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Plants influence on arsenic availability and speciation in the rhizosphere, roots and shoots of three different vegetables

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ABSTRACT

The toxicity of arsenic (As) in the environment is controlled by its concentration, availability and speciation. The aims of the study were to evaluate the accumulation and speciation of As in carrot, lettuce and spinach cultivated in soils with various As concentrations and to estimate the concomitant health risks associated with the consumption of the vegetables. Arsenic concentration and speciation in plant tissues and soils was analysed by HPLC, AAS and XANES spectroscopy. To estimate the plants influence in the rhizosphere, organic acids in lettuce root exudates were analysed by ion chromatography. The results showed that the As accumulation was higher in plants cultivated in soil with higher As extractability. Arsenate predominated in the soils, rhizosphere and root exudates of lettuce. Succinic acid was the major organic acid in lettuce root exudates. Ingestion of the tested vegetables may result in an intake of elevated levels of inorganic As.

1. Introduction

Arsenic (As) accumulation in vegetables followed by ingestion may result in a significant contribution to the daily human intake of inorganic As (Fontcuberta et al., 2011). For As accumulation in vegetables, As availability in soils is central. Clay and organic matter in soils adsorb As resulting in a reduced plant-availability (Silva Gonzaga et al., 2012). Plants may increase As availability by releasing root exudates, including organic acids, which exert their action on for example oxides/hydroxides and ion exchange sites on the soil particles where As is adsorbed (Moreno-Jiménez et al., 2012). Organic acids have a major effect on the mobilization of elements in the rhizosphere (Marschner, 1995). A study by Silva Gonzaga et al. (2012) showed a good correlation between the As concentration in soil and the As concentration in plant during the extraction of As from soil using root exudate acids, suggesting that As release by root exudates may be important in plant As uptake. Also, the root exudates of the hyperaccumulating ferns *Pteris vittata* and *Pteris biaurita* increased the plant-available As in the soil (Silva Gonzaga et al., 2009).

The toxicity of As to humans depends on As speciation. Inorganic As species, especially arsenite, are carcinogenic while organic As species like arsenobetaine are less toxic to humans (Zhao et al., 2010). In soils, the inorganic As species arsenate and arsenite usually predominates (Sadiq, 1997). Several factors, mainly redox potential and pH, but also other factors like adsorption reactions and biological activity influence As speciation (Bhumbla and Keefer, 1994). Arsenate and arsenite also exists in several forms ranging from the fully protonated arsenous and arsenic acids, to a number of different oxoanions with the same oxidation states (arsenite: III, arsenate: V), on the As atom (Sadiq, 1997). For simplicity, these two species will be referred to in this study as arsenate and arsenite, respectively. The main As-species in terrestrial plants are arsenate and arsenite (Smith et al., 2009). In terms of organic species, some reports indicate that plants are able to methylate inorganic As, even though the methylated As levels are usually low (Raab et al., 2007). However, others claim (e.g. Lomax et al., 2012) that the methylating ability of plants does not exist and that organic As in plants is produced by microorganisms and taken up from the external medium.

The distribution of As within plants can occur both in the xylem and the phloem (Ye et al., 2010). The root to shoot translocation of As species differs between plant species. In the xylem sap, arsenite predominated in tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), rice and the fern *Pteris vittata*, while arsenate

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predominated in castor bean (*Ricinus communis*), wheat (*Triticum aestivum*), Indian mustard (*Brassica juncea*) and velvet grass (*Holcus lanatus*), exposed to either arsenate or arsenite (Ye et al., 2010). In the phloem sap, arsenite predominated in *Ricinus communis* exposed to either arsenate or arsenite (Ye et al., 2010).

The internal distribution of As in plants is divided between the apoplast and the symplast. Approximately 45% of the As in the fronds and 30% in the roots was located in the apoplast of the fern *Pteris cretica* (Feng et al., 2011). In rice, approximately 60% of the total plant As was located in the apoplast of the roots (Bravin et al., 2008). Cellular uptake of arsenate is mediated by phosphate transporters (Meharg and Macnair, 1992). Cellular uptake of arsenite, MMA and DMA in rice is mediated by the aquaporin Lsi1 (Li et al., 2009). To render As harmless inside the plant cells, arsenate is reduced to arsenite, complexed with thiol-rich compounds like glutathione and phytochelatins and stored in the vacuoles (Moreno-Jiménez et al., 2012). Another detoxification mechanism used by plants is the efflux of arsenite from the cells (Xu et al., 2007).

