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Sequential Extraction of Heavy Metals in Soils Irrigated with Wastewater

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ABSTRACT

Different chemical forms of cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) were determined in agricultural soils cultivated with lettuce and celery. These soils have been irrigated for several decades with nontreated wastewaters. The chemical forms of the metals were characterized by sequential extraction analysis in five steps, the extracting solutions of which contained 200 mg L^{-1} nitrilotriacetic acid (NTA). The results showed that Cd, Pb, and Cu exhibited the highest values of extracted metal, and Cr exhibited the lowest value. Almost all of the metals in these soils are bound to iron oxides, followed by the metal complexed to organic matter. The form bound to manganese oxides is equally important to Co. Cadmium is the most mobile metal and its available forms account for 40% of the total extracted. When these metals were determined in plants, both shoots and roots, a greater concentration of Cr, Zn, and Cu was found in celery, and Cu, Ni, and Zn in lettuce, most of the metals being in roots. Only Cd and Co are similarly distributed in shoots and in roots. The relation between the metal contents in plants and the different fractions of the metal in soil was evaluated through linear correlations. Different results were obtained, the forms accounting for the metal in celery are those of the metal bound to iron oxides, while the metal content in lettuce would be related to the exchangeable, bound to manganese oxide, and carbonate forms.

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INTRODUCTION

Anthropogenic activities, such as mining operations, aerosol depositing, fertilizer application, and irrigation with industrial and domestic wastewaters can be the cause of soil contamination by heavy metals. It is not enough to know the contamination level through total metal content (Elliot and Shields, 1988), since high concentrations of these metals in soils do not necessarily imply their release and consequent assimilation by plants or contamination of ground waters. Heavy metals are distributed among the various soil components and become associated with them in different ways which thus determines their mobility and bioavailability. Knowledge of the metal distribution in the various chemical forms would help to predict or detect a possible contamination risk and consequent environmental damage, such as plant, animal, or microorganism toxicity. It has been demonstrated that anthropogenic heavy metals are more mobile and potentially available than pedogenic or lithogenic ones (Chlopecka et al., 1996). The so-called chemical forms in which heavy metals occur are the following: water-soluble metals as free cations, complexed or adsorbed in organic matter, adsorbed or occluded in oxides, and associated with clay minerals or primary minerals. Soluble and exchangeable forms are considered really mobile and assimilable by plants, and metal forms bound to the crystalline lattice of clay minerals by isomorphic substitution would be in the opposite end. The other metal forms, whether associated with iron (Fe), manganese (Mn), and aluminum (Al) oxides, complexed with organic matter, or precipitated as carbonate or sulfide could be considered mobile and assimilable, due to the variation of some physical chemical factors, such as pH and oxidation-reduction potential (Gambrell, 1994; Ahumada and Schalscha, 1993).

Sequential extraction procedures have been widely used in determining heavy metal chemical forms in soils, sediments (Han et al., 1996; Khalid et al., 1981; Mester et al., 1988), and sludge (McGrath and Cegarra, 1992; Tsadilas et al., 1995). These methods use a succession of reagents to dissolve the various soil components. This chemical fractionation has become an operational approximation in order to predict and establish the existence of a possible relation between the bioavailable fraction of the metal in soil and its content in plants (Davies, 1992; Zhu and Alba, 1993). In spite of the wide use of these sequential procedures, there are still doubts about the accuracy of these methods, since analytical results are affected by reagent selectivity (Xiao-Quan and Bin, 1993; Kheboian et al., 1987). Better results could be obtained with adequate reagent choice and sequence, in addition to using complexing agents to keep metals in solution and avoid their resorption (Howard and Shu, 1996).

The purpose of this study was to characterize the chemical forms of Cd, Co, Cr, Cu, Ni, Pb, and Zn in soils irrigated with wastewaters and to determine a possible relation between these metal fractions and metal assimilation by plants.

TABLE 1. Some properties of soils under study.

