Distribution of extractable heavy metals in different soil fractions

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ABSTRACT

Due to the difficulties of precisely characterizing environmentally contaminated soil, the effects of heavy metals on plants are studied using uncontaminated soil spiked with known quantities of heavy metals. One problem in using spiked soils is how accurately the distribution of metals mimics stabilized natural soils. We studied the distribution of cadmium, chromium, copper, lead, nickel, and zinc in soil fractions after application in soluble form. The soil samples included a control (an uncontaminated Typic Argiudoll) and two samples spiked with either a moderate or high heavy metal concentration). After application of the salts the soils were subjected to wet/dry cycles over the course of three months. The soils were fractionated using a sequential chemical extraction procedure employing: (1) $CaCl_2$, (2) NaOH, (3) Na_2EDTA and (4) HNO_3, HCl, and HF. Soil physical separation was carried out by ultrasonic dispersion. The heavy metal levels were determined using ICP-AES. Each heavy metal displayed a unique behavior when added to soil in the form of soluble salts. Cadmium and zinc remained in the soluble fraction, indicating that no equilibrium was attained, while nickel primarily appeared in the insoluble fraction. Chromium, copper and lead were distributed among various soil chemical fractions. The highest levels of all metals appeared in the clay fraction except lead which was mainly present in the silt fraction.

Keywords: heavy metals, soil, fractionation, extraction

INTRODUCTION

The contamination of soils by heavy metals is a widespread problem. Sources of metal contamination include industry, mining, agriculture, and urban life. Metals can enter the soils as a result of direct application (*i.e.* through the use of biosolids) or as an involuntary consequence of human and natural activity, such as the settling of wind-borne particles. It has long been known (Karamanos *et al*., 1975) that native metals in soils are usually in low concentration and frequently in highly immobile form. Heavy metals from anthropogenic sources often appear in more active forms and tend to be more mobile and plant-available than those from pedogenic or lithogenic sources (Chlopecka *et al*., 1996; Tack and Verloo, 1995). As a result, up to 60% of heavy metals in contaminated soils and some natural soils developed from metal-rich parent materials occur in easily labile forms (Ma and Rao, 1997; Singh *et al*., 1995; Karczewska *et al*., 1998).

When heavy metals enter the soil, reactions with soil components lead to progressive conversion to insoluble forms. Heavy metals are incorporated into soil minerals or bound to various soil phases through a variety of mechanisms, particularly absorption, ion exchange, co-precipitation, and complexation (Navas and Lindhorfer, 2003). According to Koen and Janssen (2003) the mobility usually decreases very slowly and equilibrium is attained only after decades. Consequently, the forms and availability of heavy metals in soils are strongly determined by their origin and history. Due to these difficulties simulated

contamination of soils has been used to evaluate critical uptake by crops. It is possible to use soluble heavy metal salts but they have the disadvantage of introducing conditions that do not exist in natural or contaminated soils. When soluble salts are used the bioavailability of metals is overestimated (Basta *et al.*, 2005). This overestimation was demonstrated in greenhouse experiments (Korcak and Fanning, 1985) and under field conditions (Brown *et al.*, 1998; Giordano and Mays, 1977).

An alternative is to use spiked soils. Previous reports of the equilibration time for heavy metal soluble salts added to soils have ranged from days (Martínez and Motto, 2000) to years (Podlesäková *et al.*, 2001). The chemical and physical properties of each element are different, and distribution among the various soil fractions is also dependent on the soil properties. The results of sequential analyses display soil-specific features. There are several methods of soil fractionation, but all chemical fractionation techniques progress from the weakest extractants to the strongest, separating the metals into several fractions (Tack and Verloo, 1995). Heavy metal distribution in soils may also be examined on the basis of particle size. Finer particles typically show higher concentrations of heavy metals due to increased surface area, higher clay mineral and organic matter content, and the presence of Fe–Mn oxide phases (Haque and Subramanian, 1982; Zhang *et al.*, 2003). However, some authors (*e.g.* Yarlagadda *et al.*, 1995) have measured high concentrations of heavy metal in all textural fractions.

In summary, spiked soils mimic true contaminated soils to a certain extent. However, the similarity of the heavy metal distribution between natural and spiked soils and the consequent bioavailability is still in question. To study this, we applied soluble forms of Cd, Cr, Cu, Ni, Pb, and Zn to soil samples, incubated the samples for a short time, and examined the metal content of the chemical and physical fractions.

