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FIRST PROOF

Changes in the speciation, partitioning and phytoavailability of chromium induced by organic soil amendments

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ABSTRACT

This study investigated the effect of two organic amendments (compost of cattle ruminal content and *Sphagnum*-moss peat) on the reduction of hexavalent chromium and the distribution of this metal among the main solid phases of a soil with low organic matter content treated with different levels of Cr(VI) (0–2000 mg Cr kg^{-1} soil). At the same level of added organic carbon, the peat reduced Cr(VI) added to the soil from 250 to 2000 mg kg^{-1}, with 100% efficiency. The reduction efficiency of the compost, however, decreased with the increasing dose of Cr(VI) soil. The distribution of Cr between the different soil components was evaluated by a sequential chemical extraction procedure. The concentration of water-soluble and exchangeable Cr decreased with the addition of organic amendments to the soil, whereas Cr increased in the organic fraction. The effect of added organic material on the Cr absorption was examined with two ornamental plants (*Melissa officinalis* and *Begonia semperflorens*). The increased Cr(VI) in the soil increased the Cr concentration in plant tissues. The addition of organic matter produced a greater aerial biomass for each level of added Cr in comparison with unamended soil. *Sphagnum* moss peat was more effective than the compost to decrease the total Cr and the Cr(VI) concentration in the water-soluble and exchangeable fraction of soil, thereby reducing the Cr accumulation in plants tissues and phytotoxic symptoms.

Keywords: soil, Cr(VI) reduction, fractionation, organic amendments

INTRODUCTION

The ability of plants to absorb metallic pollutants and accumulate them in roots or translocate them to the aerial biomass depends on the availability of each metal in soil. The main factor that limits metal being taken up by the roots is low transport from soil particles to the root surface. For most metals, this transport takes place in soil solution. In soil, metal solubility is limited due to adsorption to soil particles (Chaney, 1988).

The availability of metals in soils is strongly related to the soil geochemical phases to which they are linked (Maiz *et al*., 1997). The distribution of metals in the solid phases of soils may be determined by fractionation methods (sequential chemical extractions) (Filgueiras *et al*., 2002; Kozuh and Stupar, 2003) allowing the distinction between metals linked to highly mobile forms, potentially mobile forms, and residual forms with high environmental stability.

Chromium (Cr) is among the most common metal contaminants in soils. Although Cr can exist in several redox states, the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms are more stable. Hexavalent chromium contamination results from improper disposal from a variety of industrial operations including stainless steel manufacturing, chrome plating, leather tanning, wood treatments, dyes, and pigments (Proctor *et al*., 1997).

The mobility and toxicity of Cr in both terrestrial and aquatic environments depends on its oxidation state. The Cr(VI) is more toxic and more mobile than Cr(III) since under acidic to slightly alkaline conditions the $HCrO_4^-$, CrO_4^{2-} and $Cr_2O_7^{2-}$ species are strongly adsorbed in soils. On the other hand, Cr(III) can be adsorbed on clays, Fe and Mn oxides and other negatively charged surfaces of soils and sediments. Due to this, *in-situ* reduction of toxic Cr(VI) to less hazardous Cr(III) is becoming a strategy to reduce adverse environmental effects of pollution with Cr(VI) (Bolan *et al*., 2003). Commonly Cr(VI) reductants in soils are organic matter (OM) and Fe(II) (Tokunaga *et al*., 2003). Plants absorb Cr in both states of oxidation, and high concentrations may reduce growth and induce leaf chlorosis (Panda and Patra, 1997). Literature surveys show that few workers have reported ameliorative

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measures for Cr toxicity in crop plants (Shanker *et al*., 2005; Bank *et al*., 2005). This is mostly because most of the reported studies (Meers *et al*., 2005; Fengxiang *et al*., 2004) have been focused on enhancing phytoaccumulation of Cr in plants for its use in phytoremediation. The addition of OM to polluted soils can be used to modify Cr bioavailability, speciation, plant absorption and translocation processes (Al Chami, 2007; Rendina *et al*., 2006). While there are several studies on the effects of soil OM (natural or exogenous) on the Cr distribution in contaminated soils (Balasoiu *et al*., 2001; Kozuh and Stupar, 2003; Barajas-Aceves *et al*., 2007), the reduction efficiency of Cr(VI) to Cr(III) (Bolan *et al*., 2003; Kozuh *et al*., 2000; Losi *et al*., 2004; Banks *et al*., 2005) and the physiological effects of Cr in plants (Shanker *et al*., 2005), there are few studies that evaluate the effects of the addition of organic amendments to contaminated soils with Cr(VI) on the bioavailability of Cr in relation to changes in speciation and the partitioning of Cr in soil.

