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Phytoavailability and Solid-Phase Distribution of Chromium in a Soil Amended with Organic Matter

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The uptake of metals by plants may be hazardous to human health when edible plants are cultivated on contaminated soils. The ability of plants to absorb metallic pollutants and accumulate them in roots or translocate them to the aerial biomass (phytoextraction) depends on the availability of metal in soil, being its movement from soil to root the limiting step for absorption (Chaney 1988). It is widely acknowledged that the availability of metals in soils is affected by the quantity and quality of organic matter (OM) either natural or added (i.e. fulvic and humic acids), which may interact with heavy metals, forming chelates and complexes with different stability. Yuan and Lavkulich (1997) observed that the addition of humified OM to soils contaminated with bivalent metals reduced their bioavailability as a consequence of higher geochemical stability in soils. However, OM with a high content of soluble organic carbon might form soluble metallic complexes and promote their absorption by plants (Walker et al. 2003). The geochemical distribution of metals in the solid phases of soils may be determined by fractionation methods (sequential chemical extractions) allowing the distinction between metals linked to highly mobile forms, potentially mobile forms, and residual forms with great environmental stability. Chromium (Cr) is a relevant environmental pollutant as a result of industrial uses. In nature, Cr exists in two different stable oxidation states; trivalent (Cr^{III}) and hexavalent (Cr^{VI}) chromium. Both Cr^{III} and Cr^{VI} differ in terms of mobility, bioavailability and toxicity. Chromium hexavalent is more toxic than Cr^{III}, whereas Cr^{III} is required in trace amounts as an inorganic nutrient for animals. The transformation of Cr^{VI} to Cr^{III} tends to occur in anoxic soils and sediments. Chromium trivalent may be adsorbed on clays, OM, Fe and Mn oxides, and other negatively charged surfaces, as opposed to Cr^{VI} which does not significantly interact with these fractions. Plants absorb Cr in both states of oxidation, and high concentrations may reduce growth and induce leaf chlorosis (Panda and Patra 1997).

The Reconquista is a lowland river of Buenos Aires Province, and one of the most contaminated rivers in Argentina. Sediments contain high concentrations of heavy metals originated in metallurgic, tanning and dyeing industries, many of which spill effluents directly into the river. Over the last years land management work has been undertaken in order to deepen the river bed and improve rainwater drainage, preventing periodic floods. The aim of this work was to evaluate the

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effects of organic amendments on the distribution of Cr and its phytoextraction by *Lactuca sativa* var. *capitata* in a contaminated soil derived from dredged sediments.

MATERIALS AND METHODS

A soil derived from sediments dredged from Reconquista river was selected for this study. Total concentrations of metals surpassed the established limits for agricultural soils in Argentina (Law 24051, Hazardous wastes). Soil samples were collected from the upper 20 cm, air-dried and passed through a 2 mm plastic sieve. Two organic amendments were used, compost of cattle ruminal content and straw (CRC) with three-month composting time, and vermicomposted CRC with five-month vermicomposting time with *Eisenia fetida* (VCRC). Table 1 shows the main characteristics of soil (S) and amendments.

Table 1. Soil and organic amendments characteristics.

	S	CRC	VCRC		S	CRC	VCRC
pH	7.41	8.14	6.96	Ca (mg kg^{-1})	8577	35225	14550
Clay (%)	28.0	ND	ND	Mg (mg kg^{-1})	194	182.5	198.5
Silt (%)	48.2	ND	ND	Fe (g kg^{-1})	3225	1825	2940
Sand (%)	23.8	ND	ND	Cd (mg kg$^{-1)}$	3.75	nd	nd
EC (dS m^{-1})	2.20	6.72	3.30	Cr (mg kg^{-1})	1510	45.0	55.0
CEC(cmolckg^{-1})	11.9	115.5	80.8	Cr(VI) (mg kg^{-1})	<2	<2	<2
OM (%)	12.9	56.4	23.4	Cu (mg kg^{-1})	197.5	35.5	60,0
TOC (%)	6.45	28.2	11.6	Pb (mg kg^{-1})	158	13.5	53.5
C/N ratio	9	25	11	Zn (mg kg^{-1})	855	157.0	152.8

ND not determined, nd not detected, EC electrical conductivity, CEC cationic exchangeable capacity, OM organic matter, TOC total organic carbon.