The aims of the study were (1) to investigate if plant growth in soils with various As concentrations influenced the As accumulation and speciation in carrot, lettuce and spinach and (2) to evaluate the potential health risk with the consumption of these vegetables. Also, the study investigated if X-ray absorption near-edge structure (XANES) spectroscopy could be used to supplement the determination of As speciation through chemical extraction. The hypotheses were that As accumulation would be higher in soils with high extractable As compared with soils with low extractable As. Also, plants influence the As accumulation by the root exudates. In addition, based on theory (Moreno-Jiménez et al., 2012), we hypothesize that arsenite is the predominating As species in all plants resulting in a potential health risk if consuming the vegetables from this study.

2. Materials and methods

The following methods were used to provide answers about the behaviour of As in plants, and bulk and rhizosphere soil, and the influence on soil As by plant root exudates in the rhizosphere.

2.1. Soil and plant materials

Four different soils were used in the experiments: two moderately As-polluted soils, one highly polluted soil, and one greenhouse soil ("K-jord", Hasselfors Garden). The two moderately polluted soils were agricultural alum shale soil (Kinne-Kleva [N 58° 34.33′, E 013° 26.19′]) and soil from an abandoned glassworks (Flygsfors [N 56° 50.52′, E 15° 46.70′]). The highly polluted soil was obtained from an abandoned glassworks (Gadderås [N 56° 51.88′, E 15° 48.98′]. While As is natural-occurring in the alum shale soil, the primary source of As in the glassworks soils is the leaching of As-bearing glass that was dumped on the ground surface outside the glassworks. Soil characteristics are listed in Table 1. The soil fraction ≤2 mm was used in the experiments.

Three different vegetables were used in the experiments; carrot (*Daucus carota* L. "Nantaise 2"), lettuce (*Lactuca sativa* L. "Amerikanischer brauner") and spinach (*Spinacia oleracea* "Fabris").

Table 1
Soil characteristics of experimental soils. Mean concentrations are shown for total As.

	Soil			
	Alum shale (Kinne-Kleva)	Glassworks (Flygsfors)	Glassworks (Gadderås)	Greenhouse
Total As (mg kg^{-1} DW)	142.2	77.7	514	2.2
Conductivity (μS cm^{-1})	213	108	257	374
Organic matter (%)	11	2	8	40
pH	7.71	6.29	7.90	6.62
Sand (%)	56	93	68	57
Clay (%)	40–60	<5	5–15	<5

2.2. Cultivation of carrot, lettuce and spinach

Seeds of carrot, lettuce and spinach were cultivated in the moderately As-polluted agricultural alum shale soil, the highly As-polluted glassworks soil and a greenhouse soil in 1 L pots. Cultivation was performed in October–December 2009 in a greenhouse (20 ± 2 Co, RH ~65%) with a 18 h light/6 h dark cycle (Osram Daylight, HQ1-BT 400 W). Watering was performed with deionized water approximately every other day, to keep the pots constantly moist. Carrot and lettuce were cultivated for five weeks to obtain well developed edible polluted parts while spinach was cultivated for three weeks to forestall blooming before harvest. Three individual plants from three separate pots were harvested for each respective plant species. Fresh weights of the harvested plants are shown in Table 2. After harvest, the plants were washed carefully with de-ionized water for approximately 5 min until all visible soil was removed from the plants, separated into roots and shoots and stored at −20 °C until analysis for total As and As species (see Sections 2.4–2.6). Spinach roots were only analysed for total As due to a lack of plant material for As species analysis.

Lettuce samples destined for XANES analysis were freeze-dried and stored at −20 °C until analysis with XANES spectroscopy. Three individual lettuce plants from three separate pots were harvested for the analyses. The other vegetables were not analysed by XANES as previous experience had indicated that the As concentrations were too low in these matrices (see below) for definitive species determination at the I811 beam-line.

