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Polynuclear aromatic hydrocarbons (PAHs) differentially influence growth of various emergent wetland species

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ABSTRACT

The growth of emergent wetland plants may be influenced by toxic organic pollutants, which would influence the extent of phytoremediation when used in constructed wetlands. A series of glasshouse experiments were conducted to investigate the influence of polynuclear aromatic hydrocarbons (PAHs) on the growth of various emergent wetland species. The response of species to PAHs varied significantly. A significant interaction (species × PAH treatment) was observed for relative growth rates (RGRs) of *Baumea juncea*, *Baumea articulata*, *Schoenoplectus validus* and *Juncus subsecundus* in hydroponics with naphthalene, and of *B. juncea* and *J. subsecundus* in soils freshly spiked with phenanthrene and pyrene. In hydroponics, biomass of *B. articulata* significantly increased in the treatments with relatively low addition of naphthalene, whereas that of *S. validus* significantly increased with all naphthalene additions. In both hydroponics and soils, the growth of *B. juncea* increased with the PAH (phenanthrene and pyrene) additions, whereas that of *J. subsecundus* decreased in the treatments with relatively high concentrations of PAHs. The removal of PAHs from soil was not affected significantly by *J. subsecundus* after 70 days of growth and *B. juncea* after 150 days of growth. The growth of *J. subsecundus* was slightly (but not significantly) influenced by the PAH residues in soil. The effect of PAHs on wetland plant growth could be species-specific regardless of PAH types and media. The response of species to PAHs needs to be taken into account when selecting species for wetlands constructed for phytoremediation.

1. Introduction

Constructed wetlands for treating wastewater (that may frequently contain metals as well as organic pollutants) represent an increasingly important phytoremediation technology around the world [1]. Plants play a significant role in constructed wetlands [2,3]. They may facilitate organic pollutant biodegradation (i) directly in the rhizosphere by the release of root exudates, and/or (ii) indirectly by improving soil biology via build-up of organic carbon [1]. Although the removal of organic pollutants may be satisfactory in constructed wetlands, some pollutants such as PAHs may accumulate in the substrate when wetlands are exposed to wastewater over long periods of time [4]. Such organic pollutants may affect growth and pollutant removal by wetland plants, resulting in differential success of phytoremediation in constructed wetlands. While the effect of plants on the removal and degradation of contaminants in constructed wetlands is studied within the scope of phytoremediation, the knowledge about the influence of contaminants on wetland plant growth is relatively poor [5,6].

Polynuclear aromatic hydrocarbons (PAHs) are becoming increasingly prevalent contaminants in the ecosystems (e.g. wetland sediments) due to urbanization and industrial contamination [4]. PAHs in soils and waters raise great concerns due to their recalcitrance and toxicity to living organisms [7]. The toxicity of PAHs to soil-grown plants has been examined extensively [8]. Exposure to low doses of PAHs can stimulate the plant growth, but high doses of PAHs hamper and eventually inhibit plant growth [9]. Morphological symptoms of PAH stress were root and shoot growth reduction, deformed trichomes, impaired root hair initiation and growth, chlorosis, late flowering, and appearance of white spots. At the tissue and cellular levels, plant suffered from oxidative stress [10]. PAHs can penetrate through the cell membranes, decrease water and nutrient utilization efficiency, and inhibit photosynthetic activity and electron transport [9].

As improvements in design of constructed wetlands are starting to reach a plateau, species selection may be the best way to maximize pollutant removal [11]. A wide variety of wetland plants can be used in constructed wetlands designed for pollutant treatment. Commonly, however, constructed wetlands are planned as marsh-

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type wetlands and are planted with emergent macrophytes (rooted plants that anchor to the substrate media) adapted to a water-dominated environment. Frequently used macrophytes species are cattails (*Typha sp.*), reeds (*Phragmites sp.*), bulrushes *(Scirpus sp.*) and sedges (*Carex sp.*) [12].

