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Organoarsenic compounds in plants and soil on top of an ore vein

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Plants and soil collected above an ore vein in Gasen (Austria) were investigated for total arsenic concentrations by inductively coupled plasma mass spectrometry (ICP-MS). Total arsenic concentrations in all samples were higher than those usually found at non-contaminated sites. The arsenic concentration in the soil ranged from ~700 to ~4000 mg kg^{-1} dry mass. Arsenic concentrations in plant samples ranged from ~0.5 to 6 mg kg^{-1} dry mass and varied with plant species and plant part. Examination of plant and soil extracts by high-performance liquid chromatography–ICP-MS revealed that only small amounts of arsenic (<1%) could be extracted from the soil and the main part of the extractable arsenic from soil was inorganic arsenic, dominated by arsenate. Trimethylarsine oxide and arsenobetaine were also detected as minor compounds in soil. The extracts of the plants (*Trifolium pratense, Dactylis glomerata*, and *Plantago lanceolata*) contained arsenate, arsenite, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, the tetramethylarsonium ion, arsenobetaine, and arsenocholine (2.5–12% extraction efficiency). The arsenic compounds and their concentrations differed with plant species. The extracts of *D. glomerata* and *P. lanceolata* contained mainly inorganic arsenic compounds typical of most other plants. *T. pratense*, on the other hand, contained mainly organic arsenicals and the major compound was methylarsonic acid. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenic compounds; soil; plants; HPLC–ICP-MS

INTRODUCTION

The environmental chemistry of arsenic is complex, largely because of the many different chemical forms of arsenic that can occur in environmental samples.[1,2] Although in abiotic compartments arsenic is present predominantly in inorganic forms, organisms are able to biotransform inorganic arsenic into organoarsenic compounds. This is most clearly illustrated in marine systems, where arsenate in seawater is taken up by algae and converted into arsenic-containing carbohydrates (arsenosugars).[3] The situation in the terrestrial environment is different. Although arsenosugars[4,5] and other organoarsenic compounds typical of marine organisms are also found in terrestrial organisms, they occur generally as minor constituents only, and inorganic arsenic usually predominates.[6]

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The formation of organoarsenic compounds is thought to be a detoxification process to enable organisms to cope with potentially toxic inorganic arsenic. Investigations on mushrooms growing on old mining areas have revealed that those organisms concentrate high amounts of arsenic[7] and contain a variety of arsenic compounds,[8–10] often with organoarsenicals as the major arsenic constituent — as an exception to the rule in the terrestrial environment. The diversity and range of arsenic compounds in terrestrial green plants were shown in a limited number of reports.[4,11–13] However, the green plants appear to have other strategies for dealing with arsenic. This has been highlighted recently with the report that the brake fern (*Pteris vittata*) grows well on arsenic-contaminated soil, and accumulates arsenic enormously in its fronds, mainly as arsenite, a toxic form of arsenic.[14] In a related study, the break fern and another fern species (*Pityrogramma calomelanos*) were shown to be the only two plant species out of 36 species examined to accumulate such large quantities of arsenic.[15] Investigation of the arsenic in *P. calomelanos* showed that it, too, had apparently accumulated most of its arsenic burden as arsenite, and contained only

traces of organoarsenic compounds.[16] This work and some other reports about arsenic hyperaccumulating plants[17–19] has stimulated interest in how some plants can grow well in arsenic-enriched soils, and how these plants handle their arsenic exposure.

The Strassegg area in Gasen (Styria, Austria) contains extensive arsenopyrite (FeAsS) mineralization,[20,21] which has been a rich source of arsenic for several hundred years. The Strassegg was a mining area, mainly in the 15th and 16th centuries, but was abandoned in the 19th century. The ore body runs to the surface, and hence surface soil contains high concentrations of arsenic.[22] The Strassegg area is agriculturally utilized and provided an opportunity to investigate how plants deal with their 'natural' high arsenic environment. We report an investigation by high-performance liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry (ICP-MS) on the arsenic content and arsenic species in soil and plants from this site.

EXPERIMENTAL

Sampling and sample preparation

Soil and plant samples (individual plant species and mixed grass) were collected on top of the ore vein at the Strassegg in Gasen (Styria, Austria).

Soil samples (0–30 cm) were taken with a soil auger and were separated into two portions. One portion was dried in an oven at 105 °C for 24 h and extracted with aqua regia according to ÖNORM 1085[23] for the determination of total arsenic concentration; the other portion was freeze-dried for the determination of the arsenic compounds. All soil samples were sieved with a plastic mesh (mesh size ~1 mm).

