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# Arsenic Compounds in Terrestrial Organisms III: Arsenic Compounds in *Formica* sp. from an Old Arsenic Smelter Site

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**Total arsenic concentrations in the freeze-dried pulverized ants (*Formica* sp.) and material from an ant-hill collected at a former arsenic roasting facility were determined by inductively coupled plasma mass spectrometry (ICP–MS) after microwave digestion with nitric acid and hydrogen peroxide. The ants contained 12.6 ± 0.9 mg As/kg dry mass, the ant-hill material 5420 ± 90 mg As/kg dry mass. Total arsenic concentrations in needles of *Picea abies* and *Larix decidua* (spruce and larch needles) were also determined, because needles are the main constituents of the upper layer of ant-hill material. Needles of *Picea abies* contained 1.17 mg As/kg dry mass and needles of *Larix decidua* 3.71 mg As/kg. The *Formica* sp. and ant-hill material were extracted with water or methanol/water (9:1). The extracts were chromatographed on a cation-exchange and an anion-exchange column. Water extracted 20% of the arsenic from the ants and only 3% from the ant-hill material. With methanol/water (9:1) only 7% of the arsenic was released by the ants and 0.5% by the ant-hill material. The arsenic compounds in the column effluents that were introduced into the plasma via a hydraulic high-pressure nebulizer (HHPN) were quantified on-line by ICP–MS. Arsenite and arsenate were the major arsenic compounds in the extract. Dimethylarsinic acid and traces of methylarsonic acid and arsenobetaine were also detected. The extracts of the ant-hill material contained the same compounds. Additionally, traces of trimethylarsine oxide were found. The presence of arsenobetaine was confirmed by spiking an extract of the ants with synthetic arsenobetaine bromide.** 

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## INTRODUCTION

Whereas arsenic compounds in the marine environment have been investigated in detail,[1,2] comparatively little is known about arsenic compounds in terrestrial ecosystems. The arsenic metabolism in terrestrial animals has been studied in rats,[3–5] mice,[3,4,6,7,20] rabbits,[3,6–10] monkeys,[11,12] and hamsters.[13–20] The animals were dosed with inorganic arsenic,[4–12,14,17] methylarsonic acid (MA),[18] dimethylarsinic acid (DMA),[7,13] arsenobetaine (AB),[15] trimethylarsine,[20] trimethylarsine oxide (TMAO),[19] arsenocholine (AC)[3] or gallium arsenide.[16] Inorganic arsenic is reported to be metabolized to MA[4,5,8,10,14] and DMA[4–10,14,17] by all of these animals except the monkeys, in which no methylation was observed.[11,12] MA is partially metabolized to DMA by hamsters,[18] and a small amount of incorporated DMA is converted to an unidentified trimethylated arsenic compound.[13] Hamsters do not metabolize arsenobetaine.[15] Arsenocholine is partially converted to arsenobetaine by mice, rats and rabbits and also stored in the form of arsenic-containing phospholipids.[3] The metabolites of gallium arsenide detected in hamsters were inorganic arsenic, DMA and MA.[16] Trimethylarsine is oxidized to TMAO in mice and hamsters.[20] When TMAO is administered, part of the compound is excreted unchanged in the urine and the other part is reduced to trimethylarsine that is expelled with

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the expired air.[19] No data on arsenic compounds in animals other than mammals are available.

Arsenic compounds in mushrooms collected at a former arsenic smelter site in the Poellatal, Carinthia, Austria, have been previously identified by HPLC–HHPN–ICP–MS.[21,22] These organisms contained a variety of arsenic compounds. In addition to arsenite, arsenate, MA and DMA, arsenobetaine, the tetramethylarsonium cation and even arsenocholine, a compound only detected previously in marine organisms, were found in the mushrooms.[21,22] Several unknown arsenic compounds were also detected. These results indicate that terrestrial organisms metabolize arsenic just as marine organisms do. Whether terrestrial organisms other than mushrooms also contain arsenobetaine, arsenocholine, the tetramethylarsonium ion and other complex arsenic compounds is not known. Ants (*Formica* sp.) living in the area of the smelter site were analyzed for their total arsenic concentration and the arsenic compounds arsenite, arsenate, MA, DMA, AB, AC, TMAO and the tetramethylarsonium ion (TETRA) by ICP–MS and HPLC–HHPN–ICP–MS. Material from the ant-hill was also analyzed. The total arsenic concentrations of needles of *Picea abies* and *Larix decidua* (spruce and larch needles) were determined, because needles are the main constituent of the upper layer of an ant-hill.

