Phytoremediation potential of *Juncus subsecundus* in soils contaminated with cadmium and polynuclear aromatic hydrocarbons (PAHs)

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ABSTRACT

A phytoremediation potential of emergent wetland species may be influenced by co-contamination by metals and polynuclear aromatic hydrocarbons (PAHs) in soils. A glasshouse experiment was conducted to investigate effects of Cd (0, 5, 10 and 20 mg kg^{-1}) without or with PAHs (50 + 50 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion) on growth of *Juncus subsecundus*, removal of pollutant from soils and the abundance of PAH-degrading bacteria in the rhizosphere/non-rhizosphere. After 10 weeks, plant growth and biomass were significantly influenced by interaction of Cd and PAHs. The shoot concentration of Cd significantly increased by Cd additions, but not by PAHs (except at Cd treatment of 20 mg kg^{-1}). Cadmium accumulation and removal (except for Cd removal at 20 mg Cd kg^{-1}) by plants was significantly higher in Cd treatments with than without PAHs, whereas accumulation of PAHs by plants (except for pyrene in roots at 0 added Cd) and dissipation of PAHs from soils were not significantly influenced by Cd additions. The abundance of PAH-degrading bacteria in soil increased significantly in Cd treatments with PAHs, particularly in the rhizosphere. The results indicate that it is feasible to use wetland species for phytoremediation of soil co-contaminated with Cd and PAHs, but further work in naturally contaminated soils under field conditions is needed.

1. Introduction

Phytoremediation is an environmental technology in which plants are used for decontamination of organic and inorganic pollutants from soils and waters (Pilon-Smits, 2005). Soil and water contaminated with organic pollutants usually contain other pollutants as well, such as heavy metals, because they were discharged from the same sources, including vehicle emissions, industrial processes, power and heat generation and waste incineration. The presence of co-contamination in waters and soils represents a threat to biota (Sun et al., 2011). Despite many studies on phytoremediation of sites contaminated with either heavy metals or organics, little information is available on the effectiveness of phytoremediation of co-occurring metal and organic pollutants (Lin et al., 2008). The combined presence of different pollutants might influence remediation processes because different compounds may interact among themselves and/or with plants and their rhizosphere biota (Almeida et al., 2008).

The wastewater is considered one of the most important freshwater resources and has been used for agricultural irrigation in arid and semiarid regions due to increased scarcity of clean freshwater.

However, as one of unexpected side effects, large areas of soils were contaminated by heavy metals such as cadmium (Cd) and organic pollutants such as polynuclear aromatic hydrocarbons (PAHs) because of the common practice to discharge a large volume of wastewater either untreated or after minimal preliminary treatments (Sun et al., 2009). Hence, potential deleterious impacts on human and environment health associated with the use of wastewater containing pollutants necessitate wastewater treatment before use.

Constructed wetlands for treating wastewater are a growing phytoremediation technology around the world (Tel-Or and Forni, 2011). Plants play a significant role in constructed wetlands (Zhang et al., 2007; 2008). They can enhance metal removal and/or stabilization (Weis and Weis, 2004) and may also facilitate organic pollutant biodegradation (i) directly in the rhizosphere by the release of root exudates and (ii) indirectly by improving soil biology via build-up of organic carbon (Pilon-Smits, 2005). Hence, successful phytoremediation using constructed wetlands depends on the tolerance of wetland plants to the contaminants in wastewater and/or soils.

The uptake and translocation of metals in various wetland plants have been studied (Marchand et al., 2010; Weis and Weis, 2004; Zhang et al., 2010b), but few studies have focused on the uptake and translocation of organic pollutants (e.g. PAHs) in wetland species, especially under co-contaminated conditions. Although the removal of organic and inorganic pollutants may be satisfactory in constructed wetlands, some pollutants such as metals (e.g. Cd) and PAHs may

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accumulate in the substrate when wetlands are exposed to wastewater over long periods of time (Batty and Younger, 2004; Srogi, 2007). The growth and pollutant removal by wetland plants may be influenced by an interaction of Cd and PAHs. The knowledge about the influence of these interactions between co-contaminants on the phytoremediation potential of wetland plants is relatively poor.

The emergent wetland species such as *Juncus subsecundus* N.A. Wakef. (family Juncaceae) are often used in constructed wetlands (Zhang et al., 2010b, 2010c). In our previous study, an interaction between Cd and PAHs on growth of *J. subsecundus* was observed when relatively low concentrations of Cd (10 mg kg^{-1}) and PAHs (50 + 50 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion) were present in soils (Zhang et al., 2011c). However, the previous study quantified neither influence of Cd on PAH uptake and translocation in plants nor PAH dissipation and its degrading bacteria in the rhizosphere and non-rhizosphere. Hence, the objectives of this study were to investigate (1) the effect of Cd–PAHs as combined contamination on growth of *J. subsecundus*; (2) the influence of the co-contaminants on uptake and translocation of Cd and PAHs in plants and their removal from the substrate by plants; and (3) the impact of the co-contaminants on dissipation of PAHs and the abundance of PAH degraders in rhizosphere and non-rhizosphere.

