Science of the Total Environment 473–474 (2014) 350–358

Contents lists available at ScienceDirect

Science of the Total Environment

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

Rhizosphere effects of PAH-contaminated soil phytoremediation using a special plant named Fire Phoenix

Rui Liu [a,*], Nan Xiao [a], Shuhe Wei [a,*], Lixing Zhao [b], Jing An [a]

[a] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] College of Science, Northeastern University, Shenyang 110004, China

HIGHLIGHTS

- Fire Phoenix species has considerable potential to remedy PAH contaminated soil.
- After 150 days, the removal rate of ∑8PAHs was up to 99.40% using Fire Phoenix.
- There was a dramatic increase in the activity of CAT, POP and PHO after planting.
- The activity of PPO had a significant negative relation with ∑8PAHs removal rate.
- Fire Phoenix may promote growth of the flora genus *Gordonia* sp. that degraded PAHs.

ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form 4 December 2013
Accepted 5 December 2013
Available online 27 December 2013

Keywords:
Polycyclic aromatic hydrocarbons (PAHs)
Enzymatic activity
Microbial community
Rhizosphere soil
Phytoremediation

ABSTRACT

The rhizosphere effect of a special phytoremediating species known as Fire Phoenix on the degradation of polycyclic aromatic hydrocarbons (PAHs) was investigated, including changes of the enzymatic activity and microbial communities in rhizosphere soil. The study showed that the degradation rate of Σ8PAHs by Fire Phoenix was up to 99.40% after a 150-day culture. The activity of dehydrogenase (DHO), peroxidase (POD) and catalase (CAT) increased greatly, especially after a 60-day culture, followed by a gradual reduction with an increase in the planting time. The activity of these enzymes was strongly correlated to the higher degradation performance of Fire Phoenix growing in PAH-contaminated soils, although it was also affected by the basic characteristics of the plant species itself, such as the excessive, fibrous root systems, strong disease resistance, drought resistance, heat resistance, and resistance to barren soil. The activity of polyphenoloxidase (PPO) decreased during the whole growing period in this study, and the degradation rate of Σ8PAHs in the rhizosphere soil after having planted Fire Phoenix plants had a significant ($R^2 = 0.947$) negative correlation with the change in the activity of PPO. Using an analysis of the microbial communities, the results indicated that the structure of microorganisms in the rhizosphere soil could be changed by planting Fire Phoenix plants, namely, there was an increase in microbial diversity compared with the unplanted soil. In addition, the primary advantage of Fire Phoenix was to promote the growth of flora genus *Gordonia* sp. as the major bacteria that can effectively degrade PAHs.

1. Introduction

As an important type of typical chemical pollutants in the soil environment, polycyclic aromatic hydrocarbons (PAHs) are primarily generated from human activities (Zhou et al., 2004, 2005), especially from the energy utilization process (Freeman and Cattle, 1990). PAHs can directly pollute water, air and soil, with the "three wastes" emissions and dumping occurrence in industrial and agricultural production, as well as farmland wastewater irrigation. Because PAHs exhibit the "three-induced effect" of strong carcinogenicity, mutagenicity and teratogenicity (Menzie et al., 1992; Wilson and Jones, 1993), the problem of PAH pollution has become a global environmental issue. So far, more and more attention has been paid to PAH-contaminated soils and their effective remediation (Zhou and Song, 2004).

There are many important potential methods for controlling and remedying PAH-contaminated soils, including physical remediation, chemical remediation, bioremediation and phytoremediation (Zhou and Song, 2004), wherein more and more attention has been paid to bioremediation technology due to the unique advantages such as low cost, no secondary pollution and large area application. Bioremediation is one of soil remediation technologies with the greatest potential. Phytoremediation is an emerging environmental pollution control technology proposed for use after bioremediation has been implemented.

* Corresponding authors at: Institute of Applied Ecology, Chinese Academy of Sciences, No. 72, Wenhua Road, Shenyang 110016, Liaoning, China. Tel.: +86 24 83970373; fax: +86 24 83970436.
E-mail addresses: liurui@iae.ac.cn, regardsrui@yahoo.com.cn (R. Liu), shuhewei@iae.ac.cn (S. Wei).

