Copper distribution and hydrolase activities in a contaminated soil amended with dolomitic limestone and compost

A. Lagomarsino [a,c,*], M. Mench [b], R. Marabottini [c], A. Pignataro [c], S. Grego [c], G. Renella [d], S.R. Stazi [c]

[a] Agriculture Research Council, Research Centre for Agrobiology and Pedology (CRA-ABP), Piazza M. D'Azeglio 30, 50121 Firenze, Italy
[b] UMR BIOGECO INRA 1202, Ecologie des Communautés, Université Bordeaux 1, Bat B2, RdC Est, Avenue des Facultés, 33405 Talence, France
[c] Department of Agrobiology and Agrochemistry, University of Tuscia, Via S.Camillo de Lellis s.n.c., 01100 Viterbo, Italy
[d] Department of Soil Crop and Environmental Science, University of Florence, P.le delle Cascine 18, 50144 Florence, Italy

ARTICLE INFO

ABSTRACT

Chemical fractionation of copper in bulk soil and its distribution in the particle-size fractions were analyzed in a Cu-contaminated soil ($674 \pm 122\,\mu g\ Cu\ g^{-1}$, up to $1900\,\mu g\ Cu\ g^{-1}$ in the clay fraction) sampled from a wood preservation site left untreated and subsequently treated with dolomitic limestone (DL, 0.2% w/w) and compost (CM, 5% w/w), singly and in combination (DL+CM). Soil enzymatic activities of leucine aminopeptidase, cellulase, N-acetyl-β-glucosaminidase, arylsulfatase, β-glucosidase, acetate esterase, butyric esterase, and acid phosphatase were determined. Chemical speciation showed that Cu was mostly present in the acid-soluble and reducible fractions in both untreated and treated soils, whereas treatments with DL and CM reduced the soluble and exchangeable Cu fractions, due to Cu precipitation and complexation, and increased Cu bound to soil organic matter. Analysis of the particle-size fractions showed that more than 80% of Cu was in the silt and clay fractions and that treatment with CM increased the concentration of Cu in the sand size fractions. Soil treatment with DL and CM, singly or in combination, increased hydrolase activities, mainly in the clay fraction, with the largest positive effects on N-acetyl-β-glucosaminidase, leucine aminopeptidase, and β-glucosidase activities. Overall, results confirm that (1) Cu in contaminated soils is mainly bound to the silt–clay fraction, (2) CM additions change its allocation in the particle-size fractions, and (3) treatments with DL and CM singly and in combination reduce Cu solubility and its inhibitory effects on soil enzyme activities.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Identifying the most effective techniques to reduce the risk associated with soil contaminants is major challenge for the sustainable management of trace element contaminated soils (TECS). Soil remediation can remove excessive trace elements (TE) from the soil (e.g. by phytoextraction) or reduce the risk of contamination by reducing exposure to TE (in situ stabilization, phytostabilization) (Kumpiene et al., 2008; Mench et al., 2009; Vangronsveld et al., 2009; Park et al., 2011).

Copper is a common soil contaminant due to its wide use in agriculture (Alva et al., 2000), industry, and in urban and mining activities (Arias et al., 1998). Although Cu is an essential element, its excessive availability in soil is potentially toxic for plants and microorganisms, and inhibits soil enzyme activities (Bååth, 1989;

McGrath et al., 2002; Sauvé, 2006; Bes and Mench, 2008). The toxicity of Cu and of other TE in soil depends mainly on their solubility and chemical speciation, which are in turn influenced by their sorption onto clay minerals, carbonates, and reactions with soil organic matter (SOM) (Garrido et al., 2005) and with Fe and Mn oxides and hydroxides (Bradl, 2004; Kumpiene et al., 2008). Copper solubility in soils is generally low at slightly alkaline pH (Sauvé et al., 1997), and its solubility can be further reduced by incorporating alkaline materials and clay minerals and by causing co-precipitation with carbonates and phosphates (Kabata-Pendias and Pendias, 2000; Álvarez-Ayuso and García-Sánchez, 2003; Kumpiene et al., 2006). Calcium oxide (CaO) and dolomitic limestone (DL) have been successfully used as liming agents in several Cu-contaminated soils (Kiikkila, 2003; Bes and Mench, 2008; Khan and Jones, 2009). In the soil, copper is mainly associated with SOM, forming Cu–SOM complexes (Bolan and Duraisamy, 2003) particularly with non-soluble, high molecular weight organic acids (Chirenje and Ma, 1999; Balasoiu et al., 2001), although dissolved SOM may increase Cu solubility (Hsu and Lo, 2000). The dependence of Cu solubility on SOM solubility

* Corresponding author. Fax: +39 055 241485.
E-mail addresses: alessandra.lagomarsino@entecra.it,
ale.lagomarsino@gmail.com (A. Lagomarsino).

may be responsible for the contrasting results of studies on Cu bioavailability in contaminated soils (Geebelen et al., 2002; Castaldi et al., 2005; Clemente et al., 2006; Van Herwijnen et al., 2007; Bolan et al., 2011). Liming combined with the addition of organic matter has been successfully used for revegetation of Cu-contaminated vineyard soils (Delas, 1963) and barren Cu mine tailings (Hao et al., 2003), and to decrease Cu uptake by plants (Chen et al., 2000; Lombi et al., 2002) due to soil alkalinization and metal sorption. In an aided phytoremediation experiment, amendment of a Cu polluted soil with DL and compost reduced Cu availability and soil toxicity and allowed the revegetation of the area with different tree and herb species (Bes and Mench, 2008; Bes et al., 2010).

While ample information is available on Cu speciation in natural and agricultural soils (McLaughlin et al., 2000) less has been published on Cu distribution in soil particle-size fractions (Jaradat et al., 2006; Clemente and Bernal, 2006). Copper allocation in particle-size fractions has been studied in both uncontaminated (Hardy and Cornu, 2006) and contaminated soil (Ducaroir et al., 1990; Besnard et al., 2001). But little information is available on the physical fractionation of Cu in soils under aided phytostabilization, although such information may be useful for improving the remediation strategy and investigating post-remediation scenarios.

Copper is known to inhibit enzyme activities in TECS (Tyler et al., 1989) through its interactions with –SH groups of amino acids of the enzyme active site or the enzyme–substrate complex (Huang and Shindo, 2000; Acosta-Martínez and Tabatabai, 2001) either as free Cu^{2+} ion or as a metal–organic complex (Marzadori et al., 2000). Consequently, enzyme activity is one of the soil functions recovering as a consequence of metal stabilization in TECS (Mench et al., 2006; Renella et al., 2008; Kumpiene et al., 2008).