## MATERIALS AND METHODS

### Instrumentation

The *Formica* sp. and needles of *Picea abies* and *Larix decidua* were freeze-dried in an Alpha 1–4 freeze-drying system (Christ, Osterode am Harz, Germany). The dry samples were pulverized in a Retsch ZM 1000 mill (Retsch, Haan, Germany) equipped with a titanium rotor and a 0.25-mm sieve. Digestions for total arsenic determinations were performed with an MLS-1200 Mega microwave system (MLS, Leutkirch, Germany). Total arsenic was determined with a VG PlasmaQuad 2 Turbo Plus inductively coupled argon-plasma mass spectrometer (ICP–MS; VG Elemental, Winsford, UK) equipped with a Meinhard concentric glass nebulizer type TR-30-A3.

The high-performance liquid chromatography system consisted of a Hewlett–Packard 1050 solvent delivery unit (Hewlett–Packard, Waldbronn, Germnany) and a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, USA) with a 100-$mm^3$ injection loop. The separations were performed on a Hamilton (Reno, USA) PRP-X100 anion-exchange column (25 cm × 4.1 mm i.d., 10-μm styrene–divinylbenzene particles with trimethylammonium exchange sites) and a Supercosil LC-SCX (Supelco, Bellefonte, USA) cation-exchange column (25 cm × 4.6 mm i.d., 5-μm silica-based particles with propylsulfonic acid exchange sites).

The outlet of the HPLC column was connected via 60 cm of 1/16-inch (1.6 mm) PEEK (polyether-ether-ketone) capillary tubing (0.25 mm i.d.) to a Hydraulic High Pressure Nebulizer (Knauer, Berlin, Germany). The VG PlasmaQuad 2 Turbo Plus ICP–MS served as arsenic-specific detector. The ion intensity at *m/z* 75 ($^{75}As$) was monitored using the 'time-resolved' analysis software© Version 1a (Fisons Scientific Equipment Division, Middlesex, UK). Additionally, the ion intensity at *m/z* 77 ($^{40}Ar^{37}Cl$, $^{77}Se$) was monitored to detect possible argon chloride ($^{40}Ar^{35}Cl$) interferences on *m/z* 75. Prior to each HPLC–ICP–MS run, the ion intensity at *m/z* 87 (Rb added to the mobile phases) was optimized at the rate meter of the instrument. Instrumental settings used throughout this work are summarized in Table 1. The chromatograms were exported and the peak areas were determined using software written in-house. The arsenic compounds were quantified with external calibration curves established with each of the eight compounds.

### Reagents, standards and mobile phases

All solutions were prepared with NANOpure (18.2 MΩ cm) water. Concentrated nitric acid (Merck, p.a.) was further purified in a quartz subboiling distillation unit. The preparation of standard solutions was described previously.[21] The mobile phase for the anion-exchange HPLC was prepared by dissolving 2.30 g $NH_4H_2PO_4$ to 1000 $cm^3$ and adjusting the pH of this solution to 6.0 by addition of a 25% aqueous $NH_3$ solution (Merck, p.a.), the mobile phase for the cation-exchange HPLC by dissolving 1.98 g pyridine to 1000 $cm^3$ and adjusting the pH to 2.5 by addition of formic acid (~98%, Fluka, puriss. p.a.). To all mobile phases 50 $mm^3$ of a solution containing 1000 mg Rb $dm^{-3}$ (RbCl in water) was added to achieve a concentration of 50 ng Rb $cm^{-3}$.

