

Extractable copper, arsenic and antimony by EDTA solution from agricultural Chilean soils and its transfer to alfalfa plants (*Medicago sativa* L.)

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Following our research on copper, arsenic and antimony in Chilean ecosystems, a study to understand the mobility and transport of these elements from soil to plants was carried out. So, the aim of this study, which follows on from the previous work, was to demonstrate if the total concentrations of these elements or their fractions extracted by 0.05 M EDTA pH 7 from different Chilean soils correlate with the respective total concentrations in the edible tissue of alfalfa plants collected simultaneously from 20 different sites affected or unaffected by mining activities. The highest copper fractions extracted by EDTA solutions were obtained in contaminated soils from the central region (41–69%); however the northern soils presented the highest extractable fractions of arsenic (9–34%). The antimony fraction was low in all soils (0.4–8.0%). Alfalfa plants from all contaminated sites presented high copper, arsenic and antimony concentrations (19–126 mg kg⁻¹, 5.7–16.3 mg kg⁻¹ and 0.16–1.7 mg kg⁻¹, respectively). Statistically significant correlations were obtained between the total contents of copper and arsenic and their respective extractable fractions in soils. Good correlations were found between elements in alfalfa plants. Correlations were also obtained between the total concentrations of three elements in soils and in alfalfa plants. However, excepting for antimony in the northern samples, higher correlation coefficients were evaluated when the extractable fractions were considered. Samples from the north region presented the highest copper transfer factor and the lowest for arsenic, in spite of the high concentration of this metalloid extracted by EDTA solution in these soils. There was not a clear trend on the transfer factor for antimony, probably due to the low content of this element in alfalfa plants and/or the low recovery obtained for this element by EDTA.

Introduction

Contamination of the environment with arsenic from both anthropogenic and natural sources has occurred in many parts of the world and is a global problem. Elevated levels of arsenic from natural and industrial sources have been reported in several countries of America, such as Chile, Argentina, Mexico, Canada and the United States of America.¹ Elevated antimony concentrations are generally associated with high arsenic concentrations in sulfide ores. The most important anthropogenic emission sources to the environment for metalloids are the non-ferrous mining and smelting industries and the burning of coal and petroleum. Gross contamination of surface soils and plants by dust and smelter fumes from copper and lead smelters have been reported; the nearest samples to mine industries present the highest concentrations but the levels decrease with increasing distance.^{2–7} Slight contamination by these metalloids may also arise from the application of sewage sludge and fertiliser to soil. While a world-wide community of researchers work on arsenic speciation, transformation, transport, seasonal cycling, accumulation, geochemistry and toxicology, scarce information is available about the mobilisation and transformation of antimony in the different compartments of the environment. Many arsenic compounds are toxic and potentially carcinogenic, which raises much concern from the environmental, occupational and nutritional points of view according to ATSDR (Agency for Toxic Substances and Disease Registry).⁸ Because of the toxicity of its compounds, antimony is listed as a priority pollutant by the United States Environmental Protection Agency and by the Council of the European Communities.^{9,10} It is on the list of

hazardous substances under the Basel Convention concerning the restriction of transfer of hazardous waste across borders.¹¹

In agricultural soils, toxic elements have received a great deal of attention because of their long term effects on crop yields, soil quality, accumulation in crop tissue and potential source of risk to animals, farmer and consumer. But the mobility of trace elements, their bioavailability and related eco-toxicity to plants depend strongly on their specific chemical forms or types of binding rather than the total element contents. However, the determination of specific chemical species is difficult and often hardly possible. Therefore, in practice determinations of phases defined by their function *e.g.* “bioavailable forms” or “carbonate bound forms”, are usually carried out by single or sequential extraction with reagents having different chemical properties.¹² The function relating soil parameters to element availability seems to be complex, and several models have been used to approach this problem.^{13–15} The physico-chemical characteristics of soils result in important control of the adsorptive capacities and therefore will affect the availability of elements to plants.¹⁶ But these interactions vary with the element, soil type and the form in which the metal or metalloid itself is present or enters the soil.^{17–20}

Relative to antimony, Hammel *et al.* have described that in polluted soils it is mostly immobile.²¹ In contrast, in soils with high mobile antimony content, an accumulation of this element was observed in leaves of spinach grown under controlled conditions; the correlation between the mobile fraction and antimony in leaves was found.²² In general, there is scarce evidence of accumulation of antimony in food chains represented by soil–vegetation–invertebrate–insectivore pathways in grasslands.^{4,23}

In Chile, copper mining is by far the major economic activity, but in spite of the economical importance, mining has contributed to the propagations of heavy metals in the environment. In a previous paper we reported the total concentrations of copper, arsenic and antimony in affected and unaffected agricultural soils, from the north and central region in Chile.²⁴ High concentrations of these elements in soils collected near the smelters and mining industries, clearly showed the impact produced by these activities. Following our research on these elements in Chilean ecosystems, and taking into account that total elemental concentrations are not reliable indicators of environmental risk, a further study to obtain information on their mobility and transfer into plants was performed. In this paper, which follows on from the previous work, the fraction of these elements extracted by 0.05 M EDTA pH 7 from 20 different agricultural soils was determined. Their uptake by forage alfalfa plants (*Medicago sativa*) collected from the same sites where soils were sampled (total concentrations in the edible tissues) is discussed. The relationships between the total concentrations and extractable fractions of these elements in soils and their total contents in alfalfa plants were examined. The effect of some physico-chemical parameters on the extractable fraction were also considered.

Materials and methods

Reagents

High quality water (18 M Ω) obtained from a Milli-Q system (Nanopure, Barnstead) was used to prepare all solutions. Antimony(III) standard was obtained as potassium antimonil tartrate $K(SbO)C_4H_4O_6H_2O$ (Aldrich, 99.95% purity); stock solution of 1000 mg L^{-1} Sb(III) was prepared by dissolving this compound in 1% m/v ascorbic acid (Merck). Stock solutions of 1000 mg L^{-1} As(III) and Cu(II) were obtained from the respective standard solution Titrisol[®], Merck. All standard solutions were stored at 4 °C in polyethylene bottles. Standard working solutions were prepared by diluting the stock solution with deionized water as necessary, those of Sb(III) were diluted with 0.1% m/v ascorbic acid.

High purity nitric, hydrochloric, hydrofluoric and sulfuric acids (Suprapur[®], Merck) were used for digestion of soils and alfalfa samples.

For hydride generation analysis, $NaBH_4$ powder (Merck) was dissolved in 0.4% m/v NaOH solution (Merck) to provide a

concentration of 0.7% m/v. This solution was prepared daily just before use or stored for up to three days at 4 °C.

The extractant solution 0.05 mol L^{-1} EDTA pH 7 was prepared by dissolving di-sodium dihydrogen ethylene diamine tetraacetate salt dihydrate (Na_2 EDTA \times $2H_2O$ Merck). The pH solution was adjusted to 7.0 adding NH_4OH solution (trace element quality, Fisher).

Instrumentation

A GBC, model 905, Atomic Absorption Spectrometer, equipped with a copper hollow cathode lamp was used to determine copper in soil extracts and alfalfa plant solutions. The pre-diluted sample solution was aspirated in the air acetylene flame, using the instrumental operating parameters recommended by the manufacturer.