The aim of this work was to study Cu chemical speciation, its distribution in particle-size fractions and several hydrolase activities in a Cu-contaminated soil at a wood preservation site, in its untreated state and one year after soil amendment with dolomitic limestone (DL) and compost (CM), singly and in combination.

2. Materials and methods

2.1. Site description and soils

The wood preservation site (6 ha) is located in Gironde, SW France and has been used for over a century to preserve and store timbers, posts, and utility poles (Mench and Bes, 2009; Bes et al., 2010). Soil Cu contamination is mainly the result of washing treated wood. The contaminated soil is of alluvial origin (Fluvisol), sandy loam texture, salinity 0.13 dS m^{-1}, and CaCO$_3$ 2.14%. The main soil properties are listed in Table 1, more details on soil and site characteristics and established vegetation can be found in Mench and Bes (2009), Negim (2009), and Bes et al. (2010). The field trial started in May 2006 at site P1-3, which consists of 16 (1 × 3 m) plots. The trial comprised the following four treatments: untreated (UNT); 0.2% (air dried soil, w/w) dolomitic limestone (DL, containing 30% CaO and 20% MgO combined with carbonates, fineness index 80% < 0.16 mm, neutralizing power 58, Prodical Carmeuse, Orthez, France), compost (CM, 5% w/w), derived from 9- to 12-month composting of poultry manure and pine bark chips (ORISOL, Cestas, France), and DL combined with CM (DL+CM, at the same rates as DL alone), randomly replicated in four blocks. Amendments were incorporated into the soil to a depth of 25 cm. Compost characteristics are listed in Table 1. Plots are cultivated as a short rotation coppice including mycorrhizal poplar and willows (Bes, 2008). Mean rainfall rate at the site is 840 ± 99 mm. In April 2007, soil samples were collected from 0 to 25 cm soil layer from four plots per treatment using a stainless steel spade and were kept as independent replicates for all the analyses. Soils were sieved at 2 mm and stored at 4 °C prior to analysis.

2.2. Total soil Cu

The modified *aqua regia* digestion method used 0.5 g of soil dried at 105 °C for 24 h and milled, added with 1.5 mL H$_2$O$_2$, 4.5 mL HCl, and 1.5 mL HNO$_3$, following the official protocol of the Italian legislation (D.M. 13 September 1999, G.U. n. 248, 21 October 1999). After digestion, solutions were diluted with milliQ water and Cu concentration measured by flame atomic absorption spectrometry (FAAS, Perkin

Table 1
Chemical characteristics of soil and CM treatment.

	Soil (0–25 cm)	CM amendment
Sand (%)	85.8	
Silt (%)	8.3	
Clay (%)	5.9	
Organic C (g kg^{-1})	9.3	321
C/N	17.2	19.4
CEC (cmol kg^{-1})	3.5	–
P$_2$O$_5$ (g kg^{-1})	–	17.7
K$_2$O (g kg^{-1})	–	10.9
MgO (g kg^{-1})	–	4.7
CaO (g kg^{-1})	–	47.1
Na$_2$O (g kg^{-1})	–	1.4
SO$_3$ (g kg^{-1})	–	4.9
Cr (mg kg^{-1})	23	< 0.5
Cu (mg kg^{-1})	674	32.1
Ni (mg kg^{-1})	5	1.8
Zn (mg kg^{-1})	46	131
Cd (mg kg^{-1})	0.12	0.5
Pb (mg kg^{-1})	27	9.0
Hg (mg kg^{-1})	–	0.2
As (mg kg^{-1})	9.8	0.8
Co (mg kg^{-1})	< 2	–
Fe (mg kg^{-1})	6090	–
Mn (mg kg^{-1})	181	–

–: not determined.

Elmer 4000, AA flame, detection limit 20 μg L^{-1}). For each analytical batch, blanks were also prepared and analyzed.

2.3. Chemical fractionation of copper

The sequential extraction method proposed by Tessier et al. (1979) was used ($n=4$ replicates per soil sample). Aliquots (2 g) of dry soil were sequentially extracted with deionized water (soluble fraction), 0.5 M MgCl$_2$ (exchangeable fraction), 1 M sodium acetate at pH 5.0 (acid-soluble fraction), 0.08 M NH$_2$OH · HCl in 25% acetic acid (reducible fraction bound to poorly crystalline Fe and Mn oxyhydroxides), 0.02 M HNO$_3$ and 30% H$_2$O$_2$ at 85 °C (organic matter bound fraction). The residual fraction was calculated by subtracting the sum of the fractions from the total Cu content. The concentrations of Cu in the extracts were measured by FAAS in the same way as for total Cu. The different Cu fractions were extracted in capped tubes, end-over-end shaken for 30 min at 120 rpm at room temperature. The supernatants were separated by centrifugation (10^3g for 5 min) at room temperature, Cu is expressed per g of dry weight (DW) soil.

2.4. Copper distribution in physically isolated fractions

Soil samples were dispersed by low-energy sonication and the particle-size fractions were separated by a combination of wet sieving and centrifugation (Stemmer et al., 1998). Very small micro-aggregates are not necessarily disrupted with this method, and so around 20% residual clay is to be expected in the silt-size fraction (Marx et al., 2005). Twenty-five grams of moist soil were dispersed in 80 mL of cold distilled water by a probe-type ultrasonic disaggregator (50 J s^{-1} for 3 min). The coarse and medium particle-size fraction (> 250 μm) and the fine particle-size fraction (250–63 μm) were separated by manual wet sieving with maximum 700 mL of cold distilled water. During wet sieving, the slurry was gently stirred to achieve complete disruption of the macro-aggregates. Silt-sized particles (63–2 μm) were separated from the clay fraction (< 2 μm) by four repeated centrifugations at 270g for 2 min at 15 °C. After each centrifugation, the pellets were resuspended in water and centrifuged again to isolate the silt fraction. The combined supernatants were centrifuged at 5500g to obtain clay-sized particles (2 − 0.1 μm). Total Cu was determined by *aqua regia* digestion on these four size fractions as described above.

