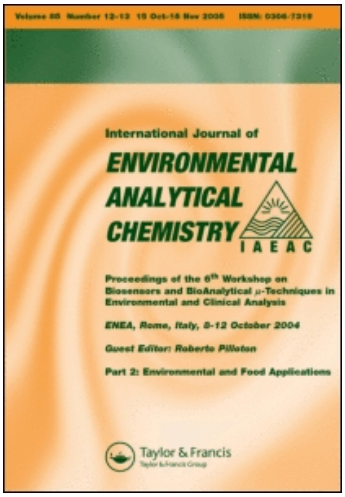

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Distribution of arsenic species in environmental samples collected in Northern Chile

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Online publication date: 17 September 2010

Intern. J. Environ. Anal. Chem.,
Vol. 83, No. 10, pp. 879–890, October 2003

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DISTRIBUTION OF ARSENIC SPECIES IN ENVIRONMENTAL SAMPLES COLLECTED IN NORTHERN CHILE

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(Received 14 May 2003; In final form 6 June 2003)

Gradient ion chromatographic separation coupled with ICP-MS was used to resolve and determine the most common arsenic species in environmental and biological samples of carrots, trout, soil, sediment and river water from Region II of Chile. The carrot and trout samples showed a concentration of 49 and 168 $\mu g\,g^{-1}$, respectively, of total As. Both concentrations are high considering the basal level. In the carrots, percentage of 45 and 31% of total As were found for As(III) and As(V) species, respectively. In the trout, the higher concentration related to AsB at 39% of the total As. As(III) and DMA were also present in relatively high concentrations. The River Loa and the soil in which the carrots are growing also present very high As(V) concentrations of 100 and 17 $\mu g\,g^{-1}$, respectively.

The ratio between the concentration for the same As species found in the living organisms (carrots and trout) and the environment in which they grow (soil and water) can provide important information about the possible absorption or biotransformation of As species in living beings. As(III) and DMA are the species in which the greatest accumulation occurs with respect to the medium in which they are present, and biotransformation also appears to take place.

Keywords: Trout; Carrots; Water; As species; Mobility; Transformation

INTRODUCTION

Biological methylation of As [1], Sb [2], Se [3], Sn [4], Te [5], Hg [6] and Pb [7,8] by the activity of bacteria, fungi, algae, etc., has been demonstrated. For example, the biological conversion of arsenic into various methylated forms takes place in soil and other anaerobic environments by the fungus *Laccaria amehystina* or its associated bacteria [9,10].

Arsenic speciation in nature is important because the different forms of arsenic have vastly different toxic effects on humans. For example, inorganic arsenic As(III) and As(V) are carcinogenic and can cause neurological, cardiovascular and haematological disorders [11], while monomethylarsonic acid (MMA) is believed to be far less toxic [12]. The long-term effects of dimethyl arsinic acid (DMA) are not fully understood [13]. Other forms such as arsenobetaine (AsB) and arsenocholine (AsC), have been considered to be essentially inert, and excreted readily by humans with little or

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ISSN 0306-7319 print: ISSN 1029-0397 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/0306731031000160330

no absorption [14]. Arsenic compounds such as arsenic metal oxides and arsenophosphates that are not readily water soluble have been shown to be less toxic than water soluble compounds because they are less bioavailable [15,16].

The environmental mobility of arsenic depends on its species. Inorganic As(V) is relatively sedentary in soil, and can be adsorbed onto clays, or precipitated with sulphur, iron, copper and aluminium [17]. Methylated As(V) is less likely to precipitate and is therefore mobilised when it comes into contact with water. As(III) species can be mobilised or volatilised more readily than As(V) species.

Currently, the US Environmental Protection Agency (EPA) is investigating the need for lowering the arsenic standard in drinking water to reduce the public health risk. Drinking water and dietary sources form the major routes of exposure to arsenic for most people exposed to these contaminants. Drinking water contains primarily inorganic forms of arsenic [18,19].

The Total Diet Study of the US Food and Drug Administration (FDA) estimates a daily total arsenic consumption of approximately 50 μg per adult [20–22].

Seafood accounts for most of the dietary arsenic ingested, and most of the arsenic species contained in it are relatively non-toxic. However, there are very few data on the chemical forms of arsenic in foods of terrestrial origin, and accurate risk assessments require studies similar to those performed for marine-origin organisms.

