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Copper accumulation and changes in soil physical–chemical properties promoted by native plants in an abandoned mine site in northeastern Brazil: Implications for restoration of mine sites

Fabio Perlatti [a,e], Tiago Osório Ferreira [b,*], Ricardo Espíndola Romero [c], Mirian Cristina Gomes Costa [c], Xosé Luis Otero [d,f]

[a] National Department of Mineral Production – DNPM, R. Dr. José Lourenço, 905, 60115-280 Fortaleza, CE, Brazil
[b] Department of Soil Science, University of São Paulo (ESALQ/USP), Av. Pádua Dias, 11, 13418-900 Piracicaba, SP, Brazil
[c] Department of Soil Science, Federal University of Ceará, Av. Mister Hull, 2977, 60440-554 Fortaleza, CE, Brazil
[d] Department of Edaphology and Agricultural Chemistry, Faculty of Biology, University of Santiago de Compostela – USC, Rúa Lope Gómez de Marzoa, s/n. Campus sur, 15782 Santiago de Compostela, Spain
[e] PhD Course of Ecology and Natural Resources, Department of Biology, Federal University of Ceará – UFC, Building 906, 60455-760 Fortaleza, CE, Brazil
[f] Departamento de Ciencias de los Alimentos y Biotecnología, Universidad Politécnica Nacional, Quito, Ecuador

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ABSTRACT

In this study, the copper-accumulating capacity of plants growing spontaneously in copper-contaminated soils in an abandoned mine site in northeastern Brazil was evaluated by calculating enrichment (EF) and translocation (TF) factors. The effects of physical and chemical changes in the rhizosphere soil on copper mobility were determined by using different compounds (Mehlich3/$MgCl_2$) to extract Cu from different types of soil samples (bulk/rhizosphere soil). Finally, the possible implications for the use of these plant species in restoring the area were assessed by calculating the balance between the Cu mobilized in the rhizosphere and the Cu absorbed by the plants. On the basis of the EF and TF values obtained (all <1), none of the species under study (*Ruellia paniculata, Bidens pilosa, Pityrogramma calomelanos* and *Combretum leprosum*) were classified as hyperaccumulators. However, consideration of readily bioavailable levels (extracted with $MgCl_2$) and the rhizosphere soils (rather than total levels and bulk soils) yielded higher correlations with the levels of metal in plant tissues. This approach therefore appears more appropriate for determining the capacity of the plants to accumulate copper. The different characteristics of the bulk and rhizosphere soils have direct effects on the concentrations of copper, which were much lower in the rhizosphere soil. In general, each species responded differently to the high concentration of Cu in soils (range 3604–9601 mg kg^{-1}). By calculating the balance between the amounts of Cu mobilized in the rhizosphere and uptake by plants, we found that the presence of such plants in the field may have antagonistic effects. Two of the species (*B. pilosa* and *P. calomelanos*) contained more Cu in their tissues than mobilized in the rhizosphere. This is a desirable characteristic for restoration purposes, as the plants can reduce the bioavailable Cu content in soils and thus act as facilitators for regeneration of the site. By contrast, the other two species (*R. paniculata* and *C. leprosum*) mobilized more Cu in the rhizosphere than they were able to take up, which may led to transfer of bioavailable Cu to the ecosystem, which is undesirable in terms of site restoration.

1. Introduction

The disposal of metallic mine waste in open pits represents a serious environmental problem worldwide as it is one of the main routes of release of toxic metals to the environment (Lottermoser, 2007). The practice may lead to unfavourable conditions for plant growth, affecting the diversity and abundance of many species and hindering the reestablishment and development of natural vegetation and, therefore, regeneration of the whole ecosystem (Bradshaw, 1997; Adriano, 2001; Bell, 2001; Hernández and Pastor,

* Corresponding author at: Department of Soil Science, University of São Paulo (ESALQ/USP), Av. Pádua Dias, 11, 13418-900 Piracicaba, São Paulo, Brazil. Tel.: +55 1934172147; fax: +55 1934345354.
E-mail addresses: toferreira@usp.br, tiago.osorio.ferreira@gmail.com (T.O. Ferreira).

