Extractability of Arsenic, Copper, and Lead in Soils of a Mining and Agricultural Zone in Central Chile

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ABSTRACT

Copper (Cu) mining activities are very important in central Chile. The present study consists in determining the distribution and availability of copper, arsenic (As), and lead (Pb) in soils near a smelting facility, in the VI Administrative Region of Chile, which are subjected to risk of contamination by emissions and by irrigation with contaminated water. In order to determine the distribution of these elements in their various chemical forms in soils, two sequential extraction methods (SEM) were applied. A five stage

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SEM was used for Cu and Pb, and a four stage one for As. Single extraction methods were also carried out using 1 M HCl, 0.005 M DTPA and 0.005 M EDTA for As, Cu, and Pb, respectively. Furthermore, a 0.1 M malic-citric acid solution (MC) was used as simple extractant for the three elements to simulate the conditions of root environment of the plant. The soils showed the following order of total metal concentration: Cu > As > Pb. Copper was extracted in higher amount than the other metals, both with the sequential and with the simple extraction method. Single correlation analysis was applied between the amount of metal in the single extraction method and the different fraction obtained with the sequential technique. Significant positive correlations were obtained, especially for copper and lead.

Key Words: Copper; Arsenic; Lead; Soil; Sequential extraction; Single extraction.

INTRODUCTION

Although mining is one of the most important sectors in Chilean economy, it constitutes one of the most environmentally hazardous activities in many parts of the country. The main environmental problems associated with the mining sector of central Chile are: air pollution due to gas and particle emissions; water pollution by liquid effluents containing particulate matter, dissolved metals and acids; soil contamination; and risks caused by abandoned mining sites and tailing dams. The metallurgical industry, especially copper smelters, is the main source of sulfur dioxide (SO_2) emissions in Chile. Copper smelters also release significant amounts of heavy metals into the atmosphere. In recent years there have been reductions in the released amounts of sulfur dioxide and arsenic, but the problem remains serious. One of the major conflicts between copper mining and agricultural activities is caused by soil deterioration detected in the Cachapoal valley near the mining copper company El Teniente.[1] Romo-Kröger et al.[2] used a series of aerosol analyses in this area and showed that a group of elements, among them Cu and As, came from copper smelters and suggested that copper mining activities are one of the biggest sources of contamination in the region. The spread of contaminants to the surrounding environment poses the greatest risk in areas of the country where one-third of the citizens live. Other previous studies[1,3] carried out in soils located in this area revealed the existence of soils with very high levels of copper. This metal, however, was found to be mainly immobile and unavailable to the plants.

Metals cause toxic effects on plants or may accumulate in their tissues and thus enter the animal and human food chain. The total amount of heavy metals and metalloids in soils and their chemical forms have an important influence on plant growth and also on animal and human health. The chemical forms of metals in the environment drive their distribution between the solid and solution phase in soil, thus affecting metal behavior in terms of mobilization, availability and toxicity to plants.[4] Different geochemical fractions are operationally defined through an extraction sequence that generally follows the order of decreasing solubility.[5] Despite uncertainties due to lack of selectivity of various proposed extractants and to the possible problems associated to readsorptions, sequential extraction methods (SEM) constitute an excellent qualitative tool to determine trace metal associations, which indirectly defines their availability.[6] This can also be estimated through single extraction procedures by utilizing diluted acids, complexing agents, such as diethylentriaminepentaacetic acid (DTPA), ethylendiaminetetraacetic acid (EDTA), or low molecular weight organic acids to simulate the conditions of root environment.[7] Unlike single extraction methods, multi-step extraction methods, although more time-consuming, give a relatively more detailed information about the status of heavy metals in soils.[8]

Various schemes for SEM have been used for metalloids such as As that attempt to allocate these elements to particular solid phases. These schemes are based on some sequential extraction procedures designed for phosphate, considering that there is some chemical similitude between As and P.[9] On the other hand, SEM of heavy metals such as Cu and Pb are based mainly on procedures by Tessier et al.[10] and by BCR (Community Bureau of Reference) of the Commission of the European Communities,[11] which have been developed to differentiate various soil fractions, and are considered useful for evaluation of availability of metals in soil.[12]

The purpose of this study was to determine soil fractions at which Cu, As, and Pb are associated within a mining and agricultural region of Central Chile in order to indirectly estimate their availability to plants. In this context, two procedures were used: sequential extraction to carry out metal chemical partitioning, and simple extraction, using selected reagents. On the other hand, a single correlation analysis was applied between the amount of metal extracted with the single extraction methods and the different fraction obtained by the sequential methodology.