2.5. Enzyme activities

β-glucosidase (βG), cellulase (CELL), acetyl esterase (AC), butyrate esterase (BUT), acid phosphatase (AP), N-acetyl-β-glucosaminidase (NAG), arylsulphatase (ARYL), and leucine-aminopeptidase (LEU) activities were measured using methyl-lumbelliferyl (MUF) and 7-amino-4-methylcoumarin (AMC) conjugated substrates (Sigma, St Louis, MO, USA) and analysis of fluorogenic MUF-derived substrates (Marx et al., 2001; Vepsäläinen et al., 2001) on both whole soil and particle-size fractions. Three lab replicates were used for each soil sample. Briefly, weighed moist soil samples equivalent to 1 g soil DW were placed in sterile jars with 50 mL

of Na-acetate buffer at pH 5.5 and homogenized using a UltraTurrax shaking plate at 9600 rev min^{-1} for 3 min. Aliquots of 100 μL were taken from each suspension and dispensed into a 96-well microplate (three analytical replicates per sample for each substrate). Finally, 100 μL of 1 mM substrate solution was added giving a final substrate concentration of 500 μM. Fluorescence was measured after 0, 30, 60, 120, and 180 min of incubation at 30 °C, with an automated fluorimetric plate reader (Fluoroskan Ascent), equipped with excitation and emission filters at 360 and 450 nm, respectively.

2.6. Statistical analysis

A randomized block design was applied using the general linear model procedure, followed by the Bonferroni post hoc test, to determine significant differences across treatments in bulk soil and fractions in the parameters analyzed. Data were tested for normality with Shapiro–Wilk statistics. Discriminant function analysis (DFA) was performed using soil enzymatic activities and catabolic responses to single substrates as variables to discriminate among groups (UNT, DL, CM, and DL+CM in bulk soil, coarse sand, fine sand, silt, and clay fractions). Squared Mahalanobis distances between group centroids were determined. Two significant discriminatory roots were derived and the results of DFA are presented graphically in two dimensions. All statistical analyses were performed using the Statistica 6.0 software package (Statsoft, Tulsa, USA).

3. Results

3.1. Chemical speciation of copper

Total Cu in the soil samples varied between 600 and 900 mg kg^{-1}, with no significant differences between treatments and UNT soil. Copper was mainly associated with the acid-soluble fraction (34%), the reducible fraction (32%), and, to a lesser extent (12%) with SOM, and with the residual fraction (16%). Low Cu concentrations (4%) were observed in both soluble and exchangeable fractions (Fig. 1). The water soluble Cu fraction was significantly higher in the UNT soil than in all the treated soils ($p < 0.01$). The exchangeable Cu fraction decreased in the DL+CM
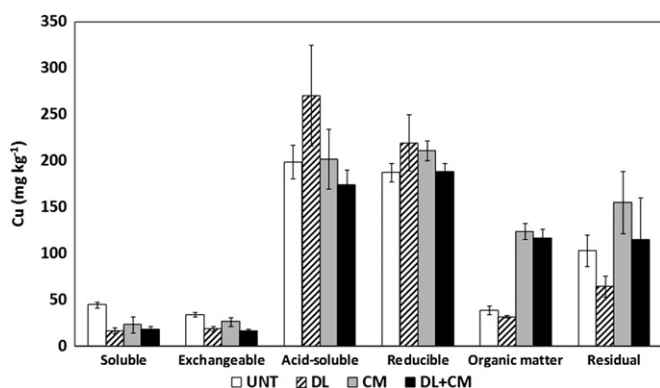
treatment, as compared to UNT soil ($p < 0.05$). Copper bound to the organic matter showed a 3-fold increase in the CM and DL+CM treatments ($p < 0.001$), whereas Cu concentrations in acid-soluble, reducible, and residual fractions were not changed by any treatment.

3.2. Recovery of particle-size fractions

The percentage distribution and recovery of the particle-size fractions are listed in Table 2. Sand size fraction accounted for more than 80% of the soil. The DL+CM treatment increased coarse sand size and decreased fine sand size fractions, as compared to UNT soil.

3.3. Copper distribution in particle-size fractions

Copper was mainly associated with the clay and silt fractions, and was, respectively, 8 and 2 times higher in the clay and silt fractions than in the bulk soil (Fig. 2). In the bulk soil, and clay and silt fractions, Cu concentration did not vary across the treatments (Fig. 2); but in the CM and DL+CM treatments, the Cu concentration in the coarse and fine sand size fractions was higher than that in the coarse and fine sand fractions of the UNT soil ($p < 0.05$ and $p < 0.001$, respectively; Fig. 2).

3.4. Soil enzyme activities

Enzyme activities were found to be associated mainly with the clay size fraction. The lowest values were generally found in coarse and fine sand fractions (Figs. 3 and 4). In the bulk soil, CM and DL+CM treatments significantly increased βG ($p < 0.05$), NAG ($p < 0.01$), and LEU ($p < 0.05$) activities (Figs. 3b and 4a, b).
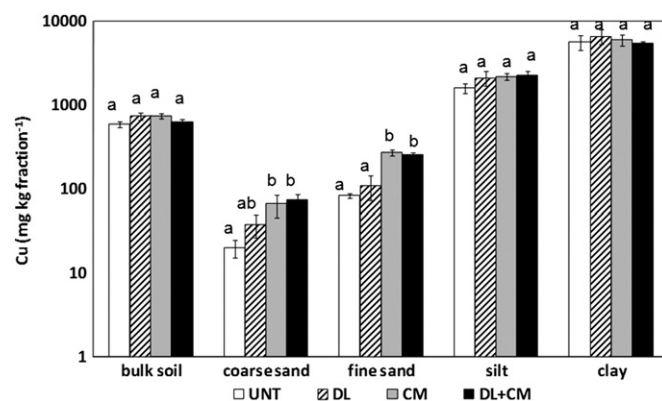

Fig. 1. Cu (mg kg^{-1}) in chemically isolated fractions. Bars represent standard errors. Different letters indicate significant differences between treatments ($p < 0.05$; Bonferroni post hoc test).

Fig. 2. Cu content in bulk soil and in the coarse sand, fine sand, silt, and clay fractions in untreated soil (UNT) and soil amended with DL, CM, and DL+CM. Logarithmic scale (log$_{10}$). Bars represent standard errors. Different letters indicate significant differences between treatments ($p < 0.05$; Bonferroni post hoc test).