There have been a few reports of arsenic speciation in vegetables grown in natural or contaminated arsenic soils [23–26]. According to the report published by Schoof *et al.* [18] on dietary exposure to inorganic arsenic, rice contains the highest concentrations of inorganic arsenic (74–110 $ng\,g^{-1}$) compared with other products tested. When the vegetables broccoli, lettuce, potato (flesh and peel) and Swiss chard were grown in soil treated with 100 $\mu g\,g^{-1}$ of arsenic, they were found to contain As concentrations ranging from very low trace levels when the soil had been treated with MMA, to the relatively high trace level of 3 $\mu g\,g^{-1}$ when the soil had been treated with As(V) [23]. Arsenic speciation in carrots growing in soils contaminated by a wood preservation plant showed that they contained As(III), As(V) and traces of trimethylarsine oxide (TMAO) [23]. The concentration response of the carrots to arsenic contamination was evident.

Arsenic contamination of soil and waters has been reported from all over the globe: USA, Mexico, China, Germany, Japan, Bangladesh, West Bengal (India), etc. [27–31]. Region II of Chile located in the pre-Andean area in the north of the country also presents an environment with high concentrations of arsenic in its water and soils. The contamination comes from various sources such as a high natural hydrogeological background, volcanic activity and anthropogenic activity mainly due to the mining of Cu metal that releases a large amount of arsenic associated with pyrite minerals. The subsequent processes of disintegration and lixiviation distribute the arsenic in the atmosphere, soil and water. In this region, 1099 $\mu g\,g^{-1}$ and 11.2 $mg\,L^{-1}$ of arsenic have been quantified in the soil and aquifers, respectively [32–34]. The average As concentration between 1955 and 1970 in drinking water in the cities of Antofagasta and Calama was about 0.8 $mg\,L^{-1}$ [34]. This contamination of natural and anthropogenic origin in Chile has had a negative effect on human health. The incidence of cardiovascular diseases, blackfoot disease and bladder and lung cancer mortality of the inhabitants of Region II of Chile is higher than in non-contaminated areas [35,36].

This study tackles the determination of As species in vegetables, such as carrots cultivated in contaminated soils [37–39], and in river fish, such as trout living in contaminated waters of Region II of Chile. The comparison of the As species found

in living beings with those found in the environment in which they are growing, contribute to a better understanding of the mechanisms involved mainly in As bioabsorption or biotransformation. It is important also from the human point of view because these living organisms can become a pathway for the entry of As into the human food chain.

EXPERIMENTAL

Instrumentation

FI-HG-AFS

A flow injection–hydride generation–atomic fluorescence spectrometer, FI-HG-AFS (Excalibur, PS Analytical Ltd, Orpington, Kent, UK, Model 10033) was used to determine the total As content. Polytetrafluoroethylene tubing (i.d. 1.6 mm) was used in all connections.

LC-ICP-MS

An inductively coupled plasma mass spectrometer, PQ3 ICP-MS (VG Elemental, Thermo Instruments, Uxbridge, UK) operating under normal multi-element tuning conditions was used as a detector after LC species separation. The column effluent was directly introduced into a Meinhard-type concentric glass nebuliser and a double-pass Scott-type spray chamber with a surrounding water jacket maintained at 5°C. Single ion monitoring at m/z 75 was used to collect the data. All signal quantification was performed in the peak area mode. The peaks were integrated using either ICP-MS Plasma Lab software or Grams/32 software (Galactic Industries, Salem, NY, USA).

For chromatographic separations, a high-pressure pump (LDC Division, Riviera Beach, Florida, USA) was used as a sample delivery system. 100-μL samples were introduced through a 0.45-μm nylon syringe filter into the injection valve Rheodyne 9125 (USA). The connections between the HPLC and the ICP-MS were made of polytetrafluoroethylene tubing (i.d. 0.5 mm).

The analytical parameters for FI-HG-AFS, ICP-MS and HPLC are summarised in Table I.

Others

Sample mineralisation was performed in PTFE reactor vessels in an oven. An I.R. distiller (Berghof, BSB-939IR, Germany) was used for HNO_3 and HCl purification.

Solvent evaporation of extracts was performed in a Univapo100H-Unijet II system (UNIEQUIP, USA). Sonication of samples was performed in a focussed ultrasonic bath (Bandelin Sonopuls HD-2200, Fungilab S.A., USA).