2. Materials and methods

2.1. Preparation of contaminated soil

Soil without detectable PAHs and Cd was collected from Gingin, Western Australia (31°46′S, 115°86′E), air-dried and sieved through a 2-mm mesh. This soil, used as media in constructed wetlands for treatment of stormwater (Zhang et al., 2010c, 2011b, 2011c), was sandy loam containing coarse sand (200–2000 μm) 873 g kg^{-1}, fine sand (20–200 μm) 79 g kg^{-1}; silt (2–20 μm) 19 g kg^{-1} and clay (<2 μm) 29 g kg^{-1}. Soil chemical properties were: pH$_{water}$ 6.4, EC 0.012 dS m^{-1}, total organic carbon 3.2 g kg^{-1}, total nitrogen 0.22 g kg^{-1} and total phosphorus 0.12 g kg^{-1}.

PAHs (phenanthrene > 96% purity, and pyrene > 98% purity; Sigma Chemical Co., Germany) were spiked into the soil at 50 + 50 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion. Phenanthrene and pyrene were dissolved in acetone and added to 25% by weight of the required amount of soil. The same amount of acetone was used in all treatments, including the control (without PAHs). After evaporation of acetone in a fume hood, the soils were thoroughly mixed with the remaining 75% by weight of the required amount of soil (cf. Brinch et al., 2002).

Cadmium (as CdCl$_2$×2^1/$_2$H$_2$O, analytical grade, AJAX Chemicals, Sydney, Australia) was dissolved in Milli-Q water and added to the PAH-spiked soils at concentrations of 0, 5, 10 or 20 mg kg^{-1}.

The basal nutrients in solution were added to all treatments at the following rates (mg kg^{-1} soil): 33.3 N, 20.5 P, 88.7 K, S 34.2, Ca 41.0, Cl 72.5, Mg 3.95, Mn 3.26, Zn 2.05, Cu 0.51, B 0.12, Co 0.11 and Mo 0.08, and were mixed uniformly. The spiked soils were placed in plastic bags and equilibrated in a dark room for one week. The soils were mixed once every day, and the moisture content was kept at 10% (w/w). The soil samples were collected after equilibration, and analysed for pH, water-extractable Cd, PAH-degrading bacteria and the initial concentrations of phenanthrene and pyrene in spiked soils.

2.2. Experimental setup

Based on the previous experiments (Zhang et al., 2010b, c), the species *J. subsecundus* was selected for this study conducted in a glasshouse at The University of Western Australia (31°58′ S, 115°49′

E) with controlled day/night temperatures of 25/20 °C under natural light conditions from late June to early September. The seedlings of *J. subsecundus* were collected from the local nursery and transplanted (with initial plant fresh weight 4.0 ± 0.2 g per pot) into 2.5-L pots (165 mm in diameter at the top and 125 mm in height) containing 3 kg spiked or non-spiked soil per pot. The pots were irrigated with de-ionized water to achieve a water layer of 15 mm above the soil surface, maintained by re-filling twice a week.

2.3. Sampling and measurements

The shoot number and the highest shoot height were measured weekly after plant establishment. The plants were harvested after 10 weeks of growth. Shoots were cut just above the soil surface and their base was washed with de-ionized water to remove any adhering sediments. Each pot was then excavated, and the roots (including rhizomes) were separated from soil by washing with running tap water over a mesh and rinsing with de-ionized water three times. All samples were dried to constant weight at 40 °C for 7 days in a forced-air cabinet, weighed for dry weight (DW) biomass and ground to pass a 0.75-mm mesh.

The soil samples from the pots were separated into the rhizosphere and non-rhizosphere soils according to a hand-shaking method (Hammer and Keller, 2002). The non-rhizosphere soil was easily shaken off the roots, whereas the soil attached relatively tightly to roots was collected as the rhizosphere soil. Both types of soil samples were analyzed for pH, water-extractable Cd, extractable phenanthrene and pyrene and PAH-degrading bacteria.

The concentration of Cd in plant tissues was determined by ICP-OES (Optima 5300DV, PerkinElmer, Shelton, USA) after digesting plant material in a heated mixture of concentrated nitric and perchloric acids (Bassett et al., 1978).

Phenanthrene and pyrene in plant samples were extracted and analyzed according to the procedure described by Gao et al. (2011). Briefly, 0.5 g plant samples were extracted by ultrasonication for 1 h in a 1:1 (v/v) solution of acetone and hexane. The solvent was then decanted, collected and replenished. This process was repeated three times. The solvents were then evaporated and exchanged for 2 mL hexane, followed by filtration through a 2-g silica gel column and elution with 12 mL of 1:1 (v/v) hexane and dichloromethane. Samples were then evaporated and exchanged for methanol to a final volume of 2 mL for HPLC analysis. The average recoveries obtained by spiking plant samples with phenanthrene and pyrene were, respectively, 86% (n = 5, RSD < 5.5%) and 85% (n = 5, RSD < 8.9%) for the entire procedure. The treated plant extracts were analyzed using an HPLC fitted with a 250-mm reverse-phase C$_{18}$ column with 4.6 mm internal diameter, using methanol as the mobile phase at flow rate of 1 mL min^{-1}. Chromatography was performed at 30 °C. Phenanthrene and pyrene were detected at 245 and 234 nm, and their detection limits were 44.1 and 50.2 pg, respectively.

Phenanthrene and pyrene in soil samples were analyzed by Australian Laboratory Services Pty Ltd, Perth, Western Australia. Briefly, 10 g of fresh soil sample spiked with surrogates (2-fluorobiphenyl, anthracene-d10 and 4-terphenyl-d14) was extracted with 20 mL 1:1 dichloromethane/acetone by an end-over-end tumbler for 1 h after sodium sulfate was added to remove any moisture from the sample. The solvent was transferred directly to a gas chromatography (GC) vial for analysis. Extracts were analysed by a Capillary GC/Mass Spectrometer in Selective Ion Mode (SIM), and quantification was done by comparison against an established 5-point calibration curve. The recoveries for surrogates were > 84%.

