# Rhizodegradation gradients of phenanthrene and pyrene in sediment of mangrove (*Kandelia candel* (L.) Druce)

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## ARTICLE INFO

## ABSTRACT

A greenhouse experiment was conducted to evaluate degradation gradient of spiked phenanthrene (Ph, $10 \, mg \, kg^{-1}$) and pyrene (Py, $10 \, mg \, kg^{-1}$) in rhizosphere of mangrove *Kandelia candel* (L.) Druce. Rhizosphere model system was set up using a self-design laminar rhizoboxes which divided into eight separate compartments at various distances from the root surface. After 60 days of plant growth, presence of the plant significantly enhanced the dissipation of Ph (47.7%) and Py (37.6%) from contaminated sediment. Higher degradation rates of the PAHs were observed at 3 mm from the root zone (56.8% Ph and 47.7% Py). The degradation gradient followed the order: near rhizosphere > root compartment > far-rhizosphere soil zones for both contaminants where mangrove was grown. Contribution of direct plant uptake and accumulation of Ph and Py were very low compared to the plant enhanced dissipation. By contrast, plant-promoted biodegradation was the predominant contribution to the remediation enhancement. The correlation analysis indicates a negative relation between biological activities (microbial biomass carbon, dehydrogenase, urease, and phosphatase activity) and residual concentrations of Ph and Py in planted soils. Our results suggested that mangrove rhizosphere was effective in promoting the depletion of aromatic hydrocarbons in contaminated sediments.

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## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants persisting in the environment. Anthropogenic inputs of PAHs from oil spills, ship traffic, urban runoff and emission from combustion and industrial processes have caused significant accumulation of PAHs in coastal mangrove wetlands especially those near urban centers and industrial cities [1,2]. Phytoremediation of PAHs is a promising alternative approach to sediment remediation due to its cost effectiveness, convenience and environmental acceptability [1,3]. There are several branches of phytoremediation identified by the USEPA (2000), including phytoextraction, rhizofiltration, phytovolatization, rhizodegradation and phytodegradation, and phytostabilization. Rhizodegradation refers to the microbial breakdown of organic contaminants in the root zone (rhizosphere) soil and sediment. This process uses the natural ability of plants to manipulate the biological, chemical and physical characteristics of the rhizosphere for reducing organic contaminant concentrations in soil and sediment [4,5]. In sediment, rhizoremediation was suggested to be the primary mechanism responsible for PAH degradation in plant-assisted remediation efforts [6,7]. In this case, roots contribute to the dissipation of hydrocarbon contaminants through an increase in the number of microbes, improvement of physical and chemical soil conditions, increased root exudates and humification, and adsorption of pollutants in the rhizosphere was investigated.

Mangrove ecosystems, important inter-tidal estuarine wetlands along coastlines of tropical and subtropical regions, are closely tied to industrial activities and are subject to contamination [8,9]. Mangrove may contribute to the dissipation of organic contaminants through an increase in the number of microbes, improvement of physical and chemical soil conditions, increased humification and adsorption of pollutants in the rhizosphere, but the impact of each process has not been clearly elucidated. A number of bacterial strains able to degrade PAHs have been isolated from surface mangrove sediment and the degradation of PAHs by these consortia and isolates in culture medium and in sediment slurry have been studied [10,11]. Nevertheless, the question of how far a mangrove rhizosphere effect on degradation of PAHs may extend has, however, never been approached, but the preferential use of plants with fibrous root systems for rhizodegradation indicates that it was rather narrow.

The production of protons, exudates and metabolites is released by plant roots in rhizosphere soil which led to significant

differences between rhizosphere and non-rhizosphere in soil properties have been reviewed in previous reports [12,13]. The rhizosphere, a layer of soil surrounding plant roots, was difficult to physically sample and manipulate with precision. Rhizosphere soil was commonly separated from plant root by gentle shaking. In an attempt to overcome some of these problems, a rhizobox was designed where soil in close proximity to roots which allowed for the harvesting of thinner consecutive sections (1–5 mm, and >5 mm) of rhizosphere soil in the lab [14].