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2008; Närhi et al., 2012). Depending on the geochemical composition of the mine waste, its disposal can have various different effects on soil quality: decreased nutrient contents (Schulz and Wiegleb, 2000; Nikolic et al., 2010); deteriorated physical quality (Shrestha and Lal, 2011); abrupt changes in pH (acidification or alkalinization) (Jurjovec et al., 2002; Aykol et al., 2003); and release of large amounts of toxic elements (Leblanc et al., 2000; Wu et al., 2011; Zornoza et al., 2012; Martínez-Sanchez et al., 2012; García-Lorenzo et al., 2012).

The vast majority of plant species do not tolerate high concentrations of metals in soil, and identification of species that spontaneously inhabit contaminated environments (autochthonous flora) is extremely valuable for restoration programs (Whiting et al., 2004; Pilon-Smits and Freeman, 2006), as well for helping scientists to understand the ecological mechanisms underlying adaptation to such environments (Boyd, 2004; Boojar and Goodarzi, 2007; Manara, 2012; Anawar et al., 2013). As primary resources for many other organisms, plants may also represent a large source of trace elements into the trophic chain. Thus, species that accumulate large amounts of trace elements in aboveground tissues (leaves, stems, fruits and flowers) are prone to release these elements throughout the environment (McLaughlin, 2001; Boyd, 2004; Peplow and Edmonds, 2005). However, species with such characteristics are suitable for use in phytoremediation programs (i.e. phytoextraction), as they can remove large amounts of contaminants by accumulating the elements in aboveground structures and then being removed from the site, and disposed at appropriate places (Reeves and Baker, 2000; Dzantor and Beauchamp, 2002; Pilon-Smits, 2005; Ernst, 2005). Contrastingly, plant species that accumulate metals in their roots, even at high levels, are not considered as metal accumulators according to the technical criteria used to classify hyperaccumulator plants (Baker and Brooks, 1989; Reeves, 2006). However, these plants are still of great interest for the remediation of contaminated areas, since they may be useful in the immobilization and/or revegetation programs. These species can immobilize contaminants in belowground tissues and/or favour metal complexation in their rhizospheres preventing metal release and limiting the bioavailability of the metal to other species. By doing so, these plants may facilitate the regeneration and phytostabilization processes (Whiting et al., 2004; Pilon-Smits, 2005; Mendez and Maier, 2008).

The specificity of the mechanisms that govern the forms of metals that plants absorb is still somewhat uncertain; however, in general, accumulation of an element in plant tissues is governed by the availability of the element in the soil solution, which is in turn determined by different variables, such as climatic, anthropogenic, biological and geochemical factors, and rhizosphere processes (Kabata-Pendias, 2004).

Different methods have been used to determine the bioavailability of metals in soils, with the aim of predicting the fraction of metal in the soil that is actually absorbed by plants. The pseudo-total concentration, which is extracted by strong acids, may comprise forms of metals that are scarcely accessible to plants, such as crystalline forms of primary minerals or very stable organometallic complexes (Fernandez-Calvino et al., 2009). The Mehlich3 extractant is widely used to extract the potentially bioavailable metal, as it extracts the readily bioavailable fraction plus the fractions that may be rendered readily available by small changes in soil conditions, such as those provided by the plant rhizosphere (Mehlich, 1984; Monterroso et al., 1999; Otero et al., 2012). The metal extracted by a salt solution such as $MgCl_2$ represents the fraction that is weakly adsorbed on soil surfaces and is therefore readily available to plants (Filgueiras et al., 2002). We propose that considering the different metal fractions extracted from bulk and rhizosphere soils, along with the metal levels absorbed and translocated by plants, may provide a more realistic approach to understand the response of plants to increase metal concentrations in soil. This has implications for phytoremediation and/or restoration programs, as well as for elucidating the mechanisms that enable these plants to survive in highly contaminated environments.

