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RESEARCH ARTICLE

Copper distribution in surface and subsurface soil horizons

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Abstract The horizons of four natural soils were treated with Cu^{2+} in an acid medium to study the retention capacity of Cu. The possible mineralogical changes arising because of the treatment were also studied. The soil properties and characteristics with the greatest influence on the metal retention and its distribution among the different soil fractions were determined. Crystalline phases of each horizon were determined by X-ray diffraction (XDR). The morphology, structural distribution and particle chemical composition of soil samples were investigated using field emission scanning electron microscopy. Cu distribution in the different geochemical phases of the soil was studied using a sequential extraction. The treatment led to an increase in the amorphous phases and the formation of new crystalline phases, such as rouaite ($Cu_2(NO_3)(OH)_3$) and nitratine ($NaNO_3$). Cu was also found superficially sorbed on amorphous hydroxy compounds of Fe that interact with albite, muscovite and gibbsite, and also on spherical and curved particles of aluminium clays. The largest amount of Cu retained was in an exchangeable form, and the smallest amount associated with the crystalline Fe oxides and residual fraction. In the surface horizons, the predominant Cu retention process is complexation in organomineral associations, while in the subsurface horizons it is adsorption.

Keywords Copper · FE-SEM · Mineral alteration · Sequential extraction · Soil · XRD

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Introduction

In recent decades, the soil has become a medium that receives potentially contaminating heavy metals, whose overall content has been significantly modified (Wei and Yang 2010). Cu is one of the metals that major concerns about their impact on the environment in general and human health in particular (Qin et al. 2004).

Cu is a relatively abundant metal in the lithosphere. It is also an essential metal for the growth and development of living organisms (He et al. 2005), although when it is introduced in the environment, depending on its concentration and speciation, may lead to problems of toxicity and changes in environmental conditions (Xue et al. 2003). The accumulation in soils is mainly due to the use of fertilizers and pesticides, the draining of acidic waters from waste spills and the production of alloys, the manufacture of electrical and fibre optic cables, its use in water pipes, in biocides for treating wood, its use in agriculture, in paint manufacture and in smelting procedures.

High levels of Cu in the soil have a negative effect on the biomass, the microbial diversity and plant growth, but they can also cause changes in the chemical properties of the soil (Andrade et al. 2005). The mobility, bioavailability and toxicity of Cu are mainly controlled by sorption–desorption processes that occur in both the organic and inorganic soil colloids (Violante et al. 2010). Therefore not only the soil organic carbon, clay and oxides contents but also other soil properties like soil pH and the cationic exchangeable capacity govern the Cu availability.

Soils are often many metres deep and several processes are responsible for the presence of identifiable horizons. Surface horizons result from the mixing of organic material and mineral material and subsurface ones are formed after accumulations, mineral transformations of specific soil components or preferential losses (Buol 2006). Each horizon deep depends on many external and internal soil factors.

The investigations on metal forms, particularly using sequential extractions, mainly concern surface soil horizons (Kabala and Singh 2001) since they are the most affected ones when pollution events like spills occur. Nevertheless, leaching occurs through the soil profile and it is required that subsurface horizons are included in this kind of studies. Surface and subsurface horizons significant differ among their soil components and they will probably react in different way when metals like Cu are in the soil profile.

In order to understand and therefore minimize the mobility and toxicity of pollutants, it is essential to identify the components and properties of the soil horizons that affect their retention. Many studies are focused on soils already contaminated by Cu (Zhuang et al. 2009; Srinivasa Gowd et al. 2010; Moon et al. 2011; Yang et al. 2011) and the problem that anthropogenic pollution can cause in not contaminated places is not well addressed.

Recent studies have shown that techniques such as field emission scanning electron microscopy (FE-SEM) and X-ray diffraction (XRD) are effective in identifying minerals (Silva et al. 2010a, b) and soil solid phases that retain metals (Beesley and Marmiroli 2011; Cerqueira et al. 2011, 2012). However, the information obtained from this type of analysis only provides qualitative results on the association between the heavy metals and the soil. The results provided by microscopy and XRD can be rounded out using other methods such as fractionation based on sequential extractions (Zhou et al. 2002; Yu et al. 2010). Conclusions about the distribution of the metals, their behaviour, mobility and bioavailability can be reached and their potential risk to the environment can be evaluated.

In this study, we simulated an industrial spill with a high concentration of Cu^{2+}. The surface and subsurface horizons of four different natural soils were Cu spiked in order to study the properties and components of the soil horizons involved in fixing this element and other possible effects of the spill on soils. The objectives were as follows: (1) to identify the properties and characteristics of the horizons with the greatest influence on the retention of Cu therefore with the least influence on its mobility; (2) to determine if the simulated spill causes changes in the mineralogy of the horizons, by means of the techniques XRD and FE-SEM and (3) to study the distribution of the Cu fixed in the soil fractions with the greatest influence on Cu sorption by means of sequential chemical extraction.

Material and methods

Selection and analysis of the soils

We selected four soils: S1 (N: 42°00′373″, O: 8°40′149″), S2 (N: 42°54′690″, O: 8°04′946″), S3 (N: 43°33′1.8″, O: 7°20′ 2.4″), and S4 (N: 42°06′52″, O: 8°49′25.2″), developed on quaternary sediments, amphibolite, slate and "two mica" granite, respectively. According to the FAO (2006), the soils are classified as Umbric Acrisol, Dystric Fluvisol, Mollic Umbrisol and Thionic Fluvisol, respectively, and the surface (S1.A, S2.A, S3.A and S4.A) and subsurface horizons (S1.B, S2.B, S3.B and S4.G) of each soil were sampled using an Eijkelkamp sampler. Six samples of each horizon were collected and then stored in polyethylene bags. The samples were pooled, air-dried, passed through a 2-mm sieve and homogenized in a Fritsch Laborette 27 rotary sample divider. Each homogenized and pooled sample was divided in six subsamples; three were used for soil analyses and three for sorption and sequential extraction experiments, XRD and FE-SEM analysis.

Soil pH was determined according to Guitián and Carballas (1976) and particle size distribution following Day (1965). Clay mineralogy was determined by powder X-ray diffractometry in a SIEMENS D-5000 Bragg–Brentano (θ/2θ) apparatus with a Cu anode, using 0.05 steps and 10 s per step.