0048-9697/$ – see front matter.
http://dx.doi.org/10.1016/j.scitotenv.2013.12.027

Phytoremediation refers to transferring, accommodating or transforming toxic and hazardous pollutants in the environmental media by utilizing remediation plants, thereby making the pollutants harmless to the environment and repairing the effects of pollution in the environment (Sun et al., 2008; Zhou and Sun, 2004). In recent years, many studies on utilizing plants for remedying PAH-contaminated soils were performed both at home and abroad. Reilley (1996) studied the degradation of PAHs and discovered that the degradation of PAHs is increased as the density of microbes in a plant root zone is increased. The study demonstrated that plants have a prominent role to enhancing the degradation of PAHs. For example, the degradation rate of phenanthrene can be increased (on average) by 0.3%–1.1%, and the degradation rate of pyrene can be increased by 2.4%–53.8% under plant growth conditions. Another study also demonstrated the enhancement of soil microbial degradation, in which the average degradation rate of the total PAHs was demonstrated to be improved by 2.0%–4.7% for the presence of alfalfa in the soil compared with the non-plant control soil (Sun et al., 1999).

Phytoremediation of PAH contaminated soils primarily involves four types of mechanisms: direct absorption of PAHs by plants; plant volatility and adsorption; plant secretions and enzyme decomposition of PAHs; and the degradation of rhizosphere strengthens microbial communities to PAHs. The enzymes released by roots into soil can directly degrade PAHs (Kraus et al., 1999; Gramms et al., 1999; Rentz et al., 2005). The enzymes released into the environment after plant death can also continue to play a role in the decomposition of PAHs (Zhou et al., 2004). Ding et al. (2008) found that the content of PPO in ryegrass rhizosphere soils increased and the degradation of benzo[a]pyrene was accelerated in studying the influence of ryegrass on the dynamic change of benzo[a]pyrene in soil. Sun et al. (1999) reported an outdoor pot-culture study on alfalfa for phenanthrene biodegradation and found that the degradation rate of phenanthrene in the soil rhizosphere of growing plants was significantly higher than that in the soil without plant growth (control). In addition, the number of microorganisms in the rhizosphere soil was significantly higher than that in the non-rhizosphere soil. These results demonstrated that the degradation of phenanthrene in soil is attributed to the joint action of plants and their rhizosphere microorganisms.

In recent years, a great progress in phytoremediation of contaminated soils has been made (Sun et al., 2008; Peng et al., 2009; Wang et al., 2012a,b; Sujit et al., 2014), and plays a very important role in improving the quality of environment (Zhou et al., 2004; Liu et al., 2010; Wei et al., 2010; Helmi et al., 2012). Extensive literature on phytoremediation has reported that the root exudates (primarily including nutrients and enzymes) and the rhizosphere microbial communities are the two main factors that affect the performance of plant phytoremediation (Cai et al., 2010). However, because the transfer and the problems involved with the mutual action mechanism in the soil and the plants are not clear, no mature technology that can apply and promote the phytoremediation of contaminated soils in large fields exists currently, thereby greatly hindering its development.

The tested plant known as Fire Phoenix was screened from 14 plants after 4-year experiments (Liu and Zhou, 2012). The objective of the study was to investigate the relationship between rhizosphere enzymes, microbial community diversity and phytoremediation performance of Fire Phoenix, in particular, to clarify their mutual response and impact and to elucidate the mechanism of PAH contaminated soil phytoremediation.

