Phytoremediation of polluted soils : recent progress and developments.

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PHYTOREMEDIATION OF POLLUTED SOILS: RECENT PROGRESS AND DEVELOPMENTS

Phytostabilisation of a copper contaminated topsoil aided by basic slags: assessment of Cu mobility and phytoavailability

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Abstract

Purpose: Basic slags are alkaline by-products of the steel industry with potential properties to ameliorate nutrient supply and metal stabilisation in contaminated soils. This study aimed at investigating the potential of a P-spiked Link Donawitz slag and a conventional basic slag called Carmeuse for the aided phytostabilisation of a Cu-contaminated soil at a wood treatment site. The effects of basic slag addition on Cu fractionation, mobility and (phyto) availability were assessed.

Materials and methods: Both slags were incorporated at 1% w/w into the Cu-contaminated soil phytostabilised with Cu-tolerant plants, using either outdoor lysimeters or a field plot. Untreated soil (UNT), amended soils with the P-spiked Link Donawitz slag (PLDS) and the conventional slag (CARM) respectively, and a control soil (CTRL) were sampled, potted and cultivated with dwarf bean. Physico-chemical analysis, determination of total soil elements and a Cu-sequential extraction scheme were carried out for all soils. Physico-chemical characteristics of soil pore water and Cu speciation (rhizon, ion selective electrode and diffusive gradient in thin film (DGT)) were determined. Shoot dry weight yield and leaf ionome (i.e. all inorganic ions present in the primary leaves) of dwarf beans were investigated.

Results and discussion: The slag incorporation at only 1 % w/w increased the soil pH from 1.5 to 2 units and electrical conductivity in soil pore water by 3 times. The residual Cu fraction increased for both slag amended soils compared to the UNT soil by 6 times in parallel to the decrease of the Cu oxidisable fraction (1.5 times) and to a less extent the reducible fraction. The incorporation of both slags did not reduce the total dissolved Cu concentration in the soil pore water but significantly reduced the real dissolved Cu concentration *ca* 5 times, the Cu labile pool as measured by DGT (at least two times) and the Cu phytoavailability. The dwarf

bean total biomass was also improved with the slag addition especially for the P-spiked Linz–Donawitz slag.

Conclusions: The addition of both slags in the contaminated soil increased Cu concentration in the residual fraction and thus reduced its potential mobility. Though the total dissolved Cu soil pore water concentration remained identical, its speciation changed as the real dissolved fraction diminished and lowered the Cu bioavailability. The addition of small amount of P-spiked Linz–Donawitz and Carmeuse slags was beneficial for this Cu-contaminated soil in the context of aided phytostabilisation.

1 Introduction

Trace elements (TE) such as As, Cu, Cr, and Zn can be in excess in contaminated soils at wood preservation sites, especially when Cu-based salts, i.e. Cu sulphate and chromated copper arsenate (CCA), have been long term used as preservatives against insects and fungi, which may result in soil phytotoxicity (Kumpiene et al. 2008). Among the feasible remediation options, phytostabilisation, singly and in combination with mineral and organic amendments (i.e. aided phytostabilisation), is a less invasive, low-cost phytotechnology able to restore (1) the physical and chemical properties of TE contaminated soils and (2) the cascade of biological processes and functions leading to remediated ecosystem services (Bolan et al. 2003, 2014; Kumpiene et al. 2008; Mench et al. 2010).

In situ (phyto) stabilisation techniques aim at decreasing both the labile TE pool and providing nutrient supply, and simultaneously promoting other beneficial process such as water retention, by incorporating organic and mineral amendments (Hattab et al. 2014). These techniques are able to enhance one or several processes such as metal adsorption through

increased surface charge, formation of organic and inorganic metal complexes, sorption on Fe, Mn, and Al oxides, and precipitation (Kumpiene et al. 2008). Further, roots of the implemented vegetation cover can modify the TE mobility by changing soil pH, electrochemical potentials, through element sorption on the root iron plaques and in their apoplast, and functioning of membrane transporters, and TE reactions with their rhizodeposition (Hinsinger 2001; Lombi et al. 2001). Among higher plants, poplars and perennial grasses possess a strong phytostabilisation capability (Marmiroli et al. 2011). The immobilization of TE in soil can also be induced through the use of soil amendments that reduce TE mobility and bioavailability (Hooda 2010). Several mineral and organic amendments such as lime, coal fly ashes, phosphates, red muds, compost, biosolids, iron grit and Fe/Mn/Al oxides can improve phytostabilisation and production of plant-based feedstock through decrease in TE bioavailability (Lombi et al. 2002; Bolan et al. 2003, 2014; Geebelen et al. 2003; Brown et al. 2004; Basta et al. 2004; Kumpiene et al. 2008; Mench et al. 2010). Several alkaline slags have been used for amending acidic soils. Linz-Donawitz (LD) slag is an alkaline by-product of the steel industry with potential properties to ameliorate nutrient supply and metal stabilisation in contaminated soils, and it consists of Ca, Al and other metal oxides (Negim et al. 2010). The LD slag incorporation into the soil increases the soil pH and electrical conductivity, and plant growth compared to the untreated soil (Marchand et al. 2011; Negim et al. 2012). It can be used as a soil additive to render metals less soluble in contaminated soils by precipitation of oxides and by adsorption on their surfaces. Additionally, in a pot experiment, a P-spiked LD slag was incorporated in a contaminated, acidic sandy topsoil from a wood preservation site to improve physical and chemical soil properties and to in situ stabilize Cu and other TE (Negim et al. 2012).