Recently, phytodegradation of PAH contaminated sediments using mangrove plants has been the subject of several studies [2,8]. However, limited by sampling techniques of rhizosphere soil in the proximity to roots, the distance-dependent microscale depletion of PAHs in root–soil interface along the rhizosphere gradient has thus far been seldom studied. Therefore, we hypothesized that the differences with distance in rhizosphere effects would coincide with the degradation gradients of PAHs. The objective of this study was therefore to investigate the rhizosphere effect on the removal process of PAHs in a specially designed rhizobox which permitted the separation of rhizosphere soil (root compartment) and soil affected by root exudation (root-free compartment). The study also attempts to reveal the effect of the increasing distance on PAH removal. 3-Ring PAHs Ph, and 4-ring PAHs Py were used as target PAHs. *Kandelia candel* (L.) Druce (*K. candel*), a common red mangrove species in China, was chosen as the model plant.

## 2. Materials and methods

### 2.1. Chemicals

Ph and Py with a purity of 99.9% were obtained from Sigma–Aldrich Co. Ltd., UK. All the other chemicals used in the study were of analytical purity.

### 2.2. Preparation of PAH-spiked sediment

Bulk samples of surface sediments were collected from Jiulong Estuary mangrove wetland, PR China, and sieved through a 0.5 cm sieve to remove coarse debris, homogenized, and then stored at 4 °C until use. The physical and chemical properties of the sediments were measured in the laboratory as follows: pH 6.63, moisture content 49.5%, total organic content 2.1% and total nitrogen amount 0.90 g kg$^{-1}$ dry sediment, total phosphate amount 0.62 g kg$^{-1}$ dry sediment and cation exchange capacity 15.8 cmol kg$^{-1}$. PAHs were detected in the sediment samples with concentration of 19.3 μg kg$^{-1}$ Ph and 24.56 μg kg$^{-1}$ Py, respectively.

Sediment was taken and spiked with PAHs as follows: a portion of the sediment was accurately weighed in the vessel. Then, a volume of the PAHs dissolved in acetone is added and allowed to equilibrate with the matrix, stored in the dark and allowed to dry. Mass balance is used to determine the evaporation of acetone. The acetone was evaporated 12 h and the portion of spiked sediment was first mixed with near 25% of total sediment, and then to mix with the remaining 75% of wet sediments followed by mechanical mixing. After aging for 7 days, the sediment was used for rhizobox experiment. The detected concentration of Ph and Py was 10 ± 0.5 and 10 ± 0.4 mg kg$^{-1}$, respectively in 7 days aged sediment. No nutrient amendments were added to the soil during the experiment.

### 2.3. Experimental design

A laboratory rhizobox modified from our previous study [15] (Fig. 1) was used to plant *K. candel*. The dimension of the rhizobox (Fig. 1) was 150 mm × 300 mm × 200 mm
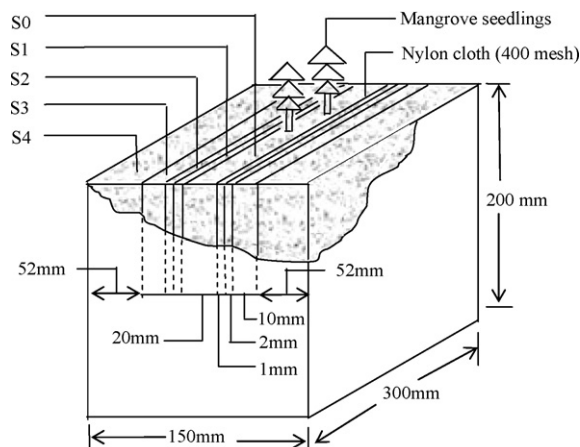


**Fig. 1.** Sketch diagram of rhizobox (modified from Lu et al. [15]). S0: sediment for seedling growth; S1: rhizosphere; S2: near rhizosphere; S3: near bulk soil; and S4: bulk soil.

(length × width × height). The rhizobox was divided into five sections from central to left or right boundary of rhizobox which were surrounded by nylon cloth (400 mesh), viz. a central zone for plant growth (20 mm in width), rhizosphere zones (1 mm in width), near rhizosphere zones (2 mm in width), near bulk soil zones (10 mm in width) and bulk soil zones (52 mm in width). In the rhizobox soil for seedlings growth, rhizosphere, near rhizosphere, near bulk soil and bulk soil zones were designated as S0, S1, S2, S3 and S4, respectively. The design successfully prevents root hairs from entering the adjacent soil zones as well as keeping the soil zones separated, while permitting the transfer of soil microfauna and root exudates between the compartments. About 12 kg of the treated sediment was added to each rhizobox, each treatment had three replicates. Five *K. candel* seedlings were planted in the central zone of the box. The plants were grown under greenhouse conditions with natural illumination and the relative humidity of 85%, the temperature ranging from 26 to 32 °C for 60 days. Sediment moisture content was adjusted to 100% of the water holding capacity by watering with fresh water to minimize drainage and simulates anoxic, waterlogged conditions. Rhizobox without plant was used as control. Rhizoboxes were arranged in a randomized design within the glasshouse and their position was rotated regularly to ensure uniform conditions. Before harvesting, rhizoboxes were withheld from watering for 2 days. Harvesting involved the sequential dismantling of each rhizobox, separating the layers of each soil zone of the rhizobox and removing the plants from the root compartment. Roots and shoots were manually separated from soils washed with deionized water, and then blotted dry with filter paper. The soil samples from different soil zones of each rhizobox were homogenized separately before analysis.