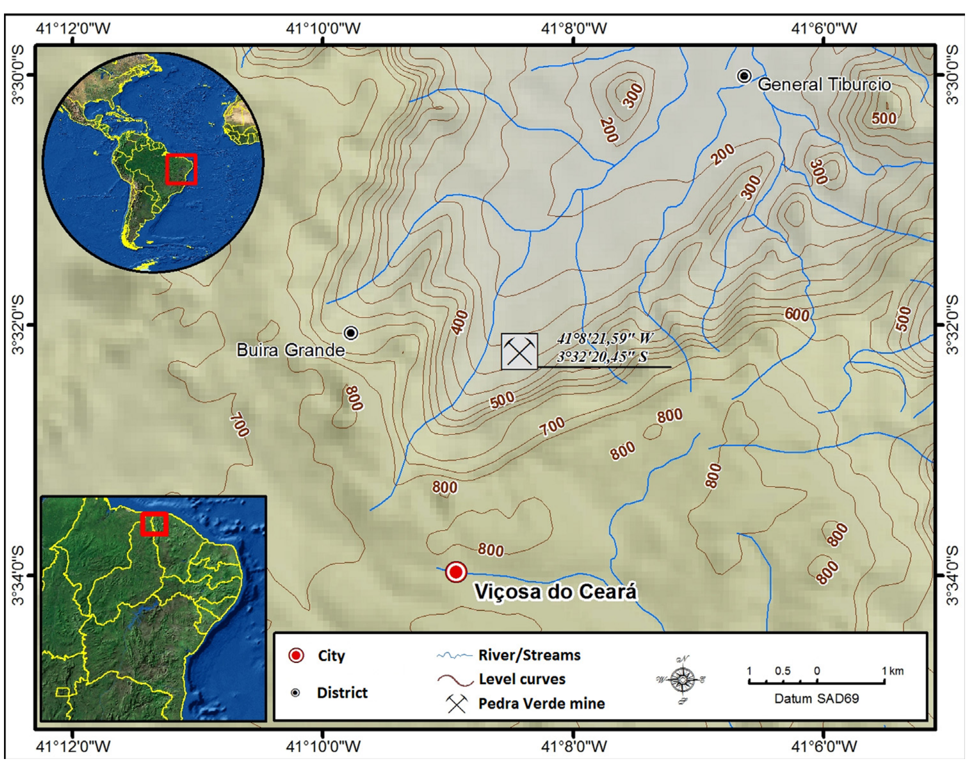

Fig. 1. Location of the Pedra Verde mine.

The specific aims of this study were to: (i) determine the total, potentially bioavailable and readily bioavailable concentrations of Cu in bulk and rhizosphere soils under plants growing spontaneously in the area surrounding an abandoned copper mine; (ii) determine the concentration of Cu in the leaves and roots of these plant species and, on the basis of the findings; (iii) identify the plant response to high concentrations of Cu in soil (accumulation/immobilization/exclusion) and thus evaluate their potential value for phytoremediation and restoration programs.

2. Material and methods

2.1. Study area

The study was conducted in the “Serra da Ibiapaba” region of northeastern Brazil (Fig. 1). The climate in the region is semi-arid warm tropical in the regions of lower altitude (<300 m; where the mine is located) and sub-humid warm tropical in the higher regions (>300 m; at the top of the slopes and “Serra da Ibiapaba” plateau). The average annual rainfall in Viçosa do Ceará (alt. 710 m) is 1470 mm and in General Tibúrcio (alt. 220 m), 960.0 mm, and it is concentrated between January and May in both areas. The average annual temperature in both areas is between 22 and 24 °C (FUNCEME, 2014).

