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# Bioaccessibility and speciation of arsenic in country foods from contaminated sites in Canada

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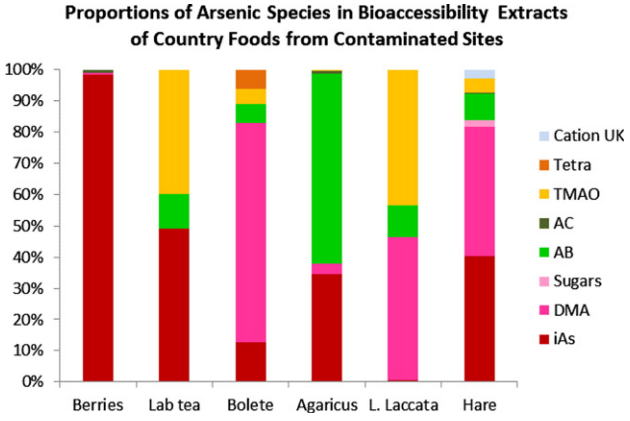
## HIGHLIGHTS

- We measured arsenic in country foods from contaminated sites.
- Bioaccessibility and arsenic species in extracts were determined.
- Plants (berries and Labrador tea) contained mainly toxic inorganic arsenic.
- Mushrooms and hare meat contained varying amounts of less toxic arsenic species.
- Variability between samples indicates that site-specific study is necessary.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Arsenic in foods obtained through foraging or hunting (country foods) in contaminated areas has not been reported; moreover the chemical form (arsenic speciation) is not known. Bioaccessibility extractions can be used to extract the arsenic from samples, giving information about the arsenic that is available for absorption into humans. Bioaccessibility of arsenic was measured in country foods (berries, other plants, mushrooms and hares) collected from contaminated sites in Canada. Arsenic speciation in the bioaccessibility extracts was also determined. Arsenic concentrations in berries ranged from 0.06 to 21 mg/kg, and Labrador tea contained 1.9 mg/kg of arsenic (all wet weight). Arsenic concentrations (wet weight) ranged up to 46 mg/kg in mushrooms, but they were much lower in hare muscle tissue (0.007 to 0.6 mg/kg). Percent bioaccessibility was lowest in berries and plants (means of 12–45%), where the arsenic species were mostly toxic inorganic arsenic. Bioaccessibility was higher in mushrooms and hare meat (means of 22–76%), where along with toxic inorganic arsenic, substantial proportions of less toxic organoarsenic species were measured, including non-toxic arsenobetaine. The speciation patterns were highly variable in both mushrooms and hare meat. Toxic forms of arsenic are present in country foods collected from contaminated areas, but the amounts vary according to and within each sample type. Therefore testing should ideally be carried out for new sample types and locations to estimate exposures to humans.



## 1. Introduction

The term “country foods” usually refers to dietary items that are obtained by hunting and gathering; in Canada, these foods often

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account for a large proportion of foods consumed by First Nations people. For example, Yukon First Nations people consume country foods (also referred to as traditional food) on 50% of the days in a year (Receveur et al., 1998). Additionally, Health Canada guidelines indicate that fish consumption for Canadian aboriginal populations is approximately double than that for the non-aboriginal Canadian population. Wild game consumption is even larger than fish consumption for aboriginal populations, but non-existent for non-aboriginal Canadians (Health Canada, 2009).

When the local environment from which country foods are harvested is near or on a contaminated site, these foods have the potential to contain elevated concentrations of contaminants such as metals and metalloids. In such cases a risk assessment, or quantification of the potential for adverse effects to consumers, may be warranted. The risks associated with the consumption of country food are quantified by estimating doses of contaminant to which consumers are exposed. This dose depends not only on the total concentration of the contaminant in the country food, but also on the contaminant bioavailability in the foods' matrices.

Bioavailability refers to the amount of a contaminant that is absorbed into the body where it can cause toxic effects, and can be estimated by bioaccessibility measurements, which simulate the human gastrointestinal environment in a laboratory test. Bioaccessibility measurements give information about the amount of a contaminant that is available for absorption into the bloodstream, but for inorganic contaminants they do not imitate the absorption. Currently, there is very little published literature on the bioavailability or bioaccessibility of contaminants in country foods. One study that examined mercury in country foods reported a wide range of bioaccessibility results (less than 1% to 60%) for various organs of caribou, walrus, arctic char, seals and whales (Laird et al., 2009), but no studies have been reported for other metal/metalloids, such as arsenic, in similar matrices.

Arsenic can be found in many different chemical forms, or species, that vary greatly in their toxicities. Thus their determination in a sample, by using analytical techniques capable of distinguishing individual compounds (speciation analysis), is crucial in understanding risk. Arsenic risk is often based on incomplete methods of speciation analysis giving information about "inorganic" (toxic) and reputedly "organic" (non-toxic) arsenic, but some of the organoarsenic compounds may be a concern (Feldmann and Krupp, 2011). For example, methylated arsenic compounds were thought to be less toxic than inorganic compounds, and their formation a possible detoxification process, but recent studies point to the acute toxicity of methylarsenic (III) species (Petrick et al., 2001; Styblo et al., 2000) and the carcinogenicity of dimethylarsinic acid (DMA, $(CH_3)_2As(O)OH$) (Arnold et al., 2006; Kenyon and Hughes, 2001). "Organic" arsenic compounds considered to be non-toxic to humans are arsenobetaine (AB, $(CH_3)_3As^+CH_2COOH$) and arsenosugars. This non-toxic classification appears to hold true for AB (Kaise et al., 1985), which is rapidly excreted by humans (Brown et al., 1990; Le et al., 1994), but arsenosugars can be metabolized to (potentially carcinogenic) DMA prior to excretion (Le et al., 1994), and the potential exists for their reduction to trivalent arsenosugars, which appear to exhibit increased toxicity (Andrewes et al., 2004).

