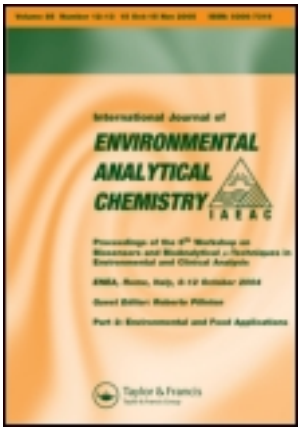

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Occurrence of methylated arsenic species in parts of plants growing in polluted soils

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Occurrence of methylated arsenic species in parts of plants growing in polluted soils

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Arsenic compounds were determined in extracts of branches, leaves and roots from plants growing in a mining contaminated area. The selected species were *Dryopteris filix-max, Quercus pubescens, Dipsacus fullonum, Alnus glutinosa, Buxus sempervirens* and *Brachythecium* cf. *reflexum*. Total arsenic content in the subsamples was analysed by ICPMS after acidic digestion. In general, concentrations in the plant parts followed the gradient roots > branches > leaves indicating that they are arsenic-resistant plants. Arsenic species were determined in water/methanol (9 + 1, v/v) extracts by HPLC-ICPMS. Different levels of organoarsenicals were found depending on plant part and plant species. Higher percentages of organoarsenic compounds were recorded in branches and leaves (up to 35% in the boxtree sample), than in roots (0.7–5.2% in the same plant species). The absence of organic arsenic species in the soil where the plants were collected and the low levels of organoarsenicals found in the roots, indicate that the studied plants have the ability to accumulate or synthesise organoarsenic compounds in relatively high percentages, and this information contributes to enlarge the knowledge of arsenic uptake and speciation in plants.

Keywords: arsenic; polluted terrestrial plants; speciation; polluted soil; HPLC-ICPMS

1. Introduction

The identification and quantification of arsenic species in terrestrial plants can contribute to the knowledge about the uptake mechanisms, translocation and transformation of arsenic compounds by plants. This information is essential to elucidate the cycling and metabolic pathway of arsenic species in the terrestrial environment, and to assess the potential risk of toxicity.

The presence of different arsenic species in terrestrial plants can derive from environmental uptake and/or synthesis within the plant. The uptake of arsenic by plants depends on many factors, such as plant species and the concentration of arsenic in the soil where they are growing. It is clearly stated by several authors that arsenate (As(V)) is taken up by the phosphate transporters in the plasma membrane of root cells [1–3]. In the case of arsenite (As(III)), since its chemical properties differ from phosphate, the uptake would not be expected to occur via the same pathway. Then, different plasma membrane systems must therefore be responsible for the uptake of arsenite and arsenate [4]. Few reports are

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available regarding the uptake of organic arsenic species, since inorganic species prevail in the soil-to-plant environment and the uptake of methylarsonate (MA) and dimethylarsinate (DMA) is generally low [5–7]. Arsenic-resistant plants are able to avoid the uptake and/or translocation of arsenic by regulating their plasma membrane systems and this is the most extended mechanism in terrestrial plants, which accumulate arsenic primarily in the root system [6].

Mobility or translocation is influenced by the type of arsenic species present in the plant. Organic arsenic species such as DMA and MA are generally poorly translocated from root to shoot systems [5,8]. The translocation of inorganic species depends on the As(V) and P(V) supply and varies greatly between plant species [9]. The process of translocation from root to shoot systems is not fully elucidated [10].

Some terrestrial organisms such as fungi [11], bacteria [11] and gilled fungi [12] can synthesise organoarsenic compounds from the inorganic forms, and high levels of organoarsenicals have been found in some of these organisms [11–14]. It is thought that these mechanisms are used as a way of detoxification, as in the complexation of arsenite with phytochelatins [15–16]. In terrestrial plants, however, the major arsenic species reported are inorganic [17–20] and only a few studies have reported the presence of organoarsenicals as major species in some plants [21–23]. Apparently this could be attributed to the inability to transform inorganic arsenic into organic species within the plants.

Mixtures of methanol/water are commonly used for arsenic species extraction from plants and due to the usually low levels of arsenic found in the extracts, the coupling HPLC-ICPMS has revealed as a robust and sensitive technique for speciation analysis of such extracts [9].

