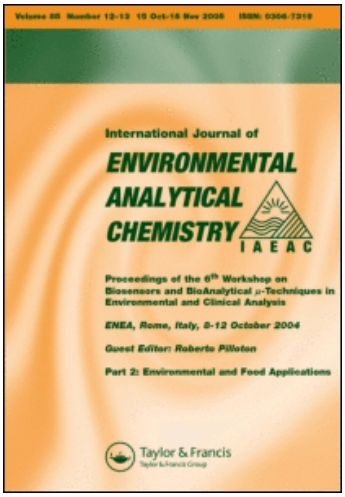

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Speciation analysis to unravel the soil-to-plant transfer in highly arsenic-contaminated areas in Cornwall (UK)

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Two areas near derelict calciners in Cornwall (UK) were chosen to study the uptake of arsenic from arsenic-contaminated soil into indigenous plants (heather, *Calluna vulgaris*; blackberry, *Rubus ulmifulmus*; gorse, *Ulex europaeus*). With total arsenic concentrations in soil ranging from 1240 to 2860 mg kg^{-1} at Site 1 (Tuckingmill), no adverse effects on the growth of the plants studied were observed. Very low soil-to-plant transfer factors (0.01 to 0.03) were found although they were much higher when the extractable soil arsenic concentrations were taken into account (0.1 to 1.1). In the central dump area at Site 2 (Bissoe, 9.78% [*w*/*w*] arsenic in soil), the only plant to grow was heather, although it was severely impaired. However, heather was thriving at the edge of the dump where higher soil arsenic concentrations were found (10.32% [*w*/*w*]), indicating that arsenic is not a growth-limiting factor in itself. Soil-to-plant transfer factors in the range $2 \times 10^{-5} - 9 \times 10^{-4}$ confirm that arsenic is indeed effectively excluded from uptake, even taking into account extractable soil arsenic concentrations ($9 \times 10^{-4} - 1.2 \times 10^{-2}$).

Extraction of bioavailable arsenic from soil using 0.05 mol L^{-1} ammonium sulphate yielded recoveries from 1.18 to 3.34% of the total arsenic, predominantly in the form of arsenate. Extraction of arsenic and its metabolites from plants was achieved with water or a water/methanol mixture yielding recoveries up to 22.4% of the total arsenic, with arsenite and arsenate the predominant arsenic species and a minor fraction consisting of methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide. The identity of the remainder of the non-extractable arsenic species still has to be revealed. Although the data suggest that higher plants synthesise organoarsenic compounds it cannot be excluded that symbiotic organisms have synthesised these compounds.

Keywords: arsenic; uptake; speciation; plant; soil

1. Introduction

As a result of former mining and mineral processing activities in Southwest England, areas in Cornwall and Devon are polluted with heavy metals and metalloids, in particular arsenic. Roasting of ores has dispersed arsenic through the air, contaminating soils and water systems, whereas dumped mine waste has created local hot spots with high arsenic

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concentrations of up to 16,000 mg kg^{-1} and more [1,2]. The Soil Guideline Value (SGV) for arsenic in soil is 20 and 500 mg kg^{-1} in residential and industrial areas, respectively [3], indicating the minimum levels of arsenic in soil which require further site investigation. However, since the SGV only takes into account the total arsenic concentrations actual risks through dispersion are not expressed. It is known, for example, that arsenate is less mobile than arsenite and prevails in soil under natural oxidising conditions. The arsenate/arsenite ratio will strongly depend on soil pH, redox potential and the presence of clay, iron and aluminium oxides and its uptake in plants will be influenced by the physico-chemical form(s) of arsenic [4,5].