Calibration curves for the HPLC–ICP–MS

 

**Table 1.** Operating conditions for the HPLC–HHPN–ICP–MS

| | | |
|---|---|---|
| Hydraulic high-pressure nebulizer | | |
| Desolvation | Heating 150°C | |
| | Cooling 1.5 °C | |
| Nebulizer gas (argon) | 1.00 $dm^3\ min^{-1}$ | |
| Back pressure | ~200 bar | |
| Inductively coupled plasma mass spectrometer | | |
| Plasma | | |
| RF power | Forward | 1.4 kW |
| | Reflected | <1 W |
| Argon gas flows | | |
| Cooling gas | 13.5 $dm^3\ min^{-1}$ | |
| Auxiliary gas | 1.1 $dm^3\ min^{-1}$ | |
| Vacuum | | |
| Expansion | 2.4 mbar | |
| Intermediate | $<1.0\times10^{-4}$ mbar | |
| Analyzer | $5.4\times10^{-6}$ mbar | |
| Ion sampling | | |
| Sample cone | Nickel, orifice 1.00 mm diameter | |
| Skimmer cone | Nickel, orifice 0.75 mm diameter | |
| Measuring parameters | | |
| Monitored signal | $^{75}As$, $^{40}Ar\ ^{37}Cl$ or $^{77}Se$ | |
| Time/slice | 0.53 s | |
| Total analysis time | Column-dependent (380–430 s) | |

measurements were obtained by chromatographing aliquots (100 $mm^3$) of solutions of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid containing 5.00, 10.0 or 20.0 ng As/$cm^3$ on the Hamilton PRP-X100 anion-exchange column, and solutions of arsenobetaine, arsenocholine, tetramethylarsonium iodide and trimethylarsine oxide containing 1.00, 5.00, or 10.0 ng As/$cm^3$ on the Supercosil LC-SCX cation-exchange column.

## Collection and treatment of the samples

The *Formica* sp., material from the upper layer of the ant-hill and needles from *Picea abies* and *Larix decidua* were collected at an old arsenic smelter site in Austria, Poellatal, Carinthia. The *Formica* sp. was mechanically cleaned from soil and rinsed with tap-water. All samples were frozen at −20 °C for storage. Before analysis, the *Formica* sp. and needles of *Picea abies* and *Larix decidua* were freeze-dried for 24 h at −10 °C and for 24 h at 10 °C at 0.1 mbar. The material from the ant-hill was dried at 40 °C in an oven. The dry samples were pulverized in the mill, and the powder was stored over silica gel in a desiccator.

## Determination of total arsenic

Aliquots of the powders (~0.2 g) were weighed to 0.1 mg into Teflon® digestion vessels. Concentrated nitric acid (5.0 $cm^3$) and 30% hydrogen peroxide (0.50 $cm^3$, Merck, p.a.) were added to each vessel. The vessels were closed, secured in the rotor, and placed in the microwave oven. The samples were digested using the following digestion program: 2 min 250 W, 0.5 min 0 W, 5 min 300 W, 0.5 min 0 W, 5 min 450 W, 0.5 min 0 W, 5 min 600 W, 7 min 500 W, 2 min 0 W (ventilation). The digests were transferred quantitatively into 50-$cm^3$ volumetric flasks. An aliquot (0.250 $cm^3$) of a solution containing 10 μg Ga $cm^{-3}$ was added to each flask. The flasks were filled to the mark. Total arsenic concentrations were determined in these solutions by ICP–MS with an external calibration curve established with arsenate solutions. All concentrations of arsenic are reported on a dry-mass basis.

## Extraction of the arsenic compounds

Aliquots (~0.2 g) of the powders of the *Formica* sp. and the ant-hill material were weighed to 0.1 mg into 50-$cm^3$ polyethylene tubes. Methanol/water (9:1 v/v, 10 $cm^3$) or NANOpure water

 

($10\ cm^3$) was added. The tubes were shaken for 14 h. The mixtures were centrifuged at 2500 rpm. The water extracts were filtered and chromatographed on the HPLC–HHPN–ICP–MS system. The methanol/water extracts were transferred into round-bottomed flasks. The residues in the centrifuge tubes were washed three times with methanol/water (9:1 v/v, $10\ cm^3$ each), the mixtures were centrifuged, and the supernatants were again transferred into the respective round-bottomed flasks. The methanol was evaporated from the supernatants on a Rotavapor (Büchi, Switzerland) at room temperature under an aspirator vacuum. To each of the evaporation residues water was added to a total mass of 10.0 g. The redissolved extraction residues were centrifuged at 3000 rpm, the supernatants filtered through 0.22-μm Millex-GS cellulse ester filters (Millipore, Bedford, USA), and chromatographed on the HPLC–HHPN–ICP–MS system.