MATERIAL AND METHODS

Six soil sites were sampled in the agricultural area in the vicinity of a mining center, Caletones Smelter. The Caletones copper smelter plant belongs to copper mining company El Teniente, located in the VI Administrative Region of Chile, in the Andes mountain range, 34° 07′ S and 70° 27′ W. Composed surface (0–10 cm) and subsurface (10–30 cm) soil samples were taken. At each site three replicates were taken, 10 × 10 m plot was drawn, and a soil sample was taken from each corner and center of the plot using a stainless-steel hand auger. The samples were mixed, reduced and processed. All samples were air dried, sieved through a 2-mm mesh-size polyethylene sieve. Portions of soil fraction <2 mm were ground in an agate mortar and stored in polyethylene sample bottles. Table 1 indicates sampling site and soil classification. Soil pH was determined as 1:2.5 soil to water (wt/wt).[13] The organic carbon (C) was determined by Walkley-Black procedure.[14] Cation exchange capacity was determined by the sodium acetate procedure at pH 7. Manganese (Mn), iron (Fe), and aluminum (Al) free oxides were extracted by Mehra Jackson procedure, using citrate-bicarbonate buffer and dithyonite, also via solubilization with ammonium oxalate at pH 3.[15] Total metal determination was done by Flame Atomic Absorption Spectrophotometry (F-AAS) after acid digestion (with a mixture of 4 mL HNO_3, 2 mL HF, and 4 mL H_2O_2) of 200 mg of soil sample, using a Milestone/mls 1200 Mega microwave oven. Copper and Pb were determined by flame AAS using an atomic absorption spectrometer Perkin-Elmer 1100 B, and arsenic measurements were made by hydride generation using a FIA system (Perkin-Elmer FIAS-200) interfaced to the atomic absorption spectrometer. A solution of 10% v/v hydrochloric acid (HCl) was used as carrier and a solution of 0.2% (w/v) sodium tetrahydroborate ($NaBH_4$), stabilized in 0.05% sodium hydroxide (NaOH), was used as reductant.

Sequential Extraction Methods (SEM)

Two different SEM were followed, one for As and another for Cu and Pb.

Table 1. Some general characteristics of soils.

						MnO_2		Fe_2O_3		Al_2O_3		Total metal		
						a	b	a	b	a	b	As	Cu	Pb
		Order	pH (H_2O)	%C	CEC ($cmol\,kg^{-1}$)	($g\,kg^{-1}$)		($g\,kg^{-1}$)		($g\,kg^{-1}$)		($mg\,kg^{-1}$)	($mg\,kg^{-1}$)	($mg\,kg^{-1}$)
Graneros														
Soil1	A	Mollisol	6.4	1.5	31	0.6	2.2	28	34	14	1.7	67	217	40
	B		6.4	0.94	31	0.6	2.2	29	34	13	1.6	69	158	20
Soil 2	A	Mollisol	5.9	1.3	33	0.7	2.3	31	38	15	1.9	75	184	48
	B		5.8	1.0	34	0.7	2.5	32	38	16	1.8	74	139	30
Carretera del Cobre														
Soil 3	A	Alfisol	6.5	1.4	26	0.8	2.8	25	28	11	1.2	38	364	34
	B		6.6	1.1	30	1.0	3.7	28	25	1.4	1.6	33	200	37
Soil 4	A	Alfisol	6.7	1.6	29	0.8	2.9	25	30	12	1.3	40	352	36
	B		6.9	1.0	30	0.9	3.2	28	26	14	1.6	35	244	38
Carretera Panamericana														
Soil 5	A	Inceptisol	6.8	0.72	18	0.5	2.0	20	54	9.7	1.0	43	947	30
	B		6.7	0.62	19	0.5	2.1	20	36	10	0.77	41	865	25
Soil 6	A	Inceptisol	6.9	2.0	29	0.6	2.5	21	34	11	1.2	38	772	37
	B		6.7	1.2	25	0.7	2.6	21	29	12	1.0	33	442	33

