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Polynuclear aromatic hydrocarbons (PAHs) mediate cadmium toxicity to an emergent wetland species

Zhenhua Zhang [a], Zed Rengel [a,*], Kathy Meney [b], Ljiljana Pantelic [b], Radmila Tomanovic [b]

[a] School of Earth and Environment, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
[b] Syrinx Environmental Pty Ltd., 12 Monger St., Perth, WA 6000, Australia

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ABSTRACT

Growth and pollutant removal by emergent wetland plants may be influenced by interactions among mixed pollutants in constructed wetlands. A glasshouse experiment was conducted to investigate interactive effects of cadmium (Cd) × polynuclear aromatic hydrocarbons (PAHs) × plant treatments on growth of *Juncus subsecundus*, Cd and PAH removal from soil and the total number of microorganisms in soil. Growth and biomass of *J. subsecundus* were significantly influenced by interaction of Cd and PAHs, significantly decreasing with either Cd or PAH additions, but with the effect of Cd on plant growth being stronger than that of PAHs. The mixture of low Cd and low PAH lessened Cd toxicity to plants, resulting in improved plant growth and increased Cd accumulation in plant tissues, thus enhancing Cd removal by plants. The dissipation of PAHs in soils was significantly influenced by interactions of Cd, PAH and plant presence or absence. The total number of microorganisms in soils was significantly increased by the PAH additions. The interactive effect of Cd and PAHs on plant growth may be linked to the changes in the abundance of microorganisms in the rhizosphere, probably via a positive effect of PAH metabolites and/or phytohormones produced by microorganisms on plant growth.

1. Introduction

Phytoremediation of mixed pollutants (organics and inorganics) is a poorly understood area, but very relevant because many soils and waters are exposed to mixed pollution [1]. Mixtures of heavy metals and polynuclear aromatic hydrocarbons (PAHs) are becoming increasingly prevalent in the ecosystems (e.g. wetland sediments) due to urbanization and industrial contamination [2]. Phytoremediation of mixed pollution (metal and organic pollutants) is a complex problem [3].

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 [4]. Previous studies have shown that biodegradation of organic pollutants can be diminished by metal toxicity in both aerobic and anaerobic co-contaminated systems [5]. In contrast to organic pollutants, the most effective, but also technically the most difficult, phytoremediation strategy for heavy metal is phytoextraction. The interactions of metal and organic pollutants could influence metal uptake and accumulation by plants. For instance, PAHs influenced the soluble copper (Cu) fraction and increased Cu uptake by *Halimione portulacoides* in elutriate, but not in the presence of sediments [4]. There were significantly higher concentrations of zinc (Zn) in shoots of Indian mustard (*Brassica juncea*) grown in soil with a mixture of pyrene and Zn [6]. Nevertheless, the effects of metal toxicity on organic pollutant biodegradation and/or organic pollutants on the phytoextraction of metal have not been adequately defined quantitatively or qualitatively in contaminated water and soil environments.

Constructed wetlands for treating wastewater (that may frequently contain metal and organic pollutants) are a growing phytoremediation technology around the world. Plants play a significant role in constructed wetlands [7,8]. They can enhance metal removal and/or stabilization [9] and may also facilitate biodegradation of organic pollutants such as PAH (i) directly in the rhizosphere by the release of root exudates, and (ii) indirectly by improving soil biology via build-up of organic carbon [1].

Although the removal of organic and inorganic pollutants may be satisfactory in constructed wetlands, some pollutants such as metals and PAHs may accumulate in the substrate when wetlands are exposed to wastewater over long periods of time [2,10]. Growth and pollutant removal by wetland plants may be influenced by interactions between organic and inorganic pollutants. However, the knowledge about these interactions is poor.

The published studies investigating the combined toxicity of metals and PAHs have indicated that toxicity may be additive, independent, synergistic (greater than additive toxicity),

* Corresponding author. Tel.: +61 8 6488 2557; fax: +61 8 6488 1050.
E-mail addresses: zed.rengel@uwa.edu.au, zhenhuaz70@hotmail.com (Z. Rengel).

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or antagonistic (less than additive toxicity). For instance, the synergistic effects were noted between a photooxidised PAH (1,2-dihydroxyanthraquinone) and Cu on photosynthesis and plant growth of *Lemna gibba* [11], whereas the negative effects of lead (Pb) and Cu on growth of *Triticum aestivum* were diminished by the simultaneous addition of fluoranthene to the metal solutions [12].

Cadmium (Cd) is a naturally occurring heavy metal with no known nutritional requirement for biota. The high concentrations of Cd are toxic to plants [13].

