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BIOAVAILABILITY, BIOACCESSIBILITY AND SPECIATION OF ARSENIC AND OTHER HEAVY METAL ELEMENTS IN CONTAMINATED AREAS OF CHILE

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Abstract: Studies on the bioavailability of As, Cr, Cu, Pb, Mn and Cd of impacted soils; the As bioaccessibility in the edible parts of carrots, beets and quinoa growing in these polluted soils thought "*in vitro*" gastrointestinal process; the As speciation both in the edible parts of vegetables and in their gastrointestinal extracts have been performed. Elemental analysis and As speciation has been performed by ICP-MS and HG-AAS; and LC-ICP-MS, respectively. The high As contents in the interchangeable and oxidized fractions of soil may be responsible for the high As species content in these high consume vegetables. Arsenic recovery after the *in vitro* gastrointestinal digestion was of 98, 90 and 40% for carrots, beets and quinoa, respectively; with no significant transformation of original As species. These studies provide a clearer understanding of the impact that As and other contaminant elements may present in the population of this high polluted Chilean region.

Keywords: arsenic, heavy metals, bioaccessibility, bioavailability, soils, carrots (*daucus carota*), beets (*beta vulgaris*), quinoa (*chenopodium*), *in vitro* gastrointestinal digestion, As speciation, ICP-MS

INTRODUCTION

Since approximately 1915, the Northern Chilean economy has been mainly supported by the exploitation of copper mining resources around the Atacama Desert. This mining activity produces heavy metals contamination of the water and aquifers of the Loa river basin and its tributaries such as the San Pedro, Salado and San Salvador rivers [1,2].

Associated with copper and the other heavy metals from the mines, is arsenic, a very dangerous environmental element. This As is present in the enargite (Cu_3AsS_4), one of the sulphur minerals of the mines. In this area, there are also copper mineral smelting plants and during the pyrometallurgical process, the As is released as As_2O_3 both in the gas phase and in fine particles [3,4]. It is believed that suspended dust particles and intake through the drinking water are the major sources of As exposure for living beings in the area. The concentration of As in the water of the Loa river's hydrography basin is very different depending on place, and changes from no contamination in the Copiapo river (snow melting, concentration <0.005 mg L^{-1}) to extreme contamination in the San Salvador river (2-2.5 mg L^{-1}) [5].

Between 1958 to 1970, the Antofagasta population had drinking water from the Loa river with a total As concentration of about 1000 μg L^{-1} (most probably in the form of As(V)) [6]. Successive water treatment has been decreasing the water concentration to an actual annual average of 50 μg L^{-1}, but not in all places. This is far from the WHO's recommended amount of 10 μg L^{-1} [7]. A study in 1992, showed the contamination of As in soil, water, and vegetables growing in the Antofagasta region [8,9].

Therefore, natural geographic contamination, anthropological action, the zone's desert climate, and the exposure of vegetables, animal and human being communities to these extremes in environmental conditions, makes for a very delicate ecological equilibrium similar to what can be seen in other well-known parts of the world such as India, China, New Zealand, etc.[10,11].Cancerous and non-cancerous diseases associated with As contamination reaches alarming levels in the Antofagasta region, as evidenced by the incidence of cardiovascular [6,12] bronchial lung, bladder diseases and renal cancer [13].

In addition to As, the characteristics of the mines themselves result in high concentrations of other heavy metals, which also have a high environmental and human impact. Indeed, the

possible contaminating elements in the region (Cu, Cr, Mn, Hg, Pb and Cd), are included on the list of priority toxic pollutants published by the World Health Organization (WHO) and the International Register of Potentially Toxic Chemicals (IRPTC) [14].

The environmental world legislation relating to soils and sediments contaminated with heavy metals is mainly based on an estimation of the total metal content using concentrated nitric, fluoride or *aqua regia* as main acids for sample mineralization. However, the total content is not useful in predicting environmental impact because of the full amount of the element is not available or cannot be mobilized. Current sequential extraction schemes able to distinguish between exchangeable, reduced and oxidized fractions, provides a better overview of the actual environmental impact of elements. Most of the commonly used sequential extraction systems are those developed by Tessier and the Community Bureau of Reference (now Standards, Measurements and Testing Program, SM&T) [15]. Such sequential schemes are currently used together with advanced analytical techniques for chemical extraction such as ultrasound probes, microwave extraction, accelerated solvent-based extraction etc., and multi-element analytical methods of detection such as ICP-OES or ICP-MS [16].

The evaluation of food from the point of view of arsenic and trace element contribution to human diet requires knowledge not only of the total element content but also their absorption rates in the gastrointestinal tract. A way to quantify the absorbable fraction in trace element bioaccessibility is the "*in vitro*" simulation of digestion processes [17].

It is also recognized that inorganic arsenic are probably the most dangerous forms of arsenic in food, being As (III) more toxic than As(V) [18]. The formation of As (III) from the As(V) species present in natural environmental conditions, obey in some case to an a initial reduction before the methylation or other bigger species formation (arsenobetain, arsenosugar, etc.), in the detoxification mechanism that happens in microorganisms and also in more complex living beings [19-21]. The methylation process first produces monomethylarsonic acid (MMA) and in a second step dimethylarsinic acid (DMA) both less toxic than inorganic forms because their interaction with tissues is weaker [22].