In order to determine the effect of organic amendment in the form of compost of cattle ruminal content and straw and *Sphagnum*-moss peat on Cr speciation and soil phase distribution, and its accumulation in plants, a greenhouse pot experiment was carried out using two ornamental plants (*Melissa officinalis* and *Begonia semperflorens*) grown on an artificially Cr(VI) contaminated soil amended or not with compost or peat.

METHODOLOGY

Pot experiment

An experiment was conducted using an uncontaminated typical Argiudoll soil of the Buenos Aires province with low organic matter content (2.2%). Three treatments were used: soil (control) without amendment (S), amended soil (50 g organic carbon kg^{-1}) with a commercially available compost from cattle ruminal content and straw (CS), and amended soil (50 g organic carbon kg^{-1}) with *Sphagnum*-moss peat (SP), from the Tierra del Fuego province. Soil and amendments were air dried and passed through a 2-mm plastic sieve. Mixtures of soil and amendments were thoroughly homogenized and then subjected to physical-chemical characterization. The reductive capacity (RC) for Cr defined as mg Cr reduced per kg of soil-amendment mixture was calculated from easy oxidisable organic carbon (OXOC) values determined by the Walkley-Black method (Walkey and Black, 1934) taking into account that 1.00 g of organic carbon reduce 5.78 g of Cr(VI) (Adriano, 2001).

The trial was conducted in plastic pots and included six repetitions per treatment. Water content was maintained during two weeks at 80% of the water holding capacity of the soil by adding deionized water. They were subsequently contaminated with 0, 250, 500, 1000 and 2000 mg Cr kg^{-1} of Cr(VI) using $K_2Cr_2O_7$ and maintaining the same moisture content for a month. After incubation, seedlings of *Melissa officinalis* and *Begonia semperflorens* were transplanted into pots. To avoid nutritional deficiencies Hoagland's solution (Hoagland and Arnon, 1950) was added once a week. At harvest (3 weeks after transplanting) the plants were separated into root and shoots (leaves plus stems) using a plastic knife. The biomass was washed with deionizer water and dried at 70°C for 24 h. The dry weights were recorded and the plant materials were ground using a Cr-free stainless steel grinder. The dry matter was digested with HNO_3 and $HClO_4$ (Miller, 1997) and Cr concentrations in the digest of plants were measured by AAS.

Fractionation and speciation of Cr in soils

Distribution of Cr in different soil phases for each treatment was determined according to the sequential extraction procedure proposed by Tessier *et al*. (1979) adapted for humid samples.

Four fractions were obtained: soluble and exchangeable (F1), bound to carbonate (F2), bound to amorphous Fe and Mn oxides (F3) and associated to OM (F4). Chromium concentrations in all the extracts were measured by AAS. In order to evaluate the reduction efficiency (RE) of Cr calculated as $100 \times [Cr(III)/Cr(VI)]$ (Bolan *et al*., 2003), the speciation of Cr in soil was carried out. The Cr(VI) of soil was extracted with H_2SO_4 0.18 M (Pettine and Capri, 2005). Additionally, the Cr speciation was determined (Cr(VI) and the Cr(III) content) in the F1 fraction. In all extracts the Cr(VI) concentration was determined colorimetrically by UV-Vis spectrophotometry with 1,5-diphenylcarbazide (EPA Method 7196A) at 540 nm, and the Cr(III) concentration was calculated as the difference between the total Cr concentration and the Cr(VI) concentration present in the F1 fraction or in the whole soil. The calculation of Cr concentrations in soils was done on dry weight basis.

Quality assurance, quality control and statistical analysis

Quality assurance and quality control measures on soils and plants included replicate analysis, metal spike and blanks. The accuracy of the analytical procedure for total Cr determination in plants was checked using SRM 1573 (tomato leaves reference material) from the National Institute of Standards and Technology, USA. Replicate analysis of this SRM showed good accuracy, with recovery rates for Cr between 94 and 103% for plant reference material. Statistical analysis was performed with Statistix 8 software (2003). Data were analysed using analysis of variance (ANOVA), and means were compared using the Tukey test at the 0.05 level of significance.

