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Review

Atriplex atacamensis and *Atriplex halimus* resist As contamination in Pre-Andean soils (northern Chile)

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HIGHLIGHTS

- *A. atacamensis* and *A. halimus* resist high levels of arsenic in soils.
- *A. halimus* accumulated higher levels of arsenic than *A. atacamensis.*
- *A atacamensis* retains arsenic in the roots and *A. halimus* translocated it to leaves.
- Both species can be recommended to generate plant cover in As-contaminated soils.

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ABSTRACT

The Pre-Andean area of Chile exhibits saline soils of volcanic origin naturally contaminated with arsenic (As), and we hypothesise that revegetation with resistant species may be a valid alternative for soil management in this area. Thus, the xerophytic and halophytic shrubs *Atriplex halimus* and *Atriplex atacamensis* were cultivated in containers for 90 days in Pre-Andean soil, As-soil, (111 ± 19 mg As kg^{-1}, pH 8.4 ± 0.1) or control soil (12.7 ± 1.1 mg As kg^{-1}, pH 7.8 ± 0.1) to evaluate As accumulation and resistance using stress bioindicators (chlorophylls, malondialdehyde (MDA) and total thiols). Sequential extraction of As-soil indicated that 52.3% of As was found in the most available fraction. The As distribution was significantly different between the species: *A. halimus* translocated the As to leaves, whilst *A. atacamensis* retained the As in roots. At 30 and 90 days, *A. halimus* showed similar As concentrations in the leaves (approximately 5.5 mg As kg^{-1}), and As increased in stems and roots (up to 4.73 and 16.3 mg As kg^{-1}, respectively). In *A. atacamensis*, As concentration was lower (2.6 in leaves; 3.2 in stems and 6.9 in roots in mg As kg^{-1}). Both species exhibited a high concentration of B in leaves (362–389 mg kg^{-1}). If the plants are used for animal feed, it should be considered that *A. halimus* accumulates higher concentration of As and B in the leaves than *A. atacamensis*. Neither plant growth nor stress bioindicators were negatively affected by the high levels of available As, with the exception of MDA in the leaves of *A. halimus*. The results indicate that these plants resist contamination by arsenic, accumulating mainly the metalloid in the roots and can be recommended to generate plant cover in As-contaminated soils in the Pre-Andean region, under saline conditions controlled, preventing the dispersion of this metalloid via wind and leaching.

Contents

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1. Introduction

The high concentration of arsenic (As) in soils of the Pre-Andean zone of northern Chile (Antofagasta region) is mainly associated with the Quaternary volcanic and geothermal activity in the Andes Cordillera (Queirolo et al., 2000; Romero et al., 2003). The concentrations of As in soils of the zone range from 53 to 448 mg kg^{-1} (Sancha et al., 1995; Diaz et al., 2011). However, the average concentration of As estimated in soil varies between 5 and 8 mg kg^{-1} (Alloway, 2010). These soils also contain high concentrations of B (Cáceres et al., 1992). Due to the climatic characteristics of the region, land for farming has low economic importance, though some indigenous populations produce vegetable crops in these locations.

The mobility of As in soil is mainly dependent of the pH, redox potential, Fe oxides, soil texture and organic matter (Fitz and Wenzel, 2002; Fayiga et al., 2007; Alloway, 2010; Moreno-Jiménez et al., 2012). In soil contaminated with As, pH is one of the major factors determining As availability (Fayiga et al., 2007). An increase in pH often results in mobilisation of As in the soil (Fitz and Wenzel, 2002). Arsenate, As (V), is generally the predominant form that exists in aerated soil environments, whilst arsenite, As (III), is only predominant in flooded soils (Moreno-Jiménez et al., 2012).

Arsenic is nonessential in plants. In woody plant growing on arsenic polluted soils (74.1–218 mg As kg^{-1}) Madejón and Lepp (2007) found concentrations of arsenic in leaves between 0.27 and 2.29 mg As kg^{-1}. Plants vary in their ability to accumulate and tolerate As, and its mobility is generally low with respect to translocation from roots to aerial part (Zhao et al., 2009) except in hyperaccumulator plants. The phytotoxic effects commonly observed following As exposure include growth inhibition, chlorophyll degradation, nutrient depletion and oxidative stress (Márquez-García and Córdoba, 2010; Moreno-Jiménez et al., 2012), this later is reflected by an increase in MDA, whilst – SH groups (thiols) can avoid this oxidative stress by the formation of complex with As (III) (Moreno-Jiménez et al., 2008).

There are many studies related with plant tolerance to As under hydroponic conditions (Srivastava et al., 2005; Aldrich et al., 2007; Moreno-Jiménez et al., 2008, 2010a, 2010b; Shaibur and Kawai, 2009; Vromman et al., 2011), whereas there are only a few studies performed in As-contaminated soils (without artificially spiking with As). Many hydroponic studies have used much higher concentrations of As than those found in soil solutions, and their environmental relevance has been questioned (Zhao et al., 2009).

The *Atriplex* genus (Chenopodiaceae), denominated saltbush, is one of the most important families of plants in the region of Antofagasta, Chile (Poblete et al., 1991; Saiz et al., 2000). These shrubs xerohalophyte are dominant in many arid and semi-arid regions of the world, particularly in saline, arid soils, and they are used for rehabilitation of degraded lands (Lutts et al., 2004; Conesa et al., 2007; Manousaki and Kalogerakis, 2009), ornamental plants and revegetating sealed landfills (Ingelmo et al., 1998) and for animal feed (Otal et al., 2010). Certain assays have shown that plants of the genus *Atriplex* accumulate B, Cd, Mo and Se (Watson et al., 1994; Lutts et al., 2004; Tapia et al., 2011). *Atriplex atacamensis* is native of northern Chile (Atacama Desert). *Atriplex halimus* is native of Mediterranean frequently encountered on marginal soils and degraded lands in southern Europe and North Africa (Lefèvre et al., 2009). There are few studies related to the accumulation of As in the species *A. atacamensis* (Diaz et al., 2011) and *A. halimus* (no references were found) growing in soils with high levels of this metalloid.