The regional vegetation, which is directly affected by climate and altitude, consists of evergreen seasonal tropical forest on top of the plateau (Serra) and a dry tropical forest (locally known as “caatinga”) in the lower parts of the slopes and on the plains (<300 m alt.) (IBGE, 2012).

The Pedra Verde ore body is of supergene origin, and the Cu mineralized zone occurs in phyllites as sulphidic deposits and in an oxidized zone at the surface as carbonated outcrops (Collins and Loureiro, 1971). The Cu sulphide minerals in the ore body include chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), bornite (Cu_5FeS_4) and covellite (CuS). In the upper part of the mineralized horizon, iron oxides predominate at levels of up to 0.1% Cu, with hematite (Fe_2O_3) as one of the main constituents, in addition to the occasional presence of cuprite (Cu_2O) (Collins and Loureiro 1971).

The oxidized zone at the surface is mainly formed by Cu-carbonates in malachite ($Cu_2(CO_3)(OH)_2$) impregnations and streaks, which are concentrated along a distance of more than 100 m in a fracture zone, at a depth not exceeding 15 m. The presence of pseudomalachite (a copper phosphate mineral) was detected in the mine (Perlatti et al., 2014). The gangue minerals mainly comprise quartz, feldspar, muscovite, sericite, chlorite and calcite (Collins and Loureiro, 1971).

The mine was exploited in the 1980s for the extraction (and processing) of Cu from sulphides (chalcopyrite and chalcocite) and carbonate (malachite) minerals. After the mine was closed in 1987, the processed minerals were piled on terraces constructed on the slope of the plateau and, as a result of the large amount of waste and the lack of maintenance of the terraces, some piles eroded, dispersing waste mine materials over distances of up to 1.5 km along the slope (Fig. 2).

The waste rock from the mine is highly heterogeneous, ranging from very fine material to boulders. It essentially comprises wall rock material (gangue material), removed to gain access to the ore, and processing waste generated by hydrometallurgical processing of the ore (i.e. malachite, chalcopyrite and chalcocite).

2.2. Sampling and analytical procedures

2.2.1. Plants

Few plant species were growing naturally in the area. For the study, only species growing directly on the waste-impacted area were selected; the species and sample locations are shown in Fig. 2.

Three specimens of each species were collected. Whole plants of *Pityrogramma calomelanos* (Fig. 2A) and *Bidens pilosa* (Fig. 2B) were collected and separated in the laboratory into leaves (approximately 20 leaves of each individual) and roots. *Ruellia paniculata* (Fig. 2C) and *Combretum leprosum* (Fig. 2D) produce larger plants, and therefore approximately 20 leaves were removed