RESULTS AND DISCUSSION

Characteristics of soil and soil amended with compost and peat

The main characteristics of control soil and amended soil with compost and *Sphagnum* moss peat are shown in Table 1. The addition of amendments did not change the texture silt-loam (USDA classification) of soil. Soil pH value was 6.1 (slightly acidic). Both amendments modified substantially the pH value of soil. The soil amended with compost was slightly alkaline (pH 8.2), and the soil

Table 1 Characteristics of unamended and amended soil with compost and *Sphagnum* moss peat

	Soil	soil + compost	soil + peat	Method
Sand (%)	5.9	12.4	15.0	Hydrometer measurement (Gee and Bauder, 1986)
Silt (%)	72.8	67.7	66.5	Hydrometer measurement (Gee and Bauder, 1986)
Clay (%)	21.2	19.8	18.5	Hydrometer measurement (Gee and Bauder, 1986)
pH	6.1	8.2	4.1	Solid to water ratio 1 : 10 (McClean, 1982)
EC (dS m^{-1})	1.31	9.50	0.72	Saturated paste method (Rhoades, 1982a)
CEC ($cmol_c$ kg^{-1})	7.5	10.2	17.1	Ammonium acetate. pH 7 (Rhoades, 1982b)
TOC g/kg)	1.1	51.9	52.2	LECO combustion technique (Tabatabai and Bremmer, 1991)
Total Cr (mg kg^{-1})	10.0	8.1	7.4	Acid digest with HNO_3-HCLO_4 (AOAC, 1990)
Cr(VI) (mg kg^{-1})	<2	<2	<2	Extraction with H_2SO_4 0.18 M (Pettine and Capri, 2005)

EC, electrical conductivity; CEC, cationic exchange capacity; TOC, total organic carbon. Results are the mean of three replicates.

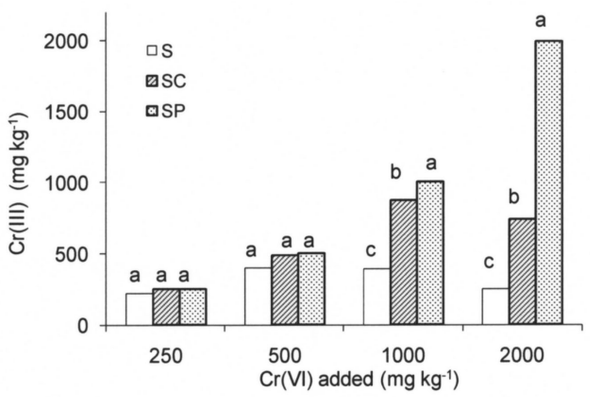

Figure 1 Cr(III) concentration for each treatment (S, unamended soil; SC, soil amended with compost; SP, soil amended with peat). Different letters indicate significant differences ($P < 0.05$) between treatments for each Cr(VI) level. Values are means ($n = 6$).

amended with peat was acidic (pH 4.2). The soil amended with compost has considerably more dissolved salts (EC = 9.5 dS m^{-1}) than the soil amended with peat (EC = 0.72 dS m^{-1}). Composts usually show a high salt and nutrient content. The organic matter added to soil increased the CEC, which would be attributed to the incorporation of organic components from compost and peat. Total organic carbon (TOC) content of the soil amended with compost and peat was 51.9 and 52.4 g kg^{-1} respectively, and showed an increase close to 50 g TOC per kg relative to control soil. Chromium total concentration (10.0 mg kg^{-1}) present in the soil was low and was representative of ''non contaminated areas'' of Buenos Aires province (Lavado, 2006). The addition of compost and peat did not modify substantially the total Cr concentration of the soil.

Hexavalent Cr reduction in soils

The addition of compost and peat to the contaminated soil produced a significantly higher Cr(III) concentration compared to the (S) control, for 1000 mg kg^{-1} and 2000 mg kg^{-1} of Cr(VI) added (Figure 1). Bolan *et al.* (2003) reported that the addition to a mineral soil of several organic amendments (at the same level of the total organic carbon) such as biosolid, compost, pig manure and poultry manure, enhanced the rate of Cr(VI) reduction in the soil. In our experiment the peat was more effective than compost in reducing 1000 and 2000 mg kg^{-1} of added Cr(VI) while there were no significant differences between both treatments at lower Cr levels.

The reduction efficiencies (RE) for treatments S and SC decreased significantly at concentrations greater than 500 mg kg^{-1} of added Cr(VI) (Figure 2), however, the RE for the SP treatment were close to 100% for all doses of Cr.

The contribution of carbon, protons, and microorganisms are considered the main factors increasing the Cr(VI) reduction in the presence of organic amendments (Losi *et al.*, 1994). Dissolved organic carbon and easy oxidisable organic carbon have been identified to facilitate the reduction of Cr(VI) to Cr(III) in soils (Jardine *et al.*, 1999; Bolan *et al.*, 2003). Kozuh *et al.* (2003) showed that the soluble Cr(VI) reduction mainly depends on the amount of insoluble OM and its accessibility to Cr(VI), although the rates of Cr(VI) reduction are highly dependent on the specific organic compound.

