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Effects of low molecular-weight organic acids and dehydrogenase activity in rhizosphere sediments of mangrove plants on phytoremediation of polycyclic aromatic hydrocarbons

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HIGHLIGHTS

- LMWOAs and dehydrogenase in the rhizosphere sediment promoted the removal of PAHs.
- LMWOAs and dehydrogenase activity changed with the levels of PAH contamination.
- Citric acid was the most dominant, followed by succinic acid, among six LMWOAs.
- *Bruguiera gymnorrhiza* in 3 mangrove plants was most efficient in removing PAHs.

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ABSTRACT

This work evaluated the roles of the low-molecular-weight organic acids (LMWOAs) from root exudates and the dehydrogenase activity in the rhizosphere sediments of three mangrove plant species on the removal of mixed PAHs. The results showed that the concentrations of LMWOAs and dehydrogenase activity changed species-specifically with the levels of PAH contamination. In all plant species, the concentration of citric acid was the highest, followed by succinic acid. For these acids, succinic acid was positively related to the removal of all the PAHs except Chr. Positive correlations were also found between the removal percentages of 4-and 5-ring PAHs and all LMWOAs, except citric acid. LMWOAs enhanced dehydrogenase activity, which positively related to PAH removal percentages. These findings suggested that LMWOAs and dehydrogenase activity promoted the removal of PAHs. Among three mangrove plants, *Bruguiera gymnorrhiza*, the plant with the highest root biomass, dehydrogenase activity and concentrations of LMWOAs, was most efficient in removing PAHs.

1. Introduction

Mangrove forests are very important woody communities in the intertidal zone of tropical and subtropical coasts, and they are flooded periodically by tidal water. The community has high biodiversity and productivity, but often is polluted by human activities (Tam and Wong, 1998; Cheng et al., 2012). One of the most common organic pollutants in mangrove sediments requiring remediation is polycyclic aromatic hydrocarbons (PAHs), because of their ubiquitous, persistence, toxicity, carcinogenicity and mutagenicity (Song et al., 2012). Some mangrove plants were able to tolerate and remove PAHs in contaminated sediments, as PAHs with low water solubility were unlikely to be absorbed into plant tissues (Ke et al., 2003). Biodegradation by the microorganisms associated with root surfaces and sediments could be an important process (Luan et al., 2006; Tam and Wong, 2008). However, published information on phytoremediation has focused mainly on the uptake and accumulation of heavy metals in terrestrial plants (Intawongse and Dean, 2006; Ranieri, 2012), such as annual grass, leguminous plants and trees in upland or agriculture soils, virtually no literature is available which refers particularly to the transformation of toxic organic contaminants in the rhizosphere of wetland plants. Little is known about the phytoremediation of toxic organic pollutants by mangrove plants in coastal environments, particularly the significance of roots and their surroundings.

The roles of roots, such as the secretion of root exudates, are complicated and differ considerably among plant species. Root

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exudates are the photosynthetic products transferred to roots and released into the rhizosphere. Root exudates consist of different carboxylic acids, strong organic acids, alcohols, carbohydrates and proteins, which might serve as carbon and nutrient sources for the growth of soil microorganisms (Yoshitomi and Shann, 2001; Rentz et al., 2005). The densities of rhizosphere bacteria could be as much as two to four orders of magnitude greater than the populations in surrounding bulk sediments and displayed a greater range of metabolic capabilities (Macek et al., 2000; Wang et al., 2012). The range of low-molecular-weight organic acids (LMWOAs) exuded by roots varied by plant, but oxalic, benzoic, maleic, succinic, lactic, malic and citric acids were frequently found in most plant species (Jones, 1998; Strobel, 2001). The release of LMWOAs from roots into the rhizosphere sediment could increase the mobilization of soluble nutrients in the sediment for microbial and plant uptake, the phytoextraction of metals by plants (e.g., iron, cadmium, aluminum), the microbial proliferation in the rhizosphere and the dissolution of soil minerals leading to pedogenesis (e.g., podzolisation) (Jones, 1998). LMWOAs released from the roots of *Kandelia obovata* (previously known as *Kandelia candel*), one of the typical mangrove plants, had positive effects on the removal of heavy metals, and the concentrations of LMWOAs, especially malic, citric and lactic acid, were varied, dependent on the composition of sediment (Lu et al., 2007). Root exudates also changed the pH and organic matters of rhizosphere sediment (Landi et al., 2006).

In the biodegradation of toxic organic pollutants, root exudates acted as surfactants and reduced the sorption of organic contaminants, thus increasing their bioavailability (Parrish et al., 2005). This feature is extremely important for the aged or bound PAHs that are strongly adsorbed onto sediments and are essentially not available to roots or microorganisms for biodegradation. Root exudates could also selectively foster the growth of degraders in the rhizosphere and cause a shift in the microbial community structure (Yoshitomi and Shann, 2001; Krutz et al., 2005). However, Rentz et al. (2005) showed that some root exudates had negative effects on the degradation of PAHs. Ciccillo et al. (2002) also reported that microbial communities were adapted to low nutrient concentrations and degradation might decrease due to excess carbon sources provided by the rhizosphere. This means that the roles of root exudates in biodegradation are still controversial.

