APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 216–220

Arsenic compounds in leaves and roots of radish grown in soil treated by arsenite, arsenate and dimethylarsinic acid†

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Received 12 June 2001; Accepted 13 December 2001

The effect of arsenite [arsenic(III)], arsenate [arsenic(V)] and dimethylarsinic acid (DMA) on the growth of radish and the concentration of arsenic compounds in the roots and leaves of radish were investigated. Radish was grown in pots on Luvisols individually amended with arsenic concentrations of 20 mg kg^{-1} in the form of arsenic(III), arsenic(V), and DMA. In untreated soil, arsenate was the dominant arsenic compound; arsenite and DMA were also present. Arsenic(III) added to the soil was oxidized to arsenic(V), so that no differences between arsenic(III) and arsenic(V) soil treatments were observed. On DMA treatment, this compound remained in soil in high concentration in soluble and plant-available states, and the sum of arsenic(III), arsenic(V) and methylarsonic acid (MA) reached only 30% of water-extractable arsenic content. A low portion of soil arsenic added as DMA was immobilized, via adsorption, compared with inorganic compounds. Arsenic(III) was the dominant compound in radish roots planted in the untreated soil, whereas in leaves most of the arsenic present was arsenic(V). DMA was also detected in both plant tissues. A similar distribution of arsenic compounds was also found on arsenic(III) and arsenic(V) treatments. On DMA treatment, this compound showed high stability and the DMA concentration exceeded the sum of the remaining arsenic compounds [arsenic(III), arsenic(V) and MA] in both roots and leaves of radish.

KEYWORDS: radish; arsenite; arsenate; dimethylarsinic acid; soil; HPLC–ICP-MS; plant availability

INTRODUCTION

Arsenic behavior and its transformations in soil and dependence on different physicochemical soil properties are widely investigated.[1] The influences of soil texture, pH, redox potential, organic matter content, inter-element interactions on arsenic sorption/desorption and plant availability have already been discussed.[1–3] However, there is very little detailed information to be found concerning individual arsenic compounds in soil. The literature suggests that arsenic in soil is present mostly in the pentavalent state, but that it can be easily changed to arsenite under reducing soil conditions.[2–5] When the redox potential of soil suspensions dropped below 0 mV, most of the arsenic was present as arsenite [arsenic(III)]. Under partially oxidizing conditions both arsenic(III) and arsenate [arsenic(V)] were present.[4] Small percentages of methylated arsenic compounds in soils were also reported.[5] Mineralization of organoarsenicals in soil under different soil conditions was also mentioned. The main metabolite from degradation of methylarsonic acid (MA) and dimethylarsinic acid (DMA) was arsenate, and DMA demethylates directly to arsenic(V) rather than through MA as an intermediate.[5] The rate of MA mineralization was slower than that of DMA under the same conditions. Transformation of arsenate to MA or of MA to DMA was negligible.[6]

Phytoavailability and phytotoxicity of individual arsenic compounds in soils were summarized by Sheppard.[7] Differences in arsenic responses were probably caused by the soil properties and the source of arsenic. Inorganic and

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†This paper is based on work presented at the 5th International Conference on Environmental and Biological Aspects of Main-Group Organometals (ICEBAMO-5) held at Schielleiten, near Graz, Austria, 5–9 June 2001.
Contract/grant sponsor: Grant Agency of Czech Republic; Contract/grant number: 511/95/0557.
Contract/grant sponsor: AKTION Östereich–Tschechische Republic; Contract/grant number: 500 12.