The present study was performed on plants collected in a mining contaminated area in Eastern Pyrenees, Spain [24]. Previous studies in the same area revealed relatively high percentages of organoarsenic species on plants growing on it [22]. Based on this finding the aim of the present study is to investigate arsenic species in different plant parts in order to obtain information about distribution patterns in the selected samples, especially for the organoarsenicals.

2. Experimental

2.1 *Standards and reagents*

All solutions were prepared with doubly deionised water (18.2 $M\Omega\,cm^{-1}$ resistivity). Concentrated nitric acid (69%, Hiperpur), methanol (HPLC-gradient), formic acid (98%, p.a.), ammonium dihydrogen phosphate (p.a.) and aqueous ammonia solution (25% p.a.) were purchased from Panreac, and pyridine (p.a.) from Scharlau. Hydrogen peroxide (31%, Selectipur®) was purchased from Merck. Standard solutions (1000 $mg\,L^{-1}$) were prepared as follows. Arsenite: prepared from As_2O_3 (NIST Oxidimetric Primary Standard 83d, 99.99%, MW 197.8414) dissolved in 4 $g\,L^{-1}$ NaOH (Merck, Suprapure). Arsenate: prepared from $Na_2HAsO_4 \cdot 7H_2O$ (Carlo Erba, MW 312.0141) dissolved in water. Methylarsonate (MA): stock solution prepared from $(CH_3)AsO(ONa)_2 \cdot 6H_2O$ (Carlo Erba, MW 292.0263) dissolved in water. Dimethylarsinate (DMA): stock solution prepared from $(CH_3)_2AsNaO_2 \cdot 3H_2O$ (Fluka, MW 214.0260) dissolved in water. Arsenocholine (AC) standard was supplied by the 'Service Central d'Analyse' (CNRS

Vernaison, France). The stock solutions were kept at 4°C and further diluted solutions for the analysis were prepared daily.

The following Certified Reference Materials were used: BCR CRM 626 Certified Reference Material. Arsenobetaine Standard Solution. $1031 \pm 6\,\mathrm{mg\,AB\,L^{-1}}$. SRM 1575 pine needles from the National Bureau of Standards with a certified total arsenic content of $0.21 \pm 0.04\,\mathrm{mg\,As\,kg^{-1}}$.

NIES CRM 09 Sargasso seaweed from the National Institute for Environmental Studies, with a certified total arsenic content of $115 \pm 9.2\,\mathrm{mg\,As\,kg^{-1}}$.

A moss sample quantified for TMAO (trimethylarsine oxide) and TETRA (tetramethylarsonium ion) with standards [22] was used in the present work as internal Quality Control for the identification, by comparison of the chromatographic peaks and the retention times of these species, from the cation exchange separation.

2.2 *Instrumentation*

Digestions for the total arsenic determinations were performed by triplicate with the microwaves digestion system Milestone Ethos Touch Control, with a microwave power of 1000 W and temperature controller. Total arsenic determination was carried out with an Agilent 7500ce inductively coupled plasma mass spectrometer (ICPMS) with a micro-flow nebuliser (Agilent, Waldbronn, Germany).

HPLC-ICPMS was used for the determination of arsenic species in water : methanol sample extracts. A Perkin Elmer 250 LC binary pump (CT, USA), equipped with a Rheodyne 7125 injector (Cotati, CA, USA) with a 50 µL loop was used. The analytical columns Hamilton PRP-X100 (250 mm × 4.1 mm, 10 µm, Hamilton, Reno, USA) and Zorbax-SCX300 (150 mm, 4.6 mm, 5 µm, Agilent, Wladbronn, Germany) were protected by guard columns filled with the corresponding stationary phases. The outlet of the HPLC column was connected via PTFE capillary tubing to a T-shape in which nitric acid 1% was added to dilute the mobile phase, and the outlet of the T-shape was connected to the nebuliser (Cross-flow type) of the ICPMS system mentioned above. The ion intensity at m/z 75 (^{75}As) was monitored using time-resolved analysis software. Additionally, the ion intensities at m/z 77 ($^{40}Ar^{37}Cl$ and ^{77}Se) were monitored to detect possible argon chloride ($^{40}Ar^{37}Cl$) interferences on m/z 75. The chromatograms were exported, and the peak areas were determined using home made software running with Matlab language.