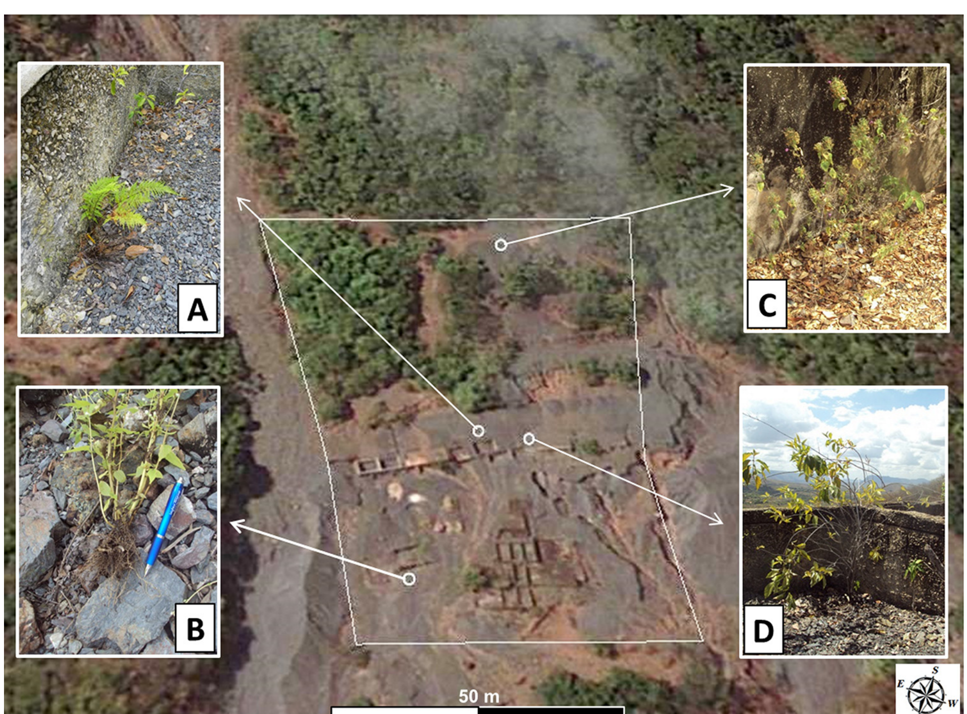

Fig. 2. Location of plant sampling and species identified. (A) *Pityrogramma calomelanos* L.; (B) *Bidens pilosa* L.; (C) *Ruellia paniculata* L. and (D) *Combretum leprosum* M.

from each specimen to form a composite sample. Root samples were collected after excavation of the samples at 0–20 cm depth.

In the laboratory, the leaves and roots were processed by washing in tap water, rinsing three times with distilled water, drying in an oven at 60 °C for 48 h, and milling in a ceramic mortar. The samples were then stored in a refrigerator prior to analysis. A subsample of the roots was pretreated with sodium dithionite and sodium citrate (DC) to eliminate any strongly adhered soil particles that may not have been removed by water. This procedure is often applied to remove iron oxides/hydroxides attached to roots (Liu et al., 2004, 2006); the procedure used was adapted from the cold DCB extraction described by Taylor and Crowder (1983) which is usually applied in order to minimize possible structural damages to more fragile roots.

For this purpose, 1.0 g of roots were placed in centrifuge tubes, to which 50 ml of a solution containing 0.03 M sodium citrate ($Na_3C_6H_5O_7·2H_2O$) and 0.8 g of sodium dithionite ($Na_2S_2O_4$) was added. The tubes were then shaken for 16 h at room temperature; the root samples were removed, rinsed three times with deionized water, dried at 60° C for 48 h and stored in a refrigerator until analysis.

To determine the concentration of copper in the plant tissues, the dry plant material was placed in Teflon tubes to which 9 ml of nitric acid (HNO_3 65%) was then added, and the samples were digested at room temperature for 4 h. Three mililiter of perchloric acid ($HClO_4$) was added to the tubes, which were then left overnight. The tubes were placed in a microwave oven (Ethos Plus Microwave Labstation – Milestone) for 35 min at a controlled temperature (200° C). The extracts were analysed (in triplicate) in an atomic absorption spectrophotometer (Perkin Elmer model 2380). For quality control, certified reference material (N° 1547, peach leaf – NIST) was also analysed; the recovery rate for Cu was 104 ± 3%.

2.2.2. Bulk and rhizosphere soils

The bulk soil around plants was collected, at least 3 cm from the roots up to approximately 30 cm from the plants and at a depth of 0–20 cm. Care was taken to ensure that the sample did not include roots. Triplicate samples were collected and combined to make a composite sample of bulk soil.

For rhizosphere soil samples, plant roots were collected and shaken to remove the loosely attached soil. The remaining soil, which was strongly attached to the roots, was then removed by vigorous shaking and by brushing with a toothbrush within approximately 2.0 mm of the root surface (for more details see Chung and Zazoski, 1994). Triplicate samples were collected.

Once the bulk and rhizosphere soil were separated, the samples were dried at 40 °C for 72 h, passed through a 2 mm sieve and stored under refrigeration (4 °C) before analysis. The pH was determined in water (soil/solution ratio, 1:2.5). Total S was measured in a LECO SC-144DR, and the total N (TN) and total C (TC) contents were measured in a LECO 2000-CNS element analyzer. Total organic carbon (TOC) and total inorganic carbon (TIC) were determined following the methods described by Cambardella et al. (2001).