DOI:10.1002/aoc.282

waste forms of arsenic were less toxic to plant growth compared with organic sources (*ride infra*). On the contrary, different patterns of arsenic compound uptake were observed in liquid cultivation solutions. The uptake of arsenic from nutrient solution by bean roots was in the order arsenic(V) > arsenic(III) > MA > DMA.[1] If rice was planted in a liquid soil suspension amended by DMA, this compound was less plant available than inorganic one arsenic.[8] The availability of arsenic for rice increased with increasing amounts of soluble arsenic(III) at lower redox potentials of the soil suspension.[4] MA was in this case twice as phytoavailable for rice than inorganic arsenic forms, and increased with decreasing pH and redox potential.[4,8]

Concerning plant availability of arsenic, the influence of arsenic compounds contained in the soil on plant yield and/or total arsenic concentrations have been evaluated,[1,4,8,9] but there is sparse data available about soil–plant relationships for the individual arsenic species. When ryegrass and barley were planted in a loam soil amended by arsenic as arsenic(III) or as arsenic(V), arsenic(III) introduced a more inhibitory effect on crop yield than arsenic(V). However, only low differences in total arsenic concentration in crops between arsenic(III) and arsenic(V) sources were observed.[9] Translocation of total arsenic in rice plants cultivated in soil solution amended by individual arsenic compounds was investigated by Marin *et al.*[8] Arsenic concentration increased more easily in shoots if DMA was added, whereas the addition of arsenic(III), arsenic(V) and MA to the soil solution led to increasing arsenic concentrations in root. In tissue cultures of periwinkles grown in a medium containing arsenate, the presence of arsenic(V), arsenic(III) and low levels of MA and DMA was determined in cell extracts.[10] These results suggested that higher plants are able to methylate arsenic compounds. Detailed evaluation of individual arsenic compounds in both soils and plants could only elucidate the biological processes in the soil–plant system, including their toxicity.

The objective of this study was to investigate the effect of three arsenic species [arsenic(III), arsenic(V) and DMA] added individually to the soil, on the growth of radish (*Raphanus sativus* L.), their individual accumulation and distribution between roots and shoots of plants, and the transformation of arsenic compounds in soil across the radish plantation.

EXPERIMENTAL

Pot experiment

Radish (var. Duo) was planted in model pot experiments on Luvisols of the following physicochemical properties: pH 6.7 (KCl), C_{ox} 1.4%, CEC 151 mval kg^{-1}, clay particles 26%, silt particles 35%, sand particles 39%, total arsenic concentration 15.1 ± 2.5 mg kg^{-1}. Aliquots (5.0 kg) of these air-dried soils were placed into plastic pots. Four treatments, each with four replicaties, were set up in this experiment. To each pot was individually added an aqueous solution containing an arsenic concentration of 10.0 mg ml^{-1}. Treatments containing arsenic(III), arsenic(V) and DMA at a level corresponding to 20 mg of arsenic per kilogram of soil were made. Control untreated pots (zero) were also included. The solution added was thoroughly mixed with the whole amount of the soil. Radish seeds were sown in the soil immediately after amendment with arsenic compounds. The soils were watered with deionized water to keep soil moisture at 60% of its maximal water holding capacity. The plants were harvested 43 days after sowing. The radish roots were freed from adhering soil by washing with deionized water. The leaves were separated from the roots with a stainless steel knife. Leaves and roots of each pot were weighed, dried at 60 °C to constant mass and then ground to a fine powder in a mixer. Soil samples were taken immediately after the harvest of the plants from the bulk, then dried in air at 20 °C, ground in a mortar and passed through a 2 mm plastic sieve. Total arsenic concentrations were determined in the roots and leaves of radish from individual pots by atomic absorption spectrometry (AAS) after a dry ashing procedure. After completion of total arsenic analyses, the samples of individual replications were mixed together to obtain representative samples of individual arsenic treatments. In the mixed samples, total arsenic concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted wet digestion. Arsenic compounds were determined in the same samples by high-performance liquid chromatography (HPLC) coupled with the ICP-MS as an element-specific detector.

Sample decomposition

Dry ashing

An aliquot (~1 g) of the dried and powdered leaves or roots was weighed to 1 mg into a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases ($O_2 + O_3 + NO_x$) at 400 °C for 10 h in a Dry Mode Mineralizer Apion (Tessek, Czech Republic).[11] The ash was dissolved in 25 ml of 1.5% nitric acid (electronic-grade purity, Analytika Ltd, Czech Republic) and kept in glass tubes until measurement. Aliquots of the certified reference material RM 12-02-03 Lucerne were mineralized under the same conditions for quality assurance of the analytical data.

