Bioavailability, Bioaccesibility of Heavy Metal Elements and Speciation of as 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 bioaccesibility 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 have been performed by ICP-MS 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 vegetables consumption. The Arsenic recovery after the *in vitro* gastrointestinal digestion was from 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.

Introduction

Since 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 *enargita* (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 hydrographic 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 and 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 reach 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) [7] 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 are able to distinguish between exchangeable, reduced and oxidized fractions; it 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].

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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, obeys in some case to an 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 tested products. 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 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].

The objectives of this study have been centralized on the polluted Chiu region located Northern Chile that is about 10 Km from Calama and 235 Km from Antofagasta city. The study performed which addressed the following points: i) The *pseudo*-total content and bioavailability of As and other heavy metals such as Cr, Cu, Pb, Mn and Cd present in impacted soil and sediments by the selective extraction for the determination of its content in the exchangeable, oxidized, reduced, and residual fractions. ii) The total As, Cr, Cu, Pb, Mn and Cd content in the edible parts of vegetables growing in the area where the above mentioned soil samples were located, and where the indigenous population live. iii) The As species present in the edible parts of lyophilized vegetables. iv) The bioaccesibility of the total As in the edible parts of these vegetables under *"in vitro"* reproduction of gastrointestinal process and finally. v) The possible transformation of original As species during the gastrointestinal process.

As far as we know, such global studies have not been performed yet in the area under investigation.

Materials and Methods

Instrumentation

An inductively coupled plasma mass spectrometer, (HP-4500, Agilen Technologies, Analytical System, Tokio, Japan) operating under normal multi-element tuning conditions was used for total arsenic and metals analysis. The main analytical parameters of the ICP-MS are the following (R.F. power: forward 1350 W; reflected 2.2 W; coolant argon flow rate 14 L min^{-1}; auxiliary argon flow rate 0.9 L min^{-1}; integration time 0.1 s per point, point per peak 3).

For As speciation the ICP-MS has been used as the detector system after LC species separation. The column effluent was directly introduced into a Meinhard-type concentration glass nebulizer and a double-pass Scott-type spray chamber with a surrounding water jacket maintained at 5°C. Single ion monitoring at m/z 75 was used to collect the data. For chromatographic separation, a high-pressure pump (LDC Division, Riviera Beach, Florida, USA) was used as a sample delivery system. Samples of 100 µL were introduced through a 0.45-µm nylon syringe filter into the injection valve Rheodyne 9125 (USA). The connections between the HPLC and the ICP-MS were made of polytetrafluoroethylene tubing (id. 0.5 mm). The HPLC column was a Hamilton PRP-X100 (10 µm, 250 mm × 4.1 mm, Torrance, CA, USA) and Phenomenex precolumn (25 × 2.3 mm 12-20 µm).

All signal quantification was performed in the peak area mode. The peaks were integrated using either ICP-MS Plasma Lab software or Grams/32 software (Galactic Industries, Salem, NY, USA). Sonopuls Ultrasonic Homogenizer (HD 2200 Bandelin Electronic Berlin, Germany) and a Selecta Vibromatic shaker were also used in the sample extraction process.

Arsenic species and heavy metals solutions: The Standard of As (III) of 1000 ± 2 mg L^{-1} was prepared in HNO$_3$ (2%, m/m), TraceCERT^{TM} Ultra (Fluka, Sigma-Aldrich, Steinheim, Germany). The standard of As (V) 1000 mg L^{-1}, was prepared in HNO$_3$ (2% m/m), CertiPUR (Merck, Darmstadt, Germany). Dimethylarsinic acid (DMA) and methylarsonic acid (MMA) standard of 1000 mg L^{-1}, were prepared from methyl disodium arseniate (Na$_2$CH$_3$AsO$_3$, 99% Supelco, Bellefonte, PA, USA)) and cacodilic sodium trihydro ((CH$_3$)$_2$AsNao$_2$.3H$_2$O) 98%, Fluka) TraceCERT^{TM}. The standards of cadmium copper, manganese and lead (1000 ± 2) µg mL^{-1} were from Fluka, Sigma-Aldrich, Steinheim, Germany. Standard solutions of cromium, (1000 ± 2) µg mL^{-1} was from Merck. These solutions were kept at 4°C in the dark. Working solutions were daily prepared and then diluted with water to the final concentration.