Arsenic speciation has been determined more frequently in aqueous extracts (e.g., in cattails meant for human consumption, Lu et al., 2009) than in bioaccessibility extracts. Bioaccessibility measurements are limited to a few studies, mostly concerning conventional food items (i.e., presumably from uncontaminated locations), specifically, fish and shellfish (Laparra et al., 2007; Moreda-Piñeiro et al., 2012), algae (Laparra et al., 2003, 2004), and rice (Laparra et al., 2005; Sun et al., 2012). This type of analysis has been reported only infrequently in food items from contaminated sites, specifically, shellfish and seaweed (Koch et al., 2007), freshwater crayfish (Williams et al., 2009), and garden vegetables (Koch et al., 2003). To address the data gaps identified here, the objective of the present study is to report the arsenic speciation in bioaccessibility extracts of a number of country foods from contaminated sites in Canada.

## 2. Materials and methods

### 2.1. Sample collection and processing

Country food samples including hares, edible mushrooms and wild berries were collected from Yellowknife, Northwest Territories in 2000, 2004 and 2010 (YK samples); and from Seal Harbour, Nova Scotia in 2004, 2005, and 2010 (NS samples). Hares were collected by snaring under the appropriate permits, with methods having been approved by territorial or provincial departments. Muscle was removed as the item most likely to be ingested, and frozen until analysis. Edible mushrooms were collected from mine tailings' locations and wooded contaminated areas, washed immediately after collection to remove soil, and frozen until analysis. Berries were collected from contaminated areas and frozen unwashed, until analysis. All samples were analyzed in their wet (fresh) form. Samples were pulverized between metal plates after being frozen in liquid nitrogen for sample homogenization.

### 2.2. Chemicals

Total arsenic matrix spikes and calibration curves were prepared from stock solutions with concentrations of $995 \pm 3$ μg/mL and $10006 \pm 25$ μg/mL total arsenic (Inorganic Ventures). If available, the source of standards used for calibration curves was different from those used for quality control (QC) calibration check solutions and matrix spikes. Trimethylarsine oxide (TMAO, $(CH_3)_3AsO$) and AB were available from both Wako and Argus Chemicals, and two sources were available for DMA (cacodylic acid > 99% from Fluka and City Chemical), As(III) (as 1000 mg/L solution, > 99.0% from Fluka and as $As_2O_3$, 99.995%, from Aldrich), and As(V) (as solutions 9775 mg/L from Aldrich and 1000 mg/L from Inorganic Ventures). Only one source was available for arsenocholine (AC, $(CH_3)_3As^+CH_2CH_2OH$) as the bromide salt (Argus Chemicals), tetramethylarsonium ion (Tetra, $(CH_3)_4As^+$) as the iodide salt (Wako), and monomethylarsonic acid (MMA, $CH_3As(O)(OH)_2$) as monosodium acid methane arsonate sesquihydrate (99.0%, Chemservice). Arsenosugars were extracted from brown algae (*Fucus vesiculosus*) collected in Nova Scotia, Canada; the extract contained Sugars 1–4 (Sugar 1 = glycerol arsenosugar, Sugar 2 = phosphate arsenosugar, Sugar 3 = sulfonate arsenosugar, Sugar 4 = sulfate arsenosugar); structures can be found in Koch et al. (2007). The reference materials were obtained from the National Institute of Standards and Technology (NIST, SRM NIST 2710) and National Research Council of Canada (NRCC, DORM-2 and DORM-3). All other chemicals were analytical grade or better and were obtained from Fisher.

### 2.3. Bioaccessibility extraction using PBET

A physiologically-based extraction test (PBET) was adapted from Ruby et al. (1996) and is carried out in two stages; the first mimics the gastric phase, and the second follows the gastric phase and mimics the intestinal phase. Solution aliquots are removed at each stage for analysis, producing two solutions per sample, the gastric solution (G) and the gastric + intestinal solution (GI). The gastric solution contains 1.25 g/L pepsin (P-7000, Sigma-Aldrich, 1:10,000,), 0.50 g/L sodium citrate, 0.50 g/L DL malic acid disodium, 420 μL/L lactic acid (85%), and 500 μL/L acetic acid, with the pH adjusted to $1.8 \pm 0.05$ using concentrated HCl.

For all samples, 10 g of homogenized sample were mixed with 100 mL of extraction solution to obtain a solvent-to-sample ratio of 10:1 (10 + 1); this was chosen as a conservative estimate of human gastrointestinal conditions when consuming a meal (e.g., a ratio of 3:1 was used in Rodriguez et al., 1999). The solutions were mixed by end-over-end rotation in an incubator set to 37 °C (Innova 4230, New Brunswick Scientific) for 1 h for the gastric phase, with pH checking and adjustment at 30 min. At the end of the gastric phase

mixing, a 10 mL aliquot was collected from the 100 mL solution for analysis (G sample); the remaining 90 mL of solutions were adjusted to pH 7.0 ± 0.2 using a saturated solution of $Na_2CO_3$, and 10 mL of a solution of bile extract (Sigma Aldrich) and pancreatin (Sigma-Aldrich) was added to give final concentrations in the 100 mL solution of 1.75 g/L of bile and 0.5 g/L of pancreatin. The intestinal phase followed, during which samples were mixed for an additional 4 h at 37 °C, with pH adjustment, if necessary, after 2 h. Following mixing, a 10 mL aliquot (GI sample) was collected. All G and GI samples were centrifuged at 3800 rpm for 10 min and then filtered (0.45 μm Millipore Millex-HV Hydrophilic PVDF filter) prior to performing dilutions and analysis.