There is a large number of native wetland plant species growing in wetlands of the south-west of Western Australia [13]. The four emergent wetland species: *Baumea juncea* (R. Br.) Palla, *Baumea articulata* (R. Br.) S.T. Blake and *Schoenoplectus validus* (M. Vahl) A. & D. Löve (all family Cyperaceae) and *Juncus subsecundus* N.A. Wakef. (family *Juncaceae*) are often used for revegetation of natural wetland as well as in constructed wetlands [14]. Even though the benefits of the plant presence and differences among species in pollutant removal have been documented [11,15,16], there is a lack of knowledge on whether the growth of native wetland species is influenced by PAHs in constructed wetlands. Hence, the objectives of this study were to investigate (1) effect of naphthalene on the growth of four wetland species (*B. juncea*, *B. articulata*, *S. validus* and *J. subsecundus*) in hydroponics; (2) difference in growth and development between two species (*B. juncea* and *J. subsecundus*) in freshly spiked PAH (phenanthrene and pyrene) soil, and PAH removal from soil; (3) response of *J. subsecundus* to PAH residues in soil.

2. Materials and methods

2.1. Experiment in hydroponics (exp 1)

2.1.1. Experimental setup

The experiment was conducted in a controlled-environment room (20/15 °C day/night temperatures, 75–85% relative humidity, 12-h photoperiod, irradiance of 375–490 μmol quanta m^{-2} s^{-1}, PAR) at the University of Western Australia (31°58′S, 115°49′E). The seedlings of *B. juncea*, *B. articulata*, *S. validus* and *J. subsecundus* were collected from the local nursery and transplanted into a solution culture system in 4-L plastic containers, 4 plants in each, in a modified Hoagland nutrient solution. The nutrient solution was constituted by: 5 mM Ca $(NO_3)_2{\cdot}4H_2O$, 1 mM NaH_2PO_4, 5 mM KNO_3, 2 mM $MgSO_4{\cdot}7H_2O$, 1 mM KOH, 0.6 mM H_2SO_4, 28 μM Na_2-EDTA·$2H_2O$, 28 μM $FeSO_4{\cdot}7H_2O$, 45 μM H_3BO_3, 9 μM $MnCl_2{\cdot}4H_2O$, 0.4 μM $CuSO_4{\cdot}5H_2O$, 0.6 μM $ZnSO_4{\cdot}7H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$. After 2 weeks of acclimation growth in the nutrient solution, two containers of each species (total 8 plants per species) were harvested. The initial biomass and the shoot number and height were recorded. The remaining 36 containers were spiked by naphthalene (30 mg L^{-1} after each addition) in three treatments with triplicates (N0: unamended control, N1: added once every day and N3: added twice a day). A stock solution of naphthalene (Laboratory grade, AJAX Chemicals, Sydney, Australia) was dissolved in acetone. A spike dose of naphthalene was added to achieve an initial concentration of 30 mg L^{-1} of naphthalene in the nutrient solution according to the method described by Maillacheruvu and Safaai [17]. The same amounts of acetone were added in each treatment. The pH was adjusted to 6.5, and the nutrient solutions were replaced weekly. Solutions were vigorously aerated throughout.

2.1.2. Sampling and measurements

The plants were harvested after 2 weeks of naphthalene treatments. The plants were washed and separated into different parts: shoots (including leaves and stems), rhizomes and roots. Fresh weights (FW) were recorded. All plant samples were dried to constant weight at 70 °C for 5 days in a forced-air cabinet and weighed for dry weight (DW).

Table 1
Concentrations (mg kg^{-1}) of extractable phenanthrene and pyrene in soils (exps 2 and 3).

	PAH treatment		
	0	50 + 50	250 + 250
Initial PAHs (phenanthrene + pyrene) in freshly spiked soil			
Phenanthrene	ND[a]	33.9	203
Pyrene	ND	37.7	222
PAH residues in no-plant soil after 150 days			
Phenanthrene	ND	ND	3 ± 0.6[b]
Pyrene	ND	1 ± 0	48 ± 6

[a] ND: below detection limit.
[b] Values are the means ± SE, $n = 3$.