Table 2
pH, relative percentage, and recovery rate of soil particle-size fractions.

	pH	Coarse sand	Fine sand	Silt	Clay	Recovery rate
		250–2000 μm (fractions, %)	63–250 μm (fractions, %)	2–63 μm (fractions, %)	0.1–2 μm (fractions, %)	(%)
UNT	7.1[a]	66.4[a]	16.7[a]	10.5[a]	5.7[a]	99.3[a]
DL	7.51[b]	67.3[ab]	15.1[ab]	12.8[b]	3.0[a]	98.1[a]
CM	7.42[ab]	67.8[ab]	15.9[ab]	11.6[ab]	3.6[a]	98.9[a]
DL+CM	7.67[b]	70.9[b]	14.1[b]	11.2[ab]	3.1[a]	99.3[a]

Different letters indicate significant differences between treatments (Bonferroni post hoc test).
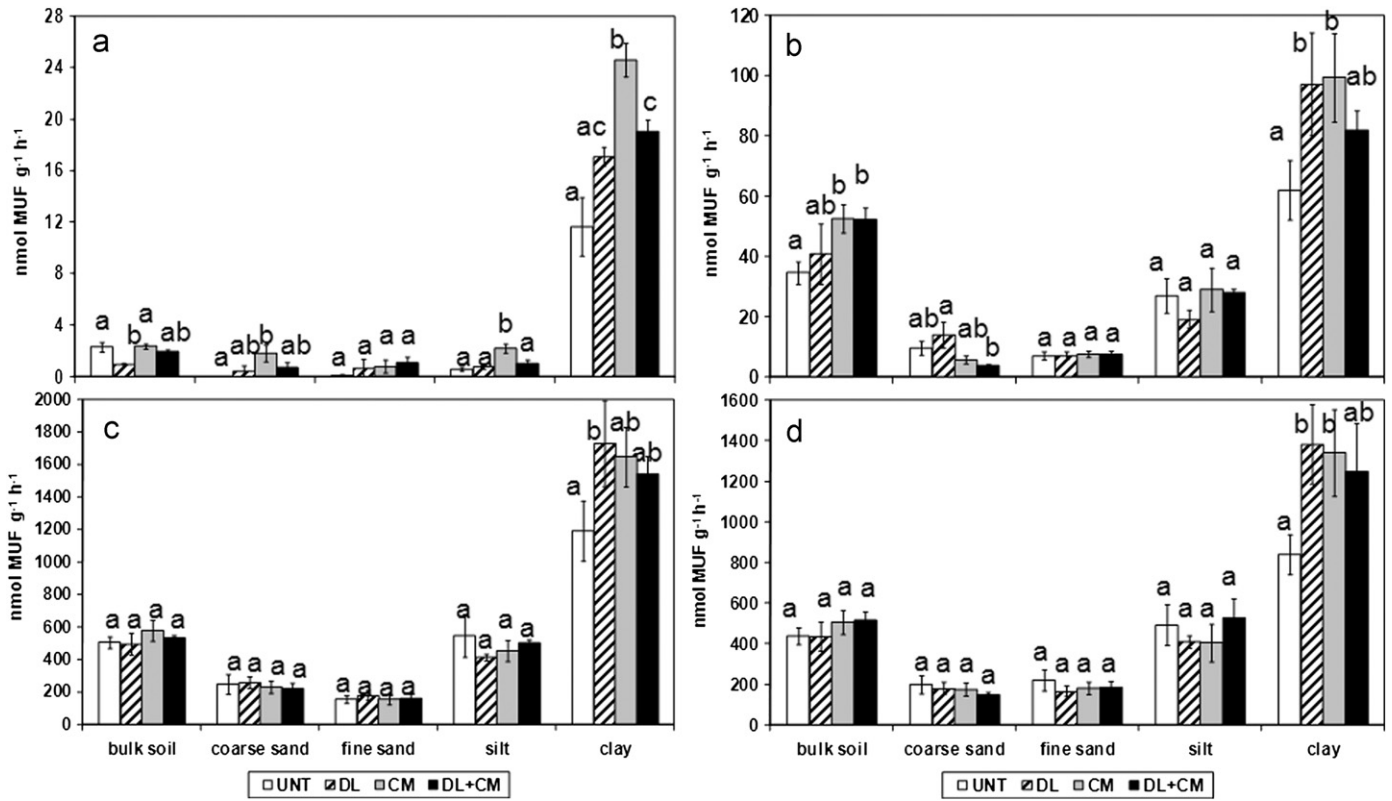
Fig. 3. Cellulase (a), β-glucosidase (b), acetyl esterase (c), and butyrate esterase (d) activities in bulk soil, coarse sand, fine sand, silt, and clay in untreated soil (UNT) and soil amended with DL, CM, and DL+CM. Bars represent standard errors. Different letters indicate significant differences between treatments ($p < 0.05$; Bonferroni post hoc test).
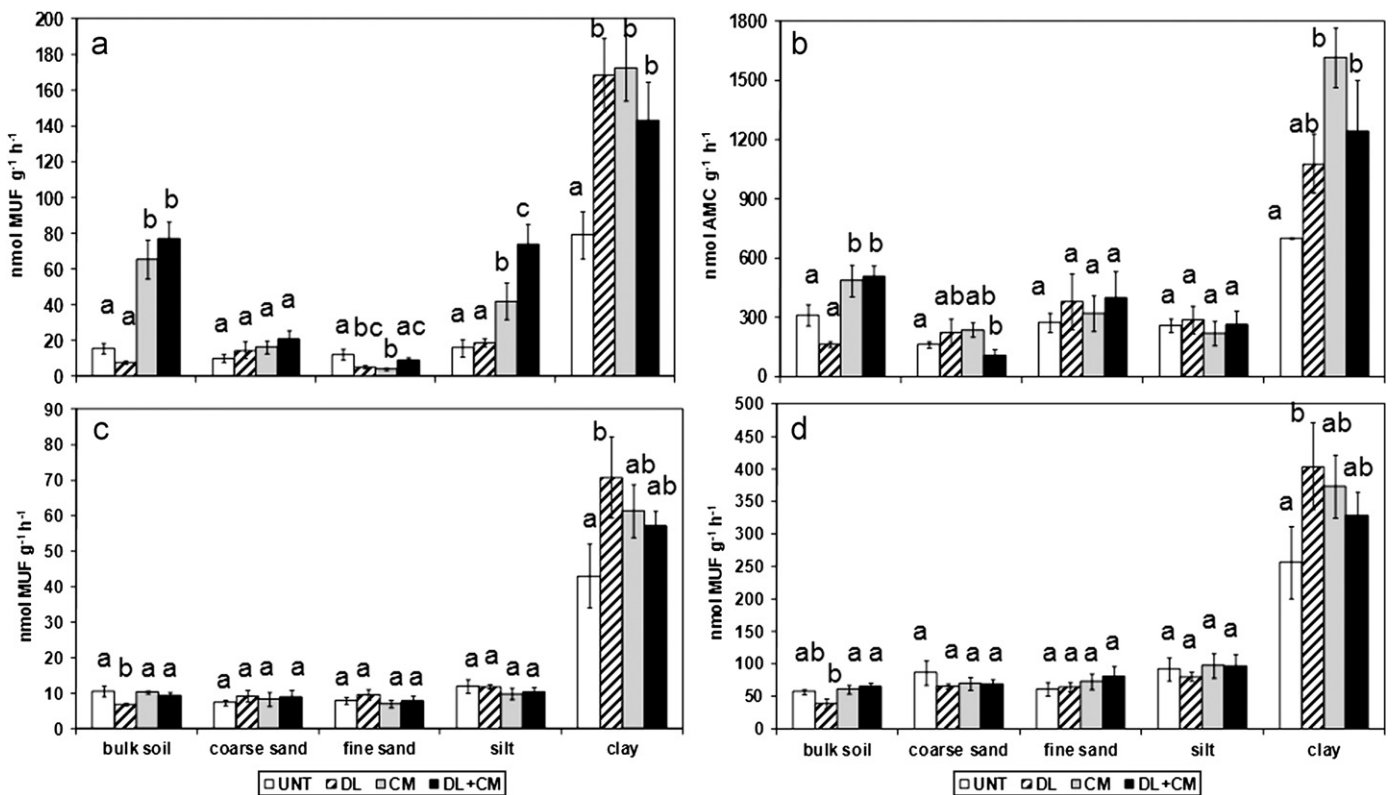

