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Arsenic Compounds in Terrestrial Organisms I: *Collybia maculata, Collybia butyracea* and *Amanita muscaria* from Arsenic Smelter Sites in Austria

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Three mushroom species from two old arsenic smelter sites in Austria were analyzed for arsenic compounds. The total arsenic concentrations were determined by ICP–MS. *Collybia maculata* contained 30.0 mg, *Collybia butyracea* 10.9 mg and *Amanita muscaria* 21.9 mg As kg^{-1} dry mass. The arsenic compounds extracted with methanol/water (9:1) from the dried mushroom powders were separated by HPLC on anion-exchange and reversed-phase columns and detected by ICP–MS using a hydraulic high-pressure nebulizer. In *Collybia maculata* almost all arsenic is present as arsenobetaine. *Collybia butyracea* contained mainly arsenobetaine (8.8 mg As kg^{-1} dry mass) and dimethylarsinic acid (1.9 mg As kg^{-1}). *Amanita muscaria* contained arsenobetaine (15.1 mg As kg^{-1}), traces of arsenite, dimethylarsinic acid and arsenate, and surprisingly arsenocholine (2.6 mg As kg^{-1}) and a tetramethylarsonium salt (0.8 mg As kg^{-1}). © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

The arsenic compounds present in the marine environment and their biotransformation have been investigated in detail,[1,2] but nothing is known about the more complex organic derivatives (arsenocholine, arsenoriboses, arsenic-containing lipids) in the terrestrial environment.[2] Simple methylated arsenic compounds (methylarsonic acid, dimethylarsinic acid) are synthesized from arsenite by terrestrial organisms, such as fungi,[2] bacteria,[2] mushrooms,[3] freshwater algae,[4] animals[5] and humans.[6] Fungi were observed to convert arsenite to volatile trimethylarsine.[7,8] Mushrooms are known to contain arsenic[3,9–12] and some accumulate arsenic to concentrations as high as 182 mg kg^{-1} dry mass.[9] Of the organic compounds identified in marine organisms, only methylarsonic acid,[13] dimethylarsinic acid[3] and recently arsenobetaine[13] were identified in extracts obtained from mushrooms. The availability of liquid-chromatographic methods for the separation of anionic and cationic arsenic compounds[14] and the very low detection limits achievable with a hydraulic high-pressure nebulizer–inductively coupled argon plasma mass spectrometer (HHPN–ICP–MS) as arsenic-specific detector[15] combine to make the search for arsenic compounds not yet identified in mushrooms possible and very probably successful.

Three mushroom species, collected at two old arsenic smelter sites in Austria with high concentrations of arsenic in the soils, were investigated with the aim of identifying all the arsenic compounds in these samples.

MATERIALS AND METHODS

Instrumentation

The mushrooms were freeze-dried in an Alpha 1–4 freeze-drying system (Christ, Osterode am Harz, Germany). The freeze-dried samples were pulverized in a Retsch ZM 1000 mill (Retsch, Haan, Germany) equipped with a titanium rotor

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and 0.25 mm sieve. Digestions for total arsenic determinations were performed in an MLS–1200 Mega microwave oven (MLS, Leutkirch, Germany).

Total arsenic was determined with a VG Plasma Quad 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, Germany) and a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, USA) equipped with a 100-mm^3 injection loop. The separations were performed on a Hamilton (Reno, USA) PRP-1 reversed-phase column (25 cm × 4.1 mm i.d., 10-μm styrene–divinylbenzene copolymer particles), a Hamilton PRP-X100 anion-exchange column (25 cm × 4.1 mm i.d., 10-μm styrene–divinylbenzene copolymer particles with trimethylammonium exchange sites), and on a Supelcosil LC-SAX (Supelco, Bellefonte, USA) anion-exchange column (25 cm × 4.6 mm i.d., 5-μm silica-based particles with quaternary aminopropyl exchange sites). The outlet of the HPLC column was connected via 60 cm of 1/16-in (1.6 mm) PEEK (polyether-ether-ketone) capillary tubing (0.25 mm i.d.) to a hydraulic high-pressure nebulizer (HHPN) (Knauer, Berlin, Germany). The VG Plasma Quad 2 Turbo Plus ICP–MS served as the 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 intensities at *m/z* 77 ($^{40}Ar^{37}Cl$, ^{77}Se) and *m/z* 82 (^{82}Se) were 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 (^{87}Rb) was optimized at the rate meter of the instrument. The chromatograms were exported and the peak areas were determined with external calibration curves using software written in-house.

