Technical Note

Effect of rhizodeposition on pyrene bioaccessibility and microbial structure in pyrene and pyrene–lead polluted soil

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HIGHLIGHTS

- Bioaccessible pyrene accounted for the largest proportion of the total removal.
- Rhizodeposits enhanced significantly pyrene bioaccessibility in spiked soils.
- Rhizodeposition greatly enhanced the property of soil microbial populations.
- The removal of associated pyrene was related with MUFAs, PUFAs and CFAs abundances.

GRAPHICAL ABSTRACT

A B S T R A C T

Phytoremediation for PAH hydrocarbons has been widely studied, but few focus on the influence of rhizodeposition on their bioaccessibility during the process. This literature revealed the effect of celery (Apium graveolens) rhizodeposition on pyrene fractionation and bioaccessibility in simulated pyrene and pyrene–lead contaminated microcosms. A sequential extraction methodology was used to quantify different morphological fractions of pyrene in the soil, and phospholipid fatty acid (PLFA) pattern to monitor shifts in microbial populations. Bioaccessible pyrene accounted for the largest proportion of the total removal. Biodegradation of both bioaccessible and associated pyrene fractions was enhanced by celery rhizodeposition in pyrene spiked soils. However, rhizodeposition promoted the removal of bioaccessible rather than associated fractions in pyrene–lead spiked soils. In contrast, the bound fraction increased over time in pyrene spiked soils without amendment, but kept relatively stable in amended microcosms. It was found that rhizodeposition facilitated the reproduction of all the subgroups of soil microorganisms through PLFA analysis. Although all the subgroups contributed to the removal of bioaccessible pyrene, only abundances of unsaturated and cyclic fatty acids were positively correlated with the removal of associated pyrene. These findings provide meaningful insights into the microecological mechanisms involved in the phytoremediation of PAH polluted sites.

1. Introduction

As widely distributed refractory pollutants, PAHs intrude into the environment via spills of petroleum or incomplete pyrolysis of carbon-containing compounds (Gomez-Eyles et al., 2012). Therefore, PAH pollutants are often accompanied by oil hydrocar-
bons or heavy metals (Cang et al., 2013; Chigbo and Batty, 2013; Wang et al., 2013). Phytoremediation, a promising remedial strategy for PAHs, depends on the metabolic potential of rhizospheric microorganisms to degrade the contaminants, which is restricted by their bioaccessibility (Megharaj et al., 2011). Bioaccessible compounds are those available to cross an organism’s cellular membrane from the environment, if the chemical is accessible to the organism (Semple et al., 2004). Not only bioavailable but also potentially bioavailable fractions are involved in bioaccessibility. The bioaccessibility of PAH to soil microorganisms can be greatly reduced by sorption onto a complex mixture of black carbon, coal, kerogen and other organic matter in soil (Cornelissen et al., 2005). It is of great importance to evaluate bioaccessible PAHs to facilitate necessary and valid remediation strategies, as well as to avoid overestimating environmental and human risks. PAH in the soil can be grouped into bioaccessible fraction, associated fraction (chemically adsorbed by soil particles and unbioaccessible until desorption) and bound fraction with decreasing potential for biodegradation based on their morphological characteristics (Gao et al., 2009). Sequential extraction approaches are used to segregate different morphological PAH fractions in the soil (Macleod and Semple, 2003; Gao et al., 2009; Ma et al., 2012).

It is generally thought that plants can enhance the biodegradation of PAHs in the rhizosphere by improving microbial activity and diversity (Xie et al., 2012). In the process of phytoremediation, plants release root exudates, secretions, mucilages, and root turnover, involving inorganic and organic compounds, into the soil through rhizodeposition (Read et al., 2003; Meng and Zhu, 2010). These compounds will significantly modify the biophysical microenvironment resulting in shifts in soil microbial communities (Kamath et al., 2004; Gébrón et al., 2011). Meng and Zhu (2010) demonstrated that PAH degraders flourished in pyrene spiked microcosms when amended with simulated celery rhizodeposits. However, little is known about how rhizodeposition affect fractionation and bioaccessibility of organic contaminants in the soil during the process of phytoremediation, and whether the potential of different microbial populations for accessing PAH fractions discriminates or not (Xie et al., 2012). Thus researches are necessary to further comprehend the influence of rhizodeposition on PAH bioaccessibility and PAH-utilizing abilities of different microbial communities.