The objectives of this study were 1) to evaluate and compare the concentrations of arsenic in the species *A. atacamensis* and *A. halimus* growing in soils naturally with high levels of arsenic in greenhouse conditions and; 2) determine the effects of arsenic in *A. atacamensis* and *A. halimus* on the levels of chlorophyll, lipoperoxides (measured as malondialdehyde, MDA) and thiols as bioindicators of stress.

2. Materials and methods

2.1. Zone of soil collection and determination of the main physico-chemical properties

Surface soil samples (0–15 cm depth) were collected from a plot (22°20′57″S, 68°38′91″W, 2535 m a.s.l.) in the village of ChiuChiu, located 35 km from the city of Calama (northern Chile), which were classified as Aridisols (Fig. 1). The zone of Pre-Andean soil collection is influenced by the Loa river and is located prior to the confluence with the Salado river. The zone is characterised by scarce/poor plant cover, saline properties of soils and high concentrations of arsenic of natural origin.

Soil collected the zone of Pre-Andean (156.3 ± 19.0 mg As kg^{-1}) showed extremely high electrical conductivity values (26.8 dS m^{-1} in 1:5 w/v water extracts). *A. halimus* showed symptoms of toxicity in soils with EC of 9.3–11.5 dS m^{-1} in saturated aqueous extracts (Manousaki and Kalogerakis, 2009). Therefore, to decrease the electrical conductivity the soil collected the zone of Pre-Andean was washed with tap water in plastic containers (4 L) by irrigation using a garden hose. To each container was required water in proportion 1:5 v/v (soil:water). In the soil washed (As-soil) the main physical and chemical properties and the procedure of sequential extraction were determinated.

Control soil (0–15 cm depth) was collected from an area located in the central zone of Chile (33°26′S, 68°39′W, 529 m a.s.l.), which were classified as Mollisols. The main physico-chemical characteristics of As-soil and control soil were determined. EC and pH were determined in water extracts (1:5 v/v) using an electrode (WTW multi 340i). The cation exchange capacity (CEC) was determined with sodium and ammonium acetate (Chapman, 1965). The organic matter content was determined by calcination at 550 °C. Nitrogen was determined by the Kjeldahl method. Finely ground dry soil samples (0.5 g) were digested with 4 mL of milli-Q H_2O, 6 mL of HNO_3 and 4 mL of H_2O_2 in an autoclave (Seepdy HL-341) (Moreno-Jiménez et al., 2010a). In the digest extracts, total P was determined using colorimetric methods with ammonium molybdate (Murphy and Riley, 1962). Cd, Cu, Fe, Mn, Na, Pb and Zn, in the digest extracts, were measured using flame atomic absorption spectrophotometry (AAS) with a Thermo Electronic Corporation AA Series apparatus (detection limit: 0.005 mg Cd L^{-1}, 0.004 mg Cu L^{-1},

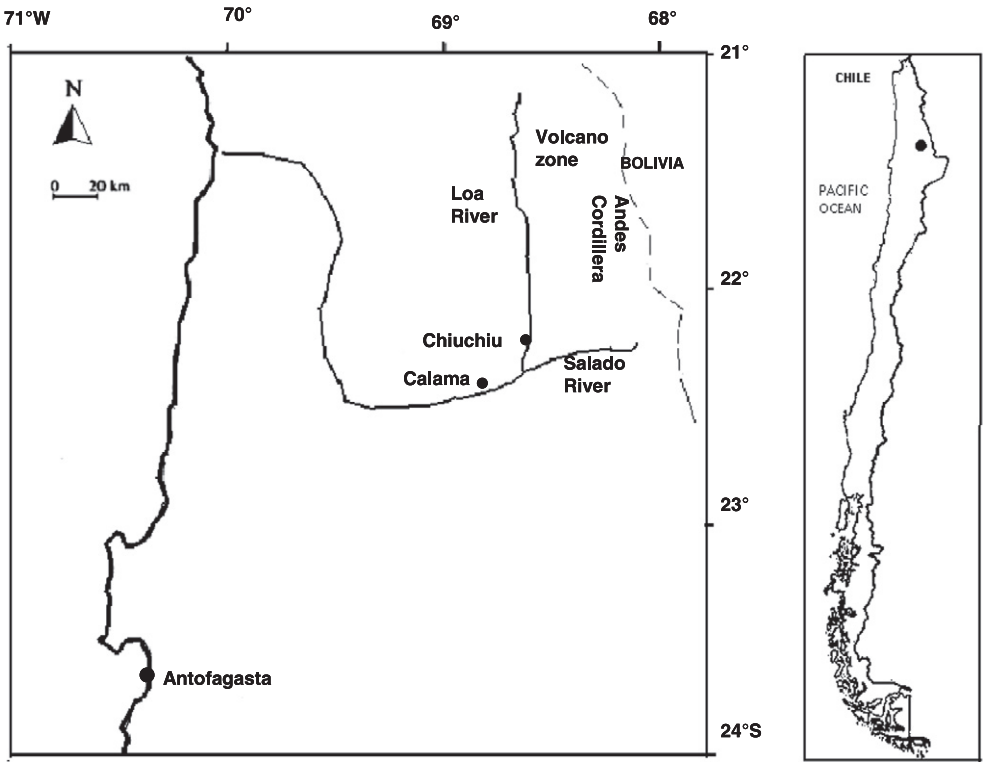

Fig. 1. Study areas, near the Loa river, where soil samples with high levels of arsenic were collected (Pre-Andean zone, Chile).