2.2. Experiment in freshly spiked soil (exp 2)

2.2.1. Preparation of contaminated soil

The Gingin loam soil located in Gingin County, Western Australia, has been used as a media in constructed wetlands for stormwater treatment [14]. Soils without detectable PAHs were collected from Gingin (31°46′S, 115°86′E). The soil was classified as 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 (in 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}. The soils were 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 0 (control; P0), 50 + 50 (low; P1) or 250 + 250 mg kg^{-1} (high; P2), with phenanthrene and pyrene in 1:1 proportion. Phenanthrene and pyrene were dissolved in acetone and added to a quarter of the required amount of soil. The same amount of acetone was added to all treatments, including the control. After evaporation of acetone in the fume-hood, the soils were thoroughly mixed with the remaining 3/4 of the required amount of soil [18]. 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 into the soils. The spiked soils were kept in a dark room for 1 week for equilibration before transplanting. Initial concentrations of PAHs in spiked soils were analyzed before commencement of the experiment (Table 1).

2.2.2. Experimental setup

Based on response of species to naphthalene in above study (experiment 1), the species (*B. juncea* and *J. subsecundus*) were selected for the subsequent experiment conducted in a glasshouse at The University of Western Australia with controlled day/night temperatures of 25/20 °C under natural light conditions from early July, 2009 to mid-December, 2009. A complete randomized block design (three PAH treatments × three planting treatments including no-plant, *B. juncea* and *J. subsecundus*) with three replicates was employed. The seedlings were collected from the local nursery and transplanted (with initial plant dry weight 0.10 ± 0.02 for *B. juncea* and 1.1 ± 0.1 g per pot *J. subsecundus*) into the pots 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.

2.2.3. 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 for *J. subsecundus* and 150 days of growth for *B. juncea*. 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 washing with running tap water, rinsing with de-ionized water three times 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 and weighed for dry weight (DW).

The soil samples were collected after 70 and 150 days of plant growth. Phenanthrene and pyrene in soil samples were analyzed by Australian Laboratory Services Pty Ltd., Perth. Briefly, 10 g of soil sample spiked with surrogate 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 residing in the sample. The solvent was transferred directly to a gas chromatography (GC) vial for analysis. Extracts were analyzed by a capillary GC/mass spectrometer in selective ion mode (SIM) and quantification was by comparison against an established 5-point calibration curve.

2.3. Experiment in soil with PAH residues (exp 3)

The experiment was setup in the no-plant treatment described above. The concentrations of phenanthrene and pyrene in the treatments after 150 days were considered as PAH residues in soils (Table 1).

According to experiments 1 and 2, the species (*J. subsecundus*), which was affected by relatively high-PAH concentration, but not by relatively low concentration, was selected for the subsequent experiment from mid-December, 2009 (i.e. immediately after experiment 2) to February, 2010. The seedlings of *J. subsecundus* were collected from the local nursery and transplanted (with initial plant dry weight 2.64 ± 0.04 g per pot) into the pots. The nitrogen was re-applied at concentration of 33.3 mg N kg^{-1} as NH_4NO_3 after plant transplantation. The plants were harvested after 72 days of growth. Other methods and measurements were the same as for experiment in soil described above.

2.4. Calculations

Relative growth rate (RGR), expressed as g kg^{-1} day^{-1}, was calculated to describe the plant growth responses [19].

$$\mathrm{RGR} = \frac{\ln \text{ final dry weight} - \ln \text{ initial dry weight}}{\text{days}} \quad (1)$$

2.5. Statistical analyses

All statistical tests were performed using SPSS version 17. Two-way analysis of variance (ANOVA) was used to determine significance of species and PAH on biomass and RGR in hydroponics and RGR in freshly spiked soils and PAH removal from soil. One-way analysis of variance (ANOVA) was used to determine significance of PAH on shoot number and height, and PAH residues on RGR. Least significant difference (LSD) was applied to test for significance between means.

3. Results

3.1. Plant response to naphthalene (exp 1)

The biomass after 14 days of growth in the treatments was recorded (Fig. 1). The biomass of *B. articulata* significantly increased in the treatments with relatively low concentrations of naphthalene, and *J. subsecundus* significantly decreased in the treatment with relatively high concentrations of naphthalene, whereas *B. juncea* and *S. validus* increased with naphthalene additions.

A significant interaction (species × naphthalene treatment) was detected for relative growth rate (RGR) after 14 days (Fig. 2). *J. subsecundus* had the highest RGR, followed by *S. validus*, *B. articulata* and *B. juncea* when they grew in control (unamended) treatment. The value of RGR for *B. juncea* was significantly higher and for *J. subsecundus* significantly lower with the addition of naphthalene twice a day compared to unamended control. The RGR for *S. validus* and *B. articulata* were not significantly different among the treatments.

