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Arsenic Species in Terrestrial Fungi and Lichens from Yellowknife, NWT, Canada

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Levels of total arsenic and arsenic species were determined in fungi collected from Yellowknife, NWT, Canada, an area that has been affected by past mining activities and elevated arsenic levels. Lichens (belonging to *Cladonia* and *Cladina* genera), as well as the mushrooms *Coprinus comatus, Paxillus involutus, Psathyrella candolleana* and *Leccinum scabrum*, were studied for the first time. Most of the fungi contained elevated arsenic levels with respect to data found in the literature for background levels. Minor amounts of arsenobetaine were found in all lichen samples. The major water-soluble arsenic species in the fungi were inorganic arsenic for lichens and *Psathyrella candolleana*, arsenobetaine for *Lycoperdon pyriforme* and *Coprinus comatus*, and dimethylarsenate for *Paxillus involutus* and *Leccinum scabrum*. A large proportion of water-soluble arsenic in *Paxillus involutus* occurred as an unknown compound, which did not co-chromatograph with any of the available standard arsenic compounds. Low proportions of water-soluble arsenic species (made evident by low extraction efficiencies) were observed in the majority of fungi studied. Arsenic that is not extracted may be bound to lipids, cell components or proteins, or might exist on the surface of the fungus as minerals.

Keywords: arsenic; mushrooms; lichens; speciation; arsenobetaine; mining

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INTRODUCTION

Yellowknife is located on Great Slave Lake, in the Northwest Territories, Canada. A major industry in the city is gold mining, with two gold mines having been in recent operation. The gold in the mined ore is associated with arsenopyrite (FeAsS), and hence arsenic waste is generated during the smelting operation. This has led to increased amounts of arsenic in the Yellowknife environment, associated with aerial emissions, tailings runoff and effluent discharge.[1,2]

Arsenic is associated with adverse effects, but its toxicity is dependent on the chemical form, or species, that it takes. For example, arsenobetaine [$(CH_3)_3As^+CH_2COO^-$] is found in marine animals and mushrooms,[3] and is much less toxic than arsenous acid [$As(OH)_3$] or arsenite. For this reason, the determination of the total amount of arsenic in a sample is not sufficient to assess environmental risk, and speciation analysis is necessary in order to determine the form of arsenic in the sample.

Interest in the arsenic content in mushrooms has increased in the last decade. Some species of mushrooms appear to accumulate arsenic and other metals from soil,[4] and their potential as biological pollution indicators has been discussed.[5] For mushrooms that accumulate arsenic and are edible, such as *Laccaria amethystina*, toxicological consequences (if any) to consumers have been of concern.[6] For these reasons, the uptake and speciation of arsenic in mushrooms has been studied. Determining the species of arsenic in mushrooms, especially those containing elevated levels, helps in toxicological risk assessment.

Chemical processes taking place in the terrestrial environment have not been studied to as great an extent as those in the marine environment. The recent findings of arsenobetaine and arsenocholine in mushrooms have led researchers to draw

similarities between marine and terrestrial pathways for the formation of arsenic compounds.[7–9] The presence of arsenobetaine in mushrooms in higher taxonomic positions (i.e. more highly evolved)[10] is similar to that of arsenobetaine in higher marine organisms, such as marine animals.[11]

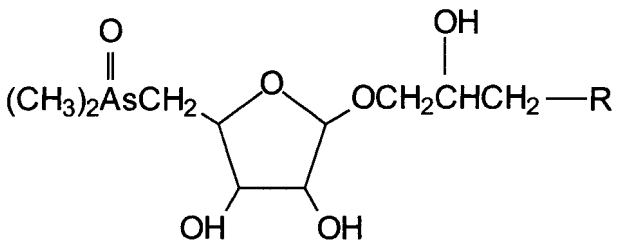
It has been proposed that the fungi producing the mushrooms are responsible for the biosynthesis of more complex arsenic forms, such as arsenobetaine.[10] This theory is favored for two reasons. One is that arsenobetaine has not been found in soil.[4] Arsenobetaine was, however, identified in ant-hill material,[12] and its presence in estuarine waters was recently confirmed.[13] The second reason is that similar chemical forms of arsenic have been seen in mushroom species collected from different locations.[6] In support of the fungus biosynthesis theory, the fungi *Agaricus placomyces* and *Pleurotus* sp. (producing edible mushrooms) methylate arsenic to a small extent.[14]

This paper summarizes new data about arsenic species in lichens, which have not yet been studied, as well as some mushroom species for which arsenic speciation has not yet been determined. The elevated levels of arsenic in the Yellowknife environment make it an ideal study area for arsenic speciation in fungi.