Due to the phytotoxic effect of the highly As-polluted glassworks soil, this soil was not used in the following experiments. Instead, the more moderately As-polluted glassworks soil was used.

2.3. Root exudates of lettuce

Seedlings of lettuce germinated in vermiculite were mounted in Styrofoam plates and placed in 1 L containers with 25% Hoagland solution for hydroponic cultivation. The nutrient solution was replenished once a week. After ten days, the Hoagland solution was increased to 50%. The containers were aerated with microtubes throughout the hydroponic cultivation. The plants were placed in a climate chamber and grown altogether for 25 days in August–September 2011. Climate chamber conditions were set to 16 h/8 h light/dark, 20 ± 2 °C and RH to 70%. The full-grown lettuce plants were then moved to a rhizobox-like, non-sterile system according to Greger (2005), containing the moderately As-polluted alum shale and glassworks soils and a greenhouse soil. Three individual lettuce plants with a mean shoot fresh weight of 57 ± 9 g were used in the experiments, for each soil. After 48 h, each plant was removed from the rhizobox-like system and the roots were rinsed in 13 ml of de-ionized water which was collected. The 13 ml solute was divided into three parts, one for As-species and pH analysis, one for oxalic acid analysis and one for the analysis of acetic, citric, formic, malic and succinic acids. The fractions for the analysis of As-species and oxalic acid were left un-altered while the third fraction was treated with NaOH to obtain a final concentration of 0.1 M NaOH. All samples were filtered through 0.45 μM syringe filter, frozen in liquid nitrogen, freeze-dried and stored in −80 °C until analysis. The plants were divided into roots and shoots, washed thoroughly in de-ionized water for approximately 5 min until all visible soil was removed, and analysed for total As and As species (see Sections 2.5–2.7).

Freeze-dried samples were re-suspended in 1 ml of de-ionized water. For the analysis of the acetic, citric, formic, malic and succinic acids, 50 μl sample were injected in an ion chromatograph. Peaks were separated using an IonPac-AS6 analytical column (Dionex corporation, Sunvalley, CA, USA) and detected using an AMMS-ICE II ion-exclusion micro membrane suppressor, an ED50A electrochemical detector and a DS3-1 conductivity cell D23 detection stabilizer. The eluent was 0.4 mM heptafluorobutyric acid with a flow rate of 0.8 ml min^{-1} and the regenerant was 5 mM tetrabutylammonium hydroxide with a flow rate of 5 ml min^{-1}. Quantifications of the organic acids represented by the peaks were performed by external calibration using standard solutions for acetic acid, citric acid, formic acid, malic acid and succinic acid. The detection limit was 0.1 mg L^{-1} for all acids.

Oxalic acid was analysed using a modified method originally developed by Mayer et al. (1979) which includes a precipitation of oxalate by mixing 1 ml sample with 0.1 M $CaCl_2$ at the ratio 1:1 in an Eppendorf tube. The tube was then centrifuged for 40 min at 13,000 *g* and the supernatant removed using a suction pump. The remaining pellet was re-suspended in 1 ml 25 mM H_2SO_4 and 50 μl of this sample was injected into the ion chromatograph. The eluent was 0.1 mM H_2SO_4 with a flow rate of 0.8 ml min^{-1}. Oxalic acid standard was added to the sample to quantify the amount of oxalic acid in the sample.

2.4. Arsenic in lettuce rhizosphere and bulk soil

Lettuce seeds were sown in the moderately As-polluted alum shale and glassworks soils and a greenhouse soil. The plants were cultivated for 40 days in May–July 2011 in a greenhouse in 1 L pots. After harvest, the soil was separated into bulk soil, i.e. the soil without any direct interference with plant roots, and rhizosphere soil, i.e. the soil in the direct vicinity of plant roots. The rhizosphere soil was collected by harvesting the plants from the pots, gently removing the bulk soil, and leaving the roots with the attached rhizosphere soil to dry for a few min in room temperature. The rhizosphere soil could then be removed by gently brushing off the