The data of Table 2 show the RC and OXOC values for each treatment. The RC for Cr(VI) followed the order:

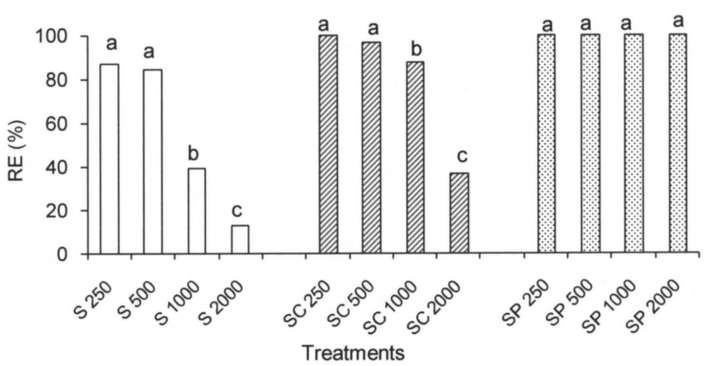

Figure 2 Reduction efficiency for each treatment (S, unamended soil; SC, soil amended with compost; SP, soil amended with peat The numbers 250, 500, 1000 and 2000 following each treatment indicate the level of Cr(VI) added in mg kg^{-1}. Different letters indicate significant differences ($P < 0.05$) between Cr(VI) the levels for each treatment. Values are means ($n = 6$).

Table 2 Reductive capacity (RC) of soils for Cr(VI). RC was calculated from easy oxidisable organic carbon (OXOC) values. Means of three replicates are presented

Treatments	OXOC (g kg^{-1})	RC (mg Cr(VI) kg^{-1})
S	8.5	49.1
SC	20.1	116.2
SP	40.0	231.2

S, unamended soil; SC, soil amended with compost; SP, soil amended with peat.

SP > SC > S. The RC of Cr in the SC treatment was 116 g of Cr(VI) kg^{-1} and in the SP treatment was 231 g Cr(VI) kg^{-1}. These values indicate that for all doses of Cr, the RE should have been 100%. However, the differences observed between both treatments (Figure 2) suggest that other factors influence in order to regulate the reduction of Cr(VI) to Cr(III). More acidic conditions in the SP treatment compared to SC (Table 1) contribute to the reduction of the metal in the soil amended with peat. The low pH of peat would favour the Cr(VI) reduction by reacting with the peat OM and also would increase the rate of release of Fe(II) mineral soil that would react with the Cr(VI) species. According to Eary and Rai (1991) the Cr(VI) can be effectively reduced in acid subsoils with very low OM content because it increases the dissolution rates of Fe(II). High reductive capacity in peat soils artificially contaminated with Cr(VI) was also reported by Kozuh and Stupar (2003). The differences found between the RE of Cr(VI) for treatments with the addition of organic amendments may also be due to the general microbial activity or the microbial population that is specific for the reduction of Cr(VI) (Losi *et al*., 1994; Bolan *et al*., 2003).

Chromium fractionation in soils

In the F1 fraction the total concentration of Cr, Cr(VI) and Cr(III) was determined. In contrast, in the F2, F3 and F4 fractions only the total concentration of Cr was measured because the metal speciation (Cr(III) and Cr(VI)) may be misleading due to the possible reduction of Cr(VI) during sequential extractions. The reduction of Cr(VI) can take place, either by direct effects of the extractants solutions used or by the reaction between the Cr(VI) and Fe(II) (formed by reductive dissolution of Fe oxides in the third step of the fractionation) (Lobau *et al*., 1997). However, in the SP treatment, the Cr(VI) would not be present in F2, F3 and F4 fractions due to high RE ($\sim$ 100%) obtained, nor in the SC treatment because the alkaline pH (8.2) may not favour the presence of mineral surfaces (*i.e*. iron and aluminum oxides) with positively charged sites that could absorb CrO_4^{2-} (Bank *et al*., 2005). Therefore, in the treatments with the addition of OM, the retention of Cr in the fractions F2, F3 and F4 would be in the form of Cr(III).

For all treatments the Cr concentration in each fraction increased with the increasing level of Cr(VI) added (Figure 3), except for the F1 fraction of SP treatment, in which Cr concentration showed a slight increase to 2000 mg kg^{-1} of Cr(VI) added (6.1 mg kg^{-1}) with respect to lower levels of Cr(VI) added, in which the concentration of Cr ranged from 3.2 to 3.7 mg kg^{-1}.