Solutions for the determination of total arsenic and of arsenic compounds

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.

For the determination of the total arsenic concentrations by ICP–MS, 0.100 cm^3 of the standard arsenate solution ($Na_2HAsO_4 \cdot 7H_2O$ in water) with 100 μg As cm^{-3} was diluted to 1 μg As cm^{-3} in a 10-cm^3 volumetric flask. Aliquots (0.1, 0.5 or 1.0 cm^3) were pipetted (pipets: Brand, Wertheim, Germany) into 10-cm^3 volumetric flasks to obtain solutions with 10, 50 or 100 ng As cm^{-3}. Concentrated nitric acid (0.2 cm^3) and a solution (0.05 cm^3) with 10 μg Ga cm^{-3} were added before filling to the mark.

The mobile phases for anion-exchange HPLC were prepared by dissolving 3.45 g $NH_4H_2PO_4$ (Merck p.a.) to 1000 cm^3 (30 mmol dm^{-3}) and adjusting the pH to 6.0 by addition of 2 M NH_3 (Merck p.a.) or to 3.75 by addition of phosphoric acid (*d* 1.70, 15 M, Fluka puriss. p.a.). To 1000 cm^3 of the mobile phase at pH 3.75, 10 g methanol was added. The mobile phase for the ion-pairing separation was obtained by dissolving 2-hydroxy-5-sulfobenzoic acid dihydrate (Merck p.a., 2.54 g) to 1000 cm^3 (10 mmol dm^{-3}) and adjusting the pH to 3.5 by addition of 30% NaOH (Merck p.a.). Rubidium was added to all mobile phases to achieve a concentration of 50 ng cm^{-3}.

Standard solutions (1000 μg As cm^{-3}) for the identification and quantification of arsenic compounds were prepared by dissolving 433.0 mg $NaAsO_2$ (Merck p.a.) [As(III)], 1041 mg $Na_2HAsO_4 \cdot 7H_2O$ (Merck p.a.) [As(V)], 460.5 mg dimethylarsinic acid (Aldrich p.a.) (DMA), 466.6 mg methylarsonic acid (MA), 864.2 mg arsenobetaine bromide (AB), 817.5 mg arsenocholine bromide (AC), 453.9 mg trimethylarsine oxide (TMAO) or 874.2 mg tetramethylarsonium iodide (Tetra) (all synthesized in-house) to 250 cm^3. Calibration curves for the HPLC–ICP–MS measurements were obtained by injecting aliquots (0.100 cm^3) of solutions containing 5, 50 or 100 ng As cm^{-3} of arsenite, arsenate, methylarsonic acid and dimethylarsinic acid for the separation on the PRP-X100 anion-exchange column; of arsenite, arsenate, methylarsonic acid, dimethylarsinic acid and arsenobetaine for the separation on the Supelcosil LC-SAX anion-exchange column; and of arsenobetaine, arsenocholine, tetramethylarsonium iodide and trimethylarsine oxide for the separation on the PRP-1 reversed-phase column.

Mushrooms

The mushrooms *Amanita muscaria, Collybia maculata* and *Collybia butyracea* were collected at two old arsenic smelter sites in Austria. *Amanita muscaria* and *Collybia maculata* were from Poellatal, Carinthia, Austria. *Collybia butyracea* was found in Rotguelden, Salzburg, Austria. The collected mushrooms were mechanically cleaned of soil, rinsed with tap-water and frozen at −20 °C for storage. Before analysis the specimens were freeze-dried for 24 h at −10 °C and for 24 h at 10 °C at 0.1 mbar. The freeze-dried mushrooms were pulverized, and the powders were stored over silica gel in a desiccator.