This study aimed at investigating the mobility and bioavailability of Cu in phytostabilised contaminated soils amended with basic slags after 5 years of treatment. A P-spiked LD slag

and a Carmeuse slag were respectively incorporated into the contaminated topsoils of large outdoor lysimeters and a field plot, cultivated with Cu tolerant plants. The effects of basic slag addition on Cu fractionation, mobility and (phyto) availability were studied for the contaminated soils with and without slag addition together with a control soil. Finally the potential of slags for aided-phytostabilisation of Cu-contaminated soils was assessed.

2 Materials and methods

2.1 Site description and soil sampling

The wood preservation site is located in southwest France, in the Gironde county, nearby the city of Bordeaux (44°43'N; 0°30'W). It consists of 2 ha remaining in activity and 6 ha of derelict areas. The industrial facility dates back to 1846. Creosote, Cu sulphate (from 1913 to 1980), aqueous solution of Cu, Cr and As salts (standard CCA type C; from 1980 to 2006), and Cu hydroxycarbonates with benzylalkonium chlorides (since 2006) were successively used (Mench and Bes 2009). Anthropogenic soils are developed on an alluvial soil in terrace (Fluvisol) containing alluvial materials from the Garonne River combined with wind deposits (Mench and Bes 2009). Copper is the main contaminant in topsoils, with total soil concentrations between 65 and 2600 mg kg^{-1}, showing considerable spatial variation, and exceeding background values for French sandy soils (3.2 – 8.4 mg kg^{-1}) and threshold values of 35 mg kg^{-1} above which a negative impact can be observed on plants and animals (Baize 1997; Baize and Tercé 2002). Arsenic and chromium did not accumulate in soil, except very slightly at a sub-site where CCA-treated posts were stacked (As = 52 mg kg^{-1} and Cr = 87 mg kg^{-1}, Mench and Bes 2009). Consequently, data for As and Cr were not reported in this study. Plant communities were assessed (Bes et al. 2010). The site has been colonized by several plant communities including mainly poplars (*Populus nigra*) and willows (*Salix caprea*, *S. viminalis*). Soil samples were taken from the P1-3 sub-site (for site details, see Mench and

Bes 2009). Total Cu concentrations in the P1-3 topsoil varied between 163 and 1460 mg kg^{-1} (Mench and Bes 2009; Marchand et al. 2011).

Soils were treated with two types of slags. The initial LD slag was furnished by the Centre Technique et de Promotion des Laitiers Sidérurgiques (CTPL, La Plaine Saint-Denis, France), and resulted from electric steel furnace, also known as basic oxygen furnace. It was chemically modified by precipitation of mineral phosphates to increase its P content, and so-called P-spiked LD slag. Details on P-spiked LD slag were done in Negim et al. (2012). This slag consisted of a complex mixture of Ca phases, i.e. Ca silicates such as larnite, Ca ferroaluminates such as brownmillerite and sreboldoskite, calcite and hydrated calcite such as portlandite, Fe oxides such as wustite, Si oxide (silica), and P phases such as oxides and phosphates. The phosphate crystals in the P-spiked LD slag mainly belonged to the analcime and apatite families. The Carmeuse slag is a steel slag from a converter produced by the Carmeuse company (norm Class IV, NF U 44-051, 2006), and highly crystallized. The crystalline phases identified by X-ray diffraction in this slag were identical to P-spiked LD slag except for the phosphate crystals (personal communication). The chemical composition of both slags was determined by atomic absorption spectrometry after mineralisation of the solid samples. The P-spiked LD slag is mainly composed of Ca, Fe, Si, P, Mg, Al, Mn. Major elements in the Carmeuse slag are Ca, Fe, Si, Mg, and Mn, whereas Al and P are present in minor amounts (Table 1). TE concentrations are below the detection limits for As, Co, Ni, Cu, Pb, and Zn, except Zn in the P-spiked LD slag (24 mg kg^{-1}), and Cu in the Carmeuse slag (13.9 mg kg^{-1}, Table 1).