There have been several reports of arsenic speciation in vegetables growing in natural or contaminated soils [23]. According Johnson et al [24], rice contains the highest concentrations of inorganic arsenic species compared with other products tested. Broccoli, lettuce, potato, carrots, etc. can concentrate arsenic when the soil or the irrigation water contains As (V). In most of the vegetables, arsenic is taken up by plant roots via macro-nutrient transporters [25, 26]. The coupling of the liquid chromatography (LC) to high sensitive detectors like ICP-MS,HG-AAS and CV-AFS, are the most frequent techniques for the detection and quantification of these inorganic and methylated As species at trace level after their smooth extraction from the samples [27].

Our study on bioavailability, bioaccessibility, As speciation and heavy metal contamination has been centralized on a small area in Northern Chile: the impacted Chiu - Chiu region, located approximately 10 km from Calama, and 235 Km from Antofagasta. In this study, we have addressed: i) the bioavailability of the As and other heavy metals such as Cr, Cu, Pb, Mn and Cd contained in impacted soil and sediments through labile, reduced, oxidized and residual fractions, ii) the total As and heavy metals content in the edible and non edible parts of vegetables growing in the area where soil

samples were taken, and where the indigenous population live, iii) the bioaccessibility of the total As in the edible parts of these vegetables under "*in vitro*" reproduction of gastrointestinal process, iv) the As speciation in the edible parts of vegetables, and finally, v) As speciation in the "*in vitro*" gastrointestinal extracts of vegetables. These studies provide us with clearer understanding of the impact that arsenic and other contaminant elements have in the region.

SAMPLE TREATMENT FOR DETERMINATION OF THE TOTAL AS, CR, CU, MN, PB AND CD CONTENT IN SOIL, SEDIMENT AND VEGETABLE SAMPLES

Chilean samples were collected from the Chiu Chiu area (Chile). About 10 Kg of soils or sediment were taken randomly following the established protocol [29]. They were taken at a depth of 5-7 cm, dried, sieved in the place to a grain size of 1.25 mm to obtain homogeneous sub-samples. Samples were sent to the laboratory in polycarbonate bags at ambient temperatures (about 20ºC). About 10 Kg of carrots (*daucus carota*), beets (*beta vulgaris*) and quinoa (chenopodium) were taken from the Chiu Chiu area (Chile) and about 2 Kg of the same vegetables from the Madrid (Spain) market place. The samples were sent fresh from origin to laboratory maintained under N_2 gas.

Pretreatments for soils, sediment and vegetables: For soils and sediment, no other pre-treatment than dry at 80ºC to constant weigh was performed before the determination and for the sequential extraction protocol. Carrots and beets were peeled with plastic knife and the edible and not edible part was blended in a titanium blender until a homogeneous mush was achieved. A fraction of about 2 Kg of flesh or skin were lyophilized, bottled and maintained at 4ºC until analysis. The rest of the fresh part was maintained fresh at minus 18ºC. No other treatment apart of maintaining at 4ºC was performed in the quinoa.

Attack of soil, sediment and vegetables for ICP-MS determination of As and Cd, Cu, Mn, Pb and Cr : 0.5 g of dried soil or sediment and lyophilized vegetables, were digested in PTFE vessels adding 5 mL of concentrated HNO_3 and 2 mL of $H_2O_{2..}$ Sediment reference material (PACS-2 NRC-CNRC), Rice Flour 1568ª NIST), White Cabbage (BCR 679) and tomato Leaves (NIST 1573a) were used.

The mixture was maintained about 4 hours in open air until vapor stoppage. Table 1 show the microwave programme applied subsequently [30, 31]. The digested samples were filtered, transferred to polyethylene containers diluted with 0.2 M HCl to an appropriate volumes and stored at 4ºC before analysis. The solid residue was discarded. Method validation was performed by triplicate with the before mentioned CRMs. Reagent blank did not show any significant contamination. The analytical characteristics of the method were as follows: detection limits (DL) in µg Kg^{-1}. As=5; Cd=0.03; Cu=0.1; Pb=0.08; Mn=2,7; Cr=2. Precision= 2-6% depending on the element.

Samples	Steps	Power (W)	Time (min.)	Temperature (ºC)	Pressure (psi)
Soil and sediments	1	800	20	100	150
	2	800	20	150	150
	3	800	20	200	150
Vegetables	Unique	600	15	200	150

Table 1, Microwave programme for soil, sediments and vegetables mineralization

Sample mineralization for As determination in vegetables by HG-AAS: About 0.5–1.0 g of sample was attacked in the PTFE reactor with 10 mL of concentrated HNO_3, 2 mL of concentrated $HClO_4$ and 2

mL of 2% m/v $Na_2S_2O_8$. Samples were pre-digested overnight at room temperature and the reactor bombs heated to 150ºC for 2 hours in a refractory oven. After cooling, 0.5 mL of concentrated H_2SO_4 was added, and the digested samples heated in an aluminium heating plate from ambient to 300ºC by semi-refluxing into a 50 mL glass Erlenmeyer flask for 2 hours until the final volume was 2 mL approximately. The digested samples were diluted to 10 or 25 mL with 0.5 M HCl. The analytical characteristics of the method are were as follows:DL As 7 μg Kg^{-1} and a precision of 4%.