Digestions for the determination of total arsenic

Aliquots (~0.2 g each) of the freeze-dried powders were weighed to 0.1 mg into Teflon digestion vessels. Concentrated nitric acid (3.0 cm^3) and 30% hydrogen peroxide (0.2 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 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 clear, colorless digests were transferred quantitatively into 50-cm^3 volumetric flasks. An aliquot (0.25 cm^3) of the 10 μg Ga cm^{-3} solution 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.

Extraction of the arsenic compounds from the mushrooms

Aliquots (~0.1 g each) of the freeze-dried mushroom powders were weighed to 0.1 mg into 50-cm^3 polyethylene tubes. A methanol/water mixture (9:1 v/v, 10 cm^3) was added to each tube. The tubes were shaken for 14 h. Then the mixtures were centrifuged at 2500 rpm and the supernatants 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), the mixtures centrifuged, and the supernatants again transferred into the 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 brownish-yellow, gummy evaporation residues water was added to a total mass of 10.0 g, the suspensions were centrifuged at 8000 rpm and the supernatants filtered through 0.2-μm cellulose nitrate filters (Sartorius, Göttingen, Germany). The filtrates were chromatographed.

RESULTS AND DISCUSSION

To facilitate the identification of arsenic compounds in terrestrial organisms, mushrooms from sites with high concentrations of arsenic in the soils were chosen. At the first site, located at approximately 1400 m altitude in Poellatal, northern Carinthia, Austria, arsenic trioxide had been produced by roasting sulfidic arsenic ores. The facility had operated for several hundred years but was closed about 100 years ago. The mushrooms had grown within a 50-m radius of the facility and on the pile of residues from the roasting operation. This pile is now covered with vegetation (grass, moss, small trees). The second site (Rotguelden, Austria) is a meadow underlain with debris of an arsenic-roasting facility. The soils at these sites are very rich in arsenic and may contain several percent of arsenic. The practically inexhaustible supply of arsenic in these soils should force the mushrooms to reveal their abilities to metabolize inorganic arsenic compounds. In addition, mushrooms growing at these sites were expected to accumulate arsenic in their tissues to concentrations that would allow the identification of arsenic compounds even when present as minor constituents.

Total arsenic concentrations in ***Collybia butyracea, Collybia maculata*** **and** ***Amanita muscaria***

Total arsenic was determined after microwave-assisted digestion of the freeze-dried and powdered mushrooms by ICP–MS. The three mushroom samples had the following average ($n = 4$) total arsenic concentrations based on dry mass:

Collybia maculata	30.0 ± 0.8 mg kg^{-1}
Collybia butyracea	10.9 ± 0.8 mg kg^{-1}
Amanita muscaria	21.9 ± 0.9 mg kg^{-1}

The concentrations are not high, considering the arsenic-richness of the soils, on which the mushrooms grew. These mushrooms are not good arsenic accumulators in comparison with Laccaria species[9,16] which were found to possess arsenic concentrations as high as 182 mg kg^{-1} although they grew on soils with only 3–27 mg As kg^{-1}. However, these concentrations of arsenic in the *Collybia* and *Amanita* species were sufficient for the easy identification of arsenic compounds by HPLC–ICP–MS.

Chromatographic separation of arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, arsenocholine, arsenobetaine, trimethylarsine oxide and tetramethylarsonium iodide

The separation of all eight arsenic compounds, which may be cationic, anionic or uncharged depending on the pH of the mobile phase, is not possible on a cation-exchange, anion-exchange or reversed-phase column. On an anion-exchange column the cationic and uncharged compounds leave the column largely unseparated from, or close to, the solvent front. The anionic and uncharged compounds suffer a similar fate on a cation-exchange column. On a reversed-phase column only species with a charge opposite to the charge of the ion-pairing reagent will be separated. Consequently, a solution containing several arsenic compounds must be chromatographed on more than one column for the identification of all arsenic compounds. The mobile phases containing sodium phosphate, found useful for the separation of 'anionic' arsenic compounds on anion-exchange columns with flame atomic absorption spectrometers as arsenic-specific detector,[17] cannot be employed when an ICP–MS is used as detector. Sodium phosphate that rapidly clogs the orifice of the sampling cone must be replaced by ammonium phosphate.