Pressurized wet ashing

An aliquot (~250 mg) of the dried and powdered leaves or roots was weighed to 0.1 mg into a Teflon® digestion vessel. Concentrated nitric acid (3.0 ml; Merck p.a. Nr. 100456), purified in an all-quartz sub-boiling distillation unit, and 30% H_2O_2 (1.0 ml; Merck Suprapur® Nr. 107298) were added. The mixture was heated in an MLS-1200 Mega (MLS GmbH, Leutkirch, Germany) microwave-assisted wet digestion system.[12] After cooling, the digest was quantitatively transferred into a 50 ml polypropylene tube. An indium solution (100 µl, [In]10 mg l^{-1}) was added. The tube

Table 1. Total extractable soil arsenic concentrations (As_{tot}) by two different extractants and distribution of arsenic species in the extracts (nd: not detected)

Treatment	Water					Phosphate buffer				
	As_{tot} (mg kg^{-1})	As(III)(%)	As(V)(%)	MA(%)	DMA(%)	As_{tot} (mg kg^{-1})	As(III)(%)	As(V)(%)	MA(%)	DMA(%)
Zero	0.13	6	91	nd	3	1.04	1.4	98	nd	0.6
As(III)	1.82	0.5	99.5	nd	nd	9.49	0.2	99.8	nd	nd
As(V)	1.78	0.5	99.5	nd	nd	10.1	nd	100	nd	nd
DMA	4.88	1	22	4	73	14.6	nd	53	4	43

was filled to the mark with Milli-Q water. Aliquots of the NIST SRM 1575 Pine Needles reference material were mineralized under the same conditions.

Sample extraction

Extraction of arsenic compounds from plant material

Aliquots (~500 mg) of the dried and powdered leaves or roots, weighed to 0.1 mg, were placed into 50 ml screw-capped polyethylene tubes. A methanol–water mixture (20 ml, 8 + 2 v/v) was added. The closed tubes were fastened to the arms of a cross-shaped rotor and turned top over bottom at 45 rpm for 14 h. The mixtures were then centrifuged for 10 min at 3000 rpm. The supernatant was transferred to a 250 ml round-bottomed flask and evaporated to dryness in a Rotavapor at room temperature under an aspirator vacuum. The residue was treated with 10 ml Milli-Q water. The resulting solution was filtered through a 0.22 μm cellulose-nitrate ester filter (Millex-GS, Milipore, Bedford, MA, USA). Aliquots of this solution (100 μl) were chromatographed.

Extraction of arsenic compounds from soils

Aliquots (~1000 mg) of the dried soil samples were extracted with (i) Milli-Q water (1 + 19 w/v); (ii) 0.1 mol l^{-1} aqueous $NH_4H_2PO_4$ solution at pH 6.0 (1 + 19 w/v) for 14 h as described for the plant material. The water and aqueous $NH_4H_2PO_4$ solution extractants were centrifuged for 10 min at 3000 rpm, filtered through 0.22 μm cellulose-nitrate ester filters and chromatographed.

Determination of total arsenic compounds in the digests

AAS

Total arsenic in the roots and leaves decomposed by the dry ashing procedure was determined by hydride generation AAS (Varian SpectrAA-300, Australia, equipped with continuous hydride generator VGA-76).

ICP-MS

Total arsenic was determined in the diluted wet digests of leaves and roots, as well as in the methanol–water extracts of plant material and water and aqueous $NH_4H_2PO_4$ solution extracts with an ICP mass spectrometer[12] (VG Plasma Quad 2 Turbo Plus, VG Elemental, UK, equipped with a Fassel-type torch, a Gilson Minipuls-3 peristaltic pump and a Meinhard TR-30-A3 nebulizer).