A two-step extraction procedure (Wu and Ma 2002) was employed to separate the OC from the organic amendments into two fractions: water soluble organic carbon (WSOC) and water insoluble organic carbon (WIOC). Samples were first extracted with water (solid to solution ratio 1:10) for 2 hrs and then centrifuged at 10000 rpm for 20 min and filtered through a 0.45 μm membrane filter. The filtrate was called WSOC. The residue of the first step (WIOC) was extracted with a 0.1 *M* NaOH solution for 24 hrs (solid to solution ratio of 1:10). The NaOH extraction was conducted by shaking the suspensions in a horizontal shaker at room temperature. At the end of shaking the suspension was centrifuged at 10000 rpm for 20 min. and filtered through a 0.45 μm membrane filter. An aliquot of the filtrate (HA+FA) was adjusted to pH = 2 with H_2SO_4 and then centrifuged at 10000 rpm for 20 min. and filtered through a 0.45μm membrane filter. The precipitate collected at the bottom of centrifuge tube was called humic acid (HA), while the filtrate containing fulvic acid and other nonhumic substances is referred to as fulvic acid (FA) (Swift 1996). The OC content of each fraction (WSOC, $WIOC_{(HA+FA)}$, $WIOC_{FA}$) was determined according to a wet dichromate oxidation procedure (Golterman 1987). Extraction and fractionation were done in a single replicate on each sample, while determination of the organic content in the extracts was performed by triplicate. The OC concentration of the $WIOC_{HA}$ fraction was calculated as the difference between $WIOC_{(HA+FA)}$ and $WIOC_{FA}$.

Mixtures of soil and amendments (air-dried and sieved) were thoroughly homogenized and placed in 500 g plastic pots. A completely randomized design was used with six replicates (pots) per treatment. Three treatments were used: soil control with no amendment (S), a compost amendment (CRC) and vermicompost amendment (VCRC). The amendments were added to the soil in order to increase OC by 2%. Pots were placed in a greenhouse (22 ± 6 °C) and humidity was maintained at 70% field capacity by adding distilled water every two days. After 90 days, 20 day-old seedlings of lettuce (*Lactuca sativa* var. *capitata*) were transplanted. Pots were fertilized every week with 1.5 mg of N, 0.5 mg of P and 0.5 mg of K in 10 ml of water, to prevent nutrient deficiencies. At harvest (4 weeks later), plants were cut just above the soil surface; aerial part and roots were separed, washed with deionized water, oven-dried at 70°C for 24 hrs and weighed. Dry matter (DM) was digested with HNO_3 and $HClO_4$ (5:1 v/v).

Distribution of Cr in different soil phases for each treatment was determined in humid samples, according to the sequential extraction scheme proposed by Tessier et al. (1979). Five fractions were obtained: soluble and exchangeable (F1), bound to carbonate (F2), bound to amorphous Fe and Mn oxides (F3), associated to OM (F4) and residual (F5). Chromium concentrations in all the extracts were measured by AAS with air/acetylene flame. The results of the analysis of the wet mixtures are quoted as dry weight of soil. Statistical analysis was performed with Statistix 8 software. Data was analysed using one-way analysis of variance (ANOVA), and means were compared using the Tukey test at the 0.05 level of significance.

RESULTS AND DISCUSSION

Organic matter fractions in CRC and VCRC are shown in Table 2. The CRC had a higher concentration of WSOC, lower C_{HA}/C_{FA} ratio and lower humification ratio ($HR=100xC_{HA+FA}/TOC$) than VCRC, indicating that worm composting produced a more humified material than CRC. Organic carbon extracted with NaOH was 30% of TOC in CRC and 42% in VCRC. The quantities of WSOC, C_{FA} y C_{HA} added to each pot in treatments with CRC and VCRC are shown in Figure 1. The addition of an equal amount of TOC determined an equal mass of WSOC in both treatments, and a mass of C_{HA} higher than C_{FA} in the treatment with VCRC, while VCRC contributed with a high mass of C_{HA} and an extremely low mass of C_{FA}

Table 2. Characteristics of organic matter in amendments.