Plant samples were taken as mixed samples of a single species: *Anthoxantum odoratum* (above-ground material), *Bellis perennis* (flowers/stems), *Dactylis glomerata* (above-ground material), *Oxalis acetosella* (flowers and leaves), *Picea abies* (needles), *Plantago lanceolata* (flowers/stems and leaves/stems), *Taraxacum officinale* (flowers/stems and leaves), *Trifolium pratense* (above-ground material), and *Rumex acetosa* (above-ground material). Individuals of the different plant species were randomly collected over the ore vein area; they were washed carefully to remove soil particles. The plant samples were freeze-dried and homogenized.

Determination of total arsenic by ICP-MS

An aliquot (~0.2 g) of freeze-dried plant powder was weighed to 0.1 mg into the Teflon® digestion vessels (90 ml) of an MLS-1200 Mega (MLS, Leutkirch, Germany) microwave digestion system. Fuming concentrated nitric acid (3.0 ml) and 30% hydrogen peroxide (0.5 ml) were added. After 15 min the vessels were closed and placed into the microwave oven. The samples were then mineralized with the following microwave heating program: 2 min at 250 W, 30 s at 0 W, 5 min at 300 W, 30 s at 0 W, 10 min at 400 W, 30 s at 0 W, 5 min at 500 W, 4 min at 600 W. The resulting digests were quantitatively transferred to 50 ml volumetric flasks. The elements gallium, indium, and rhenium were used as internal standards to correct for instrumental instabilities. Solutions of these elements were added to the analytical solutions to reach a final concentration of 50 μg l^{-1} each.

An aliquot (~2 g) of the dried soil was weighed to 0.01 g into 100 ml round-bottomed flasks, and 20 ml aqua regia was added. The mixture was allowed to stand at room temperature for 24 h and then it was refluxed for 1 h. The extracts were filtered through filter papers.[23] The filtrates were diluted (1 + 19, v/v), and nitric acid (2%) and the internal standards were added as mentioned above.

Total arsenic concentrations were determined in all samples with a VG Plasma Quad 2 Turbo Plus inductively coupled argon-plasma mass spectrometer (VG Elemental Ltd, Winsford, UK). Pine Needles, SRM 1575, National Institute of Standards and Technology (NIST), Gaithersburg, USA (certified arsenic concentration: 0.21 ± 0.04 mg kg^{-1}; measured values: 0.23 ± 0.04 mg kg^{-1}; $n = 3$) and Olive Leaves (*Olea europea*, SRM-BCR No. 62, Community Bureau of Reference, Brussels, Belgium; non-certified arsenic concentration: 0.20 mg kg^{-1}; measured values: 0.210 ± 0.003 mg kg^{-1}; $n = 3$) served as standard reference materials.

Extraction of arsenic compounds

Aliquots (~1.0 g) of the soil and plant samples (*D. glomerata, P. lanceolata, T. pratense*) were weighed into 50 ml polyethylene centrifuge tubes with screw caps and conical base. A methanol–water mixture (30 ml, 9 + 1, v/v) was added; the closed tubes were fastened to a cross-shaped rotating device and rotated overnight. The extracts were centrifuged (2600 rpm, 10 min), the supernatant collected, the residue washed with methanol–water (30 ml, 9 + 1, v/v), centrifuged (2600 rpm, 10 min), and the supernatant was removed and combined with the prior one. The washing was repeated three times. The combined supernatants were evaporated (centrifugal lyophilizer, Heto Holten, Denmark) at room temperature to dryness. The dry residue was redissolved in water (10 ml). The samples were then centrifuged in an ultracentrifuge (HETTICH, Germany) for 15 min at 9500 rpm and filtered through 0.2 μm cellulose nitrate filters (Sartorius, Göttingen, Germany). Aliquots (100 μl) of these solutions were then directly chromatographed.

Determination of arsenic compounds by HPLC–ICP-MS

For the chromatographic analysis of the samples, an HP 1100 HPLC system (Agilent, Waldbronn, Germany) was used. The separations of the soil and plant samples were performed on a Hamilton PRP-X100 anion-exchange column (25 cm × 4.6 mm i.d.; Hamilton, Reno, USA) with an aqueous 30 mM ammonium phosphate solution at pH 5.6 (adjusted

with 25% ammonia) as mobile phase and on a Zorbax 300-SCX cation-exchange column (15 cm × 4.1 mm, Agilent, Waldbronn, Germany) with an aqueous 20 mM pyridine solution at pH 2 (adjusted with formic acid) as mobile phase. An HP 4500 ICP mass spectrometer (Agilent, Waldbronn, Germany) served as arsenic-specific detector. The outlet of the HPLC column was connected via a 100 cm, 1/16 in polyether-ether-ketone capillary tubing (0.13 mm i.d.) to the Babington-type nebulizer of the ICP mass spectrometer.

