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

Zhenhua Zhang [a], Zed Rengel [a,*], He Chang [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

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

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

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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 $CdCl_2 \times 2^1/_2H_2O$, analytical grade, AJAX Chemicals, Sydney, Australia) was dissolved in Milli-Q water and added to the PAH-spiked soils at concentrations of 0, 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