### 2.4. Analyses

#### 2.4.1. Measurements of enzymatic activities

Soil microbial biomass carbon ($C_{mic}$) was determined by the chloroform-fumigation–extraction method [16,17]. Sediment dehydrogenase activity was measured by the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to 1,3,5-triphenyl formazan (TPF). Briefly, 5.0 g of freeze-dried sediment sample was incubated for 24 h at 37 °C in 5.0 mL of TTC solution (5.0 g L$^{-1}$ in 0.2 mol/L Tris–HCl buffer, pH 7.4). Two drops of concentrated $H_2SO_4$ were immediately added after incubation to stop the reaction. The sample was then blended with 5.0 mL of toluene to extract TPF and shaken for 30 min at 250 rpm (25 °C), followed by centrifugation

at 5000 rpm for 5 min, and absorbance in the extract was measured at 492 nm. Finally, soil dehydrogenase activity was calculated as $1.00 \mu g\,TPF\,g^{-1}$ dry sediment [18]. Colorimetric method was utilized to determine the urease and phosphatase activities [19]. Enzyme activities expressed as $mg\,NH_4-N$ released $kg^{-1}$ dry sediment at 37 °C and $mg\,P$ released $kg^{-1}$ dry sediment at 37 °C each for urease and phosphatase, respectively.

### 2.4.2. PAH analysis

Sediment samples were freeze-dried, meshed, and extracted with an accelerated microwave extraction system modified from Zhang et al. [20]. Briefly, 10.00 g of freeze-dried sediment was extracted with 50 mL mix solvent (n-hexane/acetone 1:1, v/v) using microwave extraction system (CEM Co., Matthews, NC, USA). The surrogates Ph-d$_{10}$ and chrysene-d$_{12}$ (Chy-d$_{12}$) (Sigma–Aldrich, UK) were added to the samples prior to extraction. Activated copper (stirring copper with 5% of iodide/acetone solution for about 10 min) was added into the extract for desulphurization, and then pre-concentrated to 2 mL by a rotary evaporator (Buchi Vac V-800, Switzerland). Concentrated extracts were fractionated with alumina/silica gel (100–200 mesh) column chromatography (40 cm × 1.5 cm i.d.) packed from the bottom with glass wool, 10.00 g neutral aluminum oxide (100–200 mesh, dried at 440 °C for 4 h), 18.00 g silica gel (100–200 mesh, dried at 170 °C for 4 h) and 2.00 g anhydrous sodium sulphate. Target analytes were eluted from the column with 150 mL of mix solvent n-hexane/methylene chloride (1:1, v/v). This fraction was then concentrated to 2 mL by rotary vacuum evaporation in a water bath at 60 °C and solvent-exchanged to n-hexane. The PAH fraction was finally concentrated to 1 mL under a gentle stream of nitrogen before GC/MS analysis. Plant samples were ground and homogenized, and extracted using the same method as to sediment.

The concentrations of the PAHs in the extracts were determined by a Hewlett-Packard 6890 gas chromatography equipped with a mass spectroscopy detector (HP5975B). The HP-5MS column (Agilent Co., USA) was 30 m in length, with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. The temperature was raised from 60 °C to 150 °C at a rate of 15 °C min$^{-1}$, increased to 220 °C at 5 °C min$^{-1}$, and increased to 300 °C at 10 °C min$^{-1}$, then held at 300 °C for 5 min. Helium was used as the carrier gas. The injector and detector temperatures were 280 °C and 300 °C, respectively. The electron-impact energy was 70 eV and the mass to charge ratio scan ($m/z$) was from 50 to 400 amu. The selected ion mode (SIM) was chosen. Detection limits derived from replicate and procedural blanks were 2.2 and 1.6 $\mu g\,kg^{-1}$ dry weight for Ph and Py, respectively.