Total organic C (TOC) and dissolved organic C (DOC) were determined with a TOC analyser-V CSH/CSN Shimadzu apparatus, by applying the principle of catalytic combustion oxidation and detection by non-dispersive IR (according to UNE-EN 1484).

The cation exchange capacity (CEC) and exchangeable cation content were determined according to Hendershot and Duquette (1986). Al, Ca, K, Mg and Na were extracted with 0.1 M $BaCl_2$, and the concentration was determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Fe, Mn and Al oxide contents were determined using the dithionite–citrate method (SCS U.S. Department of Agriculture 1972; Sherdrick and McKeague 1975): samples were shaken with a solution of sodium hydrosulphite (0.5 g per gram of soil) and sodium citrate (0.27 M), and the Fe, Al and Mn contents of the extract were determined by ICP-OES as above. Amorphous and crystalline Fe oxides were determined using, respectively, 0.25 M $NH_2OH.HCl$+0.25M HCl (50 °C, 30 min) and 0.2 M $(NH4)_2C_2O_4$+0.1 M $H_2C_2O_4$+ 0.1 M citric acid (100 °C, 30 min) as extractants (Shuman 1985) and the Fe content was analysed by ICP-OES.

Cu^{2+} spiking experiments

In order to maintain the soil pH (tested after sorption) and to study their influence on the Cu^{+2} sorption, 100 mL of a 0.5-mM $CuNO_3$ and 1-mM $NaNO_3$ at soil pH was added to 12 g soil in polyethylene tubes. They were shaken in a rotary shaker for 24 h at 25 °C and then centrifuged for 15 min at 5,000 rpm. The supernatant was filtered through Filter-Lab 1244 paper (pore size 0.45 μm), and the resulting filtrate was analysed for Cu by ICP-OES. The retained Cu was calculated as the difference between its concentrations in solution before the addition of soil and after equilibration.

After Cu^{2+} addition and shaking, samples were centrifuged and filtered and the supernatant separated. The soil pellets were dried at room temperature and then stored at 4 °C to avoid alterations until subsequent analyses of X ray diffraction, scanning electron microscope and sequential chemical extraction.

X-ray diffraction

The soil samples were measured on a Philips type powder diffractometer fitted with a Philips PW1710 control unit, vertical Philips PW1820/00 goniometer and FR590 Enraf Nonius generator (QL, 1 %). The instrument was equipped with a graphite diffracted beam monochromator and copper radiation source ($\lambda(K\alpha1)=1.5406Å$), operating at 40 kV and 30 mA. The X-ray powder diffraction pattern (XRPD) was collected by measuring the scintillation response to Cu $K\alpha$ radiation versus the 2θ value over a 2θ range of 2–65, with a step size of 0.02° and counting time of 4 s per step.

In order to determine the crystalline phases, the Reference Intensity Ratio (RIR) method of Chung (1974) was used, using corundum as a reference material. The crystalline phases present were identified and quantified using the Match! programme (© CRYSTAL IMPACT, Bonn, Germany).

Field emission scanning electron microscopy

The morphology, structural distribution and particle chemical composition of soil samples containing ultrafine particles and minerals (crystalline and/or amorphous) were investigated using a Zeiss Model ULTRA plus FE-SEM with charge compensation for all applications in both conductive and non-conductive samples. The FE-SEM was equipped with an energy dispersive spectrometer (EDS), and the mineral identification was made on the basis of morphology and grain composition using both secondary electron and back-scattered electron modes (Silva et al. 2011; Silva and Da Boit 2011). Samples were dried at 45 °C. Suspensions were prepared in hexane to prevent possible mineralogical changes. The samples were prepared on a standard aluminium slide with carbon adhesive, coating them with 20-nm-thick layers of gold/palladium.

EDS spectra were recorded in the FE-SEM image mode. The electron diffraction patterns of the crystalline phases were recorded in SAED or MBD mode, and the d spacing was compared to the International Center for Diffraction Data (ICDD. International Center for Diffraction Data 2014) inorganic compound powder diffraction file (PDF) database in order to identify the crystalline phases.

Sequential extraction experiments

In order to determine the Cu content in the different geochemical phases of the soil, the sequential extraction procedure of Shuman (1979, 1985) was used, with the modifications indicated by Fabrizio de Iorio A (2010) in the method that Chao and Zhou (1983) proposed for the solution of amorphous iron oxides. The sequential extraction was carried out in six stages (Table 1). Using 6 g of soil from each horizon treated with Cu, the sequential extraction procedure shown in Table 1 was applied. The concentration of Cu in the extracts associated with each fraction was analysed by ICP-OES (QL, 0.01 mg L^{-1}). As a blank, solutions of each extractant used for speciation were prepared and were analysed by ICPOES. Cu standards for process control were prepared in these blank solutions.

Statistical analysis

All of the experiments and analysis were performed in triplicate. The data obtained in the analytical determinations were analysed with the statistical program IBM-SPSS Statistics 19 (SPSS, Inc., Chicago, IL). The results obtained in all the determinations were the average with the standard deviation of three analyses and were expressed on a dry material basis. We applied the Kolmogorov–Smirnov test to check the normality of the data, and the Levene test for homogeneity of variances to test their homoscedasticity.

The data met conditions of normality and homoscedasticity and thus were analysed using a simple factorial analysis of variance (ANOVA) and Tukey test for multiple comparisons.

The influence of soil properties on the Cu distribution between soil fractions was determined by correlation analysis. It was verified that the residuals have shown a normal distribution.

Results and discussion

Soil characteristics

The soil characteristics are shown in Table 2. There are significant differences, mainly between those characteristics that can affect the metal retention and therefore its mobility throughout the soil profile. The soil pH ranges from nearly neutral, 7.56 (S4.G), to strongly acidic, 4.74 (S1.A), and Cu retention may be influenced since its mobility and availability decreases as pH approaches neutrality. Cu is highly mobile in acid conditions.