3.2. Plant response to soil freshly spiked with PAHs (exp 2)

3.2.1. Concentrations of extractable PAHs in soil

Initial concentrations of extractable phenanthrene and pyrene in soils were determined after equilibration, i.e. immediately before commencement of experiment (Table 1). Neither phenanthrene nor pyrene was detected in unspiked soil. In spiked soil, their detected concentrations were generally lower than the original spiked values of corresponding PAH concentrations. The PAH residues, particularly of phenanthrene, in the non-planted soils were quite low after 150 days (Table 1).

3.2.2. Plant growth

The growth of *B. juncea* and *J. subsecundus* was influenced by PAH treatments. The shoot numbers of *B. juncea* significantly increased due to PAH treatments after 45 days of growth, whereas the *J. subsecundus* shoot numbers significantly decreased after 35 days of growth. In contrast, PAH treatments caused no significant difference in the shoot height of the two species (except for *J. subsecundus* after 20 days of growth) (Figs. 3 and 4). After 70 days of growth, the

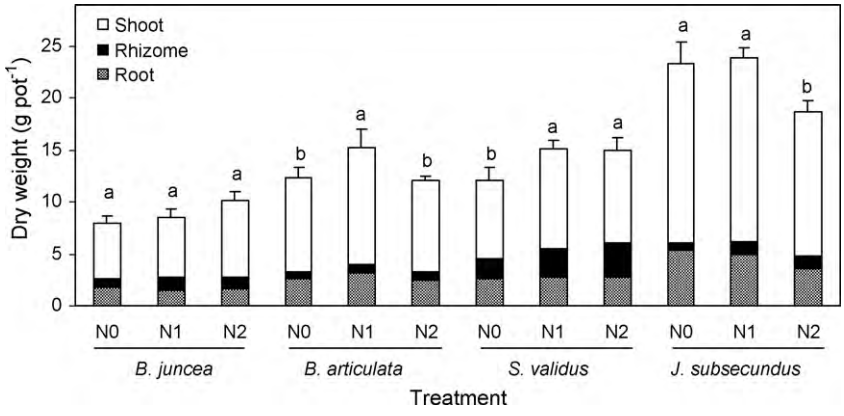

Fig. 1. The biomass of species influenced by naphthalene additions after 14 days of plant growth in hydroponics (exp 1). Bars (means + SE, $n = 3$) with different letters within species are significantly different based on LSD ($p \leq 0.05$). Treatments: N0, N1 and N2 represent unamended (control), added once every day and added twice a day.

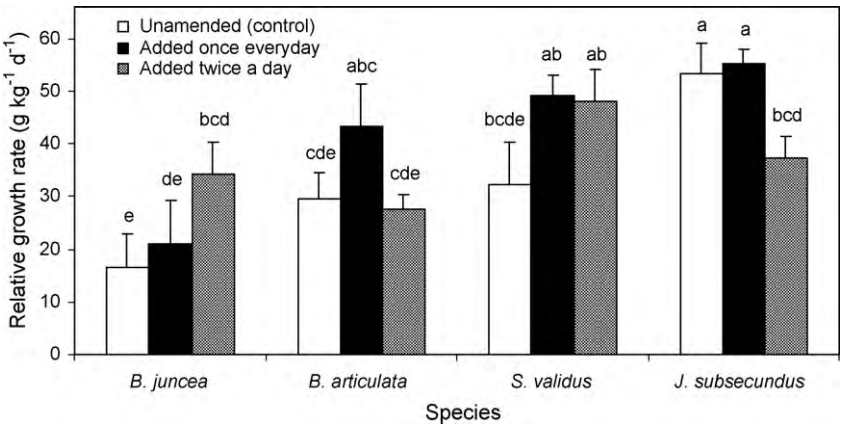

Fig. 2. The relative growth rate (RGR) of species influenced by naphthalene additions after 14 days of plant growth in hydroponics (exp 1). Bars (means + SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$).

J. subsecundus shoot numbers decreased by 11 and 19% in the low- and high-PAH treatments compared to control, but no significant difference was found between the low-PAH treatment and control (Fig. 3). After 150 days of growth, the shoot number of *B. juncea* increased by 130 and 100% in the low- and high-PAH treatments, respectively, compared to control (Fig. 4).

