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Uptake, transport and transformation of arsenate in radishes (*Raphanus sativus*)

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ABSTRACT

The localization and identification of arsenic compounds in terrestrial plants are important for the understanding of arsenic uptake, transformation and translocation within these organisms, and contributes to our understanding of arsenic cycling in the environment. High performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS), and X-ray absorption near-edge structure (XANES) analysis identified arsenite, arsenate and arsenic(III)–sulphur compounds in leaf, stem and root sections of *Rhaphanus sativus* (radish) plants grown in both arsenic contaminated mine waste, and arsenic amended liquid cultures. The total arsenic distribution was similar between the plants grown in mine waste and those grown hydroponically. Arsenate was the predominant form of arsenic available in the growth mediums, and after it was taken up by roots, X-ray absorption spectroscopy (XAS) imaging indicated that some of the arsenate was transported to the shoots via the xylem. Additionally, arsenate was reduced by the plant and arsenic(III)–sulphur compound(s) accounted for the majority of arsenic in the leaf and stem of living plants. In this study the application of synchrotron techniques permitted the identification of arsenic(III)–sulphur species which were "invisible" to conventional HPLC-ICP-MS analysis.

1. Introduction

Arsenic can be taken up by plants and subsequently introduced into a food chain. This may be cause for concern, particularly when considering that as much as 99.7% of all biomass is found in terrestrial plants; any contamination of these organisms will have far reaching effects (Trapp and McFarlane, 1995). Arsenate, arsenite, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are all arsenic compounds that can be taken up by a number of plant species either from solution or arsenic amended soils (Marin et al., 1992; Carbonell-Barrachina et al., 1998; Tlustoš et al., 2002; Tu et al., 2004).

Generally in soils with elevated arsenic levels, it is inorganic arsenate that is the dominant phytoavailable form of arsenic, and it is well established that plants can take up this compound via phosphate transporters (Ullrich-Eberius et al., 1989; Meharg and Macnair, 1992). It is not surprising then that arsenate is often one of the main arsenic compounds reported in terrestrial plants, with arsenite, another inorganic species, frequently detected (Meharg and Hartley-Whitaker, 2002). Arsenite can be found in anaerobic soils, and it was determined that rice plants actively transport arsenite into the roots (Abedin et al., 2002). In addition, arsenate can be reduced to arsenite soon after transport into the plant (Pickering et al., 2000). Further evidence for the metabolism of arsenic compounds in terrestrial plants was observed in phosphate-starved tomato (*Lycopersicum esculentum*) plants and cell suspension cultures of periwinkles (*Catharanthus roseus*) which were found to methylate inorganic arsenic to some extent (Cullen et al., 1989; Nissen and Benso, 1982). A pathway involving alternate reduction and oxidative methylation produces simple methylated arsenic compounds such as DMA and MMA identified in rice, grass (e.g. tufted hair), and trees (e.g. apple, cedar) (Meharg

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and Hartley-Whitaker, 2002). Arsenobetaine (AB) is less frequently observed in terrestrial plants, but has been reported in red clover and ribwort plantain, the latter of which was also reported to contain arsenocholine (AC) (Meharg and Hartley-Whitaker, 2002); however, the pathway for the production of these arsenic compounds is not as well understood. Many of the arsenicals noted above have been identified in both roots and shoots of plants, but the distribution of total arsenic within plants is highly variable, with the exception that fruits and seeds generally contain lower concentrations of arsenic than other parts of the plant (Marin et al., 1992; Cobb et al., 2000).

In addition to the metabolism of arsenic compounds by plants, there is the possibility of chelation of arsenic to biomolecules in plant cells. In particular, arsenite has a high affinity for sulfhydryl residues found on molecules such as glutathione (GSH) and phytochelatins (PCs) and *in vitro* studies have demonstrated the complexation of arsenic to both (Schmöger et al., 2000; Raab et al., 2004b). GSH is composed of γ-glutamate-cysteine-glycine residue (γ-Glu-Cys-Gly). In plants, the majority of GSH is found in the chloroplasts (50–70%), where it acts as a powerful antioxidant (Rennenberg, 1982). PCs are synthesized from GSH and contain additional glutamate and cysteine components ($PC_n = (\gamma\text{-Glu-Cys})_n\text{Gly}$). PCs are produced by plants in response to metal cation (e.g. Ag^+, Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}) or metalloid (e.g. $As^{(V)}$ oxy-anion) stress (Raab et al., 2004a). A wide variety of arsenic–PC complexes have been described and some of the predominant complexes include: arsenite–PC_3 in *H. lanatus*, GS–arsenite–PC_2 in *P. cretica*, PC_2–arsenite–PC_2 in *R. serpentine*, and PC_3 and homoPC_3 in *C. arietinum* (Schmöger et al., 2000; Gupta et al., 2004; Raab et al., 2004a). PCs may be important for arsenic tolerance in plants and are thought to play a role in arsenic detoxification, transport and compartmentalization.