Fig. 4. Activities of N-acetyl-β-glucosaminidase (a), leucine amino peptidase (b), arylsulphatase (c), and acid phosphatase (d) in bulk soil, coarse sand, fine sand, silt, and clay in untreated soil (UNT) and in soil amended with DL, CM, and DL+CM. Bars represent standard errors. Different letters indicate significant differences between treatments ($p < 0.05$; Bonferroni post hoc test).

In the coarse sand fraction, CM amendment increased CELL activity ($p < 0.05$, Fig. 3a) compared to UNT, whereas the DL+CM treatment reduced LEU activity ($p < 0.05$, Fig. 4b). In the fine sand fraction, NAG activity was lower in the CM and DL treatments than in the UNT one ($p < 0.05$, Fig. 4a). In the silt-size fraction, CM treatment increased CELL and NAG activities, whereas DL+CM treatment increased only NAG activity (Figs. 3a and 4a). In the clay size fraction, the soil treatments led to a significant increase of most of the enzyme activities measured (Figs. 3 and 4).

Discriminant function analysis significantly separated the clay size fractions along the root 1 axis, which explained most of the variance (Fig. 5). Bulk soil and silt-size fractions were significantly ($p < 0.01$, Mahalanobis distance) separated from the sand fractions only along the root 2 axis (Fig. 5). In the bulk soil, treatments including CM amendments were not significantly separated, but both of them were distinguished from the UNT and DL treatments ($p < 0.001$, Mahalanobis distance). In the clay size fraction, the four treatments were significantly separated ($p < 0.001$, Mahalanobis distance) with the maximum distance between UNT and treated soils. Concerning the silt-size fraction, only the CM treatment was significantly separated from the other treatments. None of the treatments were separated in the coarse and fine sand size fractions. All enzyme activities were positively correlated with the root 1 axis, whereas NAG, βG, AC, and BUT activities were negatively correlated with root 2 axis (Table 3).
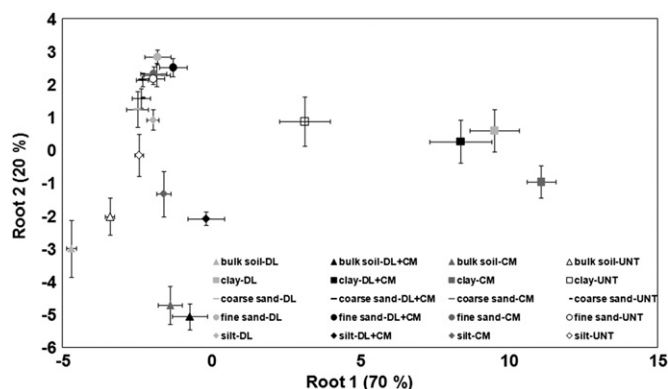

Fig. 5. Discriminant function analysis of enzyme activities (all enzymes except cellulase) in UNT soil (open symbols), DL (light gray), CM (dark gray), and DL+CM (black) in bulk soil (triangle), coarse sand (line), fine sand (circle), silt (diamond), and clay (square) fractions. Group centroids, representing the average of four replicates, are reported with standard errors. BS—bulk soil, C—clay, CS—coarse sand, FS—fine sand, and S—silt.

Table 3
Linear correlations (*r*-values) of the enzymatic activities with the root 1 and 2 axis of the discriminant function analysis reported in Fig. 5.

	Root 1 axis	Root 2 axis
LEU	0.939***	n.s.
NAG	0.929***	−0.30**
ARYL	0.932***	n.s.
βG	0.804***	−0.458***
AC	0.894***	−0.223*
BUT	0.861***	−0.263*
AP	0.929***	n.s.

n.s.: not significant.

    * Significant at $p < 0.05$.
    ** Significant at $p < 0.001$.
    *** Significant at $p < 0.0001$.