The soil pH (5:1 water/soil) was determined by a combination glass membrane electrode with a Calomel internal reference (Cyberscan 20 pH meter, Eutech Instruments, Singapore). The concentration of water-extractable Cd in soil samples was measured by ICP-OES (Optima 5300DV, PerkinElmer, Shelton, USA) after extraction and

filtration. Briefly, soil (3 g) was shaken with Milli-Q water (20 mL) for 4 h at 20 °C on an end-over-end shaker. Extract was filtered through a 0.45-μm membrane Acrodisc® syringe filter.

The most probable number (MPN) of PAH-degrading bacteria in soils was estimated by a 96-well microplate method modified based on Johnsen and Henriksen (2009). Briefly, 4 g of soil samples was mixed with 36 mL tetrasodium pyrophosphate (2 mM, pH 7.0) and placed in a rotary tumbler for 1 h. Ten-fold dilutions of the resulting supernatant (10^{-2}–10^{-8}) were prepared for each sample using BH medium (Bushnell-Haas, Difco) supplemented with 0.85% (w/v) NaCl and adjusted to pH 7.4. The 96-well microplates were prepared by adding 10 μL per well of hexane containing 500 μg mL^{-1} of each phenanthrene and pyrene. Hexane was evaporated for 30 min before adding 200 μL per well of BH media containing serial dilutions of the original soil sample. Three wells of each dilution were used for each soil sample. Wells containing only hexane were used as a control. Plates were incubated in the dark at room temperature (approx. 23–25 °C) in a fume hood, and the PAH-degrading bacteria growth was monitored by measuring changes in optical density of the medium (Multiskan® Spectrum Spectrophotometer, Thermo Fisher Scientific Inc.). The measurements of the bacterial growth and its products were done at 405 nm, whereas the reference measurements were done at 620 nm. The reference value was subtracted from each reading at 405 nm to minimize the noise caused by PAH crystals and soil particles. After 4 weeks, 50 μL of INT solution (Iodonitrotetrazolium violet 3 g L^{-1} dissolved in MilliQ water) was added to each well and the plates incubated overnight at room temperature (approx. 23–25 °C) in the fume hood. The number of growth-positive wells for each dilution was scored by visual inspection of the plates, and the MPN was calculated using MPN tables (de Man, 1983).

2.4. Data calculation

The shoot/root concentration factor (SCF/RCF) was calculated as:

$$SCF/RCF = \text{pollutant concentration in shoots or roots} \quad (1)$$
$$/\text{pollutant concentration in soils}$$

The translocation factor (TF) was calculated as:

$$TF(\%) = (\text{pollutant concentration in shoot/pollutant concentration in root}) \quad (2)$$
$$\times 100$$

Total accumulation of Cd or PAHs in plants, expressed as μg pot^{-1}, was calculated as:

$$\text{Total pollutant accumulation} \quad (3)$$
$$= (\text{pollutant concentration in shoots} \times \text{shoot DW})$$
$$+(\text{pollutant concentration in roots} \times \text{root DW})$$

The percentage removal of Cd or PAHs by plants was calculated as:

$$\text{Removal of Cd or PAH by plants } (\%) \quad (4)$$
$$= (\text{total Cd or PAH accumulation in plants}$$
$$/\text{total added Cd or total measured PAH in soil}) \times 100$$

The percentage dissipation of PAHs from soils was calculated as:

$$\text{PAH dissipation } (\%) \quad (5)$$
$$= (1-\text{concentration of extractable PAH in soil after experiment})$$
$$/\text{initial concentration of extractable PAH in soil})$$
$$\times 100$$

2.5. Statistical analyses

Experiment was set up in a complete randomized block design (4 Cd treatments × 2 PAH treatments) with three replicates. Statistical analyses were carried out using IBM® SPSS® version 19. Data on PAH-degrading bacteria, pH and water-extractable Cd in soils after experiment were analysed using three-way analyses of variance (ANOVA), including Cd, PAH and rhizosphere/non-rhizosphere as treatments. Two-way analysis of variance (ANOVA) was used to compare (i) the effect of Cd and PAHs on PAH-degrading bacteria, pH and water-extractable Cd in soils before the experiment, and on shoot, height and biomass of plants, concentrations, accumulation and translocation of Cd in plants and Cd removal by plants after the experiment; and (ii) the effect of Cd and rhizosphere/ non-rhizosphere on dissipation of PAHs from soils after the experiment. One-way analysis of variance (ANOVA) was used to detect significant effects of Cd on the concentrations, accumulation and translocation of PAHs in plants and removal of PAHs by plants. Least significant difference (LSD) was applied to test significance between means.

3. Results

3.1. Initial concentrations of extractable PAHs and water-extractable Cd, soil pH and abundance of PAH-degrading bacteria in soil

Initial concentrations of extractable phenanthrene and pyrene in spiked soils after equilibration were 35 ± 0.5 and 40 ± 1 mg kg^{-1} (means $\pm$ SE, $n = 3$), respectively, before transplanting seedlings. Neither phenanthrene nor pyrene was detected in un-spiked soil.