### Determination of arsenic compounds in the water extracts and redissolved residues from the methanol/water extracts

Aliquots ($100\ mm^3$) of the filtered water extracts and redissolved residues from the methanol/water extracts were chromatographed on the Hamilton PRP-X100 anion-exchange and on the Supelcosil LC-SCX cation-exchange column. The water extracts of the ant-hill material were diluted 1:100 prior to chromatography on the cation-exchange column and 1:10 prior to chromatography on the anion-exchange column. To ascertain the presence of arsenobetaine, aliquots ($0.500\ cm^3$) to be chromatographed on the Supelcosil LC-SCX column were spiked with $10.0\ mm^3$ of an arsenobetaine bromide solution containing 25 ng As $cm^{-3}$.

## RESULTS AND DISCUSSION

### Total arsenic concentration

The *Formica* sp. and material from the upper layer of the ant-hill (which mainly consists of needles, small pieces of brushwood and plants and resin) were collected from an area in which a facility for roasting arsenic ores had been in operation for about 500 years. This facility was closed about 100 years ago. Three ant-hills were located approximately 10 m from the outside walls of the room, in which the arsenic trioxide subliming from the hot ore had condensed. The *Formica* sp. was collected from all three ant-hills. The ant-hill material was taken from the biggest of the ant-hills. Needles of *Picea abies* were collected from trees close to the outside walls, and needles of *Larix dedicua* from trees growing on the dump of the roasting facility. The average arsenic concentration determined in the ant-hill sample after microwave digestion was $5420 \pm 90$ mg As $kg^{-1}$ (mean of four digests). The *Formica* sp. contained $12.6 \pm 0.9$ mg As $kg^{-1}$ (mean of four digests). In needles of *Picea abies* 1.17 mg As $kg^{-1}$ and in needles of *Larix decidua* 3.71 mg As $kg^{-1}$ were found (mean of two digests). Compared with the total arsenic concentrations in the *Formica* sp., in the needles and in humic soil ($\sim 700$ mg As $kg^{-1}$)[22] collected at the smelter site, the concentration in the ant-hill material is very high. Therefore, the high arsenic concentration cannot be caused by the needles, which are the main constituents of the ant-hill, or by the humic soil around the ant-hill. It is also unlikely that parts of other plants with high arsenic concentrations, that are not major constituents of the ant-hill, can cause such a high concentration.

### Arsenic compounds

The *Formica* sp. and ant-hill material were investigated using two extraction media and two chromatographic separations. On the Hamilton PRP-X100 anion-exchange column arsenate, MA and DMA can be quantified (Fig. 1a). Arsenite can only be determined in the absence of the cationic arsenic compounds AB, TMAO, AC and TETRA, which would elute with the solvent front on this column and whose signals would overlap the signal for arsenite. The retention behavior of the arsenic compounds on the Hamilton PRP-X100 was discussed previously.[21,23] On the Supelcosil LC-SCX cation-exchange column AB, TMAO, AC and TETRA can be separated (Fig. 1b). Arsenate elutes with the solvent front. The other anionic arsenic compounds leave the column after arsenate in the following elution order: arsenite, MA, DMA. The retention behavior of the eight arsenic compounds on this column was discussed in a previous publication.[22]

The *Formica* sp. and ant-hill material were

 

