Occurrence of Arsenic Species in Algae and Freshwater Plants of an Extreme Arid Region in Northern Chile, the Loa River Basin

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ABSTRACT

This study reports data on arsenic speciation in two green algae species (*Cladophora* sp. and *Chara* sp.) and in five aquatic plants (*Azolla* sp., *Myriophyllum aquaticum*, *Phylloscirpus* cf. *desserticola*, *Potamogeton pectinatus*, *Ruppia filifolia* and *Zannichellia palustris*) from the Loa River Basin in the Atacama Desert (northern Chile). Arsenic content was measured by Mass Spectrometry coupled with Inductively Coupled Plasma (ICP-MS), after acidic digestion. Liquid Chromatography coupled to ICP-MS was used for arsenic speciation, using both anionic and cationic chromatographic exchange systems. Inorganic arsenic compounds were the main arsenic species measured in all samples. The main arsenic species in the extracts of freshwater algae and plants were arsenite and arsenate, whereas glycerol-arsenosugar (gly-sug), dimethylarsinic acid (DMA) and methylarsonic acid (MA) were present only as minor constituents. Of the samples studied, algae species accumulated more arsenic than aquatic plants. Total arsenic content ranged from 182 to 11,100 and from 20 to 248 mg As kg^{-1} (d.w.) in algae and freshwater plants, respectively. In comparison with As concentration in water samples, there was hyper-accumulation (>0.1% d.w.) in *Cladophora* sp.

HIGHLIGHTS

- Loa River Basin (area of study) presents extreme environmental conditions
- Arsenic and arsenic compounds were determined in algae and aquatic plants
- Inorganic arsenic species predominated in all samples
- Arsenic content in most samples ranged from 20 to 341 mg As kg^{-1}
- One sample (*Cladophora* sp.) presented hyperaccumulation of As (11,000 mg As kg^{-1})

KEYWORDS

Arsenic speciation, LC-ICP-MS, algae, aquatic plants, Loa River

1. INTRODUCTION

The Antofagasta Region (northern Chile) has high environmental levels of arsenic (Queirolo, et al., 2000a). The only river in the region that flows into the sea is the Loa, an extremely saline river. Dissolved arsenic content in the Loa and its tributaries range from 200 to 4,400 µg As L^{-1} (seasonal maximum) (Dirección General de Aguas (DGA), 2004). The chemical composition of the Loa's water is strongly influenced by its tributaries, mostly by the Salado River, which is As-enriched by waters from the El Tatio geothermal fields with levels up to 27 mg As L^{-1} (Romero, et al., 2003). The extremely arid conditions, high evaporation and the lack of low-level arsenic tributaries maintain high concentrations of arsenic and other components (e.g. copper, boron, chloride, sulfate…) throughout the river course. Nevertheless, arsenic not only comes from natural sources such as volcanic bedrock and geothermal activity, but also has anthropogenic origins, such as smelter emissions, mining waste and enriched arsenic effluents from water treatment plants (Dirección General de Aguas (DGA), 2004). The Loa River and its main tributaries provide water to the cities and it is extensively used for agriculture and by the mining industry in the Atacama region. Adverse health effects due to high arsenic concentrations in drinking water have been reported in rural populations since 1962 (Smedley, et al., 2000). Nowadays, major cities and towns receive water that complies with Chilean legislation (< 0.010 mg As L^{-1}) (Ministerio de Salud Pública, 1969).

The Loa River is a suitable habitat for a high number of endemic flora and fauna species, particularly relevant for their adaptation to this extremely arid region. Algae and aquatic plants can be considered possible bioindicators of arsenic levels in the aquatic system. As they are able to remove inorganic arsenic from water, they could be useful for bioremediation purposes (Bird, et al., 2011; Hansen, et al., 2006; Knauer and Hemond, 2000; Robinson, et al., 2006b).

A comprehensive review on distribution an occurence of organoarsenic compounds in living organisms is available from Reimer et al. (2010). Specifically, several studies on arsenic and its compounds in marine algae around the world have been reported (Francesconi and Edmonds, 1998; Llorente-Mirandes, et al., 2010; Thomson, et al., 2007; Tukai, et al., 2002). However, few data are available for total arsenic (Hansen, et al., 2006; Vasquez and Guerra, 1996) and arsenic speciation in Chilean seaweeds (Ruíz Chancho, et al., 2010). Nor is there much information on freshwater algae and aquatic plants (Miyashita, et al., 2009; Schaeffer, et al., 2006; Zheng, et al., 2003). Although some reports are available on arsenic in water (Dirección General de Aguas (DGA), 2004; Queirolo, et al., 2000a; Romero, et al., 2003), vegetables (Muñoz, et al., 2002; Queirolo, et al., 2000a; Queirolo, et al., 2000b) and aquatic plants

(Stegen, et al., 2000) from the Loa River Basin, no study was found reporting arsenic speciation in the algae and aquatic plants of this basin.

The aim of the study is to determine total arsenic and arsenic species in algae and aquatic plants from the Loa River Basin in order to assess their contribution to overall contamination in this lotic ecosystem. This could be a motive for further bioremediation studies in the area and studies of possible bio-monitoring organisms.