In a previous study (Negim et al. 2012), the P-spiked LD slag was incorporated into the contaminated soil at increasing rates (0%, 1%, 2%, 3% and 4% in w/w), and even the 1% slag amendment ameliorated physico-chemical soil properties (pH and electrical conductivity) and metal stabilisation. Thus 1% w/w was selected for soil amendment for both P-spiked LD and

Carmeuse slags to potentially optimize the Cu immobilization without the adverse effect of over-liming. Both basic slags were carefully mixed into the topsoils (three aliquots successively incorporated to ensure a good homogenization) at 1% w/w.

For aided phytostabilisation experiments, two slag treatments were applied whether in outdoor lysimeters for the P-spiked LD slag or in open field for the Carmeuse slag. In March 2007, large vats (75 L, 0.5 m diameter) were filled with three successive layers to consists outdoor lysimeters in triplicates per soil treatment (Marchand et al. 2011): 5 cm of coarse gravels (1–3 cm, diameter), 22 cm of subsoil (from the 30–60 cm soil layer), and 25 cm of topsoil (from the 0–30 cm soil layer). Gravels and the subsoil were separated by a geotextile.

Topsoil treatments in 2007 included (i) untreated soil without plants (UNT) and (ii) soil amended with the P-spiked LD slag characterized by a granulometry less than 160 μm (1 g of slag for 100 g of air-dried soil; Negim et al. 2012), hereafter called PLDS, and phytostabilised with Cu-tolerant plants (*Agrostis gigantea* L. and *Populus trichocarpa x deltoides* cv. Beaupré). The topsoils of the UNT and PLDS were sampled in March 2012 in six lysimeters (i.e. three replicates of *ca* 1 kg for each soil treatment). In March 2012, a third topsoil sample was collected (in triplicates) in a plot (1 m x 2 m) of the P1-3 sub-site that had received the following treatment in 2007 also: (iii) incorporation of the Carmeuse slag (1 g of basic slag for 100 g of air-dried soil; granulometry < 0.160 μm) into the 0-25 cm soil layer and annual cultivation with a tobacco-sunflower crop rotation, and called CARM. Additionally, lysimeter leachates were collected in plastic bottles (1.5 L).

An uncontaminated soil, located on the same alluvial terrace at 2.3 km from the site (12-178 Chemin de Pâte, 33140 Cadaujac, France, 44.727083, -0.521458) was sampled and used as a control soil (CTRL). This area was a natural meadow.

2.2 Soil characterization

All bulk soil samples were dried at 40°C for 24 hours, and sieved to < 2 mm prior to analysis (Table 3). Particle size distribution of the < 2 mm fraction was determined by sieving at 250 and 50 μm. The soil pH and electrical conductivity (EC) of each sample were determined in distilled soil water extracts (1:2.5 w/v) (NF ISO 10390, 2005), as well as for lysimeter leachates. Total carbon contents were quantified by combustion of about 40 mg of soil sample at 1573 K using an elemental flash pyrolyser analyser (Flash 2000, Thermo Fischer Scientific). The combustion process converted any elemental carbon into CO_2 which was quantified with an infrared spectroscopy cell. Measurements were done in triplicate for each sample and the uncertainties were ± 0.5 mg g^{-1}. Total organic carbon (TOC) was determined in the soil samples by Rock-Eval pyrolysis (Rock-Eval 6 Turbo, Vinci Technologies, France). Laser ablation-inductively coupled plasma-mass spectrometer (LA-ICP-MS) analysis was performed on all soil samples. An elemental XR Thermo Specific, following the procedure described by Gratuze et al. (2001), Aries et al. (2001) and Motelica-Heino et al. (2001) was used in combination with a VG UV laser probe laser ablation sampling device operating at 213 nm. Powder samples of 350 mg were put under 8 metric ton pressure, without added binder, to form 13 mm (diameter) x 1 mm (height) pellets. The repetition rate of the laser was fixed to 10 Hz. Calibration took place with certified artificial glass, NIST-610. Measurements were replicated four times to validate the analytical precision of the technique. This procedure enabled to quantify the major and minor elements in the soils. The accuracy of the procedure was assessed with reference soil materials.

Additionally, total dissolved Cu concentrations in lysimeter leachates were determined using HR-ICP-MS (Finnigan Element XR, Thermo Electron, Germany), after filtering with cellulose nitrate Millipore 0.2 μm filters.

Sequential extraction schemes of Tessier et al. (1979) were used for all soil samples and experimental conditions are summarized in Table 2. All the reagents used to prepare the

extracting solutions were products of analytical-grade quality (Merck pro-analysis, Darmstadt, Germany). All solutions and dilutions were prepared using distilled water. Each extraction step was performed in triplicates, starting with 1g of original soil material previously sieved with the 2 mm mesh. The aqua regia step performed on the residue obtained from step 4 for was based on the ISO 11466 method (ISO norm 11466, 1995). Cu concentrations in all extracts from these sequential extraction schemes were determined by HR-ICP-MS.