TOC contents range from 144.79 g kg^{-1} in the surface horizon of S2 and 4.34 g kg^{-1} in S4.G. Organic carbon tends to form not only insoluble complexes with Cu but also soluble

Table 1 Operating conditions used in sequential extraction procedure

Stage	Fraction/bound to	Reagents	Volume	Conditions
1	Exchangeable	$Mg(NO_3)_2$ 1 M pH 7	25 mL	1—6 g soil; 2 h agitation; centrifugation 6,000 rpm (10 min); supernatant decantation 2—Bidistilled water washing (15 mL–30 min); centrifugation 6,000 rpm (10 min) 3—Washed added to supernatant
2	Organic matter	0.7 M NaClO pH 8.5	15 mL	Remaining fraction: 1—Bath 100 °C (30 min); centrifugation 6,000 rpm (10 min); supernatant decantation 2—Bidistilled water washing (15 mL–30 min); centrifugation 6,000 rpm (10 min) 3—Washed added to supernatant 4—0.5 mm sieving residue
3	Mn oxides	0.1 M NH_2OH HCl pH 2	25 mL	Remaining fraction: 1—30 min agitation; centrifugation 6,000 rpm (10 min); supernatant decantation 2—Bidistilled water washing (25 mL–3 min); centrifugation 6,000 rpm (10 min) 3—Washed added to supernatant
4	Amorphous Fe oxides	0.25 M $NH_2OH \cdot HCl$ + 0.25 M HCl	25 mL	Remaining fraction: 1—Bath 50 °C and agitation (30 min); centrifugation 6,000 rpm (10 min) 2—Bidistilled water washing (25 mL–3 min); centrifugation 6,000 rpm (10 min) 3—Washed added to supernatant
5	Crystalline Fe oxides	0.2 M $(NH_4)_2C_2O_4$ + 0.1 M $H_2C_2O_4$ + 0.1 M citric acid	25 mL	Remaining fraction: 1—Bath 100 °C (30 min); centrifugation 6,000 rpm (10 min) 2—Supernatant decantation
6	Residual	HNO_3–HCl (1:3)	9 mL	1—0.2 g remaining fraction: digestion in microwave oven

complexes that can migrate throughout the profile (Cao et al. 2004). Therefore, dissolved organic content (DOC) was analysed and the content is low, it ranges between 0.54 g kg^{-1} (S1.A) and 0.09 g kg^{-1} (S4.G). As expected, both

Table 2 Soil characteristics

		S1.A	S1.B	S2.A	S2.B	S3.A	S3.B	S4.A	S4.G
pH		4.7d±0.1	4.9d±0.1	5cd±0.1	5.4c±0.3	5.2c±0.2	5.2c±0.0	6.8b±0.0	7.6a±0.0
TOC	g kg^{-1}	63.8b±0.6	14.6d±0.4	145a±11	16.8d±0.7	50.2c±1.3	47.4c±0.5	58.8b±0.3	4.3e±0.4
DOC		0.5a±0.1	0.2c±0.0	0.4b±0.0	0.1cd±0.0	0.3c±0.0	0.2c±0.0	0.5b±0.0	0.1d±0.0
OM	%	12.8b±0.1	2.5d±0.1	29a±2.1	2.9d±0.1	8.7c±0.2	8.2c±0.1	11.8b±0.1	0.8e±0.1
FeO_x	g kg^{-1}	19.7d±0.2	21c±0.3	15.5e±0.9	4.4g±0.0	23b±0.5	29a±0.6	11.5f±0.5	3.6g±0.1
AmorphousFeO_x		5.2a±0.4	2.7d±0.2	5.4a±0.2	2.5d±0.1	3.6c±0.2	4.1b±0.3	4bc±0.3	1.1e±0.0
CrystallineFeO_x		12.1c±1	15.6b±1.3	11.4c±0.7	1.8e±0.2	16.6b±0.8	19.5a±1	6.3d±0.4	1.1e±0.0
AlO_x		0.03c±0.0	0.02d±0.0	0.11b±0.0	0.15b±0.0	0.12b±0.0	0.2a±0.0	0.05c±0.0	0.04c±0.0
MnO_x		19.5c±0.3	13.1d±0.2	35.7a±3.1	5.2f±0.2	20.3c±0.5	27.1b±1.1	9.3e±0.6	1.3g±0.1
Amorphous Fe/free Fe		0.26	0.13	0.35	0.58	0.22	0.21	0.63	1.01
ECEC	$cmol_{(+)}kg^{-1}$	5.2b±0.0	2.9e±0.1	4.5c±0.0	1.8f±0.1	3.3d±0.2	3de±0.2	19.3a±0.2	2.8e±0.3
CEC		12.3b±0.3	5.9e±0.2	10.7c±0.6	4.0f±0.3	6.2d±0.3	6.5d±0.3	19.3a±0.2	3.2g±0.3
Na^+		0.15de±0.0	0.12de±0.0	0.13de±0.0	0.07e±0.0	0.39c±0.0	0.34cd±0.0	7.9a±0.4	1.1b±0.1
K^+		0.11bcd±0.0	0.08cd±0.0	0.18b±0.0	0.03d±0.0	0.2b±0.1	0.15bc±0.0	1.32a±0.1	0.2b±0.0
Ca^{2+}		0.1c±0.0	0.8b±0.0	0.1c±0.0	0.1c±0.0	0.7b±0.1	0.1c±0.0	4.2a±0.3	0.1c±0.0
Mg^{2+}		0.2cd±0.0	0.1ef±0.0	0.1de±0.0	0.02f±±0.0	0.4c±0.1	0.2de±0.0	5.8a±0.1	1.3b±0.1
Al^{3+}		4.6a±0.1	1.9d±0.1	3.9b±0.1	1.6e±0.0	1.6e±0.0	2.1c±0.3	0.01f±0.0	udl
H^+		7.1a±0.2	2.9d±0.1	6.3b±0.6	2.2e±0.2	3d±0.1	3.5c±0.1	0.1f±0.0	0.4f±0.0
Sand	%	49g±0.6	48.7h±1	50.6f±0.9	60.3c±1	57.5e±0.8	60.9b±1.2	59.9d±1	91a±1.6
Silt		24.1f±0.4	24g±0.6	32a±0.4	28.4c±0.7	30.8b±0.3	28.3d±0.3	27.2e±0.8	5.1h±0.1
Clay		26.9b±0.5	27.3a±0.7	17.5c±0.1	11.4f±0.2	11.7e±0.2	10.8g±0.2	13d±0.6	4h±0.1

