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# The Predominance of Inorganic Arsenic Species in Plants from Yellowknife, Northwest Territories, Canada

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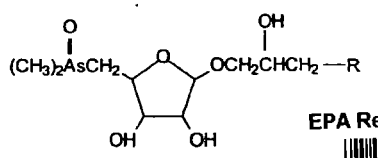
Elevated levels of arsenic in Yellowknife, NWT, Canada, from historic and recent gold mine operations, are of increasing concern to Yellowknife residents. The study of arsenic in Yellowknife plants is a part of ongoing bioavailability and food chain research. A variety of plants from Yellowknife were analyzed for total arsenic and water soluble arsenic species. The plants included vascular plants and bryophytes (mosses). Total amounts of arsenic were greatest in mosses and varied greatly within specimens of the same plant species from different locations. Mostly inorganic arsenic species were extracted from plants using methanol/water (1:1). This result is very important from a toxicological point of view, since inorganic species are relatively toxic arsenic species. Small amounts of methylated arsenic species, as well as arsenosugars, were present in some plants. On average, greater than 50% of arsenic in these plants was not extracted; the chemical and toxicological characteristics of this fraction remain a topic for further study.

## Introduction

Yellowknife is located on Great Slave Lake, in the Northwest Territories, Canada. A major industry in the city is gold mining, and two gold mines, the Royal Oak Giant Mine and the Miramar Con Mine, have 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*). The increased amounts in Yellowknife of arsenic, a known poison and carcinogen, is of great concern to residents of the city. Therefore, information pertaining to the problem of arsenic contamination in Yellowknife is directly relevant to the parties involved.

While arsenic is associated with adverse effects, 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 and is much less toxic than arsenous acid or arsenite ($As(OH)_3$). 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.

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| | |
|---|---|
| a | -OH |
| b | $-OPO_3HCH_2CH(OH)CH_2OH$ |
| c | $-SO_3H$ |
| d | $-OSO_3H$ |

**FIGURE 1. Structure for arsenosugars and numbering system (1a–d) for the different structures.**

The study of arsenic in plants is significant in two ways: (1) uptake of arsenic by plants can indicate that fraction in the soil which is bioavailable (dependent on plant species) and (2) the residual arsenic in the plant is then available to the next level in the food chain, making the determination of the form of arsenic in the plant necessary. Little is known about arsenic speciation in plants in the freshwater and terrestrial environments. In a hot springs environment three species of vascular plants contained predominantly inorganic arsenic (*3*), and carrots (*4*) and other vegetables (*5*) grown in arsenic contaminated soil under laboratory conditions also contained large amounts of inorganic arsenic. Arsenic species in vegetables meant for human consumption or grown naturally in contaminated environments have not yet been determined. In Yellowknife specifically, only total concentrations of arsenic have been determined in macrophytes (aquatic plants) (*6*, *7*) and birch leaves (*8*). Concentrations were found to be elevated compared with specimens collected from uncontaminated areas. Toxic effects of reduced plant health and lack of biodiversity were observed in areas of high arsenic content (*6*).

The previous findings of inorganic arsenic in a few plants point to the importance of determining arsenic species in a greater variety of plants from a natural environment that has been contaminated with arsenic. The concern in Yellowknife about arsenic contamination makes this study of arsenic speciation in plants from the area especially timely.

## Experimental Section

**Chemicals and Reagents.** Arsenic standards to make solutions of arsenate (As(V)), arsenite (As(III)), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were obtained from commercial sources. Trimethylarsine oxide (TMAO) (*9*) and tetramethylarsonium iodide (Tetra) (*10*) had been synthesized previously according to standard methods. Extracts of kelp powder (Galloway's, Vancouver, BC) and Nori (*Porphyra tenera*) of known arsenosugar content (*11*) were used to identify the retention times of arsenosugars; the retention times were then verified by comparison with those obtained from pure arsenosugars generously donated by K. Francesconi and T. Kaise. The structures and numbering system for arsenosugars are summarized in Figure 1. Other reagents of analytical grade or better were obtained from commercial sources.