Exposure to low doses of PAHs can stimulate plant growth, but high doses of PAHs hamper and eventually inhibit plant growth [14]. *Carex gracilis* and *Juncus effusus* did not suffer any damage when exposed to naphthalene at the concentrations of 30 mg L^{-1} (saturation concentration) in water [15]. However, plant sensitivity to metals and PAHs may be enhanced by mixture of pollutants.

The emergent wetland species such as *Juncus subsecundus* N.A. Wakef. (family *Juncaceae*) are often used for revegetation of native wetlands as well as in constructed wetlands [16,17]. Even though the benefits of the plant presence and differences among species in pollutant removal have been documented [7,18], there is a lack of knowledge on whether the growth of native wetland species is influenced by the mixture of Cd and PAHs in constructed wetlands. Hence, the objectives of this study were to investigate (1) the interactive effects of Cd and PAHs on growth and development of *J. subsecundus*; (2) Cd removal and phenanthrene/pyrene degradation when mixtures of these pollutants were present in soils; and (3) the influence of Cd and PAHs on the total number of microorganisms in soils.

2. Materials and methods

2.1. Preparation of contaminated soil

The Gingin loam soil (Gingin Shire, Western Australia) has been used as media in constructed wetlands for stormwater treatment [19] and was therefore used in the present study. Soil without detectable PAHs and Cd was collected from Gingin (31°46′S, 115°86′E). The soil 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 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}. The soil was air-dried and sieved through a 2-mm mesh.

PAHs (phenanthrene > 96% purity and pyrene > 98% purity; Sigma Chemical Co., Germany) were spiked into the soils at concentrations of 0, 50 + 50 or 250 + 250 mg kg^{-1} (i.e. *P*0, *P*1 and *P*2) 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. After evaporation of acetone in the fume-hood, the soils were thoroughly mixed with the remaining 75% by weight of the required amount of soil [20].

Cadmium (as $CdCl_2 \times 2^1/_2H_2O$, analytical grade, AJAX Chemicals, Sydney, Australia) was dissolved in Milli-Q water and added to the PAH-spiked soils at concentrations of 0, 10 or 50 mg kg^{-1} (i.e. *C*0, *C*1 and *C*2).

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 to the soils. The spiked soils were kept in a dark-room for one week of equilibration before transplanting. The concentration of PAHs in spiked soils was analysed before starting the experiment (Table 1).

Table 1
Initial concentrations of extractable phenanthrene and pyrene in soils.

Treatments	PAHs spiked (phenanthrene + pyrene, mg kg^{-1})		
	0	50 + 50	250 + 250
Phenanthrene	Not detected	34.3 ± 0.4	201 ± 2.5
Pyrene	Not detected	38.8 ± 1.0	222 ± 5.6

2.2. Experimental setup

Based on the previous experiments [16], the species *J. subsecundus* was selected for the experiment 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 early July to mid September, 2009. A complete randomized block design (three Cd treatments × three PAH treatments × two plant treatments) with three replicates was employed. The plant treatments included: (A) control (without plant); (B) planted with *J. subsecundus*. The seedlings were collected from the local nursery and transplanted (with initial plant fresh weight 5.8 ± 0.7 g per pot) into the pots (165 mm in diameter at the top and 125 mm in height) containing 3 kg 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 tallest shoot height were measured weekly, starting 2 weeks after plant establishment. The plants were harvested after 70 days 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 belowground (rhizomes including stem base and root) were separated from soil by rinsing with de-ionized water three times after washing with running tap water and collecting rhizomes and roots onto a mesh. All samples were dried to constant weight at 70 °C for 5 days in a forced-air cabinet, weighed for dry weight (DW) biomass and ground to pass a 0.75-mm mesh. The soil samples were collected and analysed for pH, extractable Cd, phenanthrene and pyrene, and total number of microorganisms.

The concentration of Cd in plant tissues was determined by ICP–OES (Optima 5300DV, PerkinElmer, Shelton, USA) after digesting plant material in a mixture of concentrated nitric and perchloric acids [21].