Sample soil	Site no.	pH	Org-C %	CEC $cmol\ kg^{-1}$	Ox Fe % Fe	Ox Mn % Mn
Cultivated with celery	1	7.2	1.6	42.2	2.0	0.083
	2	7.3	1.6	44.3	2.1	0.086
	3	7.2	1.9	49.3	2.0	0.092
	4	7.2	1.7	46.3	2.0	0.090
Cultivated with lettuce	1	7.7	1.6	42.9	2.4	0.10
	2	7.8	1.8	36.6	2.6	0.095
	3	7.8	1.6	44.0	2.6	0.10
	4	7.8	1.9	50.9	2.6	0.11

MATERIALS AND METHODS

In this study, surface soil samples were used from an area of about 5,000 m^2 cultivated with celery (*Apium graveolens*) and another of similar size cultivated with lettuce (*Latuca sativa* L.). These agricultural lands are located southwest of Santiago and have been irrigated with wastewaters of domestic and industrial origin for more than five decades. These soils are of alluvial origin and belong to the Inceptisol order and the Clayed, mixed, thermic, Vertic Xerochrepts family. A total of eight samples from four sampling sites were used for each cultivation area. In the laboratory, the samples were dried at room temperature (20-22°C), ground in a porcelain mortar, and sieved through a 2-mm plastic sieve. The pH was determined in a 1:1 soil-water suspension, organic carbon (C) percent was determined by the Walkley-Black procedure (Nelson and Sommers, 1982), cation exchange capacity (CEC) was determined by the sodium acetate procedure (Jackson, 1970). Manganese, Fe, and Al free oxides were extracted by the Mehra-Jackson procedure using dithyonite-citrate and bicarbonate. Total metal determination was done by flame atomic absorption spectrophotometry (AAS) after acid digestion [nitric acid (HNO_3), hydrofluoric acid (HF), and hydrogen peroxide (H_2O_2)] of the soil using a Milestone microwave oven. For sequential extraction, it was necessary to grind subsamples in an agatha mortar and to sieve through a 150-mesh sieve. The equivalent to 3 g of soil dried at 105°C was weighed in triplicate. The extraction procedure used is based on the Chao (1972), Tessier et al. (1979), and Miller et al. (1986) methods, according to the following scheme:

TABLE 2. Sequential extraction of some heavy metals in soil cultivated with celery.

Sample	Exch.		Carb-ocd		Mn-ocd		Organic		Fe-ocd.		Sum seq.	TM	Rec. tot. met.
	%SS	%CV	%SS	%CV	%SS	%CV	%SS	%CV	%SS	%CV	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	%
Cd													
1	20	5.5	15	6.3	7.5	13	24	8.1	33	6.1	1.10	1.03	106
2	22	8.6	16	5.8	7.9	12	22	14	33	8.4	1.16	1.02	113
3	22	7.1	17	8.0	8.0	13	21	15	33	7.1	1.12	1.09	103
4	21	13	17	10	9.0	23	17	17	36	13	1.00	1.03	96
Co													
1	n.d.		7.6	4.9	38	2.1	15	10	40	3.4	16.8	27.4	61
2	n.d.		8.3	17	38	1.2	14	11	40	4.2	16.8	32.4	52
3	n.d.		7.9	10	38	2.0	14	13	40	7.7	16.1	30.4	53
4	n.d.		9.7	13	37	1.3	13	13	40	5.4	15.9	33.6	47
Cr													
1	3.4	13	3.8	20	4.6	13	24	5.3	64	5.1	19.5	73.1	27
2	3.4	11	4.3	16	1.1	30	35	5.5	56	20	20.6	99.1	21
3	4.0	11	3.3	35	1.8	34	40	5.0	51	11	17.2	73.6	23
4	5.4	7.3	3.2	19	3.4	63	40	2.5	52	15	13.1	71.0	18
Cu													
1	18	3.5	16	2.3	4.7	0.77	22	2.6	39	3.8	313	546	57
2	18	2.1	18	2.7	5.1	2.5	22	6.1	38	4.2	296	514	58
3	19	1.3	19	1.2	5.7	1.00	22	2.6	34	7.2	281	490	57
4	19	2.0	18	1.3	5.2	1.78	22	3.9	35	8.6	285	529	54