Here we present the results for a study designed to determine arsenic speciation and distribution in radish plants, grown in two sources of arsenic: mine waste and arsenic amended liquid. Analysis involved conventional speciation techniques that employed a methanol:water extraction, as well as X-ray absorption spectroscopy (XAS) methods. XAS involves direct analysis of solid material without the need to extract arsenic, and has been particularly useful for the detection of arsenic–sulphur compounds in terrestrial plants (Pickering et al., 2000; Meharg and Hartley-Whitaker, 2002; Zhang et al., 2002). XAS imaging can be used to determine the location of arsenic compounds in an unaltered plant sample, providing information on the translocation of arsenic compounds within a plant (Pickering et al., 2006). The complementary nature of the analytical techniques used in this study allowed for a more comprehensive understanding of arsenic compounds in radish plants by combining results from both direct (XAS) and indirect (conventional) speciation analyses.

2. Materials and methods

2.1. Chemicals and standard reference materials

Chemicals used in these studies include nitric acid (Fisher Scientific, optima), methanol (99.93% HPLC grade, Aldrich), liquid nitrogen (BOC), *ortho*-phosphoric acid (85% Fluka), and ammonium hydroxide 30 wt.% (Sigma-Aldrich). Distilled deionized water (ddH_2O) had a resistivity better than 17.5 MΩcm (E-pure Barnstead). Arsenic compounds used as standards included: arsenate (Fluka KH_2AsO_4 solid and Aldrich ICP/DCP standard solution), arsenite (Fluka As_3O_3 solid and atomic spectrometry standard solution), monomethylarsonic acid (Pfaltz & Bauer and ChemService monosodium acid methane arsenate), dimethylarsinic acid (Aldrich cacodylic acid sodium salt hydrate), trimethylarsine oxide (synthesized), tetramethylarsonium iodide (synthesized), arsenobetaine (synthesized), arsenocholine (synthesized), arsenic(III)–glutathione (synthesized) and TuneA (Thermo Electron Corporation). 'Synthesized' compounds were prepared by Drs. H. Sun and W.R. Cullen, at the University of British Columbia, using standard methodologies which have been referenced previously (Smith et al., 2005). Certified reference materials included Bush Branches and Leaves, certifying body: Institute of Geophysical and Geochemical Exploration (GWB 07603), and DORM 2 (dogfish muscle), certifying body: National Research Council of Canada.

2.2. Experimental design

2.2.1. Liquid cultivation of radishes

Six hydroponic treatments were used with target arsenic concentrations of 0, 1, 2, 5, 15, 30 and 60 μg g^{-1} arsenic. These treatments were designated H-0, H-1, H-2, H-5, H-15, H-30 and H-60 respectively. Plants were grown in a phytotron growth chamber and experimental conditions were maintained at 12 h dark at 12°C, 12 h light at 18 °C, and humidity 70–90%. Pots were watered from the bottom, no visible signs of infection or algae growth were observed, and plants were harvested 31 days after sowing. Radish (var. Cherry Belle) seeds for all experiments were purchased from the Ontario Seed Company and planted in 4″ pots of Turface® (Profile™ Products). Plants were supplied with a base nutrient solution (P 15 mg L^{-1}, N 150 mg L^{-1}, K 91 mg L^{-1}, Ca 180 mg L^{-1}, Mg 50 mg L^{-1}, Fe 3 mg L^{-1}, Cu 0.1 mg L^{-1}, Mn 1.0 mg L^{-1}, Zn 0.1 mg L^{-1}, B 1.0 mg L^{-1}, Mo 0.4 mg L^{-1}) without arsenic for the first 5 days, at which point they were thinned to 3 plants per pot. Arsenic treatments contained base solution prepared with KH_2AsO_4 (Fluka). Lost solution was replaced daily throughout the growth period. Plants were harvested 28 days after sowing, and sectioned into root, stem and leaf which were thoroughly washed and scrubbed with tap water and further rinsed with distilled water before weighing and freezing. Roots were further separated into peeled root and skin before sample preparation, to determine the possible contribution of arsenic compounds adsorbed to the outer skin.

2.2.2. Mine waste cultivation of radishes

Mine waste was collected from the tailings area of the Miramar Con Mine in Yellowknife (NWT, Canada). The mine waste was sieved (Canadian metric sieve series, 5 mm opening) and the total arsenic concentration in the final product was ~1100 mg kg^{-1}. The mine waste treatments used were: undiluted and diluted. The diluted treatment was mixed with potting mix (Agro Mix Super, Fafard®) by hand until visually homogenous. A sample of soil from each treatment was taken before pots were filled for planting to determine initial soil total arsenic concentration. Plants were grown in a