2.3 *Sampling and sample pre-treatment*

Sampling sites were located in the Vall de Ribes region, on the south side of the Eastern Pyrenees (northern Catalonia, Spain). The Vall de Ribes climate can be considered as temperate and wet with an average annual precipitation of 900 mm. The Vall de Ribes area was an active mining district at the beginning of the nineteenth century, with veins of As and Sb, together with subordinate amounts of Cu, Pb and Ag. The irregularity of the veins and the geographical situation of the mines made them uneconomical and they were closed by the end of the nineteenth century [25–26].

Figure 1 shows a map of the Ripollés district where the sampling was performed. In June 2006, nine samples from six plant species (see Table 1) (vascular plants and one moss growing around mine tailings) were collected in three selected sites, most of them in Sites B and C. Only one plant was collected in Site A. Site A was located near Ribes de Freser,

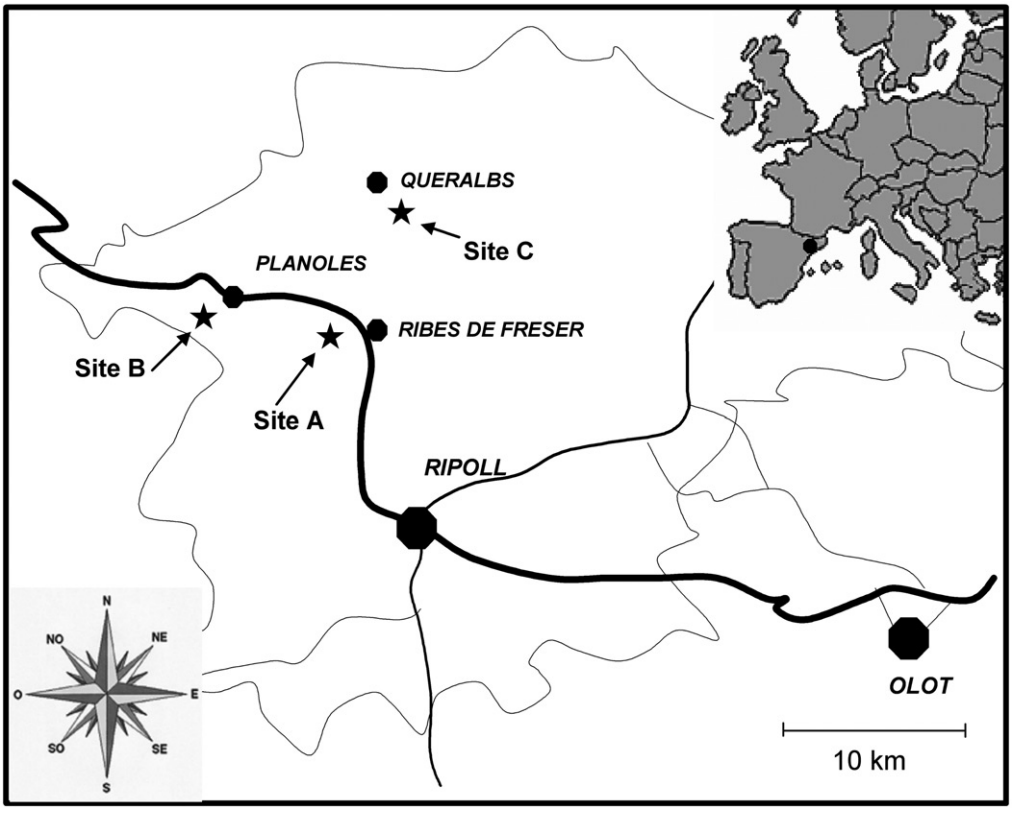

Figure 1. Map of the sampling area located in the Ripollés district (Eastern Pyrenees, Spain).

a village in the Ripollés district, where small abandoned antimony and zinc mines were located. Site B was located above an arsenic and antimony mine near Planoles, a village to the north-west of Ribes de Freser. Finally, Site C was located near Queralbs, another village to the north of Ribes de Freser, where there is an abandoned arsenic mine. Composite samples, obtained by mixing three to four different plants of each species, collected in an approximately 20 m^2 area were obtained by mixing several single plants. Arsenic concentration ranges measured in the soils, expressed as mg As kg^{-1}, corresponding to the above mentioned sites were: Site A from 51.9 to 66.8, Site B from 1886 to 6114 and Site C was 21200 [24].