Arsenic and antimony determinations were performed on a PSA Millenium Excalibur Analytical Ltd. (Orpington, Kent, UK), equipped with a continuous flow hydride generation system and the respective boosted discharge hollow cathode lamp (As or Sb BDHCL) as the radiation source of the atomic fluorescence detector. The sample solution or 1.5% m/v HCl and 0.7% m/v $NaBH_4$ were continuously pumped to the separator. The hydride compounds were purged with argon (300 mL min⁻¹), coupled *via* a commercial dryer membrane (Perma Pure product, dryer model MD-110-12 FP), to the $H_2(g)$ flame, produced during hydride formation. A supplementary H_2 flow (30 mL min⁻¹) was supplied to maintain a stable flame.

Sampling and samples treatments

Soil and alfalfa samples were collected simultaneously from the same agricultural sites (20 sites) located in three different regions, in Chile: I, II and V regions. The general description of sampling sites has been previously described.²⁴ In Table 1 are summarized the geographic location of sites where soils and alfalfa samples were collected, and the distance from the copper smelter in the area. Physico-chemical parameters were determined using standard methods. Soil pH was measured using 1:2 soil to water ratio; pE ($pE = E_h$ (mv)/59.2, where pE is the negative log of the electron activity and E_h is the difference in potential between the Pt electrode and the standard hydrogen electrode) values were calculated from the potential measured in the same solution (Pt and calomel electrodes). Organic matter was determined by dichromate oxidation, in sulfuric acid media, the dichromate excess was titred with iron(II) standard solution. Fe content was determined by

Table 1 Geographic situation of sampling sites and distances from copper smelter

Geographic situation in Chile	Site	Distance from Cu smelter/km	Sample abbreviation
Region I (Tarapacá)	Quebrada de Tarapacá	—	T1
	Quebrada de Parca	—	T2
	Mamiña	5 (E)	T3
Region II (Antofagasta)	Chiu-Chiu	35 (E)	A1
	Calama	16 (S)	A2
	Quillahua	—	A3
Region V (Valparaíso) Puchuncaví Valley	La Greda	2 (E)	P1
	Maitenes	2.6 (E)	P2
	Campiche	6 (E)	P3
	Puchuncaví	8 (E)	P4
	Nogales	26 (E)	P5
Catemu Valley	Catemu	4 (E)	C1
	San José	5.5 (E)	C2
	Santa Margarita	7 (E)	C3
Near to Quillota city	Panquehue	13.5 (E)	C4
	Rautén	—	Q1
	Quillota	—	Q2
Casablanca Valley	Pocochay	—	Q3
	Casablanca	—	CB
Near to Peñuelas lake	Las Tablas	—	LT

FAAS. Available phosphate was determined in the soil extracts by the molybdate molecular absorption method (5.0 ± 0.1 g soil was extracted with 100 mL 0.5 M NaHCO_3 , pH 8.5 according to the Olsen method).²⁵ The analysis of particle size distribution in soils was executed according to the hydrometer method.²⁶

Alfalfa plants

Approximately 3 kg of mature alfalfa plants were randomly sampled (from 25–30 different plants) in each agricultural site. Samples were rapidly transported to the laboratory and were thoroughly washed with deionized water to remove soil and superficial dust particles. The alfalfa plants were dissected to remove above ground tissues from roots and then frozen at -20°C , until treatment (only the edible tissues were considered for this study). Alfalfa samples were then crushed and homogenised using a plastic food processor (Moulinex®) especially adapted with high purity titanium blades. The homogenised samples were frozen at -20°C and then freeze-dried (Lyovac GT2). Lyophilised samples of each site were pulverised to a fine powder and preserved in a desiccator, at room temperature, until analysis.

Digestion of alfalfa plants

The digestion of alfalfa samples (or certified reference materials) was performed in a microwave oven operating system, (Microdigest A301, Prolabo) with an energy output of 0–200 W (0–100% potency, respectively) with $\text{HNO}_3/\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ mixture. The freeze-dried alfalfa samples were accurately weighed 1.0 g (± 0.1 mg) and placed into the glass microwave digestion vessel, then 8 mL of concentrated HNO_3 were added to each sample to stand overnight at room temperature. Then the vegetable samples were digested using an optimized microwave program, previously described.²⁷ After all the samples had cooled, the clear solutions remaining were diluted to 25 mL with deionized water and stored at 4°C until analysis. Analytical blanks were prepared in the same way without addition of any sample.

Extraction of soils

Extractions were carried out by weighing 2.0 g (± 0.1 mg) of soil into 50 mL Erlenmeyer vessels adding 20 mL 0.05 mol L^{-1} EDTA pH 7.0 (extracting solution). The vessel was sealed with Parafilm® and shaken for 1 h at 150 rpm at room temperature

(horizontal shaker, Junior orbit shaker, Labline instrument, Melrose Park, Illinois, USA). After extraction, the mixture was centrifuged at 4000 rpm (centrifuge, Hermle Z200 A, Hermle-Labortechnik, Germany) until a clear solution was obtained (30 min were sufficient), the decanted extracts were collected for analyses.

Determination of arsenic and antimony in alfalfa solutions and soil extracts

To determine total arsenic and antimony by HG-AFS, an aliquot of alfalfa sample solution or soil extract was placed into a volumetric flask adding 2.5 mL KI (15% m/v) + ascorbic acid (3% m/v) solution and 3 mL concentrated HCl, the final volume was made to 25 mL with deionized water. These solutions were allowed to stand for 30 min at room temperature and then the metalloids were determined by HG-AFS, as was previously described for the total determinations of these metalloids in soil samples.²⁷

Results and discussion

General physicochemical parameters and particle size distribution of soils

In Table 2 are presented the physico-chemical and granulometric characteristics of agricultural soils considered in this study. The main physico-chemical parameters determined for agricultural soils included: (i) organic matter contents ranging from 0.8 to 3.1%, with most values between 1.5 and 2%; (ii) values of pH ranging in a narrow interval (6.7–8.7), which suggested neutral to sub-alkaline conditions for all soils; (iii) pe values were between 7.38 and 9.84, which permits their classification as oxic soils.²⁸

The texture, calcium, iron and aluminium oxides contents and the presence of other interfering elements affect the sorption capacity of a soil. Sand and silt fractions show little sorption capacity due to low surface area and the predominance of quartz, while the clay fraction is the main sorber.¹⁶ As can be seen in Table 2 the particle size distribution of soils are widely ranged; very low content of clay (2%) was found in two soils from Tarapacá region (T2 and T3) and also in two soils from Puchuncaví valley (P2 and P3). Highest and relatively similar values for this fraction were found in P5, Q1–Q3 and LT, all sites from the central region, not particularly contaminated by mining and with great agricultural activity,

Table 2 Some physical and chemical characteristics of the sampled soils from different regions in Chile