SELECTIVE EXTRACTION PROCEDURES FOR AS, CR, CU, MN, PB AND CD IN SOILS AND SEDIMENT. BIOAVAILABILITY STUDIES BY ICP-MS

First step: 0.5 g of soil or sediment was extracted with 7.5 ml of 0.1M acetic acid, shaking for 16 h in a shaker at 40 rpm at ambient temperature. The first extract and solid phase were separated by centrifugation at 4.000 rpm for 30 min. The solid phase was washed with Milli-Q water. The washing water was pooled together with the first extract. This fraction is composed mainly of the exchangeable, labile and bound to carbonate elements.

Second step*:* The metals bounded to oxides (mainly Mn and Fe oxides) in the residue after the first step, was extracted with 7.5 ml of 0.5 M hydroxylamine hydrochloride, at pH 2 with HNO_3 (60%). The mixture was separated as in the first step. This is called the reduced fraction.

Third step: For the extraction of metals bounded mainly to organic matter and sulphides residues from the second step, it was treated in Teflon reactors with 6 ml of 8.8 M H_2O_2, and maintained for about one hour at ambient temperature in an open reactor. After that, the reactor was heated open air in water bath to reduce the volume up to about 1 mL. A second portion of 6 mL $H_2O_2$was added and the volume was once again reduced up to 1 ml, 10 mL of 2M ammonium acetate was added to the reactor maintained in cool conditions. The liquid and solid phases were separated as in precedent steps, being the liquid phase the oxidized fraction [15].

After convenient dilution, the total content of metals on each liquid fraction was determined by ICP-MS. The metal portion not dissolved, called the residual phase, was obtained from the difference between the total content after attack and the sum of the content in the three liquid fractions. The same procedure was applied to PACS-2. Reagent blanks showed not representative contamination for these elements.

"*IN VITRO*" GASTROINTESTINAL DIGESTION OF VEGETABLES FOR ARSENIC BIOACCESSIBILITY BY ICP-MS AND HG-AAS

SAMPLES WERE DIGESTED FOLLOWING A SIMULATED DIGESTION PROCESS WITH THREE STEPS.

Salivary digestion: About 1.0 g of lyophilized sample was placed in a 25 mL Erlenmeyer with 5 mL of salivary juice and 2 mL water and shacked for 1 min for degassing. The mix was heated for 5 min (37-39º C), shacked 5 min, heated again for 5 min and centrifuged at 14000 g during 20 min to separate salivary and solid phases.

Gastric digestion: The solid phase from first step was heated with 10 mL of the prepared gastric juice, heated for 1 h at 36-39 ºC, shacked for 30 min at 100 rpm, adducting the pH to 1.8 with HCl, and heated for 3 h more at 36-39ºC. The mixture was centrifuged at 14000 g for 20 min to separate the soluble

fraction from the solid phase.

Intestinal digestion: 10 mL of the artificial intestinal juice was added to the solid fraction obtained before and the procedure was repeated as in the gastric digestion. The residue was discarded [17].

The different extracts obtained were appropriate diluted for ICP-MS or HGAAS analysis after mineralization of the fractions as previously described.

EXTRACTION PROCEDURES FOR AS(III), AS(V), MMA AND DMA SPECIATION BY LC-ICP-MS AND HG-AAS (FIRST ORDER TECHNIQUE) IN VEGETABLES

Procedure1 for LC-ICPMS: *H_2O: methanol extraction.* To 0.5 g of the fresh homogeneous mesh of edible part of vegetables, 5 mL of methanol: water (1:1) solution was added. The mixture was heated for 30 min, at 55ºC, sonicated with a ultrasonic probe for 5 min at 30% power and centrifuged at 5000 rpm for 20 min at ambient temperature. The procedure was repeated in the solid fraction. The two liquid extracts were pooled and evaporated to a final volume of 2 mL.

Procedure 2 for LC-ICPMS: *Enzymatic extraction.* To 0.3 g of the fresh homogeneous mesh of edible part of vegetables, 10 mg alpha-amylase and 3 mL water was added. The mixture was sonicated with an ultrasonic probe for 60 s 30% power in a bath ice. After that, 30 mg protease was added, and the mixture was again sonicated with a ultrasonic probe for 120s at and centrifuged for 10 min at 4000 rpm. The solution was made up to 2 mL for analysis.

The two procedures were performed in triplicate. Table 2 shows the chromatographic conditions for As species separation.

PROCEDURE 3: AS SPECIATION BY HG-AAS A REFERENCE METHOD

(EPA, 1632A, 2001) [32].

Samples were digested in concentrated HCl at 80° C for 16 hours and made up with water to adequate volumes. An aliquot of the digest tissue was placed into a PTFE vessel, and 6M HCl was added. The $NaBH_4$ solution was added to convert inorganic arsenic (arsenite and arsenate) MMA, and DMA to volatile arsines. The trapped arsines in a cooled glass trap packed are thermally desorbed, in order of increasing boiling points, into an inert gas stream that carries them into the absorption spectrophotometer. To determine the concentration of As(III), another aliquot of digest tissue was placed in the reaction vessel and Tris-buffer is added. The concentration of As(V) is calculated by difference between the concentration of inorganic arsenic and As(III).