A significant interaction (species × PAH treatment) was observed for relative growth rate (RGR) of *J. subsecundus* after 70 days and of *B. juncea* after 150 days in freshly spiked soils (Fig. 5). *J. subsecundus* had significantly higher RGR than *B. juncea* in unamended control (no PAH added), but no significant difference was detected between two species when they grew in either low- or high-PAH treatments. The value of RGR for *B. juncea* was significantly higher in the PAH treatments than in control, whereas RGR for *J. subsecundus* was significantly lower in the high-PAH treatment than in control, but no significant difference was detected between the low-PAH treatment and control.

3.2.3. PAH removal from soil

The dissipation of PAHs was influenced by PAH and planting treatments. The percentage of phenanthrene removal in the high-PAH treatment was significantly lower with *B. juncea* compared with *J. subsecundus* and non-planted after 70 days of growth. The efficacy of phenanthrene removal was significantly higher in the low-PAH than in the high-PAH treatment after 70 days of growth, but no significant difference was detected after 150 days. The removal efficacy differed significantly between phenanthrene and pyrene (Table 2).

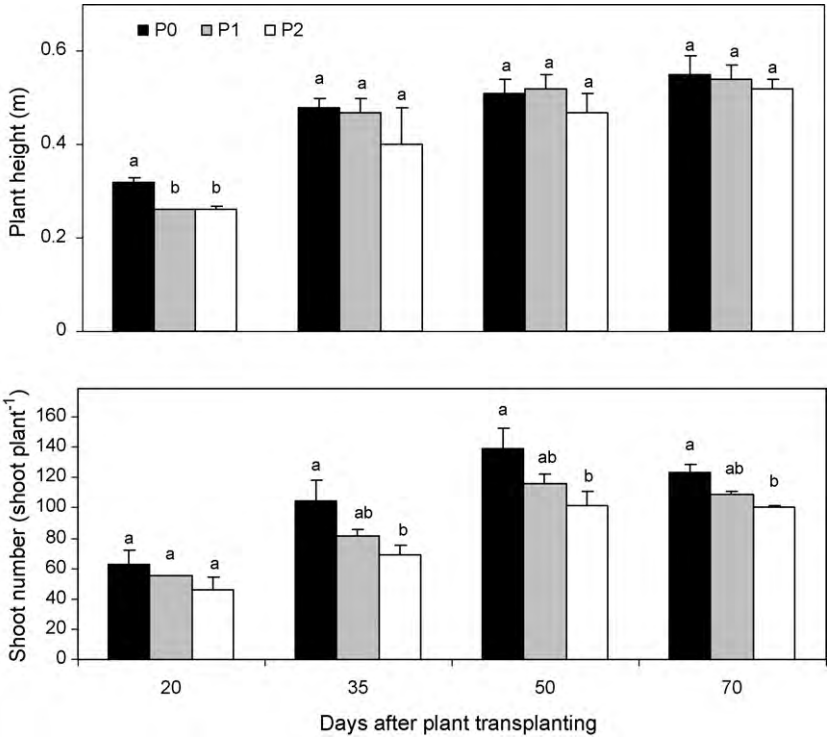

Fig. 3. The tallest shoot height and the shoot number of *J. subsecundus* influenced by PAH treatments in freshly spiked soils (exp 2). Bars (means + SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatments: P0, P1 and P2 represent 0, 50 + 50 and 250 + 250 mg kg^{-1} with phenanthrene + pyrene in 1:1 proportion.