Site	pH ^a	pe	Organic matter (%) ^a	Available phosphate/mg P kg ^{-1a}	Fe (%) ^a	%Clay	%Silt	%Sand
T1	8.71 ± 0.01	8.94	0.80 ± 0.01	7.7 ± 0.4	2.1 ± 0.1	12.0	16.0	72.0
T2	8.35 ± 0.06	9.26	1.63 ± 0.01	5.1 ± 0.2	2.5 ± 0.1	2.0	35.9	62.1
T3	7.73 ± 0.02	9.13	1.63 ± 0.04	13.8 ± 0.7	2.5 ± 0.2	2.0	33.7	64.3
A1	8.76 ± 0.07	8.28	1.62 ± 0.02	15.0 ± 0.6	1.9 ± 0.1	11.9	13.9	74.2
A2	8.96 ± 0.05	9.01	1.16 ± 0.02	8.4 ± 0.4	1.4 ± 0.1	12.0	10.0	78.0
A3	8.39 ± 0.01	9.74	2.96 ± 0.01	20 ± 1	1.8 ± 0.1	9.8	35.5	54.7
P1	6.72 ± 0.01	9.28	1.22 ± 0.09	23.2 ± 1.2	3.7 ± 0.1	11.9	6.0	82.1
P2	7.35 ± 0.03	8.96	1.46 ± 0.06	43 ± 2	4.7 ± 0.1	2.0	13.8	84.2
P3	7.86 ± 0.03	9.38	0.92 ± 0.02	77 ± 3	4.0 ± 0.2	2.0	19.9	78.1
P4	7.29 ± 0.01	8.99	3.0 ± 0.1	191 ± 10	3.8 ± 0.1	11.9	19.9	68.2
P5	7.26 ± 0.01	9.08	2.58 ± 0.03	15.0 ± 0.6	4.1 ± 0.1	21.8	29.8	48.2
C1	7.31 ± 0.02	8.92	3.1 ± 0.2	21 ± 1	5.4 ± 0.1	12.0	15.9	72.1
C2	8.04 ± 0.03	8.28	1.56 ± 0.04	16.5 ± 0.8	4.2 ± 0.1	11.9	15.9	72.2
C3	7.84 ± 0.07	8.64	2.25 ± 0.06	16.5 ± 0.6	3.2 ± 0.2	22.0	35.9	42.1
C4	8.09 ± 0.01	8.45	2.03 ± 0.04	15.0 ± 0.5	4.3 ± 0.2	12.0	19.9	68.1
Q1	6.85 ± 0.09	8.40	2.55 ± 0.08	21 ± 1	6.0 ± 0.2	15.9	29.9	54.2
Q2	7.39 ± 0.08	8.15	1.99 ± 0.08	13.5 ± 0.7	5.4 ± 0.2	20.0	13.9	61.1
Q3	7.2 ± 0.2	7.38	2.87 ± 0.06	30 ± 1	5.4 ± 0.2	21.8	23.7	54.5
CB	7.79 ± 0.05	8.69	1.50 ± 0.01	12.0 ± 0.7	3.6 ± 0.1	11.9	19.9	68.2
LT	6.87 ± 0.02	9.84	1.86 ± 0.05	7.7 ± 0.4	3.7 ± 0.1	21.9	10.0	68.1

^a Values presented as the means ± standard deviation, $n = 4$.

which increases the weathering process. P5, that has one of the lowest contents of sand, was collected at Nogales, a site located in the Puchuncaví valley at 26 km from the smelter. Q1–Q3 are soils collected at Aconcagua valley and LT is a reference site. The clay fraction in the others soils was approximately constant (10 to 12%). As can be seen, the highest percentages of silt were found in T2 and T3 soils, from Tarapacá region, A3 from Antofagasta region and C3 (Santa Margarita), site particularly contaminated in Catemu valley.

Selection of the extractant solution for soils

In a previous work, we have determined the total concentration of copper, arsenic and antimony in agricultural soils affected and unaffected by mining activities, from north and central regions in Chile.²⁴ However, to know the potential risk to plants or animals it is necessary to evaluate their mobile and/or available fraction. Many researchers have tried to find a way of measuring the plant available fraction of metals and metalloids in soils using different extraction procedures. A variety of complexing agents have been proposed to evaluate the pool of elements that is mobile or available to plants.^{12,20,22,29} These have mostly been validated in field experiments by correlating plant contents with extractable soil contents; e.g. the analysis of EDTA soil extracts are widely used in agriculture, their role is the prediction and assessment of trace element deficiency or toxicity in crops or in animals eating them. In this work 0.05 mol L⁻¹ EDTA pH 7 was chosen as extractant solution because this reagent has been recommended by the “Measurement and Testing Program” of the European Community BCR to determine the extractable or mobile fraction of heavy metals from soils and sediments.²⁹ EDTA solution is assumed to extract principally organically bound and carbonate bound fractions of metals by forming strong soluble complexes. However, this reagent can also extract metals occluded in Fe amorphous oxides.^{30,31} Quevauviller *et al.* have proposed the extraction of trace metal contents in calcareous soils with EDTA solutions, relating the extracted fraction with those bioavailable to plants.³² Moreover, some authors have used solutions of this reagent to extract arsenic and/or antimony from industrial contaminated soils, since these metalloids can react with different carboxylic acids.^{30,33,34} In fact, it has been described that Sb(III) and Sb(V) react with carboxylic acids, such as tartaric acid and EDTA forming soluble complexes.^{34–36} Anionic exchange can also occur between H₂EDTA⁻² (predominant

Table 3 Copper, arsenic and antimony concentrations (mg kg⁻¹) in 0.05 M EDTA pH 7 soil extract^{ab}

Site	Copper	Arsenic	Antimony
T1	4.0 ± 0.1 (13)	7.1 ± 0.2 (21)	0.025 ± 0.003 (1)
T2	4.8 ± 0.1 (18)	1.3 ± 0.1 (14)	0.009 ± 0.005 (1)
T3	18 ± 1 (18)	1.7 ± 0.2 (9)	0.101 ± 0.004 (4)
A1	29 ± 1 (27)	9.7 ± 0.5 (23)	0.017 ± 0.001 (3)
A2	12 ± 1 (26)	36 ± 2 (34)	0.202 ± 0.006 (2)
A3	2.4 ± 0.1 (7)	63 ± 2 (31)	0.34 ± 0.02 (3)
P1	298 ± 48 (56)	11 ± 1 (21)	0.51 ± 0.07 (8)
P2	180 ± 1 (64)	4.3 ± 0.4 (11)	0.32 ± 0.01 (5)
P3	143 ± 4 (43)	6.2 ± 0.6 (17)	0.41 ± 0.02 (7)
P4	177 ± 3 (69)	5.2 ± 0.2 (17)	0.34 ± 0.02 (6)
P5	51 ± 1 (49)	0.51 ± 0.02 (4)	0.022 ± 0.002 (1)
C1	78 ± 1 (56)	1.0 ± 0.1 (8)	0.041 ± 0.001 (1)
C2	106 ± 1 (59)	1.9 ± 0.1 (10)	0.067 ± 0.003 (2)
C3	96 ± 1 (41)	1.7 ± 0.1 (7)	0.036 ± 0.004 (1)
C4	40 ± 1 (53)	1.6 ± 0.1 (8)	0.014 ± 0.002 (0.4)
Q1	63 ± 3 (40)	0.38 ± 0.02 (2)	0.014 ± 0.001 (1)
Q2	29 ± 1 (36)	1.4 ± 0.1 (7)	0.044 ± 0.004 (2)
Q3	33 ± 1 (38)	1.6 ± 0.1 (8)	0.024 ± 0.003 (1)
CB	6.2 ± 0.4 (18)	0.07 ± 0.01 (2)	Non detected
LT	6.8 ± 0.7 (17)	0.036 ± 0.005 (1)	0.007 ± 0.001 (2)

^a Values presented as mean ± standard deviation, *n* = 4. ^b () percentage of the extracted element relative to total concentration in soils.²⁴

species of EDTA at pH 7) and the possible anionic forms of arsenic and antimony in soils.