2. STUDY AREA

The study area was restricted to the Loa River Basin in northern Chile (22°16'0''S 68°38'0''W). The location and general view of the study area are given in Figure 1. Mining activity in the Loa Basin takes place in the intensively mineralized porphyry-Cu belt with developments at three large Cu deposits: Chuquicamata, Radomiro Tomic and El Abra (Figure 1). The main tributaries of the Loa River are the San Pedro, Salado and San Salvador rivers. Two important sources of arsenic have to be considered in this basin. On the one hand, the Salado River, mainly fed by the geothermal springs of El Tatio located in the Andes, flows in an E–W direction into a canyon and cuts into volcanic rocks, mainly andesite and rhyolitic ignimbrite of the Miocene-Holocene age. On the other hand, the Chuquicamata smelter, at 2,850 MASL and 16 km from the city of Calama, producing high As content in the copper concentrates and the release of SO_2 and aerosols (containing mainly arsenic as As_2O_3 and a low proportion of Cd, Cu, Pb and Zn) into the air, contributes to the contamination of water bodies, especially saltpans (Brundenius and Göransson, 1990). The hydrologic regime of the Loa Basin is rain-dominated: the river flow increases mainly during the summer in January and February (Dirección General de Aguas (DGA), 2004). The region is extremely arid with a rainfall ranging from 300 mm per year at 3,000 MASL to 1-2 mm per year at sea level (Romero, et al., 2003) and is associated with high environmental levels of arsenic (Queirolo, et al., 2000a). Owing to the extremely arid conditions in the region, all rivers are temporal or endorrheic except for the Loa River, which is the only permanently exorrheic river in the region. It is 440 km long, covers an area of 33,570 km^2 and flows sinuously across the Atacama Desert from the Andes to the Pacific Ocean. In this basin, plants and algae grow in water with high conductivity and pH (see Table 1) and under strongly limiting conditions, such as large daily temperature variations and prolonged daily UV exposure.

Along the Loa Basin (Figure 1 and Table 1), three different sections of the river with specific chemical properties can be defined. The *Upper Loa Section* comprises the zone between the source, at the foot of the Miño volcano (UTM coordinates: 19S 541,002 7,657,055), and its confluence with the Salado River. After Lequena (Figure 1: LO-1), most of the river flow is extracted for mining and agricultural activities. The main tributary in this section is the San Pedro River, which receives water from several sources. Before the confluence with the San Pedro River, the Loa is recharged from groundwater tributaries. The *Middle Loa Section* comprises the zone between the Loa-Salado confluence near Calama (Figure 1: before LO-2) and the confluence with the San Salvador River (Figure 1: after SS-1). The origin of the Salado River is close to the El Tatio geothermal field. The Toconce River, which flows into the Salado River's upper course (Figure 1: before TO-1), has its source at the foot of the Linzor volcano (Figure 1). The *Lower Loa Section* comprises the zone between the confluence with the San Salvador River and the mouth of the river in the Pacific Ocean. The source of the San Salvador River is on the west side of Calama. The main agricultural areas in the *Lower Loa Section* are in Quillagua (Figure 1: after LO-4).

3. MATERIAL AND METHODS

3.1. Reagents and Standards

All chemicals were of analytical and/or suprapur grade. Millipore Milli-Q Plus Water (18.2 MΩ cm) was used for all solutions. Ammonium dihydrogen phosphate (Panreac, p.a.) and pyridine (Scharlau, p.a.) were used for anionic and cationic mobile phase preparation, respectively. pH was adjusted with 30% ammonia (Panreac, p.a.) and 98% formic acid (Panreac, p.a.). For sample digestion, 69% nitric acid (Panreac, Hiperpur) and 31% hydrogen peroxide (Merck, Selectipur) were used. ^{9}Be, ^{103}Rh, ^{205}Tl 20 µg L^{-1} (NIST High-Purity Standards) were used as internal standards in ICP-MS measurements.

3.1.1. Arsenic standards and Certified Reference Materials

Arsenite from As_2O_3 (NIST, USA, Oxidimetric Primary Standard 83d, 99.99%); arsenate from $Na_2HAsO_4 \cdot 7H_2O$ (Carlo Erba); methylarsonic acid (MA) as $(CH_3)AsO(ONa)_2 \cdot 6H_2O$ (Carlo Erba); dimethylarsinic acid (DMA) as $(CH_3)_2AsNaO_2 \cdot 3H_2O$ (Fluka); arsenocholine (AC) as $(CH_3)_3As^+(CH_2)CH_2OHBr^-$ supplied by the ''Service Central d'Analyse" (CNRS Vernaison, France); arsenobetaine (AB) as $(CH_3)_3\ As^+CH_2COO^-$,CRM 626, supplied by BCR (now IRMM), standard solution; and trimethylarsenic oxide (TMAO) from $(CH_3)_3AsO$ (Argus Chemicals srl) were used as arsenic standards in speciation. Standardized stock solutions of the arsenic compounds containing

about 1,000 mg $^{-1}$ were prepared in water, except for arsenite, which was dissolved in NaOH (4 g L^{-1}, Merck, Suprapure), and all were stored in the dark at 4°C to prevent decomposition or oxidation. Multispecies standard working solutions covering the range 1 - 100 µg As L^{-1} were prepared fresh daily for speciation analysis. Arsenate standard solution from NIST High-Purity Standards with a certified concentration of 1,000 ± 2 mg As L^{-1} was used for external calibration in the determination of total arsenic content with ICP-MS. An aliquot of freeze-dried extract of *Fucus serratus* dissolved in water (Madsen, et al., 2000) was used as a laboratory reference material for the identification of the major arsenosugars: phosphate (PO_4-sug), sulfate (SO_4-sug), sulfonate (SO_3-sug) and glycerol (Gly-sug). The Certified Reference Material BCR CRM 279 Sea Lettuce (*Ulva lactuca*), supplied by the Institute for Reference Materials and Measurements (IRMM) of the European Commission, with a certified value of 3.09 ± 0.20 mg As kg^{-1}, and the Standard Reference Material (SRM) 1640 for natural water were used for internal quality control purposes in total arsenic determinations.