2.3.1 *Pre-treatment*

The composite samples were divided into roots, branches and leaves subsamples, except the moss which was kept entire. All subsamples were carefully washed in the laboratory with deionised water. The moss sample required a more careful washing so it was performed through three consecutive steps. After washing all subsamples were dried in an oven at $40 \pm 2^{\circ}C$ for 24 hours. Portions of the moss resulting from every washing step were separately dried. The dried materials were finally pulverised with a tungsten carbide disc mill.

Table 1. Total arsenic in plant part and arsenic species in the water/methanol extracts. Concentrations as mg As kg^{-1} (dry weight), (mean ± SD, $n = 3$).

			Total Arsenic	Arsenic species								
			Microwave digestion mg As kg^{-1}	Arsenite mg As kg^{-1} (%)[A]	DMA mg As kg^{-1} (%)	MA mg As kg^{-1} (%)	Arsenate mg As kg^{-1} (%)	TMAO mg As kg^{-1} (%)	TETRA mg As kg^{-1} (%)	UNK[B] mg As kg^{-1} (%)	Column recovery %	Extraction efficiency %
Fern	*Dryopteris filix-max* (L.) Schott FROND (LEAVES)	Site A	1.07 ± 0.21	0.025 ± 0.003 (13.7)	0.014 ± 0.002 (7.7)	<0.010	0.144 ± 0.005 (78.6)	n.d	n.d.	–	104	16.8
		Site B	0.91 ± 0.09	0.061 ± 0.003 (27.5)	0.015 ± 0.003 (6.7)	0.024 ± 0.005 (10.8)	0.112 ± 0.005 (50.5)	0.010 ± 0.001 (4.5)	n.d.	–	101	24.0
Downy oak	*Quercus pubescens* Willd. LEAVES	Site B	1.93 ± 0.15	0.048 ± 0.004 (22.1)	0.027 ± 0.006 (12.4)	0.017 ± 0.002 (7.8)	0.094 ± 0.007 (43.3)	0.031 ± 0.003 (14.3)	n.d.	–	102	11.1
Downy oak	*Quercus pubescens* Willd. BRANCH	Site B	2.37 ± 0.11	0.063 ± 0.002 (18.5)	0.024 ± 0.002 (7.0)	0.028 ± 0.002 (8.2)	0.216 ± 0.021 (63.3)	0.010 ± 0.003 (2.9)	n.d.	–	101	14.5
Teasel	*Dipsacus fullonum* L. LEAVES	Site B	32.3 ± 0.8	0.715 ± 0.020 (7.9)	0.713 ± 0.028 (7.9)	0.049 ± 0.005 (0.5)	7.57 ± 0.12 (83.4)	0.030 ± 0.004 (0.3)	<0.008	–	93	30.6
		Site C	132 ± 14	1.17 ± 0.12 (10.8)	n.d.	0.068 ± 0.018 (0.6)	9.47 ± 0.69 (87.7)	n.d.	0.085 ± 0.008 (0.8)	–	80	10.3
Teasel	*Dipsacus fullonum* L. ROOTS	Site C	407 ± 35	12.69 ± 1.24 (21.3)	0.747 ± 0.060 (1.2)	0.543 ± 0.083 (0.9)	45.52 ± 1.70 (76.5)	n.d.	n.d.	–	93	15.8
European alder	*Alnus glutinosa* (L.) Gaertn. LEAVES	Site C	5.31 ± 0.13	0.219 ± 0.010 (28.3)	0.050 ± 0.002 (6.5)	0.064 ± 0.003 (8.3)	0.424 ± 0.016 (54.8)	0.016 ± 0.002 (2.0)	n.d.	–	96	15.2
European alder	*Alnus glutinosa* (L.) Gaertn. BRANCH	Site C	1.00 ± 0.03	0.063 ± 0.002 (26.8)	0.027 ± 0.004 (11.5)	0.030 ± 0.003 (12.8)	0.115 ± 0.003 (48.9)	<0.008	n.d.	–	110	21.5
Boxtree	*Buxus sempervirens* L. LEAVES	Site B	3.65 ± 0.10	0.376 ± 0.009 (30.0)	0.388 ± 0.016 (30.9)	0.044 ± 0.001 (3.5)	0.433 ± 0.042 (34.5)	0.013 ± 0.002 (1.0)	n.d.	–	92	37.4
		Site C	2.67 ± 0.21	0.185 ± 0.03 (31.9)	0.166 ± 0.003 (28.6)	0.010 ± 0.002 (1.7)	0.195 ± 0.012 (33.6)	0.024 ± 0.004 (4.1)	<0.008	–	111	19.9