For each parameter, values followed by different letters differ significantly with $P<0.05$

TOC total organic carbon, *DOC* dissolved organic carbon, *OM* organic matter, *FeO_x* iron oxides, *AlO_x* aluminium oxides, *MnO_x* manganese oxides, *ECEC* effective cation exchange capacity, *CEC* cation exchange capacity, *udl* undetected level contents are much lower in subsurface horizons than in superficial ones. DOC is a small fraction of TOC in all of the studied horizons (Table 2) therefore only a small proportion of the added Cu will be in the soluble organic carbon complexes. The organic matter of these soils, especially in the surface horizons, will contribute to Cu retention since it has a high affinity for this metal (Banerjee 2003; Vega et al. 2010).

Amorphous iron oxides play an important role in the sorption of Cu (Yu et al. 2004), as when removing the amorphous iron oxide fraction in soils with low organic matter content, Cu sorption decreased (Agbenin and Olojo 2004). From the studied horizons, S3.B contains the highest proportion of Fe and Mn free oxides (Table 2), and the S2.A horizon is the one with the highest Al free oxides and amorphous Fe oxides content.

The amorphous Fe/free Fe ratio (Fabrizio de Iorio A 2010) is a relative measurement of the degree of crystallinity and age of the iron oxides. A high ratio indicates low crystallinity, and that the accumulation of iron oxides is recent. This ratio is highest in the S4 soil, increasing with depth (Table 2). S4 was formed after periodic material inputs, and so the oxide accumulation is more recent than in the other studied soils. Furthermore, the S4 soil is regularly flooded and crystallization is not favoured under hydromorphic conditions. The S2 soil follows the same pattern as the S4, while S1 is similar to the S3 soil.

The ECEC is low for most of the soils (<6 cmol$_+$kg^{-1}) except for the A horizon of S4 (19.26 cmol$_+$kg^{-1}) (Table 2). S4.A has a nearly neutral pH, caused among other factors by its high content of exchangeable Ca and Mg (4.19 and 5.82 cmol kg^{-1}). The horizons with the highest clay content are S1.A and S1.B, while the smallest proportion of clay is in the S4.G horizon (Table 2).

According to the soil properties, the selected soils show different components and property values that regulate the mobility and fixation of Cu. The selected soils are suitable for studying Cu fixation, its fractionation and therefore its mobility.

Total content of fixed Cu

Figure 1 shows the total amount of Cu retained in the horizon soil samples. The concentration retained was very high, ranging between 39 mg kg^{-1} in S1.B and 240 mg kg^{-1} in S2.A (Fig. 1). The surface horizons fixed more Cu than the corresponding subsurface horizon (Fig. 1), except S3, in which there is a small difference (that is not statistically significant) between the Cu retained by both horizons.

The sequence or the horizons according to their capacity to fix Cu, from the highest to the lowest (S2.A>S4.A>S3.A≥S3.B>S1.A>S2.B>S4.G>S1.B), show that the surface horizons have the greatest capacity to retain this metal. This was already shown by Su and Wong (2004), which demonstrated the high affinity of soil organic matter for Cu.

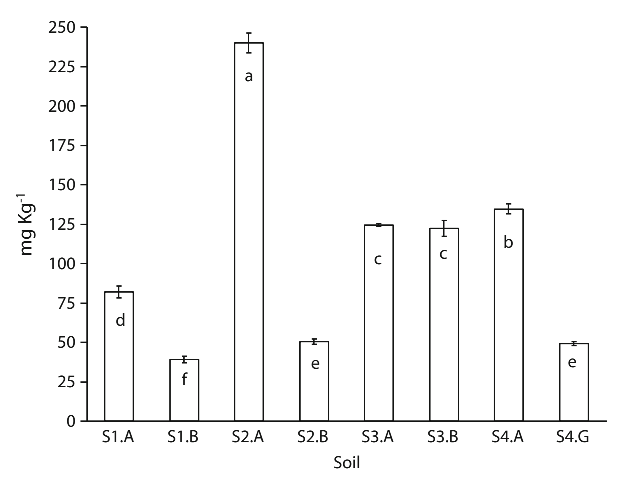

Fig. 1 Total amount of Cu retained in the soil samples (*Bars with a different letter* are statistically different using ANOVA test ($P<0.05$)

As previously indicated, S3.B has a greater fixing capacity than S1.A and it is probably due to the high content of organic matter in S3.B, as well as oxides of Fe and Mn (the highest in all of those studied, see Table 2), as they are components of soils with a high capacity to fix Cu (Ma and Rao 1997; Covelo et al. 2007; Li et al. 2007).

S2.A retains the most Cu (240 mg kg^{-1}, Fig. 1) among studied horizons. This horizon has a very high organic material content (29 %; Table 2) which has a decisive influence on the fixing of this metal (Su and Wong 2004). The highest concentration of Fe and Al oxides (Table 2) also influences the fixation of cations (Table 2).

The large difference among Cu fixed in S2 horizons is due to S2.A and has the highest content of organic matter and amorphous Fe and Al oxides, while S2.B is one of the horizons with the lowest concentration of these components (Table 2).

From the surface horizons, the one with the lowest capacity to fix Cu is S1.A (even less than the B horizon of S3). The S1.B horizon has the lowest retention capacity of all of those studied (Fig. 1, 39 mg kg^{-1}). They are also the horizons with the most acidic pH (there are no significant differences between the value of both, see Table 2) and it is well known that the pH of the soil plays a decisive role in the retention of cations.

The A and G horizons of S4 are those with the highest pH, although with significant differences between them (Table 2). The amounts of Cu fixed are very different (Fig. 1; S4.A, 134 mg kg^{-1} and S4.G, 49 mg kg^{-1}). As occurs with S2, the great difference can be attributed to the differences in their proportion of organic matter (11.75 and 0.75 %; Table 2), amorphous Fe oxides (4 and 1 g kg^{-1}; Table 2) and Al oxides (9.3 and 1.3 g kg^{-1}). Furthermore, S4 is a marsh soil, whose surface horizon mainly proceeds from the material transported by the river.