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**Sampling.** Plants were collected during four sampling periods: from June 22 to June 29, 1997, from August 23 to August 30, 1997, from July 1 to July 16, 1998, and from October 5 to October 12, 1998. The samples were collected from the locations shown in Figure 2. Sample locations 1–3 are found on the Giant Mine property and include drainage pathways from Giant Mine. Location 4 is in Back Bay near Giant Mine. Locations 5 and 6 are on the shores of Niven Lake, a former sewage pond supporting prolific plant growth. Locations 7–18 are on the Con Mine property, surrounding the tailings ponds. Locations 19–23 follow the Con Mine effluent discharge outflow (Meg Lake–Keg Lake–Peg Lake–Great Slave Lake outflow), and locations 24–30 encompass areas that are further from the tailings ponds. These include areas that are in the city of Yellowknife such as Rat Lake, Yellowknife Bay east of the city, Frame Lake, Range Lake, and Kam Lake. The plant samples consisted of vascular plants (including flowering plants, grasses, aquatic submergent plants, and emergent plants) and bryophytes (mosses).

The plant 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 and Sparkleen to remove soil and other particles, rinsed with deionized (18 MΩ) water, blotted dry, and frozen. Prior to sample preparation, plants consisting of both roots and shoots were separated into root (below soil surface) and shoot (above soil surface) subsamples. Only plant shoot samples were analyzed for this study. Aquatic plants and mosses were processed whole. Plant samples were freeze-dried and pulverized to a fine powder for analysis.

Plants were identified by using field guides (*12–16*) with assistance from experts at UBC, RMC, and the Canadian Wildlife Service (Yellowknife office). A summary of the genera and species where verification was possible, is given in the Supporting Information (Table SI-1).

**Sample Preparation and Analysis**. Details of sample preparation for the analysis of total arsenic and arsenic species are given elsewhere (*3, 11*). Briefly, for the determination of total arsenic, samples were first digested with nitric acid and hydrogen peroxide, using a glass and Teflon reflux apparatus (*17*). This acid digest was diluted appropriately and analyzed by using ICP-MS equipped 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 in a ratio of 1:1. The sample extracts were analyzed by HPLC-ICP-MS for arsenic species as described elsewhere (*3*). For the determination of total arsenic levels in some plant extracts, microwave digestion was used for sample preparation. The extracts (5 ± 0.05 g) were filtered (0.45 $\mu$m syringe filters, Millipore) into 50 mL Teflon centrifuge tubes, and 3 mL of nitric acid as well as 1 mL of hydrogen peroxide was added. The loosely capped tubes were heated in an Emerson 550W microwave oven at high for 3–4 min, three times, with a cooling period of 10 min between each heating period. The clear solutions remaining were diluted to 25 mL with deionized water. These solution were analyzed for total arsenic by using ICP-MS. See Koch et al. (*3*) for instrumental and chromatographic details.

**QA/QC**. A blank, a duplicate, a certified reference material (*fucus*, IAEA-140/TM and oyster tissue, NIST 1566a) where applicable, and a spiked sample were carried through sample preparation and analytical procedures for batches of 12–15 samples or smaller. Plasma instability and variations in $m/z$ sensitivity were monitored and corrected with the use of a rhodium internal standard during ICP-MS and HPLC-ICP-MS analysis of samples. The certified reference materials and spiked sample recoveries were within 10% of the certified or theoretical values, relative standard deviations were less than 6% for total amounts of arsenic and less than 30% (except