The level of arsenic in plants is in general low as soil-to-plant transfer coefficients (concentration in plant/concentration in soil) are typically in the range 0.01–0.1 [6,7]. Due to these low transfer coefficients most agricultural plants are found not to pose any health risk, even when grown on highly arsenic-contaminated soils [8,9] although some edible plants such as carrots [10], radish, lettuce and calabrese [11] might contain more arsenic (mg kg^{-1} range). At low concentration levels it appears that arsenic does not interfere with specific metabolic reactions [12] but at higher concentrations it may inhibit plant growth and even cause plant death [12–14]. Arsenate is a phosphate analogue and is transported across the plasma membranes via the phosphate cotransport system [15]. In the cytoplasm it competes with phosphate and can replace phosphate in ATP resulting in an unstable ADP-arsenic complex which leads to disruption of the energy flow in cells [16]. In the cells arsenate is rapidly reduced to arsenite by glutathione [17,18], subsequently reacting with sulfhydryl groups (–SH) of enzymes and tissue proteins, leading to inhibition of cellular functions and cell death [15].

Depressed crop yields were reported at 3.0–28 mg of water soluble arsenic per litre or 25–85 mg of total arsenic per kilogram of soil, clearly indicating phytotoxicity of arsenic for plants [19]. On the other hand, arsenic resistance is known for a number of plant species growing on arsenic-contaminated soils; examples are *Agrostis castellana* and *A. delicatula* [20], *A. capillaris* and *Deschampsia cespitosa* [21], *Holcus lanatus* [22,23], *Calluna vulgaris* [24], and *Leymus cinereus* [25]. If their phosphate status is raised, their resistance is increased. The reason is a higher transport preference for phosphate than for arsenate [26]. In the case of *Calluna vulgaris* its fungal symbiont *Hymenoscyphus ericae* is capable of an efflux of arsenite thereby reducing exposure of *C. vulgaris* to arsenic [24]. *Glomus mosseae* and *Glomus caledonium* were isolated from *Holcus lanatus* growing on an arsenic-contaminated mine-spoil soil. They were found to suppress high-affinity arsenate and phosphate transport into the roots of both resistant and nonresistant *H. lanatus* [27]. In a variety of plants arsenic was complexed with phytochelatins (cysteine containing tripeptids or their polymers) to reduce its toxicity [26] although some other data suggest that phytochelatins only play a minor role in arsenic detoxification [28]. In *Thunbergia alata* most of the arsenic was bound to peptides [29] and arsenic complexation with thiols was reported [30]. Some plants like arsenic hyperaccumulating ferns (*Pteris vittata*, *Pityrogramma calomelanos*) have solved the problem of arsenic toxicity in such a successful way that they have been recommended for phytoremediation purposes [31].

Arsenic speciation data for terrestrial plants are still not widely available. In a limited number of studies dealing with the subject, several arsenic compounds were measured in terrestrial plants [29,32–35]. Mushrooms contain a whole range of arsenic compounds such as the anionic species arsenite (As(III)), arsenate (As(V)), methylarsonic acid (MA) and dimethylarsinic acid (DMA) and the cationic species arsenobetaine (AsB), arseno-choline (AsC), trimethyarsine oxide (TMAO) and tetramethyl arsonium ion (TETRA)

in different ratios and combinations [36]. Higher plants in general contain a higher proportion of inorganic arsenic species. Predominantly inorganic arsenic was found in a variety of plants growing on arsenic-rich soils in Yellowknife, Canada [37] and plants from hot spring environments [38]. In another study, inorganic arsenic compounds and traces of simple methylated compounds like MA, DMA, TMAO and TETRA were found in aquatic plants from Canada [39]. *Rubus idaeus* was found to contain As(III), As(V) [37,40] and traces of TMAO, DMA and the arsenosugar glycerol ribose [40].

In this work two heavily polluted sites near the town of Camborne in Cornwall, UK, were selected to assess the uptake of arsenic from soil into autochthonous plants and the presence of arsenic metabolic products. Until the 1920s calciners on these sites were used for roasting of polymetallic ores with the intention of either removing arsenic from the ore or recovering arsenic as a commercial product (As_2O_3). As a result, the derelict remains of calciner structures and dump sites in their immediate vicinity contain extremely high levels of especially arsenic. Soil and autochthonous plant samples from such sites were collected and analysed for the presence of arsenic compounds.