Materials, Reagents and Standards

The extractant mixtures were prepared from deionised water (Milli-Q Ultrapure water systems, Millipore, USA) and HPLC-grade methanol (Merck, Darmstadt, Germany). High-purity nitric and hydrochloric acids were obtained by the distillation of the analytical-grade reagent (Merck). HF acid was suprapur grade (Merck). Other reagents were obtained from Merck. Each arsenic species stock solution containing 1000 g L^{-1} of

TABLE I Instrumental parameters for total As determination and As speciation analysis

HG-AFS				
$NaBH_4$ concentration			1% w/v	
HCl concentration			1.5 M	
Flow rate of $NaBH_4$ and HCl			1.0 mL min^{-1}	
Sample flow rate			0.8 mL min^{-1}	
H_2 flow to feed diffusion flame			60 mL min^{-1}	
Ar carrier gas flow			200 mL min^{-1}	
Ar auxiliary gas flow			100 mL min^{-1}	
ICP-MS				
R.F. power	Forward: 1350 W Reflected: 2.2 W			
Ar flow rate	Coolant: 14 L min^{-1} Nebulizer: 1.0 L min^{-1} Auxiliary: 0.9 L min^{-1}			
Measurement mode	Peak area of ^{75}As			
Integration time	0.1 s (spectrum) per point			
Points per peak	3			
HPLC				
Column	Mobile phase	pH	Flow rate	Injection
PRP-X100[a]	Phosphate/NH_3 Gradient mode (%)[b] 0–15 min (A: 100–0 and B: 0–100) 15–25 min (A: 0–100 and B: 100–0) Conditioning: 25–30 min (A: 100 and B: 0) Isocratic mode: 10 mM	6.0	1.5 mL min^{-1}	100 µL

[a]PRP-X100 column: Anion exchange, Hamilton (UK) (250 × 4.1 mm, particle size 10 µm); [b]A = 5 mM; B = 25 mM.

As was prepared by dissolving the respective amount of the pure compound in water. As(III) and As(V) standards were prepared from sodium arsenite and sodium arsenate obtained from Sigma Aldrich (St Quintin, Fallavier, France) dimethylarsinic acid (DMA) and methylarsonic acid (MMA) obtained from Merck, and arsenobetaine (AsB) and arsenocholine (AsC) obtained from Tri Chemical Laboratory Inc. (Japan). The stock solutions were kept at 4°C in the dark. Working solutions were prepared daily and then diluted with water to the final concentration. Three certified reference materials (CRMs): NIST, SRM 1568a (rice flour); NRCC DORM-2 (Dogfish muscle) and NRCC, TORT-1 (Lobster hepatopancreas) were used to validate the total As determination and for As species characterisation and/or validation.

Samples

A small area of 100 m^2 located in the agricultural smallholding of the communities of Chiu-Chiu, in which carrots were growing, was used for sampling. Chiu-Chiu is a village of about 1000 inhabitants situated in the Loa river area in the interior of Region II of Chile. This river is used to irrigate the smallholdings of cultivated land. Agriculture is the inhabitants' main economic activity. Their diet consists mainly of vegetables and meat. 1 kg of soil and carrots were randomly selected from a batch of around 5 kg each.

Trouts and sediments were obtained from the River Loa. Both water and soil in the Chiu-Chiu valley show a high arsenic concentration [33].

Five carrot and trout samples taken randomly from the same area were peeled, dried, homogenised and bottled. Soil and sediment samples were sieved through a pore size $<125\,\mu m$, and the fraction with larger particles was discarded.

Mineralization for Total Arsenic Determination Procedures

Carrots and Trout

About 0.5–1.0 g of each tested sample was placed in a PTFE reactor vessel. 10 mL of concentrated HNO_3, 2 mL of concentrated $HClO_4$ and 2 mL of 2% (m/v) $Na_2S_2O_8$ were added, and the sample was pre-digested overnight. Next, the reactor was heated to 150°C for 2 h in an oven. After cooling, 0.5 mL of concentrated H_2SO_4 was added and the digested sample was heated by refluxing in a glass beaker for about 2 h until the final volume was about 2 mL. The digested sample was diluted to 10 mL with 0.5 M HCl and each sample was analysed in triplicate. CRMs were treated in the same way as carrot and trout samples.

Soil and Sediments

About 250 mg of the sample were placed in the reactor with 5 mL of concentrated HNO_3, 5 mL of concentrated HCl and 3 mL of concentrated HF. After overnight pre-digestion, the samples were treated under conditions similar to those described above for the carrots and trout. Five subsamples and three blanks were prepared in parallel and each one was analysed in triplicate.

Extraction for Arsenic Species

Carrots and Trout

About 1.0 g of the sample and 10 mL 1:1 methanol–water were placed in a PTFE reactor. The mixture was mechanically shaken for 3 h, maintained at 55°C for 10 h and finally left in an ultrasonic focalised bath for 5 min. Next, the mixture was centrifuged and the residue was twice re-extracted with 5 mL of 1:1 methanol–water, following the procedure formerly described. The combined extracts were evaporated to an almost dry state and diluted with 5 mL deionized water and filtered through a 0.45-μm nylon syringe filter before injection into the liquid chromatograph. CRMs were extracted in the same way as the carrot and trout samples.