The water-extractable Cd in equilibrated soils was significantly increased by Cd additions (data not shown). No significant differences in pH, water-extractable Cd (except at 20 mg Cd kg^{-1}, whereby the water-extractable Cd in soils was significantly lower in the Cd treatment with than without PAHs) and PAH-degrading bacteria in soils were found between Cd treatments with and without PAHs before transplanting seedlings (data not shown).

3.2. Plant growth

The growth of *J. subsecundus* was significantly influenced by Cd, PAHs and their interaction after 10 weeks of growth (Fig. 1). After 10 weeks, the shoot number significantly increased in all Cd treatments with PAHs compared to the treatment without PAHs (except for 0 added Cd). The highest shoot height significantly increased in Cd treatments of 5 and 10 mg Cd kg^{-1} with PAHs compared to without, whereas no significant difference was detected in the other treatments. For the shoot number, the significant interaction between Cd and PAH treatments was detected after 5 weeks of plant growth, whereas the significant interaction for the highest height was observed at 10 weeks of plant growth.

3.3. Plant biomass

The plant biomass was significantly influenced by Cd, PAHs and their interaction after 10 weeks of growth. In the absence of Cd, the total biomass was significantly lower in the presence than in the absence of PAHs (Fig. 2). An addition of PAHs significantly increased the total biomass in Cd treatments with 5 and 10 mg Cd kg^{-1} (by 114 and 87%, respectively) compared to the Cd treatments without PAHs. The total plant biomass also increased (by 29%) in the highest Cd treatment (20 mg Cd kg^{-1}) with PAHs compared to the Cd treatment alone, but the difference was not significant.
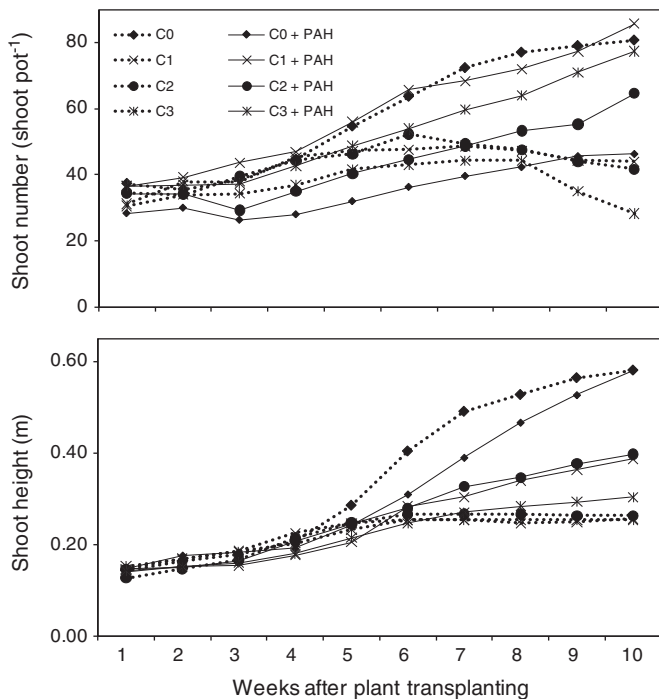
Fig. 1. The shoot number and height of *J. subsecundus* influenced by different Cd treatments with or without PAHs after plant transplanting. Treatments: C0, C1, C2 and C3 represent 0, 5, 10 and 20 mg Cd kg^{-1} and PAH represents $50 + 50$ mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion in soils.

3.4. Concentration, accumulation and translocation of Cd in plants and removal by plants

The Cd concentrations in plant tissues increased and shoot/root concentration factors (SCFs/RCFs) decreased significantly with Cd additions, but were not significantly influenced by PAHs (except for Cd concentrations in the treatment with 20 mg Cd kg^{-1}). The translocation factors (TFs) for Cd were between 84 and 151% among the treatments (except those without added Cd), but no significant difference was detected (Table 1). The total Cd accumulation in plant tissues and Cd removal (except for Cd removal in the 20 mg Cd kg^{-1} treatment) by shoots or whole plants significantly increased in the Cd treatments with PAHs. Cadmium accumulation was significantly higher in shoots than roots (Table 2).
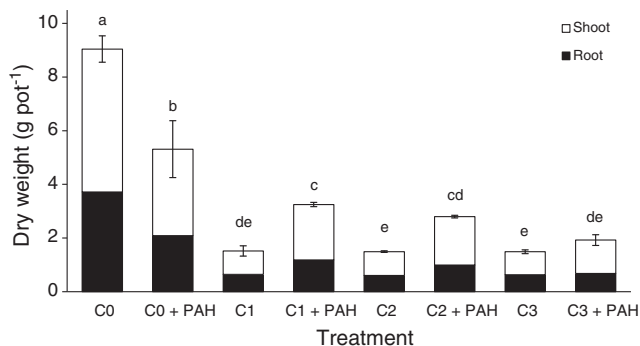
Fig. 2. The biomass influenced by different Cd and PAH treatments after 10 weeks of *J. subsecundus* growth. Bars (means ± SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatments: C0, C1, C2 and C3 represent 0, 5, 10 and 20 mg Cd kg^{-1} and PAH represents $50 + 50$ mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.

3.5. Concentration, accumulation and translocation of PAHs in plants and removal by plants

The concentrations and shoot/root concentration factors (SCFs/RCFs) of PAHs in plants (except for phenanthrene in shoots at 5 mg Cd kg^{-1}), and accumulation and removal of PAHs by shoots or whole plants were not significantly influenced by Cd additions (Tables 3 and 4). The translocation factors (TFs) of phenanthrene significantly increased with Cd additions up to 10 mg Cd kg^{-1}, but those of pyrene were not significantly influenced by Cd additions (Table 3). The accumulation of PAHs was significantly higher in roots than shoots (Table 3).