A number of enzymes were secreted by plants to degrade organic contaminants in the soil, including phenol oxidizing enzymes, laccases, peroxidases and dehydrogenase (Salt et al., 1998; Chaudhary et al., 2012). Dehydrogenase, which catalyzes a wide range of soil biological processes, is one of the most important enzymes, and has been considered as an effective indicator of general microbial activities (Killham and Staddon, 2002). Dehydrogenase is also used frequently to test the influence of various pollutants, such as heavy metals, pesticide, and crude oil, on the microbiological quality of soil or sediment (Shen, 2005). Positive correlations were found between dehydrogenase activities and the concentrations of PAHs, such as phenanthrene, fluoranthene, chrysene and dibenz[a,h]anthracene in soils collected from a military airfield (Baran et al., 2004). However, the effects of mixed PAHs on this enzyme, and how it relates to PAH biodegradation in mangrove sediments, are unknown.

The present study aims to evaluate the roles of LMWOAs from the root exudates and the dehydrogenase activity in rhizosphere sediments of different mangrove plants on the removal of mixed PAHs in contaminated sediments. The effectiveness of three mangrove plant species on the removal of mixed PAH contamination were also compared. The three species, namely *K. obovata* Sheue, *Bruguiera gymnorrhiza* (L.) Poir and *Avicennia marina* (Forsk.) Vierh., were selected because they are commonly found in mangrove swamps in Hong Kong SAR and South China (Tam and Wong, 2002).

2. Materials and methods

2.1. Regent and materials

All standards and chemicals with 99.6% purity were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). They were LMWOAs standards, including benzoic, maleic, succinic, lactic, malic and citric acids; glutaric acid (internal standard for LMWOAs); N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (derivatization regents); PAH standards, including 3-rings (Fluorene [Flu], Phenanthrene [Phe] and Anthracene [Ant]), 4-rings (Fluoranthene [Flo], Pyrene [Pyr] and Chrysene, [Chr]) and 5-rings (Benzo[a]pyrene [Bap] and Benzo[k]fluorene [Bkf]); and *m*-terphenyl (internal standard for PAH analysis). A stock solution of mixed PAHs was prepared in acetone, with each PAH compound at a concentration of 1000 mg L^{-1}, and stored in the dark at 4 °C. The other reagents, including concentrated hydrochloric acid, ethyl acetate, acetone, toluene, *n*-hexane, trichloromethane, dichloromethane, methanol, dimethylformamide and ethanol, were of analytical-reagent grades. Distilled water was processed through a Milli-Q water system (Millipore, Bedford, MA, USA) prior to use.

2.2. Plant materials and culture conditions

One year prior to the start of the present experiment, the propagules of three mangrove species, namely *K. obovata*, *B. gymnorrhiza* and *A. marina*, were collected from local mangrove swamps. Each propagule was germinated and grown in a greenhouse, in a rhizosphere bag containing 300 g fresh surface sediment collected from Sai Keng, a typical mangrove swamp in Hong Kong. The culture conditions were as follows: temperature 25–30 °C, relative humidity 70%, light intensity 280 μmoL m^{-2} s^{-1} and light–dark duration 12:12 h. The mean and standard deviation of pH, salinity and the concentrations of total organic matter, total N and total P of sediment were 7.16 ± 0.11%, 1.8 ± 0.5%, 0.18 ± 0.02%, 0.048 ± 0.002 g kg^{-1} and 0.025 ± 0.003 g kg^{-1}, respectively (n = 3). The concentration of total PAHs in Sai Keng sediments was relatively low, 1044 ± 497 ng g^{-1} (dry weight), as Sai Keng was a relatively unpolluted mangrove swamp in Hong Kong (Tam et al., 2001).

2.3. Experimental design

At the beginning of the experiment, a one-year old seedling was transplanted into a rhizsphere bag filled with Sai Keng sediment, prepared as described above. For each plant species, a total of 20 rhizosphere bags was set up and divided into five PAH treatments, that is, 0 (control)-, 3-, 6-, 12- and 24-mg of mixed PAHs spiked per bag; each treatment had four replicates. The bags were arranged in a complete randomized design. The mixed PAHs consisted of Flu, Phe, Ant, Flo, Pyr, Chr, Bap and Bkf at a ratio of 1:1:1:1:1:0.3:0.5:0.25. These PAHs were chosen based on their reported contamination values in sediments collected near industrial sites (Fismes et al., 2002). Before spiking into the rhizosphere bag, an appropriate amount of PAH stock solution, depending on the treatment level, was added into a 10 mL beaker and then vacuumed for 10 min to let the acetone completely vaporize. The beaker was rinsed with distilled water several times and the PAH suspension was poured onto the sediment surface of the rhizosphere bag. All the plants were cultured in the same greenhouse, as described above, for 2 months and irrigated daily with 30 mL of artificial seawater at a salinity of 15‰, which was prepared by