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Arsenic enhanced plant growth and altered rhizosphere characteristics of hyperaccumulator *Pteris vittata*

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ABSTRACT

We investigated the effects of arsenic species on As accumulation, plant growth and rhizospheric changes in As–hyperaccumulator *Pteris vittata* (PV). PV was grown for 60–d in a soil spiked with 200 mg kg^{-1} arsenate (AsV–soil) or arsenite (AsIII–soil). Diffusive gradients in thin–films technique (DGT) were used to monitor As uptake by PV. Interestingly AsIII–soil produced the highest PV biomass at 8.6 g $plant^{-1}$, 27% and 46% greater than AsV–soil and the control. Biomass increase was associated with As–induced P uptake by PV. Although AsIII was oxidized to AsV during the experiment, As species impacted As accumulation by PV, with 17.5% more As in AsIII–soil than AsV–soil (36 vs. 31 mg $plant^{-1}$). As concentration in PV roots was 30% higher in AsV–soil whereas As concentration in PV fronds was 7.9% greater in AsIII–soil, suggesting more rapid translocation of AsIII than AsV. These findings were important to understand the mechanisms of As uptake, accumulation and translocation by PV.

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1. Introduction

Arsenic (As), which is toxic and carcinogenic, is ubiquitous in the environment. Various anthropogenic activities such as mining, smelting, and use of As pesticides, wood preservatives and feed additives have elevated As in the environment (Zhao et al., 2010). Arsenic is mainly present in soils as inorganic form, namely arsenate (AsV) and arsenite (AsIII). Of the two, AsIII is more mobile and toxic, which binds to thiol groups and deactivates enzyme activity (Kaltreider et al., 2001). On the other hand, as an analog of phosphate, AsV inhibits oxidative phosphorylation in organisms (Tsai et al., 2009). The difference in their behaviors makes it important to understand the effects of As speciation on As biochemical processes in soils and plants.

Pteris vittata (PV), a well–known As hyperaccumulator, has a potential to be used for phytoremediation of As–contaminated soil. When grown in soil spiked with 1500 mg kg^{-1} As, PV accumulated up to 2.3% As in the fronds (Ma et al., 2001). It is unique that addition of AsV stimulates PV growth (Lou et al., 2010; Tu and Ma, 2002, 2003). Ghosh et al. (2011) hypothesized that As–induced phosphorus (P) uptake may have enhanced PV growth. In addition, PV has an extensive root system and its rhizosphere sustains diverse microbial communities (Al Agely et al., 2005; Xiong et al., 2010). Many As–resistant bacteria have been identified, some have a dual function of AsIII oxidation and AsV reduction (Ghosh et al., 2011; Huang et al., 2010; Wang et al., 2012).

Rhizosphere is a dynamic region where multiple interactions occur in plant roots–soil–microbe system (Darrah et al., 2006). Fitz and Wenzel (2002) reported that root–induced changes altered rhizospheric chemical composition and facilitated As uptake by PV. The ability of PV to exude large amounts of dissolved organic carbon (DOC) and to change rhizospheric pH enhances As bioavailability in the soil, thereby increasing its As uptake (Tu et al., 2004). For example, Gonzaga et al. (2009) observed that As–induced root exudates reduced soil pH by 0.74–0.92 units and increased DOC concentration by 2–3 times in PV rhizosphere.

However, these chemical measurements represent more equilibrium interactions than kinetic processes. A dynamic technique called diffusive gradients in thin films technique (DGT) uses a layer of binding agent to accumulate metals after diffusion through the filter and diffusive gel (Zhang and Davison, 1995). It mimics the physical process of plant metal uptake by locally lowering metal concentration in soil solution, subsequently inducing metal resupply from the labile pools in the soil solution and solid phase (Williams et al., 2011). In a pot experiment, Fitz et al. (2003) applied DGT to assess As depletion in the rhizosphere of PV growing in a rhizobox and observed a 19.3% decrease in As flux, showing some resupply of As from less available pools.

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Plant As uptake is directly influenced by As species in the medium, which has been demonstrated in hydroponic system. For example, Fayiga et al. (2005) observed that PV accumulated more monomethyl arsenic acid (MMA) than AsIII in its fronds. Similarly, Mathews et al. (2010) found that higher As in PV roots after exposing to AsV and higher As in PV fronds after exposing to AsIII. However, the impact of As species on plant As uptake in soil experiment is less obvious (Tlustos et al., 2002). This is partially due to AsIII oxidation to AsV during the experiment. Although AsIII is oxidized to AsV in aerobic soils, it still has impact on As accumulation in PV (Tu and Ma, 2002). Therefore, we hypothesized that AsIII and AsV in soils would have different impact on As uptake and translocation by PV.