3.2. Instruments

A Perkin Elmer system of Flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS), Model AAnalyst 700 and FIAS 400, was used for total As in water, under the following conditions: sample loop 0.5 mL; reducing agent, 0.5% $NaBH_4$ in 0.125% NaOH at 5 mL min^{-1}; 10% HCl, at 10 mL min^{-1}; and argon at 100 mL min^{-1} as carrier gas for the FI system. An As electrodeless discharge lamp and electric oven temperature for the quartz cell at 900°C was used in AAS.

Algae and aquatic plants and CRM Sea Lettuce were digested in a closed microwave digestion system, Milestone Ethos Touch Control. The ICP-MS analyses were performed through an Agilent 7500ce ICP-MS (Agilent, Germany) with Ari Mist HP nebulizer (Burgener, Canada). The chromatographic system consisting of an Agilent 1200 LC quaternary pump, equipped with an autosampler and degasification module, was connected to an analytical PRP-X100 (Hamilton, USA) and Zorbax SCX300 (Agilent, Germany). Both columns were protected with their respective guard column. The Instrument operating conditions of LC-ICP-MS and arsenicals that are separated with each chromatographic system are given in Table 2.

3.3 Procedures

3.3.1 Sample collection and preparation

In June 2010, the Analytical and Environmental research group of the Chemistry Department of the Católica del Norte University (Antofagasta, Chile) collected samples of water and of the

dominant species of both algae and plants from eight sites along the Loa River and its tributaries, San Pedro, Salado and San Salvador (Figure 1). The geographical coordinates and the water properties of the sampling sites are shown in Table 1. Electrical conductivity, dissolved oxygen, pH and water temperature were measured *in situ*. Water samples were acidified with 2 M HNO_3 and cooled in a refrigerator (< 5 °C) during transport to the laboratory, where they were stored at -20°C until further analysis. The taxonomic identification of the plants and algae is given in Table 3. Samples were stored in sealed plastic bags at -18°C in the laboratory until preparation for transportation. Samples were defrosted under a laminar flow clean bench, washed with deionized water to remove mud, sand and little stones, pre-dried at 45°C for 3 days and sealed in plastic bags.

Plant and algae samples were transported by plane to the Analytical Chemistry Department of the University of Barcelona. There, a stereomicroscope (Zeiss) was used to remove remaining impurities. Then, samples were dried at 40°C, crushed by hand in a glass mortar and stored in PET bottles until their analyses.

3.3.2. Determination of total As in water

Total arsenic content in water samples was determined after microwave acidic digestion, using a closed-vessel system as follows: a 45 mL water sample was placed into the pre-cleaned EasyPrep™ vessels and 9 mL of 65% nitric acid and 3 mL of 40% hydrogen peroxide were added for digestion. The program for addition was as follows: 10 min at room temperature, 10 min from room temperature to 200°C and 15 min maintained at 170°C. After cooling, digested samples were filtered through ash-free filter papers (Whatman 42) into a 100 mL volumetric flask and 5 mL of 50% HCl and 5 mL of reducing solution (5% KI + 5% ascorbic acid) were added. After 30 min, the resulting solution was diluted to volume with 50% HCl. Blanks were also prepared for each batch sample. Total As was measured by FI-HG-AAS under the conditions described in *Instruments*.

3.3.3. Extraction of arsenic compounds and speciation analysis

Homogenized, powdered samples (0.1 g) were separately weighed in polypropylene tubes in triplicate and 10 mL of water was added. The extraction procedure was performed in an end-over-end shaker overnight at 35 rpm for 16 hours at room temperature. Water extracts were centrifuged (3,000 rpm, 15 min) and the supernatants were filtered through PET syringe filters (Chromafil PET, Macherey–Nagel, 0.45 µm) before analysis. The LC-ICP-MS system previously

used (Llorente-Mirandes, et al., 2010; Ruíz Chancho, et al., 2010) was applied for the determination of arsenic compounds in algae and plant extracts, under the conditions described in Table 2. An aliquot of each extract was analyzed by anionic exchange chromatography immediately after extraction. The remaining extract was stored at -80°C for further analyses (cationic exchange and total arsenic measurements). Chromatographic peaks were identified according to their retention time by comparison with standards. Arsenic species were quantified by external calibration curves. Total As was determined in aliquots of the extracts, for mass balance calculations.

3.3.4. Determination of total As in algae, aquatic plants and the speciation extracts

Algae and aquatic plants and BCR CMR 279 were digested under a closed-vessel microwave system as follows: 0.2 g of powdered sample was weighed in the pre-cleaned TEFLON® vessels in triplicate. After addition of 8 mL of 69% nitric acid and 2 mL of 33% hydrogen peroxide, samples were digested 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 maintained for 10 min at 190°C. After cooling, digested samples were filtered through ash-free filter papers (Whatman 40) and diluted to 20 mL with water. Blanks were also prepared for each batch sample. Total arsenic content was measured by ICP-MS. The digested samples and the extracts obtained for further arsenic speciation were properly diluted with 1% nitric acid prior to measurement, to ensure that all arsenic concentrations were within the working calibration range (0–50 µg As L^{-1}). Helium was used in the collision cell to remove interferences in ICP-MS measurements and a solution of ^{9}Be, ^{103}Rh, ^{205}Tl (20 µg L^{-1}) was used as an internal standard. Samples were quantified by external calibration method. For quality control purposes, the calibration curve was run before, within and after each sample series measurement.