As a culture-independent, well-characterized and convenient technique, phospholipid fatty acid (PLFA) analysis has been demonstrated to be suitable to profile microbial community structure (Wu et al., 2009; Ben-David et al., 2011; Veresoglou et al., 2011). PLFAs are derived from phospholipids which are the major ingredients of living cell members. They decompose immediately upon the death of cells. Moreover, signature fatty acids can be used as indicators for biomass belonging to certain microbial groups, making it possible to monitor shifts in microbial populations (Kaur et al., 2005).

The aim of this study was to investigate the influence of plant rhizodeposition on pyrene fractionation and bioaccessibility, as well as shifts in microbial community structure in pyrene and pyrene–lead polluted soils. Furthermore, the abilities of various microbial communities to utilize pyrene fractions were discussed to reveal the microecological mechanisms involved in pyrene bioaccessibility and biodegradation.

2. Materials and methods

2.1. Chemicals

Pyrene (98% purity) was purchased from Aladdin Reagent. All the other chemicals (analytical grade or better) were bought from Sinopharm.

2.2. Soil

Pollution free soil (clean soil) utilized in this study was collected from the 0 to 20 cm top layer of a vacant lawn at Shanghai University, China. Its properties are listed in Supplementary Material (SM), Table SM-1. After air-drying, the soil was sieved through a 2 mm mesh to get homogenized. To minimize the influence of solvents on indigenous microorganisms, the soil was spiked with pyrene or lead in a stage-by-stage process. For the spiking process, 500 mg of pyrene was dissolved in 100 mL of dichloromethane and mixed with 1 kg of clean soil. After aging for 100 d at 4 °C in the dark, the soil was mixed with 9 kg of clean soil to make the final pyrene concentration to be 50 mg kg⁻¹. Then lead nitrate (Pb(NO₃)₂, analytical grade) was applied to the pyrene spiked soil to obtain the pyrene–lead polluted soil, and final lead concentration was 115 mg kg⁻¹. No significant pH variation was detected during the procedure of spiking and experiment.

2.3. Preparation of plant rhizodeposits

In the preliminary experiment, celery (Apium graveolens) turned out to be the most efficient in pyrene removal among the nine tested plants (Table SM-2). Previous study also proved the excellent pyrene degradation ability of celery (Yi and Crowley, 2007). Therefore, celery was selected as the model plant in this study. After germination, celery seedlings were grown for 40 d in a greenhouse, then fine roots were collected as sources of root rhizodeposits and stored at −80 °C till utilization (Meng and Zhu, 2010).

2.4. Microcosm setup

Four groups of microcosms were set up: pyrene spiked soils (P), pyrene–lead spiked soils (PL), pyrene spiked soils amended by root rhizodeposits (PP), and pyrene–lead spiked soils amended by root rhizodeposits (PPL). Spiked soils were sieved (<2 mm) and homogenized before 35 g aliquots were packed into each Erlenmeyer flask of 50 mL capacity. Fresh root tissues were ground under liquid nitrogen, then 2.8 g ground roots were added into each Erlenmeyer flask. Soils in each microcosm were then mixed and moistened to 30% moisture with sterilized deionized water prior to be incubated at 28 °C in the dark. Every setup was prepared in triplicates. Soil samples were taken at 10, 20, 30 and 40 d, and lyophilized immediately before sieved through a 0.125 mm mesh. Treated soils were stored at −80 °C till analysis.

2.5. Analytical methods

Different morphological pyrene in the soil was detected by a 3-step sequential extraction method according to previous studies. The bioaccessible fraction was extracted by vortex mixing 5 mL of butanol with 1 g of freeze-dried soils for 30 s. The mixture was centrifuged at 2200g for 20 min. Then the supernatant was cautiously removed, filtered, and concentrated to detect pyrene content (Gomez-Eyles et al., 2010). After that, the associated fraction was ultrasonically extracted from the residual solid phase by dichloromethane for 30 min, and this process was repeated 3 times. The resulting liquids were combined, evaporated, and redisolved in 1 mL of n-hexane (Ma et al., 2012). Finally, bound pyrene was extracted from the remaining soil according to Ma et al. (2012). Pyrene content was quantified by GC–MS (Agilent 6890N/5975B) equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm). The temperature was kept at 100 °C for 2 min, then 10 °C min⁻¹ to 300 °C, where it was held for 5 min. The recovery ratio is 94%.

