Environmental Pollution 159 (2011) 3398–3405

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Interactions of arsenic and phenanthrene on their uptake and antioxidative response in *Pteris vittata* L.

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ARTICLE INFO

Article history:
Received 18 May 2011
Received in revised form
11 August 2011
Accepted 17 August 2011

Keywords:
Pteris vittata L.
Arsenic
Phenanthrene
Co-contamination

ABSTRACT

The interactions of arsenic and phenanthrene on plant uptake and antioxidative response of *Pteris vitatta* L. were studied hydroponically. The combination of arsenic and phenanthrene decreased arsenic contents in fronds by 30–51%, whereas increased arsenic concentrations 1.2–1.6 times in roots, demonstrating the suppression of arsenic translocation compared to the corresponding treatment without phenanthrene. Under the co-exposure, As(III) concentrations in fronds deceased by 12–73%, and at higher arsenic exposure level (≥10 mg/L), As(V) in fronds and As(III) in roots increased compared to the single arsenic treatment. Arsenic exposure elevated phenanthrene concentrations in root by 39–164%. The co-existence of arsenic and phenanthrene had little impact on plant arsenic accumulation, although synergistic effect on antioxidants was observed, suggesting the special physiological process of *P. vitatta* in the co-exposure and application potential of *P. vitatta* in phytoremediation of arsenic and PAHs co-contamination.

1. Introduction

Arsenic and polycyclic aromatic hydrocarbons (PAHs), the two widespread priority pollutants of great environmental concern and well-characterized as highly toxic, mutagenic and carcinogenic, are commonly found in environmental media and accumulate in the soil and vegetation, which is recognized as serious threat to human health. It is reported that arsenic is defined as the second most common contaminant of concern at 568 Superfund sites in USA (US EPA, 2002). Many countries such as Bangladesh, India, Chile, America and China in the world are long-term impacted to various extents by arsenic contamination (Liao et al., 2005; Hossain, 2006). PAHs have also been widely detected in soil and vegetation and the occurrence of PAHs contamination in soil is continuously increasing (Xu et al., 2006; Honda et al., 2007). The interaction of arsenic and PAHs has been well documented in epidemiology studies and it is important to recognize that the combination of arsenic and PAHs could strongly potentiate the environmental risks with synergistic effect between arsenic and PAH-induced immunosuppression and cancer (Maier et al., 2002; Li et al., 2010).

In fact, the simultaneous accumulation of arsenic and polycyclic aromatic hydrocarbons (PAHs) are frequently found and extensively evaluated in several types of anthropogenic industry contaminated sites, such as coking & chemical industry site, mining and metallurgy industry site, lumber and wood production site, etc, even the urban residual areas (Lambert and Lane, 2004; Elgh-Dalgren et al., 2009; Kay et al., 2008). However, up to date, methods for remediation of arsenic and PAHs co-contamination are scarce to report. Phytoremediation has been proposed as a promising technology and developed well in many arsenic contaminated sites (Baker et al., 1994; Chaney et al., 2000). *Pteris vitatta* L., the first known and the most important arsenic hyperaccumulator, was reported to be tolerant of high concentrations of arsenic (up to 1500 mg/kg soil) and efficiently accumulate arsenic (13,800 mg/kg dry biomass) (Ma et al., 2001; Rathinasabapathi et al., 2006; Xie et al., 2009). In recent years, the phytoremediation of arsenic and heavy metal co-contamination is becoming an appealing research and has been documented in scientific literature. *P. vitatta* was observed to show excellent tolerance to heavy metals such as Cd, Ni, Pb, Zn, Se, Sb, etc. with effective arsenic hyperaccumulation capacity and is recognized as potential material for the co-contamination remediation (Fayiga et al., 2004; An and Huang, 2006; Srivastava et al., 2009; Feng et al., 2009, 2011).