2.3 Pot experiments

2.3.1 Germination tests

For each soil condition, three pots were each filled with 450 grams of soil (dry weight, DW) after sieving at 2 mm. During the first days, the soil moisture was manually maintained at 50% of the water holding capacity with additions of distilled water. Four seeds of dwarf beans (*Phaseolus vulgaris* L., cv. Limburgse vroege), previously germinated in wet cotton for four days, were transplanted in each pot and cultivated for 10 days in controlled conditions (16 h light/8h darkness regime at 21°C). The soil moisture was raised to 80% during this growing stage. At the end of the growing period, the plants were harvested and the DW yield of bean leaves was determined after drying at 70°C.

2.3.2 Determination of the leaf Cu concentration and amount for dwarf beans

Aliquots of primary leaves (35-150 mg DW) were wet digested into Savillex PTFE 50 mL vessels with 2 mL H_2O and 2 mL of concentrated HNO_3, and heated at 65°C for 16 hours. A volume of 0.5 mL H_2O_2 (30%) was added to each sample and left open at 75°C for 3 hours. Finally, 1 mL HNO_3 (48%) with 5 mL H_2O and 0.1 mL H_2O_2 were added, and gently warmed up. After cooling down, the mixture was completed with H_2O to a final volume of 50 mL. Cu concentration in digests was determined by HR-ICP-MS (Element 2, Thermofischer) and was

recovered according to the standard values. Cu concentrations are expressed in mg kg^{-1} on a DW basis. The Cu mineral mass in the primary leaves was obtained by multiplying their leaf DW yield with leaf Cu concentration: mineral mass of Cu (mg Cu/pot) = total biomass of bean primary leaves (mg biomass/pot) * primary leaf Cu concentration (mg Cu kg^{-1}).

2.4 Soil pore water characterization

The soil pore water (SPW) was collected before and after the plant testing with Rhizon soil-moisture samplers (SMS, model MOM, Rhizosphere Research Products, Wageningen, The Netherlands) with a pore diameter of 0.15 μm. The SMS devices were previously cleaned with 5% HNO$_3$ and then washed twice with deionised water. The capped end was inserted into each potted soil during filling. Two SMS devices were placed with a 45° angle into each potted soil to collect the SPW (~ 30 mL) before planting and after harvesting the dwarf beans, respectively. For this, a syringe needle was connected to the female lock and inserted into a 10 mL glass vacuum tube, for extracting the SPW by vacuum (Cattani 2006). The SPW samples were stored at 4°C. A fraction of each SPW sample was acidified to 0.1 mol L^{-1} HNO$_3$ for measuring the Cu concentrations by HR-ICP-MS (Element 2, Thermofischer). The rest of the solutions was kept for the following analyses: pH, EC, and dissolved organic carbon (DOC) determined by a Teckmar–Dohrman Phoenix 8000 TOC analyser. A Cu ion selective electrode (Thermo Scientific Orion) was used to determine the labile Cu concentration in the SPW samples. Knowing the soil pH and the total soil Cu concentrations, the copper activity, pCu^{2+}, was calculated with the following equation (Sauvé, 2003):

pCu^{2+} = 3.20 + 1.47pH − 1.84 log$_{10}$(total soil Cu)

2.5 Labile pool of Cu in the soils

For measuring available Cu in the soils and characterizing the soil exposure, standard cylindrical DGT (diffusive gradient in thin- film) units with Chelex-100 resin gels and an active surface area of 3.14 cm^2 were manually inserted for 24 h directly into the humid topsoil (80%) of each pot after the dwarf bean harvest (Davison et al. 2000; Ernstberger et al. 2002). DGT devices were washed after deployment with distilled water to remove the adhered soil particles. Subsequently, DGT devices were opened to remove the filter and the diffusive membrane. The chelex-100 resin gels were eluted by 1mL of 1M HNO$_3$. The eluate was further diluted 10 times and analyzed by using ICP-MS. The mass of metals accumulated in the resin gel (M, μg) was obtained following (Zhang et al. 1995):

$$M = Ce \, (V_{HNO3} + V_{gel})/fe$$

where Ce is the metal concentration in the eluent (μg mL^{-1}) measured analytically, V_{HNO3} is the volume of HNO$_3$ (1 mL), V_{gel} is the gel volume (0.15 mL) and fe is the elution factor for metals. The time-averaged concentration at the interface soil diffusive layer (C_{DGT}, μg L^{-1}), can be calculated from:

$$C_{DGT} = (M \, \Delta g)/(D \, t \, A)$$

where Δg is the diffusion layer thickness (0.094 cm), D is the diffusion coefficient of metal in the diffusion layer ($\times 10^{-6}$ cm^2 s^{-1}), t is the deployment time (s) and A is the area of the gel membrane exposed to the soil (3.14 cm^2).