Carretera Panamericana soil 1 Km 97 and soil 2 Km 99.

A*—depth 0–10 cm y B—depth 10–30 cm.

a—NH_4-oxalate extractable metal, b—dithionite extractable metal.

Arsenic

The equivalent to 1 g of soil of each sample dried at 105°C was weighed and was sequentially extracted with the following four-step procedure:

Step 1: Soluble As, 25 mL of 0.25 M KCl pH 7, shaken for 30 min at 25°C.
Step 2: Ligand-exchangeable As, 25 mL of 0.1 M KH_2PO_4, shaken for 30 min at 25°C.
Step 3: Acid-extractable As, 25 mL of 4 M HCl, heated with occasional stirring in a water bath for 45 min at 95°C.
Step 4: Oxidizable As, the residue from fraction 3 was mixed with 0.25 g of $KClO_3$ and 5 mL of 12 M HCl was slowly added for 45 min occasionally stirred.

The extract from each fraction was stored in polythene bottles. The residual As was calculated by difference between the sum of the four fractions and the total As content.

Copper and Lead

The equivalent to 3 g of soil of each sample dried at 105°C was weighed and these metals were extracted with the SEM described by Howard and Shu.[16]

Step 1: Exchangeable, 20 mL 1 M $MgCl_2$, shaken for 1 h at 25°C.
Step 2: Carbonate-occluded, 20 mL 1 M NaOAc (sodium acetate) pH 5, shaken for 5 h at 25°C.
Step 3: Mn oxide-occluded, 20 mL 0.1 M $NH_2OH.HCl$ (hydroxylamine hydrochloride) + 0.01 M HNO_3, shaken for 30 min at 25°C.
Step 4: Organically bound, 20 mL 0.1 M $K_4P_2O_7$, shaken for 24 h at 25°C.
Step 5: Fe oxide-occluded: 20 mL 1 M $NH_2OH\text{-}HCl + 25\%$ (v/v) HOAc (acetic acid), heated with occasional stirring in a water bath; 4 h at 85°C.

The extracting solutions were prepared with an equivalent to 200 mg L^{-1} nitrilotriacetic acid (NTA) to prevent redistribution of the metals.[17] After each individual step, supernatants were obtained by centrifugation; then they were filtered and placed in acid-washed polypropylene bottles.

Single Extractions

In order to estimate metal availability through single extraction procedures, three different extracting solution were used. Copper was extracted using DTPA[18] composed of 0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanol-amine adjusted to pH 7.3; lead[19] was extracted with 0.005 M EDTA; and arsenic,[20] with 1 M HCl solution. In addition, a solution composed of a mixture of 0.1 M malic acid and 0.05 M citric acid (CM) was used for the single extraction of these three elements under study.[21] The equivalent to 3 g of soil, dried at 105°C, was weighted. The single extraction procedures consisted in adding 20 mL of each solution, shaking for 6 h, centrifuging and then separating the supernatant for the determination of the elements under study. All extractions were made in triplicate.