2. Experimental

2.1 *Sample collection and preparation*

Soil samples were taken in the vicinity of two calciners (see Table 1 for details). On the partially rehabilitated and well-overgrown Site 1 (Tuckingmill) two samples were taken, one directly from the derelict flue leading from the calciner to the chimney (Site 1A) and the other two from the land adjacent to the calciner's chimney (Sites 1B and C). On the more open Site 2 (Bissoe), residual dump material was sampled in the centre of the dump where almost no plants were growing (Site 2A) and at the edge, where top soil (Site 2B) and deep soil (Site 2C) samples were taken. The following plant samples were also collected: heather (*Calluna vulgaris*) and blackberry (*Rubus ulmifulmus*) on both sites and gorse (*Ulex europaeus*) on Site 1 only.

Plant samples were washed (brief immersion in Milli-Q water, repeated 3 times with clean water; throughout the procedure Milli-Q water was used), air-dried (3 days) and oven-dried (40°C, overnight), crushed (in an agate mortar after addition of liquid nitrogen) and finally ground in a Zr planetary mill. Powdered samples were sieved through a 250 μm plastic sieve and stored in airtight containers in the dark until analysis. Soil samples were dried and ground in the same way with the omission of the liquid nitrogen crushing step.

2.2 *Extraction of arsenic species from soil and plant samples*

Soils and plants were subjected to conventional extraction procedures to release the arsenic compounds.

2.2.1 *Plant samples*

To 0.35 g of sample, 35 mL of extractant was added (1 + 1 methanol/water mixture or water) followed by shaking in a temperature-controlled reciprocal water bath for 20 h at a temperature of 20°C. Samples were centrifuged (3200 rpm, 10 min) and decanted. To the residue, 10 mL of 1 : 1 methanol/water or water, respectively, was added and shaking was resumed (2 h). Centrifuging was repeated and the two extracts were combined.

Table 1. Sampling details for soils and plants and total arsenic and scandium concentrations in the samples. Results are given as average values ± SD, $n = 4$. A sample coding of e.g. 2B/h1 represents a sample from Site 2, taken from the cca. top 20 cm soil, specifically related to the fine branches and leaves of heather. Enrichment factors (EF) indicating potential pollution with soil particles are calculated according to the formula given in the results and discussion section.

Location	Soil sample	Total As in soil (mg kg^{-1})	Total Sc in soil (mg kg^{-1})	Corresponding plant sample	Total As in plant (mg kg^{-1})	Total Sc in plant (mg kg^{-1})	EF
Site 1	A: shallow sandy soil on the collapsed wall of the flue leading to the calciner	2860 ± 100	4.07 ± 0.16	h1: heather (fine branches and leaves)	38.6 ± 1.4	0.156 ± 0.006	0.352
	B: top soil (cca. top 10 cm), dark, rich in organic matter, next to the calciner's chimney	1240 ± 40	8.13 ± 0.29	g1: gorse (fine branches and leaves), roots penetrating into the deep soil	3.3 ± 0.2	0.085 ± 0.003	0.255
	C: deep soil (cca. 10–20 cm deep), light brown, next to the chimney	1390 ± 50	9.68 ± 0.35	b1: blackberry (fine branches and leaves), roots penetrating into the deep soil	31.7 ± 1.1	0.116 ± 0.004	1.792*
Site 2	A: top soil (cca. 5 cm deep), mostly roasted remains of the ore with very little soil, centre of the dump	103,200 ± 3600	13.9 ± 0.6	h1: heather (fine branches and leaves)	94.8 ± 3.3	0.184 ± 0.007	0.069
				h2: heather (flowers)	11.9 ± 0.4	0.422 ± 0.015	0.004
				b1: blackberry (fine branches and leaves)	17.1 ± 0.6	0.265 ± 0.010	0.009
				b2: blackberry (fruits)	2.21 ± 0.08	0.021 ± 0.001	0.014
	B: top soil (cca. top 20 cm), roasted remains of the ore mixed with soil, edge of the dump	97,800 ± 3400	12.8 ± 0.5	h1: heather (fine branches and leaves), roots not penetrating into the deep soil	25.8 ± 0.9	0.513 ± 0.020	0.007
	C: deep soil (cca. 20–40 cm deep), mostly roasted remains of the ore with very little soil, edge of the dump	161,900 ± 5700	10.2 ± 0.4				

*Potentially contaminated with soil particles.