The translocation factor (TF) was calculated as [16]:

$$\mathrm{TF}(\%) = \frac{\text{Cd content in shoot}}{\text{Cd content in root}} \times 100 \quad (1)$$

Total Cd accumulation in plant, expressed as mg pot^{-1}, was calculated as:

$$\begin{aligned}\text{Total Cd accumulation} &= (\text{Cd concentration in shoots} \times \text{shoot DW}) \\ &+ (\text{Cd concentration in rhizomes} \times \text{rhizome DW}) \\ &+ (\text{Cd concentration in roots} \times \text{root DW}) \quad (2)\end{aligned}$$

The percentage removal of Cd by plants was calculated as:

$$\text{Removal of Cd by plant}\,(\%) = \frac{\text{total Cd accumulation in plant}}{\text{total Cd added in to soil}} \times 100 \quad (3)$$

The number of microorganisms in soils was determined by the plate-count method for viable cells. To enumerate the viable microbial population, aqueous extracts of 4 g soil samples were serially diluted and spread on nutrient agar. Plates were incubated for 7

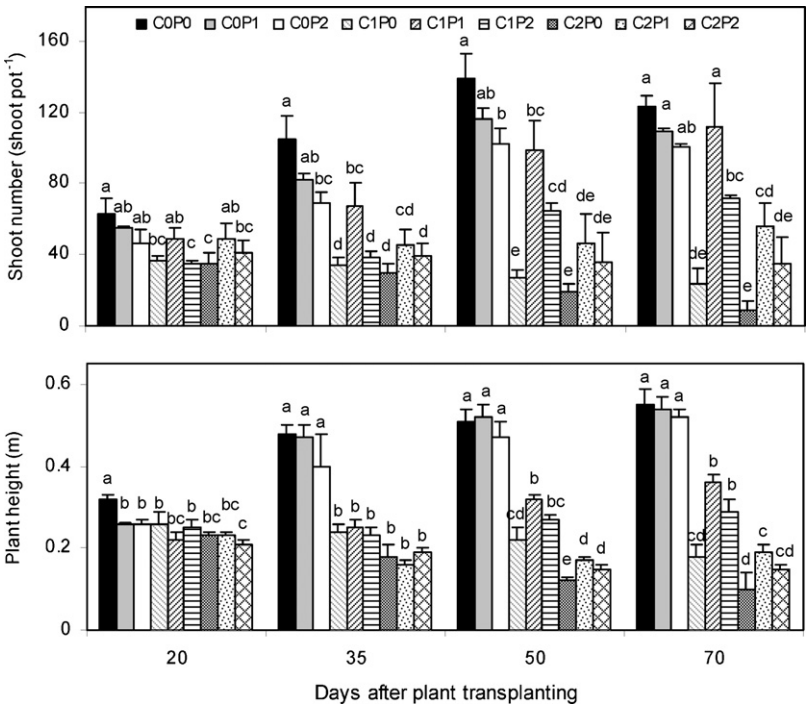

Fig. 1. The shoot number and the tallest height of *J. subsecundus* influenced by Cd and PAH treatments. Bars (means ± SE, $n = 3$) with different letters within days are significantly different based on LSD ($p \leq 0.05$). Treatments: *C*0, *C*1 and *C*2 represent 0, 10 and 50 mg Cd kg^{-1}; *P*0, *P*1 and *P*2 represent 0, 50 + 50 and 250 + 250 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.

days at 25 °C after overnight incubation at 37 °C prior to counting the numbers of colony forming units (CFU).

The pH (water and soil in 5:1) in soil were determined by a combination glass membrane electrode with a Calomel internal reference (Cyberscan 20 pH meter, Eutech Instruments, Singapore). The concentrations of water- and EDTA-extractable Cd in soil samples were measured by AAS (AAnalyst 400, PerkinElmer, Shelton, USA) after extraction and filtration. Briefly, for water-extractable Cd, 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. For EDTA-extractable Cd, soil (4 g) was shaken with 0.05 M EDTA (pH 7.0, 20 mL) for 1 h at 20 °C on an end-over-end shaker. Extract was clarified by filtration through Whatman® No. 42 filter paper [22].

Phenanthrene and pyrene in soil samples were analysed by Australian Laboratory Services Pty Ltd, Perth. Briefly, 10 g of fresh soil sample spiked with surrogates (2-fluorobiphenyl, anthracene-d10 and 4-terphenyl-d14) were extracted with 20 mL 1:1 dichloromethane/acetone by an end-over-end tumbler for 1 h after sodium sulphate 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 by comparison against an established 5-point calibration curve. The recoveries for surrogates were >81%.

The percentage of PAH removal from soils was calculated as:

$$\text{PAH removal } (\%) = \frac{\text{initial concentration of extractable PAH in soils} - \text{concentration of extractable PAH in soil after 70 days of plant growth}}{\text{initial concentration of extractable PAH in soils}} \times 100 \quad (4)$$

2.4. Statistical analyses

Statistical analyses were carried out using SPSS 17.0. Two-way analysis of variance (ANOVA) was used to compare the effects of Cd and PAHs on plant growth, biomass, pollutant concentrations and accumulation as well as translocation factor in plant tissues, Cd removal by plants, pH and concentrations of water- and EDTA-extractable Cd in soils. For the data regarding PAH removal from soils and total number of microorganisms in soil, three-way analyses of variance (ANOVA) were performed (including Cd, PAH and presence/absence of plants as treatments). Least significant difference (LSD) was applied to test for significance between means.