Reagents: All reagent were of analytical or reagent grade. HNO$_3$ 60% (Scharlau), HCl (15%) (Scharlau), HClO$_4$ (Scharlau), (acetic acid 99.8% (Panreac), hydroxylamine hydrochloride 99% (Carlo Erba), hydrogen peroxide 33% (Panreac), ammonium acetate 97% (Scharlau), ammonium phosphate 98% (Scharlau), alpha-amylase (Sigma Aldrich, Spain 11800 USP Units), pepsin (Sigma Aldrich, Spain, enzymatic activity 944 U/ mg protein), pancreatin (Sigma Aldrich, Spain, activity equivalent to 4 x US Pharmacopoeia specifications/mg pancreatin), lipase (Sigma Aldrich, Spain, 944 USP units), protease (Sigma Aldrich, Spain, 11800 USP units), bile extracts (glycine and taurine conjugates of hyodeoxycholic and other bile salts Sigma Aldrich, Spain). Deionized water (18.2 MΩ cm), obtained with a Milli-Q water system (Millipore, Inc., Spain) was used in the solutions preparation. Plastic and glassware was maintained in 10% HNO$_3$ for 24 h before use.

Simulated gastrointestinal juices: *Salivary juice* (pH 6.8 ± 0.1) was prepared by dissolving 0.15 g NaH$_2$PO$_4$. H$_2$O, 0.029 g amylase, 0.1 g KCl and 0.1 g NaCl and make up to 100 mL with water. *Gastric juice* (pH 1.8 ± 0.1) was prepared by ddissolving 6.0 g of pepsin and 0.88 g sodium chloride (Sigma) in water, adjusting pH 1.8 with HCl and making up to 100 mL with water**.** *Intestinal juice* was prepared just before use by mixing equal volumes of A and B solutions. (Solution A: 1.5 g pancreatin, 0.44 g sodium chloride, 30 mg alpha-amylase and water up to 50 mL. Solution B: 2 g bile salts, 0.44 g sodium chloride and water up to 50 mL) [28].

Sediment soils and vegetable samples: Chilean samples were collected from the 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 room temperature (about 20°C). About 10 Kg of carrots (*Daucus carota*), beets (*Beta vulgaris*) and quinoa (*Chenopodium*) were taken from the 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. The vegetables were cultivated in the mentioned soils and the sediment was taken from the deep Loa River where the water for watering the vegetables was taken.

Certified Reference Materials (CRMs): Sediment reference material (PACS-2 NRC-CNRC), Rice Flour (1568a NIST), White Cabbage (BCR 679) and tomato Leaves (NIST 1573a) were used.

Sample treatment for determination of the total As, Cr, Cu, Mn, Pb and Cd content in soil and lyophilized vegetable samples by ICP-MS analysis

For soils and sediment no pre-treatment other than drying at 80°C to constant weight was performed before the analysis. Carrots and beets were peeled with a plastic knife and the edible part was blended in a titanium blender until a homogeneous mash was achieved. A fraction of about 2 Kg of flesh were lyophilized, bottled and maintained at 4°C until analysis. No other treatment apart from drying at 80°C and maintaining it at 4°C was performed on the quinoa.

For mineralization of soil and vegetables about 0.5 g were digested in PTFE vessels adding 5 mL of concentrated HNO_3 and 2 mL of H_2O_2. The mixture was maintained for about 4 hours in open air until vapor stoppage before microwave digestion [30,31] (Table1). The digested samples were filtered, transferred to polyethylene containers diluted with 0.2 M HCl to appropriate volumes and stored at 4°C until analysis. The solid residue was discarded. Reagent blank did not show any significant contamination.

The analytical characteristics of the method were the following: detection limits (DL) in $\mu g\ Kg^{-1}$, As: 5.0; Cd: 0.03; Cu: 0.1; Pb: 0.08; Mn: 2,7; Cr: 2.0. Relative standard deviation (RSD) 2-6% depending on the element. Method validation was performed by triplicate with the above mentioned CRMs.

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, and shaken for 16 h in a shaker at 40 rpm at room temperature. The first extract and the 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 room 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 and 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 digestions of vegetables for arsenic bioaccesibility by ICP-MS

Samples were digested following a simulated digestion process with three steps (K) [28].