The combined extract was dried on a rotary evaporator at 40°C, resuspended in 5 mL of water, filtered through a 0.45 μm PVDF membrane filter and kept at 4°C until analysis.

2.2.2 *Soil samples*

To 0.100 g of sample, 25 mL of 0.05 M ammonium sulphate was added. Samples were shaken for 20 h on a rocking shaker with a frequency of 200 min^{-1} at 20°C, centrifuged at 3200 rpm (15 min), decanted, filtered (0.45 μm PVDF membrane filter) and kept at 4°C till analysis.

2.3 *Total element concentration determination*

Samples were weighed in spronk containers (SRP Systems, Lexmond, The Netherlands), irradiated in the TRIGA Mark II reactor of the Jožef Stefan Institute and analysed by instrumental neutron activation analysis (INAA) with the k_0 method [41,42].

2.4 *Arsenic speciation*

An ion exchange HPLC interfaced with a hydride generation unit to an atomic fluorescence spectrometer (HGAFS) was used for arsenic speciation in soil and in plant extracts [43,44]. For the separation an anion exchange column (Hamilton PRP-X100, 250 × 4.1 mm with an eluent consisting of 20 mmol L^{-1} KH_2PO_4 solution, pH 6.0) and a cation exchange column (Alltech Adsorbosphere SCX, 250 × 4.1 mm with an eluent consisting of 2.5 mmol L^{-1} pyridine, pH 2.65) were used for separation of the anionic and cationic arsenic species, respectively. The HPLC effluent was on-line subjected to hydride generation by addition of HCl (3 mol L^{-1}, 3.0 mL min^{-1}) and $NaBH_4$ (1.5% in 0.1% NaOH, 3.0 mL min^{-1}) and the arsines and hydrogen formed were separated from the liquid waste in a gas liquid separator and swept out of it with argon (340 mL min^{-1}). Gases were dried by passage through a Perma pure dryer and arsenic was detected in the atomic fluorescence spectrometer (Excalibur, PS Analytical, UK). A UV decomposition unit was optionally placed between the ion exchange column and the hydride generation unit to check for the presence of compounds which form hydrides with low efficiency (arsenosugars and trimethylarsine oxide) or which form no hydrides at all (arsenobetaine, arsenocholine and tetramethylarsonium ion). This consisted of a coil (Fluoroplastic PTFE/FEP) wrapped around an 8 W UV lamp from Camag (254 nm) and required the addition of 3% $K_2S_2O_8$ in 3.0% NaOH (1.35 mL min^{-1}). Since no plant reference materials exist which are certified for the arsenic compounds found in this study the only way of validation of the speciation method described above was by analysing other reference materials (IAEA-140/TM, *Fucus* sp., Sea Plant Homogenate) of which data are reported [45]. Excellent agreement was obtained between the described arsenic speciation method and an independent technique (HPLC-ICP-MS) for determination of As(III), As(V), MA and DMA in a set of urine samples [46].

3. Results and discussion

Two sites were chosen to study the arsenic soil-to-plant transfer: Site 1 (Tuckingmill), a site in the vicinity of a calciner, which was used for roasting of arsenic-bearing ores until the

1920s and partially cleaned up some years ago, and Site 2 (Bissoe), a residue dump next to a derelict calciner. Site 1 is abundantly overgrown by vegetation (trees, bushes and herbaceous plants) among which gorse, blackberry and heather represent the major vegetation. These indigenous plants are growing well on many similar polluted locations elsewhere in Cornwall which makes them suitable study objects for uptake-toxicity related studies. Site 2 is more diverse since the central part of the dump is almost bare with a few small, crooked heather plants while the edges of the dump show lush vegetation mostly of heather and blackberry. It should also be mentioned that the dump mostly consists of calcined remains of ore with very little soil making it hostile for plants.