Sediments and Soil

About 1.0 g of sample was treated with 10 mL 1 M H_3PO_4. The extract was evaporated and diluted with 5 mL 10 mM ammonium phosphate, $pH = 6$. Three subsamples and three blanks were prepared in parallel and each one was analysed in triplicate.

RESULTS AND DISCUSSION

Total As Concentration and Arsenic Species Mobility and Transformation

Six non-volatile species were considered for As speciation: arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC).

The detection limit for total As was 50 ng g^{-1} and for As species within the 3–5 ng g^{-1} range for As(III), 6–8 ng g^{-1} for As(V), 3–5 ng g^{-1} for AsB, 4–5 ng g^{-1} for MMA and 6–7 ng g^{-1} for DMA for the different samples tested.

A 100-μL injection of 5.0 ng mL^{-1} of As(V) before each chromatographic separation was used to correct any drift in the ICP-MS response.

An error is likely to occur in the As species determination by ICP-MS (^{75}As, monoisotopic element) if high chloride concentration coelutes in the chromatogram with any of the As species because of possible $^{40}Ar^{35}Cl$ formation. However, the concentration of chloride was not high enough to produce any significant interference.

Table II shows the total As content and As extraction efficiency after three consecutive extractions in the samples tested. An almost quantitative extraction efficiency was obtained in most cases and also for the CRM TORT-1 and DORM-2 materials. The As content in the blanks was negligible.

The gradient chromatographic conditions shown in Table I allowed quantification of all As species of interest in one chromatographic run using the anion-exchange column Hamilton PRP-X100. As(III) and AsB peaks that overlapped in the isocratic mode were very well resolved using the gradient elution. Figure 1(a) shows a typical gradient chromatogram in which all As species tested are resolved. The concentration distribution among species for the samples studied can be seen in Table III.

Figure 1(b)–(f) shows the chromatograms obtained for the six As species detected in Loa river water, soil, carrots, trout and river sediment. It is important to mention that final extracts were appropriately diluted in each case. The species were characterised by spiking the samples with the corresponding standard.

Some important observations are highlighted below.

The 91 μg g^{-1} concentration of As in the sediment of the Loa river is high compared with the basal level in non-contaminated rivers established as being in the 0.5–6 μg g^{-1} range [40]. As(III) and As(V) are the only species and As(III) is more predominant (about 60% of the total concentration).

The average value of As for soil was 17.2 μg g^{-1}. The species quantified were As(III), As(V), DMA, MMA and AsB, As(V) being the predominant species (50% of total As). In an earlier study, a soil sample taken near Antofagasta City showed a concentration of 3.2 μg g^{-1} [40]. The maximum recommended level of As for soil is 20 μg g^{-1}, but the level of As in polluted soils may be very high; in the Red Sea area and New Zealand concentrations of 125 and 8 mg g^{-1}, respectively, were found [17].

The As concentration in carrots and trout was 49 and 168 μg g^{-1}, respectively. A basal level of 0.01–9 μg g^{-1} for carrots has been established [40]. In an earlier study,

TABLE II Mean of total As concentration in the samples and in their methanolic extracts by HG-AFS ($n = 5$ for total As determination and for As determination in the extracts)

Sample	*Total As*	*As in methanol–water (1:1) extract*	*Extraction efficiency*
Carrots	49.1 ± 2.5	48.9 ± 2.2	99.0 ± 4.0
Trout	168 ± 11	149.1 ± 9.0	89.0 ± 4.7
Sediment	91.2 ± 3.6	90.8 ± 5.5	99.0 ± 4.0
River water	101.3 ± 3.0	–	
Soil	17.2 ± 1.1	17.0 ± 1.0	99.0 ± 5.0
DORM-2[a]	17.0 ± 0.8	16.6 ± 1.2	92.0 ± 4.7
Tort-1[b]	23.1 ± 0.8	21.5 ± 1.3	93.0 ± 4.8

Results expressed as μg g^{-1} in dry samples. [a]Certified value 18.0 ± 1.1 μg g^{-1}; [b]certified value 24.6 ± 2.2 μg g^{-1}.