3.4 Quality assessment in the determination of arsenic and arsenic species

3.4.1 Column recovery

Column recovery was calculated as the ratio of the sum of the species eluted from the chromatographic columns to the total arsenic in the extract injected into the column. Column recoveries ranged between 60% and 100% (Table 3). This parameter allows to evaluate correctly the quantification of the species and to guarantee the correct chromatographic separation.

229 *3.4.2. Certified reference material (CRM)*

230 To check accuracy, total arsenic concentration was determined in CRM BCR 279 Sea lettuce
231 (*Ulva lactuca*). The result obtained (2.9 ± 0.3 mg As kg^{-1}) was consistent with the certified value
232 (3.09 ± 0.20 mg As kg^{-1}), thereby demonstrating the accuracy of the analytical method. Our
233 results for arsenic species (As(V): 0.53 ± 0.04 mg As kg^{-1}; As(III): 0.06 ± 0.03 mg As kg^{-1}; DMA:
234 0.06 ± 0.03 mg As kg^{-1}; MA: 0.04 ± 0.01 mg As kg^{-1};AB: 0.14 ± 0.02 mg As kg^{-1}; gly-sug: 0.096
235 ±0.004 mg As kg^{-1}; PO_4-sug: 0.08± 0.01 mg As kg^{-1}; Unknown species: 0.07 ± 0.02 mg As kg^{-1};
236 Extraction efficiency: 57%; Column recovery: 81%) and those reported in the literature do not
237 disagree (Caumette, et al., 2011; Foster, et al., 2007).

238 *3.4.3. Analysis of* F. serratus *extract*

239 We used an extract from the brown seaweed *F. serratus* (Madsen, et al., 2000) to identify
240 arsenosugars present in our algae samples. For quality control purposes, we quantified As
241 species in *F. serratus* extracts. Our results[1] (DMA: 0.01 ± 0.01 µg; gly-sug: 0.07 ± 0.01 µg; PO_4-
242 sug: 0.07 ± 0.01 µg; SO_3-sug: 0.56 ± 0.04 µg; SO_4-sug: 0.37 ± 0.02 µg) confirm those reported by
243 Madsen *et al.* (2000) and other values in the literature on the same extract (Kohlmeyer, et al.,
244 2003; Llorente-Mirandes, et al., 2010; Ruíz Chancho, et al., 2008; Šlejkovec, et al., 2006).

245 *3.4.4. Quantification of arsenic species without standard*

246 Standards were not used for some arsenic species since they were not offered. Using
247 calibration curves form others species is a controversial point as nebulization efficiency might
248 be different for each compound (Entwisle and Hearn, 2006; Polya, et al., 2003); however, we
249 quantified PO_4-sug with the MA calibration curve, SO_3-sug and SO_4-sug with the As(V)
250 calibration curve, and gly-sug with the calibration curve of the AC standard as other authors
251 suggested (Francesconi and Sperling, 2005).

252 *3.4.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)*

253 LOD and LOQ were estimated. The former is the lowest concentration of an analyte that can
254 be reliably differentiated from background noise (signal-to-noise ratio greater than 3). The
255 LOQ is the lowest concentration that can be quantified (signal-to-noise ratio greater than 10).
256 For calculating LOD and LOQ, the standard deviation of the base line and the peak base of each
257 analyte multiplied by 3 or 10 (LOD and LOQ respectively) were calculated in the peak height

[1] Values for *F. serratus* extract are given as absolute amount for extract µg.

258 calibration curve. The arsenosugar LODs and LOQs was estimated through a correction factor,
259 which is the relation within the concentration of arsenosugar in *F. serratus* and the height of
260 the peak.

261

262 **4. RESULTS AND DISCUSSION**

263 ***4.1. Surface water characteristics***

264 Coordinates and water characteristics are shown in Table 1. Surface waters were characterized
265 by pH values near neutral to slightly alkaline (pH 7.27-8.42). Electrical conductivity (0.438 –
266 20.9 mS cm^{-1}) and total dissolved solids (1.84–10.61 g L^{-1}) showed wide ranges of values
267 between Loa river sections and were consistent with the location of the anthropogenic sources
268 (wastewater and mining activities). Arsenic content in the surface waters ranged from 0.220 to
269 1.40 mg As L^{-1} and varied depending on the sampling point. The results indicated that the main
270 contribution is due to the anthropogenic inputs of tributaries near the mining area of
271 Chuquicamata and Calama city. Therefore, the ecological risk of anthropogenic As from long-
272 term human activities might be mainly due to the sediments of these tributaries. An increase
273 of arsenic is observed down-stream even at a considerable distance from the confluence,
274 through the important mining area of Chuquicamata, to the mouth. The highest level of As was
275 measured in Lower Loa (LO-4), mainly polluted by mining, smelting, industrial and agricultural
276 activities. For internal quality control, the SRM 1640 was analyzed for arsenic and the results
277 obtained were within ± 5% of the reference value.