(*Continued*)

Table 1. Continued.

			Total Arsenic	Arsenic species								
			Microwave digestion mg As kg^{-1}	Arsenite mg As kg^{-1} (%)[A]	DMA mg As kg^{-1} (%)	MA mg As kg^{-1} (%)	Arsenate mg As kg^{-1} (%)	TMAO mg As kg^{-1} (%)	TETRA mg As kg^{-1} (%)	UNK[B] mg As kg^{-1} (%)	Column recovery %	Extraction efficiency %
Boxtree	*Buxus sempervirens* L. BRANCH	Site B	7.96 ± 1.38	0.180 ± 0.008 (20.9)	0.148 ± 0.004 (17.2)	0.042 ± 0.001 (4.9)	0.463 ± 0.002 (53.8)	0.015 ± 0.001 (1.7)	0.012 ± 0.001 (1.4)	–	106	10.2
		Site C	2.54 ± 0.16	0.113 ± 0.005 (28.0)	0.054 ± 0.003 (13.4)	0.010 ± 0.002 (2.5)	0.226 ± 0.017 (56.1)	<0.008	<0.008	–	105	15.1
Boxtree	*Buxus sempervirens* L. ROOTS	Site B	26.6 ± 2.4	1.16 ± 0.12 (22.0)	n.d.	0.208 ± 0.016 (3.9)	3.84 ± 0.25 (72.8)	0.069 ± 0.011 (1.3)	n.d.	–	93	21.4
		Site C	144 ± 7	34.09 ± 1.20 (49.5)	n.d.	0.219 ± 0.017 (0.3)	34.33 ± 3.39 (49.8)	n.d.	n.d.	0.278 ± 0.078 (0.4)	95	50.5
Moss	*Brachythecium* cf. *reflexum* (F.Weber & D.Mohr) Schimp	Site C	1717 ± 242	2.92 ± 0.02 (3.5)	0.195 ± 0.020 (0.2)	0.324 ± 0.015 (0.4)	79.23 ± 1.86 (95.6)	0.130 ± 0.013 (0.2)	0.066 ± 0.004 (0.1)	–	88	5.5

Notes: [A]percentage of the species with respect to the sum of species; [B]unknown species; n.d. not detected; values expressed as < indicate below LOQ.

2.4 *Procedures*

2.4.1 *Moisture determination*

All the results in the present study refer to dry mass. For this, moisture of the pulverised subsamples was determined in duplicate by drying 1 g of subsample at $100 \pm 5^{\circ}C$ to constant weight. Moisture percentages ranged from 6.4 to 11.8%.

2.4.2 *Total arsenic analysis in plant parts*

Aliquots of 0.2 g of the dried pulverised subsamples were weighed by triplicate to 0.1 mg in the digestion vessels, and 8 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added. Digestions were performed according to the following program: 10 min from room temperature to 90°C, maintained for 5 min at 90°C, 10 min from 90°C to 120°C, 10 min from 120°C to 190°C and 10 min maintained at 190°C. After cooling to room temperature, the resulting mixtures were filtered through ash-free filter papers (Whatman 40) and diluted in water up to 20 mL. The arsenic contents were determined by ICPMS with external calibration for quantification. Rh was used as internal standard.