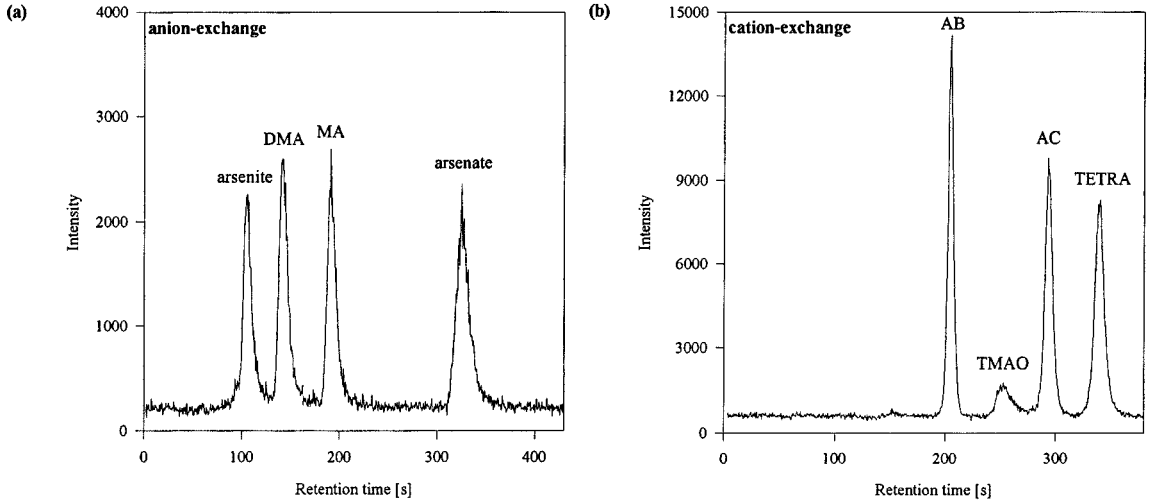


**Figure 1** Separation of arsenic compounds in standard solutions. (a) Chromatogram of a standard solution containing arsenate, arsenite, methylarsonic acid and dimethylarsinic acid (0.5 ng As each species) in distilled water on a PRP-X100 anion-exchange column (mobile phase 20 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). (b) Chromatogram of a standard solution containing arsenobetaine, trimethylarsine oxide, arsenocholine and tetramethylarsonium iodide (0.5 ng As each species) in distilled water on a Supelcosil LC-SCX cation-exchange column (mobile phase 25 mM pyridine at pH 2.5, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$).

extracted with water and with methanol/water (9:1). Samples with high concentrations of inorganic arsenic should be extracted more efficiently with water.[24] To extract inorganic and organic arsenic compounds as efficiently as possible, both extractions were performed. However, the percentages of arsenic extracted by these two solvents were rather low. With water only 20% of the total arsenic of the *Formica* sp. and only 3% of the total arsenic of the ant-hill material were extracted. With methanol/water the percentages were even lower (7% for the *Formica* sp., 0.5% for the ant-hill material). The arsenic compounds detected in the water extracts and in the redissolved residues from the methanol/water extracts are summarized in Table 2. In the water extracts of the *Formica* sp. (Fig. 2a) and ant-hill material (Fig. 3a), as well as in the redissolved residues from the methanol/water extracts of the ant-hill material (Fig. 3b), arsenate was the main arsenic compound. In these solutions arsenite, DMA and traces of MA were also detected by anion-exchange chromatography. In the redissolved residues from the methanol/water extracts of the *Formica* sp. (Fig. 2b), arsenate and arsenite were present in almost equal amounts. DMA was identified as a minor constituent and MA was present only in traces. In the redissolved residues from the methanol/water extracts of the ant-hill material, traces of TMAO and AB were also detected (Fig. 3d). These two compounds were below the detection limit in the water extract (Fig. 3c), which had to be diluted 100-fold before injection because of its high total arsenic concentration. In the water as well as in the redissolved residues from the methanol/water extracts of the *Formica* sp. (Fig. 2c, d), traces of AB but no TMAO were found. The presence of AB was successfully confirmed by spiking the sample with synthetic arsenobetaine bromide (Fig. 2d, dashed line). Water extracts arsenite and arsenate from the ant powder more efficiently than the methanol/water (9:1) mixture. No difference in extractability of arsenite from the ant-hill material exists between the two extractants. However, ten times more arsenate is extracted from the ant-hill material by water than by methanol/water (9:1). The extraction yield for DMA is almost the same with both extraction media (Table 2). The low total extraction yields achieved with both extraction media could indicate that arsenic in the *Formica* sp. and the ant-hill material is bound to the organic matrix and, therefore, it is not extractable with the mild extraction procedure employed. The way in which arsenic is linked to the organic matrix remains to be investigated.

In contrast to the mushrooms collected at the smelter site,[21,22] the *Formica* sp. contains only a few organic arsenic compounds. The concentra-

 

tion of these compounds is rather low. DMA and MA are well-known metabolites of inorganic arsenic in mammals. The *Formica* sp. consumes, as its main diet, insects and secretions of *Aphidina* sp. (aphids). Plant material is a minor constituent of the diet. Data on arsenic compounds in insects are not available. Consequently, the provenance of the methylated arsenic compounds in the *Formica* sp. cannot be established. The organic arsenic compounds could be taken up with the diet or could be the products of the biotransformation of inorganic arsenic by the *Formica* sp.