278

279 ***4.2. Total arsenic in algae and aquatic plants***

280 Results of total arsenic and arsenic species found in the algae and aquatic plants, limits of
281 quantification and detection, extraction efficiency and column recoveries are given in Table 3.
282 Each of the values shown in the tables is the mean of three replicates.
283 Total arsenic content determined in various species of algae and aquatic plants varied along
284 the river course and ranged from 20 to 341 mg As kg^{-1} (Table 3), but this range was greatly
285 exceeded in an algae sample (*Cladophora* sp.: 11,100 mg As kg^{-1}) from the Salado River (SA-1),
286 one of the most polluted sites (Dirección General de Aguas (DGA), 2004). The disparity in the
287 values found in these algae is largely attributable to the water's chemical composition in the
288 Salado River, which is strongly influenced by its origin in the geothermal field of El Tatio.
289 Nevertheless, a freshwater plant (*Phylloscirpus* cf. *deserticola*) collected at the same site (SA-1)
290 as *Cladophora* sp. had 49 mg As kg^{-1}. A similar figure was seen in a study comparing the same

algal species with some aquatic plants in a freshwater environment (Schaeffer, et al., 2006). The differences in arsenic concentration between samples might be due either to the fact that *P.* cf. *deserticola* is a vascular plant and *Cladophora* sp. is a filamentous alga, or to differences in the *habitat* where samples were collected. *Cladophora* sp. lives submerged in water, whereas the analyzed samples of *P.* cf. *deserticola* were only aerial stems, not submerged roots and stems. Data on arsenic content in algae and freshwater plants of the same genus as in the present study but from different locations are summarized in Table 4 for comparison purposes. Algae and aquatic plants growing in the Loa River Basin survive in an environment with high arsenic content, meaning that these species have developed arsenic tolerance mechanisms (which may vary between species). In general, hyperaccumulating plants can concentrate some elements in their tissues up to 0.1% of their dry weight. Of the species analyzed, *Cladophora* sp. is able to hyperaccumulate arsenic (1.11% of dry weight) and would be a good candidate for bioremediation studies. With this aim in mind, bioaccumulation coefficients (BC) were estimated as the ratios of total arsenic in the sample to the arsenic in water, according to Robinson et al. (2006a) (values shown in Table 5). *Cladophora* sp. shows remarkable differences between SA-1 (13,910) and SS-1 (152), whereas arsenic concentration in water at SA-1 is lower than at SS-1 (see Table 1). This behavior could be explained by including phosphorous, since the ratio As:P in soil and water affects intake, distribution and speciation due to the chemical analogy between arsenate and phosphate (Wang, et al., 2002). In the present study, as differences in phosphate concentration were found between the water samples (see Table 1), the highest BC (the highest uptake of arsenate) was obtained with the data from the site with low phosphate concentration. Thus, the increase in phosphate in the water appears to result in a decrease in arsenic uptake.

4.3. Arsenic speciation

Results of arsenic speciation, limits of quantification and detection, extraction efficiency and column recoveries are given in Table 3.

Extraction efficiencies (calculated as the ratio of total As in the extract to total As from acidic digestions) ranged from 5% to 126%. Rubio et al. (2010) reported a wide range of extraction efficiencies among algae and plants with different extracting agents (6%-108%). Water is a good extracting agent, since it enters the sample matrix and extracts the compounds determined in the present study, as these are very polar and soluble in water (Francesconi and Kuehnelt, 2004). Low extraction efficiencies are related to the presence of non water-soluble arsenicals like arsenolipids (Francesconi, 2003), and to arsenic bound to cell components or proteins, which are not extracted by soft extractants such as water (Koch, et al., 2000). For

example, *Cladophora* sp. (SA-1) had a total arsenic concentration of 11,100 mg As kg^{-1}, but only 5% of arsenic compounds were extracted, only as inorganic forms.

Inorganic arsenic (iAs) is the main form in the samples, representing 82% to 100% of the sum of arsenic species. High values of standard deviation in some arsenite values could be explained by the rapid oxidation of this species to arsenate (Table 3). DMA, MA and glycerol arsenosugars were found as minor compounds in several samples. Gly-sug was found in plant samples of *P. pectinatus* and *R. filifolia*, corroborating recent studies of aquatic plants (Llorente-Mirandes, et al., 2010; Ruíz Chancho, et al., 2010). AB was not detected in any sample, which indicates that the removal of epiphytes during sample pre-treatment was accurate and that microbial activity, which might be involved in the formation of such an arsenocompound (Llorente-Mirandes, et al., 2010; Ruíz Chancho, et al., 2010), is not significant in the Loa River Basin. In some chromatograms the presence of a large amount of a major arsenic compound might make it difficult to quantify minor species that elute with a similar retention time. As an example, Figure 2 shows an anionic and a cationic exchange chromatogram of extracts of *P. pectinatus* (LO-4) and *Chara* sp. (LO-2).

Column recovery values, calculated as the ratio of the sum of arsenicals eluted from the column to the arsenic injected in the column, are shown in Table 3. Anionic column recoveries ranged from 60% to 96%; and cationic ones, from 75% to 100%.

It is interesting to notice that samples from TO-1 and SA-1 present the same speciation patters despite being different *taxa* of aquatic plants. These results might suggest that arsenic uptake, transformation and accumulation in plants and algae growing under chemical stress depend on the environmental conditions rather than the biological species (Kabata-Pendias, et al., 1997). Diatoms were present in all algae (*Chara* sp. and *Cladophora* sp.) and in *P. pectinatus* (LO-3). Therefore, the possible influence of adsorbed diatoms on samples was examined. However, this seems to have had no effect on extraction efficiency, since samples had both low (5%) and high ratios (76%). Nor was any correlation between occurrence of diatoms and total arsenic and arsenical concentrations found (see Table 4).

5. CONCLUSIONS

This is the first study of arsenic speciation in algae and freshwater plants from the Loa River Basin (northern Chile). Samples had a wide range of concentrations of total arsenic, from 20 to 341 mg As kg^{-1} (d.w.), except for one algal sample with 11,100 mg As kg^{-1}, *Cladophora* sp., which can be classified as a hyperaccumulator. Inorganic arsenic predominated in all samples, accounting for 82% to 100% of the arsenicals measured. Small amounts of DMA, MA and gly-

sug were detected in several samples. This preliminary information should contribute usefully to further bioremediation assays and to the proposal for biomonitoring organisms in this extremely arid region.