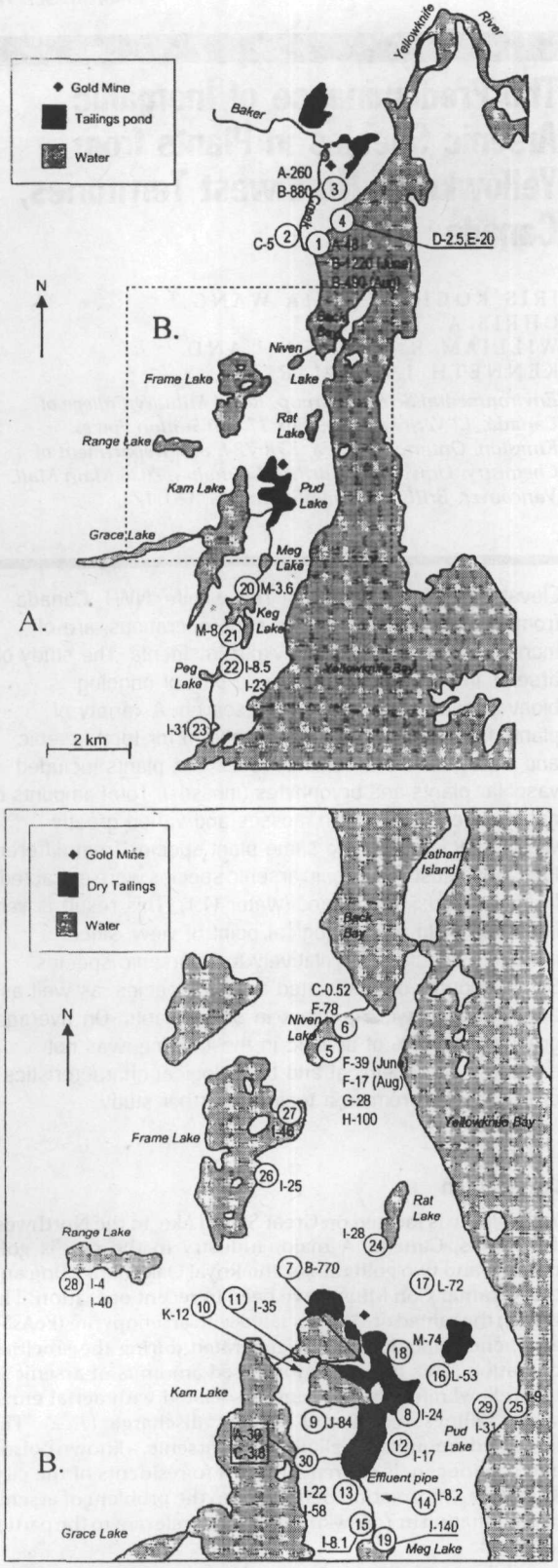


**FIGURE 2. (A) Map of Yellowknife and surrounding area, showing sample locations (circled) and total amounts of arsenic in plants. (B) Detail of Con Mine property in Yellowknife. Codes for plant types: A = *Equisetum fluviatile*; B = *Drepanocladus* sp.; C = *Typha latifolia*; D = *Sparganium augustifolium*; E = *Potomogetan richardsonii*; F = *Myriophyllum* sp.; G = *Lemna minor*; H = *Bidens cernua*; I = *Carex* sp.; J = *Rubus idaeus*; K = *Potentilla fruticosa*; L = *Agrostis scabra*; and M = *Hordeum jubatum*.**

**TABLE 1. Concentrations of Total Arsenic (ppm Dry Weight) Presented as a Range of Values and the Median Value for Groups of Plant Types**

| type of plant | range | median |
|---|---|---|
| terrestrial (grasses and shrubs) | 3.6–84 | 53 |
| all emergents | 0.52–260 | 25 |
| emergent *Carex* sp. | 3.6–136 | 24.5 |
| emergent *Equisetum fluviatile* | 30–260 | 48 |
| emergent *Typha latifolia* | 0.52–5.0 | 3.8 |
| submergents | 2.5–78 | 24 |
| mosses | 490–1220 | 825 |

in quantities near the limits of detection) for arsenic species, and blanks were less than the limit of detection, except when they were used to determine the method limit of detection (for total arsenic).

## Results and Discussion

The total amounts of arsenic in plants are shown at their respective locations on the maps in Figure 2. Mosses contain the highest levels of arsenic (490–1220 ppm dry weight, see Table 1), reflecting concentrations that are remarkably higher than those for other plants. The higher concentrations of moss compared with that of another plant (horsetail, *Equisetum fluviatile*) collected from the same location (location 1) might suggest that mosses accumulate more arsenic than other plants.

*Typha latifolia* appears to take up the least amount of arsenic (see Table 1). Dushenko et al. postulated that the lower levels of arsenic in *Typha* sp. as well as its abundance at all sampling sites (observed in the present study as well) indicates a greater tolerance to arsenic contamination by using mechanisms for the exclusion of arsenic (*6*).