3.6. Dissipation of PAHs from rhizosphere and non-rhizosphere

The dissipation of PAHs from soils was not significantly influenced by Cd additions after 10 weeks of plant growth. The dissipation of pyrene (but not phenanthrere) was significantly lower in the rhizosphere than non-rhizophere (Fig. 3). On average, 97% of phenanthrene dissipated in both rhizosphere and non-rhizosphere, whereas on average 43% and 63% of pyrene dissipated in the rhizosphere and non-rhizosphere, respectively.

3.7. PAH-degrading bacteria in rhizosphere and non-rhizosphere

The abundance of PAH degraders in soils was significantly influenced by the presence of PAHs, but not by Cd additions after 10 weeks of plant growth. PAH degraders increased significantly in both rhizosphere and non-rhizosphere, with an increase being the highest in the rhizosphere (Fig. 4).

3.8. pH and water-extractable Cd in rhizosphere and non-rhizosphere

The pH was significantly higher in the rhizosphere than non-rhizosphere soils after 10 weeks of plant growth. The pH in rhizosphere was significantly influenced by Cd additions, but not by PAHs, whereas pH in non-rhizosphere was not significantly influenced by the treatments (Table 5).

The water-extractable Cd in soils after experiment was significantly influenced by Cd additions, but not by PAHs (except for Cd treatment of 20 mg kg^{-1} in the rhizosphere). No significant difference was observed between the rhizosphere and non-rhizosphere (Table 6).

4. Discussion

Cadmium is a naturally-occurring heavy metal with no known nutritional requirement by biota. It is toxic to plant cells, even at low concentrations (Lux et al., 2011). In contrast, exposure to low doses of PAHs can stimulate plant growth, but high doses of PAHs hamper and eventually inhibit plant growth (Ma et al., 2010). In the present study, plant growth significantly decreased when either Cd or PAHs were present, but was significantly better in Cd treatments with PAHs than without (except for the treatment of 20 mg Cd kg^{-1}). Similarly, certain concentrations of pyrene could alleviate the growth inhibition in maize (*Zea mays*) caused by Cu (Lin et al., 2008) and ryegrass (*Lolium multiflorum*) caused by Pb (Khan et al., 2011). Other organic pollutants present in low concentrations could also alleviate metal-caused growth inhibition [e.g. pentachlorophenol alleviated Cu toxicity to ryegrass (*Lolium perenne*) and radish (*Raphanus sativus*)], whereas high concentrations exacerbated that toxicity (Lin et al., 2006). These results suggest that combinations of metals and organic pollutants may exert either alleviating or exacerbating effect on plant growth, depending on plant species, plant growth stages, concentrations and properties of pollutants, and soil conditions such as pH and content of organic matter. In the present study, PAHs alleviated,

Table 1

The concentrations of Cd in plant tissues, shoot/root concentration factors (SCFs/RCFs) and translocation factors (TFs) influenced by different Cd treatments with or without PAHs ($50 + 50$ mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion) after 10 weeks of *J. subsecundus* growth.

Cd treatment (mg kg^{-1})	Shoot (mg kg^{-1})		Root (mg kg^{-1})		SCF		RCF		TF (%)	
	− PAH	+ PAH	− PAH	+ PAH	− PAH	+ PAH	+ PAH	+ PAH	+ PAH	+ PAH
0	0.1 ± 0.02b*A**	0.2 ± 0.03cA	0.4 ± 0.1cA	0.9 ± 0.6dA	–	–	–	–	54 ± 28bA	53 ± 31aA
5	262 ± 46aA	236 ± 22bA	180 ± 25bA	216 ± 26cA	52 ± 9aA	47 ± 4aA	36 ± 5aA	43 ± 5aA	151 ± 32aA	111 ± 11aA
10	282 ± 14aA	270 ± 28bA	272 ± 34aA	320 ± 35bA	28 ± 1bA	27 ± 3bA	27 ± 3abA	32 ± 3abA	107 ± 13abA	84 ± 2aA
20	338 ± 29aB	444 ± 38aA	330 ± 26aB	438 ± 38aA	17 ± 1bA	22 ± 2bA	17 ± 1bA	22 ± 2bA	103 ± 10abA	102 ± 9aA

*Means (± SE, $n = 3$) followed by the same lower-case letter within columns are not significantly different according to LSD ($p \leq 0.05$).
**Means (± SE, $n = 3$) followed by the same capital letter between the treatments with and without PAHs are not significantly different based on LSD ($p \leq 0.05$).

Table 2

Cadmium accumulation in plants and percentage removal by plants influenced by different Cd treatments with or without PAHs ($50 + 50$ mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion) after 10 weeks of *J. subsecundus* growth.