### 2.4. Total arsenic

Hare samples were digested with concentrated nitric acid ($HNO_3$) using 0.5–2.0 g wet weight of sample, and re-boiled in 3 mL of 30% hydrogen peroxide as described in detail elsewhere (Smith et al., 2008). This procedure could not be used on all other samples because sample sizes were not sufficient. Consequently, total arsenic for all other samples was obtained from the sum of the extracted arsenic (bioaccessibility extraction) and residual arsenic. Residual arsenic was determined by collecting the solids from the completed (GI) bioaccessibility extract after subsamples had been removed (90 mL) on filter paper (Whatman® hardened ashless, Grade 541) and digesting the residue and filter paper with concentrated HNO . Samples were digested at 120 °C with 70% HNO being added as needed, and the digested solution was diluted to 10 mL with distilled deionized water (DDW), and filtered prior to analysis (0.45 μm Millipore Millex-HV Hydrophilic PVDF filter).

Total arsenic analyses for all samples (digests and bioaccessibility extracts) were carried out using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II) with operating conditions described elsewhere (Whaley-Martin et al., 2012). Standard mode analysis (m/z 75) gave, on average, 2600 cps per μg/L and oxide ratios of 0.033 for $CeO^+/Ce^+$ and 0.026 for $Ba^{++}/Ba^+$. Instrumental quality control (QC) tests included blanks and QC calibration checks run once every ten samples and results were accepted when blanks were below detection limits and QC calibration check recoveries were between 80 and 120%.

### 2.5. Speciation analysis of bioaccessibility extracts

Most PBET extracts were analyzed neat, but those with higher arsenic concentrations were diluted to obtain concentrations that were within the calibration curve concentrations of the instrument (1, 10,

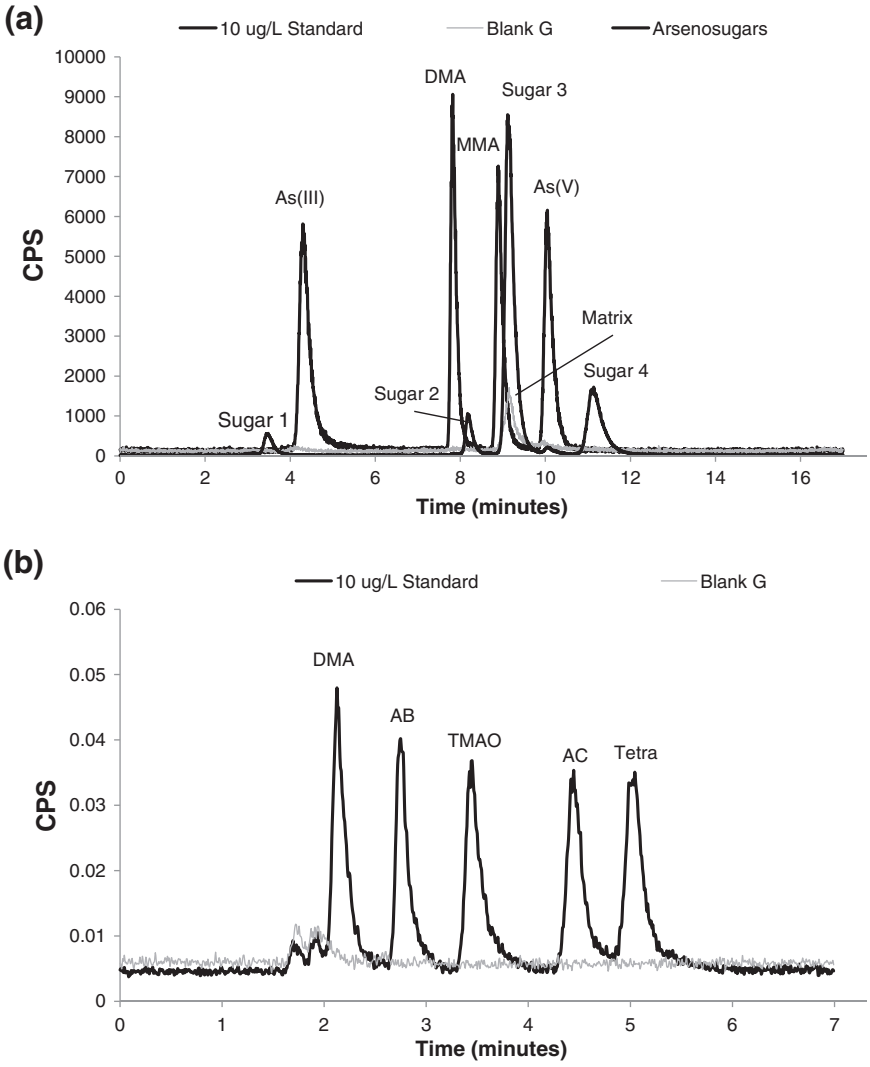


**Fig. 1.** Chromatograms of standards. (a) Anion exchange conditions, gradient elution (see text for details); 10 μg/L Standards = mix of As(III), DMA, MMA and As(V); Blank G = blank of gastric phase of PBET extraction; Arsenosugars = extract as described in the text. (b) Cation exchange conditions (see text); 10 μg/L Standards = mix of DMA, AB, TMAO, AC and Tetra; Blank G = blank of gastric phase of PBET extraction. See text for arsenic species abbreviations.