0.004 mg Fe L^{-1}, 0.004 mg Mn L^{-1}, 0.002 mg Na L^{-1}, 0.005 mg Pb L^{-1}, 0.001 mg Zn L^{-1}), and As was quantified by flow injection hydride generation atomic absorption spectrophotometry (FI-HG-ASS) using a Perkin Elmer FIA 100 apparatus (detection limit: 0.01 μg As L^{-1}). Boron was extracted with $CaCl_2$ and determined by colorimetric method with azomethine-H (Nable et al., 1997). The texture was determined using a hydrometer (Bouyoucos method). For measurement of the contents of iron and manganese oxides, soil samples were dissolved with oxalic acid and ammonium oxalate with sodium hydrosulphite as a reducing agent (McKegue and Day, 1966), and Fe and Mn were measured by AAS. All determinations were performed in triplicate and two procedural blanks were included in the analysis. Montana Soil SRM 2711 for trace element concentrations was employed to evaluate accuracy. The certified values are: As 105 ± 8; Cd 41.7 ± 0.25; Cu 114 ± 2; Mn 638 ± 28; Zn 350.4 ± 4.8 in μg g^{-1} and Fe 2.89 ± 0.06; Na 1.14 ± 0.03 in %. The values obtained were 98.19 ± 0.03 μg As g^{-1}; 38.5 ± 0.8 μg Cd g^{-1}; 110.0 ± 3.3 μg Cu g^{-1}; 589 ± 29 μg Mn g^{-1}; 1023 ± 72 μg Pb g^{-1}; 323.3 ± 25.9 μg Zn g^{-1} and Fe 2.54 ± 0.1%; Na 1.13 ± 0.1%. For boron SRM 1573a, certified value 33.3 ± 0.7 μg g^{-1} was employed and the value obtained was 32.1 ± 0.3 μg B g^{-1}. Trace metals in the procedural blanks was below the detection limits. Deionized water (18 M Ω cm) was used for the preparation of the reagents and standards. All chemicals were of pro analysi quality and certified standard solution of As and metals was used (Merck, Germany).

2.2. Distribution of arsenic in soil

Sequential extraction of As from the soil samples, in quadruplicate, was carried out in the As-soil and control soil according to the method described by Wenzel et al. (2001). Arsenic is distributed in five fractions: a non-specifically adsorbed fraction (FI), extracted with $(NH_4)_2SO_4$; a specifically adsorbed fraction (FII), extracted with $(NH_4)H_2PO_4$. The FI and FII fraction were named like that because of the extracting agent FI is extracted with sulphate, which doesn't extract selectively As, so that the name non-specifically sorbed; whilst FII is extracted with P, which effectively competes with As, so may extract As that was not extracted in the previous fraction from more specific retention places in the soil. These fractions where As is associated, can be denominated $(NH_4)_2SO_4$-extractable and $(NH_4)H_2PO_4$-extractable fraction. The FIII fraction associated an amorphous and poorly crystalline Fe and Al oxides was extracted with NH_4 buffer oxalate; the FIV fraction associated a well-crystallised Fe and Al oxides was extracted with NH_4 buffer oxalate + 0.1 M ascorbic acid, and a residual fraction (FV) was obtained by digested with HNO_3 and H_2O_2. In the FIII and FIV fractions the As is more sorbed than previous fractions and is lower the availability whilst in the FV fractions the As is strongly sorbed. The concentration of As in each fraction was determined by FI-HG-AAS.

2.3. Pot experiments

Cuttings of *A. atacamensis* and *A. halimus* were propagated in perlite for 120 days in the nursery of the Centre for Studies of Arid Zones (CEZA) of the University of Chile. *A. atacamensis* (29.7 ± 2.5 cm) and *A. halimus* (25.3 ± 2.5 cm) were transplanted in As-soil and control soil treatments (10 plants per treatment) distributed in random blocks using plastic containers of 4 L (19.5 cm diameter × 22.0 cm height) in late March of 2011 and maintained for 90 days. This assay was performed in the research greenhouse of the University of Santiago of Chile under ambient conditions and protected of the rain. The plants were irrigated twice per week with 200 mL of tap water (As concentration in tap water below the detection limit; B 0.39 mg L^{-1} and EC 1.1 dS m^{-1}) per container. During the assay, the average minimum temperature was 7.9 ± 2.8 °C, maximum temperature was 23.4 ± 4.6 °C, and relative humidity was 60.3 ± 26.5%.

2.4. Analysis of vegetal samples

Arsenic concentrations were determined at the beginning of the assay (0 days) and after 30 and 90 days. The plants (n = 3) were separated into leaves and stems (aerial part), and roots, and the soil

particles were manually removed. Plant material rinsed under tap water for 5 min and submerged in distilled water for 2 min, dried at 60 °C for 48 h, and milled to a fine powder in a grinder (Moreno-Jiménez et al., 2011). The samples (0.5 g) were digested with 10 mL of milli-Q H_2O, 3 mL of HNO_3 and 2 mL of H_2O_2 in an autoclave (Moreno-Jiménez et al., 2010a). In the digested extracts, the As concentration was determined by FI-HG-AAS. Boron was determined in leaves and roots using the azomethine-H method after calcination of vegetal samples. The bioconcentration factor (BCF) was calculated as BCF = As concentration in leaves/As concentration in soil, and translocation index (Ti) was calculated as Ti = As concentration in leaves/As concentration in roots (Ghosh and Singh, 2005), except for plant at the beginning of the assay where the aerial part (leaves + stems) was considered. Chlorophylls, thiols and MDA concentration were determined in fresh leaves at 0, 30 and 90 days of the assay (n = 3). Chlorophylls were extracted by homogenising 0.5 g fresh weight (F.W.) of leaf tissue in 80% acetone and estimated as Chl *a* (mg L^{-1}) = $12.7 \times A_{663} - 2.69 \times A_{645}$ (Moreno-Jiménez et al., 2008). Acid-soluble thiols were extracted using 0.1 g F.W. to which 0.4 mL of NaOH (0.1 M) + NaBH4 (25 mg mL^{-1}) and 0.2 mL of distilled water were added and centrifuged. The supernatant was diluted with 0.2 mL of HCl (35%). Then, 0.5 mL of 300 μM DTNB in 0.5 M phosphate buffer (pH 7.5) was added to 0.5 mL of the supernatant and heated at 30 °C for 2 min. The absorbance was determined at 412 nm. For quantification, GSH standards were used (Moreno-Jiménez et al., 2008). MDA was extracted from 0.1 F.W. to which 1 mL of colorimetric reactive TCA (0.1%) was added and centrifuged. Then, 1 mL TBA (0.5% w/v) in TCA (20% w/v) was added to 0.25 mL of the supernatant, heated at 90 °C for 30 min, cooled and centrifuged. The absorbance of the supernatant was measured at 532 nm and 600 nm. An extinction coefficient of 155 mM^{-1} cm^{-1} was used (Ederli et al., 2004). For the absorbance determination, a Shimadzu UV-1601was used.