EXPERIMENTAL

Chemicals and reagents

Arsenic standards were obtained as sodium arsenate, $Na_2HAsO_4{\cdot}7H_2O$ (Aldrich), arsenic trioxide, As_2O_3 (Alfa), monomethylarsonic acid, $CH_3AsO(OH)_2$ (Vineland Chemical) and cacodylic acid, $(CH_3)_2AsO(OH)$ (BDH) from commercial sources as indicated. They were dissolved in deionized water to make standard solutions of arsenate [As(V)], arsenite [As (III)], monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Arsenic-containing ribofuranosides (arsenosugars) were previously characterized in extracts of kelp powder (Galloway's, Vancouver, BC) and Nori (*Porphyra tenera*).[15] These extracts were used to identify the retention times of arsenosugars, which were verified by comparing them with retention times obtained from pure arsenosugars (donated by K. Francesconi and T. Kaise). The structures of the arsenosugars used in this work and their abbreviations are shown in Fig. 1. The numbering system used (**X–XIII**) follows that of Shibata *et al.*[16] Arsenobetaine $[(CH_3)_3As^+CH_2COO^-]$,[17] arsenocholine $[(CH_3)_3As^+CH_2CH_2OH]$,[18] trimethylarsine oxide $[(CH_3)_3AsO]$[19] and



Arsenosugar abbreviation	**R**
X	-OH
XI	$-OPO_3HCH_2CH(OH)CH_2OH$
XII	$-SO_3H$
XIII	$-OSO_3H$

Figure 1 Structures and abbreviations of arsenosugars.

tetramethylarsonium iodide $[(CH_3)_4As^+][I^-]$,[20] with the abbreviations AB, AC, TMAO and Me_4As^+, respectively, had been synthesized previously according to standard methods. Other reagents of analytical grade or better were obtained from commercial sources.

Study area and sampling methods

Since the major anthropogenic source of arsenic in Yellowknife is gold-mining waste, fungi were sampled near and on mine properties. Two mines were operating in Yellowknife at the time of the study: the Royal Oak Giant Mine, located north of the city, and the Miramar Con Mine, south of the city. Samples were collected during two one-week sampling trips, in June and August 1997. A lichen sample (Lichen 4, similar in appearance to frog pelt lichen *Peltigera neopolydactyla*, but not positively identified by species or genus), puffball *Lycoperdon pyriforme* and shaggy mane mushroom *Coprinus comatus* were collected from Giant Mine tailings ponds. The other samples were collected from Con Mine property: pixie cup lichen (*Cladonia* sp., PC1 and PC2), *Paxillus involutus, Psathyrella candolleana* and *Leccinum scabrum* from outflow areas west and south of the tailings ponds; pixie cup lichen (*Cladonia* sp., PC3) and *Lycoperdon pyriforme* from a tailings pond; and lichens that were growing together (Lichen 1, *Cladina* sp.; Lichens 2 and 3, *Cladonia* sp.) in a wooded area adjacent to the tailings ponds. All *Cladonia* sp. samples appeared to be different species; hence they were given different code names (PC1, Lichen 1, etc.).

The samples were picked by hand, stored in Ziploc® bags and kept cool until processing in the lab. There, they were washed thoroughly with tap water to remove soil and other particles, rinsed with deionized (18 MΩ) water, and frozen. Identification was carried out by examination of fresh and dry samples, as well as photographs, for distinguishing characteristics and by using field guide books.[21–23] Assistance was obtained from Olivia Lee (Botany Department, UBC) and James Black (Vancouver Mycological Society).