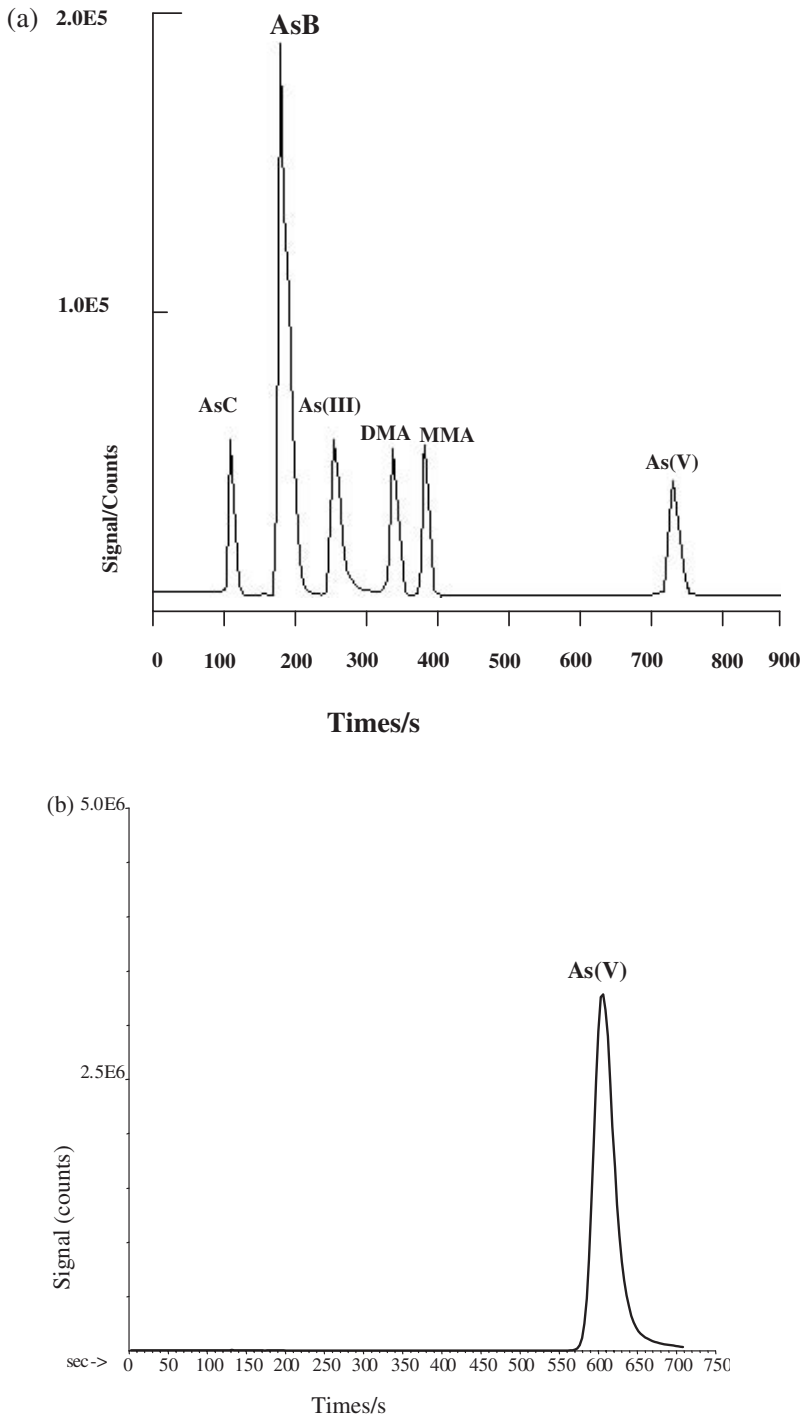
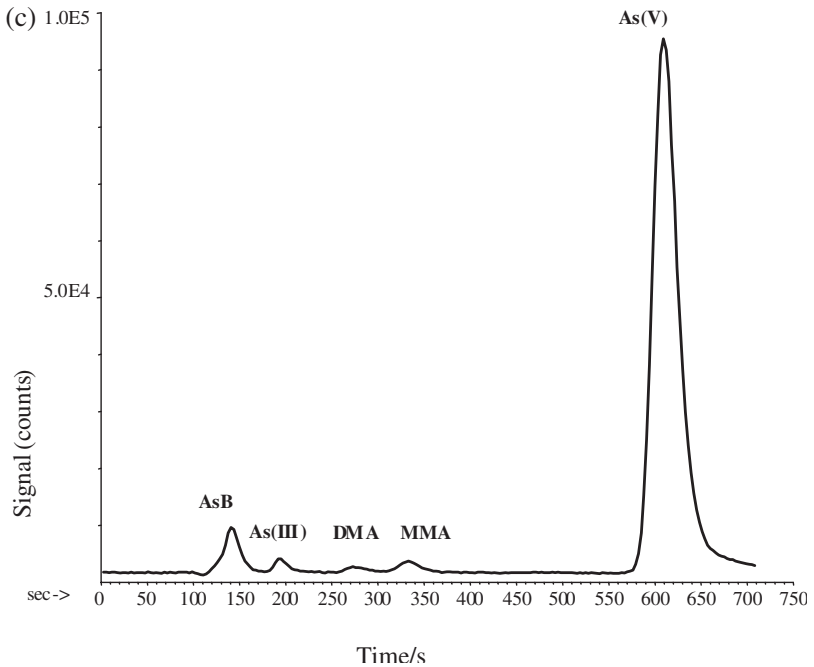