All data were subject to strict quality control procedures. Matrix spikes, laboratory sample duplicates, and laboratory blanks were processed with each batch of samples (10 samples per batch) as part of the laboratory internal quality control. The mean recoveries of deuterated surrogate were $87.2 \pm 2.1\%$ for Ph-d$_{10}$ and $90.3 \pm 1.8\%$ for Chy-d$_{12}$, respectively ($n = 3$). Spiked samples in each batch were analyzed with mean recoveries of $86.7 \pm 2.6\%$ for Ph and $89.6 \pm 1.4\%$ for Py, respectively ($n = 3$). Each extract was analyzed in duplicate form and relative standard deviations were less than 20%. Any analyses not meeting quality assurance requirements were re-analyzed.

### 2.5. Statistical analyses

All of these experiments were performed in triplicates and the results presented were average values of the three replicates. Data were analyzed statistically using analysis of variance (ANOVA) and the Duncan's multiple range tests was employed to determine the significance of the differences between the parameters. The

**Table 1**
Removal percentages of phenanthrene and pyrene in various sampling zones in planted and unplanted treatments after 60 days of *K. candel* growth.

| Zones | Treatment | | | |
|---|---|---|---|---|
| | Phenanthrene (%) | | Pyrene (%) | |
| | Unplanted | Planted | Unplanted | Planted |
| S0 | 27.1 ± 3.5Cb | 47.5 ± 4.1Aa | 23.5 ± 2.9Cb | 32.4 ± 3.7Ba |
| S1 | 26.5 ± 3.4Cb | 53.6 ± 6.4Aa | 24.4 ± 2.6Cb | 46.2 ± 5.1Aa |
| S2 | 25.6 ± 3.1Cb | 56.8 ± 6.2Aa | 21.9 ± 2.3Cb | 47.7 ± 4.9Aa |
| S3 | 26.1 ± 2.9Cb | 43.2 ± 3.4Aa | 23.2 ± 2.8Cb | 38.1 ± 4.2Ba |
| S4 | 25.5 ± 3.2Cb | 37.3 ± 3.2Ba | 20.9 ± 2.6Ca | 23.5 ± 2.6BCa |

*Note*: Values in each column followed with different capital letters (A, B, C and D) indicated significant ($p < 0.05$) differences among different distances (0, 1, 2, 4 and 6 mm) from roots, and in each row followed with different lowercase letters (a and b) indicated significant difference between planted and unplanted soils by statistically using Duncan's multiple range tests. Values represent means ± standard deviation. S0–S4 represented the distance of 0, 1, 2, 4 and 6 mm far from the root surface.

statistical package used was SPSS statistical software package (Version 11.0) and the confidence limit was 95%.

## 3. Results and discussion

### 3.1. Dissipation gradients of Ph and Py in sediment

At the beginning of the experiment, $10 \pm 0.5$ and $10 \pm 0.4\,mg\,kg^{-1}$ of the added Ph and Py in the sediment slurry were adsorbed onto the sediments, respectively. This indicates that evaporation of acetone did not cause any significant loss of the spiked PAHs in sediment slurries. At the end of 60 days experiment, the results showed initial Ph ($10.0\,mg\,kg^{-1}$) and Py ($10.0\,mg\,kg^{-1}$) concentrations significantly decreased in the planted sediment as well as in unplanted control, but a more marked rate of disappearance was evident when plants were presented. The removal percentages for Ph and Py were 37.3–56.8% and 23.5–47.7%, respectively, in different gradient zones of planted sediment, which were significantly higher compared to unplanted treatments (25.5–27.1% for Ph and 20.9–24.4% for Py) (Table 1). The PAH concentration in the sediment after growing mangrove was affected by proximity to the roots. At both spiked Ph and Py sediment with planted treatments, the general trend in the degradation of the PAHs was typically rhizosphere > compartment > far-rhizosphere apart from a slight difference in root zones (Table 1).