2.4.3 *Arsenic speciation in plant parts*

The dried pulverised subsamples (0.5 g by triplicate) were weighed to 0.1 mg in 25 mL Teflon tubes, where 10 mL water/methanol (9 + 1, v/v) were added. Arsenic compounds were extracted in an end-over-end shaker by turning the vials at 30 rpm for 16 h at room temperature. The resulting mixtures were centrifuged and the supernatants were filtered through PET filters (Chromafil® PET, Macherey-Nagel, pore size 0.22 μm).

The extracts were analysed with HPLC-ICPMS. Arsenite (As(III)), arsenate (As(V)), dimethylarsinate (DMA) and methylarsonate (MA) were measured by anion exchange chromatography on the Hamilton PRP-X100 column using an aqueous solution of 20 mM $NH_4H_2PO_4$ at pH 6.0 (adjusted with aqueous ammonia) as mobile phase at 1.5 mL min^{-1} flow rate. Quantification was performed with external curves of arsenite, arsenate, MA and DMA standards. Arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium cation (TETRA) and trimethylarsine oxide (TMAO) were measured in the extracts by cation-exchange chromatography on the Zorbax 300-SCX using an aqueous solution of 20 mM pyridine at pH 2.6 (adjusted with formic acid) as mobile phase at 1.5 mL min^{-1} flow rate. Quantification of AB and AC was performed with external curves of the corresponding standards. Quantification of TMAO and TETRA was performed with external curves of AC standard, since with ICPMS detection the species-independent calibration function is usually found [27].

The ICPMS performance was optimised with the corresponding mobile phase solution containing 10 μg As L^{-1} to give maximum response on the signal (m/z 75).

3. Results

3.1 *Quality control and quality parameters*

The accuracy of the results for total arsenic determination was assessed by analysing two Certified Reference Materials. The values obtained for NIST SRM 1575 (pine needles) and NIES CRM 09 (Sargasso seaweed) expressed as mg As kg^{-1} were 0.18 ± 0.01 and 115 ± 16 respectively, which are in good agreement with the certified values.

Mass balance calculations (see Table 1) showed that column recoveries for arsenic species (expressed as the percentage of the ratio between the sum of arsenic species eluting from the column and the arsenic injected into the column) were between 80 and 111%. Column recovery is a paramount parameter in speciation analysis with coupled techniques as a rigorous test system to assure that all the elemental species injected into the column have been eluted, so it is a good strategy as Quality Control of the chromatographic performance. This practice is used by some authors mostly in elemental speciation analysis [28–29].

Repeatability of the analysis ranged from 2 to 20% RSD(%), depending on the concentration of the species, obtaining higher values when concentration was lower.

Detection limits (DL) of the arsenic species are calculated from three times the standard deviation of blank solution ($n = 10$) divided by the sensitivity (slope of the calibration curve) for arsenite, arsenate, DMA, MA, AB, AC, TMAO and TETRA. The values for these compounds expressed as $\mu g\,L^{-1}$ are 0.078, 0.083, 0.166, 0.155, 0.120, 0.127, 0.127 and 0.127 respectively. The detection limits reported in the literature when the HPLC-ICP-MS system is used are in the range of low-ppb level [19–20,29–36] and they are in agreement with the obtained in the present study.

Quantification limits (LOQ) are calculated the same way but considering 10 times the standard deviation of blank solution.

3.2 *Arsenic content in plant parts*

The results obtained are reported in Table 1. Most of the plants studied contained elevated levels of arsenic compared with the general background levels found in plants of the same species growing in a non-contaminated area (0.06 to 0.58 mg As kg^{-1}) [22]. In the present study, the highest level of arsenic was found in the moss sample collected at Site C, which is in agreement with values reported in the literature for moss samples growing in contaminated areas [17]. However, in this particular case and due to the high levels of arsenic in the soil, the presence of some soil fine particles retained in the moss before sampling could lead to erroneously high values. For this reason, the sample was therefore subjected to three consecutive washings to evaluate the effectiveness of the washing steps. In the first and second washing 2695 and 1964 mg As kg^{-1} were obtained respectively. In Table 1 is presented the value obtained after the third washing. The obtained results show that the washing process has a strong influence on the results, thus particular care must be taken in the pre-treatment when analysing mosses.