On the Hamilton PRP-X100 anion-exchange column arsenite, dimethylarsinic acid, methylarsonic acid and arsenate were baseline-separated within five minutes with an aqueous, 30 mM ammonium phosphate buffer at pH 6.0 (Fig. 1). The retention times for the arsenic compounds increase with their apparent negative charge (with decreasing pK_a values). At pH 6.0 arsenite (H_3AsO_3) is uncharged[17] and arsenate is present as an almost equimolar mixture of $H_2AsO_4^-$ and $HAsO_4^{2-}$. Arsenobetaine has under these conditions almost the same retention time as arsenite. Tetramethylarsonium iodide and arsenocholine, compounds that are cationic irrespective of pH, leave the column with the solvent front.

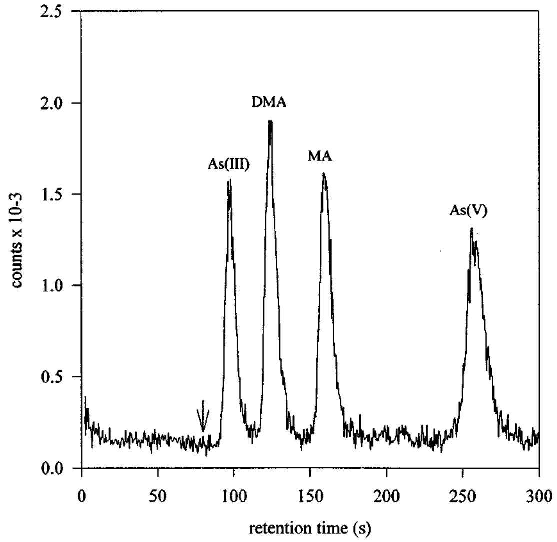

Figure 1 Chromatogram obtained with a solution of arsenite [As(III)], dimethylarsinic acid (DMA), methylarsonic acid (MA), and arsenate [As(V)] (0.5 ng As from each species) in distilled water on a Hamilton PRP-X100 anion-exchange column (mobile phase 30 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 0.100 cm^3, flow rate 1.5 cm^3 min^{-1}).

On the Supelcosil LC-SAX column the 30 mM ammonium phosphate mobile phase at pH 3.75 containing 1% methanol separates arsenite, dimethylarsinic acid, methylarsonic acid, arsenate (in the same retention sequence as with the PRP-X100 column), and arsenobetaine with a retention time (~200 s) between dimethylarsinic acid (~170 s) and methylarsonic acid (~260 s) (Fig. 2). The cationic species tetramethylarsonium iodide and arsenocholine appear with the solvent front (~110 s). At pH 3.75 of the mobile phase, anion-exchange processes can be responsible for the retention of only methylarsonic acid (pK_1=3.6; pK_2=8.2), and arsenate (pK_1=2.3) and not for the retention of arsenite (pK=9.2), dimethylarsinic acid (pK=6.2) and arsenobetaine (pK=2.2). Arsenite and dimethylarsinic acid are present as uncharged molecules and arsenobetaine largely as zwitterion.[14] The separation of these uncharged species must be effected by their hydrophobic interactions with the aminopropyl groups on the stationary phase.

The cationic compounds (arsenobetaine,

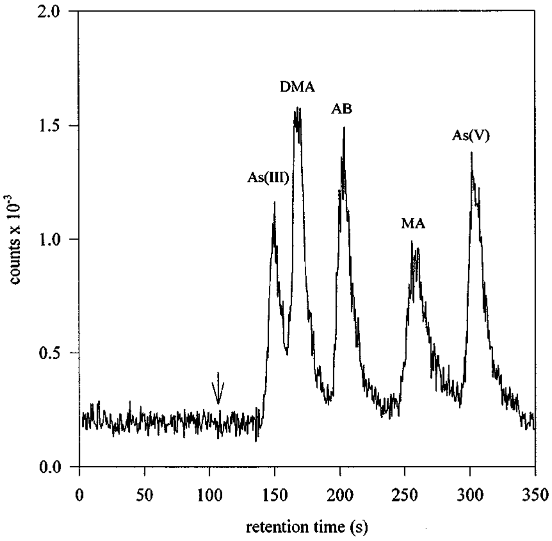

Figure 2 Chromatogram obtained with a solution of arsenite [As(III)], dimethylarsinic acid (DMA), arsenobetaine (AB), methylarsonic acid (MA), and arsenate [As(V)] (0.5 ng As from each species) in distilled water on a Supelcosil LC-SAX anion-exchange column (mobile phase 30 mM $NH_4H_2PO_4$ with 1% methanol at pH 3.75, injection volume 0.100 cm^3, flow rate 1.5 cm^3 min^{-1}).