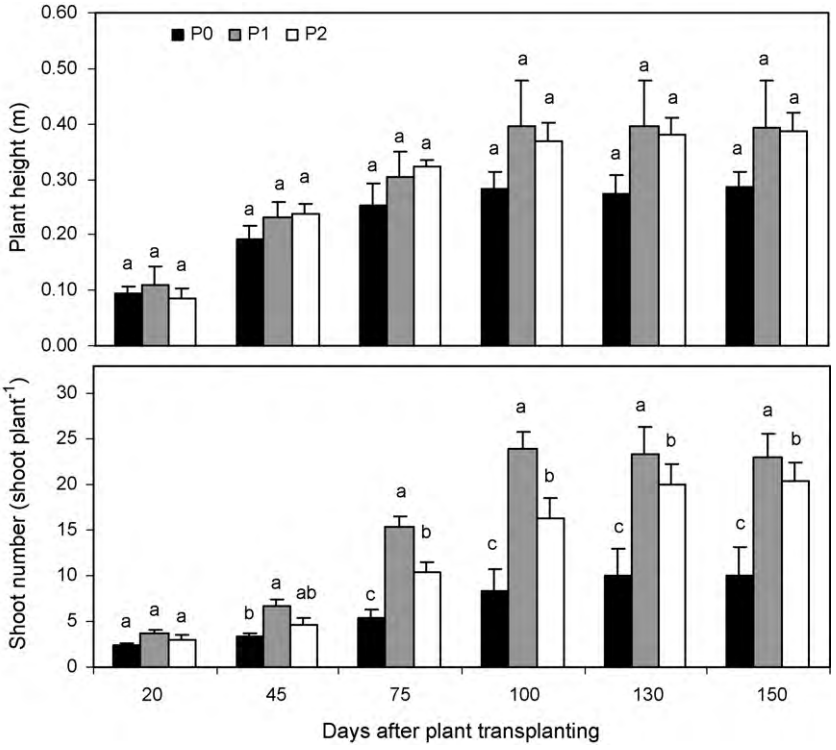

Fig. 4. The tallest shoot height and the shoot number of *B. juncea* influenced by PAH treatments in freshly spiked soils (exp 2). Bars (means + SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatment notation was the same as in Fig. 3.

Table 2
The percentages of PAH removal (%) from freshly spiked soils influenced by PAHs and planting treatments after 70 and 150 days of plant growth (exp 2).

	Low-PAH treatment			High-PAH treatment		
	Non-planted	*B. juncea*	*J. subsecundus*	Non-planted	*B. juncea*	*J. subsecundus*
After 70 days of plant growth						
Phenanthrene	97 ± 1a[a]	97 ± 0a	95 ± 1a	93 ± 2ab	71 ± 22b	95 ± 1a
Pyrene	51 ± 18a	50 ± 8a	39 ± 9a	37+3a	37 ± 2a	13 ± 3a
After 150 days of plant growth						
Phenanthrene	100 ± 0a	100 ± 0a	NA[b]	96 ± 0a	99 ± 3a	NA
Pyrene	98 ± 0a	97 ± 1a	NA	84 ± 22a	65 ± 5a	NA

[a] Means (±SE, $n = 3$) followed by the same letter within rows are not significantly different based on LSD ($p \leq 0.05$).
[b] NA: not available.

3.3. Plant responses to PAH residues (exp 3)

Although the dry weight of *J. subsecundus* increased by 9% in the treatment with residues of low-PAH concentration and decreased by 5% in the treatment with residues of high-PAH concentration compared to unamended control, there was no significant difference in relative growth rate (RGR) among the treatments after 72 days of growth (data not shown).

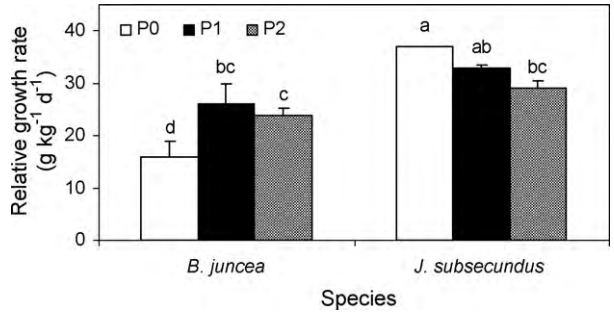

Fig. 5. The relative growth rate (RGR) of species influenced by PAH treatments after 70 days for *J. subsecundus* and 150 days for *B. juncea* in freshly spiked soils (exp 2). Bars (means + SE, $n = 3$) with different letters are significantly different based on LSD ($p \leq 0.05$). Treatment notation was the same as in Fig. 3.

4. Discussion

4.1. Plant response to PAHs

PAHs are lipophilic, meaning they mix more easily with oil than water. The larger compounds are less water-soluble and less volatile (i.e., less prone to evaporate). Phenanthrene and pyrene are larger, and less water-soluble and less volatile than naphthalene. Because of these properties, naphthalene was used in hydroponics for relatively short experiment (14 days), whereas phenanthrene and pyrene were used in soils for relatively long experiments (>70 days).