The ion intensities at *m/z* 75 (^{75}As) and *m/z* 77 ($^{40}Ar^{37}Cl$, ^{77}Se) were monitored. Arsenic compounds were identified by comparison of the retention times with standard solutions of arsenite [arsenic(III)], arsenate [arsenic(V)], methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenobetaine (AB), tetramethylarsonium ion (TETRA), arsenocholine (AC), trimethylarsine oxide (TMAO), and four dimethyl arsinoyl-ribosides phosphate (PO_4), glycerol (GLY), sulfate (SO_4), and sulfonate (SO_3) arsenosugars. Sources of arsenic standards and chemical structures of compounds were previously reported. The arsenic compounds in the samples were quantified with external calibration curves established with standard solutions of MA, DMA, arsenic(III), and arsenic(V) (anion-exchange column) or AB, TMAO, AC and TETRA (cation-exchange column).[12]

RESULTS AND DISCUSSION

Total arsenic concentrations

The arsenic concentration in the soil at the ore vein of Gasen ranged from ~700 to ~4000 mg kg^{-1} dry mass with variations in soil depth and distance to the centre of the vein. These results are in agreement with earlier reports of this area (~1800 mg kg^{-1} dry mass).[22]

The total arsenic concentrations of the different plant species were in the range ~0.4–6 mg kg^{-1} dry mass, with differences depending on plant species and plant parts (Table 1). The arsenic concentrations in the plants from the area of the ore vein are higher than those usually reported for plants from uncontaminated sites.[2,6] However, none of the plants was shown to be an arsenic hyperaccumulater.[14–19] A similar range of arsenic levels (~0.3–8.5 mg kg^{-1} dry mass) was reported from plants grown at a former arsenic smelter site.[12] The highest concentrations were found in *P. lanceolata* and *O. acetosella*, lowest in *T. officinale* and *R. acetosa*. Flowers of *P. lanceolata* and *O. acetosella* were higher in arsenic than leaves of the same species, whereas *T. officinale* showed higher amounts of arsenic in leaves than in flowers. Preliminary investigations — some plants were analysed with and without roots — had shown that, generally, plant samples with roots were higher in arsenic concentration than only above-ground plant samples of the same species. Therefore, only above-ground plant material was investigated for total arsenic and speciation of arsenic compounds, because the contamination by small soil particles on the roots can never be completely ruled out, despite intensive washing.

Table 1. Arsenic concentration in plant species

Plant species	[As] (mg kg^{-1})
Anthoxantum odoratum	3.98
Bellis perennis	1.63
Dactylis glomerata	1.62
Oxalis acetosella	
Leaves	4.48
Flowers	5.97
Picea abies needles	2.24
Plantago lanceolata	
Leaves	4.30
Flowers	5.93
Rumex acetosa	0.68
Taraxacum officinale	
Leaves	1.73
Flowers	0.36
Trifolium pratense	3.17

Arsenic compounds in plants

The extraction efficiency in the plants was relatively low (2.5–12% of total arsenic), but not unusual for plants.[12] The following discussion on arsenic compounds in all samples relates to the water-soluble compounds; arsenic remaining in the pellet after the extraction procedure remains unidentified and might be Lipid arsenic or protein-bound arsenic.

The plant species investigated contained inorganic arsenic as arsenic(III) and arsenic(V). Besides the simple methylated compounds MA and DMA, TMAO, TETRA, AB and AC could also be detected as organic arsenic compounds (Figures 1 and 2). The presence and concentrations of arsenic compounds differed depending on the plant species (Table 2). In *D. glomerata* (87%) and *P. lanceolata* (77%), the inorganic arsenic compounds dominated, which is typical for terrestrial plants.[6] On the other hand, *T. pratense* showed nearly two-thirds (62%) organoarsenicals. The main organoarsenical detected in *D. glomerata* and *P. lanceolata* was DMA, whereas *T. pratense* contained mainly MA (57%). This monomethylated compound was not present in the two other species, and it has not been reported as the major compound in any other plant species, despite being found in some yams from Taiwan.[24] *T. pratense* belongs to the family *Fabaceae*, where symbiosis with bacteria is well known; this symbiosis possibly influences the methylation of arsenic. Small amounts of AB, TMAO and TETRA were present in all three plant species, whereas AC was only detected in *P. lanceolata*. The only plant species previously reported to have contained AC is *Holcus lanatus* grown on contaminated soils.[25] No arsenosugars could be detected in the plants. Kuehnelt *et al.*[12] investigated a variety of terrestrial plants from a former arsenic roasting facility and reported the presence of DMA, MA, TMAO, AB, TETRA and an arsenosugar beside the inorganic arsenicals. Mattusch *et*