Sample preparation and analysis

Whole mushrooms were prepared for analysis. Details of sample preparation for the analysis of total arsenic and arsenic species are given elsewhere.[15,24] Briefly, for the determination of total arsenic, samples were first digested with nitric acid and hydrogen peroxide, using a glass and Teflon reflux apparatus.[25] This acid digest was diluted appropriately and analyzed by ICP–MS with a flow injection sample introduction system. To determine arsenic species in the water-soluble fractions of plants, the plants were extracted with a mixture of methanol/water (1:1, v/v). The sample extracts were analyzed by HPLC–ICP–MS for arsenic species as described elsewhere.[24]

Quality assurance (QA) and quality control (QC)

Although fully quantitative data could not be realized, appropriate QA/QC measures were undertaken. Fungi and lichens were analyzed together with a large number of biota samples from Yellowknife and elsewhere[24,26,27] and the QA/QC program was applied to this large study. A blank, a duplicate, and a certified reference material (fucus, IAEA-140/TM and oyster tissue, NIST 1566a) were carried through sample preparation and analytical procedures for batches of 12–15 samples or smaller. The certified reference materials were within 10% of the certified values. Relative standard deviations (RSD) were less than 15% for total amounts of arsenic, and less than 30% (except in quantities near the limits of detection) for arsenic species. Blanks were less than the limit of detection for arsenic species. A limit of detection of 0.07 ppm was determined for total arsenic by using the method blank.

RESULTS AND DISCUSSION

The elevated levels of arsenic in the Yellowknife environment gave us the opportunity to examine arsenic levels and species in fungi of which some had not been studied previously. Total arsenic concentrations in lichens and mushrooms from Yellowknife are summarized in Table 1. Levels of arsenic in lichens were higher than the levels in samples from a hot springs environment in British Columbia (maximum of 4.8 ppm dry weight of arsenic).[24] The highest level of arsenic in all lichens occurred in Lichen 4 from the Giant Mine tailings pond. This was also the sampling location for the puffball mushroom, *Lycoperdon* sp., which contained the highest level of arsenic among the mushrooms. The highest concentration of arsenic in the remaining lichens was observed in PC3 (*Cladonia* sp.), sampled from the Con Mine tailings pond (520 ppm); it was likely to have been submerged at times of slow drainage from the tailings pond. The lichens that were sampled

Table 1 Arsenic concentrations in lichens and fungi from Yellowknife

Sample	Location	[As] (ppm dry weight)
PC1 (*Cladonia* sp.)	West Con Mine outflow	14.3
PC1, residue[a] (*Cladonia* sp.)	West Con Mine outflow	6.4
PC2 (*Cladonia* sp.)	South Con Mine outflow	29
PC2, residue[a] (*Cladonia* sp.)	South Con Mine outflow	15.9
PC3 (*Cladonia* sp.)	Con Mine tailings pond	520
Lichen 1 (*Cladina* sp.)	Beside Con Mine tailings pond	38
Lichen 2 (*Cladonia* sp.)	Beside Con Mine tailings pond	49
Lichen 3 (*Cladonia* sp.)	Beside Con Mine tailings pond	55
Lichen 4	Giant Mine tailings pond	2300
Paxillus involutus	South Con Mine outflow	36
Psathyrella candolleana	South Con Mine outflow	13.6
Leccinum scabrum	South Con Mine outflow	8.3
Coprinus comatus	Giant Mine tailings pond	410
Lycoperdon pyriforme	Giant Mine tailings pond	1010

[a] Sample obtained when the residue remaining after MeOH/water extraction was acid-digested.

together, Lichens 1, 2 and 3, contained similar levels of arsenic, with an average of 47 ppm (RSD 9 ppm) dry weight. This may indicate that these different species of lichens accumulate arsenic to a similar extent.

Some of the mushrooms contained higher levels of arsenic than those found by others in mushrooms collected from uncontaminated areas. For example, the specimen of *Lycoperdon* sp. analyzed in the present study contained 1010 ppm dry weight of arsenic, whereas the published background values are 0.46 to 2.81 ppm dry weight for the same family of mushrooms.[10] *Lycoperdon* sp. from the Con Mine tailings pond was not analyzed for total arsenic because the sample size was sufficient only for speciation analysis. In previous studies, total arsenic levels for *Psyathyrella* sp. and *Leccinum* sp. collected from uncontaminated areas were less than 0.2 ppm dry weight[4,28] and for *Paxillus involutus* they were 5.7–5.9 ppm.[4] Arsenic levels in *Paxillus involutus, Psathyrella candolleana* and *Leccinum scabrum* in the present study were apparently elevated compared with the levels published for similar species collected from uncontaminated areas. The levels of arsenic in these three mushrooms in the present study, however, were still close to the range of background concentrations (non-detectable to 15 ppm dry weight of arsenic) for most mushrooms.[4,10] The *Coprinus comatus* mushrooms from the Giant Mine tailings pond were appreciably elevated in arsenic concentration with respect to a published literature amount of <0.1 ppm for *Coprinus micaceus*.[10] Its arsenic concentration was also elevated even when compared with the exceptionally high background levels (up to 130 ppm dry weight) found in *Laccaria* sp.[6]