50, 100 μg/L) (Fig. 1) and analyzed by high performance liquid chromatography (HPLC)–ICPMS using Chromera® Chromatography Data System. Instrument parameters and mobile phases used in the HPLC–ICPMS analysis are described elsewhere (Whaley-Martin et al., 2012); anion gradient conditions with two mobile phases A (4 mM of ammonium nitrate, $NH_4NO_3$) and B (60 mM $NH_4NO_3$ pH = 8.65) were 0–2 min A; 2–3 min A→B; 5–6.5 min B; 6.5–7.5 min B→A; 7.5–10.5 min A; 10.5–11 min A→B; 11–13 min B; 13–13.5 min B→A; 13.5–16 min A. Indium (m/z 115) was used as an internal standard (introduced in the mobile phase) for cation exchange chromatography but the high pH reached in the gradient elution program precluded the use of a suitable internal standard for the anion exchange system. The injection volume was 50 μL. All chromatographic speciation data was then analyzed with Peak Fit© Version 4.12 software. Instrumental QC tests included blanks and QC calibration checks conducted once every ten samples, and results were accepted when blanks were below detection limits and QC calibration check recoveries were between 80 and 130%.

### 2.6. QA/QC

A rigorous quality assurance/quality control (QA/QC) program was conducted throughout the sample analysis. All measured parameters were within the QA/QC limits and the results are tabulated in Table 1; anomalies or exceptions are as follows. Blank results in the bioaccessibility extract analyses (i.e., total arsenic in extracts) indicated more uncertainty in the GI extracts, probably attributable to matrix components (e.g., chloride and organic chemicals), which caused the detection limit to be 10× higher (0.05 mg/kg) than that for G extracts (0.005 mg/kg). Speciation analysis indicated the presence of a peak attributed to matrix in all samples (including blanks) appearing at the retention time of Sugar 3 (Fig. 1). Even though it consists of a different matrix from those analyzed in the present study, the soil SRM NIST 2710 was used to monitor control of the bioaccessibility extraction, since our lab has established control limits for arsenic in this sample (25–53% bioaccessibility) that are consistent with bioavailability results (37–52%) (Koch et al., accepted for publication). A fish SRM (DORM-2) with certified values for AB was also included to monitor accuracy of speciation analysis with satisfactory results. Matrix spikes (sample fortified with known concentrations of arsenic species) were also included with mostly satisfactory results although some matrix effects (enhancement of signal) may be present (Table 1). Samples were analyzed singly with appropriate replications (duplicates, except for one sample extracted in triplicate) in each batch; a total of 6 samples of the 26 studied samples were extracted in duplicate or triplicate (1 duplicate and 1 triplicate for 9 plant samples, 1 duplicate for 7 mushroom samples, and 3 duplicates for 10 hare samples). Replicates had acceptable repeatability (actual results are given in Tables 2 to 4) with relative percent different (RPD = 100% × difference in 2 values/average of 2 values) and relative standard deviation (RSD = 100% × standard deviation of 3 values/average of 3 values) results summarized in Table 1. The ranges and averages shown in Table 1 do not include a sample that duplicated poorly during the bioaccessibility extraction (Blueberries NS 4 from tailings, Table 2). The reason for the poor duplication was not clear, but may have been attributable to heterogeneity of the sample. The sample size was not sufficient to repeat the analysis. Column recovery (CR) values (Tables 2 to 4) represent the percent of arsenic in the bioaccessibility extract that was quantified by the HPLC–ICP-MS method (i.e., % CR = sum of species by HPLC–ICP-MS/bioaccessible [As] by ICP-MS). CRs close to 100% usually indicate that all the arsenic extracted was recovered from the analytical column, although an acceptable range of 70–130% takes into account the different sources of uncertainty in the comparison. For most samples % CR fell within this range but for 7 extracts a lower amount was obtained. Incomplete recovery can be a result of arsenicals forming complexes with proteins, and these complexes binding strongly to the column's stationary phase (Slejkovec et al., 2008). One extract with CR of 140% is unexplained but is likely a result of matrix effects specific to the sample, enhancing signals during the speciation analysis.

## 3. Results and discussion

The results of arsenic speciation, percent bioaccessibility, and total arsenic in all individual samples are presented in Table 2 (plants), Table 3 (mushrooms) and Table 4 (hare muscle) and are displayed graphically in Figs. 2 and 3. The berries contained predominantly inorganic arsenic (iAs = As(V) + As(III)) in the bioaccessibility extracts, which is consistent with arsenic speciation in other plant materials reported in the literature (Reimer et al., 2010). DMA was not found in large amounts in any berry samples; thus the bioaccessible fraction of contaminated berries is primarily composed of toxic arsenic species. Labrador tea (*Rhododendron groenlandicum*) was included in the present study as a plant material often used as a country food to make tea or medicinal extracts. Bioaccessibility extracts contained AB and TMAO (at low concentrations) in addition to iAs and DMA. The finding

**Table 1**
Quality control results in percent bioaccessibility (NIST 2710 and DORM-2 for bioaccessibility extraction), percent recovery (DORM-3, QC checks, matrix spikes and DORM-2 for speciation), and percent RPD/RSD (replicates), unless otherwise indicated.

| QC test | Analysis | n | Range | | Average | Comment |
|---|---|---|---|---|---|---|
| | | | P1 | P2 | | |
| Blanks (mg/kg) | Bioaccessibility extraction | 4 | <0.005 | <0.05 | NA | Acceptable |
| NIST 2710 | Bioaccessibility extraction | 4 | 39–51 | 38–49 | 44, 43 | Acceptable |
| DORM-2 | Bioaccessibility extraction | 1 | 102, 94 | | NA | Acceptable |
| Replicates | Bioaccessibility extraction | 12 | 0.1–31 | | 11 | Does not include blueberries NS 4 (see text) |
| Blanks (mg/kg) | Residue digestion | 3 | <0.005 | | NA | Acceptable |
| DORM-3 | Residue digestion | 2 | 83, 125 | | 104 | Acceptable |
| Replicates | Total As (sum) | 6 | 3.4–11 | | 7.3 | Acceptable |
| Blanks (mg/kg) | Total As hares | 1 | <0.005 | | NA | Acceptable |
| DORM-3 | Total As hares | 1 | 123 | | NA | Acceptable |
| Duplicate | Total As hares | 1 | 8.4 | | NA | Acceptable |
| Blanks (mg/kg) | Speciation | 4 | <0.005 anion<br><0.005 cation | | NA | Acceptable |
| Matrix spikes | Speciation | 3 | 95–136 anion<br>83–144 cation | | 111, 115 | Some matrix effects indicated |
| DORM-2 | Speciation | 1 | 102 | 94 | NA | Acceptable |
| Replicates | Speciation | 42 | 0.3–64 | | 17 | Does not include blueberries NS 4 (see text) |