Cd treatment (mg kg^{-1})	Shoot (µg pot^{-1})		Root (µg pot^{-1})		Removal by shoots (%)		Removal by whole plants (%)	
	− PAH	+ PAH	− PAH	+ PAH	− PAH	+ PAH	− PAH	+ PAH
0	0.7 ± 0.1b*A**	0.6 ± 0.2bA	1.3 ± 0.5cA	2.0 ± 1.7bA	–	–	–	–
5	217 ± 15aB	491 ± 64aA	85 ± 15bB	205 ± 14aA	1.4 ± 0.1aB	3.3 ± 0.4aA	2.0 ± 0.2aB	4.6 ± 0.5aA
10	252 ± 23aB	483 ± 35aA	114 ± 7abB	250 ± 35aA	0.8 ± 0.1abB	1.6 ± 0.1bA	1.2 ± 0.1abB	2.4 ± 0.2bA
20	286 ± 14aB	551 ± 62aA	154 ± 17aB	247 ± 45aA	0.5 ± 0.02bA	0.9 ± 0.1cA	0.7 ± 0.02bA	1.2 ± 0.2cA

*Means (± SE, $n = 3$) followed by the same lower-case letter within columns are not significantly different according to LSD ($p \leq 0.05$).
**Means (± SE, $n = 3$) followed by the same capital letter between the treatments with and without PAHs are not significantly different based on LSD ($p \leq 0.05$).

Table 3

Concentrations of PAHs in plant tissues, shoot/root concentration factors (SCFs/RCFs) and the root-to-shoot transfer factors (TFs) influenced by Cd treatments after 10 weeks of *J. subsecundus* growth.

Cd treatment (mg kg^{-1})	Shoot (mg kg^{-1})		Root (mg kg^{-1})		SCF		RCF		TF (%)	
	Phenanthrene	Pyrene	Phenanthrene	Pyrene	Phenanthrene	Pyrene	Phenanthrene	Pyrene	Phenanthrene	Pyrene
0	7.4 ± 1.1b*	2.7 ± 1.6a	44 ± 12a	78 ± 12a	0.65 ± 0.03b	0.07 ± 0.35a	1.3 ± 0.04a	2.0 ± 0.3a	19 ± 5c	3 ± 2a
5	11 ± 3b	1.2 ± 0.6a	37 ± 1a	79 ± 4a	0.92 ± 0.09b	0.03 ± 0.04a	1.1 ± 0.01a	2.0 ± 0.1a	29 ± 10bc	2 ± 1a
10	21 ± 4a	1.5 ± 0.4a	34 ± 1a	57 ± 9a	1.8 ± 0.1a	0.04 ± 0.04a	1.0 ± 0.01a	1.5 ± 0.2a	61 ± 11a	3 ± 1a
20	21 ± 4a	2.1 ± 1.1a	39 ± 4a	52 ± 7a	1.8 ± 0.1a	0.05 ± 0.13a	1.1 ± 0.03a	1.3 ± 0.2a	45 ± 8ab	4 ± 2a

*Means (± SE, $n = 3$) followed by the same letter within columns are not significantly different according to LSD ($p \leq 0.05$).

at least partly, Cd toxicity to the wetland species, but the mechanisms behind interactive effect of Cd and PAHs were unclear.

In the present study, the significantly better plant growth in the Cd treatments (5 and 10 mg kg^{-1}) with PAHs compared to the treatments without PAHs (Figs. 1 and 2) might not have been related to the concentration of bioavailable Cd in soils, because the concentration of Cd in plant tissues was not significantly different between Cd treatment of either 5 or 10 mg kg^{-1} regardless of PAHs. Compared to Cd treatments without PAHs, the water-extractable Cd in Cd treatment of either 5 or 10 mg kg^{-1} with PAHs was not significantly different before transplanting (data not shown), and slightly but not significantly decreased in non-rhizosphere, while increased in rhizosphere after the experiment (Table 6). The soil pH could influence

Cd bioavailability (Marchand et al., 2010), but in the present study the pH was not significantly influenced by Cd treatments with or without PAHs before (data not shown) and after the experiment (Table 5).

In the present study, the water-extractable Cd in soils after the experiment was not significantly influenced by the addition of PAHs (except for Cd treatment of 20 mg kg^{-1} in the rhizosphere). In our previous study, however, the water-extractable Cd was decreased by the PAH additions (using higher concentrations of Cd and PAHs than in the present study), whereas EDTA-extractable soil Cd after the experiment was not significantly influenced by the PAH additions (Zhang et al., 2011c). The interaction between metal and organic pollutants may influence their adsorption onto soil particles. For instance, the presence of heavy metals (Cd, Pb and Zn) could enhance

Table 4

PAH accumulation in plants and percentage removal by plants influenced by Cd treatments after 10 weeks of *J. subsecundus* growth.

Cd treatment (mg kg^{-1})	Shoot (µg pot^{-1})		Root (µg pot^{-1})		Removal by shoot (%)		Removal by whole plant (%)	
	Phenanthrene	Pyrene	Phenanthrene	Pyrene	Phenanthrene	Pyrene	Phenanthrene	Pyrene
0	24 ± 6a*	7 ± 3a	64 ± 22a	116 ± 25a	0.02 ± 0.01a	0.006 ± 0.003a	0.09 ± 0.03a	0.11 ± 0.02a
5	22 ± 7a	2 ± 1a	33 ± 2a	71 ± 7b	0.02 ± 0.01a	0.002 ± 0.001a	0.05 ± 0.01a	0.06 ± 0.01b
10	37 ± 5a	3 ± 1a	24 ± 3a	40 ± 8b	0.04 ± 0.01a	0.002 ± 0.001a	0.06 ± 0.01a	0.04 ± 0.01b
20	27 ± 7a	3 ± 1a	27 ± 4a	36 ± 5b	0.03 ± 0.01a	0.002 ± 0.001a	0.05 ± 0.01a	0.03 ± 0.00b