Arsenic compounds were identified by comparing retention times of arsenic compounds in samples with those of standard compounds. If the retention time for an arsenic compound in a sample was the same as that for a standard compound, the arsenic compound was concluded to have the same identity as the standard compound. If the presence of arsenobetaine or cationic species (such as trimethylarsine oxide, arsenocholine and tetramethylarsonium) was indicated after analysis with anion-exchange HPLC–ICP–MS, the identities of such species were confirmed by analysis with the cation-exchange HPLC–ICP–MS method. Cation-exchange chromatography was also used to confirm the presence of arsenosugar X. This analytical method separates the aforementioned peaks from other peaks that co-elute with them on other chromatographic systems. The identities of arsenosugars were confirmed by ion-pairing chromatography. Some samples were spiked with standards to confirm that retention times of arsenic compounds did not depend on matrix.

The primary purpose of the present study was to determine the identity of detectable arsenic species that could be extracted by MeOH/water (1:1) The amounts of the arsenic species found were calculated semi-quantitatively, to determine approximate levels of the compounds (i.e. major or minor components).

Table 2 Concentrations of arsenic species in Yellowknife lichens (ppm)

Sample[a]	As (III)	As (V)	MMA	DMA	Sugar X	AB	TMAO	Me_4As^+	Unknown	Sum of As species[c]	EE (%)[d]
PC1	0.8	0.8	<0.02	Trace[b]	<0.01	0.2	<0.02	<0.01	<0.01	1.8	13
PC2	6	1.4	0.26	0.4	0.6	1.31	1.8	0.039	0.3 X[e]	12	42
PC3	29	13	1.9	1.6	<0.03	1.9	<0.06	<0.03	<0.03	47	9.1
Lichen 1	6.4	3.0	<0.04	1.0	<0.02	0.6	0.5	<0.02	0.19 X	12	31
Lichen 2	4.6	1.4	0.39	<0.04	<0.02	0.79	0.5	<0.02	<0.02	7.7	16
Lichen 3	4.2	3.5	0.5	0.38	0.4	0.4	0.6	<0.02	<0.02	10	18
Lichen 4	0.5	24	1.0	0.7	<0.05	Trace	<0.10	<0.05	<0.05	26	1.1

Abbreviations: MMA and DMA, mono- and dimethylarsenic acid; AB, arsenobetaine; TMAO, trimethylarsine oxide.
[a] See Table 1 for lichen genera and locations.
[b] Trace amounts are greater than or at the limit of detection (LOD) but less than 3 × LOD.
[c] Sum of As species includes trace amounts (given an LOD value).
[d] EE = Percentage extraction efficiency, calculated as (sum of As species)/(total arsenic from Table 1) ×100%.
[e] Unknown X = peaks eluting between arsenobetaine and tetramethylarsonium ion on the cation exchange system.

The arsenic species extracted from lichens and mushrooms are summarized in Table 2 (lichens) and Table 3 (mushrooms). The major arsenic species in lichens were arsenite and arsenate, with the sum of these two species making up 62 to 93% of the total extracted arsenic for the lichen specimens analyzed.

Arsenobetaine has been found for the first time in lichens; it was present in all the lichens sampled. Chromatograms of PC1 and PC2 extracts, obtained from cation-exchange HPLC–ICP–MS analysis, are shown in Fig. 2. Arsenobetaine was identified in PC1 by spiking the sample extract with standard arsenobetaine and demonstrating co-chromatography of the suspected component with the authentic material (Fig. 2a). It was identified in PC2 (and other lichen samples) by matching the retention time of the presumed arsenobetaine peak in the sample with that of the standard (Fig. 2b).

Arsenosugar X was present in minor amounts in two lichen samples, PC2 (5% of extracted arsenic) and Lichen 3 (4% of extracted arsenic) from the Con Mine area. Arsenosugars were not detected in Lichens 1 and 2, which were growing together with Lichen 3 (Table 2).