3. Results

3.1. Plant growth and biomass

The growth of *J. subsecundus* was significantly influenced by Cd and PAH treatments and their interactions. The shoot number and the tallest shoot height significantly decreased with either Cd or PAH additions, but the effect of Cd on plant growth was much stronger compared to that of PAHs (Fig. 1). For the shoot number, the significant interactions were detected from 35 to 70 days of growth, whereas the significant interaction for the plant height was observed at 70 days of growth.

After 70 days of growth, the shoot number significantly increased by 367 and 200% in the mixture of low Cd and either low PAH or high PAH compared to the low-Cd treatment alone, and by 522% in the mixture of high Cd and low PAH compared to the high-Cd treatment alone. The shoot height significantly increased by 100 and 61% in the mixture of low Cd and either low PAH or high PAH compared to the low-Cd treatment alone, and by 90% in the mixture of high Cd and low PAH compared to the high-Cd treatment alone. Although shoot number and height increased by 289% and 50%, respectively, in the mixture of high Cd and high PAH

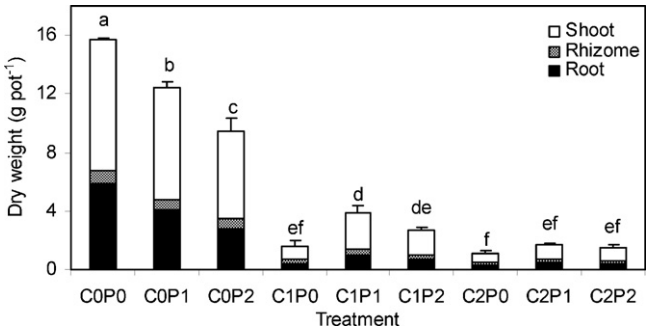

Fig. 2. Dry weight of *J. subsecundus* influenced by Cd and PAH treatments after 70 days of growth. Bars (means ± SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatment notation was the same as in Fig. 1.

compared to the high-Cd treatment alone, no significant difference was detected. There was no significant difference in shoot numbers and heights after 70 days of growth in treatments containing no added Cd regardless of PAH treatments.

The significant interactions of Cd and PAHs influenced the shoot, root and total biomass, but not rhizome biomass. After 70 days of growth, the plant biomasses were significantly reduced by Cd and PAH treatments. The influence of Cd on biomass was much stronger compared to PAHs (Fig. 2). The total biomass significantly decreased by 90 and 93% in the low- and high-Cd treatments, and by 21 and 40% in the low- and high-PAH treatments compared to the control. The total plant biomass was significantly increased (by 147%) in the mixture of low Cd and low PAH compared to the low-Cd treatment alone. Although the total plant biomass increased by 66% in the mixtures of low Cd and high PAH compared to the low-Cd treatment alone, no significant difference was detected. There was no significant difference in plant biomass among the high-Cd treatments regardless of PAH treatments.

3.2. Cd concentration, accumulation and translocation in plant tissues and removal by plants

The concentrations and accumulation of Cd in plant tissues and removal by plants were significantly influenced by Cd and PAH treatments, but no significant interaction was detected. The concentrations of Cd in different plant tissues increased with Cd additions and decreased with PAH additions (except for roots in the mixture of low Cd and low PAH), but a significant difference within the same tissues was not observed among the low-Cd treatments regardless of PAH treatments (except for shoot in the mixture of low Cd and high PAH) (Table 2). The order of Cd concentrations among the plant tissues was shoot > root > rhizome among the mixtures of Cd and PAH (except for shoot in the mixture of low Cd and high PAH).

The total Cd accumulation in plant tissues increased by 165% in the mixture of low Cd and low PAH compared to the low-Cd treatment alone, but no significant difference was found in the mixture of high Cd and low PAH compared to the high-Cd treatment alone. Although the accumulation of Cd decreased by 22 and 14%, respectively, no significant difference was observed in the mixtures of either low or high Cd and high PAH compared to either low- or high-Cd treatment alone (Fig. 3). The order of Cd accumulation in different plant tissues was shoot > root > rhizome among the treatments with added Cd.

The translocation factors (TFs) were above 100% (between 144 and 307%) among the treatments, except those without added Cd, but no significant difference was detected (data not shown).

The percentage of Cd removal from soils by plants was significantly greater in the low- than high-Cd treatments. The highest percent Cd removal from soils by plants was in the mixture of low Cd and low PAH, which increased significantly (2.6-fold) compared to the low-Cd treatment alone. The percent removal of Cd by plants was not significantly different among the high-Cd treatments regardless of PAH treatments (Fig. 3).