Ni													
1	3.3	7.4	16	2.0	19	4.3	20	11	42	3.5	14.4	24.7	59
2	3.4	13	16	6.7	21	4.4	19	15	41	7.7	15.9	29.6	54
3	3.6	21	17	0.0	22	0.0	19	4.8	39	0.0	15.6	29.1	54
4	4.9	14	19	0.0	19	0.0	16	0.0	41	0.0	13.4	28.5	47
Pb													
1	n.d.		2.6	15	3.6	7.0	25	11	69	4.1	26.4	36.3	73
2	n.d.		2.4	5.0	5.0	6.5	23	9.4	69	6.4	29.5	40.4	73
3	n.d.		2.4	11	5.1	12	23	8.9	69	5.5	27.4	38.4	70
4	n.d.		2.5	11	3.8	15	22	10	71	6.9	22.9	36.3	62
Zn													
1	13	4.0	10	4.4	5.2	3.3	17	6.0	55	6.5	95.9	183	52
2	14	1.8	12	11	6.2	2.2	16	12	51	5.0	103	205	50
3	14	4.4	12	8.2	6.2	2.1	13	14	55	6.1	106	207	51
4	14	3.0	11	5.6	5.6	32	14	13	56	11	89.6	190	47

%SS=metal percent of total sequentially extracted and mean of six replications.
%CV=variation coefficient.
TM=total metal.
Rec. tot. met.=recovery of total metal.

TABLE 3. Sequential extraction of some heavy metals in soil cultivated with lettuce.

Sample	Exch.		Carb-ocd		Mn-ocd		Organic		Fe-ocd.		Sum seq.	TM	Rec. tot. met.
	%SS	%CV	%SS	%CV	%SS	%CV	%SS	%CV	%SS	%CV	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	%
Cd													
1	20	14	15	8.7	12	1.3	25	17	28	11	1.25	1.15	109
2	20	9.7	15	19	9.0	1.0	23	9.3	34	16	1.25	1.50	83
3	21	8.6	18	13	8.5	1.0	20	15	32	9	1.32	1.24	106
4	19	3.7	18	14	7.0	0.8	28	12	28	8.8	1.28	1.22	105
Co													
1	n.d.		9.1	1.0	49	2.4	15	8.7	35	7.0	17.6	40.6	43
2	n.d.		8.9	9.0	38	2.6	14	6.2	39	7.9	17.5	34.7	50
3	n.d.		5.9	2.0	37	3.1	14	8.2	43	5.2	18.4	38.5	48
4	1.6	6.6	6.9	2.6	34	2.4	17	7.8	40	7.7	18.9	35.5	53
Cr													
1	n.d.		1.3	5.2	4.4	2.1	27	3.0	67	10	16.2	49.8	32
2	n.d.		1.2	1.4	3.2	6.4	26	6.3	70	16	22.7	54.7	41
3	n.d.		1.2	2.2	2.7	10	18	5.0	71	6.4	30.3	89.0	34
4	n.d.		1.2	5.6	3.1	15	30	4.5	66	13	23.2	64.0	36
Cu													
1	11	2.4	19	4.5	4.4	2.8	33	6.0	32	6.8	272	476	57
2	11	1.7	20	3.1	4.6	0.9	31	4.0	33	6.1	273	445	61
3	12	2.8	20	1.4	4.8	3.4	32	18	32	6.8	300	486	62
4	11	2.0	19	2.6	4.3	1.6	36	11	29	7.2	291	513	57