## CONCLUSIONS

The concentration of total arsenic in the ants at 12.6 mg $kg^{-1}$ is surprisingly low. The ants live in an extremely arsenic-rich environment. Perhaps the plants and the insects have developed a mechanism for the exclusion of arsenic compounds. Such protective mechanisms would serve the biota forced to live close to an arsenic smelter well. Concentrations of total arsenic of the same magnitude as those found in the ants are common in marine organisms. However, the marine organisms are exposed to an ambient

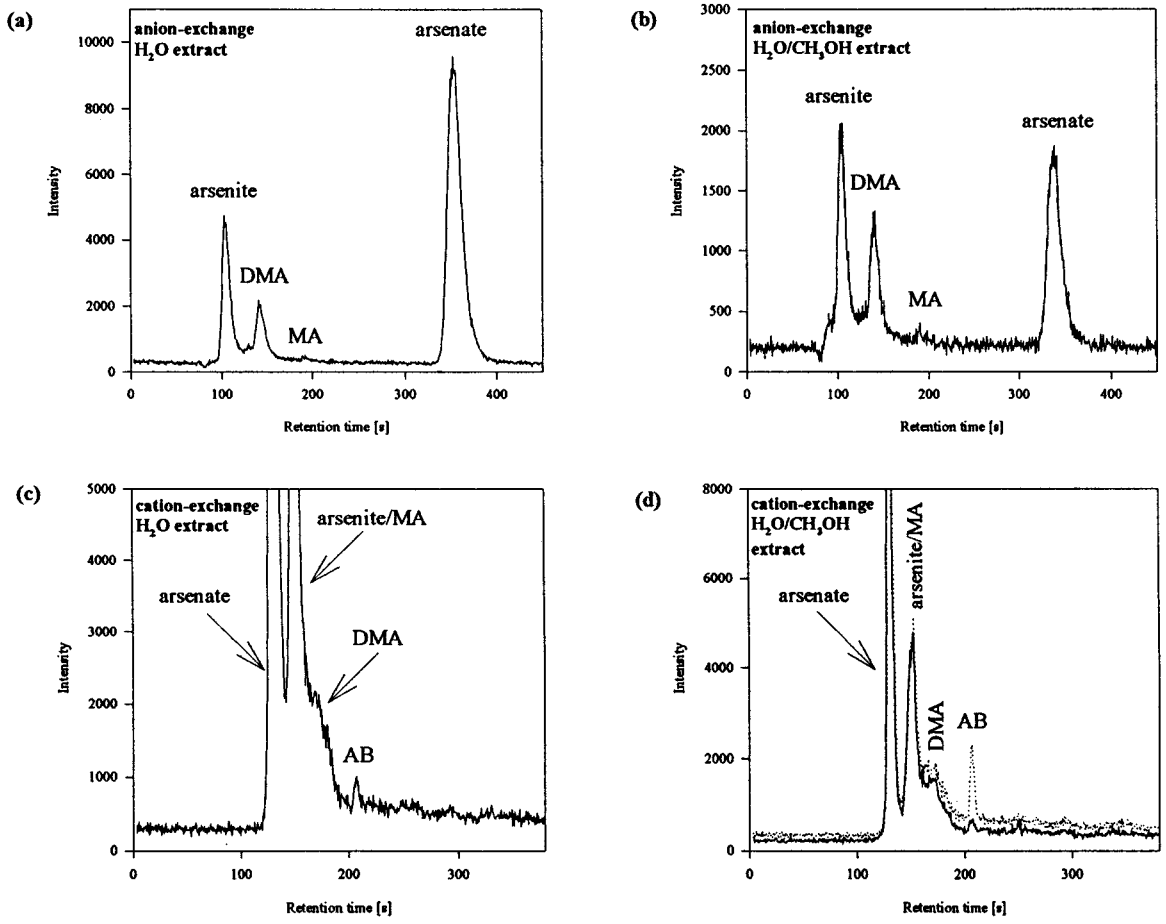


**Figure 2** Separation of arsenic compounds in the extracts from the *Formica* sp. (a) Chromatogram of the aqueous extract of the ant powder on a PRP-X100 anion-exchange column (mobile phase 20 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). (b) Chromatogram of the methanol/water (9:1) extract of the ant powder on a PRP-X100 anion-exchange column (mobile phase 20 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$. (c) Chromatogram of the aqueous extract of the ant powder on a Supelcosil LC-SCX cation-exchange column (mobile phase 25 mM pyridine at pH 2.5, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). (d) Chromatogram of the methanol/water (9:1) extract of the ant powder on a Supelcosil LC-SCX cation-exchange column (mobile phase 25 mM pyridine at pH 2.5, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). Dashed line: Same extract (100 $mm^3$) spiked with 0.49 ng As $cm^{-3}$ in the form of arsenobetaine bromide.

 

concentration in ocean water of only a few micrograms per liter and must rely on preferential uptake of arsenic from the water to achieve a thousand-fold accumulation of arsenic. A more detailed investigation of the biochemical necessities for accumulation of arsenic in a low-arsenic marine environment and for prevention of uptake in a high-arsenic terrestrial environment will provide new information about the biochemical processing of arsenic compounds and their functions in biota.

The concentration of total arsenic in the ant-hill material, at 5420 mg $kg^{-1}$, was surprisingly high. This concentration is at least five times higher than in the surrounding soil and 1000- to 5000-fold higher than in needles collected from trees growing close to the ant-hills. A likely explanation for the high concentration is a transfer of arsenic from the deeper region of the ant-hill to its surface.

Ant-hills of the *Formica* sp. consist of the hill above ground and a system of tunnels underground. Microorganisms which are present in soil are known for their ability to generate arsines.[1,25] Therefore, arsines could be formed from arsenic-containing material, which was