Although in this study mainly arsenic soil-to-plant transfer factors were targeted, INAA analysis also gave results for a range of other elements. Some of these elements found at both sites were very elevated compared to their average concentration in the earth's crust [47]: Sb (30–5000×), W (140–2800×), Sn (1500–11,000×), and As (700–70,000×); the lower numbers are from Site 1 and the higher ones from Site 2. This is not unexpected since these elements are characteristic constituents of the Cornubian ore, which was calcined and processed on the sites. Table 1 gives total arsenic concentrations of all six soil samples and of corresponding plant samples taken on both sites. In order to relate the chemical availability or mobility of arsenic in soil to the bioavailability or uptake potential in plants, soil samples were extracted with $0.05\,\mathrm{mol\,L^{-1}}$ ammonium sulphate, which represents non-specifically sorbed arsenic [48] (easily available) and correlates well with arsenic in the soil pore water [49].

Since plants had been growing on highly contaminated sites, possible contamination by soil particles had to be excluded. Removal of these particles in the sample preparation step was achieved by immersion of the plant samples in water (3 times in clean water) before drying and homogenisation. An indication of removal of these particles may be obtained by determination of the so-called enrichment factor (EF) given by [50–53]:

$$EF = \frac{(c_x/c_{Sc})_{\mathrm{plant}}}{(c_x/c_{Sc})_{\mathrm{soil}}}$$

with c_x the concentration of the element (in plant or soil) and c_{Sc} the *Sc* concentration (in plant or soil). Since *Sc* is not actively taken up by the plant it serves as a tracer to follow potential contamination by soil particles. The closer the enrichment factor value is to 1, the more likely the element in question originates from soil particles. Values much higher than 1 are indicating environmental pollution of the sample. Enrichment factors found for arsenic were in the range of 0.004–0.356 for seven out of eight plants and 1.792 for the sample of blackberry (branches, leaves) from Site 1 (Table 1). So, for most samples external contamination of the plants by soil particles could be excluded.

From Table 2 it can be seen that even though the arsenic extractability was very low (1.18–3.34%), still as much as 16–49 $\mathrm{mg\,kg^{-1}}$ of arsenic in soil (based on dry weight, Site 1) or 2210–2722 mg of arsenic $\mathrm{kg^{-1}}$ in soil (based on dry weight, Site 2) was found to be chemically available and thus potentially bioavailable. HPLC-HGAFS analysis of those soil extracts showed that arsenic is mainly present as As(V) (87–96% of the arsenic extracted, Table 2). As(III) in soil extracts was present in lower concentrations and knowing that it is more mobile than As(V) [54], and thus easier to extract, this suggests that the main (non-extracted) arsenic compound in the soils is As(V) in the form of arsenate, as found by EXAFS and XANES for a similar site in Cornwall [55]. In none of

Table 2. Soil arsenic extractability in 0.05 mol L^{-1} $(NH_4)_2SO_4$ and arsenic speciation in the resulting extracts. Results are given as average values ± SD, $n = 4$. Details on coding of the samples can be found in Table 1.

Soil sample	Total As (mg kg^{-1})	Extractability (%)	As(III) in extract (mg kg^{-1})	As(V) in extract (mg kg^{-1})
1A	2860	1.71	6.15 ± 0.17	42.7 ± 1.0
1B	1240	3.34	1.74 ± 0.09	39.8 ± 5.5
1C	1390	1.18	2.11 ± 0.11	14.3 ± 0.6
2A	103,200	2.31	469 ± 12	1913 ± 100
2B	97,800	2.26	400 ± 7	1810 ± 66
2C	161,900	1.68	205 ± 4	2517 ± 69

Table 3. Soil-to-plant transfer factors, indicating the ratio of element in plant to corresponding soil, calculated from either total arsenic in soil or extractable (available) arsenic in soil. Details on coding of the samples can be found in Table 1.