This methodology has been used for the validation of LC-ICPMS methodology

Procedure 1	Mobile Phase	$(NH_4)H_2PO_4$ (A: 5 mM; B:25 mM), pH 6
	Gradient mode	0-15 min (A: 100-0 y B: 0-100) 15-25 min (A: 0-100 and B: 100-0) Flow rate: 1 mL x min. Conditions: 25-30 min (A: 100, B::0)
Procedure 2	Mobile Phase	$(NH_4)H_2PO_4$ (10 mM), pH 6
	Isocratic	Flow rate 1 mL x min.
		Conditions: 25 min (A: 100)

Table 2: Chromatographic conditions for As speciation in the methanolic (procedure 1) and enzymatic (procedure 2) extracts of vegetables.

Statistical analysis: All experiments were performed at least in triplicate. When possible, results were obtained by two different techniques. Differences were considered significant when $p<0.05$ following the t Student´s test.

RESULTS

BIOAVAILABILITY STUDIES SELECTIVE EXTRACTION OF AS, CR, CU, MN, PB AND CD IN SOILS AND SEDIMENT.

Table 3, reports the metal concentration of the analyzed soils, sediment and the PACS-2 CRMs by the *pseudo total attack* in which the material is not completely dissolved. However, it is important to highlight that the portion of metals not dissolved by the HNO_3/H_2O_2 attack is not from the environmental point of view. As can be seen through the CRMs, the release of total Cu, Mn and Cr need drastic conditions using HF to liberate the whole metals content. Similar behavior could be expected in the analyzed soils and sediment.

Table 4 shows the ERL and ERM US NOAA`s sediment quality guidelines for some of our studied elements [33]. ERL represents chemical concentrations in mg Kg^{-1} dry weight below adverse biological effects were rarely observed (generally lower than 10%), while the ERM represents concentrations in mg Kg^{-1} dry weight over effects were more frequently observed (generally higher than 75%).

	Cd	Cu	Pb	As	Cr
ERL	1.2	34	47	8.2	81
ERM	9.6	270	218	70	370

Table 4: US NOAA´S ERL y ERM sediment quality guidelines (mg Kg^{-1})

Table 5 shows the total content of the elements As, Cd, Cu, Pb, Mn and Cr in flesh and peel of carrots, beets and quinoa from Chilean area and Spanish market, and the ratio of metal concentration in Chilean / Spanish samples.

Table 6 shows results for the white cabbage, rice flour 1568 and tomato leaves (NIST 1573a) CRMs, used to validate the total metals determination.

Table 7 shows the results of As after gastrointestinal digestion by ICP-MS (direct extract analysis) and by HG-AAS (after digestion of the extracts)

AS SPECIATION IN CARROTS, BEETS AND QUINOA

In the speciation studies, an important step is the extraction efficiency of the species present in the sample**.** Table 8 shows the total As concentration in the vegetables under study referred to fresh flesh and the extraction efficiency in the methanol water 1:1 extract and in the enzymatic extracts, by ICP-MS and HG-AAS procedures. As can be seen, methanolic extraction is appropriate for extraction of the total As species in carrots and quinoa, but not for beets, in which is necessary the enzymatic extraction.

Vegetables	Fresh sample (µg Kg^{-1})	Methanolic Extract (%)	Enzymatic Extract (%)
Carrots	63 ± 5(1) 70 ± 7(2)	94 ± 4(1) 97 ± 5(2) (45 ± 7) As(III) (43 ±5) As(V)	18 ± **4**(1) 12 ± 5(2)
Beets	74 ± 7(1) 81 ± 9(2)	0%(1) 10 ± 2(2)	93 ± 6(1) 95 ± 9(2). (20±5)% As(V))
Quinoa	176 ± 21(1) 171 ± 22(2)	104± 8(1) 99 ± 6(2) 15 ± 4) As(III) (82 ±9) As(V)	10.5 **± 0.8** 7.4 ± 1(2)

Table 8: Methanolic and enzymatic extraction efficient for both total As and As speciation by HG-AAS.

ICP-MS; (2) HG-AAS

Figure 3 (a-h) shows the chromatograms obtained for AsC, As(III), DMA, MMA and As(V) in a standard (5 ng mL^{-1}) and the species detected in the methanolic (for carrot and quinoa) and enzymatic (for beet) extracts by LC-ICP-MS. Extracts were appropriately diluted in each case. The species for beets, were characterized by spiking the sample with 5 µg L^{-1} of As(III) and As(V). For carrots, only