We used three extractants with the aim of obtaining different forms of Cu in soil. The readily bioavailable copper was extracted with 1 M $MgCl_2$ at pH 7.0 (Cu–$MgCl_2$). The potentially bioavailable Cu was extracted with a Mehlich3 solution (2 M CH_3COOH, 0.25 M NH_4NO_3, 0.015 M NH_4F, 0.013 M HNO_3 and 0.001 M EDTA) (Cu–Me) and the (pseudo) total Cu concentration was determined after digestion in a microwave with aqua regia solution (HNO_3 and HCl, proportion 3:1) (Cu p-total).

Two samples of certified reference soils were included in the batch as analytical controls. Reference soils SO-2 and SO-3, from the Canadian Centre for Mineral and Energy Technology/Ontario were analysed, and the percentage recovery of Cu was 108 ± 6% and 98 ± 7%, respectively. All extracts were analysed by flame atomic absorption spectrometry (model Perkin Elmer 2380), and the detection limit (d.l.) for Cu was 0.05 mg kg^{-1}.

2.2.3. Statistics and data analysis

Differences between rhizosphere and bulk soil samples were established by one-way ANOVA followed by a Tukey test, or Kruskal–Wallis one way analysis of variance on ranks for non-normally distributed data. The Minitab® statistical package, version 16.2.4 (Minitab Inc.), was used to calculate Spearman's correlation coefficients to explore the relationships between different forms of Cu in soils and plants.

The enrichment (EF) and translocation (TF) factors were calculated to evaluate the potential of plants to accumulate and translocate copper. The EF represents the ratio between the concentrations of Cu in soil and leaves, and the TF is the ratio between the concentrations of Cu in roots and leaves (Branquinho, 2007).

3. Results and discussion

3.1. General properties and Cu contents of bulk and rhizosphere soils

The general characteristics of the bulk and rhizosphere soils are shown in Table 1. The pH of the soil under *R. paniculata* was close to neutral, with no difference between bulk and rhizosphere soils. The concentrations of organic and inorganic carbon, nitrogen and sulphur were higher in the rhizosphere than in the bulk soil. For *B. pilosa*, there were no significant differences in the parameters measured in the bulk and rhizosphere soils.

Some differences were observed between the bulk and rhizosphere soils of *P. calomelanos* and *C. leprosum*. The TOC, TN and TS contents were higher in the rhizosphere than in the bulk

Table 1
Selected soil properties from bulk and rhizosphere soils (means ± s.d.).

Plant species	Soil location[a]	pH (H_2O)	TOC (%)	TIC (%)	TN (%)	TS (%)
R. paniculata	Rhizosphere	6.2 ± 0.3	24.7 ± 3.43	0.56 ± 0.10	1.54 ± 0.14	0.13 ± 0.01
	Bulk	6.3	3.18	0.30	0.23	0.03
B. pilosa	Rhizosphere	5.1 ± 0.1	1.19 ± 0.50	0.23 ± 0.00	0.11 ± 0.03	0.02 ± 0.01
	Bulk	5.0	0.89	0.23	0.12	0.01
P. calomelanos	Rhizosphere	5.9 ± 0.2	2.24 ± 1.48	0.25 ± 0.02	0.16 ± 0.10	0.13 ± 0.06
	Bulk	5.0	0.48	0.37	0.02	0.05
C. leprosum	Rhizosphere	5.6 ± 0.2	13.3 ± 6.05	0.34 ± 0.08	0.80 ± 0.35	0.12 ± 0.04
	Bulk	5.0	0.48	0.37	0.02	0.05

TOC: total organic carbon; TIC: total inorganic carbon; TN: total nitrogen; TS: total sulphur.
[a] Rhizosphere ($n = 3$), bulk ($n = 1$).