According to the industry field survey, our laboratory have found that *P. vitatta* showed tolerance to high level of arsenic and PAHs co-contamination (Arsenic and PAHs reaching 228 and 34 mg/kg, respectively) with efficient arsenic accumulation

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doi:10.1016/j.envpol.2011.08.045

(1276 mg/kg dry biomass) (data not published), which might provide novel insight into the subject. In fact, the hyper-accumulation of arsenic, together with the enhanced PAHs dissipation in rhizosphere to a certain extent, is supposed to be an ideal remediation approach. Thus, whether *P. vitatta* would be a potential remediation material for arsenic and PAHs co-contamination is deserved to discuss and if confirmed and optimized, could have enormous implications for phytoremediaton. To simplify the investigation system, study on hydroponically *P. vitatta* was conducted. The objective of this research was to (1) determine the tolerance capacity of *P. vitatta* to the combination of arsenic and PAHs; (2) examine the arsenic and PAHs distribution and speciation in *P. vitatta* as well as in growth media under the co-exposure. The results of this research would provide valuable information for the application of *P. vitatta* in arsenic and PAHs co-contamination remediation.

2. Materials and methods

2.1. Experiment setup

Spores were collected from fertile fronds of *P. vitatta* in southern China and were germinated and propagated in Arsenic- and PAHs-free seed base for 5 months. *P. vitatta* seedlings with similar size and age were cultured in 1/5 strength Hoagland solution with pH adjusted to 6.5. The seedlings continued to grow in greenhouse at 20–25 °C during daytime and at 15–20 °C during night. Four weeks later, the plants with 7–8 fronds were prepared for experiment.

Phenanthrene with purities greater than 98% was selected as representative of PAHs (Acros Organics (USA)). Arsenic was added in the form of $Na_2HAsO_4{\cdot}7H_2O$. A group of two *P. vitatta* plants were transferred into culture solution spiked with 0 and 1.0 mg/L phenanthrene, and 0, 2.5, 5, 10, 20 mg/L arsenic. There were three replicates for each treatment. The loss of solution in beakers was replenished with the same amount of Hoagland solution without arsenic or PAHs. After 10 d of growth under treatment, the plants were harvested and prepared for analysis.

2.2. Phenanthrene analysis

Certain amounts of freeze-dried plant fragment were extracted by ultrasonication with acetone and dichloromethane mixture. The extracts were concentrated and then transferred to silica gel column for cleanup by washing with hexane and dichloromethane mixture. The eluate was concentrated and analyzed by Agilent GC–MS (USA). The average recoveries of phenanthrene was 95–110% ($n = 6$, RSD $< 2.61\%$). The detection limit for phenanthrene was 1 ng/g. Phenanthrene in hydroponic solution was extracted by hexane after shaken for 1 h, and then allowed to stand to separate into two layers. The supernatant was transferred to silica gel column for cleanup. The eluate was concentrated and analyzed as described above.

2.3. Total arsenic and arsenic speciation analysis

Certain amounts of freeze-dried plant fragment were extracted by ultrasonication with H_2O/MeOH mixture. The extracts were combined and concentrated, and then diluted to 50 mL for analysis (Ruiz-Chancho et al., 2008). After filtration through 0.45 μm filter units, arsenic speciation was analyzed by AFS-LC. The sampled hydroponic solution was filtration and analyzed as described above.

The dried and ground plant samples were digested with HNO_3–$HClO_4$ (US EPA 3050), and the concentration of arsenic was determined using AFS. Accuracy of the elemental analysis was verified by standard reference material.

2.4. Reduced glutathione (GSH)

Reduced glutathione (GSH) of the fronds was measured according to the method of Guo (2006) with a little modification. Leaf tissues (0.5 g) were homogenized by 5 mL 5% (w/v) trichloroacetic acid (TCA), followed by centrifugation at 15,000 rpm for 15 min. Then 2.6 mL phosphate buffer (pH 7.7, 0.15 mM) and 0.18 mL DTNB were added in 0.25 mL supernatant, respectively. Absorbance was assessed at 412 nm.