	Sediment	Soil 1	Soil 2	Soil 3	PACS-2 certificated	PACS-2 Experimental
As	44 ± 2	48 ± 1	97 ± 3	48 ±	26± 1	22 ± 2
Cd	0.5 ± 0.1	0,7± 0,1	4,0 ± 0,3	5,7 ± 0,2	2,1 ± 0.1	2.3 ± 0.1
Cu	20 ±1	60 ± 3	70 ± 6	51 ± 2	310 ± 12	248 ± 4
Cr	1.1± 0.1	1,8 ± 0,1	13,4 ± 0,9	17,0 ± 3,2	91 ± 5	64.5 ± 3.7
Mn	189 ±18	212 ± 2	234 ± 15	208 ±14	440 ± 19	262 ± 7
Pb	5.9 ± 0,7	12,7 ± 0,2	15 ± 2	22 ± 3	183 ± 8	188 ± 8

Table 3: Total concentration of As, Cr, Cu, Mn, Pb and Cd in three soils a sediment and the PACS-2 CRMs. (mg Kg^{-1})

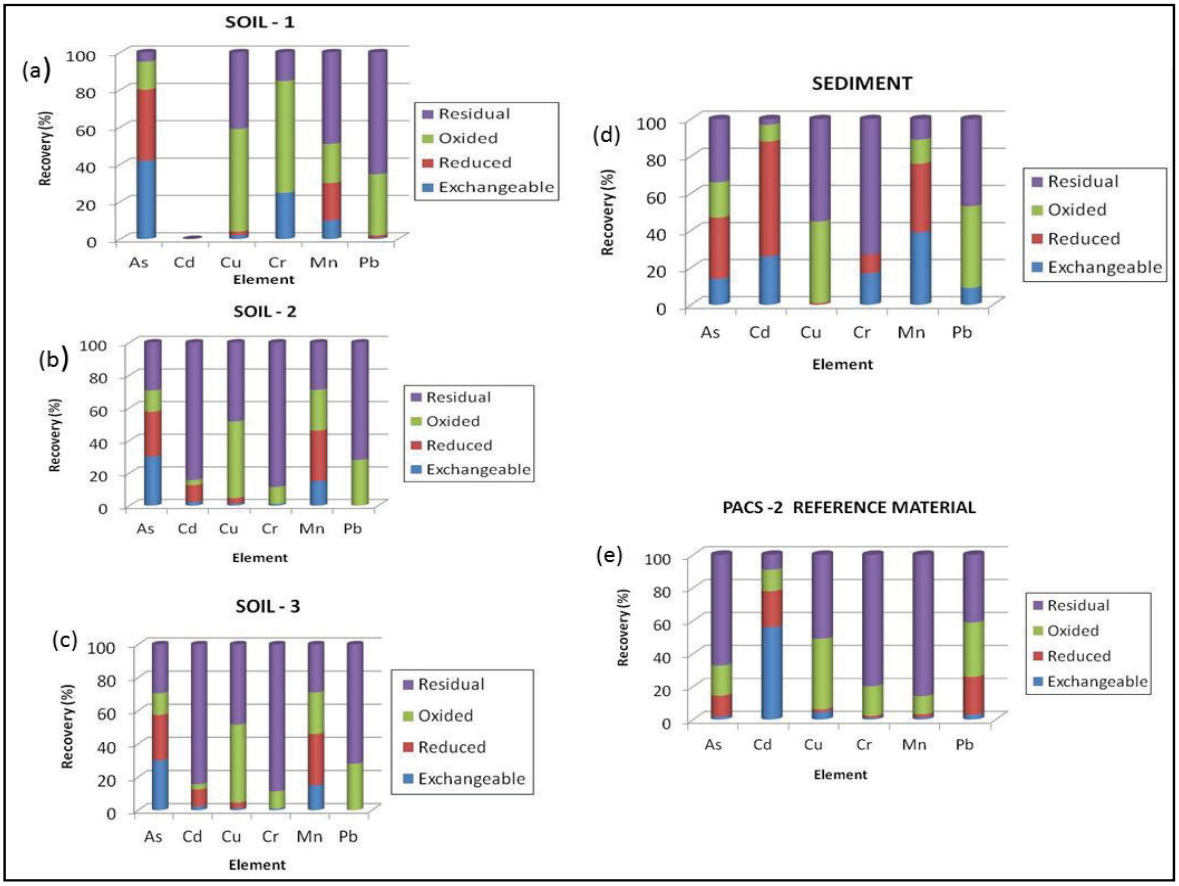

Figure 1 (a-e) shows the concentrations of these metals in the exchangeable, oxidized and reduced fractions.

Determination of total content and bioaccessibility of metals in carrots (*Daucus carota*) beets (*Beta vulgaris*) and quinoa *(Chenopodium quinoa)*