MATERIALS AND METHODS

Characteristics of the soil

The soil was a Typic Argiudoll, a conspicuous soil in the Pampas Region, Argentina, (Diaz-Zorita and Buschiazzo, 2006). Its main physical and chemical properties, using standard analytical techniques (Sparks *et al.*,1996) were: sand $171\,g\ kg^{-1}$; silt $571\,g\ kg^{-1}$; clay $258\,g\ kg^{-1}$ (pipette method of sedimentation); pH (1: $2.5\,w/v$) 6.1; organic matter (Walkley and Black method, wet digestion with sulfuric acid and potassium dichromate) $39.0\,g\ kg^{-1}$; total nitrogen (Kjeldahl method, wet digestion with sulfuric acid) $2.2\,g\ kg^{-1}$; extractable phosphorus (Bray and Kurtz method, extraction with ammonium fluoride and hydrochloric acid and colorimetric determination) $12.5\,mg\ kg^{-1}$; and exchangeable potassium $1.35\,mol\ kg^{-1}$ (extraction with ammonia acetate and atomic adsorption spectrophotometry determination). Torri and Lavado (2008) worked on the same soil and they found the mineralogy of this soil was dominated by nearly unweathered volcanic material such as plagioclases, glasses and lithic fragments. Minor components included quartz and orthoclase, as can be seen in the X-ray analysis (XRD) and SEM-EDS. In the clay fraction, Torri and Lavado (2008) found that the predominant mineral is illite with some montmorillonite and a lesser proportion of interstratified illite/montmorillonite.

Experimental

The treatments included (i) a control consisting of the uncontaminated Typic Argiudoll (C), (ii) a soil spiked with moderate levels of heavy metal (ML), and (iii) a soil spiked with high levels of heavy metal (HL). The ML samples were treated with $0.57\,g\ kg^{-1}$ chromic acid, $0.027\,g\ kg^{-1}$ $CdNO_3$, $0.54\,g\ kg^{-1}$ $CuCl_2$, $0.32\,g\ kg^{-1}$ $NiSO_4$, $0.80\,g\ kg^{-1}$ $Pb(NO_3)_2$, and $1.33\,g\ kg^{-1}$ $ZnSO_4$. The HL group received twice the quantity of the same salts as the soil in the ML treatment. After heavy metals application, the soils were subjected to three wet and three dry cycles over the course of three months.

The chemical fractionation of the soils was performed using the sequential extraction procedure described by McGrath and Cegarra (1992):

Fraction I (water-soluble and exchangeable materials): the samples were shaken with $0.1\,M$ $CaCl_2$ for 16 h at room temperature, and centrifuged at 3600 rpm. The weight of the tube and its contents were recorded and the supernatant was decanted and filtered through Whatman No. 42 filter paper. The weight of the wet residue in the tube was also recorded.

Fraction II (organic matter-bound fraction): the residue from fraction I was shaken with $0.5\,M$ NaOH for 16 h at room temperature, centrifuged as before, filtered and the weight recorded. Because

this reagent also extracted organic matter, the supernatant was digested in aqua regia.

Fraction III (inorganic, carbonate forms, and/or species associated with Fe and Mn oxides): A solution of 0.05 M Na₂EDTA was added to the residue of fraction II and was shaken for 1 h, centrifuged, filtered, and weighed as described previously.

Fraction IV (residual fraction mainly containing silicate minerals): This fraction was determined from the difference between the concentrations in the previous fractions and the total heavy metals concentration of the entire soil sample. Total heavy metal concentration in soils was obtained by acid digestion with a $1-1-1$ mixture of concentrated HNO_3, HCl, and HF (Shuman, 1979).

Soil physical separation was carried out by ultrasonic dispersion. The soil dispersion was performed using only distilled water: no dispersing agents or inorganic salts were added. The solution was stirred during the procedure (21.5 Hz and 300 mA) to obtain a homogeneous soil distribution, and the entire process was conducted at constant temperature. The $>53\,\mu m$ soil particles (sand fraction) were separated from the soil suspension by wet sieving and washed until the percolating water was clear. Particles smaller than 53 μm (clay and silt fractions) were obtained by sedimentation, applying the Stokes law of sedimentation. After collection, the fractions were oven dried for 48 h at 60°C. The heavy metals were extracted using a mixture of concentrated HNO_3, HCl, and HF (Shuman, 1979).

The heavy metals concentrations (Cd, Cr, Cu, Ni, Pb, and Zn) were measured using a Baird 20/70 inductively coupled plasma atomic emission spectrometer (ICP-AES). Three replicates were measured in all cases. Background correction was obtained using blank samples. All of analyses were checked against standard reference materials from NIST (# 1646– estuarine sediment).