Different authors stated that the Cu addition may cause pH changes in marine sediments due to high salinity and redox conditions (Hutchins et al. 2007, 2009). In our work, where the Cu^{2+} solution was added at pH 7, significant changes in soil acidity were not observed, although the most labile mineral fractions were altered. This effect is mainly due to soil buffer capacity because of their high CEC. In addition, under environments close to neutrality, the alteration and weathering of alkaline minerals generate basis (mostly Ca^{2+}, Mg^{2+} and OH^{-}). This basicity is neutralized by the H_3O^+ released by the exchangeable aluminium when it is hydrolysed. Moreover, in agreement with other studies, basic ions are exchanged by Cu, which is partly retained by adsorption (Vega et al. 2009, 2010).

Soil mineralogical analysis

Table 3 shows the results of the mineralogical analysis performed to both the unpolluted soil samples and those obtained after adding Cu.

Quartz is the main mineral in all of the horizons, varying between 27.8 % in S2.B and 66 % in S1.A (Table 3). Although to a lesser extent, the soils were also found to contain albite (between 5.8 and 29 %), muscovite (between 6.1 and 27.4 %) and kaolinite (between 3.1 and 11.9 %). Gibbsite is only present in the horizons of soils S1 and S2, varying between 3 and 14.2 % in the surface and subsurface horizons of S2, respectively (Table 3). Soil S3 is the only one without microcline in its horizons, and together with horizon A of S2, those that contain chlorite, with a very similar proportion in all of them: 6, 9.8 and 8 %, respectively (Table 3).

After the treatment with Cu, the mineralogy of the soils underwent different changes. New crystalline phases formed in all of the horizons, such as nitratine (between 1.3 % in S1.B and 14.4 % in S2.A; Table 3). This is not only due to the use of $NaNO_3$ as a background electrolyte and Cu nitrate in the added solution but also to the presence of silicates with low stability that contain Na and which are altered in an acid medium. After the soils were treated, although not in quantifiable phases, rouaite ($Cu_2(OH)_3NO_3$) was also detected in all of the horizons (Table 3).

There are some minerals such as quartz and microcline whose proportion increased in the majority of the horizons studied after adding Cu. As a result, the treatment led to the alteration of the most labile crystalline phases, and it was found that in general, the content of the least stable clay minerals decreased, such as kaolinite (following treatment, it varied between undetectable phase and 9 %), albite (between 0.3 and 17.2 %), muscovite (3.2 and 17.1 %) and gibbsite (less than 2.6 %) (Table 3). Horizons from S4 soil show similar proportions of the minerals than before treatment because the neutral pH minimized the alteration caused by the acid treatment. Surface horizons, especially S1.A and S4.A and in lesser extent S2.A, are those with less changes in the mineral composition after the treatment with Cu. They are the horizons with the high TOC and amorphous Fe oxides and these soil components are known as high reactive surfaces that are able

Table 3 Mineralogical analysis before and after Cu sorption

	Q	Alb	Mcl	Mus	Ka	Gb	Chl	$NaNO_3$	Ro
S1.A	66a±2.3	5.8c±1.2	6.7c±1.8	10.1b±0.5	7.4bc±2.7	4.0d±1.5	up	up	up
S1.A+Cu	82.3a±0.9	2.1d±0.5	5.3b±0.5	4.3c±0.5	1.3e±0.5	1.9d±0.5	up	2.8d±0.7	iup
S1.B	59.1a±3.2	3.5e±1.7	up	19.2b±1.3	11.9c±1.5	6.4d±1.8	up	up	up
S1.B+Cu	81a±0.9	0.3f±0.1	5.1c±0.6	7.8b±0.5	1.6e±0.5	2.6d±0.5	up	1.3e±0.5	iup
S2.A	35.6a±3.7	29.1b±3.4	10.9c±2.4	9.8cd±1.5	3.7e±0.5	3.0e±0.8	8.0d±1.0	up	up
S2.A+Cu	40 a±0.9	17.2 b±0.7	11.1d±0.6	6.3e±0.4	4.7f±0.5	up	4.3f±0.5	14.4 c±0.8	2.1g±0.6
S2.B	27.8 a±2.5	11.7d±2.4	13.7 c±3.4	23.4b±1.7	9.2e±1.9	14.2c±2.3	up	up	up
S2.B+Cu	29.7a±0.9	14.2b±0.7	35.7a±0.8	7.6 c±0.5	4.9d±0.5	2.5e±0.6	up	4.7d±0.7	0.9f±0.5
S3.A	43.2 a±3.2	20.1c±3.1	up	24.7 b±1.4	6d±1.3	up	6d±0.3	up	up
S3.A+Cu	52.4a±0.9	9.2d±0.7	up	16b±0.5	2.2f±0.5	up	5.7e±0.5	13.6c±0.8	0.9g±0.6
S3.Bw	45.7a±2.9	13.6c±1.9	up	27.4b±2.7	3.5e±0.6	up	9.8d±0.4	up	up
S3.Bw+Cu	48.6 a±0.9	9.5d±0.7	up	17.1b±0.6	3.7f±0.45	up	7.8 e±0.5	12c±0.8	1.3g±0.7
S4.A	38.7a±2.2	24.8b±2.5	17.4c±2,6	15.4d±2.1	3.6 f±2.6	up	up	up	up
S4.A+Cu	42.9a±0.9	12.8c±0.7	20.6b±0.8	5.3e±0.4	6.2d±0.5	up	up	10.9c±0.7	1.2f±0.5
S4.G	54.8 a±4.4	20.2b±1.8	15.9c±1.8	6.1d±0.6	3.1e±0.4	up	up	up	up
S4.G+Cu	58.4a±0.9	13.8c±0.7	19.7b±0.8	3.2e±0.4	up	up	up	4.4d±1.2	0.6f±0.4

For each soil, values followed by different letters differ significantly with $P<0.05$

Q quartz, *Alb* albite, *Mcl* microcline, *Mus* muscovite, *Ka* kaolinite, *Gb* gibbsite, *Chl* chlorite, *Ro* rouaite, *up* undetected phase, *iup* identified, but unquantified phase to retain and neutralize pollutants as heavy metals (Li et al. 2005). They also determine the cation exchange capacity and the high CEC of these surface horizons as well as the high Mn oxide content which also influence the fewer changes in the mineralogical composition after Cu treatment (Table 3).