FIGURE 1 HPLC-ICP-MS chromatogram for As species in: (a) mixture of As species containing 15 $\mu g\,L^{-1}$ of AsB and 5 $\mu g\,L^{-1}$ of the other As species; (b) Loa river water; (c) soil from Chiu-Chiu area; (d) carrots growing in contaminated soil from Chiu-Chiu area; (e) trout living in contaminated Loa water and river sediments; (f) sediment taken from the Loa river.



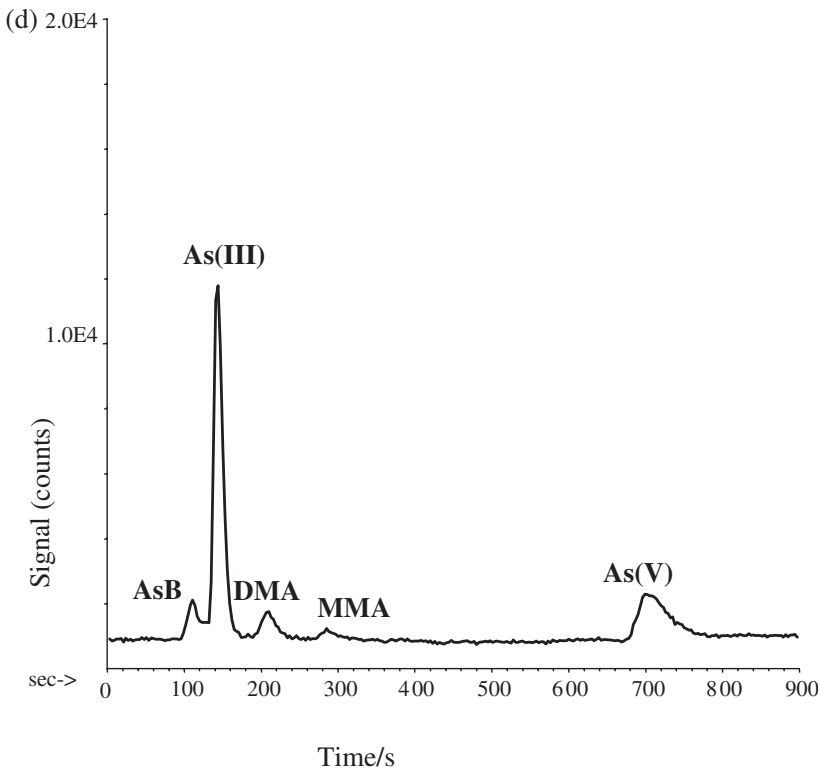
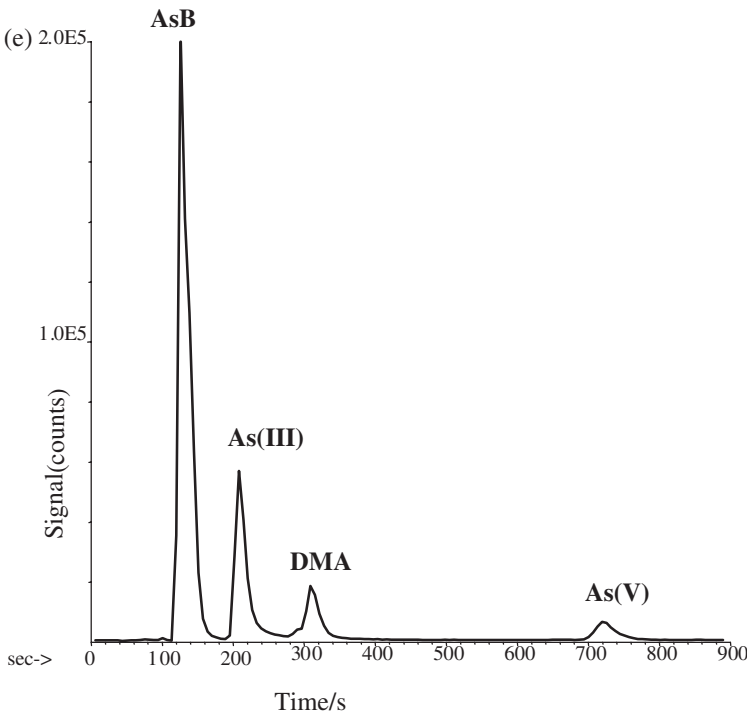


FIGURE 1 Continued.