The data were analyzed using the Statistix 8.0 program. Significant differences between the treatments were distinguished using Analysis of variance (ANOVA). In cases where F-test results were significant, the mean values were compared using the Tukey test, with $P < 0.05$ considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1 lists the results of the sequential chemical extraction procedure and the percentage of each

fraction in relation to total concentration of the studied heavy metals. This percentage gives a rough idea about heavy metal bioavalilability. In the control soil Cr, Cu, Ni, and Zn mostly appeared in the residual fraction and Cd and Pb were present in relatively similar proportions in all fractions. The total concentrations of heavy metals in the control soil were similar to concentrations measured in uncontaminated soils in the area (Lavado *et al.*, 2004). The distribution of heavy metals between the chemical fractions in the control soil resembled the distribution found in previous local studies (Lavado and Porcelli, 2000; Torri and Lavado, 2002). Sequential extraction of both spiked soils revealed that both behaved similarly and that the added heavy metals were located in different fractions independent of the applied heavy metal quantity. When soluble salts are initially applied to the soil, all of the metals are contained in Fraction I, which is considered mobile and easily absorbed by plants (Tack and Verloo, 1995). Most soluble metals are adsorbed on the soil surface in exchangeable form and then slowly transformed into stable forms with time (McLaughlin, 2001; Madrid *et al.*, 2001). Cadmium and Zn were predominantly located in Fraction I. The availability of both elements is confirmed by the fact that Cd in Fraction I meant 40 to 50% of total Cd, while Zn in Fraction I meant 50 to 65% of total Zn (Table 1). Instead, the greater portion of Cu was found in Fractions II and III. In Fraction II, Cu could form complexes with several of the functional groups found in humic and fulvic acids (Stevenson, 1982). It is well known that Cu remains linked to organic matter when it is applied to soils containing an organic matrix such as biosolids or manure. Copper can also be incorporated into the organic matter fraction when it is applied to soil in inorganic form (Torri and Lavado, 2008). Lead was mainly associated with Fraction III, the fraction linked to carbonates and/or oxides of Al, Fe, and Mn. The metal-binding activity of this fraction and the organic matter fraction depend on the physical and chemical characteristics of the soil, including soil pH and the content and type of clays, organic matter, and oxides (Ma and Rao, 1997; Karczewska *et al.*, 1998). Nickel was bound in similar proportions to Fractions I, II, III, and IV, while Cr was mainly located in Fraction IV, the insoluble soil fraction mainly comprising structural, silicate minerals. This fraction is generally considered inert and in this case it comprises 80– 90% of total Cr (Table 1). The presence of Cr in this fraction is in agreement with the fact that the native

Table 1 Cadmium, Cr, Cu, Ni, Pb and Zn concentrations (mg kg^{-1}) and percentage in each fraction obtained after the chemical sequential extractions