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Figure captions

Figure 1. Sampling sites location at the Loa River and at its main tributaries: San Pedro, Salado and San Salvador.

Figure 2. Anionic (a) and cationic (b) exchange chromatograms of a *Chara* sp. (LO-2) extract. *P. pectinatus* (LO-4) chromatograms from anionic (c) and cationic (d) exchange systems.

Figure 1
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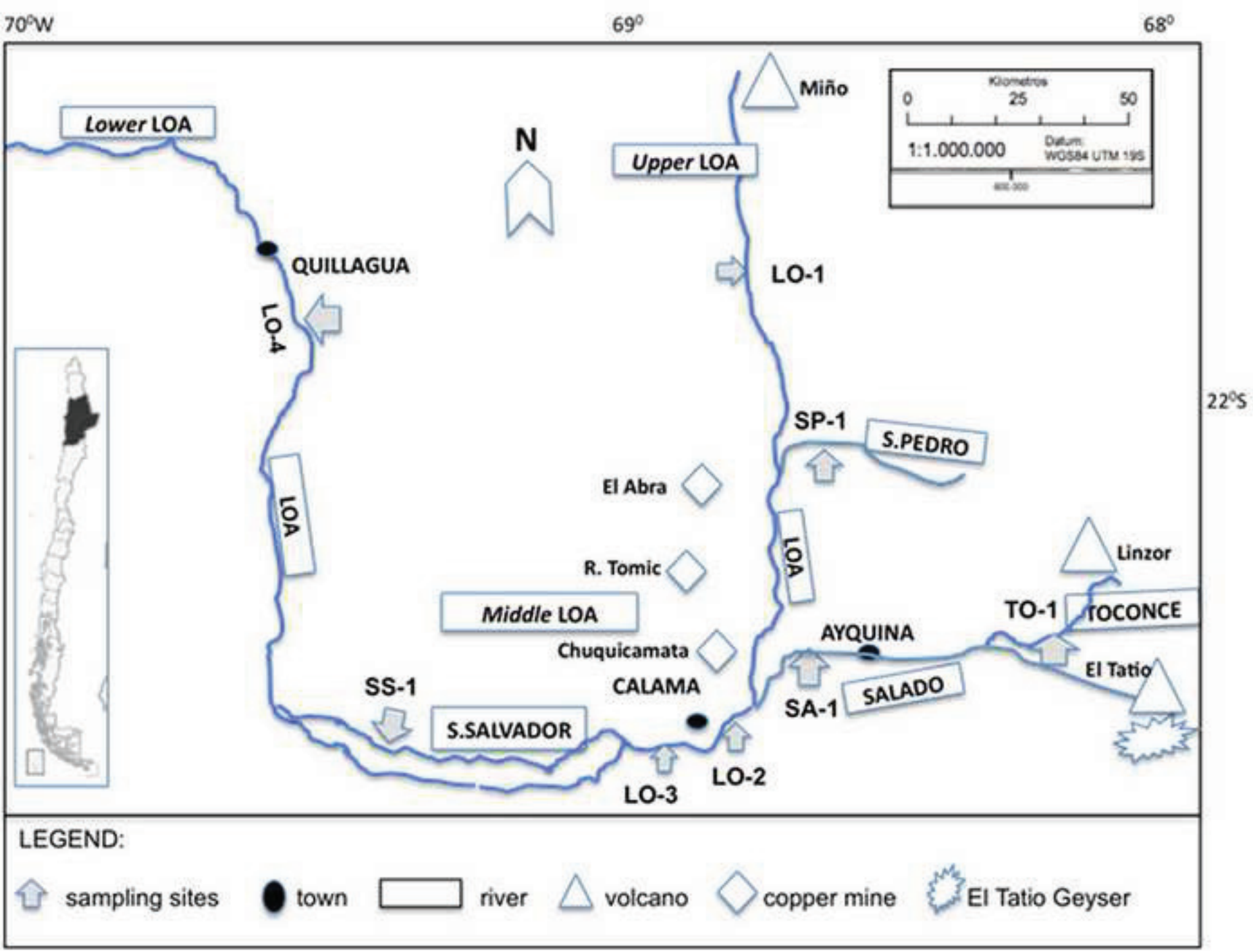