2.5. Statistics analysis

The statistical analysis was based on one-way analysis of variance (ANOVA) of the mean values of concentrations of As and B, BFC, Ti, dry weight and plant height for *A. atacamensis* and *A. halimus* as well as the concentrations of chlorophyll, thiols and MDA for plants cultivated in As-soil and control soil. To check for statistically significant differences Duncan test at $p \leq 0.05$ was used. All statistical tests were carried out with the SPSS 13.0 software package.

3. Results

3.1. Soil characteristics and As distribution

Arsenic and boron concentrations were notably higher in As-soil than in control soil. These concentrations were higher than those cited in references for soil that varies between 5 and 8 mg kg^{-1} for As (Alloway, 2010) and 10 mg kg^{-1} for B (Yazbeck et al., 2005). The As-soil presented an alkaline pH, a higher EC and, consequently, a higher concentration of Na than in control soil (Table 1). The values of organic matter, CEC, N and P were lower in As-soil than in control soil as also the concentrations of Cu, Fe, Mn, Pb and Zn. The concentration of Cd in both soils was below detection limit. The concentration of Fe_2O_3 and MnO_2 was low in As-soil. A higher percentage of sand and a lower percentage of clay showed As-soil than in control soil. The results of the sequential extraction indicate that the highest concentration of As in As-soil was associated with the non-specifically adsorbed soil fraction (FI), equivalent to 52% of the total (Fig. 2), indicating that the metalloid is highly available for plants. In control soil, the highest concentration of As was found in the fraction associated with amorphous oxides of Fe and Al (FIII), whilst in FI and FII fractions the concentration of As was below detection limit and very low in FIV fraction.

Table 1
Main physico-chemical properties of control soil and As-soil (values are means ± standard deviations, n = 3).

	Control soil	As-soil
pH (1:5 w/v water extract)	7.8 ± 0.1	8.4 ± 0.1
EC (dS m^{-1} 25 °C) (1:5 w/v water extract)	1.7 ± 0.2	2.6 ± 0.4
CEC (cmol 1 + kg^{-1})	35.0 ± 6.5	19.0 ± 4.5
Total P (%)	0.21 ± 0.00	0.06 ± 0.01
N-Kjeldahl (%)	0.27 ± 0.02	0.21 ± 0.01
Organic matter (%)	6.34 ± 0.10	0.81 ± 0.10
As (mg kg^{-1})	12.7 ± 1.1	111 ± 19
B (mg kg^{-1})	1.13 ± 0.06	79.6 ± 0.98
Cd (mg kg^{-1})	<DL	<DL
Cu (mg kg^{-1})	431 ± 21	33.2 ± 1.0
Fe (g kg^{-1})	30.0 ± 2.5	11.0 ± 0.2
Mn (mg kg^{-1})	665 ± 32	137 ± 7.0
Na (mg kg^{-1})	784 ± 20	1263 ± 72
Pb (mg kg^{-1})	302 ± 109	<DL
Zn (mg kg^{-1})	1700 ± 52	43.2 ± 4.1
Fe_2O_3 (g kg^{-1})	16.4 ± 1.2	7.9 ± 1.0
MnO_2 (mg kg^{-1})	85.8 ± 10.4	<DL
Clay (%)	16	13
Sand (%)	50	64
Silt (%)	34	23

EC: electrical conductivity. CEC: cation exchange capacity. DL: detection limit.

3.2. Accumulation of arsenic in plants

Initially, the concentration of As was observed to be at levels commonly found in plants (Table 2). At 30 days, in *A. halimus* cultivated in As-soil, the concentration of As in the leaves was significantly higher than in *A. atacamensis*, whilst the concentrations of As in stems and roots were similar between two species. After 90 days, *A. halimus* cultivated in As-soil maintained the concentration of As in the leaves, increase the concentration of As in stems and roots, which was the highest concentration of As found in this assay (16.3 ± 3.3 mg As kg^{-1}). *A. atacamensis* maintained the As in the roots and showed similar levels of As in the leaves and stems between plants grown in As-soil and control soil, indicating that this species did not translocate the As to the aerial part. In control soil, the concentrations of As in leaves, stems and roots at 30 and 90 days did not show significant differences between the two species. At the end of the assay, the content of As in the whole plant was significantly higher in *A. halimus* than in *A. atacamensis* (Fig. 3).

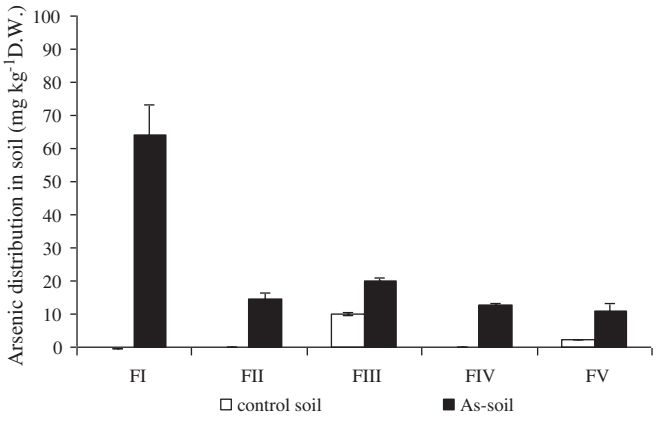

Fig. 2. Arsenic distribution (mg kg^{-1} D.W.) in control soil and As-soil: non-specifically adsorbed (FI), specifically adsorbed (FII), amorphous and poorly crystalline Fe and Al oxides (FIII), well-crystallised Fe and Al oxides (FIV), and residual (FV) (lines on bars correspond to standard deviation, n = 4).

Table 2
Arsenic concentrations (mg kg^{-1} D.W.) in *A. atacamensis* and *A. halimus* cultivated in control soil and As-soil (values are means ± standard deviations, n = 3).