These processes of transformation and alteration led also to the subsequent formation of new crystalline and amorphous phases, whose presence was verified by FE-SEM, and whose results are indicated in the following section. Both the high concentration of Cu that was added and probably together with the acidic medium affected the mineralogical composition of all of the horizons.

Field emission scanning electron microscopy

In order to complete the mineralogical study, images of the samples from the horizons before and after adding Cu were obtained by FE-SEM. Figure 2a shows Cu^{2+} sorbed on amorphous hydroxy compounds of Fe associated with albite, muscovite and mainly gibbsite in S1.B, and Fig. 2b shows Cu deposited superficially on the horizon S4.G. Bradl (2004) already stated that the association of hydroxy compounds of Fe with the clay fraction increases the sorption capacity of heavy metals in the soil.

Amorphous phases of aluminium clays were also detected in the horizons of soils S2 and S4 (Fig. 2d–f). These spherical and curved particles of aluminium clays are a metastable form of kaolinite with low crystallinity (Tomura et al. 1985). Cu^{2+} precipitates were found on the surface of these particles. The structure of these particles was partially altered because of the acid medium leading to partially decomposed materials and amorphous mixtures of kaolinite where the added Cu was also fixed (Fig. 2e). Pores were also observed, formed when these particles decomposed, in which Cu^{2+} is also deposited (Fig. 2f). It was also found, as shown

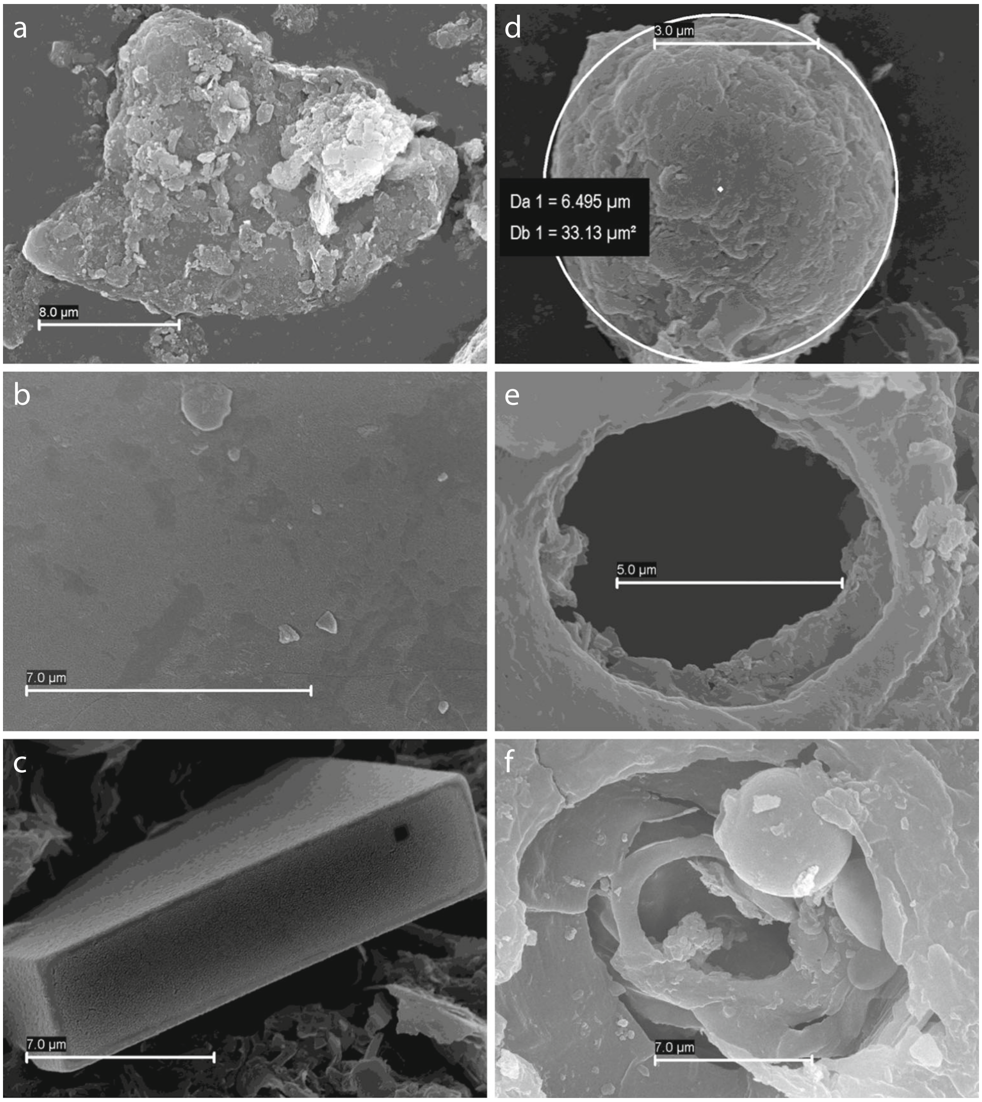

Fig. 2 **a** Cu sorbed on amorphous hydroxy compounds of Fe associated with albite, muscovite and gibbsite in S1.B. **b** Surface retention of particles of Cu in S4.G. **c** Microphotograph of a neoformed salt (nitratine) in S4.G. **d** Spherical aluminium clay in S2.A. **e** Pore with sorbed Cu in S2.A. **f** Amorphous sphere and pore of a spherical aluminium clay with sorbed Cu in S4.G

in Fig. 2c, that nitratine formed in the treated horizons, as a result of the contribution of Na^+ and nitrates to the medium.