2.6 Statistical analyses

Results were analyzed with the SPSS statistical software package (SPSS, Chicago, IL, USA). Averages were expressed with their standard deviation (± SD) and were compared by ANOVA. In each case the number of replicates (n) is indicated. Statistical tests were considered significant at $P \leq 0.05$.

3 Results and discussion

3.1 Soil properties

Main physico-chemical properties of the soils are summarized in Table 3. The aided-phytostabilised soils are similar to the UNT soil, in terms of their texture and chemistry, as the PLDS and CARM soils are composed of 99 wt% UNT and 1 wt% slag. All the studied soils had a sandy texture, their coarse fraction representing 67 to 83% of the 2000-250 μm fraction. The UNT soil was slightly acidic with a pH value of 6.4, while the aided-phytostabilised soils, PLDS and CARM, had higher pH values ranging from 7.9 to 8.4, due to the addition of basic slags. The CTRL soil had a lower pH value (5.4), characteristic of a sandy soil from an alluvial terrace. The EC values of the UNT and the CTRL soils were similar and low, related to their texture. The EC increased significantly with the incorporation of both slags (*ca* 3 times). The UNT and CTRL soils were poor in organic matter, with a mean total organic carbon content of 5 (UNT) and 10 (CTRL) mg kg^{-1} soil. Total soil Cu reached a high value in the UNT soil with 1460 mg kg^{-1}. The CTRL soil contained 96.5 ± 7.8 mg Cu kg^{-1}. This value is slightly higher than the background Cu values in French sandy soils, which varied from 3.2 to 8.4 mg kg^{-1} (Baize 1997), and may reflect a former vineyard activity. Our results are coherent to previous studies. Basic slags have been used extensively and for a long time to improve the characteristics of soils in the context of agronomical studies (e.g. improvement of the soil pH of acidic soils, soil fertilization; Chichilo et al. 1954). In particular several alkaline slags have been used for amending acidic soils (Ali and Shahram 2007). However research on the use of basic slags for the remediation of metal(loid)s contaminated soils is more limited. Basic slag, which consists of Ca, Al and other metal oxides, is an alkaline by-product of steelmaking or a residue of incineration processes. It can be used as a soil additive to render metals less soluble in contaminated soils by precipitation and adsorption on the surface of metal oxides (Negim et al. 2010, 2012). Other liming materials are indeed available on the

market and have been extensively used can improve soil properties such as pH, soil electrical conductivity (EC), cation exchange capacity (CEC), exchangeable Ca, Mg, and metal availability to plant, and also increase the crop yield. These include for instance phosphogypsum, calcitic limestone, biosolids and coal fly ashes as beringite (Negim et al. 2010, 2012). However slag treatments increased more soil pH than converter sludge treatments (Forghani et al. 2006). For Ali and Shahram (2007), the increasing rate of soil pH was proportional to the slag amount used. Additionally, the addition of phospho-gypsum can improve soil properties such as pH and soil electrical conductivity but to a lower extent (Alva and Sumner 1990). The application of calcium silicate slag reduced the soil acidity and increased available P, Si and exchangeable Ca in soil (Barbosa Filho et al. 2004). Blast furnace slag has also been used to correct soil acidity (Carvalho-Pupatto et al. 2004). Our previous work with basic slag and phosphate basic slag on similar soils (Negim et al. 2010, 2012) shows the potential of these materials to mitigate metal contamination in acidic soils by reducing the mobility and bioavailability of the contaminants.

3.2 Cu soil fractionation

Extractable Cu concentrations obtained by sequential extractions (procedure in Table 2) are presented in Fig 1. In the UNT soil, Cu extractability peaked for the oxidisable fraction (60.3 ± 8.0 %), so Cu was mainly associated with Fe- and Mn- (hydr)oxides. Another significant Cu part was located in the reducible fraction (20.5 ± 1.4 %), which likely corresponds to the fraction linked with organic matter. Both slag-amended soils had similar Cu distribution in the extractable fractions. Copper was mainly located in the residual fraction, with 44.2 ± 1.2 % for PLDS and 46.0 ± 8.0 % for CARM, then in the oxidisable fraction (37.7 ± 0.3 % for PLDS and 37.4 ± 2.3 % for CARM). The slag addition increased significantly the non remobilised Cu fraction. Copper immobilisation after incorporation of clays, organic matter,

carbonates, phosphates, and Fe oxides into the soil was reported with precipitation of Cu carbonates and oxy-hydroxides, ion exchange and the formation of ternary cation–anion complexes on the surface of Fe and Al oxy-hydroxides (Kumpiene et al. 2008). The application of calcium silicate slag reduced the soil acidity and increased available P, Si and exchangeable Ca in soil (Barbosa Filho et al. 2004). Copper is assumed to precipitate with hydroxide dissolved from the slag rather than to be adsorbed on the slag surface (Kim et al. 2008). Moreover blast-furnace slag is an effective sorbent for Cu, Zn, Ni, and Pb ions for a wide range of ion concentrations and pH values (Dimitrova 1996; Dimitrova and Mehandgiev 1998).