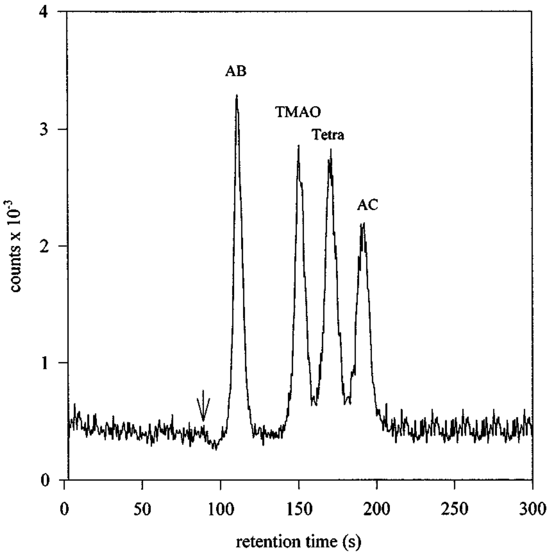

Figure 3 Chromatogram obtained with a solution of arsenobetaine (AB), arsenocholine (AC), trimethylarsine oxide (TMAO) and tetramethylarsonium iodide (Tetra) (0.5 ng As from each species) in distilled water on a Hamilton PRP-1 reversed-phase column (mobile phase 10 mM 2-hydroxy-5-sulfobenzoic acid at pH 3.5, injection volume 0.100 cm^3, flow rate 1.5 cm^3 min^{-1}).

arsenocholine, tetramethylarsonium iodide) and the potentially cationic species trimethylarsine oxide[14] ($pK = 3.6$) were separated on a Hamilton PRP-1 reversed-phase column with a 10 mM solution of the ion-pairing reagent 2-hydroxy-5-sulfobenzoic acid (Fig. 3). The pH of 3.5 for the mobile phase is required to make trimethylarsine oxide cationic.[14] Arsenite, arsenate, methylarsonic acid and dimethylarsinic acid, species that are uncharged or negatively charged under these conditions, do not interact with the ion-pairing reagent, are not retained, and consequently leave the column with the solvent front.

The ‘method determination limit’ for the

Table 1. Concentrations of arsenic compounds (mg As kg^{-1} dry mass) from two extractions in the three mushroom species determined by HPLC–ICP–MS

Mushroom species	Arsenite[a]	DMA[a]	AB[a]	MA[b]	Arsenate[a]	AC/Tetra[a]	Tetra[c]	AC[c]	Unidentified	Total extract[d]	Total digest
Collybia	0.06	0.2	43.8	<0.05	<0.05	0.2[e]	n.d.[f]	n.d.		44.3	30.0
maculata	0.04	0.2	42.2			0.2[e]				42.6	
Collybia	<0.05	2.1	9.7	<0.05	0.2	0.1[e]	n.d.	n.d.	0.2[a,e]	12.3	10.9
butyracea		1.7	7.8		0.2	0.1[e]			0.2[a,e]	10.0	
Amanita	0.3	0.4	14.0	<0.05	0.2	6.1[e]	0.8	2.5	0.4[c,g]	18.6	21.9
muscaria	0.4	0.7	16.2		0.3	6.2[e]	0.7	2.7	0.4[c,g]	21.4	

[a] Determined on the Supelcosil LC-SAX anion-exchange column.
[b] Determined on the Hamilton PRP-X100 anion-exchange column.
[c] Determined on the Hamilton PRP-1 reversed-phase column.
[d] Sum of species, difference to total in digest is caused by matrix.
[e] Quantified with DMA calibration curve.
[f] n.d., not determined.
[g] Quantified with AC calibration curve.

quantification of each of the eight arsenic compounds using the chromatographic signals is approximately 0.05 ng As, corresponding to a concentration of 0.5 μg As dm^{-3} (6.7×10^{-9} M) when 0.100 cm^3 of solution are injected.[15] The 'method determination limit' is defined as the concentration of arsenic, from a particular arsenic compound, at which five replicate injections produce an average result with a 20% relative standard deviation. Because approximately 100 mg of dried mushroom powder were extracted producing 10 cm^3 of extract, from which 0.100 cm^3 was chromatographed, the 'method determination limit based on dry mushroom powder' is 50 μg As kg^{-1}.