In the present study, a pot experiment was conducted to evaluate the effects of soil As species (AsIII and AsV) on As accumulation by PV and biochemical processes in PV rhizosphere. The specific objectives were to: (1) examine the impact of different As species on As distribution and growth in PV, and (2) determine the changes in PV rhizosphere using traditional approaches combined with DGT technique. The results from these investigations are important to better understand the mechanisms of As accumulation by PV.

2. Materials and methods

2.1. Plant propagation and soil preparation

PV plants were propagated using spores from University of Florida, Gainesville, USA. After germination, young ferns were kept in a seedbed until they grew into sporelings with 2–3 fronds. They were then transplanted into 10 cm–diameter plastic pots with potting mixture (40% soil, 40% organic matter, and 20% quartz sand). They were allowed to grow until they had 5–6 fronds and ~10 cm in height.

Surface soil (0–15 cm) was collected from Nanjing, China. Soil pH was measured with a pH electrode in a suspension of 1:5 (v/v) soil to 0.01 M $CaCl_2$ solution after 1 h shaking; soil organic carbon (OC) was determined using a TOC analyzer (Element arvario TOC cube, Germany); total soil As, Mn and Fe concentrations were analyzed using inductively coupled plasma mass spectrometry (ICP–MS; PerkinElmer NexION 300X, USA) after digestion using the EPA method 3050B. The soil properties were as follow: pH 7.02, 0.98% OC, 6.79 mg kg^{-1} As, 673 mg kg^{-1} Mn, and 27.5 g kg^{-1} Fe (data not shown).

2.2. Pot experiment

The soil was air–dried, sieved through a 2–mm mesh and mixed thoroughly with 200 mg kg^{-1}AsV ($Na_2HAsO_4{\cdot}7H_2O$) or AsIII ($NaAsO_2$) solution. Basal fertilizers (120 mg kg^{-1} N as NH_4NO_3, and 30 mg kg^{-1} P and 75.5 mg kg^{-1} K as K_2HPO_4) and 1% organic matter were added to the soil and mixed thoroughly. The treated soil was set at ~50% field capacity for one week before transplanting. The soil (3 kg) was placed into 20 cm–diameter plastic pots, with 500 g in the rhizosphere and 2500 g in the bulk soil. A nylon mesh bag (diameter of 7 cm) was placed in the center of each pot to separate the rhizosphere from the bulk soil. The nylon mesh is 24 μm, which allowed the transport of water and dissolved nutrients but not the roots.

PV plants were transplanted into rhizo–bags, including control with no As, 200 mg kg^{-1} AsV (AsV–soil) and 200 mg kg^{-1} AsIII (AsIII–soil), with 3 replicates. Soil without PV plants was included as a control. After the plants were established, rhizo–samplers (Rhizon MOM, 10 cm length, 2.5 mm OD, Rhizosphere Research Products, the Netherlands) were embedded into the soil in the middle of pots to collect rhizosphere soil solution at a depth of ~8 cm. The pots were randomly arranged in a growth chamber with a 16 h light photoperiod and a light density of 350 μmol m^{-2} s^{-1} in the daytime. The room was kept at 25 °C on average with a relative humidity of 70%. Milli–Q water was added daily as required and the pots were rearranged randomly every week.

The plants were grown for 60 d during which the rhizosphere soil and soil solution were collected at 0, 15, 30, and 60 d after transplanting. At the end of the experiment, the plants were harvested and washed thoroughly with Milli–Q water. All samples were stored at −80 °C until analyses.

2.3. Total As concentration and speciation in soil and plant samples

The soil samples were freeze–dried for 48 h and sieved to 2 mm. Each plant was separated into roots, rhizomes, and fronds, followed by freeze drying for 48 h. The dry weights were recorded. Dried plants were ground using liquid nitrogen to fine powder in a ceramic mortar. After grinding, the samples were freeze–dried for an additional 48 h. The samples were analyzed for total As and As speciation.

For total As analysis, soil and plant samples were digested using EPA Method 3050B for the Hot Block Digestion System (Environmental Express, USA) and then determined by ICP–MS. A certified reference material for soil sample (D056–540), for plant sample (GSB–21, Chinese geological reference materials) and blanks were included for quality assurance. Repeated analysis of D056–540 (certified value 264 ± 30 mg As kg^{-1}) and GSB–21 (certified value 4.5 ± 0.5 μg As kg^{-1}) gave the satisfactory recovery of 266 ± 3.76 mg As kg^{-1} and 4.66 ± 0.34 μg As kg^{-1}. Standard solution at 20 μg L^{-1}As was measured every 20 samples to monitor the stability of ICP–MS.

For soil As speciation, the soil samples were extracted following Giral et al. (2010). A solution of 30 ml consisting of 1 M phosphoric acid and 0.5 M ascorbic acid was added to 0.2 g of sample in a 50 ml digestion vial. The mixture was then maintained at 105 °C for 10 min in the Hot Block Digestion System. After digestion, the extracts were centrifuged at 4000 rpm for 10 min and then filtered through 0.22–μm filters. The filtered samples were diluted with Milli–Q water and immediately analyzed for As speciation.