		Transfer factor	
Sampling location	Sample description	Total As plant/ total As soil	Total As plant/ extractable As soil
1A	h1 = Heather/branches and leaves	0.0135	0.7894
1B	g1 = Gorse/branches and leaves	0.0251	0.1140
1B	b1 = Blackberry/branches and leaves	0.0241*	1.0950*
2A	h1 = Heather/branches and leaves	0.0009	0.0400
2A	h2 = Heather/flowers	0.0001	0.0050
2A	b1 = Blackberry/branches and leaves	0.0002	0.0072
2A	b2 = Blackberry/fruits	0.00002	0.0009
2B	h1 = Heather/branches and leaves	0.0003	0.0117

*Potentially contaminated with soil particles.

the soils organoarsenic compounds such as MA and DMA were found (< 0.03 mg kg^{-1}), although they have been reported to be leached from some soils [56,57].

Uptake factors, indicating the ratio of the element in the plant to the corresponding soil, can be calculated from either total arsenic in soil or extractable (bioavailable) arsenic in soil. It is evident that calculation from total arsenic in soil will give (much) lower uptake factors in comparison to calculation from extractable arsenic in soil. From Table 3 it is clear that the soil-to-plant uptake of arsenic is very low for all plants studied; transfer factors (from total arsenic in soil) are in the range 2×10^{-5} (Site 2) to 2.5×10^{-2} (Site 1) and are similar to the ones published previously using the same type of calculation [6,7]. Soil-to-plant transfer factors are much lower for Site 2 where extremely high total arsenic concentrations are found. Since most of the arsenic is expected to be tightly bound in secondary minerals, as found in a study of Van Elteren *et al.* [58] for a similar site in Cornwall, it can be assumed that the ammonium sulphate extractable arsenic gives an adequate representation of the potentially bioavailable fraction. Calculating soil-to-plant transfer factors (from extractable arsenic in soil) the following

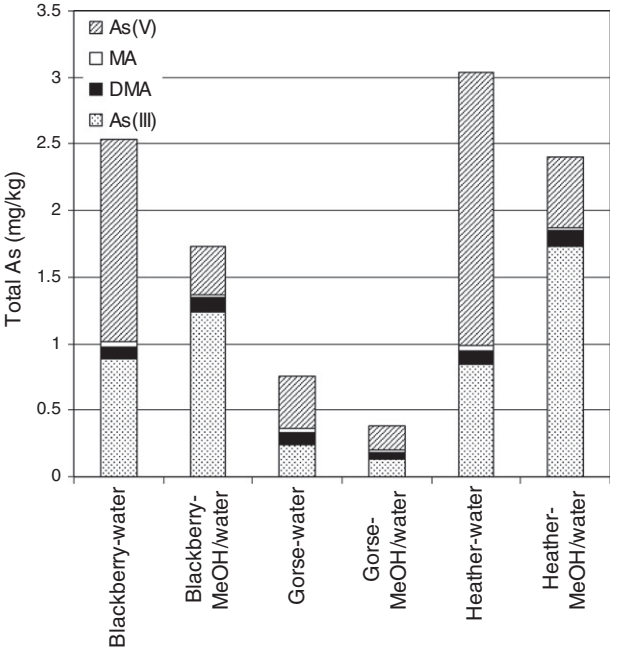

Figure 1. Extractability of arsenic species from plants from Site 1 into either water or a 1 : 1 mixture of methanol/water. In both instances 0.35 g of sample was extracted with 35 mL of extractant.

factors were found: 0.11–1.09 for Site 1 and $9 \times 10^{-4} - 4 \times 10^{-2}$ for Site 2. While active 'exclusion' of arsenic seems obvious for the heavily contaminated area (Site 2), there is not much evidence for it in the other area (Site 1), except maybe for gorse (0.11), which was found to exclude arsenic from its shoots in a study of Craw *et al.* [59] as well. Comparing arsenic concentrations in fine branches and leaves with arsenic concentrations in fruits or flowers of the same plants, cca.10 times less arsenic was found in blackberry fruits and heather flowers. Root samples, expected to contain even more arsenic [60], were not analysed due to external contamination by the highly arsenic-contaminated soils, which were not removed completely during the washing procedure.