In other studies (*18*), terrestrial plants on mine waste have been observed to contain higher levels of arsenic (up to 3470 ppm dry weight) than those observed here. Nevertheless, the arsenic levels in most of the Yellowknife plants in this study appear to be elevated compared with general background levels found in plants (nondetectable to 3 ppm dry weight (*18–20*)).

The water soluble fractions of plants were analyzed for arsenic species, and the results for the 13 different plant genera are summarized in Table 2 (details for individual plant samples are given in Supporting Information, Table SI-2). The results indicate that for all the terrestrial plant species studied, the major water soluble compounds are inorganic As(V) and As(III). This is very important from a toxicological perspective, since inorganic arsenic is relatively toxic. Previous studies have shown that carrots (*4*) and other vegetables (*5*) also contain predominantly inorganic arsenic, but this is the first study to show conclusively that inorganic arsenic predominates in a wide variety of plants that were sampled at different locations and times, from a natural environment.

The effect of these levels of toxic arsenic on consumers of the plants are unknown, especially since episodes of wildlife poisoning by arsenic have been documented only rarely (*18*). Domestic sheep that were fed diets containing lake weed (288 ppm arsenic) for 3 weeks showed no ill effect. On the other hand, a generalized feeding level of 50 ppm arsenic was linked to adverse affects in mammals. A prescribed limit of <4 ppm fresh weight (corresponding to about 20 ppm dry weight) total arsenic in feedstuff containing plants for domestic livestock was stated in the literature (*18*). Therefore for plants containing greater than 20 ppm dry weight of arsenic, the possibility that wildlife may be affected cannot be excluded.

The difference between arsenate and arsenite distributions for *Myriophyllum* sp. between June and August is interesting to note. The sample collected in August contains the same proportion of arsenate as the June sample contains of arsenite (approximately 70%). In general, submergent plants collected in August contain predominantly As(V) (see Table 2), and therefore the trend observed for *Myriophyllum* sp. may extend to other submergent plants. Throughout the growing season, the submergent plants probably take up arsenic from the surrounding water as arsenate, which is the predominant form of arsenic in Yellowknife waters (*21*).

**TABLE 2. Percent Arsenic Species of Sum of Arsenic Species (Standard Deviation) and Percent Extraction Efficiency (EE) for Different Plants[a]**

| plant | *n* | % As(III) | % As(V) | % methyl | % sugars | % EE |
|---|---|---|---|---|---|---|
| **Terrestrial (Vascular)** | | | | | | |
| *Rubus idaeus* | 1 | 47 | 53 | 0 | 0 | 25 |
| *Potentilla fruticosa* | 1 | 27.5 | 72.5 | 0 | 0 | 46 |
| *Hordeum jubatum* | 2 | 34 (12) | 63 (14) | 3.6 (2.4) | 0 | 43 (6) |
| *Agrostis scabra* | 2 | 24 (4) | 76 (4) | 0.6 (0.5) | 0 | 50 (19) |
| **Emergents (Vascular)** | | | | | | |
| *Carex* sp. | 18 | 18 (9) | 79 (9) | 3.3 (3.4) | 0 | 63 (18) |
| *Bidens cernua* | 1 | 8 | 88 | 4 | 0 | 16 |
| *Equisetum fluviatile* | 3 | 51 (11) | 49 (11) | 0 | 0 | 72 (50) |
| *Typha latifolia* | 3 | 24 (3) | 71 (10) | 5.3 (7.0) | 0 | 87 (28) |
| **Submergents (Vascular)** | | | | | | |
| *Lemna minor* (Aug) | 1 | 17 | 63 | 9 | 11 | 23 |
| *Potomogetan richardsonii* (Aug) | 1 | 12 | 79 | 3.5 | 5.9 | 13 |
| *Sparganium augustifolium* (Aug) | 1 | 30 | 70 | 0 | 0 | 37 |
| *Myriophyllum* sp. (Aug) | 1 | 26 | 67 | 5.5 | 0.9 | 44 |
| *Myriophyllum* sp. (June) | 2 | 71 (12) | 25 (14) | 3.4 (2.2) | 0 | 47 (6) |
| **Mosses (Bryophytes)** | | | | | | |
| *Drepanocladus* sp. | 4 | 31 (13) | 67 (15) | 1.7 (2.0) | 0 | 9.8 (5.4) |

[a] Methyl = MMA + DMA + Tetra, sugars = arsenosugar 1a + 1b; *n* = number of sample.