In the S treatment, the Cr distribution among the fractions varied according to the level of Cr added. The Cr concen-

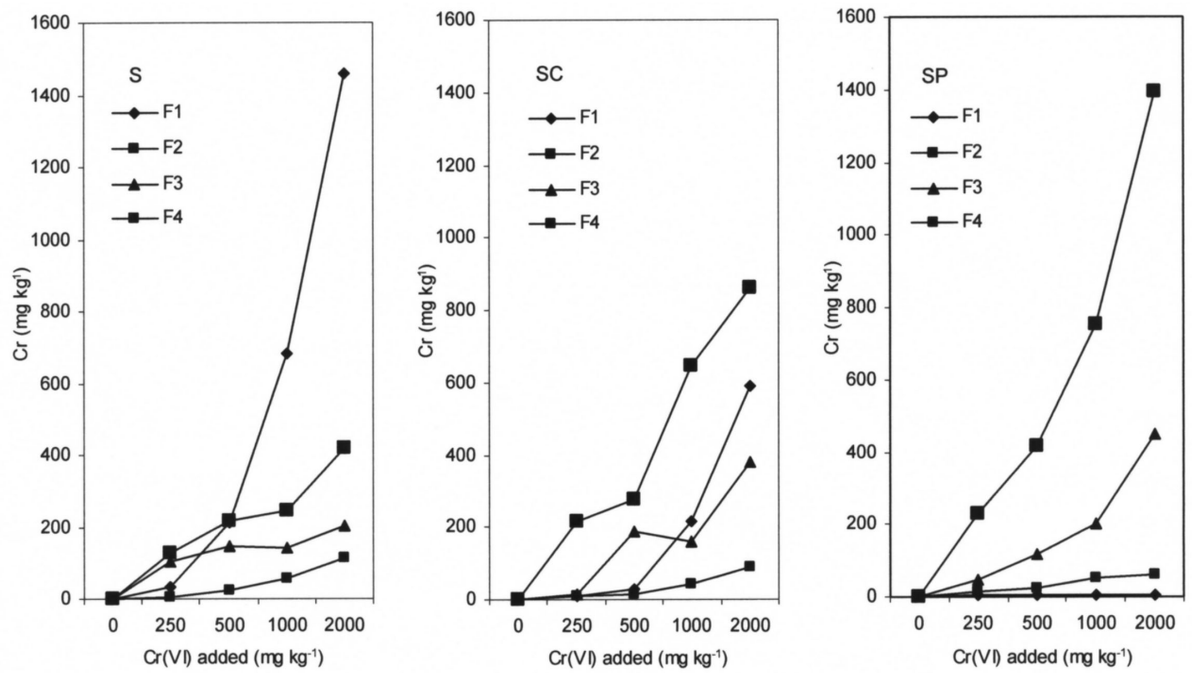

Figure 3 Chromium distribution in soil for each treatment (S, unamended soil; SC, soil amended with compost; SP, soil amended with peat) and each level of Cr(VI) added (0, 250, 500, 1000 and 2000 mg kg^{-1}). The Cr concentrations in each fraction are expressed as means ($n = 6$). F1 = soluble and exchangeable, F2 = bound to carbonate, F3 = bound to Fe/Mn oxides and F4 = bound to organic matter.

tration followed the order: F4 > F3 > F1 > F2 for 250 mg Cr kg^{-1}, F4 > F1 > F3 > F2 for 500 mg Cr kg^{-1}, and F1 > F4 > F3 > F2 for 1000 and 2000 mg Cr kg^{-1}. These results indicate that, at low Cr(VI) concentrations, the metal was preferentially retained on the natural OM by complexation. In contrast, at high concentrations of Cr(VI), the metal was found mainly in soluble and exchangeable forms (F1). This may be due to the saturation of specific binding sites for Cr in soil OM. In the SC treatment, for 250 and 500 mg Cr kg^{-1} the distribution followed this order: F4 > F3 > F1 > F2, and for 1000 and 2000 mg Cr kg^{-1} the order was: F4 > F1 > F3 > F2. Whereas in the SP treatment the order was: F4 > F3 > F2 > F1 for all levels of added Cr. These results show that in both treatments with added OM, F4 was the main Cr retention phase for all Cr levels. The concentrations of Cr in F4 ranged between 217–861 mg kg^{-1} and 228–1293 mg kg^{-1} for SC and SP respectively (Figure 3). In contrast, the total Cr concentration in F1 was substantially lower and ranged between 11.6–588 mg kg^{-1} and 3.2–6.1 mg kg^{-1} for SC and SP respectively. This suggests that the added OM contributed to reduce the solubility of Cr in soil and presumably its bioavailability due to the metal retention as inner sphere complexes with humic substances rather than retained electrostatically. Other studies have found evidence that peat retains metals mainly by complexation (Brown *et al*., 2000). Radmila and Stupar (1995) conducted the Cr fractionation in peat soils that were mixed with tannery wastes and concentration of total Cr between 1500 and 2000 mg kg^{-1}. Their results showed that both, the total Cr concentration and the Cr(VI) concentration in the soluble and exchangeable fraction decreased because the metal was redistributed into poorly soluble fractions of soils. In tannery waste amended sand and clay soils, most of the Cr (70%) was found to be in the reducible fraction, however in tannery waste amended peat soil the Cr distribution was primarily in the organic (35%) fraction.