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 in a vibromatic Pselecta (508 U/min) for 5 min for degassing. The mix was heated for 5 min in a water bath (37-39°C), shacked 5 min, heated again for 5 min and centrifuged at 5000 rpm during 20 min to separate salivary and solid phases.

Gastric digestion: 10 mL of the prepared gastric juice, was added to the solid fraction after salivary digestion, heated for 1 h in a water bath, shacked for 30 min in the vibromatic Pselecta (508 U/min), adjusting the pH to 1.8 with HCl (15%), and heated 3 h more at 36-39°C. The mixture was centrifuged at 5000 rpm for 20 min to separate the soluble and solid fractions.

Intestinal digestion: 10 mL of the prepared intestinal juice was added to the solid fraction obtained before and the procedure was repeated as in the gastric digestion. The residue was discarded. The three liquid extracts after digestion were mineralized in a microwave oven (Table 1) and appropriately diluted before analysis.

Extraction procedures for As (III), As (V), MMA and DMA speciation by LC-ICP-MS in vegetables

Procedure 1 for LC-ICPMS: Methanol: water 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 55°C, and then sonicated with an 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 at 30% power in an ice bath. After that, 30 mg protease was added and the mixture was again sonicated for 120 s and centrifuged for 10 min at 4000 rpm. The solution was made up to 2 mL for analysis [32].

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

Samples	Steps	Power (W)	Time (min.)	Temperature (°C)	Presion (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.

Procedure 1	Mobile Phase	$(NH_4)H_2PO_4$ (A: 5 mM; B: 25 mM), pH 6
	Gradient	0-15 min (A: 100-0 y B: 0-100) 15-25 min (A: 0-100 and B: 100-0) Flow rate: 1 mL × min. Conditioning: 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 × min. Conditioning: 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. Differences were considered significant when $p<0.05$ following the t Student's test.

Results and Discussion

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 it 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%). Figures 1a-e shows the distribution of the tested elements in the three soils, sediment and Pack-2 reference material under study amongst the exchangeable, oxidized, reduced and residual fractions. It is really representative of the labile or exchangeable fraction of As; its presence in all fractions, is probably due to a wide sources of pollution. Cu is mainly present in the oxidized (associated with the sulphide mineral of the mines) and residual fraction. Cd is mainly present in the residual fraction, therefore it has a low bioavailability and only about 15% could be associated with Fe and Mn oxides. Mn, with high content in the mineral of the mine exploitation, is present in all fractions. Pb has an analogous behaviour than Cu and it is mainly present in the oxidized and residual fractions, but its total concentration is very low. Cr is mainly present in none the bioavailable fractions with a 70% of the total content.

It may conclude that among all the elements studied, As is the element of major concern because of its toxicity, its relatively high concentration in the analysed soils and also its high bioavailability.

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

Table 5 shows the total concentration of As, Cd, Cu, Pb, Mn and Cr in the lyophilized flesh of carrots and beets and the fresh quinoa growing on these contaminated soils; the concentration of the same vegetables bought in the Spanish market, and the metal concentration rate between Chilean and Spanish samples. The concentrations of all tested elements in the Spanish samples are in the level reported for vegetables growing in none contaminated soils [34].

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 total As content and the As concentration in the different extracts of the *"in vitro"* gastrointestinal digestion process. In order to check the possible interference of chloride in the ^{75}As signal due to the formation of ^{40}Ar^{35}Cl$^+$ during ICP-MS analyses, the signal at m/z 75 of salivary, gastric and intestinal juices were registered. It was observed that the signal intensity for the three extracts was negligible and did not differ from that of a blank solution, which indicates that the procedure is free of interference by chloride.

The comparison of the total As content and total extracted As shows that practically the whole As is bioaccesible from carrots and beets (98% and 90% respectively). However, for quinoa, only about 40% of As is bioaccesible. Fresh carrots and beets have a water content of about 85%, while in quinoa it is only about 10%. It has been reported that the average consumption per inhabitant of quinoa in the Andean countries is about 1.15-2.35 Kg per year [35,36] and about 2-6 Kg per year for carrots and beets. 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 and the As bioaccesibility, the calculated accessible doses of As expressed as µg As per year, is about 470 for carrots, 550 for beets and 180 for quinoa. Therefore, quinoa seems to be the vegetable with the lower toxicological risk from the bioaccesible point of view.