Speciation analysis also revealed that small amounts of methylated arsenic compounds are present in some plants, including MMA, DMA, and tetramethylarsonium ion (Tetra) as well as arsenosugars **1a** and **1b**. Chromatograms of standard solutions containing arsenic compounds are shown in Figure 3. Chromatograms of methylated compounds and arsenosugars in duckweed (*Lemna minor*) using three different chromatographic methods to confirm the chromatographic identification of arsenicals are shown in Figure 4. When the sample is subjected to anion exchange chromatography (Figure 4a), peaks are seen that are attributed to the following: a cationic species; As(III) and/or sugar **1a**; DMA; MMA; sugar **1b** (proposed); and As(V). When cation exchange chromatographic conditions are used (Figure 4b), the cationic species in Figure 4a is determined to be tetramethylarsonium ion (Tetra), and a small peak with the same retention time as sugar **1a** appears. To confirm the identity of the arsenosugars, ion-pairing chromatography results in the elution of sugars **1a** and **1b** after the other peaks (Figure 4c), as demonstrated by kelp extract containing arsenosugars (Figure 3c). Note that although sugars **1b** and **1c** are not resolved from each other on the ion-pairing system, the peak at the retention time for **1b/1c** in Figure 4c is nevertheless identified as sugar **1b**, since the presence of sugar **1c** is not evident in Figure 4a.

The existing evidence (*22–24*) indicates that these compounds may be present as a result of uptake from the

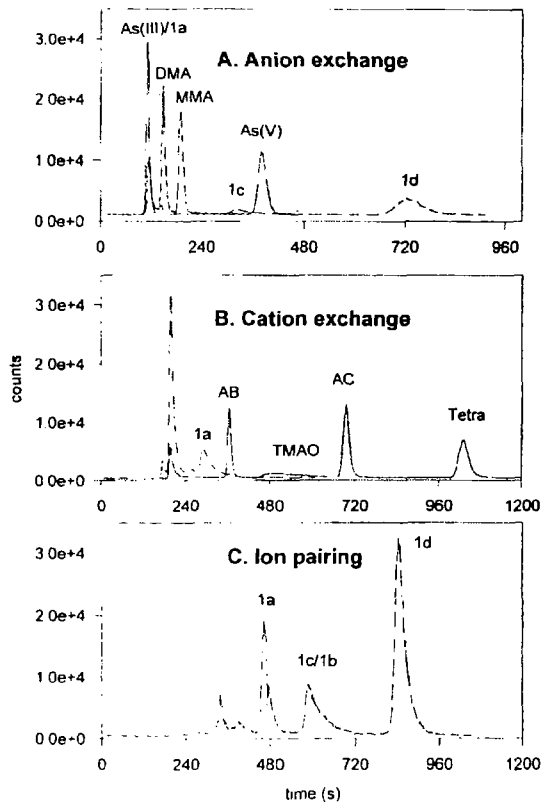


**FIGURE 3. Chromatograms of standard solutions of arsenic compounds (— commercial standards; - - kelp extract). A. Anion exchange chromatographic conditions: Hamilton PRP-X100 250 × 4.6 mm column, 20 mM ammonium phosphate, pH 6.0 at 1.5 mL/min. B. Cation exchange chromatographic conditions: Whatman SCX Partisil 5, 250 × 4.6 mm column, 20 mM pyridinium formate, pH 2.7 at 1 mL/min. C. Ion-pairing chromatographic conditions: GL Sciences ODS 250 × 4.6 mm column, 10 mM TEAH, 4.5 mM malonic acid, 0.1% methanol, pH 6.8 at 0.8 mL/min.**

