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Arsenic uptake by the Douglas-fir (*Pseudotsuga menziesie*)†

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The Douglas fir (*Pseudotsuga menziesie*) growing in an arsenic-rich gold-bearing region contains elevated arsenic concentrations in new-growth stems (374 ppm dry weight (dw)) and needles (257 ppm dw). Speciation of methanol–water extracts by using high-performance liquid chromatography–inductively coupled plasma mass spectrometry show that arsenite is the major species in needles but arsenate is more dominant in stems. Only traces of methylarsenicals are present. Arsenic concentrations in other tree species growing in the region are generally much lower; dimethylarsinate was extracted from a spruce cone. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: arsenic; accumulation; Douglas fir; gold mine; HPLC–ICP-MS

INTRODUCTION

The Bridge River district is located north of Whistler in British Columbia, Canada. In the first half of the 1900s many gold-bearing quartz veins were found in this area, and some of the more profitable ones were located close to the small town of Bralorne.[1] The Bralorne–Pioneer gold camp, which included the Bralorne, King and Pioneer mines, was eventually abandoned in 1971 because of low gold prices. The ore of these mines consists of gold in quartz veins containing small amounts of sulfides. Most of the sulfide is present as pyrite (FeS_2) and arsenopyrite (FeAsS), which are usually associated with the gold in the mined ore.[1,2] Arsenopyrite is an ore mineral that, on weathering, slowly releases arsenic into the environment.[3] Mining activity and mineral processing results in the acceleration of this process. Thus, arsenic is often a waste product from the mining of metals and it results in the contamination of many areas.

Arsenic has been used as a pathfinder element in the search for precious-metal deposits because of the well-established association of arsenic with gold and other metals.[4] Thus, soil samples were commonly analysed for arsenic in attempts to locate gold-rich veins, but biogeochemical techniques were introduced in the 1950s in order to solve problems associated with soil analysis and sampling.[5] Tree roots have the advantage of sampling at great depth and over wide areas. Warren *et al*.[4] analysed plants for arsenic as indicators of gold mineralization and unexpectedly discovered that the Douglas-fir (*Pseudotsuga menziesii*) had a remarkable affinity for arsenic. They report arsenic concentrations of over 1000 ppm in the 'ash' of the most recent growth of trees situated within 60 m of mineralization.

More recently Ma *et al*.[6] have found that the Chinese brake fern (*Pteris vittatta* L.) is a hyper-accumulator of arsenic. The element can amount to 2.3% of dry plant weight in plants grown in arsenic-rich soil. Another fern from Thailand (*Pityrogramma calomelanos*)[7] is also an arsenic hyper-accumulator: the fronds contain up to 8350 ppm arsenic on a dry weight basis.

This paper summarizes some results obtained from a reinvestigation of the arsenic-accumulating ability of the Douglas fir, and a determination of the arsenic species involved.

MATERIALS AND METHODS

Materials

Tree samples of Douglas-fir (*P. menziesii*), spruce (*Picea engelmannii*) and fir (*Abies amabilis*) were collected in May 2001 from three sites in the Bridge River district: Moha, Mission Dam, and Pioneer Mine.[8] A second sampling trip to

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the Pioneer mine was made in early September 2001. Tree branches, needles and cones were collected by hand from ground level and stored in Ziploc® or black plastic bags. All samples were kept cool following collection, and upon return to the laboratory were kept at 4 °C until processing. The identification of trees was carried out by reference to a guide book[9] and with help from a botanist, Olivia Lee, UBC.

The needles and cones (if present) of each tree sample were separated from the stems. Samples from the second collection were split into two sub-samples, one part of which was freeze-dried and the other part kept fresh. All samples from both collections were finely ground using a coffee grinder prior to further processing.

Chemicals and reagents used included methanol (HPLC grade, Fisher), tetraethylammonium hydroxide (TEAH, 20 wt%, Aldrich), malonic acid (BDH), nitric acid (68–71%, sub-boiling distilled, Seastar Chemicals), sodium borohydride (Aldrich), acetic acid, hydrochloric acid, and hydrogen peroxide (30%, Fisher). Water, with resistivity better than 18 MΩ cm, was used to make up all standard solutions, mobile phases, and sample solutions for extraction and digestion.

Standard working solutions of arsenite (arsenic(III)), arsenate (arsenic(V)), methylarsonic acid ($CH_3AsO(OH)_2$, MMA), dimethylarsinic acid ($(CH_3)_2AsO(OH)$, DMA), trimethylarsine oxide ($(CH_3)_3AsO$, TMAO), and arsenobetaine ($(CH_3)_3As^+CH_2COO^-$, AsB) were made by diluting previously prepared stock solutions with deionized water as necessary. MMA, AsB, and TMAO were prepared in our laboratories by Dr Hongsui Sun. A rhodium stock solution was made from $RhCl_3$ for use as an internal standard in total digests and in the TEAH mobile phase used in high-performance liquid chromatography (HPLC) analysis.