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 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 the enzymatic extraction is necessary. Figures 2a-e 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 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 in previous work [27] (Table 8). 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 methanol - water but almost 100% in the enzymatic hydrolysis.

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 bioaccesibility, a speciation analysis has been performed in the salivary, gastric and intestinal juice of carrots and quinoa.

	Sediment	Soil 1	Soil 2	Soil 3	PACS-2 certificated	PACS-2 Experimental
As	44 ± 2	48 ± 1	97 ± 3	48 ± 3	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 ± 4 298 (HF)
Cr	1.1± 0.1	1.8 ± 0.1	13.4 ± 0.9	17.0 ± 3.2	91 ± 5	64.5 ± 3.7 85.7 (HF)
Mn	189 ±18	212 ± 2	234 ± 15	208 ±14	440 ± 19	262 ± 7 462 (HF)
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}).

	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}).

Vegetables		As	Cd	Pb	Cu	Mn
Carrots	Flesh-C Flesh-S Factor	0.52 ± 0.04 0.02 ± 0.01 24	0.05 ± 0.01 0.07 ± 0.01 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 0.02 ± 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 0.01± 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

C: Chilean; S: Spanish. Factor: Metal concentration in Chilean /Spanish samples. Cr: below the DL in all samples analyzed.

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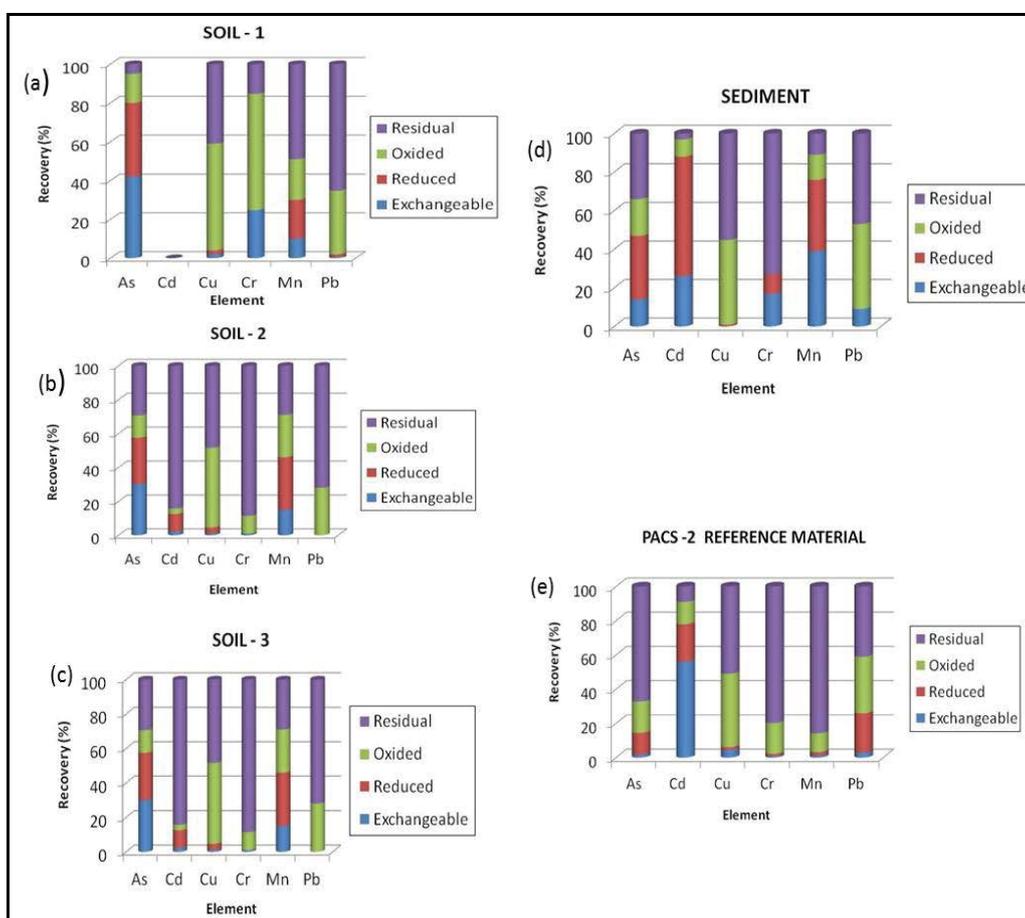

Figure 1: Selective extraction of As, Cu, Cd, Cr, Mn and Pb in soils, sediment from the Chiu area and Pack-2 reference material. Fractionation in soils, sediment.