The extractability of arsenic from plants was studied with two extractants, viz. a water/methanol mixture (1 + 1) and water. The water/methanol mixture is very often used in arsenic speciation analysis and is mostly aimed at less polar arsenic compounds such as arsenobetaine, the main compound in the majority of samples of marine origin. Water, on the other hand, is a better choice when inorganic arsenic compounds are expected. A just published study of Zheng and Hintelmann [61] shows that water is actually the extractant of choice for plants, minimising the risk of As(III) oxidation and at the same time yielding among the highest recoveries. In Figure 1 the extractability of arsenic compounds from three plants (heather, blackberry and gorse) from Site 1 into water or a 1 : 1 mixture of methanol and water is given. The aqueous extraction yields 27–95% more arsenic than the water/methanol extraction. The difference may be due to the better extractability of inorganic arsenic (As(III) and As(V)), while the extractability of the organic arsenic compounds MA and DMA remained roughly unchanged. From Table 4 we can observe that using the optimised aqueous extraction resulted in extraction yields from 7.1%

Table 4. Extractability of arsenic from plant (parts) in water and arsenic speciation in the extracts. Results are given as average values ± SD, $n = 4$. Details on coding of the samples can be found in Table 1.

Sampling location	Sample description	Total As ($mg\,kg^{-1}$)	Extractability (%)	As(III) ($mg\,kg^{-1}$)	DMA ($mg\,kg^{-1}$)	MA ($mg\,kg^{-1}$)	As(V) ($mg\,kg^{-1}$)	TMAO ($mg\,kg^{-1}$)
1A	h1 = Heather/branches and leaves	38.6 ± 1.4	7.9	0.847 ± 0.034	0.098 ± 0.019	0.042 ± 0.004	2.05 ± 0.09	< 0.01
1B	g1 = Gorse/branches and leaves	3.3 ± 0.2	22.4	0.244 ± 0.017	0.085 ± 0.022	0.030 ± 0.015	0.392 ± 0.026	< 0.01
1B	b1 = Blackberry/branches and leaves	31.7 ± 1.1*	8.0	0.887 ± 0.126	0.090 ± 0.013	0.035 ± 0.010	1.52 ± 0.04	< 0.01
2A	h1 = Heather/branches and leaves	94.8 ± 3.3	7.6	3.20 ± 0.08	< 0.01	0.022 ± 0.003	4.03 ± 0.10	0.08 ± 0.01
2A	h2 = Heather/flowers	11.9 ± 0.4	7.1	0.46 ± 0.06	< 0.01	< 0.005	0.38 ± 0.02	0.002
2A	b1 = Blackberry/branches and leaves	17.1 ± 0.6	10.2	1.60 ± 0.04	< 0.01	0.018 ± 0.002	0.12 ± 0.02	< 0.01
2A	b2 = Blackberry/fruits	2.21 ± 0.08	12.9	0.23 ± 0.01	< 0.01	0.020 ± 0.004	0.035 ± 0.003	< 0.01
2B	h1 = Heather/branches and leaves	25.8 ± 0.9	9.2	0.69 ± 0.03	< 0.01	0.013 ± 0.001	1.68 ± 0.04	0.03 ± 0.01

*Potentially contaminated with soil particles.