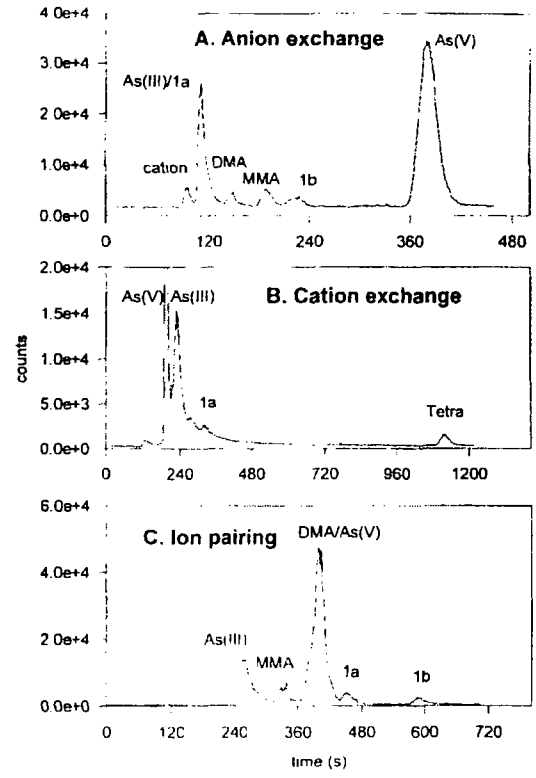


**FIGURE 4. Chromatograms of an extract of duckweed (*Lemna minor*). A. Anion exchange chromatography. B. Cation exchange chromatography. C. Ion-pairing chromatography; for details see caption for Figure 3.**

environment, synthesis by the plant, or both. Additionally, arsenicals may be adsorbed onto the outside surface of the plant. All the plants were thoroughly washed, but they were not subsequently examined under a microscope to ensure that all other organisms had been removed. The characterization of arsenic (especially with respect to the finding of minor constituents) in all plants is therefore better regarded as characterization of a plant community. While keeping this in mind, the discovery of arsenosugars in some of these plants (amounting to as much as 11% of extracted arsenic in *Lemna minor*) represents the first conclusive association of arsenosugars with higher terrestrial plants. Halophytes (salt marsh plants) were proposed to contain an arsenosugar, but no identification (i.e., comparison with known arsenosugar compounds) was carried out (*25*). Interestingly, only arsenosugars **1a** and **1b** are observed in the present study, which was also the case for other findings of arsenosugars in terrestrial environmental samples (*3*, *11*, *26*, *27*). The arsenosugars are found only in submergent plants, which are most likely to be contaminated with other organisms; for example, *Myriophyllum* sp. and *Lemna minor* were growing in physical contact with algae, which may be a possible source of arsenosugars.

Tetramethylarsonium ion (Tetra) was found in a vascular plant from Meager Creek (*3*), and its presence in some samples in the current study, albeit at very low levels, indicates a wider distribution than might otherwise be expected, considering its rare occurrence in sediments or waters (*28*, *29*). One other study has suggested the presence of tetraalkylated arsenicals in plants, which was attributed to the uptake of similar compounds from the surrounding water (*25*). Tetramethylarsonium ion occurs in *Drepanocladus* sp. sampled from location 1 in June and August but does not appear in the same species of moss sampled from the two different locations 3 and 7. The arsenic species extracted from moss may be specific to the its growing environment and affected by factors such as chemical species available in the water/soil as well as organisms present in or on the moss.

Extraction efficiencies are included in Table 2 and detailed in Supporting Information, and they range from 3 to 100%, with a median value of 49%. These results indicate that a large fraction of arsenic in many plant samples is not water soluble. The chemical and toxicological characteristics of this fraction remain unknown. Unextracted arsenic could be bound to lipids or to cell wall components, including insoluble cellulose, calcium or magnesium pectates, or lignin. Research to determine the bioavailability and nature of this arsenic is ongoing.

The present study verifies the ubiquity of predominantly inorganic arsenic species in the extractable (and potentially bioavailable) portion of plants from a natural terrestrial environment impacted by mining activities. Future research should address the bioavailability of plant arsenic as well as its impact on the food chain.

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plants. Bert Mueller was invaluable in providing technical assistance with ICP-MS.

## Supporting Information Available

Tables SI-1 (common and scientific names for Yellowknife plants) and SI-2 (amounts of arsenic species, sum of species, total arsenic extracted, total arsenic in ppm dry weight, and percent extraction efficiency for plants from Yellowknife). This material is available free of charge via the Internet at http://pubs.acs.org.

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