The mass balance results suggest that although the spiked PAHs were adsorbed tightly onto the sediments at the beginning of the experiment, PAH-degraders had the ability to utilize and degrade the sorbed PAHs efficiently (Table 2). The loss of the PAHs from mangrove sediment could be due to biotransformation,

**Table 2**
Mass balance of phenanthrene and pyrene in rhizobox sediment after 60 days of *K. candel* growth.

| PAH (mg) | | Non-vegetation | Plant-treatment |
|---|---|---|---|
| Ph | Input | 100.0 ± 5.0 | 100.0 ± 5.0 |
| | Leachate | ND | ND |
| | Plant uptake | NA | NA |
| | Remain in sediment | 74.2 ± 4.5 | 59.8 ± 3.7 |
| | Losses | 25.8 ± 1.7 | 40.2 ± 2.3 |
| Py | Input | 100.0 ± 4.0 | 100.0 ± 4.0 |
| | Leachate | ND | ND |
| | Plant uptake | NA | NA |
| | Remain in sediment | 78.4 ± 5.2 | 72.3 ± 4.4 |
| | Losses | 21.6 ± 1.2 | 27.7 ± 1.8 |

*Note*: ND, not detected; NA, not applicable because no plants were grown in the rhizobox or PAHs uptake was negligible. Values represent means ± standard deviation.

biodegradation, plant uptake, or abiotic dissipation, including leaching and volatilization [21,22]. In this study, abiotic losses by leaching were insignificant because non leachate was produced during the experiment. The losses of Ph and Py via volatilization from sediment are also unlikely to occur due to whole water covered condition and low vapor pressure of the PAHs ($10^{-1.00}$ and $10^{-2.05}$ L atm mmol$^{-1}$ for Ph and Py, respectively). Our data showed that mangrove plant only accumulate a little PAHs (detailed in Section 3.4), thus, the loss of the PAHs from soil by plant uptake/accumulation can be assumed to be negligible. In rhizosphere, Reilley's results suggested that abiotic dissipation (chemical degradation and irreversible sorption) was not a possible pathway of loss of anthracene and Py [23]. Therefore, our results indicated that the enhanced dissipation of the PAHs might be caused by increased rhizosphere microbial density and activity compared to unplanted soil, since the root exudates and plant litter could enhance the bioavailability of the contaminant, provide more substrate for co-metabolic degradation, and modify the soil environment to be more suitable for microbial transformation. The roots are known to release some organic compounds, such as amino acids, organic acids, sugars, enzyme and complex carbohydrates, providing carbon source and energy for the growth of rhizosphere microorganisms [13,24]. The increased dissipation of PAHs in the rhizosphere may also be due to the decreased extractability of the PAHs with the formation of bound residues. The rhizosphere could stabilize pollutants by polymerization reactions such as humification [12,25]. The PAH degradation gradients observed in the rhizobox showed that the dissipation of Ph and Py was higher in the sediment only receiving root exudates than the soil with root exudates and plant roots. This is not consistent with the gradients in root exudates and plant enzymes or the depletion zones of the most diffusion limited mineral nutrients in lots of reports [26,27]. This is an important but interesting conclusion. It might be the result of the competition between plant roots and soil microbes for soil nutrients influencing the activities of soil microbes, especially in low organic matter soil such as sediment used in this study. In addition, the Ph of suberization induced by root senescence might render the PAHs more hydrophobic and potentially interfere with their availability through adsorption [28]. While PAHs accumulated in plants only accounted for a small amount of removed PAHs, whether this plant *K. candel* itself was able to produce enzymes for PAHs degradation is unknown. However, the synergism mechanism still need to be confirmed by further studies, in which more plants should be involved and different plant species should be studied.