Using the Standard Reference Material 2710 from NIST (Montana soil), the accuracy of total metal determination was checked with the following results:

Elements	Certified values ($\mu g\,g^{-1}$)	Determined values ($\mu g\,g^{-1}$)
As	626 ± 38	619 ± 4
Cu	2950 ± 130	2958 ± 15
Pb	5532 ± 80	5503 ± 14

RESULTS AND DISCUSSION

Some general characteristics of the soils under study are shown in Table 1. In this respect, similarities can be observed with common soils of different land areas. Table 1 also shows total metal concentration found in the soils of the VI Region. Soils 1 and 2 from Graneros exhibited the highest Pb and As values, while Cu concentrations were remarkably higher for Carretera Paramericana soils, ranging from 442 to 947 $mg\,kg^{-1}$. Copper is clearly more concentrated at surface level for all the soils under study. According to Badilla-Ohlbaum et al.[1] this behavior is characteristically found in soils with a high copper levels of this zone. Copper higher concentration in the surface layer of a soil is an indication of soil additions from smelters, fertilizers, sewage sludges and other wastes, fungicides or bactericides, or manures.[22] With respect to arsenic, this shows similar concentrations at both levels. Lead shows an intermediate behavior, being more clearly concentrated at surface level only in Graneros soils. The main potential metal pollution sources in this region are (a) suspended air particles coming from the smelter facility, which eventually deposit on the soil surface[2] and (b) mining wastes discharged into the rivers which are used downstream for irrigation.[1] These results suggest that pollution sources vary for each one of these elements and also insinuate that pollution depends on each selected sampling location. In any case, for all sampling locations, the concentration level determined in soils for each element was higher than those levels for non-polluted soils.[23]

Sequential Extraction

Sequential extraction procedures were applied in order to determine the nature of the chemical forms in which the elements under study are present in this kind of polluted soils. According to Table 2, arsenic was sequentially extracted from all the soils in similar amounts, ranging from 51 to 67% of total As. No significant variations between soil layers were observed for As content. When total As sequentially extracted, the prevailing fraction is the HCl soluble one, ranging from 41 to 62% of total, which would suggest that most of the element could be associated to Al, Mn, or Fe oxides in addition to the metal associated to readily hydrolyzable organic matter (OM) with HCl. Arsenic associated to the humus fraction in all of the soils represent 2 to 3% of total As. Reynolds et al.[24] found that arsenic fixation is controlled largely by Fe oxides and oxihydroxides in aerated soils. Similarly, Oken and Adriano[25] observed that in arsenic-contaminated soils with high concentration of Fe, most As is bound to iron oxides. In relation to exchangeable As, it was observed that only a small proportion of total is found in this form. In the soils with the highest total content of As (soils 1 and 2), this form accounts for only 2% of total As.

The soils with the highest concentration of copper are soils 5 and 6. All soils vary in Cu content between layers, the highest values being observed in the surface horizon. A high percentage (66 to 92%) of total Cu is sequentially extracted (Table 3), and in the case of soil 5 almost all

Table 2. Arsenic distribution in alluvial soils in Region VI.

		As sol. (mg kg^{-1})	RSD[b]	As exchang. (mg kg^{-1})	RSD	As extr. with acid (mg kg^{-1})	RSD	As oxidizable (mg kg^{-1})	RSD	Total As ES[c] (%)
Graneros										
Soil 1	A[a]	0.028	0.07	1.5	1.7	42	1.3	1.5	7.0	67
	B	0.007	21	1.4	1.6	38	1.0	1.3	9.1	59
Soil 2	A	0.016	18	1.4	4.8	34	4.0	1.8	9.2	49
	B	0.006	24	1.5	4.4	37	3.0	1.8	7.4	54
Carretera del Cobre										
Soil 3	A	0.079	0.24	2.2	2.3	16	1.7	1.2	5.9	51
	B	0.017	7.9	1.4	4.0	16	32	1.2	14	56
Soil 4	A	0.033	5.3	2.0	2.2	17	1.0	1.2	15	50
	B	0.007	23	1.3	2.3	16	3.2	1.0	5.4	53
Carretera Panamericana										
Soil 5	A	0.082	2.9	2.2	1.5	20	2.2	1,0	7,5	53
	B	0.059	5.4	2.2	3.2	21	0.30	1,1	6,9	59
Soil 6	A	0.160	2.0	1.6	2.0	19	2.9	1,1	11	58
	B	0.106	5.3	1.9	3.9	19	0.17	1,1	3,0	67

Carretera Panamericana soil 1 Km 97 and soil 2 Km 99.