Extractable fractions of copper, arsenic and antimony by EDTA solutions from agricultural soils

Total concentrations of copper, arsenic and antimony in EDTA agricultural soil extracts are listed in Table 3. The percentage of extracted elements relative to total content in soils is also included. Results show that the highest available fractions of these elements were obtained from soils that have the highest total concentrations.²⁴ In fact, statistically significant correlations of total concentrations of Cu and As in soils and those in the EDTA extracts were found (Fig. 1). For antimony this behavior was not so clear, probably due to the low mobility of this metalloid in soils and/or to a low efficiency of the EDTA solution to extract this fraction (Fig. 1). The extractable concentration of copper in soils from unaffected sites ranged from 2.4 to 12 mg kg⁻¹ (7 to 26% of the total content). However, in contaminated soils from the different regions (A1, P1–P5, C1–C4, Q1–Q3) significant high extractable fractions of copper were found, ranging from 29 to 298 mg kg⁻¹ (26 to 69% of the total content). These values are higher than the proposal for maximal acceptable concentration for this element that is considered as phytotoxic in agricultural

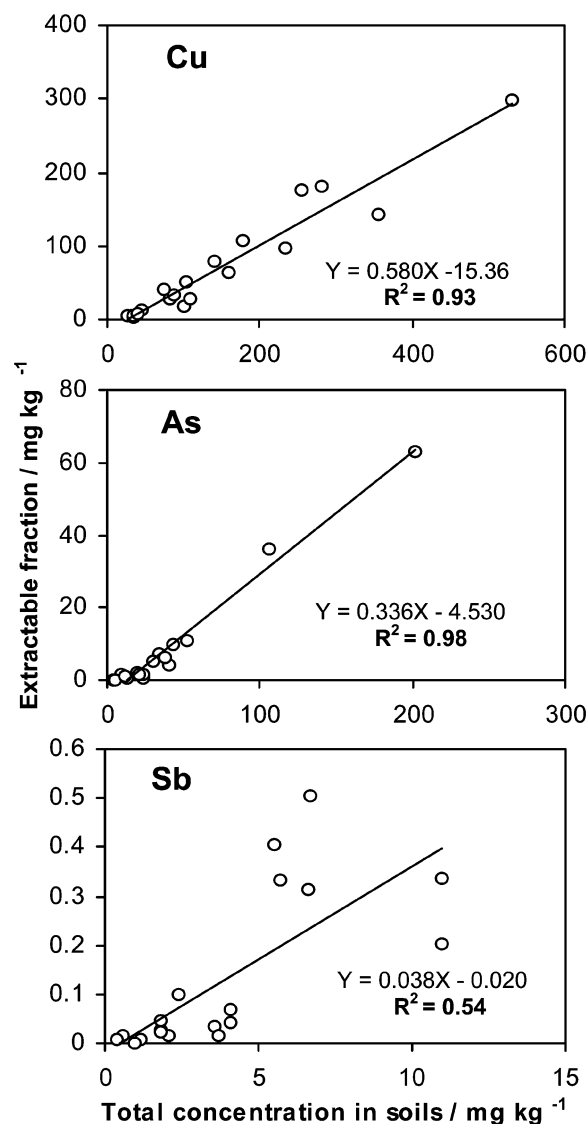


Fig. 1 Relationships between the fractions of copper, arsenic and antimony extracted by 0.05 mol L⁻¹ EDTA pH 7 solution and their total concentrations in soils.

soils.³⁷ These results demonstrate that an important fraction of exogenous copper arriving to soils from smelter stacks or another anthropogenic source remain preferentially associated with carbonaceous compounds, presenting, in this manner, high mobility. Badilla *et al.*³⁸ have reported that only 1.4–2.5% of total copper contents in contaminated soils from the central region in Chile was extracted by the acid leaching procedure. The authors attributed this behavior to the fact that the high copper contents in these contaminated soils came from tailing wastes containing highly insoluble metals, including all mineral particles that were not solubilized by milling, crushing and the concentration processing operation of copper ores.

In general, copper in soils from Antofagasta region presented higher mobility than in soils from Tarapacá region. In Antofagasta region, Chiu-Chiu and Calama soils, two sites affected by copper smelter activities²⁴ presented the highest copper extractable fractions (29 and 12 mg kg⁻¹). Flynn *et al.* have also reported high copper concentration in aqueous extracts from other Antofagasta soils affected by copper smelters.³⁹ The low organic matter and high percentage of sand in soils from this region are factors that must contribute favorably to the high copper mobility.

The extractable fraction of arsenic and antimony in unaffected soils from Valparaíso region, 2% of the total contents, was approximately similar to those of antimony in soil from the north region (1 to 4%). However, these last soils showed high amounts of arsenic associated with the extractable fraction, ranging from 9 to 34% of the total content; values similar to those determined in contaminated soils from Puchuncaví and Catemu valleys. In fact, soils from the north region in Chile have naturally high arsenic concentrations due to the presence of volcanic parental rocks rich in this element, furthermore agricultural soils in this zone are irrigated with water containing this element or with water contaminated by copper smelting activities.^{24,40} Recently, Flynn *et al.* have reported high recovery of arsenic by water in soils from salt flats in Antofagasta region, showing that arsenic in these soils is highly soluble.³⁹ Results of this study confirm the high mobility of arsenic in soils from the north region in Chile, especially in the Antofagasta region, increasing, in this manner, the presence of arsenic in the different compartments of the environment in this Chilean zone.

In impacted soils from Puchuncaví and Catemu valleys arsenic was more extracted than antimony (4–21% and 0.4–8%, respectively), but was less extracted than copper. Results can be explained by taking into account that arsenic and antimony released to soils from exogenous sources or weathering processes from parental rocks, are mostly bound to low mobile Fe–Mn oxides and to a lesser degree to organic matter or carbonaceous compounds of soil.^{20,33} EDTA solution practically cannot extract these metalloids from Fe oxides due to the low conditional solubility of these compounds in EDTA solution pH 7.⁴¹ Moreover, soils from the central region have the highest iron content (see Table 2) and presented low mobility of arsenic. In fact, as can be seen in Fig. 2, the extractable fraction of arsenic presented an inverse correlation with the total content of iron in soils, but with antimony there was no correlation. These results are in accordance with those reported by Kavanagh *et al.* who showed that arsenic present in agricultural soils collected 1.4 km from a smelter (Tamar valley in England), was predominantly bound to the relatively low mobile Fe oxides and organic matter.²⁰ Moreover, Turpeinen *et al.* showed that an aging process and sequestration of arsenic compounds occurs in contaminated soils, becoming progressively less available with time.⁴²

The major efficiency of EDTA solution to extract arsenic rather than antimony from these contaminated soils reflects the presence of different compounds of each metalloid present in soils or their different chemical properties. Though the fraction of antimony extracted by EDTA solution from contaminated