**Table 2**
Total arsenic and arsenic species (average ± SD for replicate extractions; n = 2 except where indicated) in bioaccessibility (BA) extracts (mg/kg wet weight) of plant country food samples. Blank cells are less than the detection limit of 0.005 mg/kg. Tr = values between 0.005 and 0.010 mg/kg. na = not applicable. G = gastric; GI = gastric + intestinal.

| Sample | [As] in sample | [As] in BA extract | iAs | DMA | AB | TMAO | Sum | CR % | % BA |
|---|---|---|---|---|---|---|---|---|---|
| Blueberries NS G | 0.059 | 0.026 | 0.016 | | | | 0.016 | 61 | 45 |
| Blueberries NS GI | 0.059 | <0.05 | | | | | | na | <100 |
| Blueberries (tailings) NS 1 G | 14 | 3.4 | 2.4 | 0.018 | | | 2.4 | 71 | 24 |
| Blueberries (tailings) NS 1 GI | 14 | 4.6 | 3.8 | | | | 3.8 | 82 | 32 |
| Blueberries (tailings) NS 2 G[a] | 8.0 | 2.3 | 1.7 | 0.020 | | tr | 1.8 | 77 | 29 |
| Blueberries (tailings) NS 2 GI | 8.0 | 2.9 | 2.1 | 0.034 | | | 2.2 | 73 | 37 |
| Blueberries (tailings) NS 3 G | 19 | 4.9 | 3.3 | 0.019 | | | 3.3 | 68 | 26 |
| Blueberries (tailings) NS 3 GI | 19 | 6.9 | 5.0 | 0.023 | | | 5.1 | 73 | 37 |
| Blueberries (tailings) NS 4 G[a] | 21 ± 2 | 2.6 ± 2.5 | 1.9 ± 1.6 | tr | | | 1.9 ± 1.6 | 83 ± 16 | 13 ± 13 |
| Blueberries (tailings) NS 4 GI | 21 ± 2 | 3.7 ± 3.4 | 3.6 ± 3.0 | tr | | | 3.6 ± 3.0 | 103 ± 12 | 18 ± 18 |
| Raspberries NS G | 0.16 | 0.069 | 0.028 | | | | 0.028 | 41 | 44 |
| Raspberries NS GI | 0.16 | 0.093 | 0.053 | | tr | | 0.053 | 57 | 59 |
| Blackberries NS G[b] | 0.067 ± 0.003 | 0.024 ± 0.007 | 0.020 ± 0.002 | tr | | | 0.024 ± 0.002 | 106 ± 25 | 36 ± 12 |
| Blackberries NS GI[b] | 0.067 ± 0.003 | 0.055[c] | 0.023 ± 0.003 | tr | | | 0.031 ± 0.005 | 65[c] | 79[c] |
| Cranberry YK G | 0.11 | 0.020 | 0.021 | | | | 0.021 | 101 | 19 |
| Cranberry YK G | 0.11 | 0.066 | 0.052 | | | | 0.052 | 79 | 60 |
| Labrador tea YK GI | 1.9 | 0.19 | 0.16 | tr | 0.024 | 0.087 | 0.027 | 140 | 10 |
| Labrador tea YK GI | 1.9 | 0.32 | 0.14 | tr | 0.047 | 0.17 | 0.36 | 110 | 17 |

[a] Blueberries (tailings) NS 2 G contained 0.015 mg/kg of AC; Blueberries (tailings) NS 4 G contained 0.014 mg/kg of Tetra.
[b] n = 3.
[c] Value reported for one sample replicate; other replicate was non-detect.

of AB in a plant sample is highly unusual and has been seen in plants only rarely, and in small concentrations, in other studies (Geiszinger et al., 2002; Mattusch et al., 2000). Plants could therefore provide a food source of this compound for terrestrial consumers, albeit in small amounts. Future studies are planned to determine if AB is found in samples of this plant from other contaminated locations.

The speciation of mushrooms differed according to the species, which is consistent with the literature (Kuehnelt and Goessler, 2003). The differences between mushroom species are highlighted in Fig. 3, which summarizes the proportions of arsenic species found in the bioaccessibility extracts. "Bolete" mushrooms (with pored caps; in Fig. 3 this group includes *Suillus luteus*) contain a large proportion of DMA. Meanwhile, *Agaricus* mushrooms (the genus to which common white button mushroom, *Agaricus bisporus*, belongs) contain predominantly AB. Both bolete and *Agaricus* sp. also had significant levels of toxic iAs. *Laccaria laccata* contained predominantly DMA, as well as a large proportion of TMAO; these results contrast with the predominance of iAs reported in the same mushroom species (Slekovec et al., 1999), but are similar to those reported for *Laccaria amethystina* (Larsen et al., 1998). Since these results differ from those seen in the literature, and are based on a single sample in the present study, generalizations for this mushroom may not be possible at this point. The results in the present study confirm that mushrooms must be collected and analyzed from each new site and study.

