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Response of bacterial community structure to disappearance of phenanthrene and pyrene from sediment with different submerged macrophytes

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ABSTRACT

Disappearance of phenanthrene and pyrene (on the basis of butanol-extractable fraction) from sediment with different submerged macrophytes, *Hydrilla verticillata* (H), *Myriophyllum verticillatum* (M), *Vallisneria spiralis* (V) and *Potamogeton crispus* L. (P), was investigated. A relationship between the remedial effect and the bacterial community structure was analyzed using PCR–DGGE. During a 54-day experimental period, removal efficiencies of the two contaminants were the highest in V treatment, followed by M treatment, H and P treatments, and then unplanted treatment. Moreover, only a small part of the removal increments was caused by plant accumulation, indicating that the enhanced removal was mainly due to microbial degradation. DGGE profile analysis indicated that bacterial community in sediments was relatively stable after planting. PAH-degrading bacteria were widely distributed in all of the sediment samples but species differences among the samples were obvious. Moreover, the DGGE band-based similarity between *V. spiralis* and the other three plants was the highest in M treatment, followed by H treatment, and then P treatment, which is similar to the trend of removal efficiencies of the two contaminants. Therefore, it can be concluded that the difference between the four submerged macrophytes in the removal of phenanthrene and pyrene from sediment might be largely due to the changes in the microbial community structure.

1. Introduction

In aquatic environments, sediments often serve as the major sink for hydrophobic organic compounds (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), which are mainly associated with organic matter in sediments as a result of their high hydrophobicity and slow degradation rate (Haritash and Kaushik, 2009). Phytoremediation, a low-cost remedial technology taking advantage of the root systems of plants to remediate contaminated soils and sediments, has been extensively researched in recent years (Haritash and Kaushik, 2009; Meng and Chi, 2015). For HOCs, plant-accelerated microbial degradation was considered to be the main mechanism increasing their disappearance from soils and sediments (Perelo, 2010; Meng and Chi, 2015).

It has been known that phytoremediation efficiency is influenced by plant types and varieties (Lee et al., 2008). Plants accelerate the remediation of contaminants in soil and sediment through secreting exudates and enzymes which stimulate microbial and biochemical activity in the rhizosphere. Analyses of microbial communities showed that microbial communities were clearly different between the rhizosphere and the non-rhizosphere samples, and were also influenced by plant species. For example, Kaimi et al. (2007) selected twelve plant species and found that eight plant species enhanced the removal of total petroleum hydrocarbon from soil, which was significantly correlated with the dehydrogenase activity in soil. Wang and Chi (2012) found that *Phragmites australis* and *Typha orientalis* could enhance biodegradation of phthalate acid esters (PAEs) in sediments. The enhancement of PAE biodegradation was mainly the result of changes in the microbial community structure for different plant species.

Organic contaminants in soil and sediment can be categorized into two forms: the extractable and non-extractable fractions (Northcott and Jones, 2000). The extractable fraction is normally considered to be readily bioavailable with stronger toxicity to organisms as well as higher degradation rate, while the second form has lower availability in soil and sediment. Mild extracting solvents, such as *n*-butanol, are widely utilized to extract the bioavailable fraction of HOCs in soil and sediment (Sun et al., 2013; Meng et al.,

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2015). For example, there was a good correlation between PAHs extracted by *n*-butanol and PAHs absorbed by earthworms and plants (Gomez-Eyles et al., 2010). Ma et al. (2012) found that the decline of PAH content in rice rhizosphere was faster than that in the bulk soil, and the variation of total PAHs was largely due to that of *n*-butanol-extracted fraction. Therefore, extraction with mild extracting solvents provides a helpful tool for the investigation of phytoremediation effect in soils and sediments.

Polymerase chain reaction (PCR) has been used to detect and characterize bacterial species in environmental samples for a decade (Kuske et al., 2006). Combined with denaturing gradient gel electrophoresis (DGGE), the products of 16S rDNA amplified via PCR can be separated, and PCR–DGGE is a useful way to study the diversity and dynamics of the microbial community in environmental samples. In this study, *n*-butanol-extractable PAH fractions in sediments were determined. Four typical widespread submerged macrophytes (including *Hydrilla verticillata, Myriophyllum verticillatum, Vallisneria spiralis* and *Potamogeton crispus* L.) were use to find out the differences between the four submerged macrophytes in the removal of phenanthrene and pyrene from sediment. Furthermore, the correlation between the remedial effect and the bacterial community structure was analyzed based on the PCR–DGGE.