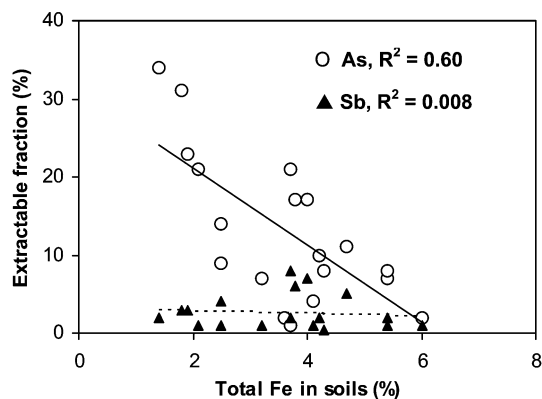


Fig. 2 Relationships between the fraction of arsenic and antimony extracted by EDTA solution and the total content of iron in soils.

soils was not so high as arsenic, it was higher than those reported by Hammel *et al.*,²² who described that antimony extracted by 1 M NH₄NO₃ from contaminated soils was less than 0.59% of the total content. The authors attributed this low mobility of antimony to the presence of insoluble antimony sulfide compounds originating from mining dumps rather than oxides originating from smelter processes, activities similar to those realized at Puchuncaví and Catemu valleys.

Since there is no regulation about mobile or available content of metalloids in soils, we have compared the extractable fraction of copper, arsenic and antimony determined in these soils with ranges of variability of these elements for unpolluted soils estimated at the world scale, or with criteria given by some organisms. Copper extracted from all soils collected in Catemu, Aconcagua and especially the Puchuncaví valleys (143–298 mg kg⁻¹), largely exceed the means range calculated for this element in unpolluted soils (13–24 mg kg⁻¹).^{43,44} Soils from Calama and Quillagua have higher extractable arsenic concentration than the soil quality criteria set by EPA for total arsenic in soils (20 mg kg⁻¹).⁴⁵ Soils contaminated by arsenic above this concentration are not recommended for sensitive use such as cultivation of vegetables for human consumption. On the other hand, all soils presented extractable fraction of antimony comparable or lower than the mean range of this element in unpolluted soils 0.3–0.9 mg kg⁻¹.

The effect of pH, organic matter content, percentage iron and percentage clay on the percentages of copper, arsenic and antimony extracted by EDTA was realized by multiple regression analysis. Results showed that the percentage Fe was the only parameter that has an effect on the extraction of arsenic by EDTA solution ($p < 0.05$). Owing to the narrow range of pH (6.72–8.96) and organic matter content (0.8–2.96%) in soil samples, these parameters had no effect on the extraction efficiency of these elements.

Total copper, arsenic and antimony concentrations in alfalfa

To understand the mobility and transport of copper, arsenic and antimony to plants, these elements were determined in alfalfa plants, collected from all the 20 sites where soil samples were collected. Alfalfa constitutes the major portion of diet of livestock (cows and sheep) grazing in these sites. The accumulation of copper, arsenic and antimony in industrial polluted soils and its subsequent uptake by plants represent a direct pathway of these elements into the human food chain, which is a major concern.

Determination of trace elements in environmental samples requires quality control of the analytical methodology employed. For this purpose, two certified reference materials (CRM) that match as closely as possible the alfalfa matrix were analyzed (Virginia Tobacco leaves CTA-VTL-2 and Orchard leaves SRM1571 from NIST). Recoveries of copper, arsenic

Table 4 Certified and obtained copper, arsenic and antimony concentrations in certified reference materials

CRM	Element	Certified value/mg kg ⁻¹	Obtained value/mg kg ⁻¹
Virginia Tobacco Leaves (CTA-VTL-2)	Cu	18.2 ± 0.9	18.4 ± 0.3
	As	0.979 ± 0.072	0.96 ± 0.03
	Sb	0.312 ± 0.025	0.32 ± 0.02
Orchard Leaves (NIST 1571)	Cu	12 ± 1	12.1 ± 0.5
	As	10 ± 2	11.1 ± 0.7
	Sb	2.9 ± 0.3	3.0 ± 0.2

^a Values presented as means ± standard deviation, *n* = 4.

and antimony from these CRM are listed in Table 4. As can be seen, the experimental values are in agreement with the certified values for these elements and were considered satisfactory to perform the analysis of alfalfa samples. The concentrations of copper, arsenic and antimony in alfalfa plants, on the basis of dry weight, are presented in Table 5.

Although the copper concentrations in alfalfa are widely ranged, most samples, excepting those from A1 (Chiu-Chiu), P1–P4 from Puchuncaví valley and C2, C3 from Catemu valley, contained levels between 7.1 and 19 mg Cu kg⁻¹, fitting within the normal range reported for herbaceous plants (5–20 mg kg⁻¹).⁶ Copper concentrations in alfalfa from Puchuncaví and Catemu valleys were four to eleven times higher than the average value of 11 mg kg⁻¹ of the other alfalfa plants. In the north region, alfalfa collected in Chiu-Chiu had the highest copper concentration 28 mg kg⁻¹, this value is statistically similar to the extracted copper concentration in soils from this site. In the previous work,²⁴ it was described that Chiu-Chiu is a site affected by mining activities developed in Chuquicamata copper mine and this contamination was also reflected in the alfalfa plants grown in this site.

An exceptional situation in terms of copper concentration was presented by the alfalfa samples from Campiche (P3) and Santa Margarita (C3), these alfalfas contain copper concentrations as high as 125 mg kg⁻¹ and are clearly outliers of the trend shown by the other alfalfas collected in both valleys. We have not a clear explanation for this behavior, but Campiche has a particular topography (it is deeper than the around sites), and Santa Margarita has roads crossing the site that has been filled with solid wastes from the Chagres smelter. Results from alfalfa corroborate the impact produced by the industrial activities developed in both valleys, especially at Puchuncaví

Table 5 Copper, arsenic and antimony concentrations in alfalfa (mg kg⁻¹) collected in sites from different regions in Chile

Site	Copper	Arsenic	Antimony
T1	9.0 ± 0.2	0.93 ± 0.11	0.054 ± 0.005
T2	14 ± 1	0.16 ± 0.01	0.026 ± 0.001
T3	19 ± 1	0.37 ± 0.01	0.042 ± 0.002
A1	28 ± 2	2.0 ± 0.1	0.059 ± 0.002
A2	13 ± 1	2.6 ± 0.2	0.155 ± 0.006
A3	7.1 ± 0.2	5.7 ± 0.3	0.14 ± 0.02
P1	65 ± 2	4.8 ± 0.2	0.42 ± 0.01
P2	47 ± 1	4.5 ± 0.2	0.34 ± 0.02
P3	125 ± 3	16.3 ± 0.4	1.7 ± 0.1
P4	50 ± 1	4.1 ± 0.1	0.43 ± 0.01
P5	10.4 ± 0.2	0.55 ± 0.04	0.065 ± 0.006
C1	17.6 ± 0.4	0.86 ± 0.04	0.024 ± 0.002
C2	35 ± 1	1.7 ± 0.1	0.061 ± 0.008
C3	126 ± 1	2.3 ± 0.1	0.09 ± 0.01
C4	12 ± 1	0.30 ± 0.03	0.088 ± 0.006
Q1	14.3 ± 0.1	0.13 ± 0.02	0.020 ± 0.003
Q2	12.7 ± 0.1	0.23 ± 0.03	0.022 ± 0.003
Q3	16.0 ± 0.2	0.25 ± 0.04	0.024 ± 0.002
CB	8.7 ± 0.1	0.47 ± 0.02	0.065 ± 0.003
LT	11 ± 1	0.10 ± 0.01	0.017 ± 0.003

^a Values presented as means ± standard deviation, *n* = 4.

valley. In spite of the high copper contents extracted from Quillota soils (Q1–Q3), ranging from 29 to 63 mg kg⁻¹, the concentration of this element in alfalfa from these sites was only slightly higher than that in unaffected sites.