Some similarities exist for all lichen species that were sampled from the Con Mine drainage system (PC1, PC2, PC3, Lichen 1, Lichen 2, Lichen 3), summarized in Table 4. The proportion of As (III) was similar for all samples (42–61% of extracted arsenic, see Table 4), but a broader range was observed for the relative amounts of As (V) in the lichens (12–44% of extracted arsenic). Amounts of arsenobetaine ranged from 4 to 11%, and similar

Table 3 Concentrations of arsenic species in Yellowknife mushrooms (ppm)[a]

Sample	As (III)	As (V)	MMA	DMA	Sugar XI	AB	AC	Me_4As^+	Unknown	Sum of As species	EE
Paxillus sp.	1.1	0.7	<0.05	16	0.8	<0.01	<0.01	0.11	0.3X,[b] 11Y[c]	30	83
Psathyrella sp.	1.4	4.4	0.14	0.6	<0.01	0.30	<0.07	0.17	<0.07	7.0	51
Leccinum sp.	0.17	0.08	<0.01	6.5	<0.01	<0.07	<0.07	<0.07	<0.07	6.8	85
Coprinus sp.	2.0	4 (1)	<0.02	1.7	<0.02	60	0.3	<0.1	0.4X	68	17
Lycoperdon sp. (Giant Mine)	3.3	21 (1)	6	10	<0.2	30	0.6	0.5	<0.1	71	7.1
Lycoperdon sp. (Con Mine)	12	1.2	<0.2	2.5	<0.2	60	0.6	0.30	0.5X	77	nd[d]

See Table 1 for mushroom locations, and Table 2 for abbreviations and notes on the column headings.
[b] Unknown X, peaks eluting between arsenobetaine and tetramethylarsonium ion on the cation-exchange system.
[c] Unknown Y, a broad peak that eluted slightly before arsenobetaine on the cation-exchange system.
[d] nd, not determined, because the total arsenic concentration was not determined.

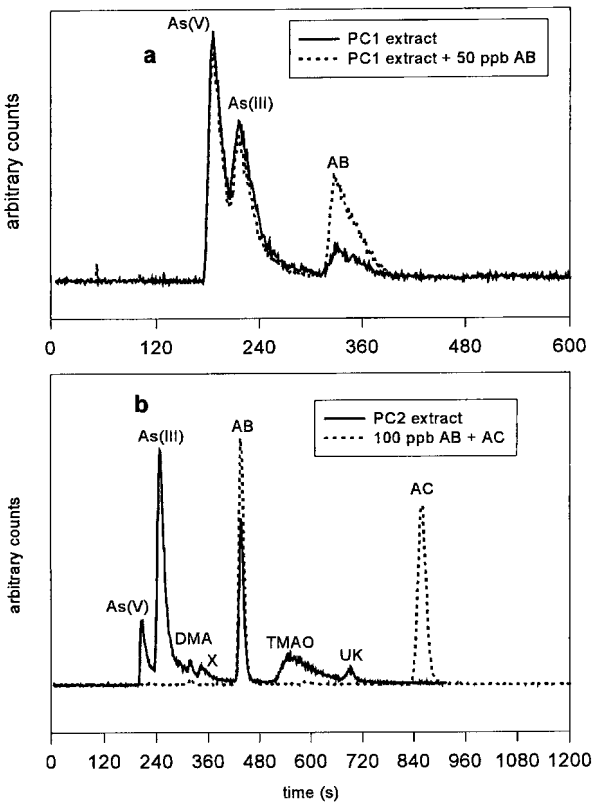


Figure 2 Arsenobetaine (AB) in lichens analysed by cation-exchange HPLC–ICP–MS (Supelcosil LC-SCX, 250 mm × 4.6 mm, 20 mM pyridinium formate, pH 2.7) (a) Chromatograms of Yellowknife lichen (PC1) extract and extract spiked with 50 ppb (parts per 10^9) AB standard. (b) Chromatograms of Yellowknife lichen (PC2) extract, and of 100 ppb AB and AC standards. UK, unknown compound.

amounts of TMAO were observed for the three lichen species (Lichens 1, 2 and 3) that were growing together (4–6.5 % of extracted arsenic). Similarities in arsenic speciation between these three lichens might be expected, due to their common environment.