2. Materials and methods

2.1. Materials

Four submerged macrophytes were selected for the experiment. Two of them (*M. verticillatum* and *P. crispus*) were obtained from Tianjin, China. *H. verticillata* was collected from Zhejiang, China. A seed culture technique was used to cultivate *V. spiralis*. Sediments were sampled from the surface layer (0–30 cm; pH 7.47, 1.86% OC) of Haihe River, Tianjin, China. The sediments were air-dried, manually crushed, and then sieved with a 2-mm mesh to take out plant residues and stones. High purity phenanthrene and pyrene (equal volume mixing) dissolved in acetone were then spiked into a small portion (1/6) of the sediments. After evaporation of acetone, the sediments were fully mixed with the residual sediments. To make sure the homogeneity of the sediment samples, the sediments were sieved with a 2-mm mesh again. The finial PAH contents (on a dry weight basis) were determined to be 10.4 mg kg^{-1} for phenanthrene and 11.2 mg kg^{-1} for pyrene.

2.2. Experimental design

Portions of 100 g spiked sediment samples were put into 200-mL glass beakers. Five similar apical shoots (10–15 cm long) were selected out, weighted, and then planted in each beaker. The beakers were then transferred into glass microcosms (0.6 m long × 0.3 m wide × 0.4 m high) under controlled conditions of 24 ± 1 °C, 2200 ± 100 lx and a 12:12 h light-dark cycle. Submerged pumps were used to stir water in the microcosms to keep homogeneity. The total experimental time was 54 days. For each sampling time (i.e. days 18, 36 and 54), 3 replicate beakers per treatment were taken from the microcosms.

After removing the supernatant water from the beakers, plant samples were removed from sediment, and then washed to get rid of any sediment particles adhering to the roots. After dried with filter paper, one gram of the plant samples was transferred to a histoid grinding tube for PAH analysis. Sediment samples were divided into two portions. One was for PCR–DGGE analysis; the other was stored at −20 °C for PAH analysis after being freeze-dried.

2.3. Analytical methods

2.3.1. PAH analysis

PAH analysis in sediment and plant samples was carried out using the method by Meng et al. (2015). In brief, the PAHs in sediments and plants were firstly extracted by *n*-butanol and dichloromethane, respectively, followed by purification on a chromatographic column. PAHs in the purified extracts were determined by GC–MS in a SIM mode.

2.3.2. DNA extraction and PCR–DGGE

Sediment bacterial DNA was extracted by the FastDNA® Spin Kit for Soil (Mpbio, CA, USA). Amplification of bacterial 16S rDNA was carried out using the universal primer pair (338F: 5′-CCTACGGGAGGCAGCAG-3′ and 518R: 5′-ATTACCGCGGCTGCTGG-3′). A GC clamp (5′-CGCCCGGGGCGCGCCCCGGGGCGGGGCGGGGGCGCGGGGGG-3′) was connected to the 5′ terminal of primer 338F. These primers were obtained from Beijing Bio-ulab Company. The PCR and DGGE were conducted according to the procedure by Ye et al. (2015).

2.3.3. DNA bands purification, cloning and sequencing

Typical bands were excised from the gels and re-amplified by using universal bacterial primer pair 338F and 518R. The re-amplification of bacterial 16S rDNA was performed using the method by Ye et al. (2015). The purified PCR products were then cloned into a pMD18-T vector for sequence identification (Bio-ulab, Beijing, China). The 16S rDNA sequences of the strains have been deposited in GenBank. The accession numbers were KP791979, KP791976, KP791981–KP791988 and KP791989–KP791991 (Table 1).

2.3.4. Statistical analysis

Data were compared using analysis of variance and Duncan's test ($p < 0.05$); SPSS 20.0 software was used. DGGE band profiles were analyzed by Quantity One 4.6.2 software (Bio-Rad). Similarity matrix of DGGE band profiles was assessed using the Dice coefficient. The clustering algorithm of UPGMA was used to generate a dendrogram.

3. Results and discussion

3.1. PAH contents

During the experiment, contents of phenanthrene and pyrene in sediments declined with time in all of the treatments (Fig. 1). The loss of the PAHs from sediments was the highest for *V. spiralis* and the lowest for unplanted sediments. At the end, the removal efficiencies were in the order of *V. spiralis* (86.7% for phenanthrene and 84.6% for pyrene) > *M. verticillatum* (69.6% and 63.5%) > *P. crispus* (59.1% and 57.5%) and *H. verticillata* (53.6% and 52.4%) > unplanted control (40.8% and 38.9%). The removal enhancement of the four plant species was 45.9%, 28.8%, 18.3% and 12.8% for phenanthrene, and 45.8%, 24.6%, 18.6% and 13.5% for pyrene, respectively. It can be seen that the enhancement of removal efficiencies was the highest in *V. spiralis* treatment. The results indicate that all of the four submerged macrophytes could promote PAH dissipation in sediments and their interspecific differences in PAH removal from sediments were significant. It can also be seen that the overall extent of PAH loss from sediments was clearly compound-dependent, exhibiting higher removal ratio of phenanthrene than that of pyrene because of lower water solubility of phenanthrene (Fig. 1). This phenomenon is consistent with that previously reported (Lee et al., 2008; Meng et al., 2015).

Contents of the PAHs in the four submerged macrophytes (on a fresh weight basis) were low, ranging from 0.12 to 0.37 mg kg^{-1}