The analysis was performed by LC-ICPMS under the same conditions than performed in original samples. Figures 2f-h shows the chromatograms obtained for carrots. Similar results were obtained in quinoa sample (chromatograms not shown). The percentages are given respect to the whole content in the corresponding extract.

The application of selective sequential extraction procedures to soil and sediments is the recommended way to determine the source of the contaminants and their capability of interacting 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 area are in general, between the ERL y ERM values (Table 4), and therefore for these elements toxicological and environmental effects could exist. However, the effects are mainly associated with the bioavailability. From the bioavailability studies
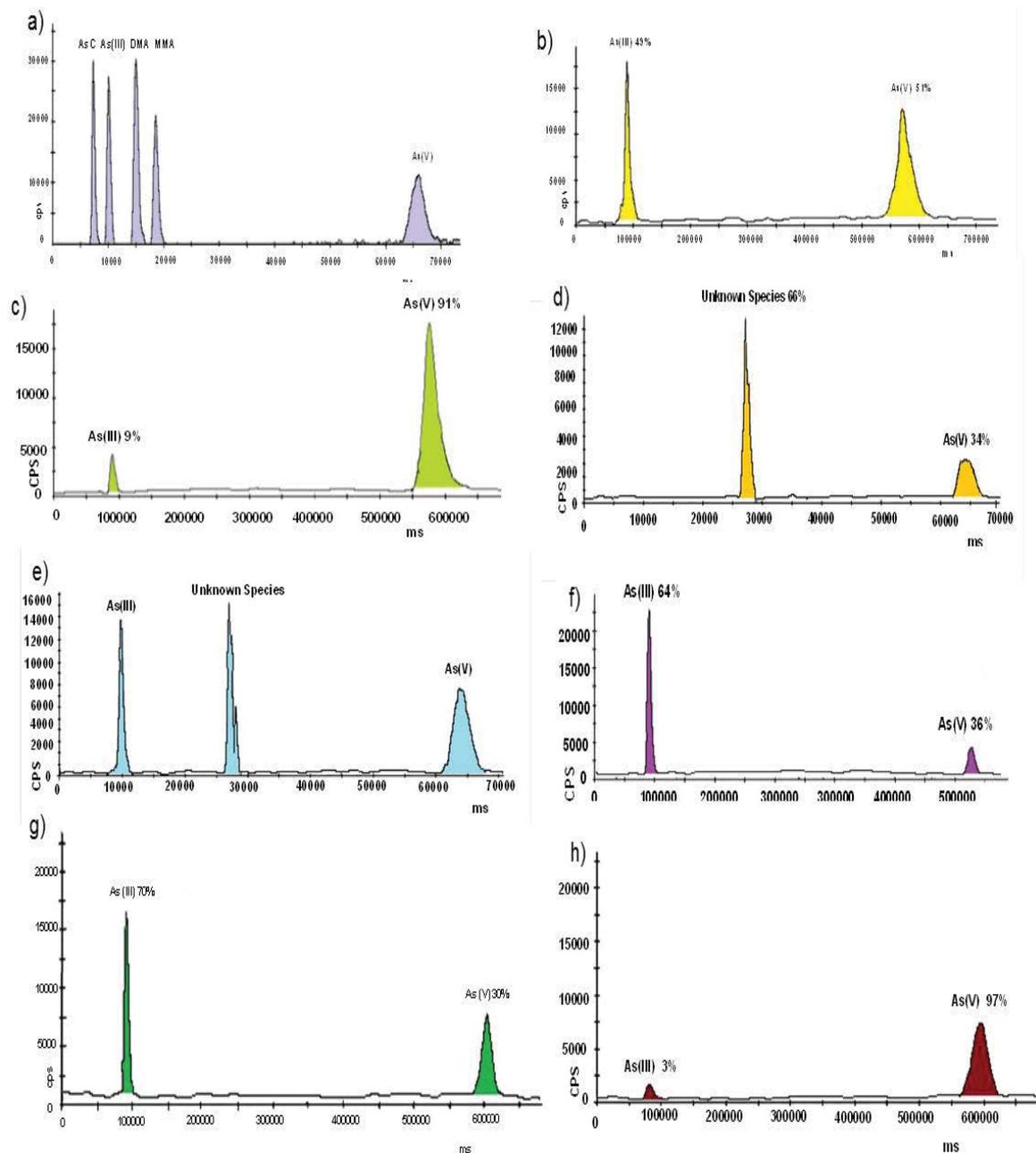
Figure 2: Chromatograms obtained in the methanolic and enzymatic extracts by LC-ICP-MS. a) Chromatogram of standards As species: As C, As (III), DMAs, MMAs, As (V), b) As species in carrots, c) As species in quinoa, d) As species in beet, e) As species in beet spiked with 5 µg/L As (III) and As (V), 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.