Figure 2

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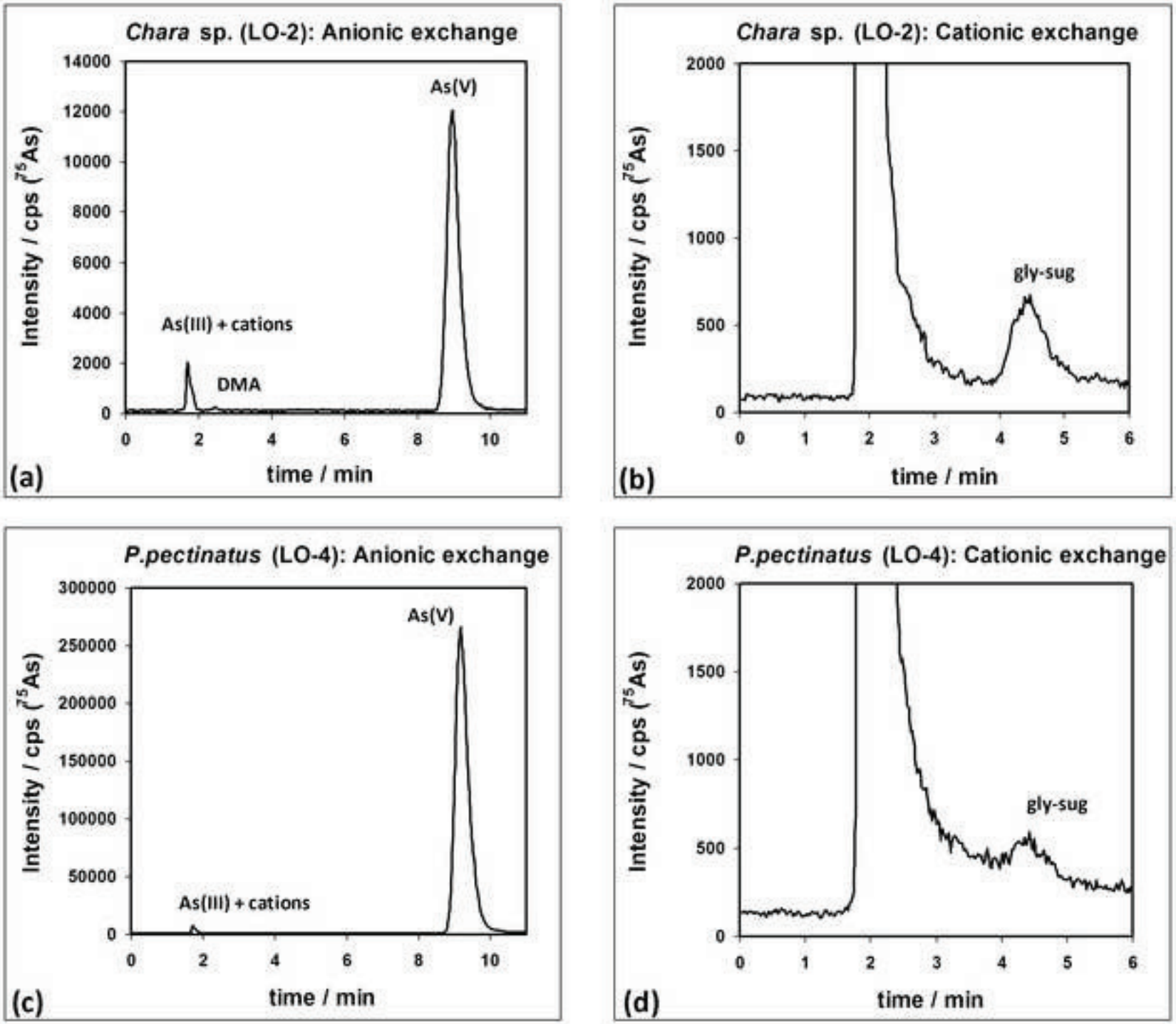

Table

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Table 1. Coordinates and water properties of the sampling sites

Sampling Site	Code	UTM Coordinates Zone 19S		Height MASL	Date	Temperature °C	pH	Electrical Conductivity mS cm^{-1}	Dissolved Oxygen mg L^{-1}	TDS g L^{-1}	Hardness mg $CaCO_3$ L^{-1}	Total As mg L^{-1}	PO_4^{3-} mg L^{-1}
		East	North										
UPPER LOA													
Loa river in Lequena	LO-1	535264	7604060	3,250	3-Jun-2010	9.4	8.42	0.669	9.2	1.90	490	0.220	<0.778
San Pedro river in Parshall 1	SP-1	565449	7570727	3,700	3-Jun-2010	20.4	7.41	0.850	12.6	2.58	610	0.456	-
SALADO RIVER													
Toconce river before Sendos Dam	TO-1	588204	7536667	3,445	4-Jun-2010	7.7	8.30	0.438	9.6	1.84	504	0.670	-
Salado river in Sifón de Ayquina	SA-1	567504	7534956	2,980	4-Jun-2010	11.1	7.27	5.19	10.1	3.98	690	0.798	<0.078
Middle LOA													
Loa river in Escorial	LO-2	510530	7518137	2,450	2-Jun-2010	9.2	8.05	7.15	8.7	4.98	778	0.710	0.346
Loa river in La Finca	LO-3	504192	7511789	2,100	2-Jun-2010	12.2	8.21	7.65	8.4	5.30	1,300	0.897	0.299
San Salvador river before junction with Loa River	SS-1	446248	7523414	1,238	2-Jun-2010	11.6	8.27	8.50	6.3	6.21	1,380	1.20	1.790
LOWER LOA													
Loa river before agricultural area of Quillagua	LO-4	443087	7605780	802	1-Jun-2010	13.2	7.85	20.9	5.5	10.61	2,160	1.40	1.017

TDS= total dissolved solids; < =below detection limit

Table

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1

Table 2. Chromatographic conditions used for arsenic speciation.

2

	Anion exchange	Cation exchange
Column	PRP-X100 (250 mm x 4.1 mm, 10 µm) (Hamilton, Reno, USA)	Zorbax SCX300 (250 mm x 4.6 mm, 5 µm) (Agilent, Waldbronn, Germany)
Pre column	PRP-X100 (20 mm x 2.0 mm, 10 µm)	Zorbax SCX300 (12.5 mm x 4.6 mm, 5 µm)
Mobile phase	$NH_4H_2PO_4$ 20 mM	Pyridine 20 mM
pH	5.8	2.6
Injection volume	20 µL	20 µL
Flow rate	1.5 mL min^{-1}	1.5 mL min^{-1}
Column temperature	Room temperature	Room temperature
As species	As (III), DMA, MA, As (V), PO_4-sug, SO_3-sug and SO_4-sug	AB, TMAO, AC and gly-sug

Table

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Table 3. Levels of total As and arsenic species in algae and aquatic plants (*mean* ± standard deviation, *n*=3, d.w.), extraction efficiency and column recoveries.