Using this technique, it was possible to verify the presence of the new crystalline phases detected by XRD, and that part of the Cu was sorbed in the surface

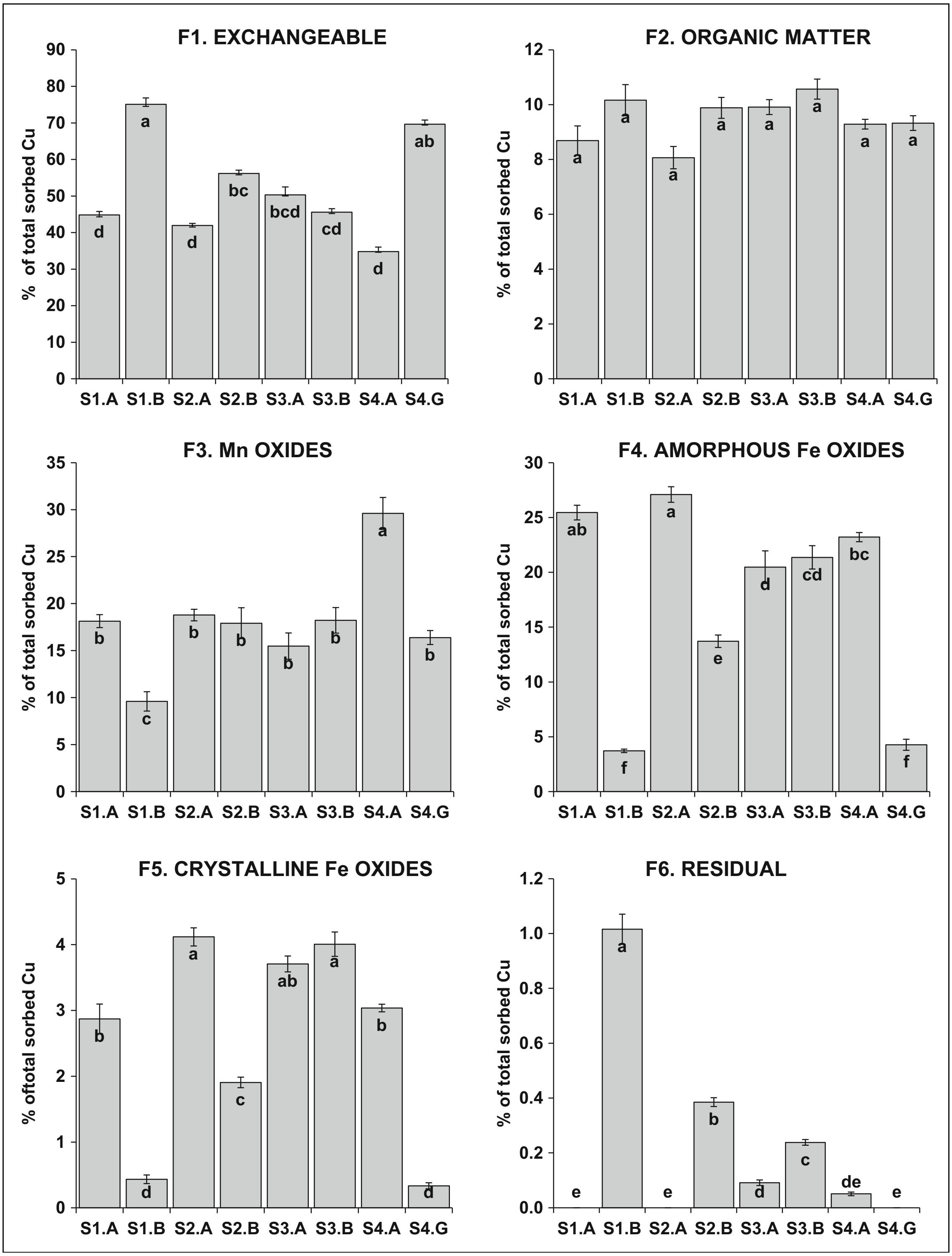

Fig. 3 Percentage of each fraction of copper in each horizon. (In each fraction, *different bold letters* indicate values significantly different ($P<0.05$))

of the crystalline and amorphous phases of the different horizons.

Distribution of Cu between the different geochemical phases

The surface retention of Cu^{2+} was verified with the previous results, nevertheless quantification was not possible. In order to be able to establish the distribution of Cu between the soil components and to determine the amount of metal associated with the different soil phases, a sequential chemical extraction was performed. This procedure provides information on which soil components the sorbed Cu is bonded. However, care must be taken since each of the reagents used may have a more or less intense effect on any of the components and the Cu associated with them. Figure 3 shows the proportion of Cu in each horizon that is associated with each of the fractions, after the successive extractions indicated in Table 1.

The highest percentage of Cu is in the exchangeable fraction of all of the horizons (Fig. 3), varying between 34.82 % in S4.A and 75.06 % in S1.B. The Cu in this fraction is considered to be the most mobile, and generally that which can give rise to toxicity problems, as it can easily be released as an ion (Roy et al. 2004) and therefore be more bioavailable.

In absolute terms, the highest quantity of Cu associated with the exchangeable fraction was found in the S2.A horizon, and the smallest quantity in S2.B (95.79±2.61 and 26.45±0.46 mg kg^{-1}, respectively). And it is always higher in the surface horizon than in the subsurface horizon in all of the soils.

In line with the findings of other authors, most of soil components (organic matter, clay fraction, oxides, etc.) directly influence the exchangeable fraction, by retaining the metal weakly by cationic exchange (Németh et al. 2010). A close positive correlation has been established between the exchangeable Cu and various features of the horizons studied, such as the ECEC, TOC and amorphous Fe oxides (Table 4). It can be deduced that soils with high contents of organic matter and amorphous Fe, such as S2.A and S4.A (Table 2), have high capacity to retain Cu in exchangeable sites (Fig. 1).