Ni													
1	6.7	11	17	4.4	20	2.9	24	5.4	32	11	15.0	41.9	36
2	7.1	14	16	5.1	18	3.0	22	8.1	36	12	17.2	36.9	46
3	5.6	14	14	2.3	23	3.7	17	12	40	8.2	20.4	49.0	42
4	5.8	7.2	16	5.1	17	3.9	26	12	35	11	17.4	45.1	39
Pb													
1	n.d.		1.4	36	4.2	6.5	27	5.4	68	7.6	26.9	45.7	59
2	n.d.		1.6	31	3.8	9.2	24	3.4	70	5.9	31.7	38.3	83
3	n.d.		1.6	14	3.8	6.1	22	5.0	72	0.15	44.4	46.4	96
4	n.d.		1.3	13	3.0	12	29	8.5	67	7.7	36.2	37.7	96
Zn													
1	10	15	12	8.[illegible]	8.1	15	33	9.0	37	14	89.2	201	44
2	9.6	10	13	12	8.6	22	27	4.7	41	19	109	216	50
3	10.5	9	12	6.8	8.5	8.1	27	29	42	7.5	159	254	63
4	9.6	14	11	26	6.7	10.1	33	11	39	11	134	237	56

%SS=metal percent of total sequentially extracted and mean of six replications.
%CV=variation coefficient.
TM=total metal.
Rec. tot. met.=recovery of total metal.

Sequential extraction of heavy metals in soils

Steps	Forms	Extractants*	Equilibrium
1	Exchangeable	$1M$ $MgCl_2$	1 hour
2	Carbonate-occluded	$1\ M$ NaOAc	5 hours
3	Mn oxide-occluded	NH_2OH-HCl + 0.01 M HNO_3	30 minutes
4	Organically-bound	$K_4P_2O_7$	24 hours
5	Fe oxide-occluded	NH_2OH-HCl + 25% (v/v) HOAc	4 hours to 96°C

* 20 mL of each extractants solution was used.

The extracting solutions were prepared with an equivalent to 200 mg nitriloacetic acid (NTA) L^{-1} according to a study done by Howard and Shu (1996) to avoid the resorption effect of heavy metals.

Lettuce and celery plant samples were extracted from each sampling site in triplicate, divided with a plastic knife into shoot and root, washed, and dried at 60°C, then ground in an agatha mortar. For plant metal analysis, 0.500 g was weighed, acid digestion was performed in a microwave oven with a mixture of 4 mL HNO_3 and 2 mL H_2O_2, and then reduced to a volume of 20 mL. Metal determination in the digest was by flame atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Some general characteristics of the soils under study are shown in Table 1. In general, it may be observed that the values are similar except for the soil cultivated with lettuce where slightly higher values may be seen for pH and content of Fe and Mn free oxides.

In relation to the total metal content in the soils (Tables 2 and 3), it was found that Cu, Zn, and Cr occur in the highest concentration, and Cd in the lowest concentration of all the metals under study.

Sequential Extraction

The results show that with the application of this extraction methodology, Cd, Pb, and Cu exhibited the highest values of extracted metal in relation to total metals, with 83 to 109% for Cd, 57 to 97% for Pb, and 57 to 62% for Cu. Chromium had the lowest percent of extraction, 18 to 42%, indicating that most of the metal would be associated with silicates and in forms that cannot be identified with this methodology.

The Cd in the smallest concentrations in the soils is the most mobile and it may be found distributed in all of the fractions in the following order: occluded in iron oxides, associated with organic matter, exchangeable, carbonate, and associated with manganese oxides. Cobalt is mainly occluded in iron and manganese oxides followed by the form linked to organic matter and as carbonate. The small amount of Cr that may be extracted from these soils is associated mainly with iron oxides and to a smaller extent with organic matter. Copper is distributed in all of the