Table 2
Concentrations of Cd (mg kg^{-1}) in plant tissues influenced by Cd and PAH treatments after 70 days of plant growth.

Treatment[a]	Concentration of Cd (mg kg^{-1})		
	Shoot	Rhizome	Root
C0P0	0.22 ± 0.10f[b]	0.29 ± 0.27d	0.74 ± 0.30e
C0P1	0.05 ± 0.07f	0.08 ± 0.12d	0.55 ± 0.21e
C0P2	0.17 ± 0.17f	0.12 ± 0.11d	0.89 ± 0.71e
C1P0	458 ± 50d	114 ± 22c	287 ± 14d
C1P1	436 ± 100d	89 ± 9c	386 ± 39cd
C1P2	135 ± 54e	70 ± 2c	231 ± 14d
C2P0	1280 ± 18a	555 ± 137a	1110 ± 154a
C2P1	1099 ± 81b	366 ± 10b	857 ± 148b
C2P2	824 ± 25c	280 ± 30b	611 ± 79c

[a] Treatments: *C*0, *C*1 and *C*2 represent 0, 10 and 50 mg Cd kg^{-1}; *P*0, *P*1 and *P*2 represent 0, 50 + 50 and 250 + 250 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.
[b] Means ± SE ($n = 3$) followed by the same letter within columns are not significantly different based on LSD ($p \leq 0.05$).

3.3. PAH removal from soil

The percentage of PAH removal from soils was significantly influenced by the interaction of Cd, PAH and planting/non-planting treatments, but the removal of phenanthrene (87–90%) was significantly greater than that of pyrene (11–70%). The removal of phenanthrene was significantly influenced by Cd and PAH additions as well as the presence of plants, whereas pyrene was influenced only by PAH additions. The percentage of PAH removal was significantly higher in the low- than high-PAH treatments. The removal of phenanthrene decreased with Cd additions, which was more obvious in the non-planted treatments. There was no significant difference between planted and non-planted treatments without added Cd regardless of PAH treatments (except for pyrene in the high-PAH treatment) or when the mixture of high Cd and high PAHs was applied. Compared to the non-planted treatments, phenanthrene removal significantly increased by 4, 6 and 7%, respectively, in the planted mixtures of low Cd and either low or high PAH, and mixture of high Cd and low PAH. Pyrene removal increased by 170% in the planted mixture of low Cd and low PAH compared to the equivalent non-planted treatment. Pyrene removal was not significantly different among either planted or non-planted high PAH treatments regardless of Cd additions (Fig. 4).

3.4. Total number of microorganisms in soil

The total number of microorganisms in soils after 70 days of plant growth was significantly influenced by the PAH treatments and the presence of plant, but not by Cd treatments. A significant interaction of planting and PAH treatments on the total number of microorganisms was detected, but there was no significant interaction between Cd and PAHs or planting treatments. The number of microorganisms in soils was significantly higher in the PAH treatments compared to the non-PAH ones (Fig. 5).

3.5. pH and extractable Cd in planted soil

The pH in soils was not significantly different among the treatments (data not shown). The concentration of water-extractable