Although 12 hare samples were available, results are reported only for the 10 samples that had detectable levels of arsenic (Table 4). The two samples that did not have detectable levels of arsenic (<0.005 mg/kg) came from a contaminated site (sampled in 2005) in Nova Scotia. For the remaining hares, results are variable, which is likely associated with differences in food sources, contamination level, geographical location (Yellowknife vs. Nova Scotia), trapping time, etc. The arsenic species identified in the bioaccessibility extracts were iAs, DMA, TMAO, AB, AC, Sugar 4 (Fig. 2) and a cationic unknown compound that eluted from the column between TMAO and AC. The unknown compound is suspected to be trimethylarsonium propionate ($(CH_3)_3As^+CH_2CH_2COO^-$), but this identification needs to be verified with detailed spiking and molecular mass spectrometry experiments with an authentic standard.

The major arsenic species found in the hare bioaccessibility extracts was DMA. The highest concentrations of total bioaccessible arsenic (0.32 mg/kg, corresponding to 54% bioaccessibility), were observed in the hare with the ID YK1 2010 (collected from a contaminated site in

**Table 3**
Total arsenic and arsenic species (average ± SD for duplicate extractions) in bioaccessibility (BA) extracts (mg/kg wet weight) of edible mushroom country food samples. Blank cells are less than the detection limit of 0.005 mg/kg. Tr = values between 0.005 and 0.010 mg/kg. na = not applicable. G = gastric; GI = gastric + intestinal.

| Sample | [As] in sample | [As] in BA extract | iAs | DMA | AB | TMAO | AC | Tetra | Sum | CR % | % BA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bolete YK G | 2.3 | 1.0 | 0.065 | 1.1 | | | | | 1.1 | 110 | 45 |
| Bolete YK GI | 2.3 | 1.0 | 0.061 | 1.00 | | 0.043 | | | 1.1 | 107 | 45 |
| *Agaricus* sp. YK 1 G | 4.8 | 2.7 | 0.39 | 0.022 | 0.43 | | | | 0.84 | 31 | 58 |
| *Agaricus* sp. YK 1 GI | 4.8 | 2.8 | 1.7 | 0.025 | 0.38 | | tr | | 2.1 | 75 | 59 |
| *Agaricus* sp. YK 2 G | 1.2 | 1.1 | 0.41 | 0.070 | 0.49 | | | | 0.96 | 86 | 91 |
| *Agaricus* sp. YK 2 GI | 1.2 | 1.2 | 0.42 | 0.059 | 0.51 | | | | 0.98 | 85 | 94 |
| *Agaricus* sp. YK 3 G | 2.7 | 1.8 | 0.16 | 0.053 | 1.3 | | | | 1.5 | 84 | 69 |
| *Agaricus* sp. YK 3 GI | 2.7 | 1.8 | 0.26 | 0.047 | 1.1 | 0.011 | 0.022 | tr | 1.5 | 80 | 68 |
| *Agaricus* sp. YK 4 G | 4.2 | 3.4 | 0.43 | 0.030 | 1.6 | | | | 2.1 | 63 | 80 |
| *Agaricus* sp. YK 4 GI | 4.2 | 3.6 | 0.44 | 0.030 | 1.7 | | | | 2.2 | 62 | 84 |
| *Laccaria laccata* NS G | 46 | 9.3 | 0.036 | 3.4 | 1.00 | 4.3 | | | 8.7 | 94 | 20 |
| *Laccaria laccata* NS GI | 46 | 9.9 | 0.056 | 4.3 | 0.73 | 3.1 | | | 8.3 | 83 | 22 |
| *Suillus luteus* NS G | 0.50 ± 0.03 | 0.193 ± 0.003 | 0.0391 ± 0.0001 | 0.12 ± 0.02 | 0.0138 ± 0.0001 | 0.011 ± 0.003 | tr | 0.014 ± 0.002 | 0.21 ± 0.02 | 109 ± 10 | 40 ± 2 |
| *Suillus luteus* NS GI | 0.50 ± 0.03 | 0.25 ± 0.07 | 0.059 ± 0.001 | 0.14 ± 0.04 | 0.017 ± 0.002 | 0.014 ± 0.001 | tr | 0.015 ± 0.001 | 0.25 ± 0.04 | 101 ± 10 | 50 ± 10 |

**Table 4**
Total arsenic and arsenic species (average ± SD for duplicate extractions) in bioaccessibility (BA) extracts (mg/kg wet weight) of hare muscle country food samples. Blank cells are less than the detection limit of 0.005 mg/kg. Tr = values between 0.005 and 0.010 mg/kg. na = not applicable. C = contaminated site, UC = uncontaminated site. G = gastric; GI = gastric + intestinal.