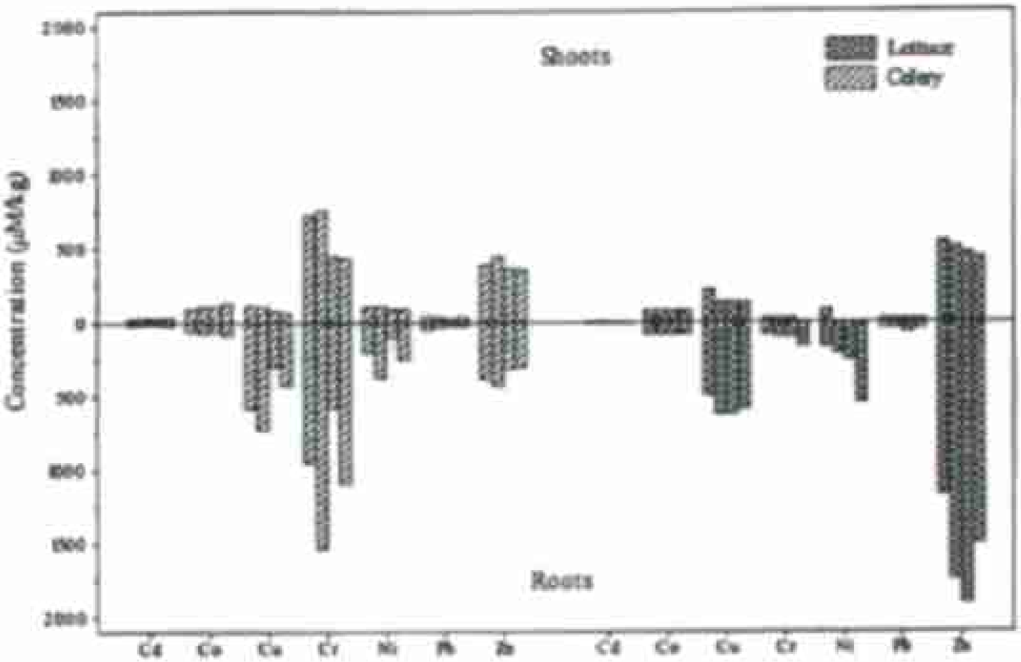

FIGURE 1. Heavy metal determinations in roots and shoots of celery and lettuce.

fractions and a difference is observed in the distribution of the metal in the soils under study. In the soil cultivated with celery, the fraction of Cu bound to iron oxides is predominant, while in the soil cultivated with lettuce, the fraction of the metal associated with organic matter prevails (Table 3). A difference may also be observed in the exchangeable fraction of this metal in the soils: exchangeable Cu occurs twice as much in the soil cultivated with celery which would decrease the amount of Cu associated with organic matter in this soil (Table 2). Nickel is also distributed in all of the fractions in increasing order from the exchangeable form to the Ni form associated with oxides which is the prevailing one. Adamo et al. (1996) found that Ni was less mobile than Cu since it occurs mostly in the residual form; similar results were found in this study, especially in one of the soils. Lead occurs mainly in two fractions, the same as Cr, associated with Fe oxides and with organic matter. This last aspect is in contrast with the findings of other authors who state that the metal has a high affinity with the surfaces of manganese oxides (Howard and Shu, 1996) which may be attributed to the low concentration of manganese oxides in these soils. Zinc is distributed in all of the fractions but the prevailing form is that associated with iron oxides and to organic matter in the case of the soil cultivated with lettuce, while Zn associated with iron oxides prevails in the soil cultivated with celery. Similar results were found in previous studies of these soils (Ahumada et al., 1997). It should be pointed out that in the case of

TABLE 4. Linear correlation between heavy metals found in roots and shoots of celery and the same metals found in the different forms in soil.

Element	Chemical form	R	Linear equation (n = 24)
		Root	
Co	Carbonate	0.54 ***	Y=58.2X - 1.08
Cu	Ox Fe	0.68***	Y=0.62X - 0.049
	Organic matter	0.54**	Y=1.28X - 0.79
Zn	Organic matter	0.79***	Y=1.39X + 0.042
		Shoot	
Cu	Organic matter	0.61***	Y=0.083X - 0.026
	OxFe	0.76***	Y=0.049X - 0.083
Cr	Ox Fe	0.67***	Y=2.66X + 0.088
Zn	Organic Matter	0.71***	Y=0.82X + 0.202

,*Statistically significant at probability level 0.01 and 0.001, respectively.
R=correlation coefficients.