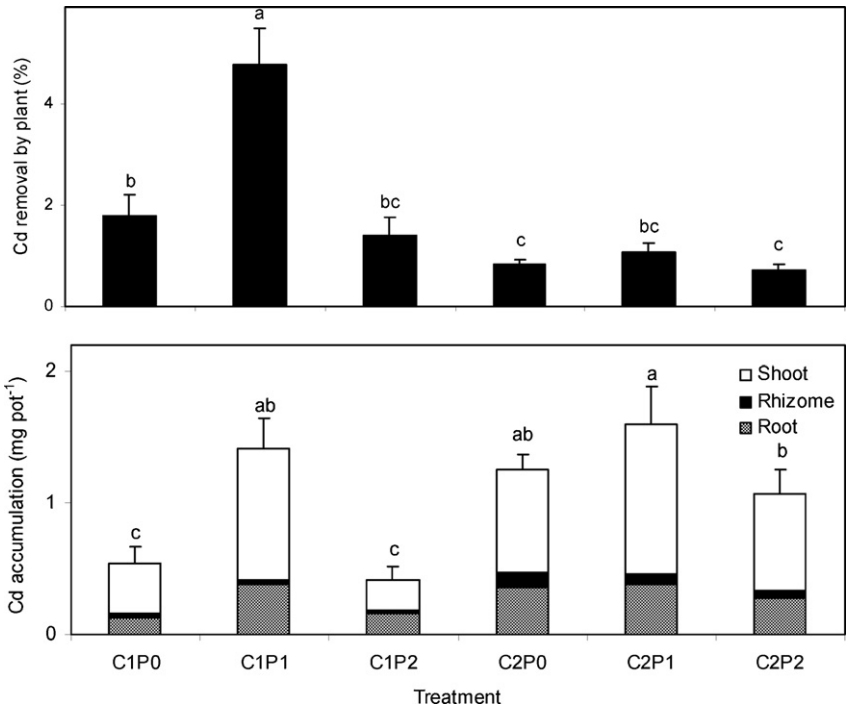

Fig. 3. Total Cd accumulation in plant tissues and percentage of Cd removal from soils by whole plants influenced by Cd and PAH treatments after 70 day of growth. Bars (means ± SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatments: *C*1 and *C*2 represent 10 and 50 mg Cd kg^{-1}; and *P*0, *P*1 and *P*2 represent 0, 50 + 50 and 250 + 250 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.

Cd in soils was significantly lowered by PAH additions, whereas the concentration of EDTA-extractable Cd was not significantly different among the PAH treatments. The water-extractable soil Cd decreased in treatments with low Cd mixed with either low (by 45%) or high PAH (by 65%) compared to the low-Cd treatment alone. A significant decrease (by 42%) was also found in the mixture of high Cd and high PAH, but not in the mixture of high Cd and low PAH compared to the high-Cd treatment alone (Table 3).

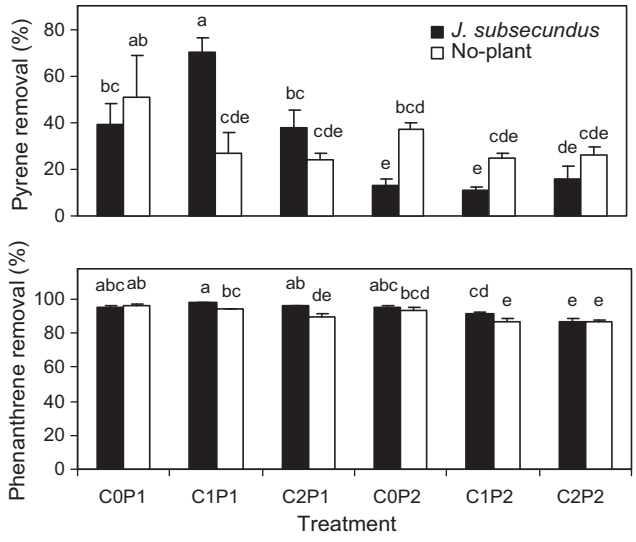

Fig. 4. The percentage of PAH removal from soils influenced by Cd, PAH and plant treatments after 70 day of growth. Bars (means ± SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatments: *C*0, *C*1 and *C*2 represent 0, 10 and 50 mg Cd kg^{-1}; *P*1 and *P*2 represent 50 + 50 and 250 + 250 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.

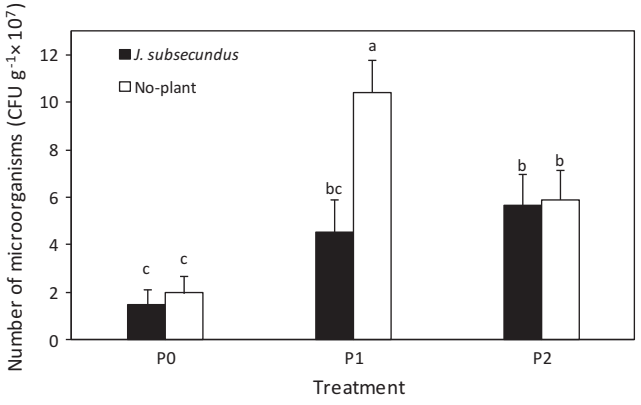

Fig. 5. Total number of microorganisms in soils, averaged over Cd treatments, as influenced by PAH and plant presence after 70 days of plant growth (There was no significant interaction between Cd and PAHs or planting treatments). Bars (means ± SE, $n = 9$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatments: *P*0, *P*1 and *P*2 represent 0, 50 + 50 and 250 + 250 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.

Table 3
Water- or EDTA-extractable soil cadmium in different Cd and PAH treatments after 70 days of plant growth.