Sample	Sampling Site	As Total mg As kg^{-1}	As (III)[A] mg As kg^{-1}	DMA mg As kg^{-1}	MA mg As kg^{-1}	As (V) mg As kg^{-1}	gly-sug mg As kg^{-1}	iAs[B] mg As kg^{-1}	Extraction efficiency	Anionic column recovery	Cationic column recovery[D]
Zannichellia palustris L.	LO-1	79 ± 5	13.5 ± 1.4	-	-	6.7 ± 0.8	-	20 ± 2	32%	80%	94%
Azolla sp.	LO-1	199 ± 12									
Myriophyllum aquaticum L.	SP-1	209 ± 11	56 ± 1	-	-	80 ± 1	-	136 ± 3	67%	96%	86%
Potamogeton pectinatus L.	TO-1	20 ± 2	0.9 ± 0.4	0.2 ± 0.01	-	7.5 ± 0.2	0.84 ± 0.04	8.4 ± 0.6	80%	60%	97%
Ruppia filifolia Skottsb.	TO-1	23 ± 2	0.6 ± 0.1	0.53 ± 0.05	-	4.1 ± 0.3	0.52 ± 0.04	4.7 ± 0.4	34%	73%	93%
Phylloscirpus cf. *deserticola* (Phil.) Dhooge & Goetgh.	SA-1	49 ± 3	12 ± 4	-	-	36 ± 6	-	49 ± 3	127%	78%	80%
Cladophora sp.	SA-1	11,100 ± 300	2 ± 1	-	-	389 ± 7	-	391 ± 8	5%	67%	75%
Chara sp.	LO-2	341 ± 6	3.88 ± 0.09	0.14 ± 0.01	-	28.2 ± 0.8	0.93 ± 0.02	31 ± 2	13%	73%	97%
Potamogeton pectinatus L.	LO-3	134 ± 1	15.7 ± 0.9	0.17 ± 0.01	-	57 ± 4	detected	73 ± 4	77%	71%	100%
Cladophora sp.	SS-1	182 ± 7	4 ± 1	detected	0.31 ± 0.02	64 ± 4	-	68 ± 5	53%	73%	92%
Potamogeton pectinatus L.	LO-4	248 ± 2	4.6 ± 0.2	-	-	130 ± 2	-	135 ± 1	58%	94%	92%
Limit of detection		0.003	0.02	0.03	0.05	0.08	0.15				
Limit of quantification		0.01	0.07	0.11	0.15	0.28	0.49				

[A] As (III) as the subtraction of the sum of cationic species from the front of anionic exchange chromatograms. As(III) peak coelutes with cationic arsenicals.

[B] Inorganic arsenic (iAs) as the sum of arsenate and arsenite.

Table
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Table 4. Data reported for similar freshwater algae and plants from different locations.

Sample	Sampling site	As in water ($\mu g\ L^{-1}$)	As in sample ($mg\ kg^{-1}$)	Speciation[A]	Reference
Cladophora sp. (fresh)	Danube River, Hungary	1.1 ± 0.2	9.33	+	Schaeffer, et al., 2006
Cladophora sp. (sun-dried)	Danube River, Hungary	1.1 ± 0.2	5.06	+	Schaeffer, et al., 2006
Cladophora glomerata Pilg.	Hayakawa River, Japan	17	18	+	Miyashita, et al., 2009
Cladophora glomerata Pilg.	Wadi Hanifah, Riyadh, Saudi Arabia	-	0.45 – 18.48	-	Al-Homaidan, et al., 2011
Myriophyllum aquaticum (Vell.) Verdc.	Loa River, Chile	-	90-900	-	Stegen, et al., 2000
Myriophylum sp.	Danube River, Hungary	1.1 ± 0.2	5.42	+	Schaeffer, et al., 2006
Chara vulgaris L.	Chelpa River, Iran	150	212.5 ± 0.4	-	Ghassemzadeh, et al., 2007
Scirpus sp.	Meager Creek hot springs, Canada (1996)	303	7.1	+	Koch, et al., 1999
Scirpus sp.	Meager Creek hot springs, Canada (1997)	286	4.5	+	Koch, et al., 1999
Zannichellia palustris L.	Loa River, Chile	-	600 - 800	-	Stegen, et al., 2000

A: (+) Reported (-) Not reported

Table 5
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Table 5. Bioaccumulation coefficients (BC) estimated as the ratios of total arsenic in the sample to the arsenic in water

Sampling point	Alga, aquatic plant	As (dw) mg As kg^{-1}	As in water mg As kg^{-1}	BC
LO-1	*Zannichellia palustris*	79	0.220	359
	Azolla sp.	199		905
SP-1	*Myriophyllum aquaticum*	209	0.456	458
TO-1	*Potamogeton pectinatus*	20	0.670	30
	Ruppia filifolia	23		34
SA-1	*Phylloscirpus* cf. *deserticol*	49	0.798	61
	Cladophora sp.	11100		13910
LO-2	*Chara* sp.	341	0.710	480
LO-3	*Potamogeton pectinatus*	134	1.897	71
SS-1	*Cladophora* sp.	182	1.20	152
LO-4	*Potamogeton pectinatus*	248	1.40	177