### 3.2. Microbial biomass and enzyme activities of the sediment

The content of soil microbial biomass carbon and activities of soil dehydrogenase, urease and phosphatase were measured to evaluate the gradient effect of the rhizosphere on the PAH degradation. The different gradient sediments from roots displayed different responses to the presence of the PAHs in the rhizobox. Overall, in the unplanted sediment, microbial biomass measured as total $C_{mic}$ was the same in various compartments but was lower than the planted sediment (Fig. 2). Likewise, $C_{mic}$ was 16–234% greater with, than without, plants. The largest $C_{mic}$ concentration (12.65 mg kg$^{-1}$ for Ph and 10.68 mg kg$^{-1}$ for Py, respectively) at either gradient zone was found in the rhizosphere (S1). The activities of soil dehydrogenase, urease and phosphatase in the planted soils were higher than those of unplanted treatments over the process of 60 days biodegradation (Figs. 3–5). In our study, it was shown that the relative lower concentrations of Ph and Py (10.0 mg kg$^{-1}$) had a stimulatory effect on enzyme activity in sediment. However, other researchers found that higher concentration of PAHs could inhibit the enzyme in the soil [10]. Our data indicated that rhizosphere effects caused the increased response characteristics in the
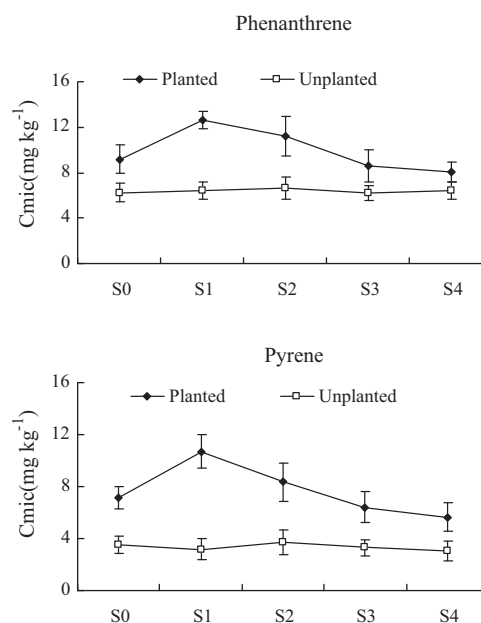
**Fig. 2.** The amount of microbial biomass carbon ($C_{mic}$) in various distances proximity to *K. candel* roots grown in the sediment treated with phenanthrene and pyrene. Bars are the standard error of means of three replicates.

sediment with plants compared to unplanted treatments. In the PAHs-treated sediment, enzyme activities were largest in the rhizosphere or root compartment, and then decreased with increased distance from the root surface (Figs. 2–5). However, enzyme activities did not decrease with distance in unplanted soils. This matches well the PAH degradation data (Table 1). The most probable number of PAH degraders was influenced by planting regime. Our microbial biomass data support the hypothesis that micro-organisms were responsible for the observed PAHs degradation. Planted treat-
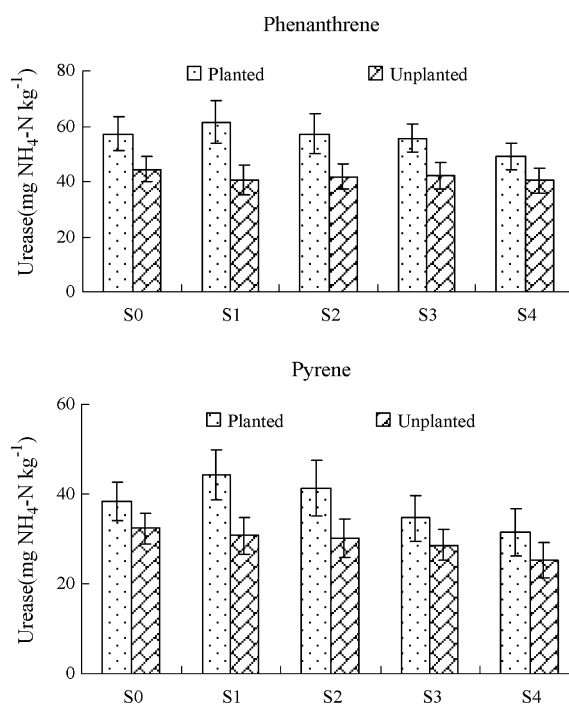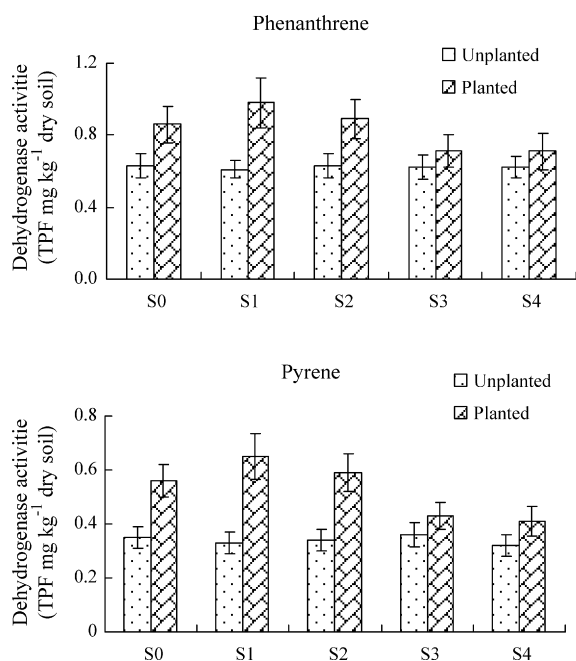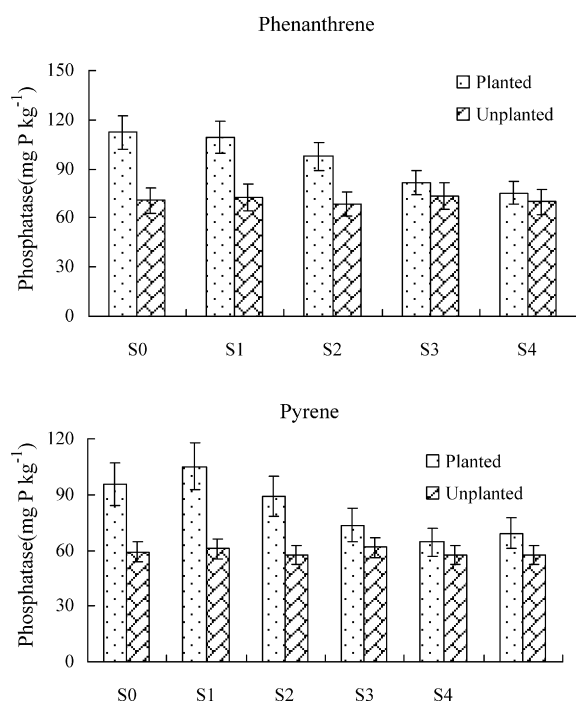
**Fig. 3.** Urease activities in various distances proximity to *K. candel* roots grown in the sediment treated with phenanthrene and pyrene. Bars are the standard error of means of three replicates.