4. Discussion

4.1. Chemical speciation

Chemical speciation showed that Cu was mainly present in the acid-soluble (e.g. hydroxides, carbonates, etc.) and reducible (e.g. Fe and Mn oxides) fractions, confirming previous findings (Jenne, 1968; Bradl, 2004; Bhattacharyya et al., 2008). The metal sorption capacity of Al and Mn oxides mainly depends on the pH dependent charge of functional groups and exchange ion-bearing sites at the surface (Bradl, 2004), which make these minerals major contributors to Cu retention in soils. Not all chemical speciation protocols separate the acid-soluble and reducible fractions, and so comparing our results with previous findings (e.g. Saha et al., 1991; Alva et al., 2000; Yin et al., 2002) was not easy. A trend toward an increase in Cu associated with the acid-soluble fraction was revealed in the DL treatment, although the differences were not significant due to data variability (Fig. 1). The speciation of Cu in calcareous soils is controlled by $CaCO_3$ (McBride and Bouldin, 1984; Madrid and Diaz-Barrientos, 1992), and Minkina et al. (2008) suggested the formation of Cu carbonates in neutral soils. The acid-soluble fraction may include the 2% $CaCO_3$ analyzed in soil P1-3 (Negim, 2009). Copper associated with OM fraction accounted for 12% of total soil Cu and increased more than 3-fold after compost was added. This increase reflects the high affinity of Cu for OM (Elzinga et al., 1999) and its fractions, including humic substances, plant and microbial cell debris (McBride, 1989; Schnitzer and Kodama, 1992; Spark et al., 1997) and particulate organic matter (Balabane et al., 1999; Besnard et al., 2001). Copper retention after CM amendment likely mirrored the formation of Cu–OM complexes (Shuman, 1999; Bolan and Duraisamy, 2003; Clemente and Bernal, 2006), which likely occurred with the humified OM portion of the Orisol compost. In contrast, fresh organic matter such as animal manure rich in soluble organic compounds can facilitate Cu mobility and bioavailability due to dissolved complexes (Kızılkaya, 2004). The stabilization of Cu by OM added in the CM treatments paralleled the reduction in both soluble and exchangeable Cu fractions and confirmed previous findings (Alva et al., 2000; Bhattacharyya et al., 2008). Shuman (1986) and Saha et al. (1991) reported that OM may reduce the retention of Cu by $CaCO_3$ in calcareous soils. In the present study, CM incorporation did not significantly influence the acid-soluble fraction in the DL+CM treatment. Besides CM, DL amendment reduced both water soluble and exchangeable fractions, as a result of increased soil pH (Table 2; Fig. 1). Alva et al. (2000) showed decreased Cu concentrations in soluble and exchangeable forms with an increase in soil pH above 6.5.

4.2. Physical fractionation

Silt and clay fractions retained the largest proportion of Cu, due to their large specific surface area and reactive sites (Fig. 2). The capacity of clays to adsorb and transport metal in soils has already been discussed (Bradl, 2004; Jaradat et al., 2006; Wang et al., 2006). Data from four soil profiles at this wood preservation site, including Site P1-3, showed no correlation between the concentration of Cu in the soil and the percentage of sand fractions but showed a positive correlation with clay content (Negim, 2009). Cuprite (Cu_2O), an arsenic copper compound ($AsCu_9$), and plancheite ($Cu_8(Si_8O_{22})(OH)_4 \cdot H_2O$) were detected by X-ray diffraction in the silt fraction at Site P7 (0–25 cm soil layer, 630 mg Cu kg^{-1}) of this wood preservation site while in the clay fraction, Cu was found in the forms of cuprite (Cu_2O), an arsenic copper compound ($AsCu_9$), and malachite ($Cu_2(CO_3)(OH)_2$). Sand-sized fractions have only weak binding affinities to Cu (Fig. 2), but its concentration in

these fractions was significantly affected by CM amendment, singly and in combination with DL. The Orisol compost included pine bark chips composted with poultry manure, suggesting that part of the added OM accumulated in coarser fractions as partially decomposed residues. As a result, the positive effect of CM amendment on Cu retention was more apparent in the sand fraction, rich in compost particles and not strongly bound with clay minerals, but this effect may be only brief. The use of organic amendments in the soil requires testing of the long-term changes in available Cu (Weber et al., 2007). After compost degradation, the sorbed metals could be released and become available again (Van Herwijnen et al., 2007). At site P1-3, Negim (2009) found a linear correlation between total soil Cu and OM content in the soil profile.

Copper and other TE can inhibit pure enzymes (Blum and Schwedt, 1998), mostly by interacting with the active catalytic site or with –SH groups, or by changing the molecule conformation. Reports in the literature mostly point to a general decrease in enzyme activity in metal-contaminated soils (Tscherko and Kandeler, 1997; Renella et al., 2008; Kumpiene et al., 2008), but information on the inhibition of enzymes associated with different soil particles is scarce. Copper has shown a greater inhibitory capacity to enzymes adsorbed onto clays than Zn and Cd (Huang and Shindo, 2000), and Cu can inhibit some enzyme activities even if it is present in the form of metal–organic complexes (Marzadori et al., 2000).

4.3. Enzyme activities

In the bulk soil, NAG, LEU, and βG activities significantly increased in soils treated with CM singly and in combination with DL, suggesting reduced Cu toxicity (Figs. 3 and 4). Moreover, the added OM supplies energy-rich substrates to soil microorganisms, thus fostering the positive effect of the lower Cu availability in CM- and DL+CM-treated soils. An increase in hydrolase activities in soils under aided phytostabilization has been reported (Kumpiene et al., 2006; Mench et al., 2006; Renella et al., 2008). In the soils concerned, most of the measured enzyme activities increased in the clay fraction, whereas the effects of the treatment on enzyme activity differed in the coarse and fine sand fractions. One possible explanation is that enzymes are more physically and chemically protected by the (organo)clay complexes (Kandeler et al., 1999; Marx et al., 2005). The increase in CELL activity in the CM treatment may reflect the presence of cellulose residues in the compost. Hydrolase activity in the silt and clay fractions, where the maximum distinction between functions occurred across the treatments, and the increase in CELL and LEU by CM and of AP, AC, and ARYL by DL, paralleled the Cu stabilization, and thus demonstrated the efficacy of the amendments in reducing Cu toxicity.

4.4. Conclusion

Amending a highly Cu-contaminated soil with DL and CM led to Cu stabilization by reducing the water soluble and exchangeable fractions. This resulted in the lower inhibition of several hydrolase activities. Moreover, the chemical speciation and the distribution in the particle-size fractions revealed allocation of Cu associated with OM derived from compost amendment to the sand-size fractions. These Cu fractions need to be monitored as, in the long term, Cu solubility may change as the compost degrades.

Acknowledgments

References

Acosta-Martínez, V., Tabatabai, M.A., 2001. Tillage and residue management effects on arylamidase activity in soils. Biol. Fert. Soils 34, 21–24.

Alva, A.K., Huang, B., Paramasivam, S., 2000. Soil pH affects copper fractionation and phytotoxicity. Soil Sci. Soc. Am. J. 64, 955–962.

Álvarez-Ayuso, E., García-Sánchez, A., 2003. Removal of heavy metals from waste waters by natural and Na-exchanged bentonites. Clay Miner. 51, 475–480.

Arias, M., Nunez, A., Barral, M.T., Diaz-Fierros, F., 1998. Pollution potential of copper mine spoil used for road making. Sci. Tot. Environ. 221, 111–116.

Bååth, E., 1989. Effects of heavy metals in soil on microbial processes and populations (a review). Water Air Soil Pollut. 47, 335–379.