Chromatographic system

The HPLC system for the separation of the arsenic compounds consisted of a Hewlett Packard 1050 solvent delivery unit (Germany) and a Rheodyne 9125 six-port injection valve (USA) equipped with a 100 μl loop. The arsenic compounds were separated on a Hamilton PRP-X100 (USA) anion-exchange column (250 mm × 4.1 mm i.d., spherical 10 μm particles of a styrene–divinylbenzene copolymer with trimethylammonium exchange sites). An aqueous 0.020 mol l^{-1} $NH_4H_2PO_4$ solution at pH 6.0 served as mobile phase at a flow rate of 1.5 ml min^{-1}. The column effluent was routed to a hydraulic high-pressure nebulizer (HHPN) through a 700 mm poly-ether-ether-ketone capillary (0.13 mm i.d.). The aerosal produced by the HHPN was introduced into the plasma of the ICP mass spectrometer[13] for arsenic-specific detection.

Statistics

The plant yield and total arsenic concentrations in plants from individual pots were evaluated by ANOVA (Statgraphics 4.0) at a significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Soil

The extractability of arsenic from soil was affected by both the extraction medium used and the soil arsenic treatment (Table 1). Total water-extractable arsenic concentrations represented, in the case of the control untreated sample, 0.9% of total arsenic in this soil, whereas phosphate buffer was able to extract 6.9% of total soil arsenic. Water-soluble arsenic compounds introduced readily available amounts of arsenic for plants; phosphate solutions are effective for extraction of specifically sorbed arsenic from mineral surfaces, and also cover parts of the labile forms of the element determined. Phosphates in soil are able to release arsenates from the adsorption sites because of their smaller size and higher charge density.[14] The addition of arsenic compounds to the soil led to increasing extractable arsenic

Table 2. Average yield of radish and total arsenic concentrations (As_{tot}) determined in individual treatments. ANOVA: the treatments marked by the same letter did not significantly differ at $\alpha = 0.05$

Treatment	Roots				Leaves			
	Yield (g/pot)	ANOVA	As_{tot} (mg kg^{-1})	ANOVA	Yield (g/pot)	ANOVA	As_{tot} (mg kg^{-1})	ANOVA
Zero	290 ± 35	a	0.25 ± 0.06	a	96 ± 9	a	0.60 ± 0.04	a
As(III)	288 ± 17	a	5.82 ± 2.42	b	100 ± 3	a	4.27 ± 0.41	b
As(V)	258 ± 22	a	5.50 ± 1.46	b	92 ± 4	a	4.43 ± 0.90	b
DMA	124 ± 37	b	4.87 ± 2.20	b	43 ± 14	b	4.75 ± 1.42	b

concentrations compared with controls in the order DMA > arsenic(V) = arsenic(III) treatments in both extractants. A comparison of the proportions of arsenic compounds leached by individual extracting agents (Table 1) showed that the phosphate buffer can release arsenic(V) more preferably than organoarsenicals. Pure water seems to respect the existing ratio of arsenic compounds in soil, and the extracted amount of arsenic represents approximately the element fraction present in the soil solution and the readily plant-available arsenic.

Arsenic(V) was confirmed as the dominant arsenic compound in control soil (Table 1), in agreement with the literature,[2–5] and its proportion was not changed during the experiment. Arsenic(III) added to the soil was oxidized to arsenic(V) during plant cultivation, and the proportions of the arsenic compounds did not differ between arsenic(III) and arsenic(V) treatments at the end of the experiment. The transformation of arsenic(III) to arsenic(V) in the soil under oxidizing conditions, even within 2 days, has also been described.[15] Finally, the only difference between the control sample and the inorganic arsenic treatments was in the higher concentration of plant-available arsenic(V) in treated soils.