The separations of the arsenic compounds on the anion-exchange and reversed-phase columns, within the short time of approximately 6 min, will be successful only when the arsenic compounds producing neighboring signals in the chromatogram are present in the solutions at approximately equal concentrations. For example, if arsenobetaine were the major and methylarsonic acid the minor component in a solution to be chromatographed on a Supelcosil LC-SAX column, the small signal for methylarsonic acid would very probably be an unquantifiable shoulder on the large arsenobetaine signal or it might completely disappear under the arsenobetaine signal. Consequently, all extracts were checked for methylarsonic acid on the Hamilton PRP-X100 anion-exchange column, from which arsenobetaine elutes almost with the solvent front and therefore does not overlap with the signal for methylarsonic acid.

Arsenic compounds in ***Collybia maculata, Collybia butyracea*** **and** ***Amanita muscaria***

The arsenic compounds can be extracted from the mushrooms almost quantitatively with the methanol/water (9:1) mixture. The results of the HPLC–ICP–MS determinations of the arsenic compounds in these extracts are summarized in Table 1. All three mushroom species contain mainly organic arsenic compounds (*Collybia maculata* 99.9%, *Collybia butyracea* 98.2%, *Amanita muscaria* 97.0%). *Collybia maculata* and *Collybia butyracea* contain the same arsenic compounds. The concentration of dimethylarsinic acid is much higher in *C. butyracea* than in *C. maculata* (Figs 4a and 4b). The Supelcosil LC-SAX chromatogram of *Amanita muscaria* shows two major signals (Fig. 4c). At the solvent front a very sharp signal is present with a shape typical of arsenocholine.[18] The second major signal attributable to arsenobetaine has a shoulder on the low-retention-time side. The arsenic