Treatment[a]	Water-extractable Cd (mg kg^{-1})	EDTA-extractable Cd (mg kg^{-1})
C0P0	Not detected	Not detected
C0P1	Not detected	Not detected
C0P2	Not detected	Not detected
C1P0	0.40 ± 0.04c[b]	9.47 ± 0.43b
C1P1	0.22 ± 0.01d	9.34 ± 0.08b
C1P2	0.14 ± 0.04d	9.30 ± 0.34b
C2P0	3.12 ± 0.14a	47.1 ± 0.38a
C2P1	2.13 ± 0.40ab	47.8 ± 1.23a
C2P2	1.81 ± 0.39b	46.9 ± 2.45a

[a] Treatment notation was the same as in Table 2.
[b] Means ± SE ($n = 3$) followed by the same letter within columns are not significantly different based on LSD ($p \leq 0.05$).

4. Discussion

4.1. Interaction of Cd and PAHs influencing plant growth

The symptoms of Cd toxicity have been studied in several plant systems growing under various conditions [23]. The inhibiting effects of Cd on biomass accumulation, height, root length and other biometric parameters of wetland plants were reported [16,24,25]. In the present study, plant shoot number and height (Fig. 1) as well as dry weight (Fig. 2) of *J. subsecundus* were significantly reduced by Cd additions after 70 days of growth in soils.

PAHs, without being activated, are only toxic to higher plant when applied at high concentrations [26]. The effect of PAHs on wetland plant growth could be species-specific regardless of PAH types and media [17]. An increase in phenanthrene concentration decreased dry weight and shoot length of *Phalaris arundinacea* and *Phragmites australis*, but increased the number of young shoots [27]. In the present study, the shoot number and the tallest shoot height of *J. subsecundus* were not significant different from the control after 70 days of growth (Fig. 1), but biomass (Fig. 2) was significantly reduced by PAH additions.

An interactive effect of Cd and PAHs on plant growth was observed in the present study. In the mixture of low Cd and low PAHs, plants grew better than in the low-Cd treatment alone (Figs. 1 and 2), implying that PAHs could at least partly alleviate Cd toxicity effects. The existing literature reports both antagonistic and synergistic effects of metals and PAHs in co-contaminated soil on plant growth. For instance, the certain concentration of pyrene could alleviate the inhibition by Cu to maize (*Z. mays*) [28], but the pyrene combined with Cd could not alleviate Cd toxicity to *Z. mays* [29]. The interactive effect was also observed for other organic pollutants with metals. For example, the alleviating effects on growth of *Lolium perenne* and *Raphanus sativus* were found when a relatively low concentration of pentachlorophenol (PCP) was combined with copper (Cu) contamination in soils; whereas the additive effects were observed when a relatively high concentration of PCP was combined with Cu [30]. These results suggest that combinations of metal and organic pollutants may exert either antagonistic or synergistic effects on plant growth and performance, depending on plant species, plant growth stages, concentrations and characteristics of pollutants, and soil conditions such as pH and content of organic matter.

The translocation factors for *J. subsecundus* were greater than 100% among the treatments, except in the treatment with no Cd added, indicating efficient Cd transfer from roots to shoots and greater accumulation in shoots than roots, which is in contrast with the previous report [16]. The variations were possibly caused by the difference in the concentrations and durations of exposure to Cd and the different growth media. In general, wetland species are not metal hyperaccumulators; instead, they store metals in belowground tissues. However, plants growing in metal-enriched environments usually take up metals to varying degrees in response to external and internal factors. The accumulation of Cd was higher in shoots than in roots when canola (*Brassica napus*) was grown in soil supplemented with 15 mg Cd kg^{-1} [31]. Nevertheless, in the present study, the relatively high Cd concentrations (Table 2) and accumulation (Fig. 3) as well as translocation in tissues of *J. subsecundus* indicate that wetland species are innately tolerant to metals, and can be used for phytoextraction in constructed wetlands with polluted waters or soils.

The relatively better growth of plants in the mixture of low Cd and low PAH compared to the low-Cd treatment alone appears not to be related to the concentration of extractable Cd in soils (Table 3), because plants had similar Cd concentrations in the two treatments (Table 2), and higher accumulation in the mixture of low Cd and low PAH compared to the low-Cd treatment alone (Fig. 3). Although the water-extractable Cd was decreased by the PAH additions, EDTA-extractable Cd in soils (which is more related to plant growth) was not influenced by the PAH treatments (Table 3). It could not be excluded that the aromatic ring system of PAHs has chelating capacities for metals in solutions [12], but it was unlikely that ionic metals directly bind with the hydrophobic hydrocarbons in sediments [32]. The pH in soils was not changed by the treatments in the present study. There was no significant difference between the treatment with only Cd contamination and the treatment with Cd and pyrene (co-contaminated soil) with respect to the percentage of Cd fractions [29].