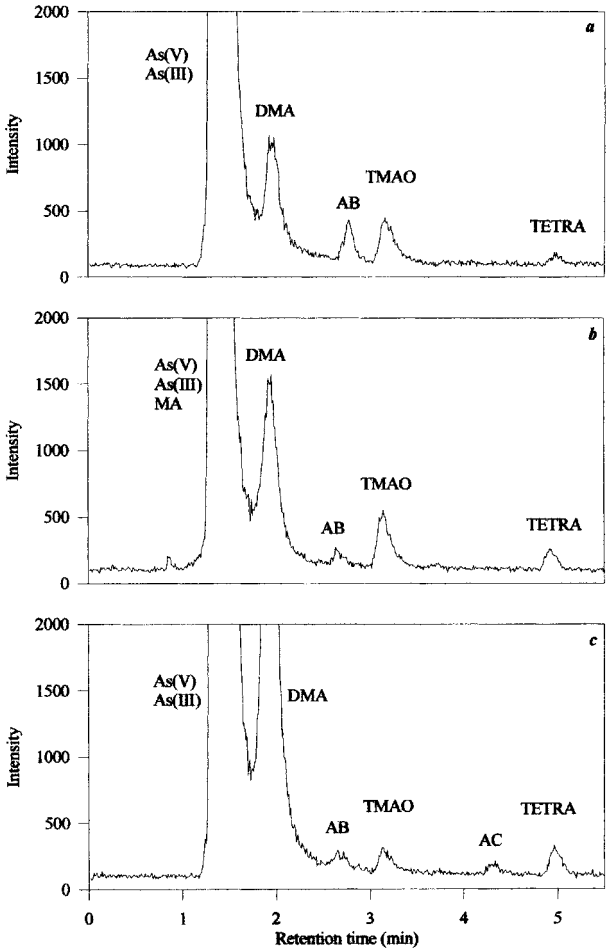

Figure 1. HPLC–ICP-MS cation-exchange chromatograms (Zorbax LC-SCX, 20 mM pyridine, pH 2, 1.5 ml min^{-1}, 30 °C) of plant extracts: (a) *D. glomerata*; (b) *T. pratense*; (c) *P. lanceolata*.

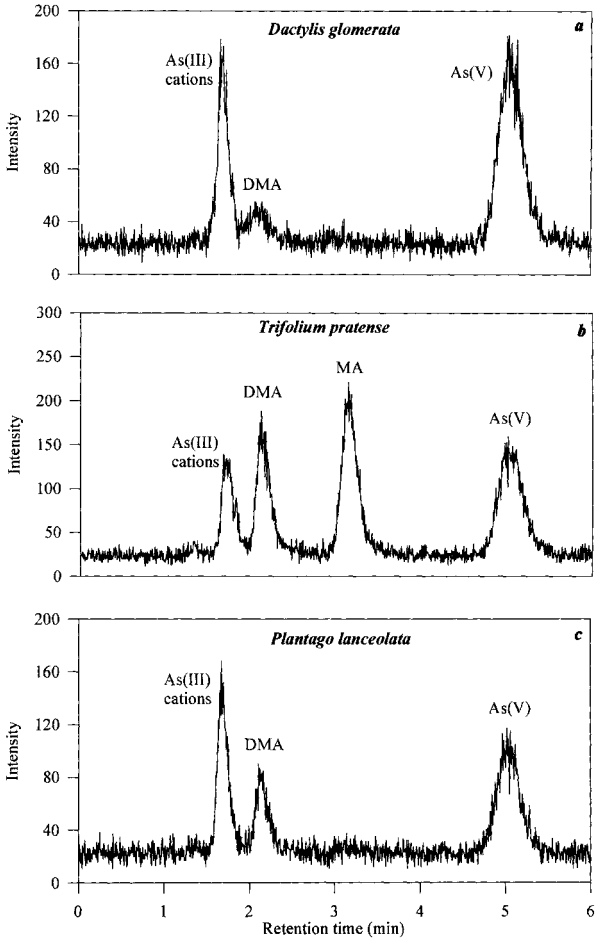

Figure 2. HPLC–ICP-MS anion-exchange chromatograms (Hamilton PRP X-100, 30 mM ammonium phosphate, pH 5.6, 1.5 ml min^{-1}, 40 °C) of plant extracts: (a) *D. glomerata*; (b) *T. pratense*; (c) *P. lanceolata*.