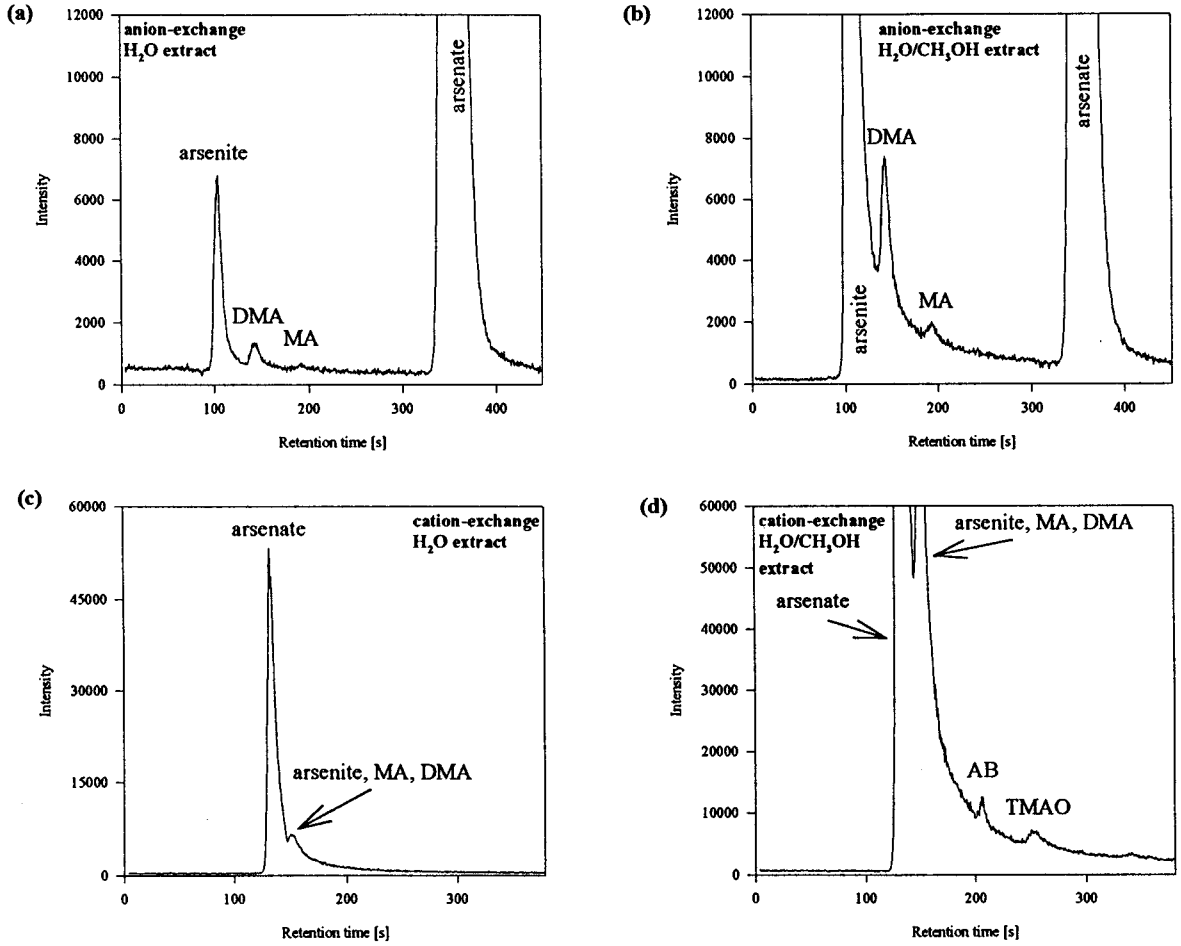


**Figure 3** Separation of arsenic compounds in the extracts from the ant-hill material. (a) Chromatogram of the aqueous extract of the ant-hill powder diluted 1:10 on a PRP-X100 anion-exchange column (mobile phase 20 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). (b) Chromatogram of the methanol/water (9:1) extract of the ant-hill powder on a PRP-X100 anion-exchange column (mobile phase 20 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). (c) Chromatogram of the aqueous extract of the ant-hill powder diluted 1:100 on a Supelcosil LC-SCX cation-exchange column (mobile phase 25 mM pyridine at pH 2.5, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$). (d) Chromatogram of the methanol/water (9:1) extract of the ant-hill powder on a Supelcosil LC-SCX cation-exchange column (mobile phase 25 mM pyridine at pH 2.5, injection volume 100 $mm^3$, flow rate 1.5 $cm^3\ min^{-1}$).

 

**Table 2.** Concentrations of arsenic compounds in extracts of ants and ant-hill material determined by HPLC–HHPN–ICP–MS

| Sample | Arsenic concentration (mg As/kg dry mass) Arsenite[a] | Arsenate[a] | MA[a] | DMA[a] | AB[b] | TMAO[b] | Sum of species | Total[c] |
|---|---|---|---|---|---|---|---|---|
| Ants | | | | | | | | |
| water extract | 0.69, 0.56 | 1.64, 1.50 | Trace | 0.30, 0.27 | Trace | <0.05 | 2.62, 2.33 | 12.6±0.9 |
| methanol/water 9:1 extract | 0.37, 0.33 | 0.32, 0.35 | Trace | 0.19, 0.21 | Trace | <0.05 | 0.88, 0.89 | |