Vegetables		As	Cd	Pb	Cu	Mn
Carrots	Flesh-C Flesh-S Factor	0,52 ±0.04 (0,54± 0,06) 0,02 ± 0,01 **24**	0,05 ±0,01 0.07 ±0,0 1 **0.7**	0,12 ± 0,02 0.09 ± 0,01 **1.3**	7,75 ± 0,91 8,82 ± 0,59 **0.9**	4,75 ± 0,51 2,28 ± 0,18 **2**
	Peel-C Peel -S Factor	1,62 ±0,02 0,15 ± 0,01 **11**	0,08 ± 0.01 0,12 ± 0,02 **0.7**	0,21 ± 0.02 0,23 ± 0,03 **0.9**	16,3 ± 1,5 17,3 ± 0,9 **0.9**	9,95 ± 0,20 13,7 ± 0,9 **0.7**
Beets	Flesh-C Flesh-S Factor	0,62 ± 0.05 (0,64 ± 0,07) 0,02±0,00 **26**	0,09 ±0,00 0.05±0,00 **1.8**	0,36 ±0,01 <LD (0,006)	6,71 ± 0,95 3,82±2,1 **1,7**	8,38 ±0.16 3,84±0,16 **2,2**
	Peel-C Peel-S Factor	3,20±0,06 0,22±0,01 14	0,11 ± 0,01 0,03±0,01 3,6	0,31 ± 0,05 <LD (0,008)	28,6 ± 0,5 3,8±1,2 **7**	21,8 ± 0,2 11,1± 0,3 **2**
Quinoa –C Quinoa -S Factor		0,20 ± 0,02 (0,21 ± 0,04) 0.01± 0,01 **20**	0,38 ± 0,07 <LD	0,04 ± 0,02 0,09 ± 0,01 **0,4**	7,89 ± 0,73 1,14 ± 0,16 **7**	13,5 ± 2,4 4,11 ± 0,11 **3**

Table 5: Total As, Cd, Pb, Cu, Mn y Cr content in Chilean and Spanish lyophilized vegetables. Results expressed in mg Kg^{-1}

	Element	Certified value (mg Kg^{-1})	Experimental value (mg Kg^{-1})
White Cabbage BCR 679[(1)]	As	7,0 ± 3,8	8.5 ± 2,0
	Cd	1,66±0,07	1,72 ± 0,22
	Cu	2,89 ± 0,12	2,62 ± 0,24
		certified value (mg K^{-1})	experimental value (mg K^{-1})
Rice Flour 1568[a(1)]	As	0,29 ± 0,03	0,29 ± 0,04
	Cd	0,022±0,002	0,033±0,002
	Cu	2,4 ± 0,3	2,3±0,1
	Mn	20,0 ± 1,6	18,5±0,8
Tomato Leaves (NIST 1573a)[(2)]	As	0.112 ± 0.004	0,115 ± 0,006

Table 6: Comparison of CRMs values *versus* experimental values for some vegetables.

Analysis performed [(1)] By ICPMS. [(2)] By HG-AAS

		Carrots	Beets	Quinoa
Extracted As (μg Kg^{-1})	Salivary	313 ± 18[(1)] 320 ± 24[(2)]	289 ± 28[(1)] 295 ± 22[(2)]	34 ± 4[(1)] 52 ± 7[(2)]
	Gastric	135 ± 12[(1)] 140 ± 11[(2)]	186 ± 21[(1)] 191 ± 14[(2)]	31 ± 4[(1)] 29 ± 5[(2)]
	Intestinal	65 ± 9[(1)] 60 ± 13[(2)]	84 ± 10[(1)] 76 ± 12[(2)]	13 ± 6[(1)] 15 ± 4[(2)]
Total extracted As (μg K^{-1})		513 ± 45[(1)] 520 ± 35[(2)]	559 ± 38[(1)] 562 ± 25[(2)]	78 ± 15[(1)] 96 ± 18[(2)]
Total As (μg K^{-1})		520 ± 41[(1)] 536 ± 46[(2)]	616 ± 57[(1)] 638 ± 59[(2)]	196 ± 24[(1)] 209 ± 28[(2)]
Recovery (%)		98 ± 3[(1)] 97 ± 5[(2)]	90 ± 4[(1)] 88 ± 7[(2)]	40 ± 4[(1)] 46 ± 6[(2)]

Table 7: Bioaccessibility of As in the digestion process of Chilean samples by HG-AAS and ICP-MS. Results expressed in lyophilized vegetables.

n= 3. (1). ICP-MS; (2) HG-AAS

As(III) and As(V) are present. Relative peaks quantification, show about a 50% distribution between the two inorganic As. These results agree with results obtained by HG-AAS (see Table 8) and previous work [27]. For quinoa, 90% of the As is present as As(V), been the As(V) concentration of As(III) about 10 %. No other species are found in the methanolic fraction. Beets show a different behavior. As species present in this vegetable is only slightly extracted in H_2O-methanol but almost 100% in the enzymatic hydrolysis.

a)Chromatogram of standards As species: AsC, As(III), DMAs, MMAs, As(V), b) As species in carrots, c) As species in quinoa, d) As species in beet, e) As species in beet spiked with 5µg /L As (III) and As (V), f) As speciation in the salivary juice digestion of carrots, g) As speciation in the gastric juice after digestion of carrots, and h) As speciation in the intestinal juice after digestion of carrots

AS SPECIATION IN THE SALIVARY, GASTRIC AND INTESTINAL EXTRACTS FOR CARROTS AND QUINOA

In order to know if the digestion process could change the original species present in the food, changes in their bioaccessibility, it has been performed the speciation analysis in the salivary, gastric and intestinal juice of carrots and quinoa.

The analysis was performed by LC-ICPMS under the same conditions than performed in original samples. Figure 2 (g-h) shows the chromatograms obtained for carrots. Similar results were obtained in quinoa sample (chromatograms not shown).The percentages are given respect the whole content in the corresponding extract. In parallel, arsenic species were quantified by HG-AAS. Table 9 shows results.