Material	TOC (g/kg)	WSOC(g/kg)	C_{FA} (g/kg)	C_{HA} (g/kg)	HR	C_{HA}/C_{FA}
CRC	269.9	2.2	78	2.0	29.6	0.02
VCRC	95.7	1.0	9.4	31	42.2	3.3

Chromium hexavalent is not thermodynamically stable in soils, becoming rapidly reduced to Cr^{III} (Cary et al. 1977). Speciation of Cr in soil (Table 1) showed that it is mainly present as Cr^{III}, probably due to Cr^{VI} reduction in the anoxic

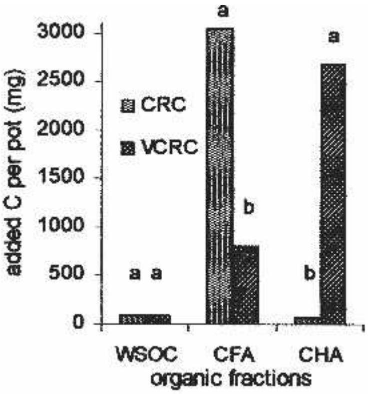

Figure 1. Mass (mg) added to each pot of soluble organic carbon, carbon in fulvic acids, and carbon in humic acids. Different letters denote significant differences between treatments ($p<0.05$).

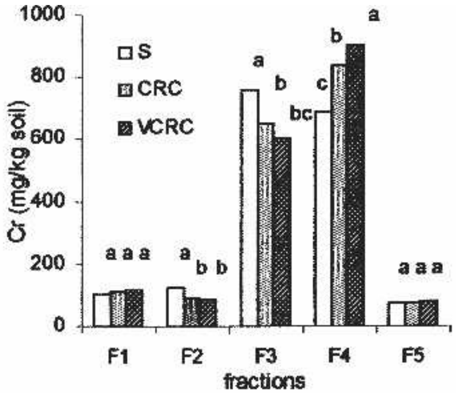

Figure 2. Distribution of Cr in soil for each treatment. Values of Cr-F1 and Cr-F2 shown in graphic are ten times the measured concentration of Cr. Different letters (in same fraction) denote significant differences between treatments ($p<0.05$).

environment of river sediments prior to dredging. Chromium in CRC and VCRC was also found in its reduced form.

The pattern of Cr distribution in non residual fractions of the control treatment (Figure 2) was the following: F3>F4>F2>F1. The elevated concentration of Cr in the reducible fraction (F3) is in accordance to Rantsar-Kalio et al. (2001) who found that immobilization mechanisms for Cr^{III} were precipitation or adsorption on Fe oxide surfaces. Chromium concentrations in F1 fraction in CRC and VCRC treatments (1.14 and 1.16 mg kg^{-1} respectively) did not differ significantly from control (1.02 mg kg^{-1}), although values were slightly higher in all treatments with addition of OM. This may be due to the increase of cation exchange capacity (CEC) in treatments with the addition of OM (21.4 and 24.8 $cmol_c kg^{-1}$ in CRC and VCRC respectively) regarding the control (11.8 $cmol_c kg^{-1}$) or to the formation of metallic complexes with dissolved OM (Kalbitz and Kaiser 2003). Antoniadis and Alloway (2002) found that the addition of WSOC increased metal availability for plants, particularly for sandy soils. Though in soils with a high proportion of thin fractions, as the soil of this study (Table 1), the added WSOC can have been fixed on sorption surfaces, for which it did not increase significantly the Cr solubility. Another factor that might disable the formation of soluble organo-metal Cr^{III} complexes would be the high calcium content in soil and amendments (Table 1), for which the mass effect would favor the saturation of the union sites with calcium ions (Walker et al. 2003).

On the other hand, Cr in F2 and F3 decreased in treatments with amendments (Figure 1) and increased in the OM fraction. These results might suggest that the addition of OM causes a redistribution of Cr reducing its mobility in soils. The

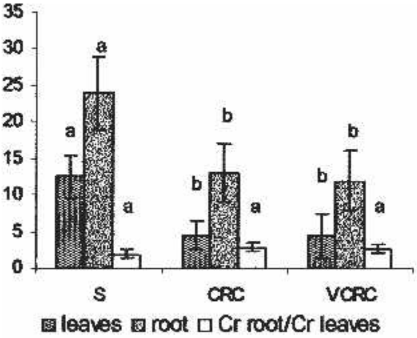

Figure 3. Concentration of Cr (mg/kg) in leaves and roots and ratio Cr_{root}/Cr_{leaves} of lettuce for each treatment. Different letters denote significant differences between treatments ($p<0.05$).