Days	Treatment		*A. atacamensis*	*A. halimus*
			Arsenic (mg kg^{-1} D.W.)	
0	–	Shoot	0.74 ± 0.22	0.99 ± 0.30
		Root	1.53 ± 0.46	0.79 ± 0.24
30	Control soil	Leaves	1.16 ± 0.29	0.96 ± 0.11
		Stem	0.87 ± 0.24	0.96 ± 0.07
		Root	1.25 ± 0.22	0.66 ± 0.23
	As-soil	Leaves	1.37 ± 0.38a	5.45 ± 1.76b
		Stem	1.46 ± 0.40	1.50 ± 0.20
		Root	7.12 ± 0.64	6.54 ± 0.55
90	Control soil	Leaves	2.26 ± 0.07	2.62 ± 0.58
		Stem	3.15 ± 0.74	2.33 ± 0.37
		Root	3.51 ± 0.28	3.21 ± 0.85
	As-soil	Leaves	2.57 ± 0.68a	5.59 ± 1.78b
		Stem	3.16 ± 0.93	4.73 ± 0.48
		Root	6.91 ± 1.24a	16.3 ± 3.3b

Different letters in rows indicate significant differences among the species according to the Duncan test at $p \leq 0.05$.

The BCF was significantly higher in *A. halimus* than in *A. atacamensis* (Fig. 3). For *A. halimus* BCF remained constant (0.05) at 30 and 90 days, because the concentration of the metalloid in leaves did not change in this period (Table 2). In *A. atacamensis*, the BCF increased significantly with increasing time from 0.007 to 0.023.

At start of the assay *A. halimus* showed Ti to aerial part higher than *A. atacamensis* (Fig. 3). At 30 days, the Ti of *A. halimus* was four times higher than in *A. atacamensis*. At 90 days, there were no differences between the two species, because *A. halimus* maintained the concentration of As in the leaves and increased the concentration in the roots (Table 2).

Both species, cultivated in As-soil, accumulated high concentrations of boron in leaves at 90 days (Table 3). *A. halimus* presented a higher concentration of boron in leaves than *A. atacamensis*, with significant differences for control soil.

3.3. Growth parameters

A. atacamensis and *A. halimus* cultivated in As-soil grew normally and did not show visual symptoms of toxicity. Additionally, neither the dry weight nor height of both plants cultivated in As-soil was negatively affected (Fig. 4). At 30 and 90 days, *A. halimus* cultivated in As-soil showed a dry weight and height significantly higher than the plants cultivated in control soil. At the end of the assay, there was no significant difference between the weight or height of *A. atacamensis* cultivated in the control soil compared to *A. halimus* cultivated in the As-soil.

3.4. Bioindicators of stress

In general, the concentration of chlorophyll in both species at 30 and 90 days was significantly higher in plants cultivated in As-soil than in control soil (Table 4). The results indicate that chlorophyll levels were not adversely affected in plants grown in As-soil. The levels of thiols were also not negatively affected in plants grown in As-soil. However, the MDA concentration in *A. halimus* was significantly higher in plants cultivated in As-soil than in control soil at 30 and 90 days. This indicates the existence of oxidative stress in this species. In *A. atacamensis*, the MDA concentration showed no significant differences between plants cultivated in As-soil and control soil. At 30 and 90 days, in plants cultivated in control soil chlorophyll levels, MDA and thiols were relatively stable and in similar levels than in the plants at start of assay (0 days).

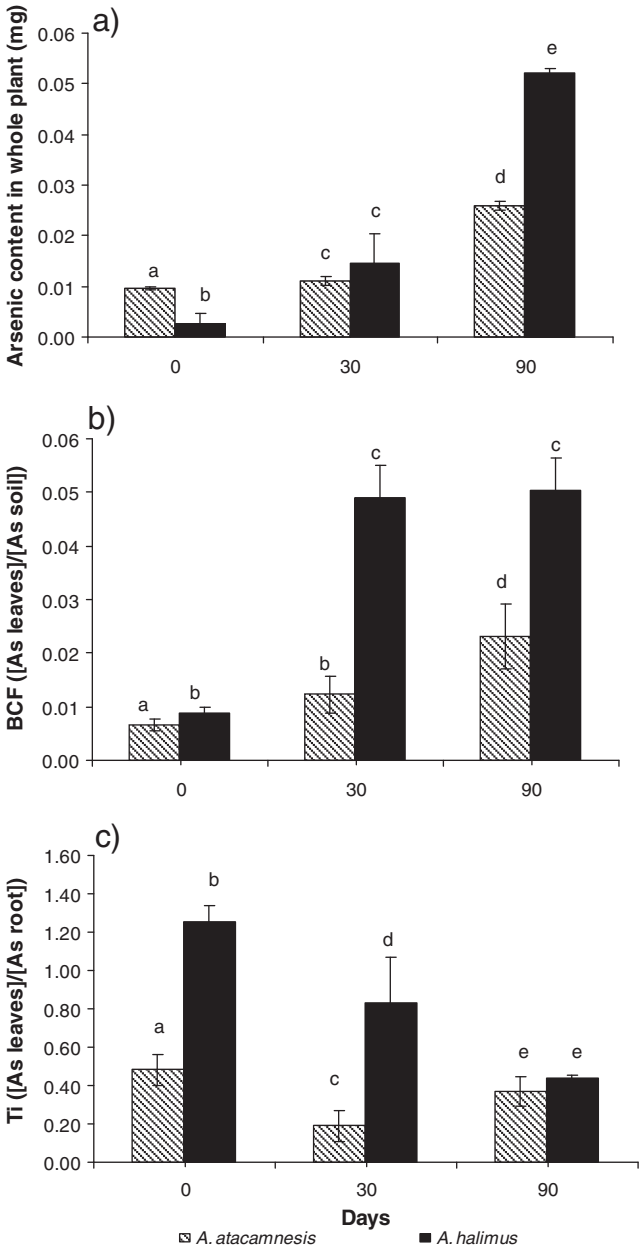

Fig. 3. Arsenic content in whole plant (mg) (a), bioconcentration factor (BCF) (b), and transport index (Ti) (c) of *A. atacamensis* and *A. halimus* cultivated in As-soil (lines on bars correspond to the standard deviation, n = 3. Different letters indicate significant differences according to the Duncan test at $p \leq 0.05$).

Table 3
Boron concentration (mg kg^{-1} D.W.) in *A. atacamensis* and *A. halimus* cultivated in control soil and As-soil (values are means ± standard deviations, n = 3).