2. Materials and methods

2.1. Experimental design

The aged PAH-contaminated soil used for this study was obtained (sampled to a depth of 250 mm) from the Shengli Oil Field in Dongying City, Shandong Province, China. The soil analysis was performed by the Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China. The contaminated soil had been classified as a drained brown soil, pH 7.66, in which the carbon (C), phosphorus (P), nitrogen (N) and available P concentrations were 45.77, 0.65, 0.73 and 0.002 $g \cdot kg^{-1}$, respectively. The uncontaminated reference soil samples were collected from the Wanliutang Park, Shenyang, China. And it had been classified as a drained brown soil, pH 6.70, in which the concentrations of C, P, N and available P were 12.82, 0.44, 0.80 and 0.011 $g \cdot kg^{-1}$, respectively. The range of the concentrations of PAHs in the contaminated soil collected was 228–398 $mg \cdot kg^{-1}$. The collected soil samples were sieved through a 4.00 mm sieve to ensure homogeneity. According to the pre-test results, all the plants tested could not grow in the aged PAH contaminated soil directly. Through the addition of uncontaminated reference soil, the contaminated soil collected was diluted to the range of 70.80–79.81 $mg \cdot kg^{-1}$ according to the experimental design. And its composition of $\sum$8PAHs was 1.19–1.47 $mg \cdot kg^{-1}$ of fluoranthene, 2.39–3.42 $mg \cdot kg^{-1}$ of pyrene, 2.71–2.88 $mg \cdot kg^{-1}$ of benzo(a)anthracene, 1.27–3.68 $mg \cdot kg^{-1}$ of chrysene, 7.57–9.90 $mg \cdot kg^{-1}$ of benzo(b)fluoranthene, 1.90–4.88 $mg \cdot kg^{-1}$ of benzo(k) fluoranthene, 12.35–18.17 $mg \cdot kg^{-1}$ of benz(a)pyrene and 38.18–40.06 $mg \cdot kg^{-1}$ of dibenzo(a, h)anthracene, respectively.

The tested plant known as Fire Phoenix is a combined Poaceae species including *Festuca arundinacea*, *Festuca elata* Keng ex E. Alexeev, and *Festuca gigantea* (L.) Vill.

The tested seeds of Fire Phoenix were purchased from the Kelaowu Seeds Company, Beijing, China.

The tested soil (2.5 kg) samples were added to 20 cm diameter pots. A disk of filter paper was placed at the bottom of each pot to prevent the dry soil from escaping out from the drainage holes, and the pots were placed on saucers. To each pot, the tested plant treatments ($n = 9$) were introduced when the germination of each seed was at 15 days.

Next, the plants studied in three replicates in the contaminated soil ($\sum$8PAHs = 70.80–79.81 $mg \cdot kg^{-1}$) were harvested after a 60-day, 120-day or 150-day culture. The three replicates of the control (no plants and only soil) were also maintained simultaneously with the same contaminated soil. The soils in the control were processed identically at the time of watering plants, and all treatments were processed during 150 days. The plants were sown in a growth chamber with a cycle of 16 h/25 °C in day and 8 h/15 °C at night. These treatments were watered every second day to maintain approximately 25% gravimetric water content. The experiments were performed from April 28th 2009 to September 29th 2009 and lasted for 150 days. The roots were shaken to dislodge loose soils and the attached rhizosphere soil samples were archived. The soil samples were transported to a laboratory on crushed ice in a cooler. The collected samples were stored at 4 °C for the determination of PAHs and enzymes, and at −70 °C for DNA extraction.

2.2. PAH extraction and analysis

Integrated extraction and cleanup were performed by the pressurized liquid extraction (PLE) with a Dionex ASE 200 accelerated solvent extractor. Briefly, 5 g of soil samples was ground in a mortar with approximately 5 g of diatomite. A 33 mL extraction cell was packed with two cellulose filters and 5 g of activated silica gel (for clean-up). The homogenized and dried sample was then transferred quantitatively to the extraction cell, and 200 μL of the surrogate standard solution was added directly on top of the sample and left undisturbed for 20 min to ensure full percolation throughout the sample. The remaining cell volume was filled with Ottawa sand (20–30 mm mesh) from AppliChem (Darmstads, Germany) as an inert matrix. The Ottawa sand was precleaned by heating at 450 °C over night.

The PLE program was described as follows. A mixture of n-pentane and dichloromethane (9:1 v/v) was used as a solvent at a pressure of 1.5 kPa and a temperature of 100 °C; the oven heat ramptime was 7 min, and the program had two extraction cycles with a 5 min static