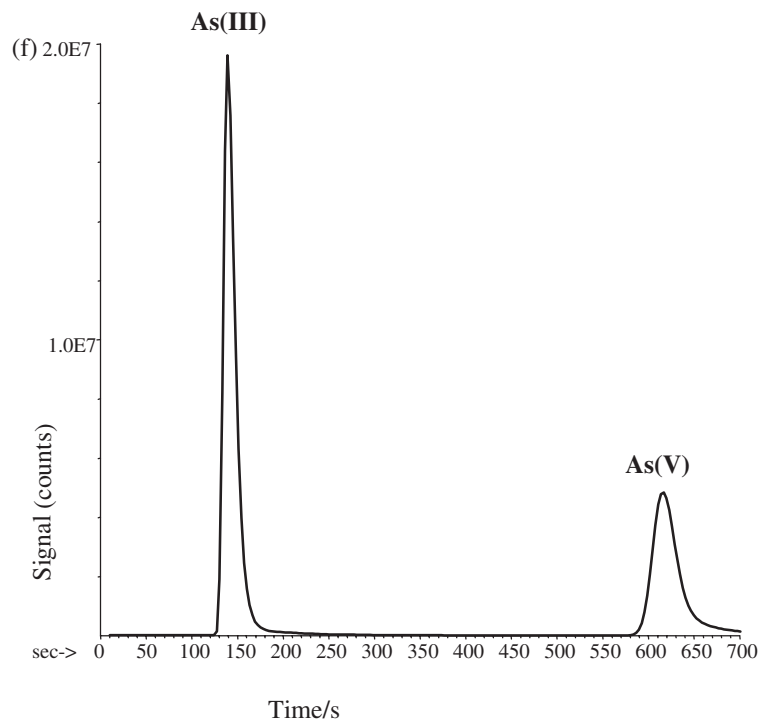


FIGURE 1 Continued.

TABLE III Concentration of As species in the samples analysed and reference materials

Sample	*As(III)*	*As(V)*	*DMA*	*MMA*	*AsB*
Carrots	22.1 ± 1.4	15.0 ± 1.0	5.2 ± 0.6	1.8 ± 0.5	3.6 ± 0.8
Trout	30.0 ± 1.1	16.0 ± 0.9	23.1 ± 1.1	nd	65.0 ± 1.6
Sediment	59.3 ± 1.5	30.6 ± 1.3	nd	nd	nd
River	nd	100.0 ± 2.0	nd	nd	nd
Soil	2.2 ± 0.4	8.2 ± 0.9	1.1 ± 0.3	1.8 ± 0.4	3.1 ± 0.6
DORM-2	nd	nd	0.66 ± 0.04	nd	15.09 ± 0.5[a]
TORT-1	nd	0.30 ± 0.03	2.01 ± 0.06	< 0.05	16.8 ± 1.1

nd: not detected or below the detection limit. Results expressed as $\mu g\,g^{-1}$. [a]Certified value of AsB in DORM-2: 16.4 ± 1.1.

the concentrations found in San Pedro de Acatama for carrots and beet were within the 30–40 $\mu g\,g^{-1}$ range [33]. The main As content was made up of toxic species, mainly As(III) and As(V). These species represent 45 and 31% of the total As, respectively. AsB, DMA and MMA are present in lower concentrations.

The total As concentration in trout, although very high (100 times higher than those reported in freshwater fish), is similar to concentrations found for certain marine organisms (living in non-contaminated environments). However, whereas AsB is the most predominant (when not unique) species in marine organisms, in trout it represents only 39% of the total concentration. It is known that the AsB content in fish and seafood is mainly derived from the consumption of algae containing arsenosugars (and probably AsB). Although fish are definitely not good bioindicators of inorganic arsenic contamination, the extremely high concentration of inorganic As in trout flesh reflects a serious effect of the As river contamination.

In addition, the trout samples analysed showed malformation problems in their guts and fins, although it is not known whether As was responsible for these problems.