[a] A—0–10 cm depth and B—10–30 cm depth.

[b] RSD (%)—Relative standard deviation ($n = 3$, replicates of three different samples).

[c] % of total As sequentially extracted.

Table 3. Copper distribution in alluvial soils in Region VI.

		Cu sol. + exchang. (mg kg^{-1})	RSD[b]	Cu-CO$_3$ (mg/kg^{-1})	RSD	Cu-MnOx (mg kg^{-1})	RSD	Cu-Organic (mg/kg^{-1})	RSD	Cu-FeOx (mg kg^{-1})	RSD	Total Cu ES[c] (%)
Graneros												
Soil 1	A[a]	70	0.7	31	2.9	4.4	2.4	19	0.4	54	3.3	82
	B	32	0.3	16	0.6	4.1	6.8	17	2.1	42	2.8	70
Soil 2	A	63	1.4	27	5.2	4.3	4.2	19	1.9	46	0.5	81
	B	26	0.4	14	1.6	3.0	4.2	14	2.6	35	2.8	66
Carretera del Cobre												
Soil 3	A	95	1.8	67	1.9	10	4.4	32	4.3	88	4.8	80
	B	57	2.8	35	2.0	6.0	3.7	19	0.3	52	2.0	86
Soil 4	A	107	0.8	66	5.2	10	7.1	31	0.3	93	3.4	87
	B	74	0.9	38	3.1	7.7	3.7	24	1.4	70	4.2	88
Carretera Panamericana												
Soil 5	A	138	0.7	160	2.4	55	0.8	123	3.4	365	1.1	89
	B	126	0.3	154	1.3	64	8.7	112	8.8	327	2.2	91
Soil 6	A	222	4.3	140	5.3	26	1.3	93	5.7	168	3.3	84
	B	141	2.2	74	1.9	15	6.3	51	2.5	113	15	90

Carretera Panamericana soil 1 Km 97 and soil 2 Km 99.

[a] A—0–10 cm depth and B—10–30 cm depth.

[b] RSD (%)—Relative standard deviation ($n = 3$, replicates of three different samples).

[c] % of total Cu sequentially extracted.

Cu is sequentially extracted. The copper that could become easily available, i.e., the soluble, exchangeable and associated-to-carbonate forms, in most cases constitutes almost 50% of total Cu, except for surface and subsurface horizons of soils 5 and subsurface horizons of soils 1 and 2. In the soils from Carretera Panamericana with the highest content of Cu, the prevailing copper fraction is that associated to Fe oxides and to OM, with a similar percent distribution in both fractions. Studies with other similar Chilean soils[26] have found that in soil with a baseline level of Cu, this is predominantly associated to iron oxides and whereas in samples spiked with Cu, exchangeable and associated-to-carbonates forms are prevailing.[27] Therefore, the high Cu concentration found in soils of Carretera Panamericana could be the result of a permanent enrichment from irrigation with contaminated water rather than deposition from air pollution.[28]

Unlike Cu, Pb is sequentially extracted in smaller amounts (Table 4). Most Pb in these soils is part of the residual fraction (not sequentially extracted), in most cases more than 50% of the total Pb content. Only a small proportion of Pb is spread in forms that could become available, the prevailing Pb fraction being associated to Fe oxides. Other studies in similar soils have also found that Pb is associated to chiefly Fe and Mn oxides.[29] Ma and Rao,[5] instead, found that Pb in contaminated soil was associated to the carbonate and Fe-Mn oxide fractions, smaller amounts being associated to either the organic or the residual fraction. A smaller amount of Pb is present in the soluble and exchangeable fractions.