The concentration of arsenic and antimony in the edible parts of plants is generally low, even when the vegetables are grown on contaminated soils.^{45,46} However, some researches have found high contents of the elements in plants grown on contaminated soils.⁴⁷ As can be seen in Table 5, there were very wide differences between the total concentration of arsenic in alfalfa plants sampled in different sites. Alfalfa A1–A3 from Antofagasta region, P1–P5 and C1–C4 from Puchuncaví and Catemu valleys, respectively, have higher arsenic concentrations than in unaffected sites. The highest value in the north region was found in Alfalfa from Quillagua A3 (5.7 mg kg⁻¹), a site that had the highest arsenic concentration in the EDTA soil extracts (63 mg kg⁻¹). The high total content of arsenic in this soil (202 mg kg⁻¹) was attributed to irrigation of soils with water naturally contaminated with arsenic due to its genetic relation with the geothermal field El Tatio.²⁴ Other authors have also demonstrated that vegetables cultivated in sites from Antofagasta region (Socaire and Talabre) presented high arsenic concentrations; *i.e.* 1.8 mg kg⁻¹ in corn and 0.86 mg kg⁻¹ in potatoes.⁴⁸ Comparatively, alfalfa plants, considered in this study, are better accumulators of arsenic than these other vegetables.

All alfalfa plants collected in Puchuncaví valley contained higher arsenic concentrations than those from unaffected sites, with the exception of alfalfa from Nogales (P5). However, what was most noticeable in this valley was the arsenic content in alfalfa collected at Campiche (P3) 16.3 mg kg⁻¹, which clearly outlies the trend showed by alfalfa in this valley. This value is four times greater than those obtained in the other samples. This alfalfa is also the sample where the highest copper and antimony concentrations were found and this fact was attributed to the topography of the site.

Arsenic concentrations in alfalfa samples from Tarapacá region, Quillota and unaffected sites in Valparaíso region were clearly lower than the impacted sites, fitting these levels in the range of natural reference values (0.02–7 mg kg⁻¹). Thus arsenic concentrations in alfalfa samples decreased in the order Puchuncaví valley > Antofagasta region > Catemu valley > Tarapacá region > Quillota ≈ Casablanca > Las Tablas.

Inter-element relationships provide interesting information on trace element sources and pathways. As can be seen in Fig. 3A copper in alfalfa shows a significant correlation with arsenic (R^2 0.81) and to a lesser extent with antimony (Fig. 3B, R^2 0.71). Arsenic is more significantly correlated with antimony (Fig. 3C, R^2 0.92), showing that both metalloids have a similar pattern in alfalfa samples. These results could indicate common natural or anthropogenic contamination sources for these elements. Furthermore, this coefficient was even more significant when only the alfalfa samples from the affected Puchuncaví valley were considered (Fig. 3D, R^2 0.99). These results confirm the similar anthropogenic contamination source for arsenic and antimony in this valley and show a similar behavior of alfalfa to uptake and to metabolize both metalloids. As can be expected, the antimony concentrations in alfalfa were lower than those of arsenic (0.017 to 1.690 mg kg⁻¹). The highest values (0.342–1.690 mg kg⁻¹) in alfalfa from Puchuncaví valley (samples P1–P4), exceed the reported normal range for antimony content in plants (0.2 mg kg⁻¹).⁶ It is important to remark that the arsenic/antimony concentrations ratio in alfalfa was approximately 10, especially in contaminated sites, a ratio that is naturally found for these metalloids in the environment.⁴⁹

In general, arsenic and antimony concentrations found in this study in alfalfa plants grown on contaminated sites are similar to those described by Li and Thornton,⁵⁰ who reported that most of the herbage grown on mining and smelting areas in the UK

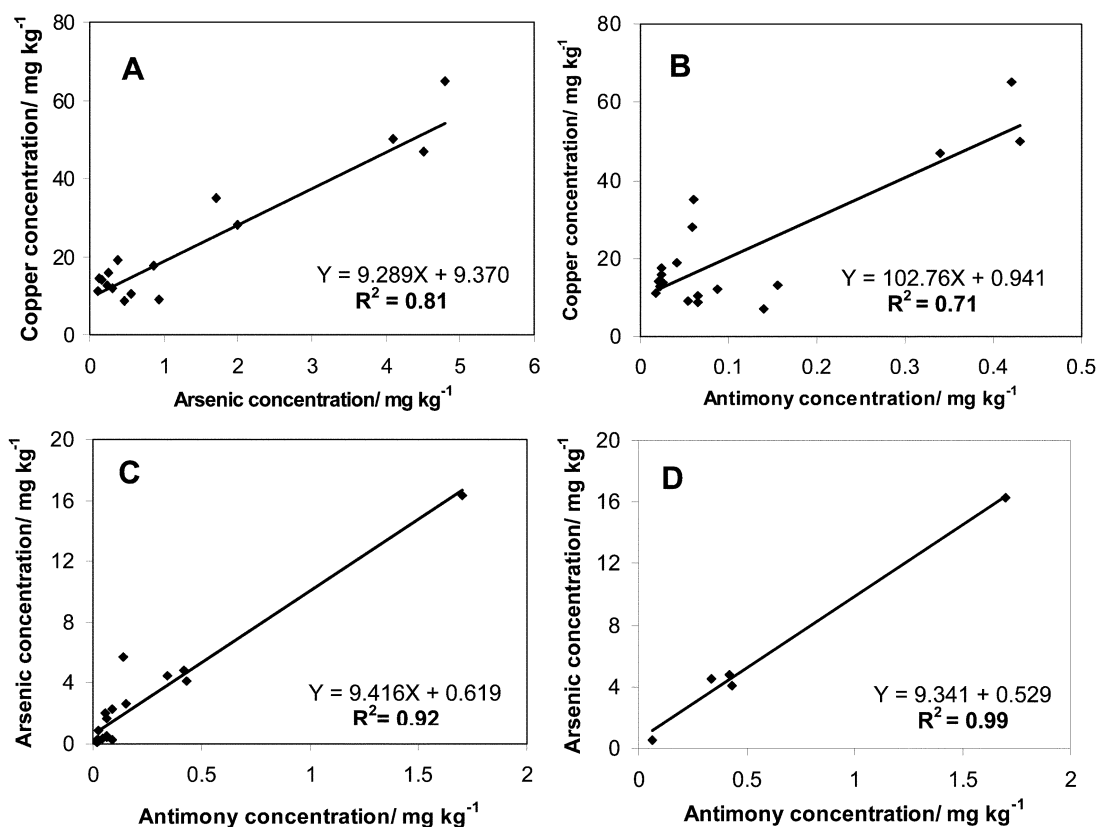


Fig. 3 Inter-element relationships in alfalfa plants: A between the total concentration of copper and arsenic, B between copper and antimony, C between arsenic and antimony in all alfalfa plants and D between arsenic and antimony in alfalfas from Puchuncavi valley.