When DMA was added to the soil, this compound remained as the dominant arsenic compound in the soil extracts. DMA immobilization was provided more via adsorption than via demethylation, and relatively high amounts of this compound remained in an easily soluble state after the radish harvest. Decomposition of organoarsenicals in soil is also possible,[6] but this process seems to be long term and strongly dependent on soil properties.[16]

Plants

The plant yields and total arsenic concentrations in the roots and leaves of radish are summarized in Table 2. The yield of the plant biomass was not significantly affected if inorganic forms of arsenic were added to the soil, whereas DMA significantly reduced the growth of leaves and roots as well. Similar results were found for radish[16] and turnip.[17] Total arsenic concentrations in both leaves and roots of radish increased significantly in treated soils irrespective of the arsenic compound added. As described above, the available concentration of DMA in soil remained higher compared with inorganic arsenic, and this concentration was phytotoxic, but it did not lead to a corresponding increase in arsenic concentrations in plants compared with arsenic(III) and arsenic(V) treatments. If plants are cultivated in a liquid medium in which the same arsenic concentrations are available, then DMA was always less available compared with inorganic arsenic compounds.[1,8] If radish plants were cultivated in soil-less culture conditions, phytoavailability of individual arsenic compounds followed the trend DMA $\leq$ arsenic(V) $\leq$ arsenic(III) $\ll$ MA.[18] In our case, the toxic effect of DMA on plants was caused by a lower binding ability of this compound than arsenic(III) and arsenic(V).

The arsenic concentrations released by the methanol–water extraction procedure did not exceed 30% (leaves) and 43% (roots) of the total arsenic concentration from untreated plants and the plants treated by arsenic(III) and arsenic(V), and showed a lower capacity of plant organic compounds to bind arsenic in the roots of radish (Table 3). In DMA-treated soil, the arsenic concentration released exceeded 75% of the total arsenic in plants. A significantly higher extractability of

Table 3. Total extractable (methanol–water) arsenic concentrations (As_{tot}) in roots and leaves of radish and distribution of arsenic species in the extracts (nd: not detected)

Treatment	Roots					Leaves				
	As_{tot} (mg kg^{-1})	As(III)(%)	As(V)(%)	MA(%)	DMA(%)	As_{tot} (mg kg^{-1})	As(III)(%)	As(V)(%)	MA(%)	DMA(%)
Zero	0.10	63	20	nd	17	0.04	40	42	nd	18
As(III)	1.64	56	40	nd	4	0.94	15	83	nd	2
As(V)	1.59	57	39	nd	4	1.14	21	77	nd	2
DMA	4.74	5	2	4	89	3.56	3	6	3	88

DMA from both leaves and roots compared with other treatments, showed the poor ability of plants to transform an excess of readily available DMA. No differences in the ratio of arsenic compounds were observed between roots and leaves in this case.

Different plant parts showed different abilities to accumulate arsenic species. Arsenic(III) was the dominant compound in roots, whereas arsenic(V) was mostly translocated to leaves. On DMA treatment, arsenic uptake by radish was not transformed and stayed non-decomposed in both parts of the plants. A higher percentage of DMA in plants (17% in roots and 18% in leaves) compared with soil suggested that radish plants are more easily able to take up organically bound arsenic than inorganic arsenic or to methylate the available arsenic compounds.[10] The occurrence of individual arsenic compounds in higher plants and their distribution into different plant tissues are also strongly dependent on plant species.[15,19] As described above, the ratio of plant-available arsenic compounds in soil treated by arsenic(III) and arsenic(V) were comparable because of oxidation of arsenic(III) in this soil. As expected, the behavior of arsenicals in plants from both arsenic(III) and arsenic(V) treatments was comparable. Similarly, as in the case of soil, the ratios of arsenic compounds in radish treated by inorganic arsenic compounds was not significantly different from the control samples and was only slightly affected by higher amounts of available arsenic(V).

The results showed that inorganic arsenic compounds are the most important ones in the soil–plant system. Methylated arsenic compounds are also present in natural soils, but chemical and microbial transformation of a large excess of DMA seemed to be difficult for both the soil and the plant. The pattern of arsenicals in roots and leaves of radish suggested that there is transformation or translocation mainly of inorganic arsenic compounds among different plant tissues, and various distributions of these compounds can be expected among other terrestrial plant species. Further investigations of transformations of arsenic species in soil–plant systems is necessary to elucidate the behavior of different compounds of this element in the terrestrial ecosystem.

Acknowledgements

Financial support for these investigations was provided by Grant Agency of Czech Republic, Project No. 511/95/0557, and AKTION Österreich–Tschechische Republik, Project 5w 12.

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