Methods

Nitric acid digestion was performed on freeze-dried samples, wet samples and standard reference materials for total arsenic. The method used was similar to that described previously.[10,11] Hydrogen peroxide (2 ml) was added to each sample to ensure complete digestion. Digested samples were redissolved in 5 ml of 1% nitric acid solution that contained 5 ppb rhodium, vortex mixed, filtered (0.45 μm) and then stored at 4 °C until ready for analysis. A VG Plasma Quad 2 Turbo Plus ICP mass spectrometer was used to analyse the digests for total arsenic.

Samples and reference material were extracted using a multiple methanol/water (1:1, v/v) extraction method similar to that described by Lai and co-workers.[10,11] The extracts were stored at −20 °C to preserve sample integrity until ready to be analysed. Speciation analysis was performed on the extracts by using ion-pairing HPLC–inductively coupled plasma mass spectrometry (ICP-MS). Arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of standard arsenic compounds. Quantitative analysis was done by using external calibration curves for each compound corresponding to a matching standard.

The apparatus and operating parameters for the analytical procedures have been reported previously.[10]

The following standard reference materials were used for quality assurance/control: fucus (IAEA-140, Monaco), oyster tissue (NIST-1566a), DORM-2 (NRC, Canada) and pine needle Standard Reference Material (NIST-1575). All samples, including standards, were digested in duplicate and analyses were performed on each sample in triplicate.

RESULTS

The concentrations of arsenic in the acid-digested Douglas-fir tissues collected from the Moha and Mission Dam sites range from 0.81 ± 0.08 to 3.1 ± 0.7 ppm.[8] The tissues of small Douglas-fir trees (less than 10 m high) sampled from the Pioneer mine showed much higher levels of arsenic, e.g. 1.5 ± 0.8 to 374 ± 10 ppm in freeze-dried stems. The total arsenic content of the needles of a Douglas-fir (257 ± 11 ppm) located adjacent to the mine had approximately 500 times that of a neighbouring spruce tree of equal height (0.4 ± 0.01 ppm).

Speciation analysis was performed only on samples from the Pioneer mine site because the acid-digestion analysis revealed low arsenic levels in the Moha and Mission Dam samples. The arsenic speciation results of several Douglas-fir and spruce tree samples collected from the mine are shown in Tables 1 and 2. Concentration data from all the samples are available.[8] Inorganic arsenic was found to be the main species present in all Douglas-fir trees sampled from the Pioneer mine. The extractable arsenic in the needles is found mainly as arsenic(III): arsenic(V) is more dominant in the extracts of stems. A typical chromatograph is shown in Fig. 1.

Only trace amounts of the methylated arsenic species MMA and DMA were found in some Pioneer-mine tree samples. Spruce cone samples showed atypical results; DMA was found to be a major species in the extract, second to that of arsenic(V). A chromatogram of one of these samples is shown in Fig. 2.

DISCUSSION

The soil concentrations of arsenic in the mine sampling area ranged from 35 to 1623 ppm, which would be expected for a mine-impacted site.[7] The arsenic concentration at the other two sites, Moha and Mission Dam, was in the range 2.2 to 14 ppm. All these soil samples were taken for another project and the results cannot be correlated with the results from the trees, which often grow on surfaces best described as rock.[8]

Typical concentrations of arsenic in terrestrial plants are <1 ppm in uncontaminated areas;[12] however, studies have shown that some plants can accumulate much higher arsenic concentrations than this from contaminated soils. In the 1960s it was unexpectedly discovered by Warren *et al*.[4] that Douglas-fir trees had an affinity for arsenic. The arsenic content of needles and stems ranged from 8 to 1550 ppm, with the highest concentration being in the new growth. King

Table 1. Concentration of the arsenic species[a] in Douglas-fir tree samples from Pioneer mine (ppm wet weight; errors are reported as standard error of the mean (SEM) at the 95% confidence level (CL))