3.3 *Arsenic speciation in plant parts*

Arsenic species were determined in extracts of the plant parts by applying the procedure described above. The results obtained are summarised in Table 1, in which plant samples are organised according to plant species.

Arsenic species were determined in parts of different plants collected in the studied area. Plant subsamples were extracted with water/methanol (9 + 1, v/v) and the arsenic compounds in the extracts were measured by HPLC-ICPMS. Extraction efficiencies were relatively low, ranging from 5.5 to 50.5%. The extraction efficiency varies according to the plant species and to the plant part. Similar percentages of extraction have been reported in terrestrial plants when water or methanol/water mixtures have been used as

extractans [21,37]. The lowest extraction efficiency was obtained for the moss sample, being similar to extraction efficiencies reported in the literature [38]. There is a relatively high percentage of unextracted arsenic which could be bound to lipids or cell-wall components [17]. The results do not quantitatively account for all the possible arsenic species in the plant samples. However, the extracted species represent the most labile arsenic fraction which is useful for understanding the mobility and transformations of arsenic in plants.

Regarding the presence of the arsenic compounds in the extracts, it can be observed that inorganic arsenic is measured in all cases in concentrations higher than those of organic species, with a relative amount ranging from 64.5 to 99%, depending on the plant part and/or plant species. Arsenate generally showed higher percentages than arsenite, except in the roots of boxtree (*Buxus sempervirens*), in which similar percentages of both inorganic species were found. The predominance of inorganic species in terrestrial plants would be in agreement with some reported studies [29,39]. DMA, MA, TMAO and TETRA were measured in the majority of the plants at trace level, with a significant amount being present in some cases.

From the overall results, it can be observed that for some plants there are differences in the levels of organoarsenicals according to the plant part or sampling site. The most striking case was the boxtree sample (*Buxus sempervirens*), in which an increase of the percentage of organoarsenicals (sum of MA, DMA, TMAO and TETRA percentages) in leaves and branches was observed when compared with roots. Only 0.5–5.2% was present as organic arsenic in the roots, whereas in the branches it ranged from 15.9 to 25.2% and the leaves showed an increase of up to 35% in organoarsenicals. As an example of this behaviour, chromatograms of the extracts of the boxtree sample collected at Site C are shown in Figure 2. In this figure, the chromatograph corresponding to the root extracts when the cation exchange column was used shows an unknown compound, with a retention time of 660 s. This compound could not be identified on the basis of existing data on arsenic species detected in plant samples.

Only branch and leaf extracts were studied in the downy oak sample (*Quercus pubescens*), but it showed the same behaviour as the boxtree, with an increase in organoarsenicals (mainly DMA and TMAO) from 18% in the branches to 35% in the leaves. As additional information, the results obtained in a previous study [22] showed that in leaves extracts of the same plant species (downy oak) collected in the same area, but in a different season (autumn), the main species present was MA, and significant levels of TMAO were also found. In the sample of the present study MA was detected at lower levels. The differences observed could be attributed to varying seasonal activity in plants. Similar behaviour has been reported in moso bamboo shoots with higher levels of organoarsenicals in winter than in spring [23].

The number of organoarsenic compounds determined is generally higher in branches and leaves than in roots. As an example, the percentage of organoarsenicals, calculated as it has been mentioned before (sum of MA, DMA, TMAO and TETRA percentages), in the teasel sample (*Dipsacus fullonum*) were quite similar in all the parts of the plant, ranging from 1.4 to 8.6%. However, only the simple methylated compounds (DMA and MA) were found in the roots, whereas TMAO and TETRA were detected in the leaves. AB and AC were only detected at trace level in a few samples, and in all cases below the quantification limit. AB, which was previously thought to be present only in marine environments, seems to be widely distributed in the terrestrial environment and it has been found in significant amounts in lichen and mushroom samples [13].