Cd as well as Cu the forms that could be readily bioavailable, exchangeable and carbonate, make up about 40% of the total extracted. This would indicate that these metals in those forms would be absorbed by plants, which would constitute a risk since they could be transferred through the trophic chain to human beings. The prevailing form for all the metals is the one associated with iron oxides. This surface would be the reserve "pool" of heavy metals which could be released by a change in oxidation-reduction potential to become bioavailable forms.

Metals in Plants

A greater amount of Cd was found in celery than in lettuce and the contents of this element in roots as well as in shoots are similar. The same is observed for Co in lettuce, but in celery the Co content in the aerial part is slightly higher (Figure 1). Copper and Ni concentrate mostly in the radicular part. Similar results concerning Ni were found by Quian et al. (1996) in a study with wheat. They report that a small portion of Pb, Ni, and Co reaches the aerial part and that Co accumulates in the roots. In this study, Cu accumulation in the root is four to five times as great as in shoot. A greater content of Cr was found in celery than in lettuce, the highest concentrations being found in roots. Lead in celery is about equally distributed in shoots as in roots, while in lettuce there is twice the amount of this metal in the radicular part as in the aerial part. In relation to Zn, instead, differences are observed in its content in both parts, but lettuce shows a greater difference, most of the element being concentrated in the radicular part of the plant.

TABLE 5. Linear correlation between heavy metals found in roots and shoots of lettuce and the same metals found in the different forms in soil.

Element	Chemical form	R	Linear equation (n = 24)
		Root	
Cd	OxMn	0.70***	Y=3.02X + 0.011
Cu	Exchangeable	0.50**	Y=0.88X + 0.140
	Carbonate	0.68**	Y=0.92X - 0.217
	OxMn	0.57**	Y=2.31X + 0.119
Cr	Organic matter	0.53**	Y=0.60X + 0.042
Ni	Organic matter	0.78***	Y=11.5X - 0.469
Pb	Carbonate	0.70***	Y=0.032X + 9.05
	OxMn	0.91***	Y=0.0068X + 7.7
	OxFe	0.87***	Y=0.012X + 0.36
Zn	Exchangeable	0.63***	Y=4.17X + 0.80
	Carbonate	0.71***	Y=4.26X + 0.61
	OxMn	0.69***	Y=5.48X + 0.76
	OxFe	0.64**	Y=0.84X + 0.979
		Shoot	
Co	OxMn	0.80***	Y=0.08X + 0.037

,*Statistically significant at probability level 0.01 and 0.001, respectively.

R=correlation coefficients.

The relation between the different metallic forms and the metal content in the shoot and the root of the plants grown in these soils was determined through linear correlations (Table 4). Results in celery differed from those in lettuce (Table 5). Cobalt as carbonate in soil was found to correlate significantly with the content of this in celery roots; in addition, Cr contained in the aerial part correlated directly with the form of this metal bound to iron oxides. In lettuce, instead, Cu and Zn were found in the exchangeable form; Cu and Pb, as carbonate; Cd, Pb, and Zn, associated with manganese oxides; Cr and Ni complexed with organic matter and Pb and Zn bound to Fe oxides correlated significantly with these metals contained in roots. On the other hand, only Co contained in the aerial part of lettuce and the Co form linked to manganese oxides in soil correlated significantly.

CONCLUSIONS

It was found that, of the metals under study, those in the greatest concentration in soil are Cu, Zn, and Cr, while Cd is in the lowest concentration. Through the use of the applied sequential methodology, Cd, Pb, and Cu are extracted the most, while Cr is extracted the least, which indicates that most of this metal would be associated to silicates and in forms that cannot be identified with this extraction method. Cadmium is the most mobile metal, being distributed in almost all of the fractions to a significant extent. Most of the metals are associated with iron oxides and organic matter, and for Co the form bound to manganese oxides is also important. Metals in plants occur mainly in the radicular part, except Co and Cd that are similarly distributed in shoot and root. Evaluation of the relation of the metal in plants with the metal in the different fractions in soil performed through the study of linear correlation gave different results for lettuce and celery.

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