Figures 1a-e it 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 the microorganisms present in the soil are frequently involved, and the proper structure of the vegetal [37,38]. Results obtained considering the soils bioavailability and the concentration found in the edible (and not 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. Some contamination of Cu can be detected in the Chilean beets and quinoa in comparison with Spanish ones, probably due to the uptake from the interchangeable fractions of the soils. The Mn shows also some accumulation in the Chilean 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 [39,40]. It is relevant that only As (III) and As (V)

	Element	Certified value (µg Kg^{-1})	Experimental value (µg Kg^{-1})
White Cabbage BCR 679	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 1568a(	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)	As	0.112 ± 0.004	0.115 ± 0.006

Analysis performed by ICPMS.

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

		Carrots	Beets	Quinoa
Extracted As (µg Kg^{-1})	Salivary	313 ± 18	289 ± 28	34 ± 4
	Gastric	135 ± 12	186 ± 21	31 ± 4
	Intestinal	65 ± 9	84 ± 10	13 ± 6
Total extracted As (µg K^{-1})		513 ± 45	559 ± 38	78 ± 15
Total As (µg K^{-1})		520 ± 41	616 ± 57	196 ± 24
Recovery (%)		98 ± 3	90 ± 4	40 ± 4

Table 7: Bioaccesibility of as in the digestion process of Chilean samples by ICP-MS. Results expressed in lyophilized vegetables (n=3).

Vegetables	Fresh sample (µg Kg^{-1})	Metanolic Extract (%)	Enzymatic Extract (%)
Carrots	63 ± 5	94 ± 4	18 ± 4
Beets	74 ± 7	0%	93 ± 6
Quinoa	176 ± 21	104 ± 8	10.5 ± 0.8

Table 8: Methanolic and enzymatic extraction efficient for both totals as by ICP-MS.

are present in carrots and quinoa (Figures 2a-e). This is not the case of beets, were As (V) and mainly un unknown As species partially overlapped (Figures 2d-e) are presented. Sacarose is the sugar extracted from the beets; therefore an As-sacarose 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 [41]. It is known than the terrestrial plants in general have very low efficient mechanisms of biometilation [42]. Probably also the drastic conditions of these soils from the environmental point of view make it difficult for the microorganisms present in the soil, to develop biometilation mechanisms giving methylated species able to be absorbed through the roots of these vegetables as proposed by Ye et al. [43].

It is well known than the main contribution of contaminant (and also nutrient) elements to living beings arise 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 bioaccesibility 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 pre-concentrate about 3 times more As that the others, but its bioaccesibility 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 bioaccesibility of a contaminant is useful for estimating potential human health risks. However, it is even more important to know the species under which the contaminant is liberate to bloodstream. Figures 2f-h shows 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 et al. [44] show the transformation of As(V) to As (III) after gastrointestinal digest when other vegetables, different to that of this study, are contaminated 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 levels 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 [45]. The studies of Ferreccio et al. [46] reports concentrations of As slightly higher than reported in this study for soil in the region.

Conclusion

From the bioavailability studies performed for As, Cu, Cd, Mn and Cr in the soils of the Chiu area, can be concluded that As has a really high content in the labile or exchangeable fraction of the three analysed 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 bioaccesibility 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. Bioaccesibility 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 bioaccesibility 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.

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