; BaA, benzo(a)anthracene; Chr, chrysene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene.



**Figure 4**

The removal rate of polycyclic aromatic hydrocarbons (PAHs) on day 150. A-P stands for plants growing in the contaminated soil A (PAH content: 90.02~113.31 mg·kg<sup>-1</sup>); B-P stands for plants growing in the contaminated soil B (PAH content: 128.38 ~149.95 mg·kg<sup>-1</sup>); NP stands for treatments without plants. Letters indicate statistical difference ( $P > 0.05$ ; LSD test). Ant, anthracene; Pyr, pyrene; BaA, benzo(a)anthracene; Chr, chrysene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene.

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