Balabane, M., Faivre, D., van Oort, F., Dahmani-Muller, H., 1999. Mutual effects of organic matter dynamics and heavy metals fate in a metallophyte grassland. Environ. Pollut. 105, 45–54.

Balasoiu, C.F., Zagury, G.J., Deschenes, L., 2001. Partitioning and speciation of chromium, copper, and arsenic in CCA contaminated soils: influence of soil composition. Sci. Tot. Environ. 280, 239–255.

Bhattacharyya, P., Tripathy, S., Chakrabarti, K., Chakraborty, A., Banik, P., 2008. Fractionation and bioavailability of metals and their impacts on microbial properties in sewage irrigated soil. Chemosphere 72, 543–550.

Bes, C., 2008. Phytoremédiation des sols d'un site de traitement du bois contaminés par le cuivre. Ph.D. Dissertation, Ecole Doctorale Sciences & Environnement, Spécialité Ecologie Evolutive, Fonctionnelle et des Communautés. Université de Bordeaux 1, Talence, France, p. 293.

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization. Environ. Pollut. 156, 1128–1138.

Bes, C.M., Mench, M., Aulen, M., Gasté, H., Taberly, J., 2010. Spatial variation of plant communities and shoot Cu concentrations of plant species at a timber treatment site. Plant Soil 330, 267–280.

Besnard, E., Chenu, C., Robert, M., 2001. Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyard soils. Environ. Pollut. 112, 329–337.

Blum, U., Schwedt, G., 1998. Inhibition behaviour of acid phosphatase, phosphodiesterase I and adenosine deaminase as a tool analysis and speciation. Anal. Chim. Acta 360, 101–108.

Bolan, N.S., Adriano, D., Senesi, N., Kunhikrishnan, A., James, T., McDowell, R., 2011. Dissolved organic carbon: biogeochemistry, dynamics and agro-environmental significance in soils. Adv. Agron. 110, 1–75.

Bolan, N.S., Duraisamy, V.P., 2003. Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: a review involving specific case studies. Aust. J. Soil Res. 41, 533–555.

Bradl, H.B., 2004. Adsorption of heavy metal ions on soils and soils constituents. J. Colloid Interface Sci. 277, 1–18.

Castaldi, P., Santona, L., Melis, P., 2005. Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. Chemosphere 60, 365–371.

Chen, H.M., Zheng, C.R., Tu, C., Shen, Z.G., 2000. Chemical methods and phytoremediation on soil contaminated with heavy metals. Chemosphere 41, 229–234.

Chirenje, T., Ma, L.Q., 1999. Effects of acidification on trace metal mobility in a papermill ash amended soil. J. Environ. Qual. 28, 760–766.

Clemente, R., Escolar, A., Bernal, M.P., 2006. Fractionation of heavy metals in a contaminated soil amended with organic materials of different mineralisation rates. Bioresource Technol. 97, 1894–1901.

Clemente, R., Bernal, M.P., 2006. Fractionation of heavy metals and distribution of organic carbon in two contaminated soils amended with humic acids. Chemosphere 64, 1264–1273.

Delas, J., 1963. La toxicité du cuivre accumulé dans les sols. Agrochimica 7, 258–288.

Ducaroir, J., Cambier, P., Leydecker, J.P., Prost, R., 1990. Application of soil fractionation methods to the study of the distribution of pollutant metals. Z. Pflanzenernähr. Bodenkd. 153, 349–358.

Elzinga, E.J., van Grinsven, J.J.M., Swartjes, F.A., 1999. General purpose Freundlich isotherms for cadmium, copper and zinc in soils. Eur. J. Soil Sci. 50, 139–149.

Garrido, F., Illera, V., Garcia-Gonzalez, M.T., 2005. Effect of the addition of gypsum- and lime-rich industrial by-products on Cd, Cu and Pb availability and leachability in metal-spiked acid soils. Appl. Geochem. 20, 397–408.

Geebelen, W., Vangronsveld, J., Adriano, D.C., Poucke, L.C.V., Clijsters, H., 2002. Effect of Pb-EDTA and EDTA in oxidative stress reactions and mineral uptake in Phaseolus vulgaris. Physiol. Plant. 115, 377–384.

Hao, X.Z., Zhou, D.M., Wang, Y.J., Cang, L., Chen, H.M., 2003. Effect of different amendments on ryegrass growth in copper mine tailings. Pedosphere 13, 299–308.

Hardy, M., Cornu, S., 2006. Location of natural trace elements in silty soils using particle-size fractionation. Geoderma 133, 295–308.

Hsu, J.H., Lo, S.L., 2000. Characterization of copper, manganese and zinc in swine manure composts. J. Environ. Qual. 29, 447–453.

Huang, Q., Shindo, H., 2000. Effects of copper on the activity and kinetics of free and immobilised acid phosphatase. Soil Biol. Biochem. 32, 1885–1892.

Jaradat, Q.M., Massadeh, A.M., Zaitoun, M.A., Maitah, B.M., 2006. Fractionation and sequential extraction of heavy metals in the soil of scrapyard of discarded vehicles. Environ. Monit. Assess. 112, 197–210.

Jenne, E.A., 1968. Controls on Mn, Fe, Co, Ni, Cu, and Zn concentrations in soils and water: the significant role of hydrous Mn and Fe oxides. In: Baker, R.A. (Ed.), Trace Inorganics in Water, Advances in Chemistry Series, No. 73. American Chemical Society, Washington, pp. 337–387.

Kabata-Pendias, A., Pendias, H., 2000. Trace Elements in Soils and Plants, third ed. CRC Press Inc., Boca Raton, USA.

Kandeler, E., Palli, S., Stemmer, M., Gerzabek, M.H., 1999. Tillage changes microbial biomass and enzyme activities in particle size fractions of a Haplic Chernozem. Soil Biol. Biochem. 31, 1253–1264.

Khan, M.J., Jones, D.L., 2009. Effect of composts, lime and diammonium phosphate on the phytoavailability of heavy metals in a copper mine tailing Soil. Pedosphere 19, 631–641.

Kiikkila, O., 2003. Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu–Ni smelter, in SW Finland. Silva Fenn. 37, 399–415.

Kızılkaya, R., 2004. Cu and Zn accumulation in earthworm L. terrestris L. in sewage sludge-amended soil and fractions of Cu and Zn in casts and surrounding soil. Ecol. Eng. 22, 141–151.

Kumpiene, J., Ore, S., Renella, G., Mench, M., Lagerkvist, A., Maurice, C., 2006. Assessment of zerovalent iron for stabilization of chromium, copper, and arsenic in soil. Environ. Pollut. 144, 62–69.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments—a review. Waste Manage. 28, 215–225.