| Hare muscle | [As] in sample | [As] in BA extract | iAs | DMA | AB | TMAO | AC | Sugar 4 | Cation UK | Sum | CR % | % BA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C YK1 2010 G | 0.60 | 0.32 ± 0.01 | 0.037 ± 0.002 | 0.17 ± 0.03 | tr | tr | | | 0.017 ± 0.003 | 0.245 ± 0.006 | 77 ± 1 | 52 ± 2 |
| C YK1 2010 GI | 0.60 | 0.33 ± 0.02 | 0.080 ± 0.010 | 0.20 ± 0.02 | tr | tr | | | 0.020 ± 0.002 | 0.31 ± 0.03 | 95 ± 3 | 54 ± 4 |
| C YK2 2010 G | 0.55 | 0.15 | tr | 0.077 | | 0.025 | | 0.011 | tr | 0.11 | 76 | 27 |
| C YK2 2010 GI | 0.55 | 0.17 | 0.020 | 0.089 | | 0.027 | | tr | 0.010 | 0.15 | 93 | 30 |
| C YK3 2010 G | 0.54 | 0.20 ± 0.01 | 0.019 ± 0.001 | 0.06 ± 0.02 | 0.030 ± 0.009 | 0.026 ± 0.004 | 0.010 ± 0.003 | 0.0121 ± 0.0002 | 0.027 ± 0.002 | 0.19 ± 0.02 | 96 ± 4 | 36 ± 2 |
| CYK3 2010 GI | 0.54 | 0.200 ± 0.009 | 0.042 ± 0.002 | 0.093 ± 0.003 | 0.021 ± 0.002 | 0.023 ± 0.004 | tr | tr | 0.022 ± 0.002 | 0.200 ± 0.003 | 99 ± 6 | 37 ± 2 |
| C YK4 2010 G | 0.58 | 0.20 | 0.026 | 0.081 | | 0.025 | | 0.010 | 0.013 | 0.16 | 79 | 34 |
| C YK4 2010 GI | 0.58 | 0.20 | 0.048 | 0.12 | | 0.027 | | tr | 0.014 | 0.20 | 99 | 36 |
| UC YK 2010 G | 0.18 | 0.063 | 0.010 | 0.038 | | | | tr | | 0.048 | 77 | 34 |
| UC YK 2010 GI | 0.18 | 0.076 | 0.016 | 0.063 | | | | | | 0.079 | 104 | 42 |
| UC YK1 2000 G | 0.102 ± 0.006 | 0.054 ± 0.006 | 0.014 ± 0.003 | tr | 0.052 ± 0.006 | | tr | | | 0.066 ± 0.009 | 120 ± 4 | 53 ± 5 |
| UC YK1 2000 GI | 0.102 ± 0.006 | 0.078 ± 0.001 | 0.018 ± 0.008 | 0.012 ± 0.001 | 0.062 ± 0.005 | | tr | | tr | 0.09 ± 0.01 | 119 ± 17 | 76.4 ± 0.1 |
| UC YK2 2000 G | 0.016 | <0.02 | 0.033 | 0.092 | | | | | | 0.16 | na | <30 |
| UC YK2 2000 GI | 0.016 | <0.02 | 0.032 | 0.014 | | | | tr | | 0.046 | na | <30 |
| C NS 2010 G | 0.021 | <0.02 | tr | | | | | | | tr | na | <100 |
| C NS 2010 GI | 0.021 | <0.02 | 0.014 | | | | | | | 0.014 | na | <100 |
| UC NS1 2005 G | 0.44 | 0.20 | 0.053 | 0.094 | tr | | | 0.030 | | 0.18 | 90 | 45 |
| UC NS1 2005 GI | 0.44 | 0.23 | 0.018 | | | | | | | 0.018 | 87 | 52 |
| UC NS2 2005 G | 0.007 | <0.02 | 0.012 | | | | | | | 0.012 | na | <100 |
| UC NS2 2005 GI | 0.007 | <0.02 | 0.016 | | | | | | | 0.016 | na | <100 |

Yellowknife, in the summer). The bioaccessibility extracts for this hare contained predominantly DMA (0.2 mg/kg) and iAs (0.079 mg/kg) and a small amount of AB (0.010 mg/kg).

The highest percent bioaccessibility (76%) was found in a hare from an uncontaminated site in the fall (ID YK1 2000), although it only corresponded to 0.078 mg/kg of bioaccessible arsenic. The majority of the arsenic found in this extract was AB, and the largest proportions of AB were measured in hares collected in the autumn. In autumn, mushroom growth is abundant, especially in Yellowknife, and mushrooms from Yellowknife have been reported to contain AB (Koch et al., 2000). We therefore propose that AB is present in hare tissue after being ingested, absorbed and retained; this compound is known to be retained in muscle tissue of rabbits (Vahter et al., 1983). Mushrooms are not normally reported as part of a hare's diet in the literature (e.g., Wolff, 1978), but the possibility exists that hares consume them when they are available, according to anecdotal reports on the internet. The AB proportion shown in Fig. 3 (based on the mean of all samples, approximately 7%) ranges from 0 to 79%.

Overall the bioaccessible arsenic in hares is primarily composed of the more toxic compounds iAs and DMA, in approximately equal proportions. This appears to be true for Nova Scotia hares, since a smaller range of arsenic species (mostly only iAs and DMA), along with lower arsenic concentrations, were found, compared with those from Yellowknife. The hare samples in the present study were analyzed uncooked, but cooking is a likely step prior to their consumption. Organoarsenic species may be stable at lower cooking temperatures (e.g., boiling) based on previous studies but changes can occur at higher temperatures, such as those used for frying, including the conversion of AB to TMAO and Tetra (Devesa et al., 2008). Future studies in this area would be warranted.

The percent bioaccessibility values for most samples were less than 100%. For most samples an increase was seen in bioaccessibility when the extraction time was extended to the intestinal phase. This increase was statistically significant ($\alpha = 0.05$) when all samples (detectable results only) were considered together (paired *t* test, 2 tails, $n = 22$, $p < 0.05$) and for plants only (paired *t* test, 2 tails, $n = 8$, $p < 0.05$), but not for mushrooms (paired *t* test, 2 tails, $n = 7$, $p = 0.102$) and hares (paired *t* test, 2 tails, $n = 7$, $p = 0.072$). This may indicate that in the absence of in vivo results to which bioaccessibility values can be compared, future bioaccessibility studies of food items should include an intestinal phase in the method to ensure conservative (highest) estimates. An increased bioaccessibility value in the intestinal phase, and with increased extraction time, has been noted for arsenic in some types of soil and tailings matrices as well (Meunier et al., 2010, 2011). For the most part, the species extracted in the intestinal phase in the present study were the same as those extracted in the gastric phase. Mean bioaccessibilities are summarized in Table 5. The highest bioaccessibilities were measured in *Agaricus* sp. samples, which also had the largest proportion of AB.

The comparison of bioaccessibility tests with in vivo bioavailability measurements has not been carried out for contaminants in food and only three studies have reported bioavailability of arsenic in food (Juhasz et al., 2008; He and Zheng, 2010; Juhasz et al., 2006). Therefore the suitability of bioaccessibility extractions in representing in vivo bioavailability measurements is still unknown and merits further study.