Single Extraction

Single extraction procedures were applied to the different soils under study in order to determine the potential availability of the elements to plants cultivated in polluted soils of the region and to evaluate the possibility of metal movement through soil profile. A large number of single extractants for soils have been developed.[30] These have been empirically derived but validated by field experiments correlating plant contents with extractable soil contents. According to the literature, DTPA for Cu,[18] EDTA for Pb,[19] and HCl for As[20] were selected. All these reagents have been traditionally used as extracting agents for the determination of available trace metals in soil, because significant positive correlations have been observed between their concentrations in these solutions and the contents determined in the plant species cultivated in the corresponding soils. On the other hand, Jones[31] has shown that low molecular weight organic acids play a very important role

Table 4. Lead distribution in alluvial soils in Region VI.

		Pb sol. + exchang. (mg kg^{-1})	RSD[b]	Pb-CO$_3$ (mg kg^{-1})	RSD	Pb-MnOx (mg kg^{-1})	RSD	Pb-Organic (mg/kg)	RSD	Pb-FeOx (mg kg^{-1})	RSD	Total Pb ES[c] (%)
Graneros												
Soil 1	A[a]	0.89	16	0.73	1.6	0.75	4.3	0.48	9.0	9.0	4.6	29
	B	0.68	5.6	0.33	5.9	0.80	15	0.54	3.9	8.5	1.7	54
Soil 2	A	0.46	6.8	0.59	8.4	0.54	15	0.51	8.7	8.5	2.2	22
	B	0.41	24	0.38	13	0.70	18	0.45	3.8	7.9	1.3	32
Carretera del Cobre												
Soil 3	A	0.26	4.9	0.46	4.4	1.2	4,6	1.07	8.4	13	13	47
	B	0.37	17	0.29	5.1	0.73	15	0.65	8.5	10	1.2	33
Soil 4	A	0.42	2.6	0.43	9.3	1.2	10	1.2	9.8	13	3.5	45
	B	0.15	6.9	0.49	4.8	0.78	22	0.75	11	11	2.2	33
Carretera Panamericana												
Soil 5	A	0.12	14	0.52	3.0	1.7	2.5	1.4	12	8.5	15	42
	B	0.08	16	0.55	13	1.6	3.4	1.3	22	11	2.3	58
Soil 6	A	0.14	4.1	0.63	4.0	1.5	6.9	2.5	6.2	12	2.3	45
	B	0.21	2.2	0.53	4.8	1.4	13	2.2	4.9	12	1.3	49

Carretera Panamericana soil 1 Km 97 and soil 2 Km 99.

[a] A—0–10 cm depth and B—10–30 cm depth.

[b] RSD (%)—Relative standard deviation ($n = 3$, replicates of three different samples).

[c] % of total Pb sequentially extracted.

in plant-root physiological activities. The organic acids secreted as metabolic products of plant roots would decrease the rhizosphere pH and, therefore, metals thus mobilized could become easily available for plants.[7] These acids on the other hand, can also act as complexing agents towards trace metals.[32] It has been demonstrated that plant roots secrete organic acids such as malic acid, citric acid, succinic acid, acetic acid, and oxalic acid; malic and citric acid would be of some predominant acids.[31] Thus, a solution of 0.1 M malic-citric (MC) was also tried as an extractant in order to assess its effect.

Table 5 shows that As extracted with 1M HCl was similar in both horizons; there were some differences only in soils from Carretera del Cobre. The highest As contents were observed in Carretera Panamericana soils. The Cu extraction with 0.005 M DTPA, presented differences between both layers, the highest values being in the superficial level; on the other hand, some small differences were observed for Pb extraction with 0.005 M EDTA between layers in both Carretera del Cobre soils and in Soil 6 from Carretera Panamericana. Lead is reported to be the least mobile among other heavy metals due to its low solubility and there is little probability that Pb is readily lost from soil profiles by leaching.[33] In these soils Pb has been found predominantly associated to Fe oxides and an important proportion of total Pb in these soils could be mainly bound to clay minerals. On the other hand, copper contrary to lead, has been found in more mobile forms and the higher values found in the surface layer could be due to the continuous enrichment by irrigation with polluted waters.