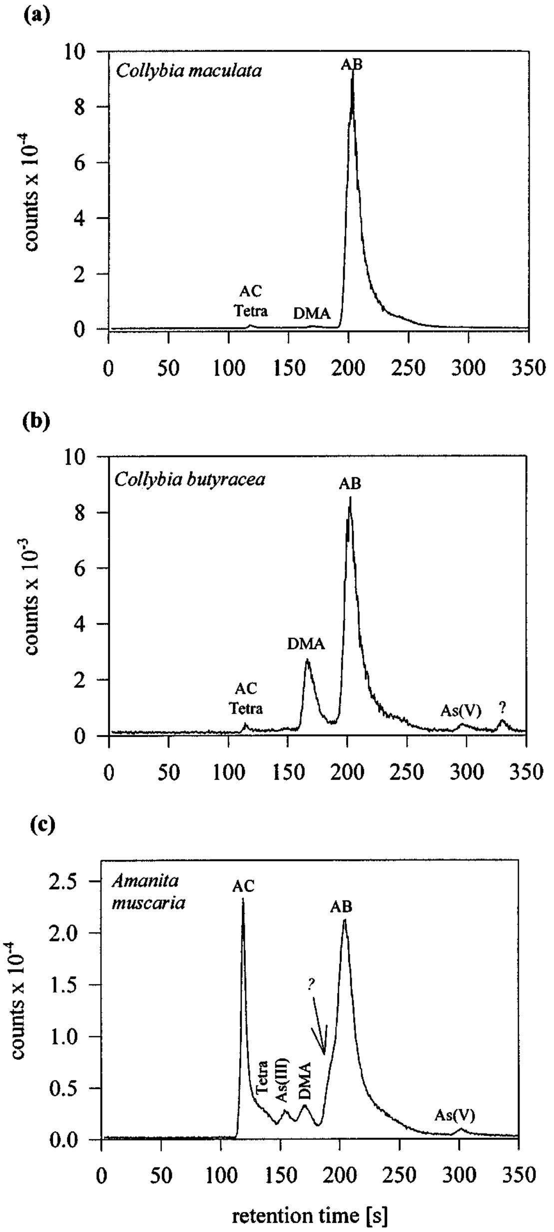

Figure 4 Chromatograms obtained on a Supelcosil LC-SAX anion-exchange column (mobile phase 30 mM $NH_4H_2PO_4$ with 1% methanol at pH 3.75, injection volume 0.100 cm^3, flow rate 1.5 cm^{3}min^{-1}). For abbreviations see Figs 1–3. (a) Extract of *Collybia maculata;* (b) extract of *Collybia butyracea*; (c) extract of *Amanita muscaria.*

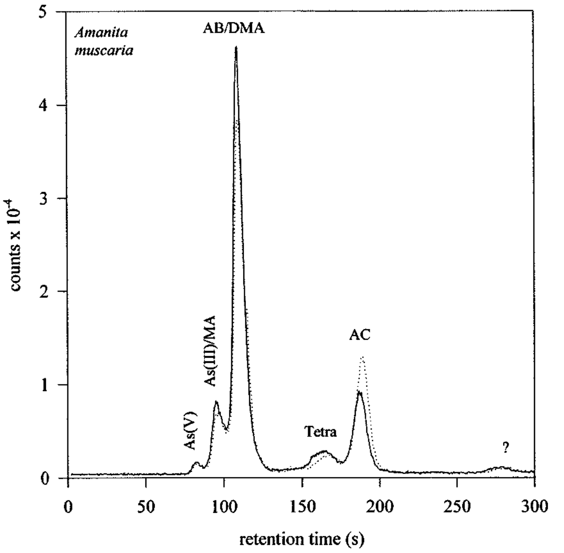

Figure 5 Chromatograms obtained on a Hamilton PRP-1 reversed-phase column (mobile phase 10 mM 2-hydroxy-5-sulfobenzoic acid at pH 3.5, injection volume 0.100 cm^3, flow rate 1.5 cm^3 min^{-1}). For abbreviations see Figs 1–3. [—], extract of *Amanita muscaria*; . . . , same extract spiked with 2.4 ng As in form of arsenocholine.

compound responsible for this shoulder could not be identified. To confirm the surprising presence of arsenocholine, the extract was chromatographed on the PRP-1 reversed-phase column. Signals with retention times characteristic of arsenocholine and the tetramethylarsonium cation are present in the chromatogram (Fig. 5), as verified by the coincidence of retention times with standards and by chromatography of extracts spiked with arsenocholine bromide (the dotted line in Fig. 5). An unidentified arsenic species, that might have caused the shoulder on the arsenobetaine peak in the Supelcosil LC-SAX chromatogram, is responsible for the broad signal with a retention time of ~280 s.

Our results indicate that all three mushrooms take up arsenic from the soil. The presence of arsenobetaine as the major arsenic compound in the mushrooms is not unexpected. However, the presence of arsenocholine in *Amanita muscaria* is surprising. This arsenic compound had been detected previously only in very few marine samples.[19–28] The tetramethylarsonium ion apparently present in *Amanita muscaria* had also been previously identified in marine samples.[25,26,29–31] Whether the biotransformation of these organic arsenic compounds occurs in the mushrooms themselves or is carried out by microorganisms cannot be deduced from our work.

Arsenic cycling in the marine environment has been investigated in detail.[2] The presence of arsenocholine and arsenobetaine, a prominent arsenic compound in the marine arsenic cycle, in the terrestrial environment strongly indicates that the terrestrial arsenic cycle is similar to the marine arsenic cycle.