Table 4 Correlation coefficients

	ECEC	TOC	Amorphous FeO_x	AlO_x
Exchangeable Cu	0.80**	0.90**	0.64**	Nc
Cu bound to organic matter	0.90**	0.89**	0.73**	0.81**
Cu bound to Mn oxides	0.83**	0.85**	0.68**	0.57**
Cu bound to amorphous Fe oxides	0.92**	0.98**	0.80**	0.81**

**Significant correlation at 0.01 (bilateral)

Nc no correlation

Even all horizons show high proportion of Cu in the exchangeable fraction, it is noticeable that S1.B, S4.G and in lesser extent S2.B are those with the highest percentage of Cu in F1 (Fig. 3). The properties of the subsurface horizons probably have a lower influence or lesser capacity to strongly fix Cu. Organic matter is one of the soil components that prevail on surface horizons compared to subsurface ones (Table 2) and it has a high affinity for Cu^{2+}, forming complexes that directly affect its availability (Borchard et al. 2012). OM also plays an important role in the sorption of Cu, immobilizing it and reducing its availability (Banerjee 2003; Su and Wong 2004; Vega et al. 2010); nevertheless, there are no statistical differences among horizons when compared the proportion of Cu associated to this fraction (Fig. 3). Although in absolute terms the Cu associated with the organic material (F2) varied between 3.90 mg kg^{-1} in S1.B and 18.43 mg kg^{-1} in S2.A, in all cases this fraction (F2) comprises around 10 % of the total Cu fixed.

Other soil components that prevail on surface horizons compared to subsurface ones are the amorphous Fe oxides (Table 2). In this case, there are clear differences among horizons comparing the proportion of Cu associated to this fraction (F4) after sequential extraction (Fig. 3). The trend is even similar to the one of total Cu fixation (Fig. 1). Except for S3 horizons, the proportion of Cu associated to F4 fraction is higher in surface horizons than in subsurface ones. The results indicate that the higher the oxide content, the higher the amount of Cu^{+2} sorbed, and that it is higher in the surface horizons (Table 2). The A horizon of S2 is the one that sorbs the most Cu in this fraction (61.88 mg kg^{-1}), and as the amount of oxides decreases as in S1.A, S3.A, S3.B and S4.A, the Cu in this fraction also decreases (between 20 and 30 mg kg^{-1}), and finally, those with the lowest content, such as S1.B, S2.B and S4.G fix 1.43, 6.46 and 1.96 mg kg^{-1}, respectively. These results concur with those of Agbenin and Olojo (2004), who noted that the Cu associated with amorphous Fe oxides is directly related to their abundance in the soil, as their large specific surface favours sorption (Jackson and Miller 2000).

Similar trends, although less concentration of Cu, were found for F5 fraction (crystalline Fe oxides). The Cu associated to F5 varied between 0.17±0.01 and 9.41±0.63 mg kg^{-1} in S1.B and S2.A, respectively, and its proportion in relation to the total Cu sorbed is always higher in the surface horizons that in the subsurface horizons. Nevertheless, it does not represent more than 5 % of the total retained in any of the horizons (Fig. 3). Therefore, only a small part of the Cu is sorbed in the crystalline Fe oxides, and in line with the findings of Jackson and Miller (2000), the reason for this low retention capacity is the small surface area of these oxides.

The soil fractions that are less capable of retaining Cu, in all of the horizons studied, are the residual fraction (F6) and the crystalline Fe oxides (F5), which have the lowest proportion of retained Cu (Fig. 3). The highest concentration of Cu associated with the residual fraction is in the S1.B horizon (0.39±0.04 mg kg^{-1}), and was not detected in the surface horizons of S1, S2 and S4.G.

Although these soils have a very low Mn oxide content (Table 2), these have a major affinity for Cu (Negra et al. 2005; Covelo et al. 2007), which coincides with the results obtained in the fractionation carried out, indicating high Cu^{2+} sorption. Therefore, despite the relatively low presence of Mn oxides in the soil (Table 2), the percentage of Cu associated to F3 is high, varying between 9.60 % in S1.B and 29.60 % in S4.A (Fig. 3). Even the amount of Mn oxides in S4.A is not the highest (Table 2), the proportion of Cu in this fraction (F3) is very high, almost similar to the one associated to F1 in this horizon. The high influence of Mn oxides in the retention of Cu in S4.A is also due to the almost neutral pH (6.89, Table 2) (Zhang et al. 1997). The amount of Cu^{2+} associated with these oxides varied between 3.68 mg kg^{-1} in S1.B and 42.92 mg kg^{-1} in S2.A, and in this fraction (F3) is always greater in the surface horizons than in the subsurface horizons, except in S3.

It can be generalized that Fe and Mn oxides (F3 and F4) have a greater influence on the fixing of heavy metals in the surface horizons, this is probably due to the formation of organomineral complexes with the organic material, and the fact that complexation is the predominant process in the fixing of Cu in these horizons. However, in the subsurface horizons, which have a lower organic matter content, adsorption predominates, contributing towards the higher proportion of exchangeable Cu^{2+}, which is more mobile and available. Furthermore, a highly significant correlation has been established between the organic matter content and amount of Cu sorbed in this fraction, but also with the amount associated with the fractions of Fe and Mn oxides (Table 4). As a result, part of the Cu that is retained is held in the organomineral associations that contribute towards increasing the Cu sorption capacity (Borchard et al. 2012).

The sequential extraction results combined with FE-SEM/EDS show that organic matter, Fe oxides and gibbsite play a very important role in the fixation of Cu^{2+}. These results are in agreement with those of previous studies (Cerqueira et al. 2011) where it was found that Cu^{2+} is sorbed in the gibbsite, whose presence and abundance in the soil significantly increases the sorption capacity of this catión. It is important to note that also in these previous studies, the association of Cu^{+2} with hematite has been proved and also that several studies have shown that iron oxides significantly contribute to Cu^{2+} sorption (Kabata-Pendias 2001; Vega et al. 2010).

Conclusions

Cu is sorbed superficially on the associations resulting from the formation of amorphous hydroxy compounds of Fe that interacted with gibbsite, albite and muscovite.

The largest amount of the Cu retained was in an exchangeable form, and the smallest amount associated with the crystalline Fe oxides and residual fraction.

In the surface horizons, the predominant Cu retention process is complexation in organomineral associations, while in the subsurface horizons it is adsorption.

FE-SEM-EDS studies combined with sequential extraction and statistical analyses are an effective tool to check the affinity of the soil components for copper.

This study will serve as a basis for further scientific research on the soil retention of heavy metals, as field emission scanning electron microscopy with energy dispersive X-ray spectroscopy makes it possible to check which soil components retain heavy metals.

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