For plants As speciation, ground freeze–dried plant samples were extracted following Zhang et al. (2002). Fifty mg samples were ultrasonically extracted with 10 ml 1:1 methanol/water for 2 h and then centrifuged at 4000 rpm for 10 min. The supernatant was decanted into a 50–ml centrifuge tube. The procedure was repeated and the two extracts were combined. The residue was rinsed three times with 5 ml Milli–Q water and all supernatants were mixed, and then diluted to 50 ml with Milli–Q water. After filtering through 0.22–μm filters, the samples were diluted and then determined for As speciation using HPLC–ICP–MS.

Arsenic speciation was carried out using high performance liquid chromatography (HPLC; Waters 2695, USA) coupled with ICP–MS. Inorganic AsIII and AsV were separated by an anion–exchange column (PRP–X100, 10 μm, Hamilton, UK) fitted with a guard column (Hamilton, UK). The mobile phase was a solution consisting of 8.0 mM $(NH_4)_2HPO_4$ and 8.0 mM NH_4NO_3 (pH 6.2) at a flow rate of 1.0 ml min^{-1}. Before flowing into chromatographic columns, it was sonicated and filtered through 0.22 μm membrane (Zhu et al., 2008). Quality assurance was obtained through the blanks, standard curves, and spiked samples. Stock solutions of As species were prepared from $NaAsO_2$ (Sigma–Aldrich, ≥90%) and $Na_2HAsO_4{\cdot}7H_2O$ (Sigma–Aldrich, ≥98%) with Milli–Q water. The stock solution at 1000 mg As L^{-1} was stored at 4 °C until use. All standard solutions were diluted from stock solution on the day of analysis. The recoveries for the spike of arsenite and arsenate added to plant samples were 83–97%. Since AsIII and AsV are predominant in the soil and in PV biomass, other As species were not considered in this study.

2.4. Plant P concentration and characterization of soil porewater

To determine the potential role of P in enhancing plant growth, total P was measured spectrophotometrically (UV–2550, Shimadzu, Japan) at 880 nm using the modified molybdenum–blue method. Due to AsV interference, the samples digested by EPA Method 3050B were first incubated with L–cysteine (5% w/v in 0.6 M HCl) at 80 °C for 5 min (Ghosh et al., 2011). The solution was cooled to room temperature followed by pH adjustment to 7 ± 0.2, and P was then determined.

Soil pore water was extracted by applying suction to the rhizo–samplers, and then filtered through 0.45 μm membrane filters. Soil solution pH was measured immediately after collection by using a pH electrode. DOC (dissolved organic carbon) concentration was determined by a TOC analyzer. Total As concentration was measured by ICP–MS. Total P concentration was determined by modified molybdenum–blue method. As speciation was determined by HPLC–ICP–MS.

2.5. DGT measurements and calculations

In this study, a 0.4 mm–thick ferrihydrite gel was used as a binding layer for As. Diffusive and binding gels were prepared followed the standard procedure of gel making by Luo et al. (2010). A subsample of each soil (20 g, 3 replications) was wetted to 40% field capacity and equilibrated for 48 h and then raised to 90% for additional 24 h incubation (Zhang et al., 2001). Thereafter, the DGT devices were pushed slightly into the soil. After maintaining at ~25 °C for 12 h, they were retrieved from the soil and rinsed with Milli–Q water to wash off the soil particles adhering to the filter membrane. The binding gel was retrieved from the devices and eluted with 1 ml of 1 M HNO_3 for 24 h. After DGT retrieval, soil solution was collected by centrifuging at 4500 g for 15 min and filtered through 0.45 μmfilters. Finally, both the eluant from DGT ($^{As}C_{DGT}$) and the soil solution were analyzed for As concentration by ICP–MS.

According to Fick's law of diffusion, As concentration at the interface between the DGT device surface and the soil can be calculated using Eq. 1.

$$^{As}C_{DGT} = M\Delta g/(DAt) \quad (1)$$

Where $^{As}C_{DGT}$ is the concentration of labile As in soil measured by DGT and reflects As supply to porewater from both solid phase and solution (Williams et al., 2011); M is the As mass accumulated on the binding gel; Δg is the thickness of the diffusive gel (0.8 mm) and that of filter membrane (0.14 mm); A is the surface area (2.51 cm^2); t is the deployment time; and D is the diffusion coefficient of As (5.18×10^{-6} cm^2 s^{-1} at 25 °C) (Luo et al., 2010).

The ratio of C_{DGT}/water–soluble As is termed R value. It is a quantitative measure of the ability of soil solid phase to resupply As to the pore water in response to As depletion induced by DGT sink. The larger the R value the greater a soil's ability to resupply As to the soil solution.