**Fig. 4.** Dehydrogenase activities in various distances proximity to *K. candel* roots grown in the sediment treated with phenanthrene and pyrene. Bars are the standard error of means of three replicates.

**Table 3**
The linear regression between residual PAH concentrations ($Y$) in sediment and different biological parameters in rhizosphere (S1) ($Y = ax + b$).

| Spiked-PAHs | Indexes ($x$) | $a$ | $b$ | $r^2$ |
|---|---|---|---|---|
| Phenanthrene | $C_{mic}$ | −0.36 | 8.79 | 0.792[*] |
| | Urease | −0.15 | 13.43 | 0.693[*] |
| | Dehydrogenase | −5.76 | 10.01 | 0.749[*] |
| | Phosphatase | −0.03 | 8.50 | 0.508[*] |
| Pyrene | $C_{mic}$ | −0.41 | 9.37 | 0.645[*] |
| | Urease | −0.17 | 12.69 | 0.745[*] |
| | Dehydrogenase | −7.22 | 10.05 | 0.761[*] |
| | Phosphatase | −0.04 | 9.77 | 0.459[*] |

[*] Significant ($p < 0.05$) difference between residual PAHs concentrations and biological parameters.

of the soil biological status [29]. In our experiment, although the responses of activities of urease and phosphatase were different and inconsistent to some extent with the degradation of PAHs, it could still be concluded that increased urease and phosphatase activity occurred in the planted soil, especially in the rhizosphere, compared to the unplanted soil. The reason for different enzyme activities in different soil zones might relate to the gradient impact of root exudates.

### 3.3. Correlation between microbial activities and PAH dissipation in rhizosphere

The successful application of rhizoremediation is largely dependent on the capacity of contaminant degraders or plant growth promoting microbes to efficiently colonize growing roots. Table 3 lists the relationships between microbial activities and the PAH dissipation in rhizosphere after 60 days of cultivation. A significant negative correlation was found between residual contaminant concentrations and soil enzymes in the rhizosphere. Statistical correlations ($r^2$) of both spiked PAHs, especially for $C_{mic}$ and dehydrogenase two indexes, had better values ($r^2_{C_{mic}} = 0.792$ and 0.645, $p < 0.001$; $r^2_{\text{dehydrogenase}} = 0.749$ and 0.761, $p < 0.001$). For phosphatase, it showed a relatively poorer correlation. Some plant species appear to increase the numbers of degradative microbes in a large volume of soil that extends beyond the rhizosphere.