Samples	Species	% Rec in salivary	% Rec. in gastric	% Rec. in intestinal
Beets	As(V)	31 ± 4	40 ± 6	10 ± 2
Quinoa	As(III)	60 ± 7	75 ± 7	15 ± 5
	As(V)	29 ± 4	23 ± 5	65 ± 8
Carrots	As(III)	55 ± 8	59 ± 8	8 ± 3
	As(V)	30 ± 4	28 ± 4	79 ± 6

Table 9: Recovery of As species respect the total As content in the extracts of salivary, gastric and intestinal digestion *EPA, 1632A, 2001*[32]

DISCUSSION

It has been accepted that selective sequential extraction procedures in soils and sediments, are the best way to determine the source of the contaminants and the capability of interaction of the elements with living organisms. Contaminants of anthropogenic origin are mainly extracted in the exchangeable, sulphides or organic matter fractions and contribute more to the toxicity because they can be easily removed by chemical or biological activity, while lithogenic metals are mainly content in the residual fraction.

Pseudo total concentrations of most of the analyzed elements in the soils and sediment of the Chiu Chiu area are in general, between the ERL y ERM values (Table 4), and therefore for these elements could exist toxicological and environmental effects. However, the effects are mainly associated with the bioavailability. From the bioavailability studies (Figure 1) can be seen that As is present in all fractions probably due to a big diversity of sources.

When vegetables grow in contaminated soils, the bioavailable part of the elements present in the soil can pass to the vegetable through intake mechanisms in which frequently are involved the microorganisms present in the soil, and the proper structure of the vegetal [34,35]. Results obtained considering the soils bioavailability and the concentration found in the edible (and not

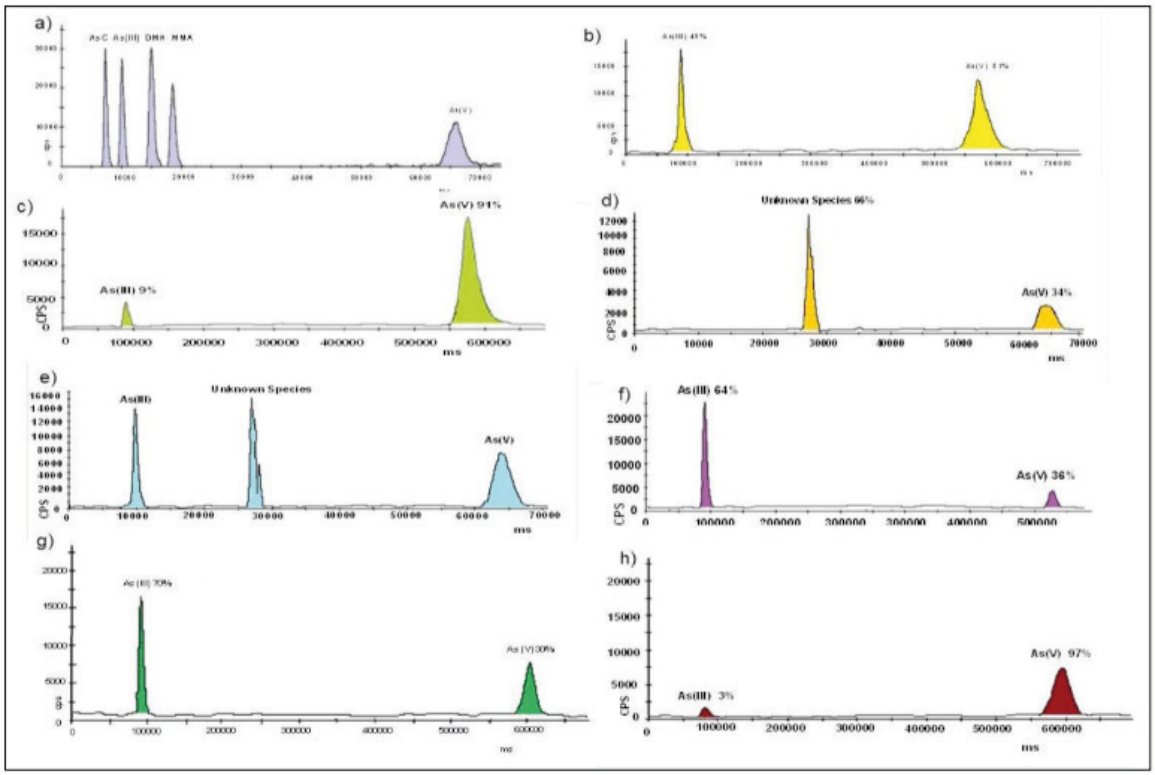

Figure 2: Chromatograms obtained in the methanolic and enzymatic extracts by LC-ICP-MS