| Ant-hill | | | | | | | | |
| water extract | 9.06, 7.29 | 161, 155 | Trace | 1.55, 1.32 | <0.50 | <5.00 | 172, 164 | 5420±90 |
| methanol/water 9:1 extract | 10.6, 10.7 | 16.7, 17.3 | Trace | 1.27, 1.32 | Trace | Trace | 28.6, 29.3 | |

[a] Determined with the Hamilton PRP-X100 anion-exchange column.
[b] Determined with the Supelcosil LC-SCX cation-exchange column.
[c] Mean of four digests.

transported by the *Formica* sp. into the tunnels of the ant-hill. When gases that may contain arsines at very low concentrations rising in the ant-hill come in contact with air in the outer layers of the hill, arsines may be oxidized and the oxidation products deposited on the ant-hill material such as needles and small twigs. If an ant-hill exists for several years, arsenic may accumulate in the upper layer of the hill and may react with functional groups in the organic compounds making up the plant material, and thus become difficult to extract. Only 3% of the total arsenic in the ant-hill material could be extracted with water. Almost all of the extractable arsenic was identified as inorganic arsenic. When the ant-hills become accessible again, new materials from the surface and the deeper layers will be collected. Attempts will be made to release the arsenic compounds with enzymatic methods and to determine the distribution of arsenic within the arsenic-rich needles with a laser microprobe mass analyzer.

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