Sample (trip#-site#)	Tissue Type	As(III)	As(V)	DMA	MMA	Sum of As species
1-1	Stem	2.86	15.62	n.d.	n.d.	18.48
	Needle	78.21	21.74	n.d.	n.d.	99.95
1-2	Stem	0.73	3.99	0.16	n.d.	4.88
	Needle	10.93	10.97	n.d.	n.d.	21.90
2-1	Cone	8 ± 1	4.9 ± 0.8	n.d.	n.d.	12.9
	Needle	182 ± 5	28 ± 2	n.d.	n.d.	210
	Stem	19 ± 5	18 ± 5	n.d.	n.d.	37
2-1	Cone	4.2 ± 0.1	8.3 ± 0.6	n.d.	n.d.	12.5
	Needle	154 ± 4	22.1 ± 0.1	n.d.	n.d.	176.1
	Stem	29 ± 7	36 ± 9	n.d.	n.d.	65
2-2	Needle	34 ± 2	5 ± 2	n.d.	n.d.	39
	Stem	130 ± 2	46 ± 20	n.d.	n.d.	176
2-3	Needle	27.6 ± 0.5	4 ± 2	n.d.	n.d.	31.6
	Stem	21 ± 2	13 ± 3	n.d.	n.d.	34

[a] n.d.: less than detection limit (DL).

Table 2. Concentration of the arsenic species[a] in spruce tree samples from Pioneer mine (ppm wet weight; errors are reported as SEM at a 95% CL)

Sample (trip#-site#)	Tissue type	As(III)	As(V)	DMA	MMA	Sum of As species
1-1	Needle	trace	0.11	n.d.	trace	0.11
	Stem	trace	0.13	n.d.	trace	0.13
1-3	Cone	n.d.	0.11	0.05	n.d.	0.16
	Needle	trace	trace	trace	trace	trace
	Stem	trace	0.06	trace	n.d.	0.06
1-3	Cone	trace	0.20	0.08	trace	0.28
	Needle	trace	trace	trace	trace	trace
	Stem	trace	0.07	trace	trace	0.07
2-1	Needle	trace	0.019 ± 0.004	n.d.	n.d.	0.02
	Stem	trace	0.036 ± 0.006	n.d.	n.d.	0.04

[a] Trace: greater than or equal to DL but less than limit of quantification; n.d.: less than DL.

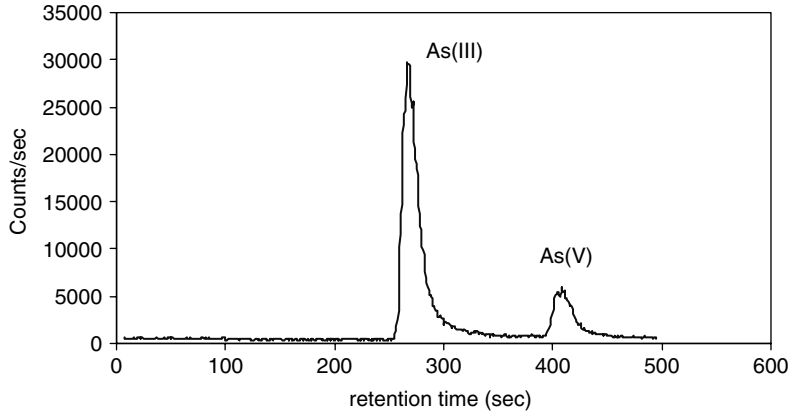

Figure 1. Chromatogram of a needle extract from a Douglas-fir tree.