According to these results, the availability order would be $Cu > As > Pb$. With respect to differences among the soils, those from Carretera Panamericana exhibited the highest percentages of extraction for the three metals, while the soils from Graneros showed the lowest ones. The same availability order was obtained when a 0.1 M MC solution was used (Table 6). However, if both single extractions are compared, the amount extracted will depend on the trace metal, and thus HCl extracts 2 to 5 times more As than the MC solution. On the other hand, without considering the Soil 5, both solutions extracted very similar quantities of copper, EDTA solution extracts higher amounts of Pb than MC solution. Although the results differ when compared with other soil extractants, DTPA and EDTA have been used for many years for determining plant-available trace elements in soils,[34] in this case, the similar results between CM and DTPA may be due to the fact that Cu is found in more mobile forms. Concerning Pb, this element in these soils is found in less soluble forms, difficult to dissolve with CM, but a little more soluble in EDTA. This chelating agent could dissolve Pb associated

Table 5. Extraction of As, Cu, and Pb with 1M HCl, 0.005M DTPA and 0.005M EDTA respectively ($n = 3$).

		As HCl (mg kg^{-1})	Total metal (%)[b]	RSD[c]	Cu DTPA (mg kg^{-1})	Total metal (%)	RSD	Pb EDTA (mg kg^{-1})	Total metal (%)	RSD
Graneros										
Soil 1	A[a]	14	21	0.5	73	34	0.42	3.1	7.9	1.8
	B	13	18	1.6	40	25	0.14	3.0	15	5.1
Soil 2	A	13	17	2.1	64	35	1.6	2.3	4.9	6.5
	B	12	16	0.8	32	23	0.0	2.4	8.1	6.3
Carretera del Cobre										
Soil 3	A	12	31	3.2	96	26	0.0	5.1	15	3.0
	B	6.4	19	1.9	55	27	1.8	4.2	11	3.8
Soil 4	A	14	35	0.15	115	33	0.87	5.0	14	5.6
	B	7.1	20	1.3	67	28	0.86	3.6	9.4	6.8
Carretera Panamericana										
Soil 5	A	18	41	0.90	139	15	0.0	4.5	15	2.2
	B	18	44	0.89	120	14	2.1	4.0	16	8.1
Soil 6	A	15	40	4.6	242	31	1.0	6.9	19	3.6
	B	15	46	2.4	143	32	0.7	5.6	17	8.5

Carretera Panamericana soil 1 Km 97 and soil 2 Km 99.

[a] A—0–10 cm depth and B—10–30 cm depth.

[b] %—As percentage referred to total metal contents in soils.

[c] RSD (%)—Relative standard deviation ($n = 3$, replicates of three different samples).

Table 6. Extraction of As, Cu, and Pb with a solution made up of a mixture (2:1) of 0.1 M malic-citric acid ($n = 3$).

		As ($mg\,kg^{-1}$)	Total metal (%)	RSD	Cu ($mg\,kg^{-1}$)	Total metal (%)	RSD	Pb ($mg\,kg^{-1}$)	Total metal (%)	RSD
Graneros										
Soil 1	A[a]	3.0	4.5	1.9	84	39	0.7	1.2	2.7	6.0
	B	3.1	4.3	1.6	43	27	1.7	0.38	2.1	7.6
Soil 2	A	2.5	3.2	1.4	71	37	0.5	0.53	1.2	7.1
	B	2.5	6.1	2.4	33	24	0.06	0.43	1.3	9.4
C. del Cobre										
Soil 3	A	5.3	7.2	1.7	166	45	1.1	1.64	4.4	0.35
	B	2.6	7.8	1.3	86	41	3.4	0.59	2.0	4.5
Soil 4	A	5.0	13	4.7	167	48	0.8	0.96	3.0	6.6
	B	2.6	7.4	3.6	107	45	1.2	0.48	1.1	6.7
C. Panamericana										
Soil 5	A	8.3	19	4.1	600	69	1.1	2.7	8.9	2.2
	B	8.2	20	3.3	544	72	0.3	2.4	9.5	6.6
Soil 6	A	7.3	18	0.8	387	50	0.7	2.0	5.7	8.2
	B	7.0	22	3.1	232	54	2.4	1.4	4.6	8.5

Carretera Panamericana soil 1 Km 97 and soil 2 Km 99.