Microbial community abundances were assessed by PLFA pattern. PLFAs were extracted from 3 g of freeze-dried soils by
15.8 mL the mixture of citrate buffer solution, methanol, and chloroform (0.8:2.0:1.0, v/v) according to previous studies (Yao and Wu, 2010; Zhang et al., 2012). Then their contents were analyzed by GC–MS. The temperature was kept at 80 °C for 1 min, then 50 °C min⁻¹ to 150 °C and 2.5 °C min⁻¹ to 195 °C, held for 3 min, finally 2.5 °C min⁻¹ to 240 °C, where it was held for 2 min. Each individual fatty acid was quantified with methyl nonadecanoate as the internal standard substance and expressed as nmol g⁻¹.

2.6. Statistical analysis

In this paper, each data was the mean value (±SD) of three replicates. SPSS 17.0 was used for ANOVA and Pearson correlation matrix. Significant differences in the main effect were further analyzed by paired comparisons, with the Duncan’s multiple range tests.

3. Results and discussion

3.1. Bioaccessibility of pyrene

Bioaccessible fraction exhibited progressive biodegradation over time and took the largest proportion of total pyrene removal in all groups (Fig. 1). Rhizodeposition significantly enhanced the biodegradation of bioaccessible pyrene in both pyrene and pyrene–lead polluted soils (P < 0.05). When amended with rhizodeposits, the bioaccessible fraction further decreased 12.7 and 9.4 mg kg⁻¹ in pyrene and pyrene–lead spiked soils, respectively. Similarly, it was found that the pyrene removal in the rice rhizosphere resulted mainly from the evident degradation of bioaccessible fraction (Macleod and Semple, 2003; Ma et al., 2012).

In contrast, associated pyrene showed poorer potential for biodegradation (Fig. 1). Addition of rhizodeposits promoted associated pyrene to further decrease 1.7 mg kg⁻¹ (P < 0.05) in pyrene spiked soils (Fig. 1a). However, the biodegradation of associated pyrene could not be accelerated by celery rhizodeposits in pyrene and lead contaminated microcosms (Fig. 1b). This phenomenon can be explained that the presence of heavy metals enhanced PAH sorption in the soil (Luo et al., 2010). Plants could affect various activities (adsorption, desorption, migration, transformation and degradation) of organic pollutants in the soil through rhizodeposition. In the mucilages of mainz, lupin and wheat, Read et al. (2003) identified phospholipid surfactants which promoted phosphate desorption in the soil. Yi and Crowley (2007) found that linoleic acid, one of the main components in celery rhizodeposits, enhanced pyrene biodegradation to a large extent. Gao et al. (2010) proved that citric and oxalic acid promoted the desorption of pyrene and phenanthrene in the soil.

To better understand the biodegradation of the bioaccessible and potentially bioaccessible pyrene fractions in the soil, the first-order kinetics was used to characterize their removal rates (Table 1). Biodegradation of the bioaccessible fraction fitted in with the first-order model much better than the associated fraction with higher correlation efficient. Furthermore, biodegradation rate constants of the bioaccessible fraction were 2–6 times higher than those of the associated fraction. It deserves to mention that the dissipation rates of bioaccessible and associated fractions were significantly relevant with each other, with Pearson correlation coefficient of 0.595 (P < 0.01). This demonstrated that the degradation of the bioaccessible fraction facilitated the desorption and removal of the associated fraction in spiked soils.

Though bound residual pyrene has a content less than 0.1 mg kg⁻¹ in this study, they were vital for the assessment of the pyrene sequestration in polluted soils. Bound residual pyrene to further increase 1.7 mg kg⁻¹ (P < 0.05) in pyrene spiked soils (Fig. 1a). However, the biodegradation of associated pyrene could not be accelerated by celery rhizodeposits in pyrene and lead contaminated microcosms (Fig. 1b). This phenomenon can be explained that the presence of heavy metals enhanced PAH sorption in the soil (Luo et al., 2010). Plants could affect various activities (adsorption, desorption, migration, transformation and degradation) of organic pollutants in the soil through rhizodeposition. In the mucilages of mainz, lupin and wheat, Read et al. (2003) identified phospholipid surfactants which promoted phosphate desorption in the soil. Yi and Crowley (2007) found that linoleic acid, one of the main components in celery rhizodeposits, enhanced pyrene biodegradation to a large extent. Gao et al. (2010) proved that citric and oxalic acid promoted the desorption of pyrene and phenanthrene in the soil.