contained 0.03–4.68 mg As kg⁻¹ and <0.02–0.45 mg Sb kg⁻¹. However, arsenic and antimony levels in alfalfa are higher than those found by Jung *et al.* in crop plants sampled around the Dalsung Cu W mine, in Korea.⁵¹

Relationships between total and extractable Cu, As and Sb in soils and its content in alfalfa

Concentrations of elements in plants can be influenced by various factors including total or extractable contents of the elements in soils, soil pH, organic matter content of soils, soil texture, climatic conditions, plant species, plant parts and age and the metabolism pathway that present some plant species for toxic elements. To demonstrate if the total contents of copper, arsenic and antimony in soils from different regions in Chile or the fractions extracted by EDTA presented different behaviors on their transfer to alfalfa plants, the relationship between these concentrations and their total content in alfalfa was studied with reference to the different regions. The analysis was made, in this manner, taking into account the different physico-chemical characteristics of soils from the different regions (see Table 2). The northern soils are sandy-loam, with low organic matter and iron contents and slightly alkaline. Those of the central region are in general both sandy-loam and loamy-sand (see Table 2).

As can be seen in Table 6 statistically significant correlations ($p < 0.05$) were determined between the total concentration in the edible tissues of alfalfa and the total concentrations in soils, especially for arsenic and antimony in samples from the north region (R^2 0.96 and 0.92, respectively) and for copper in samples from the central region (R^2 0.87). When these relations were evaluated considering the extractable fraction, (Fig. 4), similar or higher correlation coefficients were found, except for antimony in the northern samples. In these analyses, results from Campiche and Santa Margarita samples were not considered, due to their outlier pattern. These results demonstrate

that in this case, as can be expected by the correlations found between the extractable fraction of copper and arsenic and the respective total contents in soils, the content of these elements in alfalfa correlated with both concentrations. A different behavior was observed for antimony, especially for the northern samples; where better correlations were obtained between the total concentration in alfalfa and the total concentration in soils than with the extractable fraction. This fact can be probable due to the little variation of antimony in alfalfa plants and/or to the low extractability of antimony compounds by EDTA solution.

It is important to remark the different behavior on the uptake of copper, a metallic element, and the metalloid arsenic from soil collected in different regions. Comparing the slope presented in Table 6 and in Fig. 4A and B, it can be observed that the uptake of copper for alfalfa plants in the north region was different from those from the central region. This fact showed that copper compounds in the northern soils are more effectively absorbed by alfalfa plants. On the contrary, the absorption of arsenic was lesser effective in alfalfa from the

Table 6 Relationships between the total concentrations of copper, arsenic and antimony in alfalfa plants and the respective total concentrations in agricultural soils from Chile

Element	Relationships $Y = \text{concentration in alfalfa}$, $X = \text{concentration in soil/mg kg}^{-1}$	
	North region	Central region
Copper	$Y = 0.184X + 4.483$ $R^2 = 0.77$	$Y = 0.129X + 3.906$ $R^2 = 0.87$
Arsenic	$Y = 0.028X + 0.070$ $R^2 = 0.96$	$Y = 0.112X - 0.850$ $R^2 = 0.70$
Antimony	$Y = 0.010X + 0.030$ $R^2 = 0.92$	$Y = 0.063X - 0.083$ $R^2 = 0.70$

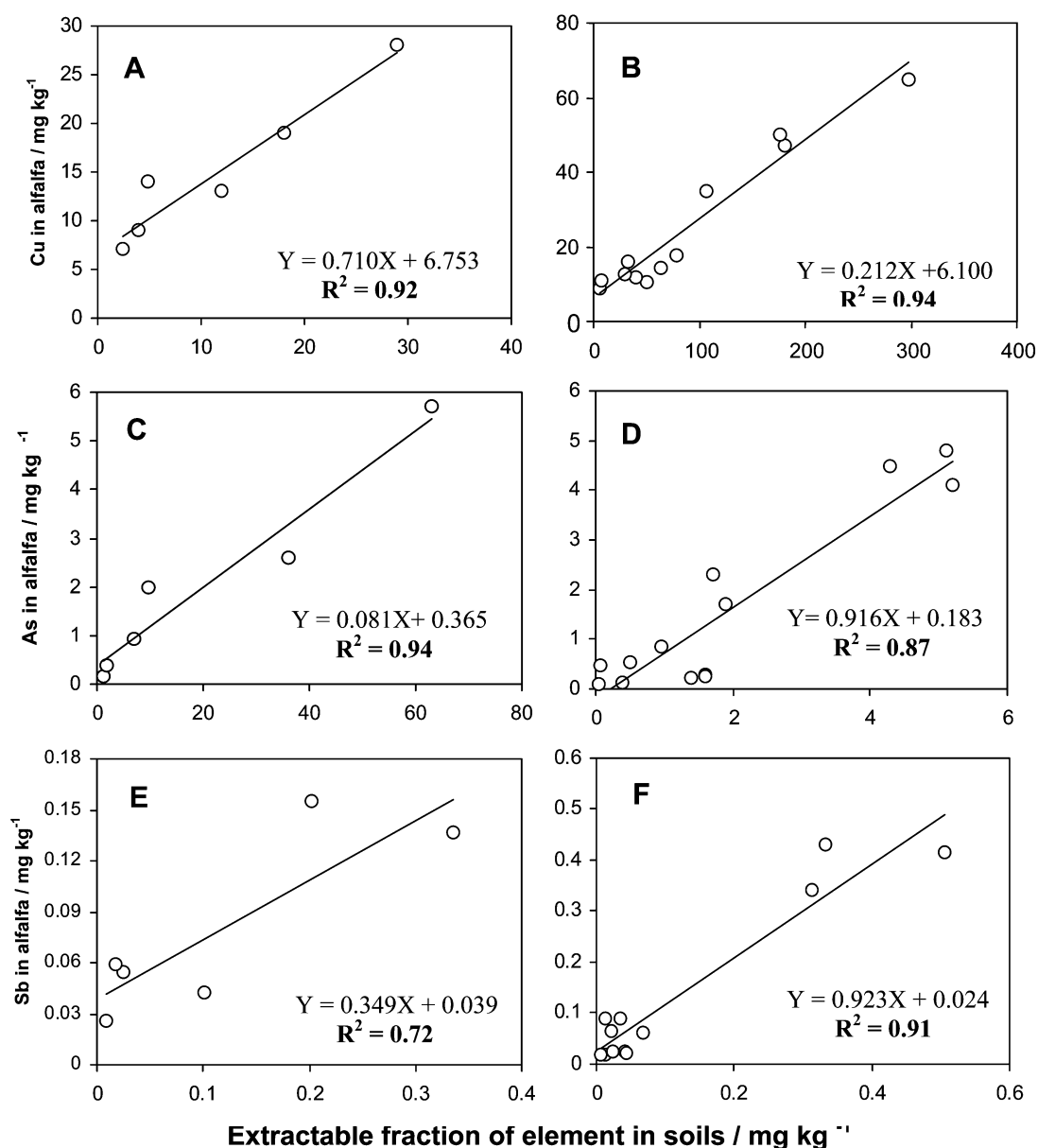


Fig. 4 Relationships between the total concentrations of copper, arsenic and antimony in alfalfa plants and the respective concentrations in the EDTA agricultural soil extracts. A, C and E plots for samples from the north regions. B, D and F plots for samples from Valparaiso region.

north region than from the central region. A similar behavior was observed for antimony.