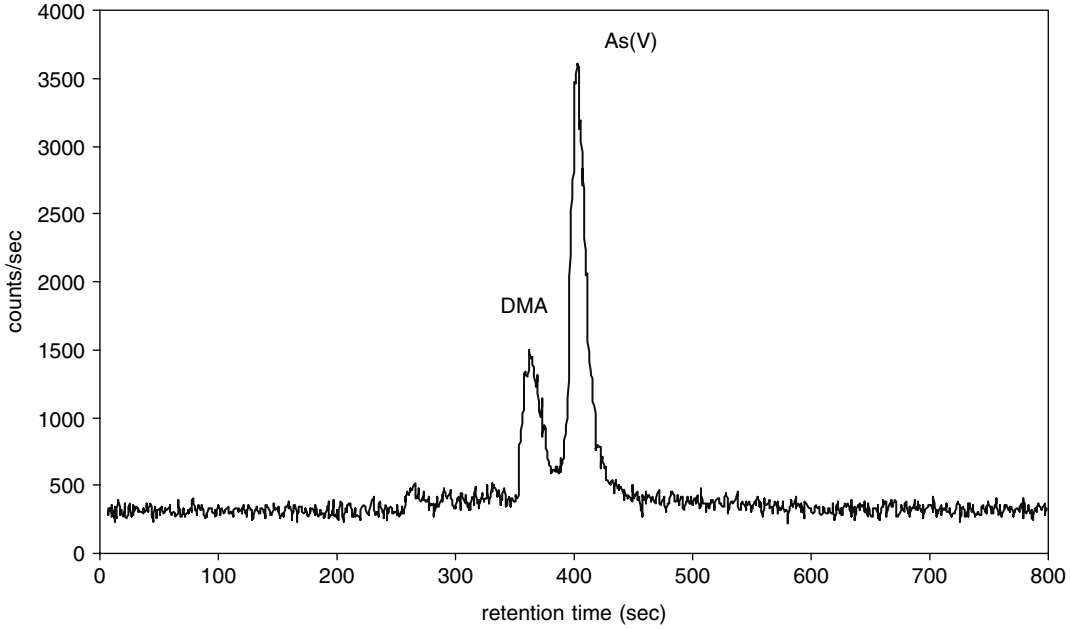

Figure 2. Chromatogram of a cone extract from a spruce tree.

et al.[13] reported that Douglas-fir trees grown in soil rich with arsenopyrite had arsenic concentrations >5000 ppm in all parts of the tree. The results of the present study support the finding that Douglas-fir trees have an affinity for arsenic; however, more work is necessary to establish whether the trees are hyper-accumulators, a term that has been defined as plants that can accumulate metals to concentrations above 1000 ppm.[14] Mature Douglas-firs are very large trees (over 80 m tall) and we assume that the 1000 ppm criterion applies to the whole tree, and so would have to be met at all heights.

Not unexpectedly, the arsenic concentration in the new-growth Douglas-fir stems is greater than in the old, e.g. 207 ± 2 ppm dry weight (dw) versus 78 ± 11 ppm dw; the concentration in the old needles, however, is greater than in the new, e.g. 380 ± 10 ppm dw versus 195 ± 8 ppm dw. The results suggest that translocation of arsenic in the Douglas-fir is from the roots to the needles via the stems. The arsenic accumulates in the needles with time, possibly providing a route for the tree to eliminate its arsenic burden.

Although the speciation of arsenic in the marine environment has been studied extensively[12,15] much less is known about the terrestrial situation. In the case of plants it appears that arsenic(V) is taken up from soils to be transformed to other arsenic species, possibly with the help of phytochelatins (PCs).[16,17] PCs are metal-binding peptides rich in thiol groups. These compounds tend to be produced by plants in response to intracellular metal stress, such as inorganic arsenic exposure.[16] Glutathione, which is a constituent of PCs, readily reduces arsenic(V) in plant cells to arsenite.[18] The presence of arsenic(III) in the tree extracts of the present study indicates that this same reduction may be occurring within the trees. It has been suggested that arsenic(III), bound to the PCs, is transported from the roots to the shoots and needles/fronds and stored in vacuoles and that the PCs are then recycled by the plant for further transport duty.[18] However, in order to do this there would have to be some mechanism for arsenic release. There is some NMR evidence that arsenicals may be accumulated in plant cell vacuoles.[19]

So, predictably, arsenic(III) is the major species extracted from some hyper-accumulating ferns[7,20] and studies indicate that the arsenic(III) is primarily present in aqueous form, i.e. is oxygen coordinated.[21] In contrast, the arsenic in another hyper-accumulating fern, *P. cretica*, is bound to PCs.[22] The arsenic(III) species in the shoots of the Indian mustard plant is also bound to sulfur donors, probably from glutathione or PCs.[23] The arsenic is easily extracted from fern species: the extraction efficiencies are high. The same is found for the Douglas-fir needles and stems, 96% for freeze-dried stems ($n = 4$); thus, the chemistry is probably similar, involving oxygen coordination. The extraction efficiencies of samples for the other trees are low, e.g. less than 25% for the spruce needles.[8] Even if the arsenic was stored as PC–As complexes in the Douglas-fir, methanol–water extraction or the analytical procedure would result in the break up of these complexes,[22] releasing arsenic(III), which would be detected as such. These speciation results are listed for supposedly 'fresh' samples, not freeze-dried, but moisture is rapidly lost from the samples on storage, so a comparison between the two sets of results would not be very meaningful.

The major arsenic species extracted from the Bridge River trees was inorganic arsenic. However, as has been described by others,[7,20,24,25] trace amounts of methylated species were found in some samples: the occurrence of DMA in the spruce

cone (Fig. 2) is noteworthy. Signals were seen at the retention time of AsB in a few Douglas-fir tree samples, but the identification is not secure. This arsenical has been found in some terrestrial plants.[25] Arsenic accumulators are being considered for use in remediation of contaminated sites. Plant species, such as the brake fern discovered by Ma *et al.*,[6] have been shown to thrive on arsenic-rich soils, efficiently extracting and translocating the arsenic from the soils into its biomass.[6,7,26] However, the Douglas-fir seems an unlikely candidate for such an application, as it grows very slowly.

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