edible) part of the Chilean vegetables, show the following: In soils, most of the Cd, Pb and Cr are under reducible and residual forms, and therefore very little transport to vegetables can be expected. This assumption seems to be corroborated by the low content of these elements in the Chilean vegetables. The most important part of Cu is also in the oxidized and residual fractions of soil, but some contamination can be detected in Chilean vegetables in comparison with Spanish one, probably due to the uptake from oxidized and interchangeable fractions of the soils. The Mn shows also some preconcentration in vegetables. Finally, the high content of As in the interchangeable and oxidized fractions, higher than 40% in soil, can be responsible for the high contamination of As in the three vegetable tested in comparison with the Spanish one. The Spanish samples are in the level reported for plants growing in non-contaminated soils [36, 37]. It is relevant that only As(III) and As(V) are present in carrots and quinoa (Figure 2 and Table 9). This is not the case of beets, were As(V) and mainly un unknown As species partially overlapped (Figure2) are presented. Sucrose is the sugar extracted from the beets; therefore, an As-sucrose or derivatives could be the unknown species. Considering that inorganic species are the most toxic ones, beet could be the vegetable with the lower concern [38]. It is known than the terrestrial plants in general have very low mechanisms of biometilation [39]. Probably also the drastic conditions from the environmental point of view of the soil make difficult that the microorganisms present in the soil, could develop biometilation mechanisms giving methylated species able to be absorbed through the roots as proposed by

Ye and col. [40].

It is well known than the greatest contribution to contaminant (and also nutrient) an element to the organism comes from consumption of food and water. This happens also for arsenic. As can be seen, the "*in vitro*" digestion process carried out in the studied vegetables liberates the whole As from carrots and beets (Table 7), and therefore this vegetables intake could be dangerous if concentration in the samples is high enough to be a toxic doses. Quinoa behavior is different and only about 40% of As is liberated. For the three vegetables, about 50-60% of the total concentration extracted is released in the salivary juice. It is important to highlight that for beets and carrots, results about total concentration of As, and the sum of the bioaccessibility fractions of As under the digestion process are very similar. However, the quinoa behavior is different and only is recovered after the whole digestion process about a 40%. Fresh carrots and beets have a water content of about 85%, while quinoa is only of about 10%. Concentration of As referred to fresh vegetables shows that quinoa preconcentration about 3 times more As that the others, but its bioaccessibility is about half of the carrots and beets, therefore not mayor concern than carrots and beets can be expected for this vegetable considering a similar intake.

Knowledge of oral bioaccessibility of a contaminant is useful for estimating potential human health risks. However, more important is to know the species under which the contaminant is liberate to bloodstream. Figure 2 and Table 9 show that not significant transformation of As species after "in vitro" gastrointestinal digestion occurs in comparison to the original species. As(III) is extracted mainly in the two first extracts and As(V) mainly in the last for quinoa and carrots. Previous studies of Calatayud M et al. [41] show the transformation of As(V) to As(III) after gastrointestinal digest when other vegetables, different to that of this study, are contamined with As(V) after soaking or boiling.

Although some others studies can be found about the determination of As in soils and vegetable in the II Chilean Region, the diversity of the places where different level of contamination can be found, the different sources of contamination and the different As water concentration depending on the river and the section, made difficult to compare our results with the content in the Chilean bibliography. A precedent work performed by us ten years ago, showed a higher concentration of As in carrots growing in the same area [42]. The studies of Ferreccio C. et al. [43] reports for soil in the region concentrations of As slightly higher than reported in this study.

CONCLUSION

From the bioavailability studies performed for As, Cu, Cd, Mn and Cr in the soils of the Chiu-Chiu area, can be concluded that As has a really high content in the labile or exchangeable fraction of the three analyzed soils, and therefore can be taken by the vegetables growing in the soils. Cu and Mn are also present in relatively high concentrations.

The "*in vitro*" digestion process has shown that As species and As bioaccessibility in the carrots, beets and quinoa growing in the same soil contaminated by the element differ between vegetables. Inorganic As (III) and As (V) are present in carrots and quinoa. Unknown species are the main components of As in beets, probably arsenosugars derivatives. Bioaccessibility of As in carrots and beets was almost, 100%, while for quinoa was about 40%. Considering the maximum intake of 2.5 Kg per year of quinoa and of 6 Kg per year of carrots and beets; the As concentration in the lyophilized vegetables; the water content

of 10% in the quinoa and 85% en beets and carrots, the As bioaccessibility obtained and the main As species present in the vegetables, the accessible doses of As is about 470 µg per year for carrots, about 550 µg per year for beets and about 180 µg per year for quinoa. Therefore, quinoa seems to be the vegetable with lower toxicological implication. The As speciation in the salivary, gastric and intestinal extracts of carrots showed that no significant transformation of As species after "*in vitro*" gastrointestinal digestion occurs. As (III) is mainly found in the salivary and gastric extracts and As (V) mainly in intestinal extract for the tested vegetables. More research is necessary to determine the species present in the beets where identification is limited by both the availability of standards and the complexity of the matrix.

Although some others studies can be found about the determination of As in soils and vegetable in the II Chilean Region, the diversity of the places where different level of contamination can be found, the different sources of contamination and the different As water concentration depending on the river and the section, made difficult to compare our results with the content in the Chilean bibliography. A precedent work performed by us ten years ago, showed a higher concentration of As in carrots growing in the same area [42]. The studies of Ferreccio C. et al. [43] reports for soil in the region concentrations of As slightly higher than reported in this study.

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