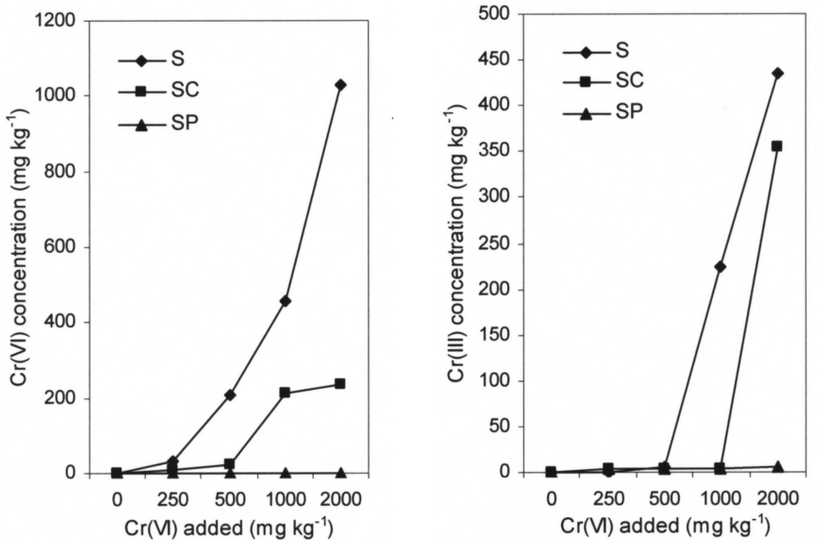

Figure 4 Concentration of Cr(VI) and Cr(III) in the soluble and exchangeable fraction (F1) for each treatment (S, unamended soil; SC, soil amended with compost; SP, soil amended with peat) and each level of Cr(VI) added (0, 250, 500, 1000 and 2000 mg kg^{-1}). Results are expressed as means ($n = 6$).

Our results also show that the Cr sorption on Fe–Mn oxides (reducible fraction, F3) is an important retention mechanism in the soils (with and without added OM), in agreement with published data (Balasoiu *et al*., 2001; Bolan *et al*., 2003; Kozûh *et al*., 2003).

The concentrations of Cr(VI) and Cr(III) in the F1 fraction (Figure 4) increased with the increasing levels of Cr(VI) in the S and SC treatments. The presence of both species Cr(VI) and Cr(III) indicate that the reduction of Cr(VI) in these treatments was not complete. In contrast, in the SP treatment Cr(VI) was not detected for any level of added Cr and the concentrations of Cr(III) were lower (3.2–6.1 mg Cr kg^{-1}) compared with the values in the S and SC treatments (5.2–434 mg Cr kg^{-1} and 3.4–354 mg Cr kg^{-1} respectively). Because under slightly acidic to alkaline pH conditions (pH between 6 and 8), most soils have a net negative charge and adsorb greater quantities of Cr(III) than of Cr(VI) (Rai *et al*., 1987; Zachara *et al*., 1989), the Cr(VI) present in the F1 fraction, both in S and in SC would be found mainly in soluble form rather than retained by electrostatic attraction to the surface of soil particles.

Growth and Cr accumulation in plants

The toxic effects of Cr were more pronounced in the *Begonia* than in the *Melissa* plants. The *Begonia* leaves exhibited visual symptoms of necrosis in the S treatment with 250 mg Cr kg^{-1} and in the SC treatment with 500 mg Cr kg^{-1}, whereas at higher doses the plants died. *Melissa* plants also showed necrosis of leaves in the S treatment with 250 and 500 mg Cr kg^{-1} and in the treatment with SC 250, 500 and 1000 mg Cr kg^{-1}; and at 2000 mg Cr kg^{-1} a total loss of plants occurred. In contrast, in the SP treatment there was no damage on leaves or plants loss at any level of added Cr for either species.