3.3 Cu speciation in the soil pore water and soils

Physico-chemical characteristics of the soil solutions, before and after bean cultivation, for the UNT, PLDS and CARM soils are listed in Table 4. Slag amendment into the soils had a significantly increased pH and EC in the SPW. The slag addition also increased the DOC in the SPW, with a maximum value for CARM (9.14 ± 0.36 mg L^{-1}) compared to the UNT soil (3.78 ± 0.37 mg L^{-1}). The total dissolved Cu concentrations varied from 5.7 to 10.2 μmol L^{-1} on average with fairly large variations notably before the plant testing. No significant difference was found between the UNT soil and both slag-amended soils. Total dissolved Cu concentrations in the SPW were similar before and after the bean cultivation. The Cu^{2+} concentrations were low in all SPW, with mean values between 0.08 and 0.72 μmol L^{-1}, with a significant decrease for the PLDS and CARM SPW as compared to the UNT one.

The lower pCu^{2+} is, the more Cu is dissolved, so potentially present as ionic form in SPW, and available for root uptake and soil food web. The pCu^{2+} presented similar values in both amended soils, much higher than in the UNT soil ($pCu^{2+} = 6.79$; Table 4). Such computed pCu^{2+} data agreed with changes in Cu^{2+} concentrations obtained by the specific Cu electrode.

Cu speciation in the soil solutions (Fig 2) can be described by the real dissolved Cu fraction (i.e. Cu^{2+} concentration measured by the specific Cu electrode), the available Cu fraction (i.e. labile pool determined by DGT), and the colloidal Cu fraction. The colloidal fraction represents the difference between the total dissolved Cu concentration and Cu^{2+} concentration, i.e. the Cu complexed with dissolved organic matter and inorganic colloids, as the ion Cu specific electrode measures Cu aquaions only (truly dissolved fraction). In both the untreated and slag-amended soil, the Cu^{2+} concentration in the SPW was largely smaller compared to the total dissolved Cu concentration, so Cu was mainly present in complexed forms, likely with colloids and dissolved organic matter. Labile Cu pool measured by DGT was numerically lower in slag-amended soils compared to UNT ($P \leq 0.05$) (Fig 2). However, no significant differences were found between both slag-amended soils (PLDS and CARM). The real dissolved Cu concentration in the SPW was significantly lower in both slag-amended soil compared to the UNT soil ($P \leq 0.05$), but no difference was observed between PLDS and CARM. Therefore, both slag amendments significantly reduced the available Cu fraction.

3.4 Cu phytoavailability

On average, the total biomass of the bean primary leaves varied between 3.4 (UNT) and 336.7 (PLDS) mg DW/pot (Table 5). The bean growth was very low on the UNT soil and increased on the slag-amended soils. The bean biomass for the CTRL soil presented an intermediate growth, this low biomass was not surprising, because of the relatively high Cu concentration (*ca* 100 mg kg^{-1}) in this control soil, which may inherit a past vineyard activity, which has not been fertilized since a long time. In fact, shoot DW of dwarf beans grown on a kitchen garden soil from the same alluvial terrace was *ca* 200 mg DW (Bes and Mench 2008). In this previous study, the application of alkaline materials, organic matters, phosphates, alumino-silicates, iron grit, basic slag (3.9%) and compost of sewage sludge (5%) combined with iron

grit were the most efficient to promote shoot production and limit foliar Cu accumulation in dwarf beans cultivated in a highly Cu-contaminated soil (Bes and Mench 2008). In combination with NPK fertilizers the application of a LD slag resulted in highest crop yields and nutrient concentrations in plants (Lopez et al. 1995). Both calcitic limestone and basic slag applied in Brazil sugarcane fields generated a beneficial residual effect in the correction of soil acidity, the increase of base saturation, and the yield of sugar cane ratoon (Prado et al. 2003).

Foliar Cu concentrations of beans from the UNT soil were very high and variable, from 1408 to 6581 mg kg^{-1} (Table 5). In contrast, foliar Cu concentrations of PLDS and CARM beans were significantly lower (P $\leq$ 0.05), five times lower in average. As expected, the leaf Cu concentrations were significantly much lower in the CTRL beans (16 $\pm$ 3 mg kg^{-1}, P $\leq$ 0.05). In a previous study on nearby soils, foliar Cu concentrations of *ca* 60 mg kg^{-1} were found for beans in a contaminated soil and 12 mg kg^{-1} in a control garden soil respectively (Bes and Mench 2008). Upper critical threshold values for foliar Cu concentrations are below 20 mg kg^{-1} (Kabata-Pendias and Pendias 2001) and were thus exceeded. Typical foliar Cu concentrations for plants from uncontaminated soils are between 5 and 10 mg kg^{-1} (Sanders et al. 1987) and were also exceeded.