*Means (± SE, $n = 3$) followed by the same letter within columns are not significantly different according to LSD ($p \leq 0.05$).
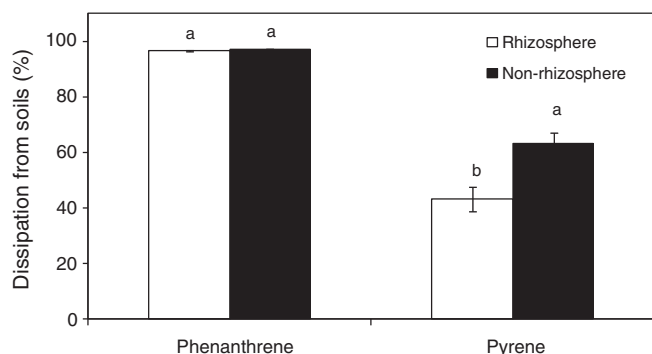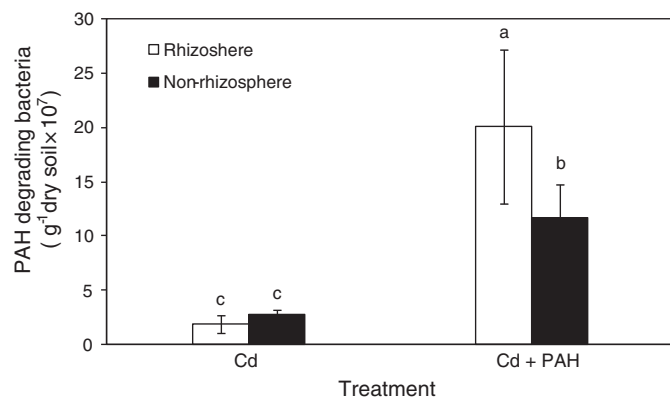
Fig. 3. The percentage of PAH dissipation, averaged over Cd treatments, in the rhizosphere and non-rhizosphere after 10 weeks of *J. subsecundus* growth (there was no significant interaction between Cd treatments and rhizosphere/non-rhizosphere). Bars (means ± SE, $n = 12$) with different letters are significantly different based on LSD ($p \leq 0.05$).

phenanthrene adsorption in soils (Gao et al., 2006; Zhang et al., 2011a), whereas phenanthrene (ranging between 100 and 300 mg kg^{-1}) suppressed Pb sorption to a certain extent (Zhang et al., 2010a). However, the presence of 1,4-dichlorobenzene (50 mg L^{-1}) in soils had little effect on Cd and Cu sorption (Sun and Zhou, 2010). There was no significant difference in the soil Cd fractions between the treatment with only Cd contamination and the treatments with co-contamination of Cd and pyrene (Zhang et al., 2009). These results indicated that an interaction between metals and organic pollutants influencing their adsorption onto soil particles was dependent on their concentrations and properties, soil conditions such as pH and/or types of co-pollutants presented in the media.

In the present study, the wetland species took up simultaneously metals (e.g. Cd) and organic pollutants (e.g. PAHs) from soils, as also found by others (e.g. Mattina et al., 2003; Sun et al., 2011). However, the shoot/root concentration factors (SCFs/RCFs) were much higher for Cd than PAHs (Tables 1 and 3), and Cd accumulation was significantly higher in shoots than roots, whereas the opposite was true for PAHs (Tables 2 and 4). Cadmium is relatively mobile in soil and is readily taken up by plants through membrane transporters for essential nutrients, such as Fe, Zn and Ca (Zheng et al., 2011). In contrast, plant uptake of hydrophobic organic chemicals, such as PAHs, could be described as partitioning between the soil aqueous solution and plant roots, with translocation of PAHs from root to shoot being highly restricted (Gao et al., 2011).

The interaction between metals and organic pollutants could influence metal uptake and accumulation by plants. Increased Zn

Fig. 4. The most probable number (MPN) of phenanthrene and pyrene degraders, averaged over Cd treatments, as influenced by PAH additions in the rhizosphere and non-rhizosphere after 10 weeks of *J. subsecundus* growth (there was no significant interaction between Cd treatment and PAHs or rhizosphere/non-rhizosphere). Bars (means ± SE, $n = 12$) with different letters are significantly different based on LSD ($p \leq 0.05$).

Table 5

The pH in non-rhizosphere and rhizosphere influenced by different Cd treatments with or without PAHs ($50 + 50$ mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion) after 10 weeks of *J. subsecundus* growth.

Cd treatment (mg kg^{-1})	Non-rhizosphere		Rhizosphere	
	− PAH	+ PAH	− PAH	+ PAH
0	5.2 ± 0.08ab*A**	5.4 ± 0.07aA	5.2 ± 0.06bA	5.1 ± 0.05bA
5	5.3 ± 0.02aA	5.4 ± 0.03aA	4.9 ± 0.03bB	5.0 ± 0.03bAB
10	5.2 ± 0.01aA	5.4 ± 0.02aA	4.9 ± 0.11bB	4.9 ± 0.03bB
20	5.1 ± 0.07aA	5.2 ± 0.03aA	4.8 ± 0.14bB	4.8 ± 0.01bB

*Means (± SE, $n = 3$) followed by the same lower-case letter within columns are not significantly different according to LSD ($p \leq 0.05$).
**Means (± SE, $n = 3$) followed by the same capital letter between the treatments with and without PAHs are not significantly different based on LSD ($p \leq 0.05$).