REFERENCES

1. K. A. Francesconi and J. S. Edmonds, *Oceanogr. Mar. Biol. Annu. Rev.* **31**, 111 (1993).
2. W. R. Cullen and K. J. Reimer, *Chem. Rev.* **89**, 713 (1989).
3. A. R. Byrne, M. Tusek-Znidaric, B. K. Puri and K. J. Irgolic, *Appl. Organomet. Chem.* **5**, 25 (1991).
4. S. Maeda, Biotransformation of arsenic in the freshwater environment. In: *Arsenic in the Environment, Part I: Cycling and Characterization,* Nriagu, J. O. (ed.), John Wiley, New York, 1994, pp. 155–187.
5. M. Vahter and E. Marafante, Metabolism of alkyl arsenic and antimony compounds. In: *Metal Ions in Biological Systems,* Vol. 29, Sigel, H. and Sigel, A. (eds), Marcell Dekker, New York, 1993, pp. 161–184.
6. J. P. Buchet, R. Lauwerys and H. Roels, *Int. Arch. Occup. Environ. Health* **48**, 71 (1981).
7. F. Challenger, C. Higginbottom and L. Ellis, *J. Chem. Soc.* 95 (1933).
8. M. Hiroki and Y. Yoshiwara, *Soil Sci. Plant Nutr.* **39**, 237 (1993).
9. A. R. Byrne and M. Tusek-Znidaric, *Chemosphere* **12**, 1113 (1983).
10. T. Stijve, E. C. Vellinga and A. Herrmann, *Persoonia* **14**, 161 (1990).
11. T. Stijve and B. Bourqui, *Deutsche Lebensm. Rundschau* **87**, 307 (1991).
12. J. Vetter, *Z. Lebensm. Unters. Forsch.* **189**, 346 (1989).
13. A. R. Byrne, Z. Slejkovec, T. Stijve, L. Fay, W. Gössler, J. Gailer and K. J. Irgolic, *Appl. Organomet. Chem.* **9**, 305 (1995).
14. J. Gailer and K. J. Irgolic, *J. Chromatogr. A* **730**, 219 (1996).
15. W. Goessler, D. Kuehnelt and K. J. Irgolic, Determination of arsenic compounds in human urine by HPLC-ICP-MS. In: *Arsenic: Exposure and Health Effects*, Chapter 3, Abernathy, C. E. (ed.), Chapman & Hall, London, 1997, in press.
16. M. Slekovec-Golob and K. J. Irgolic, *Chem. Spec. Bioavail.*, in press.
17. J. Gailer and K. J. Irgolic, *Appl. Organomet. Chem.* **8**, 129 (1994).
18. R. Rubio, I. Peralta, J. Alberti and G. Rauret, *J. Liq.*

Chromatogr. **16**, 3531 (1993).

19. H. Norin, R. Ryhage, A. Christakopoulos and M. Sandström, *Chemosphere* **12**, 299 (1983).
20. J. F. Lawrence, P. Michalik, G. Tam and H. B. S. Conacher, *J. Agric. Food Chem.* **34**, 315 (1986).
21. H. Norin, A. Christakopoulos, L. Rondahl, A. Hagman and S. Jacobsson, *Biomed. Environ. Mass Spectrom.* **14**, 117 (1987).
22. B. P. Y. Lau, P. Michalik, C. J. Porter and S. Krolik, *Biomed. Environ. Mass Spectrom.* **14**, 723 (1987).
23. D. Beauchemin, M. E. Bednas, S. S. Berman, J. W. McLaren, K. W. M. Siu and R. E. Sturgeon, *Anal. Chem.* **60**, 2209 (1988).
24. A. Christakopoulos, B. Hamasur, H. Norin and I. Nordgren, *Biomed. Environ. Mass Spectrom.* **15**, 67 (1988).
25. E. H. Larsen, G. Pritzl and S. H. Hansen, *J. Anal. At. Specrom.* **8**, 1075 (1993).
26. E. H. Larsen and S. Stürup, *J. Anal. At. Spectrom.* **9**, 1099 (1994).
27. S. Caroli, F. LaTorre, F. Petrucci and N. Violante, *Environ. Sci. Pollut. Res.* **1**, 205 (1994).
28. J. S. Edmonds, Y. Shibata, R. I. T. Prince, K. A. Francesconi and M. Morita, *J. Mar. Biol. Ass. U.K.* **74**, 463 (1994).
29. M. Morita and Y. Shibata, *Anal. Sci.* **3**, 575 (1987).
30. K. Shiomi, Y. Kakehashi, H. Yamanaka and T. Kikuchi, *Appl. Organomet. Chem.* **1**, 177 (1987).
31. Y. Shibata and M. Morita, *Anal. Chem.* **61**, 2116 (1989).