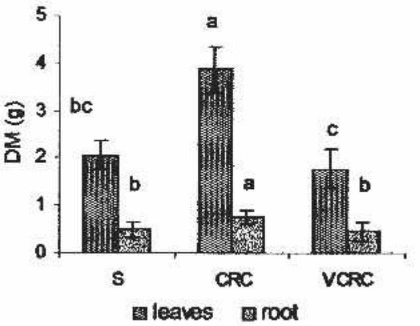

Figure 4. Dry matter production in leaves and root of lettuce for each treatment. Different letters denote significant differences between treatments ($p<0.05$).

treatment with VCRC produced a significantly higher concentration of Cr in the organic matter fraction than the treatment with CRC, indicating that an elevated humic acid to fulvic acid ratio in the organic materials (Figure 1) enhance Cr complexation. The low percentage of Cr in the soil residual fraction (3% of total Cr) indicates the prevailing anthropogenic origin of this element. Addition of OM did not modify Cr concentration in this fraction, in agreement with published data indicating that Cr is not released from this phase in normal conditions.

Levels of Cr in plants growing in non-contaminated soils range from 0.02 to 0.2 mg kg^{-1} (Kabata-Pendias and Pendias 1992). Although Cr^{III} is an essential dietary component in extremely low concentrations, low toxicity of ingested Cr^{III} is under discussion (Goyer 1995). The concentration of Cr in the leaves of lettuce in the control treatment was 12.5 mg kg^{-1}. The addition of amendments depleted it by 56% and 64% in treatments with CRC and VCRC respectively (Figure 4), being the concentrations of Cr in treatments with amendments lower than the toxicity level of 6-30 mg kg^{-1} for vegetables (Kabata-Pendias and Pendias 1986). Some studies showed that Cr^{III} and Cr^{VI} absorbed from nutritive solutions was mostly retained in roots and scarcely translocated to stems and leaves (Zayed et al. 1998), with a ratio $Cr_{root}/Cr_{leaves} \sim 100$. Though Cary et al. (1977) have observed that leaf vegetables that accumulate Fe are efficient in translocating Cr from roots to leaves. In our experiment the relation Cr_{root}/Cr_{leaves} had a value of 2.4 (the three-treatment average) being the Fe concentration in lettuce of 99.9 mg kg^{-1} (dry weight).

According to Xian (1989), the bioavailability of heavy metals is controlled by its chemical forms in soil, being the forms exchangeable and carbonates the most important in metal absorption for plants. In this work, the Cr concentration decrease in the aerial biomass of lettuce (Figure 3) in CRC and VCRC treatments, it's consistent with the decrease of Cr associated to carbonates and amorphous oxides and with the increase of it associated to humic substances.

Dry matter production in leaves and root of lettuce (Figure 4) was greater in the CRC treated soil than in the rest of the treatments. The differences in growth of *Lactuca sativa* between the CRC and the VCRC do not relate to the tissue chromium level which did not show significant differences between these two treatments. In addition, nutrient concentrations in leaves (Table 3) were similar for all treatments, indicating that differences in biomass were not related to mineral nutrition.

Table 3. Nutrient concentrations in leaves (dry weight basis) of lettuce.

Treatment	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Fe mg/kg
S	3.4 ab	0.72 a	6.1 a	0.50 a	1.6 a	102.1 a
CRC	4.1 a	0.69 a	5.3 a	0.60 a	2.1 a	99.8 a
VCRC	3.9 a	0.78 a	5.1 a	0.63 a	1.8 a	101.8 a

The same letter in each column does not show significant differences between treatments ($p>0.05$).

The greater biomass obtained with CRC in our experiment, might be related to the high concentration of fulvic acids in the CRC amendment, regarding VCRC (Figure 1). The humic substances are taken up into plant tissues, and those of low molecular mass are more easily absorbed and promote plant growth (Nardi et al. 2002). However, the remarkable increase in aerial biomass in CRC treatment (compared to control without OM addition) did not result in a greater uptake of Cr (25.7 µg/plant and 17.4 µg/plant in S and CRC respectively).

The results suggest that soil amendments with exogenous organic matter from composted and vermicomposted slaughter house wastes could constitute a short-term, cost-effective alternative for reutilization of chromium contaminated soils in marginal areas. Subsequent work is necessary to be performed to determine if the obtained results in short-term green-house experiments can also be obtained in field conditions in a long-term.

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