The Giant Mine lichen (Lichen 4) extract contained predominantly As(V) and a small proportion of arsenobetaine (0.5%) (Table 4). Differences in arsenic speciation between lichens may have been caused by differences in lichen species (and hence metabolism of arsenic), and/or differences in their environments. For example, the major form of arsenic was probably as arsenate adsorbed onto ferric hydroxide in the Giant Mine tailings,[29] which may have led to the predominance of arsenate in the tailings water. Unlike Lichen 4, the Con Mine lichen samples were less likely to be submerged in tailings pond water.

The mushroom *Lycoperdon* sp. from the Giant Mine tailings pond also contained a proportionally higher amount of arsenate, although the major arsenic species in both specimens of *Lycoperdon* sp. was arsenobetaine. In a previous study, arsenobetaine was also observed to be the major arsenic species extracted from *Lycoperdon echinatum* (78%), *Lycoperdon perlatum* (88%) and *Lycoperdon pyriforme* (62%); minor components included As(V), As (III), MMA and DMA.[10] A greater variety of arsenic species was observed in the *Lycoperdon* sp. from Giant Mine tailings pond than in *Lycoperdon* sp. from the Con Mine tailings pond (see Table 3). The specimen from the Giant Mine tailings pond was observed to be in a more mature form, and this may account for the differences in speciation observed. Again, the microbial environment influencing the fungus may also have caused differences in arsenic speciation.

The arsenic speciation was determined for water-

Table 4 Proportions of total arsenic extracted (% of arsenic species) for lichens and mushrooms

Sample	Location	As(III)	As(V)	Methyl + sugars	AB	TMAO + cations
PC1	Con Mine	44	44	1.9	11	0
PC2	Con Mine	50	12	10	11	18
PC3	Con Mine	61	27	7.4	4.0	0
Lichen 1	Con Mine	55	26	8.6	5.1	5.9
Lichen 2	Con Mine	60	18	5.1	10.3	6.5
Lichen 3	Con Mine	42	35	13	4.0	6.0
Average for	Con Mine lichens	52 (8)[a]	27 (11)	7.7 (3.9)	7.5 (3.5)	6.1 (6.5)
Lichen 4	Giant Mine	1.9	91	6.5	0.5	0
Lycoperdon sp.	Giant Mine	4.6	29	22	42	7.8
Lycoperdon sp.	Con Mine	16	1.6	3.2	78	1.8

[a] Standard deviation of proportions of each compound for PC1, PC2, PC3, Lichen 1, Lichen 2, and Lichen 3.

soluble species in the shaggy mane mushroom *Coprinus comatus* for the first time: the major arsenic compound was arsenobetaine (88% of extracted arsenic). Minor components included inorganic arsenic, DMA, arsenocholine and an unknown arsenic compound.

Paxillus involutus grew in abundance next to the Con Mine effluent stream. Its arsenic content was unusual because a major proportion of arsenic extracted (36%) was in an unidentified form (unknown compound Y). The peak for unknown Y was broad and its retention time was near that of arsenobetaine on the cation-exchange system (see chromatogram, Fig. 3). Neither unknown Y, nor a minor component, unknown X, could be identified as any of the arsenic compounds available to us as standards; the chromatogram of some of the cationic arsenic standards is shown in Fig. 3. An unknown peak possessing a retention time similar to that for unknown Y, on an almost identical chromatographic system, was observed in mussels by Larsen *et al.*[30] The authors established that the compound was neither 2-dimethylarsinylethanol [$Me_2As(O)CH_2CH_2OH$] nor glycerylphosphoryl-arsenocholine [$Me_3As^+CH_2CH_2O—PO_2^-—OCH_2CHOHCH_2OH$].

The other major arsenic compound extracted from *Paxillus involutus* was DMA (53% of extracted arsenic). As(III) and As(V), Me_4As^+, another unknown species (unknown X) and a small amount of arsenosugar XI (2.6% of arsenic extracted) occurred as minor components.

The major arsenic compound found in *Psathyrella candolleana* was arsenate, making up 63% of the total arsenic extracted. The next most abundant compound was arsenite, and minor components were MMA, DMA, arsenobetaine and Me_4As^+. This mushroom species is classified in the same family as *Coprinus* sp. (Coprinaceae).[28] Similarities have been observed in arsenic contents of different mushroom species within the same family,[10] and thus it is not surprising to find some of the same compounds (e.g. arsenobetaine) in *Psathyrella candolleana* and *Coprinus comatus*. Both mushrooms are edible but the major water-soluble arsenic species in *Psathyrella candolleana*, As (V) and As (III), are toxic to humans, whereas in *Coprinus comatus* the major species, AB, is not.