The values of dry weight (DW) on *Begonia* (Table 3) showed no significant differences between treatments for each level of added Cr, although DW of roots exhibited slightly higher values in SC and SP treatments. Root DW values also showed no differences between Cr levels for each treatment. On the other hand, there was a significant decrease in DW *Melissa* roots only in S and SC treatments to the highest concentrations of Cr(VI) added to the plants which survived (500 and 1000 mg Cr kg^{-1} respectively), and in SP Treatment at 2000 mg Cr kg^{-1} added. Under high concentrations of Cr, the reduction in root growth

Table 3 Dry weight (g plant $^{-1}$) of shoots and roots of Melissa and Begonia grown in soil (S) and soil treated with compost (SC) and Sphagnum moss peat (SP). The values are means of six replicates ± SD

Cr added (mg kg^{-1})	*Melissa* shoot DW (g plant $^{-1}$)			*Begonia* shoot DW (g plant $^{-1}$)		
	S	SC	SP	S	SC	SP
0	2.0472 ± 0.3016 bA	2.8240 ± 0.1347 aA	2.7200 ± 0.4021 aA	1.8256 ± 0.1701 bA	3.3576 ± 0.4726 aA	3.9232 ± 0.4747 aA
250	0.9272 ± 0.1791 cB	1.9384 ± 0.1253 bB	2.3968 ± 0.2296 aAB	0.4736 ± 0.1485 cB	1.9632 ± 0.1934 bB	2.6632 ± 0.2669 aB
500	0.4864 ± 0.1801 cC	1.1776 ± 0.1210 bC	2.2720 ± 0.3227 aAB	Nd	0.7784 ± 0.3047 bC	2.5816 ± 0.1825 aB
1000	Nd	0.6832 ± 0.1694 bD	1.8400 ± 0.7636 aB	Nd	Nd	1.4815 ± 0.2136 B
2000			0.6928 ± 0.6184 C	Nd	Nd	1.1951 ± 0.1725 C
	Melissa root DW (g plant $^{-1}$)			*Begonia* root SW (g plant $^{-1}$)		
0	1.2332 ± 0.1761 aA	1.3514 ± 0.2459 aA	1.4657 ± 0.4350 aA	0.3390 ± 0.0984 aA	0.4060 ± 0.0907 aA	0.4563 ± 0.1570 aA
250	1.1975 ± 0.0869 aA	1.1092 ± 0.2154 aA	1.3335 ± 0.1288 aA	0.2379 ± 0.0793 aA	0.3612 ± 0.1880 aA	0.4309 ± 0.1164 aA
500	0.2673 ± 0.2244 bB	0.7713 ± 0.4218 aAB	1.0292 ± 0.3317 aA	Nd	0.3473 ± 0.0627 aA	0.4482 ± 0.1425 aA
1000	Nd	0.3824 ± 0.1288 bB	1.1109 ± 0.1983 aA	Nd	Nd	0.4555 ± 0.1401 aA
2000		Nd	0.3866 ± 0.1235 B	Nd	Nd	0.3209 ± 0.1191 aA

Different lower case letters within a row mean significant differences between treatments ($P < 0.05$) and different upper case letters within a column mean significant differences between Cr levels ($P < 0.05$).
Nd: no data because the plants did not survive.

could be due to the direct contact of roots with Cr in the soil causing a collapse and subsequent inability of the roots to absorb water from the soil (Shanker, 2005).

The DW of shoots of both plant species decreased as the application rate of Cr increased (Table 3). The decrease in DW might be due to the reduction in photosynthesis and chlorophyll synthesis in excess Cr conditions (Vazquez *et al*., 1987) or to the effect of Cr addition on the absorption of various nutrients (Turner and Rust, 1971).

Compared to the DW controls, the addition of OM produced a greater aerial biomass for each level of Cr added. The high DW in the SC and SP treatments could be related to (1) lower Cr(VI) concentration in the culture medium, (2) lower Cr content in the more available soil fractions, and (3) beneficial effects of humic substances of the amendments on plant growth, which have been attributed to the increased availability of micronutrients, to the avoidance of soil compaction or to the direct effects on the metabolism of plants (Nardi *et al*., 2002). The DW of shoots of both species showed no difference between the SC and the SP treatments without Cr addition, however, when the metal was added, the SP treatment yielded greater DW than the SC treatment. This may be related to the fact that in the SP treatment, the majority of Cr in the soil contaminated with Cr(VI) was transformed into Cr(III) form which has limited availability and phytotoxicity compared to Cr(VI) (Zayed and Terry, 2003).

Cr levels in plants range from 0.02 to 1 mg kg^{-1} over a wide range of soil Cr concentrations (Kabata-Pendias and Pendias, 1992; Kai-Tsun-Tsia *et al*., 2007). In our work, the levels of Cr in shoots and roots of plants in the controls were lower than 1 mg kg^{-1} (Table 4), which is consistent with very low metal levels determined in each soil fraction (< 0.6 mg kg^{-1}). Roots accumulated between 2.6 and 14 times higher Cr than the shoots in *Melissa* plants, and between 2.1 and 23.4 times in *Begonia* plants in the treatments with Cr addition. The poor translocation of Cr from root to shoot is one of the possible detoxification mechanisms of plants (Adriano, 1986).