concentrations were found in shoots of Indian mustard (*Brassica juncea*) grown in soils contaminated with a mixture of pyrene and Zn (Batty and Anslow, 2008). The PAHs increased Cu uptake by a salt marsh plant (*Halimione portulacoides*) in elutriate, but not in the presence of sediments (Almeida et al., 2008). In the present study, uptake of Cd by plants was not significantly influenced by PAHs (except for Cd treatment of 20 mg kg^{-1}) and vice versa (except for phenanthrene in shoots at Cd treatment of 5 mg kg^{-1}), but Cd accumulation in plants was significantly influenced by PAHs. The phytoextraction potential of Cd (i.e. removal of Cd by shoots) was enhanced by the presence of PAHs together with Cd (Table 2), whereas the accumulation of PAHs in shoots was relatively small, and removal by shoots accounted for 0.02–0.04% and 0.002–0.006% for phenanthrene and pyrene, respectively (Table 4). The results suggested that the wetland species may be effective in Cd removal in the Cd–PAHs co-contaminated soils. Nevertheless, uptake of PAHs by plants was not a major pathway compared with biodegradation of PAHs; moreover, uptake of PAHs might occur from the air rather than just from soils (Gao and Zhu, 2004; Sun et al., 2010).

The mechanisms underlying dissipation of PAHs in soils are biodegradation, photodegradation, volatilization, plant uptake and metabolism, and incorporation into soil organic material (Ma et al., 2010). The pyrene was more persistent in soils than phenanthrene (Gao and Zhu, 2004). In the present study, the efficacy of PAH dissipation from soils differed significantly for phenanthrene and pyrene (Fig. 3), as found by others (e.g. Gao and Zhu, 2004; Sun et al., 2010). The relatively slower dissipation of pyrene in the rhizosphere compared to non-rhizosphere in the present study might suggest that plants could accumulate such hydrophobic compounds in the rhizosphere, but they would dissipate with time (Liste and Alexander, 2000a).

Plants have been shown to promote microbial biodegradation of PAHs (Liste and Alexander, 2000b). Phytoremediation of PAHs is considered to reflect the increased presence and activity of PAH

Table 6

Water-extractable Cd (mg kg^{-1}) in non-rhizosphere and rhizosphere influenced by different Cd treatments with or without PAHs ($50 + 50$ mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion) after 10 weeks of *J. subsecundus* growth.

Cd treatment (mg kg^{-1})	Non-rhizosphere		Rhizosphere	
	− PAH	+ PAH	− PAH	+ PAH
0	Undetected	Undetected	Undetected	Undetected
5	0.13 ± 0.02a*B**	0.13 ± 0.01aB	0.19 ± 0.04aB	0.27 ± 0.03aB
10	0.43 ± 0.05aB	0.30 ± 0.01aB	0.34 ± 0.06aB	0.57 ± 0.02aB
20	1.42 ± 0.53bA	1.07 ± 0.23bA	1.41 ± 0.38bA	2.00 ± 0.13aA

*Means (± SE, $n = 3$) followed by the same lower-case letter within columns are not significantly different according to LSD ($p \leq 0.05$).
**Means (± SE, $n = 3$) followed by the same capital letter between the treatments with and without PAHs are not significantly different based on LSD ($p \leq 0.05$).

degraders in the rhizosphere (Balcom and Crowley, 2009). In the present study, PAH degraders in soils, especially in rhizosphere, significantly increased in abundance due to addition of PAHs (Fig. 4). In our previous study, the total number of microorganisms in soils was significantly higher in the PAH compared to the non-PAH treatments (Zhang et al., 2011c). An increase in microbial numbers, enhancement of microbial activity and/or modifications in the microbial community structure in the rhizosphere as a result of the input of easily degradable organic substances such as PAHs may improve the plant resistance to the pollutant stress and improve plant acclimation rate and biomass production (Khan et al., 2009). The pyrene contamination resulted in significant shifts in the composition of rhizosphere bacterial communities (Balcom and Crowley, 2009). The rhizobacteria isolated from soil contaminated with petroleum and heavy metals showed plant growth-promoting traits, including indole acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and siderophore(s) synthesis (So-Yeon et al., 2010). Plant-growth-promoting rhizobacteria could accelerate phytoremediation of metalliferous soils (Ma et al., 2011). An IAA-dependent increase in the root-surface area led to improved sorghum (Sorghum bicolor) growth under phenanthrene stress (Golubev et al., 2011). Hence, the enhanced plant growth in the Cd–PAHs co-contaminated soils as observed in the study presented here is likely linked to increased abundance/activity of PAH degraders in rhizosphere, but further study is needed to confirm the plant–microbe interactions in the co-contaminated wetland soils.

5. Conclusions

The emergent wetland species *J. subsecundus* could take up and translocate Cd and PAHs, but accumulation of Cd in plants was much higher than that of PAHs. Soil contamination with Cd and PAHs could partly lessen Cd toxicity to plants, resulting in improvement of plant growth and increased Cd removal by plants. PAH dissipation from soils and the abundance of PAH degraders was not significantly influenced by Cd additions. The present study indicated that *J. subsecundus* had the potential for phytoremediation of Cd–PAHs co-contaminated soils. Nevertheless, different potential of various wetland species to take up Cd with and without PAHs present suggests that the specific criteria for phytoremediation need to be devised for Cd–PAHs co-contaminated soils and waters.

Acknowledgments

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