Days	Treatment		*A. atacamensis*	*A. halimus*
			Boron (mg kg^{-1} D.W.)	
0	–	Leaves	26.1 ± 1.6a	45.3 ± 2.8b
		Root	18.0 ± 8.1b	19.8 ± 8.9b
90	Control	Leaves	35.0 ± 0.4a	88.7 ± 23.9b
		Root	24.3 ± 4.7	16.9 ± 4.1
	As-soil	Leaves	362 ± 2.8	389 ± 81
		Root	77.8 ± 11.5	80.0 ± 14.9

Different letters in rows indicate significant differences among the species according to the Duncan test at $p \leq 0.05$.

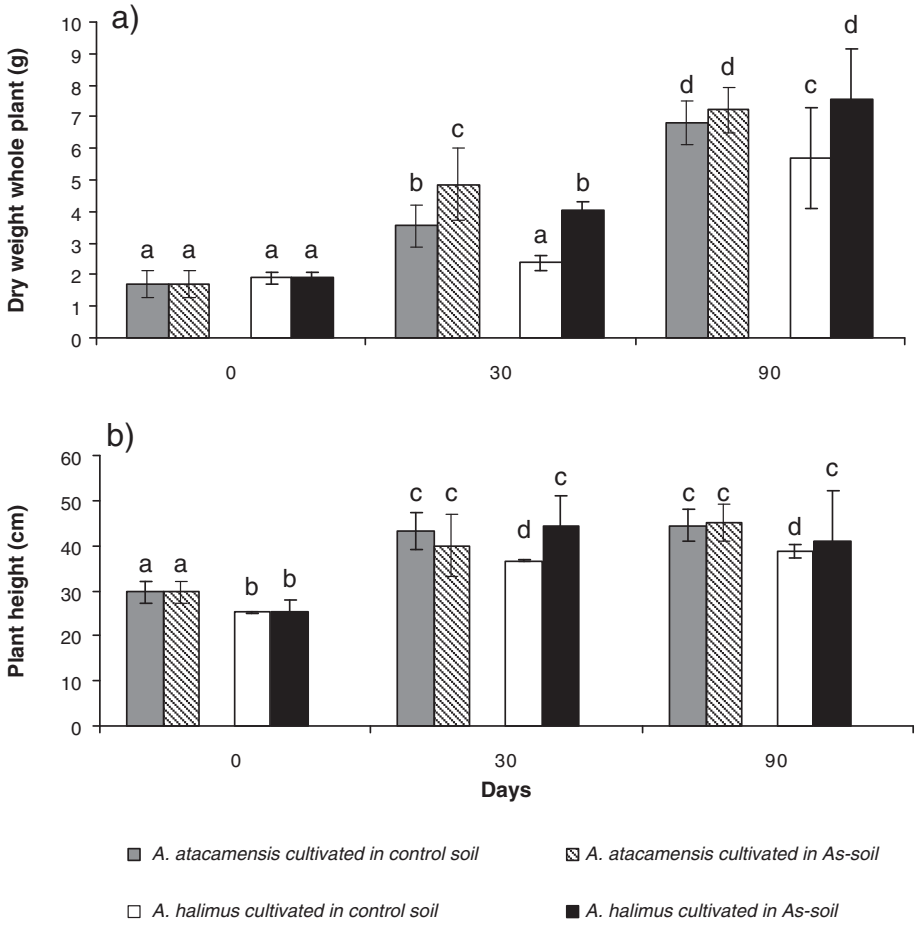

Fig. 4. Dry weight of the whole plant (g) (a) and height (cm) (b) of *A. atacamensis* and *A. halimus* plants cultivated in control soil and As-soil (lines on bars correspond to the standard deviation, n = 3. Different letters indicate significant differences according to the Duncan test at $p \leq 0.05$).

4. Discussion

The high concentration of As (111 ± 19 mg kg^{-1}) and also of B (79.6 ± 0.98 mg kg^{-1}) found in As-soil is attributable to the fact that these soils were formerly flooded by the river Loa that has been associated with high salinity and B contents (Cáceres et al., 1992). Different regions of the Loa Basin exhibit sediments with As concentrations ranging from 120 to 700 mg kg^{-1} and stream waters with contents of 300 to 8900 μg L^{-1} of As and B, respectively (Romero et al., 2003). The concentration of As in As-soil, exceeded the common range in soil of various world regions, 0.1 and 55 mg kg^{-1} (Alloway, 2010). Total B concentrations in soil of 96 mg kg^{-1} are considered high, and levels > 5 mg kg^{-1} may be toxic for some plants (Nable et al., 1997).

Table 4
Chlorophyll (mg kg^{-1} F.W.), thiol and malondialdehyde (μg kg^{-1} F.W.) concentrations in leaves of *A. atacamensis* and *A. halimus* cultivated in control soil and As-soil (values are means ± standard deviations, n = 3).

Days		Soil treatment	Chlorophyll mg g^{-1}	Thiols μg g^{-1}	Malondialdehyde μg g^{-1}
			F.W. leaves		
0	*A. atacamensis*	–	0.34 ± 0.03	27.3 ± 1.0	0.83 ± 0.17
	A. halimus		0.47 ± 0.06	35.5 ± 3.2	0.41 ± 0.15
30	*A. atacamensis*	Control soil	0.38 ± 0.01a	29.9 ± 1.3	0.91 ± 0.22
		As-soil	0.63 ± 0.04b	26.2 ± 0.7	0.90 ± 0.11
	A. halimus	Control soil	0.48 ± 0.11	36.3 ± 4.2	0.42 ± 0.07a
		As-soil	0.63 ± 0.01	37.5 ± 2.3	0.96 ± 0.22b
90	*A. atacamensis*	Control soil	0.37 ± 0.00a	29.6 ± 5.1	0.79 ± 0.16
		As-soil	0.62 ± 0.03b	28.9 ± 6.4	1.11 ± 0.19
	A. halimus	Control soil	0.40 ± 0.00a	37.2 ± 16.4	0.42 ± 0.09a
		As-soil	0.64 ± 0.00b	39.6 ± 7.2	0.67 ± 0.06b

Different letters in columns indicate significant differences among soil treatments according to the Duncan test at $p \leq 0.05$.