Lombi, E., Zhao, F.J., Dunham, S.J., McGrath, S.P., 2002. Phytoremediation of heavy metal contaminated soils: natural hyperaccumulation versus chemically-enhanced phytoextraction. J. Environ. Qual. 30, 1919–1926.

Madrid, L., Diaz-Barrientos, E., 1992. Influence of carbonate on the retention of heavy metals in soils. Eur. J. Soil Sci. 43, 709–721.

Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorometric assay for the study of enzyme diversity in soils. Soil Biol. Biochem. 33, 1633–1640.

Marx, M.C., Kandeler, E., Wood, M., Wermbter, N., Jarvis, S.C., 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biol. Biochem. 37, 35–48.

Marzadori, C., Francioso, O., Ciavatta, C., Gessa, C., 2000. Influence of the content of heavy metals and molecular weight of humic acids fraction on the activity and stability of urease. Soil Biol. Biochem. 32, 1893–1898.

McBride, M.B., Bouldin, D.R., 1984. Long-term reactions of copper (II) in a contaminated calcareous soil. Soil Sci. Soc. Am. J. 48, 56–59.

McBride, M.B., 1989. Reactions controlling heavy metal solubility in soils. Adv. Soil Sci. 26, 1–56.

McGrath, S.P., Zhao, F.J., Lombi, E., 2002. Phytoremediation of metals, metalloids, and radionuclides. Adv. Agron. 75, 1–56.

McLaughlin, M.J., Hamon, R.E., McLaren, R.G., Speir, T.W., Rogers, S.L., 2000. Review: a bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. Aust. J. Soil Res. 38, 1037–1086.

Mench, M., Bes, C., 2009. Assessment of the ecotoxicity of topsoils from a wood treatment site. Pedosphere 19, 143–155.

Mench, M., Renella, G., Gelsomino, A., Landi, L., Nannipieri, P., 2006. Biochemical parameters and bacterial species richness in soils contaminated by sludge borne metals and remediated with inorganic soil amendments. Environ. Pollut. 144, 24–31.

Mench, M., Schwitzguébel, J.P., Schröder, P., Bert, V., Gawronski, S., Gupta, S., 2009. Assessment of successful experiments and limitations of phytotechnologies: contaminant uptake, detoxification and sequestration, and consequences for food safety. Environ. Sci. Pollut. Res. 16, 876–900.

Minkina, T.M., Motuzovab, G.V., Nazarenkoc, O.G., Kryshchenkoa, V.S., Mandzhieva, S.S., 2008. Combined approach for fractioning metal compounds in soils. Eurasian Soil Sci. 11, 1171–1179.

Negim, O.E.A., 2009. New technique for soil reclamation and conservation: in situ remediation of trace elements in contaminated soils. Ph.D. Dissertation, University Bordeaux 1, Géosciences Hydrosciences Matériaux Construction, Talence, France, 179 pp.

Park, J.-H., Panneerselvam, P., Lamb, D., Choppala, G., Bolan, N., 2011. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. J. Hazard. Mater. 185, 549–574.

Renella, G., Landi, L., Ascher, J., Ceccherini, M.T., Pietramellara, G., Mench, M., Nannipieri, P., 2008. Long-term effects of aided phytostabilization of trace elements on microbial biomass and activity, enzyme activities, and composition of microbial community in the Jales contaminated mine spoils. Environ. Pollut. 152, 702–712.

Saha, J.K., Mondal, A.K., Hazra, G.C., Mandal, B., 1991. Depthwise distribution of copper fractions in some ultisol. Soil Sci. 151, 452–458.

Sauvé, S., 2006. Copper inhibition of soil organic matter decomposition in a seventy-year field exposure. Environ. Tox. Chem. 25, 854–857.

Sauvé, S., McBride, M.B., Norvell, W.A., Hendershot, W.H., 1997. Copper solubility and speciation of in-situ contaminated soils: effects of copper level, pH and organic matter. Water Air Soil Pollut. 100, 133–149.

Schnitzer, M., Kodama, H., 1992. Interactions between organic and inorganic components in particle-size fractions separated from four soils. Soil Sci. Soc. Am. J. 56, 1099–1105.

Shuman, L.M., 1986. Effect of liming on the distribution of manganese, copper, iron and zinc among soil fractions. Soil Sci. Soc. Am. J. 50, 1236–1240.

Shuman, L.M., 1999. Organic waste amendments effect on zinc fractions of two soils. J. Environ. Qual. 28, 1442–1447.

Spark, K.M., Wells, J.D., Johnson, B.B., 1997. The interaction of a humic acid with heavy metals. Aust. J. Soil Res. 35, 89–101.

Stemmer, M., Gerzabek, M.H., Kandeler, E., 1998. Organic matter and enzyme activity in particle-size fractions of soils obtained after low energy sonication. Soil Biol. Biochem. 30, 9–17.

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.

Tscherko, M., Kandeler, E., 1997. Ecotoxicological effects of fluorine deposits on microbial biomass and enzyme activity in grassland. Eur. J. Soil Sci. 48, 329–335.

Tyler, G., Pahlsson, A.M.B., Bengtsson, G., Bääth, E., Tranvik, L., 1989. Heavy metal ecology of terrestrial plants, microorganisms and invertebrates. Water Air Soil Pollut. 47, 189–215.

Van Herwijnen, R., Hutchings, T.R., Al-Tabbaa, A., Moffat, A.J., Johns, M.J., Ouki, S.K., 2007. Remediation of metal contaminated soil with mineral-amended composts. Environ. Pollut. 150, 347–354.

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ. Sci. Pollut. Res. 16, 765–794.

Vepsäläinen, M., Kukkonen, S., Vestberg, M., Sirviö, H., Niemi, R.M., 2001. Application of soil enzyme activity test kit in a field experiment. Soil Biol. Biochem. 33, 1665–1672.

Wang, H.H., Li, L.Q., Wu, X.M., Pan, G.X., 2006. Distribution of Cu and Pb in particle size fractions of urban soils from different city zones of Nanjing. China J. Environ. Sci. 18, 482–487.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Licznar, S., Jamroz, E., Kocowicz, A., 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. Soil Biol. Biochem. 39, 1294–1302.

Yin, Y., Impellitteri, C.A., You, S.J., Allen, H.E., 2002. The importance of organic matter distribution and extract soil:solution ratio on the desorption of heavy metals from soils. Sci. Tot. Environ. 287, 107–119.