2.5. Superoxide dismutase (SOD) and membrane lipid peroxidation

Leaf tissues (0.2 g) were ground thoroughly using phosphate-buffered solution (PBS) buffer (50 mM, pH 7.8) containing 1% polyvinylpyrrolidone (PVP), followed by centrifugation at 10,000 rpm at 4 °C for 15 min. The supernatant was collected to determine the SOD activities and MDA contents. SOD activity was assessed by nitro-blue-tetrazolium (NBT) photoreduction method. The MDA content was determined using the thiobarbituric acid colorimetry (Guo, 2006).

2.6. Statistical analysis

Statistical analysis was performed using SPSS 13.0. Results were expressed as means followed by corresponding standard deviations. One-way analysis of variance was to compare the effects of arsenic on phenanthrene concentration in plant and growth media. Multiple comparisons were made by the LSD test at $P < 0.05$ level. Two-way analysis of variance was used to compare the interaction of arsenic and phenanthrene in plant biomass, arsenic distribution and speciation and the antioxidants.

3. Results

This study examined the interactions of arsenic and phenanthrene on plant growth, plant uptake and antioxidative response of *P. vitatta*. Background arsenic and phenanthrene concentrations of *P. vitatta* were determined prior to the experiment. The average content of arsenic was 10.46 mg/kg and no phenanthrene was detected, which was demonstration for the clean plant to experiment.

3.1. Plant biomass

After 10 d exposure with arsenic and phenanthrene, no visible toxic symptom was observed for the fern in all treatments. The fronds and roots biomass of *P. vitatta* greatly varied with the concentrations of arsenic and phenanthrene (Table 1). Compared with control treatment (plant grown without arsenic or phenanthrene), various concentrations of arsenic had no significant effect on fronds or roots biomass ($P > 0.05$). Especially, the presence of 1 mg/L phenanthrene had a positive effect on roots biomass (increased by 13–81% at various arsenic levels, $P < 0.05$) but no significant effect on fronds biomass ($P > 0.05$). The maximum root biomass was observed in the presence of 2.5 mg/L arsenic and 1 mg/L phenanthrene. When the spiked arsenic concentration increased to 20 mg/L, beneficial effect for plant growth in the presence of phenanthrene was not much obvious but still a slight increase was observed (15%).

3.2. The plant uptake of arsenic

3.2.1. Arsenic concentration and accumulation

The interactive effect of arsenic and phenanthrene on plant uptake of arsenic in roots ($F = 7.423$, $P < 0.001$) and fronds ($F = 38.05$, $P < 0.0001$) was observed (Fig. 1). What is interesting to us is that phenanthrene addition significantly suppressed arsenic uptake in fronds by 30–51% with the maximum decrease occurred at 5 mg/L arsenic exposure, whereas dramatically enhanced arsenic

Table 1
Plant biomass of *P. vitatta* exposed to arsenic and phenanthrene after 10 d (g, DW).

Phenanthrene exposure (mg/L)	Arsenic exposure (mg/L)	Fronds biomass	Roots biomass
0	0	0.46 ± 0.18	0.77 ± 0.19
	0.5	0.60 ± 0.14	0.96 ± 0.12
	2.5	0.50 ± 0.15	0.73 ± 0.11
	5	0.41 ± 0.10	0.81 ± 0.16
	10	0.49 ± 0.15	0.70 ± 0.29
	20	0.51 ± 0.15	0.81 ± 0.25
1	0	0.48 ± 0.16	0.72 ± 0.25
	0.5	0.60 ± 0.09	1.09 ± 0.13
	2.5	0.60 ± 0.14	1.32 ± 0.33
	5	0.52 ± 0.06	1.05 ± 0.13
	10	0.63 ± 0.17	1.15 ± 0.33
	20	0.55 ± 0.19	0.93 ± 0.44
Significance of:			
Arsenic		$F = 0.76$ $P = 0.58$	$F = 1.20$ $P = 0.33$
Phenanthrene		$F = 1.80$ $P = 0.19$	$F = 6.53$ $P = 0.017$
Arsenic × phenanthrene		$F = 0.19$ $P = 0.96$	$F = 1.48$ $P = 0.23$