The ratio between the concentration of As species found in carrots and the environment in which they were growing (soil) may provide important information about the possible absorption or biotransformation mechanisms involved (Fig. 2). The data obtained highlight the following: As concentration ratios carrots/soil of about 1 : 1 were found for AsB and MMA. The low concentration of AsB found in carrots seems to confirm that non-marine plants are not producers or accumulators of this species. The AsB in carrots seems to be due to an absorption process because this species was also present in the soil as a result of microbiological activity in the soil. The 1.8 : 1 ratio of As(V) in carrots with respect to soil is also considered to be relatively low, taking into account that As(V) is the most abundant species in the soil (and in the water used for irrigation). It is known that the presence of phosphate may decrease the plant's arsenate absorption through a competitive mechanism for both analytes. Other possible mechanisms could be the biotransformation of absorbed As(V) into As(III) or methylated species. The high ratios of As(III) and DMA species, 10 : 1 and 4.7.1:, respectively, detected for carrots seem to indicate the biotransformation of the As absorbed by the plant into these species. These findings are similar to those found for other vegetables. For example, in rice grown in non-contaminated areas, the As concentrations are relatively high, As(III), DMA and As(V) being the most important species present [41,42]. Other studies performed on carrots watered with As(V) standard solutions showed that As(III) was the most abundant species, followed by As(V) [43], and for carrots growing in a soil with an arsenic content of 400 mg kg^{-1}

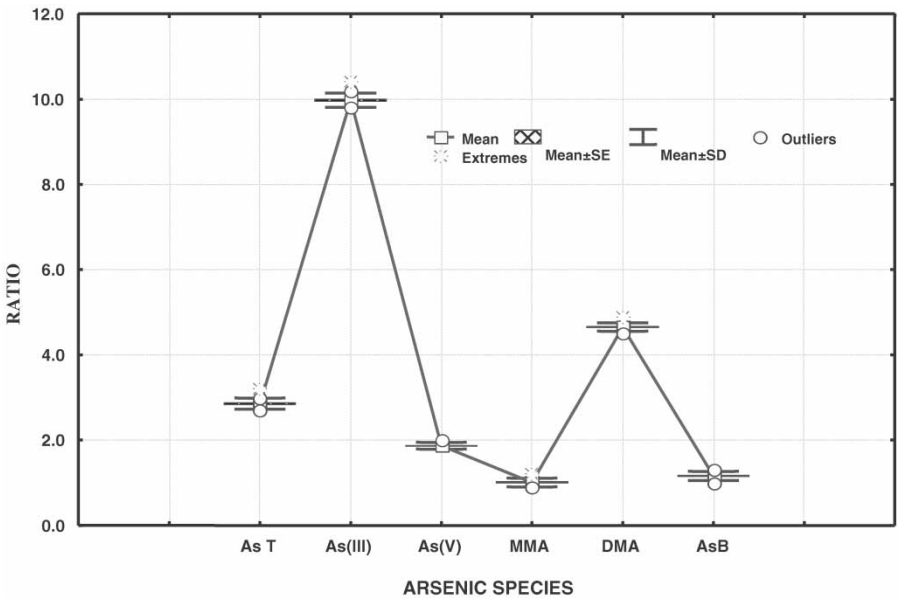


FIGURE 2 Ratio of carrots/soil for total As and species.

As(III) and As(V) were the main species. A low content of MMA was detected and no data for AsB was reported [44].

The presence of DMA in trout could be due its detoxification mechanisms. It is known that biomethylation mechanisms are always preceded by an As(V) to As(III) oxidation–reduction process. The high As(III) concentration found in trout and carrots with respect to DMA concentrations could indicate that reduction is a more favoured mechanism than methylation for high As(V) concentrations. This seems to be confirmed by the fact that As(III), and no other methylated species, is found in the sediments. The inorganic nature of these sediments (leaching from the mine) is probably why the microorganisms' biomethylation action is hindered.

CONCLUSIONS

Separation of the most common As species in the biological and environmental samples tested using gradient anion chromatography has been achieved.

It has been demonstrated that trouts and carrots living and growing in an As-contaminated area have the ability to accumulate high concentrations of toxic As species. Considering the toxicity of inorganic arsenic for human beings and animals, the consumption of the fish and vegetables grown in these contaminated areas is highly risky.

Acknowledgements

This research was supported by a grant under the RTD programme of the EU and by the BQU 2002-01348 project. We would also like to thank Professor Domingo Roman from the Antofagasta University (Chile) for providing the river sediments.

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