The release of compounds or enzymes from roots is presumed to be associated with rhizosphere biodegradation and plant types vary in the nature and quantity of compounds released, it follows that the plant species used could be a significant factor influencing the efficacy of phytoremediation. Parrish et al. [30] reported that after 12 months of plant growth, the PAH degrading microbial populations in vegetated treatments were more than 100 times greater than those in unvegetated controls. This microbial consortia can provide various benefits to plants, including the synthesis of compounds that protect the plants by decreasing plant stress hormone levels; chelators for delivering key plant nutrients; protection against plant pathogens; and degradation of contaminants before they can negatively impact the plants [31]. Therefore, differences between rhizosphere soils and nonrhizosphere soils could be explained by the rhizosphere effect.

### 3.4. Accumulative potential of Ph and Py in plant tissues

When using spiked sediments for remediation experiments, the focus has often been on the ability of a given plant to accumulate a specific compound and can be removed along with the biomass for sequestration or incineration. In order to acquire a comprehensive understanding about the mechanisms of the PAH degradation, the

ments, especially rhizosphere, contained a significantly increased and large microbial biomass that could mediate the enhanced degradation of PAHs. The differences observed between soil with and without plants, as well as among various sampling zones in proximity to roots of the planted soils, were expected on the basis of microbial growth and community structure modified by both PAHs and root exudates. Overall microbial activity, as determined by dehydrogenase, urease and phosphatase activities is indicator



**Fig. 5.** Phosphatase activities in various distances proximity to *K. candel* roots grown in the sediment treated with phenanthrene and pyrene. Bars are the standard error of means of three replicates.

**Table 4**
Phenanthrene and pyrene concentrations (mg kg$^{-1}$) and concentration factors (CFs) in plant after 60 days of plant growth.

| Treatments | Root | RCFs | Stem | SCFs | Leaf | LCFs |
|---|---|---|---|---|---|---|
| Phenanthrene | 0.83 ± 0.11 | 0.18 | 0.55 ± 0.07 | 0.12 | 0.32 ± 0.04 | 0.07 |
| Pyrene | 1.56 ± 0.25 | 0.29 | 1.83 ± 0.24 | 0.34 | 0.59 ± 0.07 | 0.11 |

uptake of Ph and Py by *K. candel* was measured (Table 4). The concentrations of Ph and Py in root were higher than those in the stem and leaf, and the concentrations of Ph in leaf were lowest among plant tissues (Table 4).

Plant concentration factors (CFs) were calculated as the ratio of the PAH concentrations in plant tissues (root, shoot and leaf) and in sediments on a dry weight basis. The results also indicated that root concentration factors (RCFs) of Ph (0.18) were much lower than those of Py (0.29) treatment. It might be explained by the higher $K_{ow}$ (octanol–water partition coefficient) value of Py than Ph [32]. It was demonstrated that hydrophobic compounds with log $K_{ow}$ > 4 are not readily taken up by plants through transpiration due to their hydrophobicity; log $K_{ow}$ for Ph and Py was 4.17 and 5.13, respectively [33]. Our data indicated that *K. candel* was not a hyper accumulation plant for PAHs (CFs from 0.07 to 0.34). There were no significant correlations between concentration of Ph and Py in roots and the dissipation of Ph and Py from rhizosphere sediments (S1) as well as other gradient sediments (S2–S4) were found. This indicated that accumulation of Ph and Py by roots was not the major factor contributing to the removal of PAHs from soil. This result is similar to previous report [34] which indicated contribution of *K. candel* accumulation and plant uptake to the removal of Py from contaminated sediments was insignificant.

## 4. Conclusion

We investigated the rhizodegradation gradient of mangrove plant *K. candel* for the PAH contaminated sediment. The presence of mangrove plant significantly increased the dissipation of Ph and Py in contaminated sediment. Effect of root proximity was important in the removal process of Ph and Py, which was depended on the distance from the root surface. Enhanced dissipation rate in different gradients of planted versus unplanted sediment was 11.8–29.9% for Ph and 2.9–25.8% for Py. Accumulation of the PAHs in plant parts showed negligible contributions to the total remediation. Plant root-promoted dissipation was the predominant contribution to the remediation enhancement for sediment Ph and Py in the presence of *K. candel*. Our results suggested that the enhancement of Ph and Py disappearance is caused by an increase in the rhizosphere biological activity compared to root free sediment. Moreover, there is a scope for future work particularly regarding underlying mechanisms responsible for observed rhizoremediation outcomes. These future directions include elucidation of the complex processes at the interface of soil, microorganisms and roots. More effort also should be made to investigate of root exudates being deposited into the rhizosphere and involved microbe activities during the remediation process, and the achievable outcome using the mangrove under field trials.

## Acknowledgements

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