The sequential extraction of As in soils provides useful information about the mobility, migration and potential toxicity of this element. The As associated with the FI, non-specifically adsorbed, indicates that the As is retained in a labile form within the soil matrix and more available for the plant. Arsenic is often associated with oxides and hydroxides in soil (Moreno-Jiménez et al., 2012), as was found in control soil. In our assay, the high pH value, low concentrations of Fe_2O_3 and MnO_2 and of organic matter and high sand content of As-soil are factors that favoured the availability of As in this soil. Probably, in As-soil the concentration of As in FI was higher, considering that this soil was washed for to decrease the high electrical conductivity.

In other assay, soil spiked with As (total As of 73 mg kg^{-1}, pH 7.8), Ascar et al. (2008) found 19 mg As kg^{-1} in FI. In Loa sediments (total As 320 mg kg^{-1}, pH 7.7), Romero et al. (2003) found the As mainly associated with Fe–Mn oxides and residual phases, and a portion of the As (approximately 20%, i.e., 64 mg As kg^{-1}) was associated with readily available fraction. Moreno et al. (2005) found low As availability (0.17%) in soils with total As of 1544 mg kg^{-1}, but with a pH of 4.87 and higher contents of organic matter (4.33%) and Fe (2.02%). Generally, the contamination of soil with As is associated with the presence of other metals (Fayiga et al., 2007). However, the concentrations of Cu, Fe, Mn, Pb and Zn in As-soil were lower than in control soil.

At 30 and 90 days of assay the concentration of As in leaves of *A. halimus* exceeded the levels of As found in other woody plants growing on arsenic polluted soils, that ranged 0.27 to 2.29 mg As kg^{-1} (Madejón and Lepp, 2007). At 30 days *A. halimus* translocated the metalloid to the leaves to similar levels than in the roots, however most plants preferentially accumulate As in the roots (Moreno-Jiménez et al., 2012) as showed by *A. atacamensis*. This could indicate that *A. atacamensis* shows an exclusion mechanism of As more effective than *A. halimus* that could be related with mechanisms that restricts the arsenic transport, as the formation of complexes in the vacuoles of the cells of the roots.

The capacity As translocation of *A. halimus* to leaves has also been demonstrated for Mn, Pb and Zn (Tapia et al., in press). However if *A. halimus* is destined for animal feed, the capacity of translocate contaminants to aerial part should be considered and, would be more sustainable to use *A. atacamensis* because this species preferentially maintains the As in the roots.

In other works, Diaz et al. (2011) in *A. atacamensis* collected from their natural habitat in the Pre-Andean zone of Chile, found 6.3 mg As kg^{-1} in leaves, 2.5 mg As kg^{-1} in stems and 1.7 mg As kg^{-1} in roots, in soil containing 54 mg As kg^{-1} and pH of 8.3. This finding suggests that *A. atacamensis* requires time to translocate the metalloid to aerial part, or there exist an adaptation mechanism involved. Under hydroponic conditions, *A. atacamensis* accumulates high concentrations of As mainly in the roots (1500 mg As kg^{-1} in roots and approximately 25 mg As kg^{-1} in aerial part) when this species was exposed 100 μM arsenate for 28 days (Vromman et al., 2011). However, it is known that the dynamics in the soil is very different to hydroponic conditions. In another species, *Atriplex patula*, growing naturally on mineral-processing tailing ponds (1225 mg As kg^{-1}, pH 7.2), the concentration of As was of 37.6 mg kg^{-1} in leaves and 21.9 mg kg^{-1} in roots (Baroni et al., 2004). In woody plants collected in soil polluted with As (52–218 mg As kg^{-1} and pH 5.6–7.4), the levels of As ranged from 0.27–2.29 mg As kg^{-1} in leaves, 0.11–1.09 mg As kg^{-1} in stems and 1.37–3.78 mg As kg^{-1} in roots (Madejón and Lepp, 2007). The higher As content observed in *A. halimus* than in *A. atacamensis* in whole plants at the end of the assay is attributable to the increased concentration of the metalloid in stems and roots, i.e., *A. halimus* continued accumulating the As.

The higher BCF value found in *A. halimus* than *A. atacamensis* is due to the greater ability of *A. halimus* to accumulate As in the leaves. However, the BCF remained constant, and the Ti was similar to *A. atacamensis* at the end of assay. This suggests that *A. halimus* stopped the translocation of the metalloid to leaves. A similar behaviour was observed in assays on a substrate contaminated with Cd, in which *A. halimus* rapidly translocated Cd to leaves, and the Cd concentration showed no significant differences between 35 and 70 days (Tapia et al., 2011). It appears *A. halimus* presents a mechanism for rapid translocation of elements to leaves up to certain levels, and then regulation mechanisms are activated.

The BCF of As (0.05) found in *A. halimus* was higher than in other species, such as *A. patula* (BCF = 0.03), that grow on mineral-processing tailing ponds (Baroni et al., 2004). The Ti values of As obtained for both species were higher than those found in other species, which are usually <0.1 (Moreno-Jiménez et al., 2008; Zhao et al., 2009). The BCF and Ti values obtained in the present assay for *A. atacamensis* and *A. halimus* indicate that both species accumulates the As in moderate concentration preferentially in roots.

Saline soils generally present high levels of B, and halophyte plants tend to accumulate this element (Watson et al., 1994). In general, the B concentrations in roots remain relatively low compared with the leaves even under a very high B supply (Nable et al., 1997). Levels of B as high as 610 mg kg^{-1} were found in *Atriplex canescens* inhabiting surface-coal mine spoils (Severson and Gough, 1983). In various species of the genus *Atriplex* (*A. canescens*, *Atriplex undulata*, *Atriplex deserticola*, *Atriplex nummularia* and *Atriplex polycarpa*) irrigated with saline drainage water (B levels ranging from 5 to 10 mg L^{-1}, EC of 17 dS m^{-1}), the mean concentration of this element in aerial part was 129 mg B kg^{-1} (Watson et al., 1994). In our assay, the concentration of B and the EC value in the irrigation water were low. Therefore, the high levels of B found in *A. atacamensis* and *A. halimus* at 90 days are attributed to uptake of this element from the soil. Planting of B-tolerant species, such as plants of the genus *Atriplex*, may be a strategy of revegetating soils containing elevated levels of soluble B (Nable et al., 1997). However, if species of the *Atriplex* genus are destined for use as animal feed, the accumulation of B should be considered.