Copper removal by bean primary leaves (so-called Cu mineral mass) was obtained by multiplying the foliar DW biomass with the foliar Cu concentration. The mineral mass of the UNT, PLDS, CARM and CTRL plants varied from 33.7 $\pm$ 28.1, 77.7 $\pm$ 29.8, 39.2 $\pm$ 27.4, to 4 $\pm$ 1 μg Cu for 4 plants, respectively (Fig 3). As expected, the mineral mass was the lowest for the CTRL plants. Concerning both slag-amended soils, the PLDS plants had a Cu mineral mass twice higher than in the CARM plants. This was a direct consequence of the higher leaf biomass for the PLDS plants, since foliar Cu concentrations were similar for both soils. The UNT plants had an intermediate mineral mass, due to a low leaf biomass combined with high

foliar Cu concentration. Our results corroborate previous results on slag addition in acidic soils for other metallic cations. For instance the incorporation of LD slag in acidic soils managed under pastures increased the soil pH with and without NPK fertilization (Pinto et al. 1995), increased the exchangeable soil Ca and Mg whereas exchangeable soil Al, Mn, Cu and Zn decreased (Besga et al. 1996). As for our results this is related to the increase of soil pH and the decrease of the acid soluble and oxidable fractions in the slag amended soils. Blast furnace slag has also been used to produce maximum root growth in acidic soils (Carvalho-Pupatto et al. 2004).

4 Conclusions

Changes in soil physico-chemical properties, Cu fractionation and potential mobility, SPW concentration and speciation, and Cu phytoavailability were assessed at a wood preservation site in topsoils of outdoor lysimeters and a field plot phytostabilised with Cu-tolerant plants and amended with a P-spiked Linz–Donawitz and a Carmeuse slag, respectively.

The slag incorporation into the Cu-contaminated soil at only 1 % in weight increased the soil pH from 1.5 to 2 units and electrical conductivity in soil pore water by 3 times. Increased soil pH may favor the sorption of free copper cations onto the slag surfaces as indicated by increased Cu concentration in the residual soil fraction and thus reduce the potential Cu mobility. In fact the residual Cu fraction significantly increased for both slag amended soils compared to the untreated soil in parallel to the decrease of the Cu oxidisable fraction and to a less extent the reducible fraction. The incorporation of both slags into the Cu-contaminated soil did not reduce the total dissolved Cu concentration in the SPW but significantly reduced the real dissolved Cu concentration and also the Cu labile pool as measured by DGT. No significant difference was observed between the effects of both slags. The incorporation of both slags into the Cu-contaminated soil reduced the Cu phytoavailability as the Cu

concentration in the dwarf beans leaves decreased significantly and this in the same order of magnitude for both slags. Additionally the dwarf bean total biomass was improved with the slag addition especially for the P-spiked Linz–Donawitz slag and even more than for the CTRL soil. Therefore the addition of a small amount of P-spiked Linz–Donawitz and Carmeuse slags is beneficial for this Cu-contaminated soil in the context of aided phytostabilisation.

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Table 1 Main characteristics of the P-spiked Link Donawitz and Carmeuse slags

Parameters	P-spiked Link Donawitz slag[a]	Carmeuse[c] slag
pH (in water)	11.6	12.7
EC[b] (μS cm^{-1})	2100	10700
SiO$_2$ (g kg^{-1})	146	111
Al$_2$O$_3$ (g kg^{-1})	56	14
Fe$_2$O$_3$ (g kg^{-1})	214	366
CaO (g kg^{-1})	307	448
MgO (g kg^{-1})	95	64
Na$_2$O (g kg^{-1})	n.d.	2
MnO (g kg^{-1})	25	42
P$_2$O$_5$ (g kg^{-1})	140	12
Cu (mg kg^{-1})	< 5	13.9

[a]Negim et al. 2012; [b]EC = electrical conductivity in water; [c]standardized product (Class IV, norm NF U 44-051, 2006); [d]n.d. = not determined

Table 2 Extraction conditions of the modified scheme of Tessier et al. (1979)

Fraction	Extractants and conditions
Exchangeable	1 M $MgCl_2$, pH 7, 8 mL, 1 h
Acid soluble	1 M NaOAc, pH 5, 8 mL, 1 h
Oxidizable	0.267 M $Na_3C_6H_5O_7$ and 0.111 M $NaHCO_3$, 50 mL, 10 min, 80°C
	then 200 g L^{-1} $Na_2S_2O_4$, 3 mL, 30 min, 80°C
Reducible	30% H_2O_2, 5 mL and 0.02 M HNO_3, 3 mL, 2 h, 85°C
	then 30% H_2O_2, 3 mL, 3 h, 85°C
	finally 3.2 M NH_4OAc, 1 h, 5 mL