## 4. Concluding remarks

For the first time, bioaccessible arsenic species are reported for a number of different country foods from Canadian contaminated sites. Bioaccessible arsenic was generally less than 100%, with the lowest bioaccessibilities observed for berries and plants. In these samples the species were mostly toxic inorganic arsenic. This speciation information

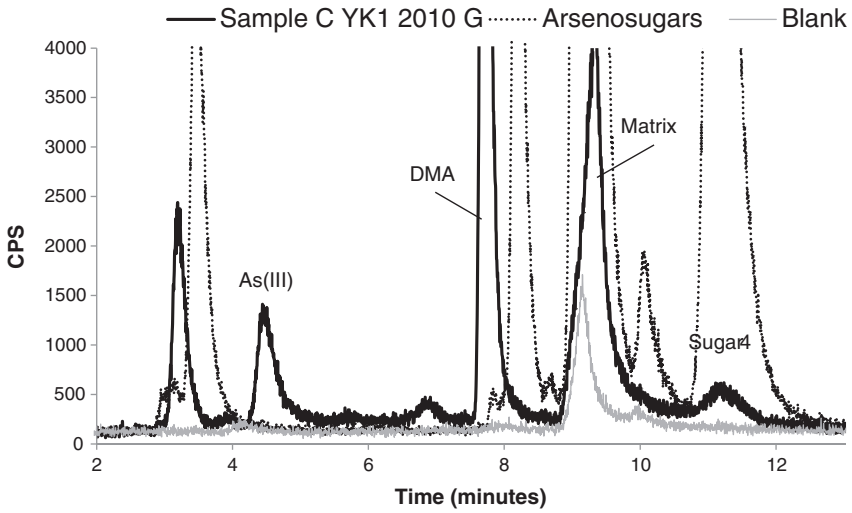


**Fig. 2.** Anion exchange chromatogram of hare tissue PBET extract (gastric) showing a small amount of Sugar 4.

would not refine exposure estimates that assume all arsenic present is in a toxic form, but the low bioaccessibilities would lower such exposure estimates. Hence conducting bioaccessibility measurements for these sample types is worthwhile when estimating risk to populations eating berries or plants. Higher bioaccessibilities were observed for mushrooms and hare muscle, where less toxic species were found (along with inorganic arsenic). However, great variability was seen in these sample types suggesting that generalizations cannot be made at this point about their arsenic speciation. This again suggests that measuring bioaccessibility, together with arsenic speciation, is necessary.

When conducting speciation analysis for risk assessment, an extraction method with physiological relevance, such as a bioaccessibility test, is more valuable than incomplete extractions using solvents that do not have any physiological relevance. Overall the incorporation of bioaccessibility and arsenic speciation data into the exposure assessment can further refine and improve the risk assessment process. Future work will address the identification of unknown arsenic species, as well as the effect of preparation techniques for human consumption (e.g., cooking) on bioaccessibility and speciation.

## Conflict of interest statement

We declare that no conflict of interest affects this work.

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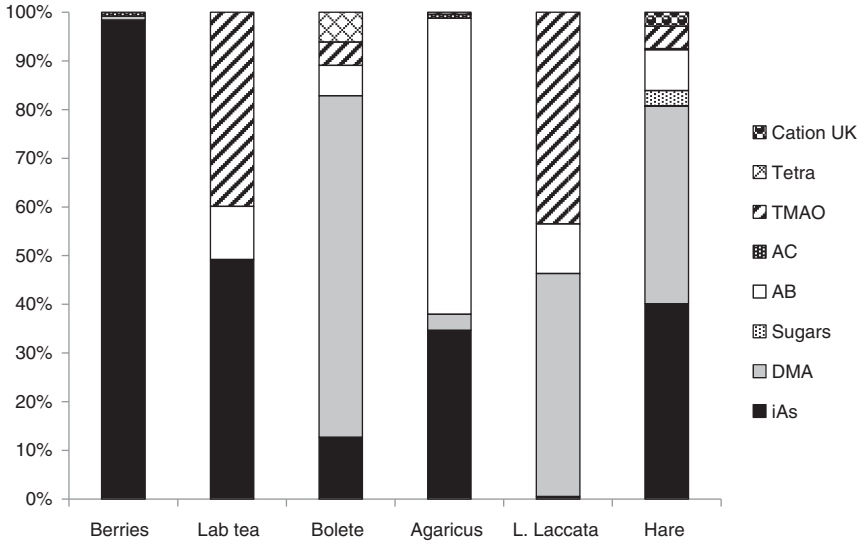


**Fig. 3.** Mean proportions of arsenic species in bioaccessibility extracts of country foods. Lab tea = Labrador tea (*Rhododendron groenlandicum*); Bolete = Bolete and *Suillus luteus*; L. Laccata = *Laccaria laccata*. Bolete, *L. laccata* and *Agaricus* are all mushrooms. Cation UK is peak appearing in the cation exchange chromatogram that does not match any available standards.

**Table 5**
Mean percent bioaccessibility (BA) (detectable values only) ± standard deviation. N in () indicates detectable values. Bolete/Suillus, *Agaricus* sp. and *Laccaria laccata* are all mushrooms.

| Sample | Mean % BA G | Mean % BA GI | N samples |
|---|---|---|---|
| Berries | 29 ± 12 | 45 ± 21 | 8 (8 for G, 7 for GI) |
| Lab tea | 10 | 12 | 1 (1) |
| Bolete/Suillus | 29 ± 23 | 31 ± 21 | 2 (2) |
| *Agaricus* sp. | 74 ± 14 | 76 ± 16 | 4 (4) |
| *Laccaria laccata* | 21 | 22 | 1 (1) |
| Hare | 40 ± 10 | 47 ± 17 | 12 (7) |

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