On the basis of these results, individual transfer factors of the extractable fraction of elements in soil to the respective alfalfa plants, defined as the ratio between the concentration in alfalfa plants and the respective concentration in the EDTA soil extracts, were evaluated (see Table 7). As can be observed, transfer factors of copper in soils from the north were higher than those from the central region. This fact showed that the uptake of copper by alfalfa plants from the north is more efficient, due probably to the particular climate of this extreme arid zone. Since both temperature and light influence plant growth, one can explain the effect of these two parameters on the copper uptake as a combination of effects caused by favorable growth and direct effect of high temperature and light of these regions in Chile.²⁴

With respect to the metalloids it is interesting to observe the different behavior presented in the north by arsenic compared to antimony. While the fraction of arsenic extracted by EDTA solution in these soils was largely higher than antimony, arsenic presented the lowest transfer factors. This fact shows that although arsenic presents a high mobility in these soils only a

limited fraction is transferred to alfalfa plants. This last phenomenon is favorable when taking into account that alfalfa is one of the principal forms of forage to animals that are consumed by humans, decreasing the possibility of arsenic intake by this route.

When a similar and comparative analysis is performed for the transfer factor in samples from the affected valleys, in the central zone, several remarks can be made. The transfer factors for copper and arsenic in these samples have significantly different values to those obtained in samples from the north region. Furthermore, while the copper factors decrease, the arsenic factors increase. Taking into account the high mobility of copper in these contaminated soils and the significant correlation between copper in alfalfa plants and soils, a high transfer factor could be expected for this element. However, results clearly showed the poor copper uptake by alfalfa, demonstrating that alfalfa plants grown in these soils act as non-accumulators of this element.

In spite of the high arsenic concentrations in the northern EDTA soil extracts, the transfer factors were very low (transfer factor means 0.14) compared to those obtained for samples from Puchuncaví and Catemu valleys (transfer factor means $\cong 1$).

Table 7 Transfer factors (concentration in alfalfa/concentration in EDTA soil extract) of copper, arsenic and antimony in soils to alfalfa plants

SITE	Copper	Arsenic	Antimony
T1	2.25	0.13	2.16
T2	2.92	0.12	2.89
T3	1.06	0.22	0.42
A1	0.97	0.21	3.47
A2	1.08	0.07	0.57
A3	2.96	0.09	0.41
Transfer factor means \pm sd	1.9 \pm 0.9	0.14 \pm 0.06	1.7 \pm 1.4
P1	0.22	0.94	0.82
P2	0.26	1.05	1.09
P3	0.87 ^a	2.63 ^a	4.17 ^a
P4	0.28	0.79	1.29
P5	0.20	0.98	2.95
C1	0.23	0.91	0.59
C2	0.33	0.89	0.91
C3	1.31 ^a	1.35	2.44
C4	0.30	0.19 ^a	6.29 ^a
Transfer factor means \pm sd	0.26 \pm 0.05	0.9 \pm 0.3	1.4 \pm 0.9
Q1	0.23	0.34	1.43
Q2	0.45	0.16	0.50
Q3	0.48	0.16	1.00
Transfer factor means \pm sd	0.4 \pm 0.1	0.2 \pm 0.1	1.0 \pm 0.5
CB	1.40	7.23	—
LT	1.62	2.78	2.43

^a Values excluded in the means.

This fact shows the great uptake of arsenic by the edible tissues of alfalfa plants grown in these contaminated sites. Low transfer factors of arsenic were presented by samples from agricultural sites in the Aconcagua valley (Q1–Q3), showing low absorption of extractable arsenic compounds by alfalfa plants growing in these sites. Although the total arsenic concentrations were relatively high, only a proportion of this metalloid was translocated to the edible part of alfalfa.

Finally, results obtained for antimony and arsenic showed the different behavior presented by these metalloids in the agricultural ecosystems considered in this study, due probably to the presence of different compounds or chemical characteristics presented in these soils. While the EDTA extractable fraction of arsenic ranged from 1 to 34% of the total contents, the fractions of antimony ranged only from 1 to 8%.

Conclusions

The physico-chemical characteristics determined showed that the northern soils are neutral or slightly alkaline, sandy-loam, with low organic matter and iron contents. Those of the central region are in general both sandy-loam and loamy-sand and have approximately two-fold greater iron content and similar organic matter content.

Results demonstrated that the fraction of copper and arsenic extracted by EDTA solution, from the agricultural soils considered was a function of their respective total concentrations. However, in the case of antimony this relationship was not so clear. Copper presented high mobility in all soils, especially in those from contaminated sites, in the central region. The copper concentrations extracted by EDTA solution in most soils from Puchuncaví and Catemu valleys were higher than the proposal for maximal acceptable concentration for this element, that is considered as phytotoxic in agricultural soils. On the other hand, the northern soils presented the highest extractable fraction of arsenic. Antimony was the element less extracted in all soils. Similar copper concentrations were found in alfalfa from the north region and from non-contaminated sites from central Chile. Although the copper concentrations in alfalfa are widely ranged, most samples contained levels between 7.1 and 14.3 mg Cu kg⁻¹, values

fitting the normal range reported for herbaceous plants (5–20 mg kg⁻¹). Alfalfa samples from Antofagasta region, Puchuncaví and Catemu valleys have the highest arsenic and antimony concentrations, reflecting natural and/or anthropogenic contamination by both elements. However, in these zones only in alfalfa from Campiche, did arsenic and antimony exceed the average normal concentration in herbaceous vegetables. Significant correlations were obtained between the total content of copper, arsenic and antimony in soils and the respective total content in alfalfa plants. Better correlations were evaluated when the extractable fraction of these elements were considered, except for antimony in the northern samples. Comparatively, the northern samples presented the highest copper transfer factor, showing that alfalfa plants from this region are more efficient uptakers of this element than those cultivated in contaminated sites in the central zone. On the contrary, in spite of the high extraction efficiency of arsenic by EDTA solution in the northern soils, the lowest transfer factors were obtained for these samples. For antimony, a clear trend in the transfer factors was not observed. Finally, other studies are needed on the fractionation of copper, arsenic and antimony in these soils to identify the soil fraction to which these elements are mostly associated. Speciation analysis of arsenic and antimony in the extractable fraction of soils and in alfalfa extracts are also considered with the aim of identifying the species of these elements and their distribution in these matrices and to demonstrate which of them are better taken up and/or metabolized by the alfalfa plants.

Acknowledgements

The authors gratefully acknowledge the financial support of Fondecyt through the research project number 1000283 and the VRIEA, Pontificia Universidad Católica de Valparaíso (project 125.723). The Program ECOS-CONICYT (Scientific Cooperation between France and Chile), through the Action C01E010 is also gratefully acknowledged.

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