The Cr concentration in plants went up with the increased level of added Cr (Table 4). Plants can absorb both Cr(III) and Cr(VI) (Shanker *et al*., 2005). Hexavalent Cr is easily absorbed by an active mechanism in the roots of plants. However, Cr(III) is absorbed by plants to a lesser degree through a passive mechanism and retained by cation-exchange sites on cell walls. The addition of amendments significantly decreased the concentration of Cr in shoots and roots of both species compared with the controls. The low Cr absorption by plants in the SC and SP treatments compared with S may be related to the positive effect of organic matter on reducing Cr(VI) to Cr(III), leading to a metal redistribution between the solid soil phases from the more available forms to the less available forms in soil (Figure 3). Fengxiang X. Han *et al*. (2004) consider that the low Cr absorption by Indian mustard could be related to the strong Cr binding to the organic matter in soils contaminated with Cr(III), and in soils contaminated with Cr(VI) in which the reduction of Cr(VI) to Cr(III) occurs. In most

Table 4 Chromium accumulation in plants. Values are means of six replicates ± SD

Cr added (mg kg^{-1})	*Melissa* shoot Cr (mg kg^{-1} DW)			*Begonia* shoot Cr (mg kg^{-1} DW)		
	S	SC	SP	S	SC	SP
0	< 1	< 1	< 1	< 1	< 1	< 1
250	244 ± 8 aB	7.2 ± 4.0 bC	8.3 ± 4.6 bD	1457 ± 12 a	52.4 ± 12,6 bB	8.3 ± 3.7 cC
500	1780 ± 20 aA	104 ± 14 bB	23.8 ± 7.4 cC	Nd	180 ± 7 aA	6.7 ± 3.0 bC
1000	Nd	905 ± 11 aA	41.7 ± 10.6 bB	Nd	Nd	28.2 ± 4.0 B
2000	Nd	Nd	410 ± 12 A	Nd	Nd	36.9 ± 7.8 A
	Melissa root Cr (mg kg^{-1} DW)			*Begonia* root Cr (mg kg^{-1} DW)		
0	< 1	< 1	< 1	< 1	< 1	< 1
250	2985 ± 21 aB	98.6 ± 31.1 bC	116 ± 20 bD	3122 ± 17 a	78.4 ± 7.6 bB	76.1 ± 15.0 bC
500	4600 ± 54 aA	1020 ± 19 bB	213 ± 30 cC	Nd	1064 ± 18 aA	157 ± 12 bB
1000	Nd	6250 ± 47 aA	369 ± 26 bB	Nd	Nd	407 ± 12 A
2000	Nd	Nd	1610 ± 40 A	Nd	Nd	419 ± 9 A

Different lower case letters within a row mean significant differences between treatments ($P < 0.05$) and different upper case letters within a column mean significant differences between Cr levels ($P < 0.05$).
Nd: no data because the plants did not survive.

cases, the Cr content in plants was lower in the SP treatment than in the SC treatment. This result is consistent with the lower content of Cr(VI) and total Cr present in the soluble-exchangeable fraction (Figure 4) of the peat-amended soil.

CONCLUSIONS

The results of this study indicate that the addition of organic matter to the mineral soil contaminated with Cr(VI) favoured the reduction of Cr(VI) to Cr(III) for high levels of metal (1000 and 2000 mg kg^{-1}). The peat addition had 100% efficiency to reduce Cr(VI) between 250 and 2000 mg kg^{-1}. However, with the addition of compost, the reduction efficiency decreased with the increasing dose of Cr(VI) from soil, while for 2000 mg kg^{-1} it was only of 37%. The incomplete reduction of the metal determined the coexistence of Cr(VI) and Cr(III) in soil. The differences observed in the extent of the metal reduction were not associated with the easy oxidisable organic carbon content on amended soils. The changes produced by the added organic matter on the Cr speciation changed the metal distribution between the solid soil phases. The Cr content decreased in the soluble and exchangeable fraction of soil and increased in the OM fraction. The retained metals in the organic fraction are not considered very mobile or available and can remain in the soil for long periods without being mobilised. However, the degradation processes of exogenous organic matter under oxidising conditions can lead to the release of metals associated to this phase and thus enhance its bioavailability. Under the experimental conditions of this study, the addition of *Sphagnum* moss peat was more effective than the compost addition in decreasing the total Cr and the Cr(VI) content in the water soluble and exchangeable fraction of soil, thereby reducing the accumulation of Cr in the shoots and roots of plants and phytotoxic symptoms.

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