Fig. 1. Remaining contents of bioaccessible and associated fractions in pyrene spiked (a) and pyrene–lead co-spiked (b) soils at 0, 10, 20, 30 and 40 d. Error bars denote the standard deviations.

Table 1

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<th>Bioaccessible pyrene</th>
<th>Associated pyrene</th>
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<tr>
<td></td>
<td>k [a]</td>
<td>r² [b]</td>
</tr>
<tr>
<td>P</td>
<td>0.020 ± 0.002</td>
<td>0.96</td>
</tr>
<tr>
<td>PP</td>
<td>0.056 ± 0.001</td>
<td>1.00</td>
</tr>
<tr>
<td>PL</td>
<td>0.026 ± 0.001</td>
<td>1.00</td>
</tr>
<tr>
<td>PPL</td>
<td>0.065 ± 0.001</td>
<td>0.96</td>
</tr>
</tbody>
</table>

[a] k, First-order rate constant calculated by the values (mean of three replicas) of bioaccessible or associated fractions.
[b] r², Correlation efficient.

Fig. 2. Remaining pyrene in the bound fraction at 0, 10, 20, 30, and 40 d. (●) only pyrene, (○) pyrene + rhizodeposits, (▲) pyrene + lead, (×) pyrene + lead + rhizodeposits; error bars denote the standard deviations.
was correlated with the fractions remaining in fulvic acids, humic acids, and humin after exhaustive extraction (Tao et al., 2010). The content of bound pyrene increased remarkably over time in pyrene spiked soils without amendment, and reached to 0.05 mg kg\(^{-1}\) at 40 d (Fig. 2). However, it remained relatively stable, ranging from 0.02 to 0.03 mg kg\(^{-1}\), when amended with celery rhizodeposits. In pyrene–lead spiked soils, the residual fraction remained relatively stable no matter celery rhizodeposits were added or not. The inhibited formation of residual pyrene, as well as enhanced degradation of the bioaccessible and associated fractions, demonstrated that plant rhizodeposition improved significantly pyrene bioaccessibility in the soil. The various components of plant rhizodeposits could lead to multiple influences on the bioaccessibility of pyrene. Gao et al. (2010) demonstrated that artificial root exudates enhanced the desorption of phenanthrene and pyrene in soil, which contributed to the enhancement of their bioaccessibility.

### 3.2. Microbial community structure

A total of twenty-nine PLFAs were detected in all the samples, among which 16:0, 16:1\(\_\_9t\), and 18:2\(\_\_6,9\) were dominants (Fig. 3). PLFAs, which are in feature structure responding to their genetic species, provide quantitative measurement of the total microorganisms and certain subgroups in the soil (Kaur et al., 2005). Monounsaturated fatty acid 16:1\(\_\_9t\) is generally regarded as sensitive indicator for Arbuscular mycorrhizal fungi (Yao and Wu, 2010). Other common PLFAs 18:2\(\_\_6,9\) and 18:1\(\_\_9t\) are also regarded as fungal indicators (Kaur et al., 2005; Joergensen and Wichern, 2008).

Generally, rhizodeposition enhanced significantly (\(P<0.05\)) the abundances of PLFA subgroups, including monounsaturated fatty acids (MUFAs), cyclopropyl fatty acids (CFAs), polyunsaturated fatty acids (PUFAs), straight-chain saturated fatty acids (SSFAs) and branched-chain saturated fatty acids (BSFAs) in both pyrene and pyrene–lead polluted soils (Fig. 4). SSFAs, BSFAs and MUFAs have relatively higher proportions in the total amount of PLFAs compared with CFAs and PUFAs. Positively correlated with the amount of microbial biomass, the total PLFAs (TPLFAs) content is a good indicator for the whole biomass of soil microbes in the soil (Blåth and Anderson, 2003). The increase in abundances of all the

**Fig. 3.** Chromatogram model of PLFAs obtained in full-scan mode from the soil samples. (1) 12:0; (2) i13:0; (3) 14:0; (4) i15:0; (5) a15:0 (6) 15:0; (7) i16:0; (8) 16:1\_9t; (9) 16:1\_7c; (10) 16:1\_7t; (11) 16:1\_5c; (12) 16:0; (13) 10Me16:0; (14) 17:1\_6,9; (15) i17:0; (16) a17:0; (17) 17:1\_7c; (18) 17:1\_5c; (19) cy17:0; (20) 17:0; (21) 18:2\_6,9; (22) 18:1\_9t; (23) 18:1\_7c; (24) 18:1\_5c; (25) 18:1\_7t; (26) 10Me18:0; (27) cy19:0; (28) 19:0 (internal standard); (29) i20:0; (30) 20:0.