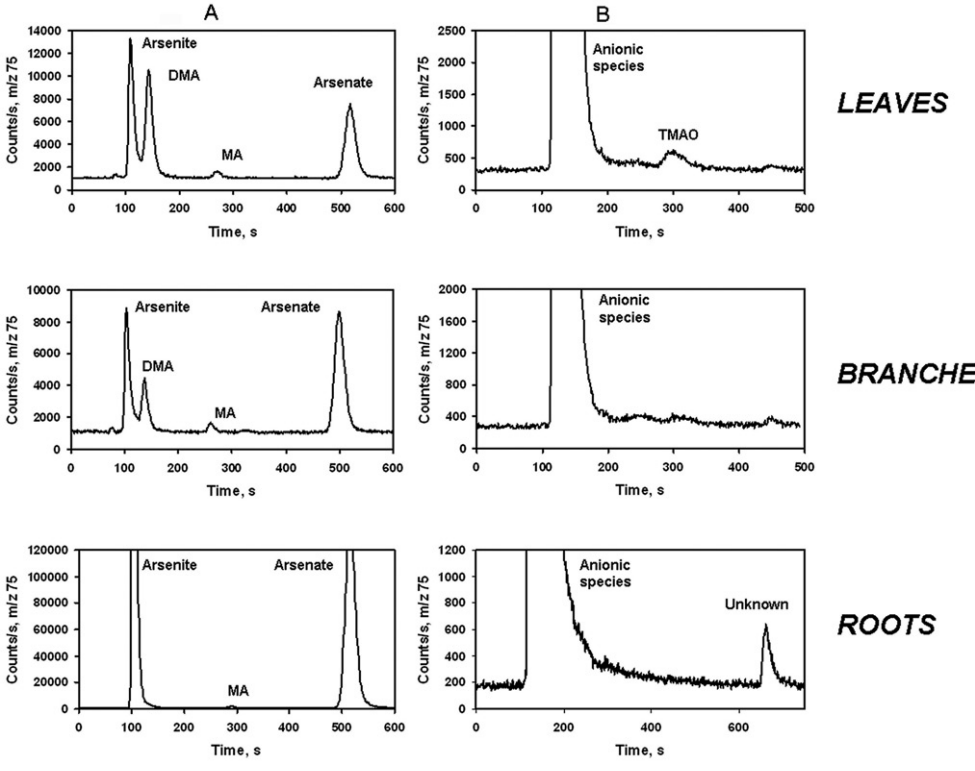

Figure 2. Chromatograms obtained for the different parts of the boxtree sample growing at Site C with (A) anion-exchange separation with PRP-X100 and 20 mM phosphate buffer and (B) cation exchange separation with Zorbax SCX300 and 20 mM pyridine aqueous solution.

4. Discussion

Regarding the total arsenic concentrations in plant parts presented above, they followed the concentration gradient roots > branches > leaves. This behaviour indicates that the plants accumulated arsenic mainly in the root system, with relatively low quantities of arsenic being translocated to the shoot system. High levels of arsenic in both leaves and roots were found in teasel (*Dipsacus fullonum*) (132 mg As kg^{-1} and 407 mg As kg^{-1} respectively), a vascular plant growing in the most contaminated soil (Site C). This behaviour is in agreement with some studies reporting that higher concentrations of arsenic in the roots than in the above-ground parts [6,19,31,40–41]. Regarding the number of arsenic species, the present study found widespread organic arsenic species in the water/methanol extracts of plant parts growing in a contaminated area. Higher proportions of organoarsenicals were found in branches and leaves than in roots in the majority of the studied plants. The presence of these compounds in plants may be attributed to the direct uptake from the soil solution and further translocation to the shoot system, to the synthesis by the plant or to both processes. The fact that only inorganic arsenic was found in the soils where the plants have grown [24], together with the low levels of methylated compounds found in the roots, supports the hypothesis of a possible transformation of

inorganic to organic compounds within the plant. However, it is reported that methylation in plants may be attributable to microbial activity in the vicinity of the root system [11,42] and subsequent translocation from roots to shoots although the uptake and translocation of methylated arsenic compounds in plants is low [5–8].

The results reported here showed that the studied plants can accumulate or synthesise relatively high percentages of organoarsenicals. These results contribute to enlarge the knowledge of arsenic behaviour in the soil-plant environment. However, exactly why plants transform arsenic, and through which mechanisms, are still major questions which suggest an interesting topic for further studies.

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