Heavy Metals	Fraction	Treatments					
		Control	%	Medium L.	%	High L.	%
Cd	I	0.2 ± 0.0	18.2	7.0 ± 0.4	39.1	12.0 ± 0.6	48.2
	II	0.3 ± 0.0	27.3	1.9 ± 0.1	10.7	1.9 ± 0.0	7.6
	III	0.2 ± 0.0	18.2	4.0 ± 0.2	22.3	6.4 ± 0.3	25.7
	IV	0.4	36.3	5	27.9	4.6	18.5
	Total	1.1	100	17.9	100	24.9	100
Cr	I	0.1 ± 0.1	0.5	1.3 ± 0.3	0.3	1.9 ± 0.8	0.1
	II	0.5 ± 0.1	2.3	14 ± 0.8	3.4	24.4 ± 3.0	2.9
	III	1.0 ± 0.1	4.7	59.8 ± 9.8	14.6	83.5 ± 33.5	10.2
	IV	19.7	92.5	335.3	81.7	725.0	86.8
	Total	21.3	100	410.4	100	834.8	100
Cu	I	0.5 ± 0.1	1.9	4.4 ± 0.1	1.2	31.1 ± 1.0	3.3
	II	6.8 ± 0.1	25.5	147.8 ± 11.7	39.9	301.9 ± 4.5	32.2
	III	6.2 ± 0.2	23.2	145.7 ± 6.1	39.2	395.1 ± 8.6	42.0
	IV	13.2	49.4	74.2	19.9	211.2	22.5
	Total	25.5	100	372.1	100	939.3	100
Ni	I	0.3 ± 0.0	2.1	20.8 ± 1.9	19.4	46.0 ± 4.0	19.3
	II	0.9 ± 0.1	6.3	26.5 ± 0.8	24.6	43.0 ± 1.1	18.0
	III	1.1 ± 0.2	7.7	35.2 ± 2.0	32.7	58.7 ± 1.8	24.6
	IV	12.0	83.9	25.1	23.3	90.8	38.1
	Total	14.3	100	107.6	100	238.5	100
Pb	I	4.1 ± 0.6	18.7	14 ± 0.5	2.6	94.5 ± 8.2	6.9
	II	3.4 ± 0.2	15.5	19.4 ± 2.1	3.6	94.8 ± 6.7	7.0
	III	7.9 ± 0.5	36.1	488.3 ± 11.9	89.7	1095.8 ± 185.1	80.7
	IV	6.5	29.7	22.6	4.1	73.5	5.4
	Total	21.9	100	544.3	100	1358.6	100
Zn	I	0.9 ± 0.1	1.8	225.8 ± 11.7	53.2	802.5 ± 36.1	65.1
	II	0.7 ± 0.1	1.3	23.4 ± 2.5	5.5	62.3 ± 1.9	5.1
	III	6.3 ± 0.3	12.3	156.6 ± 7.8	36.9	273.4 ± 14.1	22.2
	IV	43.5	84.6	18.6	4.4	94.0	7.6
	Total	51.4	100	424.4	100	1232.2	100
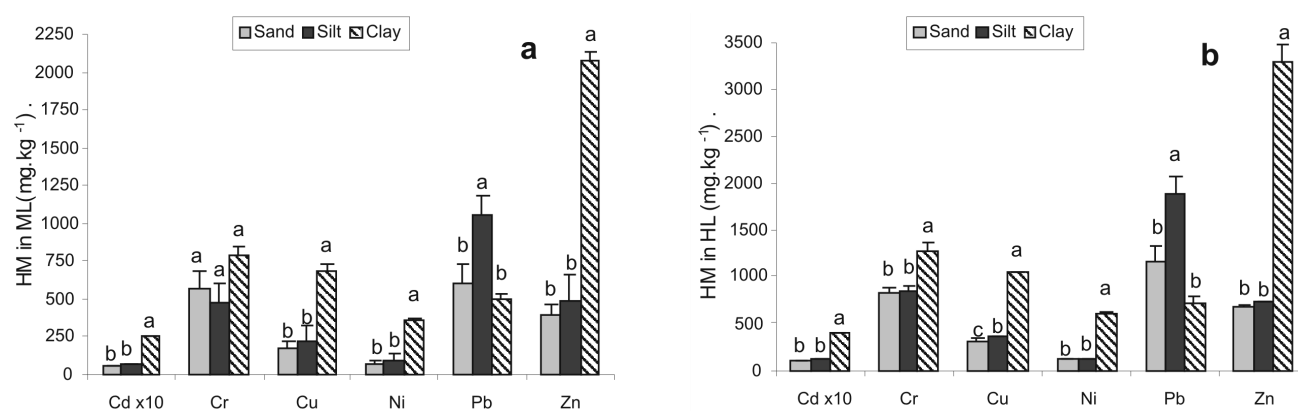
Figure 1 Distribution of Cd, Cr, Cu, Ni, Pb and Zn among soil physical fractions (clay, silt and sand). (**a**) Medium level treatment; (**b**) high level treatment.

minerals of Cr are practically inert (Pais and Benton Jones, 1997).

Figure 1 illustrates that Cd, Cu, Ni, and Zn were clearly located in the clay fraction for both spiking levels, although the concentration changed with the applied dosage. Chromium was also mainly associated with the clay fraction but was found more evenly distributed in other fractions than the remaining elements. This may be attributed to its large specific surface area and concomitant increase in the proportion of reactive substrates. However, other experiments have demonstrated that heavy metal sorption is more related to clay mineralogy than clay quantity (Appel and Ma, 2002). In soil treated with high levels of heavy metals, Pb was primarily located in the silt fraction. The occurrence of reactive components such as sesquioxides in the silt fraction would account for the binding of Pb in this fraction (Sutherland, 2003). This also agrees with the location of Pb in the Fe–Mn oxide fraction.

CONCLUSIONS

Each heavy metal displayed a unique behaviour when added to soil in the form of soluble salts. Cadmium and Zn remained in the soluble fraction, which would indicate that equilibrium was not attained, while at the other extreme most of the Cr was located in Fraction IV. Copper, Ni, and Pb were distributed among several soil chemical fractions. The concentrations of Cd, Cr, Cu, Ni, and Zn were highest in the clay fraction, while Pb was mainly located in the silt fraction. The results of the chemical and physical fractionation experiments were not completely correlated. In the spiked soil the added metals were located in different fractions depending on the nature of the metal. The behavior of each heavy metal, including the bioavailability, can not be compared with other metals. This fact must be taken into account in soil/plant studies.

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