**Fig. 4.** Abundances of SSFAs (a), BSFAs (b), MUFAs (c), PUFAs (d), CFAs (e) and TPLFAs (f) in pyrene and pyrene–lead spiked soils. Different letters indicate statistically significant differences in the abundances of PLFA subgroups among treatments (Duncan test, \(P<0.05\), \(n=3\)).
PLFA subgroups when amended with rhizodeposits indicated that plant promoted the growth of microbial populations through rhizodeposition.

It is typically thought that heavy metals exerted an adverse influence on PAH biodegradation through inhibiting microbial activity and changing PAH lability (Sandrin and Maier, 2003; Thavamani et al., 2011). However, Thavamani et al. (2012) discovered cadmium did not diminish the PAH-degrading ability of microbial consortium in the soil. Similarly, lead did not either depress microbial populations nor inhibit the degradation of pyrene in the present study.

3.3. Correlation between the pyrene removal and PLFA abundances

Abundances of not only TPLFAs but also all the subgroups were positively correlated with the degradation rate of bioaccessible pyrene (Table 2). However, the removal rate of associated pyrene was positively correlated with abundances of MUFAs, PUFAs and CFAs, rather than SSFAs and BSFAs. The increase in MUFAs, PUFAs and CFAs content must have contributed to the enhanced degradation of the bioaccessible and associated pyrene in amended soils (Figs. 1 and 4). The removal of bound residual pyrene had no significant correlation with any PLFA subgroups (Table 1). Abundances of SSFAs and BSFAs are generally considered to be positively correlated with bacterial biomass, PUFAs with fungal biomass, MUFAs and CFAs with the biomass of Gram-negative and aerobic microorganisms (Zornoza et al., 2009; Zhang et al., 2010; Cao et al., 2012). Gram-negative bacteria, aerobes and fungi are common PAH-biodegrading microbes in the soil (Zhang et al., 2011). Su and Yang (2009) found that Gram-negative bacteria were more tolerant with PAH than other microbes. The positive correlation between the degradation of associated pyrene and abundances of MUFAs, PUFAs and CFAs indicates that Gram-negative bacteria, aerobes and fungi get access to and degrade adsorbed pyrene more easily.

4. Conclusions

Plant rhizodeposits improved the biodegradation of not only bioaccessible but also associated fractions of pyrene in spiked soils. The presence of lead and celery rhizodeposits both inhibited the formation of bound residual pyrene. It was found that rhizodeposits enhanced the prosperity of microbial populations through analysis of PLFAs. Though the removal rate of bioaccessible pyrene was positively correlated with abundances of all the PLFA subgroups, that of associated pyrene was with contents of MUFAs, PUFAs and CFAs rather than SSFAs and BSFAs. To our knowledge, this is the first literature that explores the influence of rhizodeposition on PAH fractionation and bioaccessibility and tries to expound the microbial ecological mechanisms involved. These results provide valuable information to better understand the mechanisms of rhizoremediation in both PAH and PAH-heavy metals spiked sites.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.09.105.

Table 2

<table>
<thead>
<tr>
<th>Different fractions of pyrene</th>
<th>TPLFAs</th>
<th>SSFAs</th>
<th>BSFAs</th>
<th>MUFAs</th>
<th>PUFAs</th>
<th>CFAs</th>
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<tbody>
<tr>
<td>Bioaccessible fraction</td>
<td>0.595</td>
<td>0.336</td>
<td>0.460</td>
<td>0.722</td>
<td>0.768</td>
<td>0.586</td>
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<tr>
<td>Associated fraction</td>
<td>0.353</td>
<td>0.265</td>
<td>0.241</td>
<td>0.403</td>
<td>0.421</td>
<td>0.351</td>
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<tr>
<td>Bound residual fraction</td>
<td>−0.021</td>
<td>−0.011</td>
<td>0.067</td>
<td>−0.102</td>
<td>−0.072</td>
<td>0.049</td>
</tr>
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</table>

* Different levels of significance were marked with asterisks.

** P < 0.05.
*** P < 0.01.

References