PAHs, without being activated, are toxic to higher plants only when applied at high concentrations [20,21]. For instance, the addition of PAHs at concentrations of 100–400 mg kg^{-1} significantly decreased the rice (*Oryza sativa*) biomass [22], but root and shoot biomass of *Oryza sativa* was not significantly affected by PAH concentrations of less than 25 mg kg^{-1} in soils [23]. In the

present study, all species tended to increase growth in the relatively low-PAH concentrations and decrease it in the relatively high concentrations (except for *B. juncea*). However, different species responded differently to PAHs in both media (hydroponics and soil) and with different PAH types (naphthalene or mixture of phenanthrene and pyrene) (Figs. 2 and 5). The tallest shoot height of *J. subsecundus* after 70 days of growth and *B. juncea* after 150 days of growth, respectively, were not significant different from the control soil (Figs. 3 and 4), but the shoot number (Figs. 3 and 4) and RGR (Fig. 5) for *J. subsecundus* were significantly reduced, while these for *B. juncea* were significantly increased by the mixture of phenanthrene and pyrene treatments. The toxicity tests on germination and growth of *Phalaris arundinacea* and *Phragmites australis* have found that an increase in phenanthrene concentration decreased plant growth (dry weight and shoot length), but increased the number of young shoots [21]. In another study, PAH (phenanthrene and pyrene) treatment resulted in a 70% decrease in the biomass of *Panicum bisulcatum* and a 22% increase in the biomass of *Aeschynomene indica* compared to unamended control [24]. There are other reports showing no effect of PAHs on plant growth [25–27], which might be dependent on the concentrations used as well as other experimental conditions [9]. For example, Wand et al. [25] observed that *Carex gracilis* and *Juncus effusus* did not suffer any damage when exposed to naphthalene at the concentrations of 30 mg L^{-1} in water.

4.2. PAH removal from soil

The efficacy of PAH removal was relatively high, but differed significantly for phenanthrene and pyrene (Table 2). The pyrene was more persistent in soils than phenanthrene. These findings agreed with other reports [7,26]. The mechanisms underlying dissipation of PAHs in soil are biodegradation, photodegradation, volatilization, plant uptake and metabolism, and incorporation into soil organic material [28]. In many studies, plant uptake and accumulation of PAHs was small; moreover, uptake of PAHs might occur from the air and not just from the soil [29]. However, most studies have reported that plants could facilitate PAH removal from soils [24,26], but that was not the case in the present study as well as in some other reports [30,31]. Increased PAH degradation in planted over unplanted pots may take 6 months [32]. The presence of easily degradable soil organic matter in vegetated treatments was likely responsible for the slower rate of pyrene dissipation [33,34].

The contribution of plants to PAH dissipation by immobilization, removal and degradation is strongly dependent on the rhizosphere processes [1] and would therefore vary across plant species [9]. However, the effect of plants on PAH dissipation may be influenced not only by intra- and inter-species variation, but also by PAH types and experimental conditions such as period of aging, planting, dose rate, soil properties and water content in soil. In addition, influence of PAHs on wetland plant growth may affect removal of other pollutants from wastewater, including nutrient removal [2,3] and metal phytoextraction and phytostabilization in constructed wetlands [35].

5. Conclusions

The growth of wetland plants was significantly influenced by PAHs regardless of media (water or soil) and PAH types (naphthalene or mixture of phenanthrene and pyrene), but the species responded differently. The growth of *B. juncea* increased with PAH additions, but growth of *J. subsecundus* decreased in the media with the relatively high concentrations of PAHs. The relative growth rates for both *B. articulata* and *S. validus* were insignificantly affected by naphthalene additions in hydroponics. The removal of PAHs from soil was not significantly different between planted and non-planted treatments. The species-specific effect of PAHs on wetland plant growth is an important finding for wetland plants used in constructed wetlands. More studies on the response of different wetland species to PAHs are needed in order to confidently select species for wetlands constructed for phytoremediation, and provide relevant information and candidate species to study the tolerance mechanisms to PAH stress.

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