Table 3 Main soil characteristics (0 - 0.25 m soil layer)

Parameters	UNT	PLDS	CARM	CTRL
2000-250 μm (%)	83	n.d.[d]	n.d.	67
50-50 μm (%)	14	n.d.	n.d.	24
< 50 μm (%)	3	n.d.	n.d.	9
Soil pH	6.4 ± 0.4	8.3 ± 0.1	8.0 ± 0.1	5.4 ± 0.4
Soil EC[a] (μS cm^{-1})	61.2 ± 19.8	184 ± 11.9	201.3 ± 5.5	86.3
Total C (g kg^{-1})	32.6 ± 3.5	27.5 ± 4.1	23.4 ± 7.4	83.9 ± 26.2
TOC[b] (g kg^{-1})	4.9 ± 0.4	n.d.	n.d.	10.4
Na_2O (g kg^{-1})	1.3 ± 0.9	n.d.	n.d.	1.9 ± 1.1
MgO (g kg^{-1})	3.9 ± 0.6	n.d.	n.d.	4.3 ± 1.9
Al_2O_3 (g kg^{-1})	165.1 ± 29.3	n.d.	n.d.	132.1 ± 66.8
SiO_2 (g kg^{-1})	661.4 ± 55.9	n.d.	n.d.	673.1 ± 149.2
P_2O_5 (g kg^{-1})	2.4 ± 0.3	n.d.	n.d.	2.2 ± 0.9
CaO (g kg^{-1})	15.8 ± 21.8	n.d.	n.d.	3.2 ± 0.4
TiO_2 (g kg^{-1})	2.7 ± 0.2	n.d.	n.d.	10.5 ± 14.2
MnO (g kg^{-1})	0.8 ± 0.1	n.d.	n.d.	0.9 ± 0.9
Fe_2O_3 (g kg^{-1})	40.8 ± 7.9	n.d.	n.d.	41.8 ± 20.7
Cu (mg kg^{-1})	1460	n.d.	n.d.	96.5 ± 7.8

[a]EC = electrical conductivity; [b]TOC = total organic carbon; [c]n.d. = not determined

Table 4 Physico-chemical characteristics of the soil solutions before and after bean cultivation (n=3; ± standard deviation)

Parameters	Time	UNT	PLDS	CARM
pH	before	6.0 ± 0.8	7.2 ± 0.1	7.5 ± 0.2
	after	7.4 ± 0.5	7.4 ± 0.0	7.7 ± 0.1
EC (μS cm^{-1})[a]	before	406 ± 91	994 ± 31	1673 ± 791
	after	359 ± 63	n.d.[d]	1271
DOC (mg L^{-1})[b]	before	3.78 ± 0.37	6.77 ± 0.20	9.14 ± 0.36
Cu^{2+} (μmol L^{-1})[c]	before	0.67 ± 0.86	0.13 ± 0.02	0.08 ± 0.02
	after	0.72 ± 0.16	0.11	0.13 ± 0.13
pCu^{2+} [e]	before	6.79 ± 0.15	9.58 ± 0.15	9.14 ± 0.15
Total dissolved Cu	before	8.9 ± 3.3	10.2 ± 4.7	6.4 ± 4.6
concentration (μmol L^{-1})[f]	after	5.7 ± 0.7	10.0 ± 3.5	5.8 ± 0.6

[a]EC = electric conductivity; [b]DOC = dissolved organic carbon values; [c]Cu^{2+} measured by the Cu specific electrode; [d]n.d. = no data; [e]$pCu^{2+} = 3.20 + 1.47pH - 1.84\log_{10}$(total soil Cu) (Sauvé, 2003); [f]total dissolved Cu concentration analyzed by ICP-MS; time: before or after bean cultivation

Table 5 Total biomass of bean primary leaves per pot and Cu concentration in the bean primary leaves after 14 days (n=3; ± standard deviation). Different letters indicate a significant difference between soils (P ≤ 0.05)

	UNT	PLDS	CARM	CTRL
Total biomass of bean primary leaves (mg/pot)	3.4	336.7	210 130	
Cu (mg kg^{-1})	3365 ± 2807	77 ± 29	62 ± 43	16 ± 3
	a	b	b	c

Figure captions

Fig. 1 Extractable Cu concentrations by sequential extractions (n = 3, ± standard deviation). Different letters on bar graphs indicate a significant difference between soils for a given extraction (P ≤ 0.05)

Fig. 2 Cu speciation in the soil solutions (n = 3, ± standard deviation). Different letters on bar graphs indicate a significant difference between soils for a given soil solution fraction (P ≤ 0.05)

Fig. 3 Cu mineral masses in the bean primary leaves (n = 3, ± standard deviation). Different letters on bar graphs indicate a significant difference between soils (P ≤ 0.05)