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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