al.[13] investigated the arsenic species in several plants grown on a mixture of reference soil and contaminated soil from a mine tailing. All those plants contained the bulk of arsenic as inorganic arsenic and DMA as minor compound; in some of the plant species, AB and AC and/or TMAO (not resolved) were also detected as minor compounds, whereas MA only occurred in one plant species.

Arsenic compounds in soil

Only traces (<1%) of the total arsenic in the soil were extractable with the methanol–water mixture. This low extraction efficiency from the soil is not unusual[26] and may reflect the fact that arsenic is mainly bound in the mineral phases of the soil (as FeAss).

The major part of the methanol–water-extractable arsenic was inorganic arsenic (~80%), dominated by arsenate (~67%). The organoarsenicals were minor compounds, TMAO accounted for ~17% and AB for ~3% of the arsenic in the extracts (Fig. 3). Most investigations about soil mainly report inorganic arsenic.[4,11,27] However, reduction, methylation and demethylation may occur depending on the soil type, the microbes, and the conditions they are exposed to in the soil, and the occurrence of MA and DMA as minor constituents[28] or traces of TMAO[11] in soil have occasionally been reported. AB in soil was only reported from an aerobic incubated soil with high arsenic concentrations;[29] no AB was detected in untreated soil and the occurrence of AB was related to microbes in the soil.

Table 2. Concentrations of arsenic compounds in plant extracts

	Compound concentration (μg kg^{-1} dry mass)							
Plant species	As(III)	As(V)	DMA	MA	AB	TMAO	AC	TETRA
D. glomerata	45	120	15	–	3.5	5	–	trace
T. pratense	55	85	90	130	1.5	7	–	1
P. lanceolata	50	60	30	–	0.5	0.7	trace	0.7

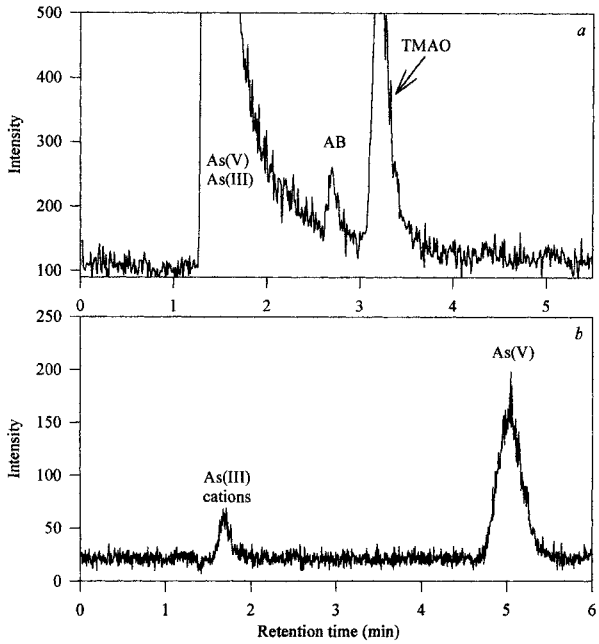

Figure 3. HPLC–ICP-MS chromatograms of soil extracts: (a) cation-exchange column (Zorbax LC-SCX, 20 mM pyridine, pH 2, 1.5 ml min^{-1}, 30 °C); (b) anion-exchange column (Hamilton PRP X-100, 30 mM ammonium phosphate, pH 5.6, 1.5 ml min^{-1}, 40 °C).

The dominance of arsenopyrite in Gasen plays a significant role for the composition of arsenicals in the soil. During the weathering of pyrite, sulfur appears in different oxidation states, so that different sulfoarsenates might be present. Several microbes are typical for ores, which possibly might contribute to arsenic transformation processes.

The few investigations undertaken so far have revealed a great variety of different arsenic compounds. The present investigation contributes further to demonstrating the diversity of arsenic in the terrestrial environment. More effort will be necessary to investigate the possible parallels of arsenic compounds in related species and to elucidate the cycling and metabolization of arsenic in the terrestrial environment.

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