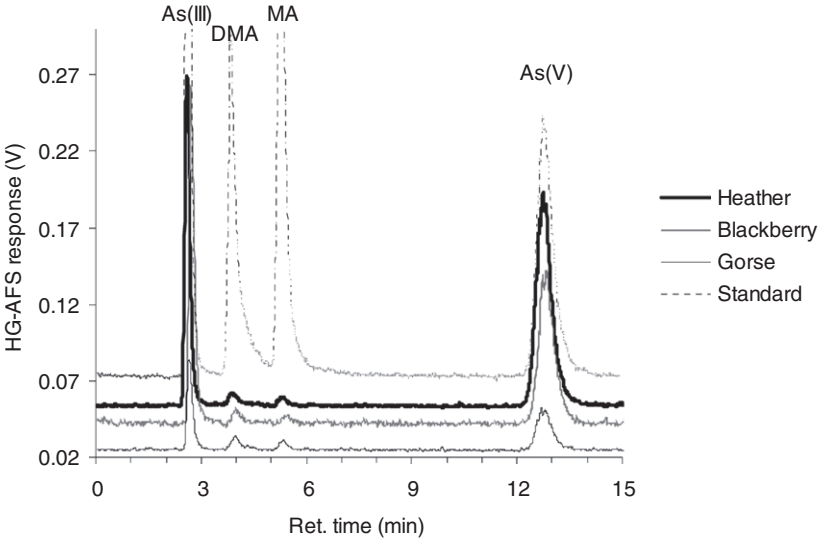

Figure 2. Anion exchange chromatogram of arsenic compounds in autochthonous plants from Site 1 and in a mixed standard solution (100 ng mL^{-1} of each compound).

to 22.4%. These results are in agreement with those of Zheng *et al*. [39], who extracted 6.3–16.1% of arsenic from water plants growing in polluted waters in Canada, using a water/methanol mixture. Koch *et al*. [37] extracted between 9.8% and 87%, with an average arsenic extraction yield from plants of $41 \pm 23\%$ and Ruiz-Chancho *et al*. [33] extracted from 3.0–41.4% of arsenic from a variety of plants from contaminated sites. The low arsenic extraction yield from plants is a known problem and may be impossible to overcome, since using a 'stronger' extractant may result in speciation alteration.

The main arsenic species found in the extracts (both water/methanol mixture and water) were As(III) and As(V), with minor amounts of DMA and traces of MA and TMAO. Arsenic species were identified on the base of identical retention times between arsenic standard compounds and arsenic compounds in samples on both anion and cation exchange columns (comparative analysis). In Table 4 quantitative arsenic speciation data are given and in Figure 2 a typical HPLC-HGAFS chromatogram is shown. This may explain why the arsenic extractability in water is so much higher than in the water/methanol mixture, since the solubility of inorganic arsenic compounds in water is higher. Neither arsenosugars nor cationic arsenic compounds (AsB, AsC and TETRA) were found in the plant extracts, similar to the findings of Koch [37,38] and Zheng [39], who found predominantly inorganic arsenic compounds (As(III) and As(V)) in several plant species from polluted aquatic and terrestrial environments and only traces of simple methylated compounds like MA, DMA, TMAO and TETRA [39]. In this study organoarsenic compounds were only found in plants and not in soil indicating that heather, blackberries and gorse (or the hyphae of symbiotic fungi if present) are able to metabolise part of the inorganic arsenic taken up from the soil. In the less polluted Site 1 up to 16% of the extractable arsenic was found in the form of MA and DMA in the case of gorse while on heavily polluted Site 2 the sum of the methylated compounds in the extracts did not exceed 7% in the case of blackberry fruits and was below 2% for all other plants investigated. Although arsenic methylation is believed to be a detoxification mechanism in

humans and animals, this may not be the case for plants as reported by Meharg and Hartley-Whitaker [26].

The reason for a refractory arsenic fraction of 78–93% in plants remains unsolved for the present, but it is speculated that this fraction may be attributed to arsenic bound to proteins [29,60], thiols [30] or lipids [62]. These non-polar compounds are unextractable with commonly used extraction protocols; however, due to instrumental developments (reduction of interferences from organic solvents) new protocols applying organic solvent extraction procedures are in the making.

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