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

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ABSTRACT

Chemical fractionation of copper in bulk soil and its distribution in the particle-size fractions were analyzed in a Cu-contaminated soil (674 ± 122 μg Cu g^{-1}, up to 1900 μg 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.

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

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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_2O_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 Elmer 4000, AA flame, detection limit 20 μg L^{-1}). For each analytical batch, blanks were also prepared and analyzed.

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_2O_5 (g kg^{-1})	–	17.7
K_2O (g kg^{-1})	–	10.9
MgO (g kg^{-1})	–	4.7
CaO (g kg^{-1})	–	47.1
Na_2O (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.

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 $NH_2OH \cdot HCl$ in 25% acetic acid (reducible fraction bound to poorly crystalline Fe and Mn oxyhydroxides), 0.02 M HNO_3 and 30% H_2O_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 ($10^3 g$ 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 270*g* 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 5500*g* 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 methylumbelliferyl (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