Halophytes may exhibit growth stimulation associated with a certain level of salinity (Belkheiri and Mulas, in press). In the case of *A. halimus*, the dry weight and height were significantly greater when plants were cultivated in As-soil. In other assay, a slight stimulating effect of As on biomass production was found in *Brassica rapa*, though the reason for this was not clearly understood (Shaibur and Kawai, 2009). Additionally, the levels of chlorophyll increased in *A. atacamensis* and *A. halimus* cultivated in As-soil. In other works, *Erica andevalensis* (2.5–4.4 mg As kg^{-1} in leaves) collected in zones with high levels of As (193–518 mg kg^{-1}) showed visibly healthy, and chlorophyll concentrations in these plants (0.38–1.5 mg g^{-1}) reflected normal plant growth (Márquez-García and Córdoba, 2010). Furthermore, in *B. rapa*, in hydroponic conditions, an increase of the As concentration increased the chlorophyll index (Shaibur and Kawai, 2009). However, Moreno-Jiménez et al. (2008) detected a decrease in the chlorophyll levels in leaves of *Macrozamia communis*, *Arbutus unedo* and *Rorippa spherocarpa* when the As supply was increased from 5 to 250 μM, although an increase in chlorophyll levels was observed in *M. communis* at 5 μM. These data indicate that in some plants, chlorophyll is not a good indicator for reflecting stress upon exposure to As, especially in plants of the genus *Atriplex*, likely due to their halophytic character and C4 photosynthesis mechanism (Akhani et al., 1997).

The levels of thiols obtained in *A. atacamensis* (equivalent to 0.09 μmol g^{-1}) and *A. halimus* (equivalent to 0.19 μmol g^{-1}) in this assay were lower or similar compared to those obtained under hydroponic conditions for *A. atacamensis* (0.2 μmol g^{-1} and 50 mg As kg^{-1} in leaves) exposed to 100 μM As (Vromman et al., 2011), where the thiol levels remained constant between 14 and 28 days. In our assay the level of thiols for each plant also remained constant. In other assay with *Sesuvium portulacastrum*, a facultative halophyte, cultivated in sand exposed to 1000 μM As (V) for 30 days, Lokhande et al. (2011) also found that the levels of thiols (3 μmol g^{-1} and 155 mg As kg^{-1} in aerial part) were not significantly affected. However, in non-halophytes (*M. communis*, *A. unedo* and *R. spherocarpa*) cultivated under hydroponic conditions exposed to 5–50–250 μM As, the levels of thiols in leaves (ranging from 0.18 to 0.5 μmol g^{-1} and 5.83 to 23.9 mg As kg^{-1} in aerial part) increased in response to an increasing As supply (Moreno-Jiménez et al., 2008). These data indicate that the thiol levels in plants vary widely in response to exposure to As and appear not be affected in plants halophyte as the genus *Atriplex*. Thiols preferentially form complexes with As (III), and the complexation of As (III) in the roots presumably decreases the rate of transport of As from roots to aerial part (Aldrich et al., 2007; Zhao et al., 2009). In our assay, *A. halimus* translocated As from roots to aerial part, and the levels of thiols in leaves were unaffected compared to the control soil. Vromman et al. (2011) reported that in *A. atacamensis*, As (V) reduction occurs at a lower rate, and could exist in other strategies of the plant distinct to the complexation with sulfhydryl groups. Recently there is a debate on the form in which As is transported from roots-to-aerial part (Tripathi et al., 2007).

The levels of MDA were increased in *A. halimus* cultivated in As-soil (equivalent to 0.019–0.031 nmol MDA mg^{-1}). In *E. andevalensis* collected in different soils with high levels of As (193–518 mg kg^{-1}), the levels of MDA were between 0.018 and 0.057 nmol mg^{-1}, though the data reported for many plant species ranged from 2 to

40 nmol MDA mg^{-1} (Márquez-García and Córdoba, 2010), such as those obtained by Moreno-Jiménez et al. (2008) for woody shrubs under hydroponic conditions. In other studies, in *A. halimus* was measured the activity of guaiacol peroxidase to evaluate stress and statistically significant differences were found only in plants treated with high levels of two metals under high salinity conditions (Manousaki and Kalogerakis, 2009). In our assay, the concentrations of As and the EC level in the soil, and the concentrations of As in leaves of *A. halimus* were not particularly high. Thus, it is possible to hypothesise that other factors together with the As may have caused the increased of levels of MDA in *A. halimus*, as the high concentration of B. *A. atacamensis* also showed high concentrations of B in the leaves, but this species did not showed translocation of As to the leaves.

The tolerance of plants of the genus *Atriplex* to the ionic components has been related to various mechanisms, such as the synthesis of osmoprotectants (glycebetaine) and polyamines (amino acids) and the excretion of trichome, which can accumulate high levels of ions (Na^+, Cl^-, Cd^{2+}) (Lutts et al., 2004; Walker et al., 2008; Lefèvre et al., 2009; Vromman et al., 2011). However, studies in *A. atacamensis* treated with As, under hydroponic conditions, indicate that synthesis of osmoprotectants and trichome excretion did not contribute to As resistance, whereas polyamines involved in the reduction of oxidative stress and the maintenance of the plant water status, could interact with As (V) (Vromman et al., 2011). In our assay, *A. atacamensis* accumulated As in the roots, and *A. halimus* translocated the metalloid to the aerial part, which indicates that these plants exhibit different mechanisms of tolerance to cope high concentrations of As in soil.

5. Conclusions

The shrubs *A. atacamensis* and *A. halimus* grew without visual symptoms of toxicity and without exhibiting decreased growth in natural soil with high levels of available arsenic. Chlorophyll and thiols levels were not affected negatively in either species, only *A. halimus* showed an increase of malondialdehyde levels in the leaves. *A. halimus* accumulated higher levels of arsenic in leaves than *A. atacamensis*. Both species accumulated the highest arsenic levels in roots. If these plants are used for animal feed, it should be considered that *A. halimus* accumulates higher concentration of As and B in the leaves than *A. atacamensis*.

The results indicate that these plants resist contamination by arsenic and can be recommended to generate plant cover in As-contaminated soils in the Pre-Andean region, under saline conditions controlled, preventing the dispersion of this metalloid via wind and leaching.

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