An increase in microbial numbers (Fig. 5), 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 formation [33,34]. Phytohormones that are produced by plant-associated bacteria, such as auxins, cytokinins and gibberellins, can frequently stimulate growth and indeed have been considered the causal agents for altered plant growth and development [3]. Fluoranthene might counteract the effect of Pb on inhibition of indolyl-3-acetic acid (IAA) production; moreover, fluoranthene itself behaved as a phytohormone [12]. These findings may imply that the interactive effect of Cd and PAHs on plant growth is possibly linked to the influence of PAH degradation by microorganisms and PAH uptake by plants on Cd tolerance and detoxification in plants.

Response to Cd stress in higher plants is a complex phenomenon. Cd evokes a response of parallel and/or consecutive events, rapid physiological and slow morphological processes, in which every mechanism could be at the same time cause and effect of metabolic changes, directly or indirectly related to the management of Cd stress [35]. The effect of mixture between Cd and PAHs on plant growth may be complicated, justifying further intensive studies to elucidate the underlying mechanisms.

4.2. Interaction of Cd and PAHs on PAH removal

The dissipation of phenanthrene in soils was much faster than that of pyrene (Fig. 4). The results agreed with other reports [33,36]. The mechanisms underlying dissipation of PAHs in soil are biodegradation, photodegradation, volatilization, plant uptake and metabolism, and incorporation into soil organic material [28]. The roles of abiotic (leaching, volatilization, photodegradation, irreversible sorption, chemical degradation, etc.) and biotic (microorganisms and plants) degradation were not separated in the present study. The contribution of abiotic losses of soil phenanthrene (amounting to 83% of the initial amount) and pyrene (57%) was the greatest among the four pathways (i.e. abiotic losses, microbial degradation, plant uptake and promotion from root exudates) [36]. In the study presented here, the abiotic losses such as volatilization and photodegradation might have also been high. Although the leaching of PAH was prevented by the sealed bottom of pots, the study was conducted under interchange between aerobic and anaerobic conditions, which simulated stormwater treatment in constructed wetlands, and exposure to the sunlight was not prevented during the experiment.

In many studies, plant uptake and accumulation of PAHs was small; moreover, uptake of PAHs might occur from the air rather than from the soil [33]. Hence, plant uptake of PAHs was not quantified in the present study. PAH uptake of *Lolium perenne* was weak, with roots impeding the dissipation of PAHs (the dissipation of PAHs was higher in the soil amended with root exudates only compared with the soil with growing roots exuding organic substances) [36]. This might suggest that the fast PAH removal by the planted treatments in the present study was related to the rhizosphere soil

microflora that is vitally important in degradation of xenobiotics, including PAHs. The contribution of plants to PAH dissipation by immobilization, removal and degradation is strongly dependent on the rhizosphere processes [1].

A negative effect of Cd on dissipation of PAHs (especially phenanthrene) was observed in the present study. It is well documented that the presence of metals such as Cd can inhibit a broad range of microbial processes [37]. Interaction of metals and PAHs with respect to degradation of PAHs can be negative or positive depending on type and concentration of both metals and PAHs. In a study of Pb and pyrene remediation, the presence of plants significantly promoted the degradation of pyrene in the soil due to enhanced bacterial community, with an increased number of pyrene degraders. Pyrene dissipation was accelerated by Pb in both rhizospheric and non-rhizospheric soils [34]. However, there was a 5-day longer lag period for phenanthrene degradation in the presence of 1 and 2 mg Cd L^{-1} and complete inhibition at 3 mg Cd L^{-1} [38]. In the study presented here, the planted treatments significantly increased phenanthrene dissipation when Cd was added, but that was not the case in the treatment with the mixture of high Cd and high PAHs (Fig. 4). The additions of Cd and PAHs could influence the plant–microbe interactions in the rhizosphere. The relatively high Cd and PAH concentrations might be toxic to specific groups of microorganisms, and the presence of PAHs might enhance Cd toxicity to microorganisms [39]. However, in planted treatments, plants might alleviate Cd toxicity to microorganisms and vise versa. Further intensive research is needed to better understand the mechanisms of PAH dissipation in soils co-contaminated with the mixture of metals and PAHs.

5. Conclusions

Interaction between low Cd load and PAHs was confirmed, whereby the presence of PAHs partially alleviated Cd toxicity to a wetland species *J. subsecundus*, probably via a positive effect of PAH metabolites and/or phytohormones produced by microorganisms on plant growth. In addition to abiotic loss, the PAH removal may be influenced by interactions of plants and microorganisms in the rhizosphere. Further research on the interaction between PAH and Cd determining PAH degradation and Cd toxicity needs to underpin practical use of phytoremediation for environments contaminated with metal and organic pollutants. The results suggest that the criteria for pollutant assessment and remediation need to be adapted if the mixed pollutants are present.

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