[a] A—0–10 cm depth and B—10–30 cm depth.

[b] %—As percentage referred to total metal contents in soils.

[c] RSD (%)—Relative standard deviation ($n = 3$, replicates of three different samples).

to: poorly crystalline Fe(hydr)-oxides (and associated metals), carbonate minerals and to Pb chemically bound inner-sphere surface complexes.[35]

Table 6 shows the extraction values with this mixture of organic acids for the different soils, ranging from 6 to 22% for As, 24 to 70% for Cu, and 1 to 9% for Pb in relation to the total content of these metals.

As mentioned above, single extractants have been used to estimate the available trace metal pool in soils, whereas SEM has been used for the same purpose and also to assess the chemical affinities of these trace metals to different soil components. In this context, a comparison was made through simple correlations between both methods in order to estimate in which form trace metals could be available to plants. Single correlation analyses are shown in the Table 7. Only the soluble and exchangeable forms of As correlated with the single As extraction performed with HCl and MC. Arsenic extracted with MC correlated better ($r = 0.8$) than extracted with HCl ($r = 0.6$). Thus, it may be said that only soluble and exchangeable As forms would be available. On the other hand, the best correlation for Cu was obtained between Cu-DTPA and the Cu-exchangeable fraction ($r = 0.99$). When MC solution was used, highly significant correlation coefficients were obtained with the Cu fraction bound to carbonate (0.97), to the organic matter (0.98), and to

Table 7. Correlation between heavy metals content obtained by single extraction (0.1 M citric-malic acid, 1 M HCl, 0.005 M DTPA, 0.005 M EDTA) and amount recovered at each step of sequential extraction ($n = 36$).

Metal fraction (y)	Linear equation	r
As		
Soluble	$y = 0.015\text{AsCM} - 0{,}022$	0.77^{a}
	$y = 0.0072\text{AsHCl} - 0.043$	0.60^{a}
Exchangeable	$y = 0.11\text{AsCM} - 1.2$	0.78^{a}
	$y = 0.059\text{AsHCl} - 0.94$	0.62^{a}
Cu		
Exchangeable	$y = 0.94\ \text{Cu DTPA} + 3.0$	0.99^{a}
Carbonate	$y = 0.24\ \text{CuCM} + 15$	0.97^{a}
MnOx	$y = 0.10\ \text{CuCM} - 3.3$	0.98^{a}
Organic	$y = 0.18\ \text{CuCM} + 6.2$	0.98^{a}
FeOx	$y = 0.51\ \text{CuCM} + 8.7$	0.98^{a}
Pb		
MnOx	$y = 0.46\ \text{PbCM} + 0.49$	0.91^{a}
Organic	$y = 0.44\ \text{Pb EDTA} - 0.72$	0.87^{a}

[a]Significant at $P < 0.01$.

the Fe-Oxides (0.98), which would indicate that 0.1 M MC acid solution extracted both the labile forms defined in SEM and other forms mentioned above. In relation to the Pb extracted with MC, this form correlated better with Pb associated to MnOx (0.9) and EDTA correlated better with Pb organic form (0.87).

The concept of availability is once again difficult to define only via physico-chemical means. Studies involving metal distribution, availability and mobility should be complemented with others using plants or other living organisms.

CONCLUSIONS

The soils under study exhibited the following order of total concentration: Cu > As > Pb. The results suggest that a small proportion of As and Pb would be available to plants. Most of these metals occurred in less mobile forms, as primarily associated to Fe-Oxides fractions. However, Cu was also found in forms that could become available. Therefore, considering the three elements under study, Cu appeared to be the most readily soluble, which makes it potentially available. A similar result was obtained using single extractions. The extraction with MC acid solution that simulates the conditions of root environment could be a good availability index only for As. For the other metals, this extraction system would consider forms that possibly are not easily available according to SEM. This aspect, however, further studies with plants, in order to derive more pertinent information.

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