Leccinum scabrum contains mostly DMA with minor amounts of inorganic arsenic. It has been suggested that in more 'primitive' genera of mushrooms arsenobetaine occurs much less frequently than in genera that are more highly evolved, such as puffballs.[10] On this basis, *Paxillus involutus* and *Leccinum scabrum* may be more primitive mushrooms.

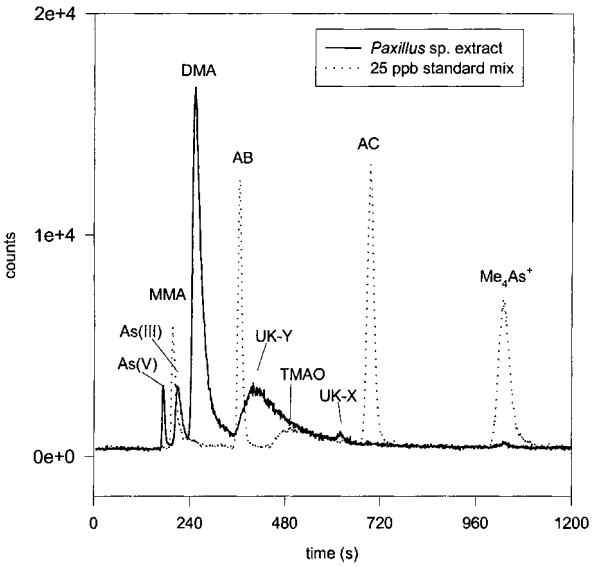


Figure 3 Chromatogram of *Paxillus involutus* extract (diluted ten fold), showing the presence of unknown compounds X and Y (UK-X and UK-Y, respectively), as well as DMA, inorganic arsenic, and a small amount of Me_4As^+. Standards (25 ppb) of MMA, AB, TMAO, AC and Me_4As^+) did not co-chromatograph with UK-X and UK-Y. Cation-exchange HPLC–ICP–MS was used: Whatman SCX Partisil 5, 250 mm × 4.6 mm, 20 mM pyridinium formate, pH 2.7.

The presence of arsenosugar XI in *Paxillus involutus* represents one of the first reports of arsenosugars in mushrooms. In another study, arsenosugar XI was tentatively identified in an extract of *Laccaria amethystina*, but further chromatographic confirmation was considered to be necessary.[6]

Small amounts (less than 1% of extracted arsenic) of arsenocholine were found in *Coprinus comatus* and *Lycoperdon* sp. Arsenocholine has been observed only in small amounts in marine samples, even though studies have demonstrated that this compound can be readily biotransformed into arsenobetaine by sediments.[31] However, arsenocholine has been observed previously in mushrooms, being one of the major extracted arsenic species in *Amanita muscaria*[9] as well as in *Sparassis crispa*.[10]

Extraction efficiencies for lichens and fungi range from 1.1% for Lichen 4 from the Giant Mine tailings pond to >80% for *Paxillus involutus* and *Leccinum scabrum* from the Con Mine outflow area. Unextracted arsenic may be in a non-

extractable form, e.g. in a mineralized form on the outside of the specimen, or bound to chitin or other cell components of the fungus. Residues of two extracted lichens were analyzed for total arsenic (see Table 1) and extraction efficiencies (in percentage values) from these results were calculated as the difference between total arsenic and the arsenic in the residue, divided by total arsenic. Based on this calculation, 55% of total arsenic was extracted from PC1, which differed from the extraction efficiency of 13% in Table 2 (calculated by summing the arsenic species detected and dividing by total arsenic). An extraction efficiency of 45% was calculated by using the arsenic concentration in the PC2 residue, which agrees quite well with the 42% reported in Table 2. The difference in extraction efficiencies for PC1 indicates that some arsenic that was extracted was not observed by the chromatographic systems available.

Research is ongoing to elucidate the identity of unknown compounds in the samples studied (e.g. *Paxillus* sp.) and to determine the chemical and toxicological nature of the unknown, unextracted fractions in fungi.

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