Table 2
Readily bioavailable copper extracted with 1 M $MgCl_2$ (Cu–$MgCl_2$), potentially bioavailable copper extractable with Mehlich3 solution (Cu–Me), and (pseudo) total copper extracted with aqua regia (Cu p-total) in bulk and rhizosphere soils.

Plant species	Type of soil sample[a]	Cu–$MgCl_2$ (mg kg^{-1})	Cu–Me (mg kg^{-1})	Cu p-total (mg kg^{-1})
R. paniculata	Rhizosphere	20.7 ± 9.57	358 ± 25.0	6310 ± 2248
	Bulk	65.2	1281	9601
B. pilosa	Rhizosphere	205 ± 82.4	804 ± 112	3143 ± 555
	Bulk	246	880	3601
P. calomelanos	Rhizosphere	38.0 ± 33.1	281 ± 50.2	3701 ± 456
	Bulk	298	1081	41302
C. leprosum	Rhizosphere	15.9 ± 6.65	292 ± 5.66	1180 ± 450
	Bulk	298	1081	4302
Phytotoxic threshold	10.0[b]	40–60.0[c]	60–140[d]	

[a] Rhizosphere (n = 3), bulk (n = 1).
[b] Yang et al. (2002).
[c] Monterroso et al. (1999).
[d] (CEC, 1986; CONAMA, 2009; Kabata-Pendias, 2010).

soil. The TIC was lowest in the rhizosphere of *P. calomelanos* and highest in the *C. leprosum* rhizosphere. The action of microorganisms and the biological activity of roots may considerably alter the soil properties under its influences (Hisinger et al., 2009), with direct consequences for the nutrient and metal dynamics. Soil pH and TOC are key factors in copper dynamics (Brun et al., 1998; Song et al., 2004; Bravin et al., 2012).

The data show that for *R. paniculata* and *B. pilosa* there were no differences in pH, but that for *P. calomelanos* and *C. leprosum*, the pH was higher in the rhizosphere than in the bulk soil.

The presence of carbonates in waste rock (i.e. malachite and calcite) may have increased the pH, as the action of microorganism or roots exudates may enhance the dissolution of carbonates, which would, in turn, release $HCO3^-$ and OH^- ions, thus buffering the acidity produced by rhizosphere processes (Bravin et al., 2009).

The concentration of Cu p-total, Cu–Me and Cu–$MgCl_2$ in bulk and rhizosphere soils are shown in Table 2 and indicate a high degree of soil contamination. In general, the concentration of Cu in the rhizosphere soil was lower than the concentrations of Cu in the bulk soils.

The concentration of Cu p-total greatly exceeded those usually considered as limits for toxicity or as a soil quality parameter (range 60–140 mg kg $^{-1}$) (CEC, 1986; CONAMA, 2009; Kabata-Pendias, 2010), ranging between 3601 and 9601 in the bulk soils and between 1180 and 6310 mg kg^{-1} in the rhizosphere soils.

The concentrations of potentially bioavailable Cu were also higher than those considered in the literature as the phytotoxic threshold. For example, Monterroso et al. (1999) reported that phytotoxic levels of Cu–Me range between 40.0 and 60.0 mg kg^{-1}. Although the levels of potentially bioavailable Cu are lower in the rhizosphere soil of all plant species considered, the Cu values were higher than the phytotoxic limits in both bulk and rhizosphere soils. This was also reflected in the levels of Cu–$MgCl_2$, which were similar to those of Cu–Me, with a decrease in the concentration in the rhizosphere soil, but with values exceeding phytotoxic levels. Yang et al. (2002) showed that concentrations higher than 10 mg Cu L^{-1} of Cu in solution were sufficient to significantly inhibit the shoot growth of various plant species.

The concentrations of Cu were generally lower in the rhizosphere than in the bulk soil. This may indicate that plants mobilize copper in the rhizosphere zone, either through the action of microorganisms, or via the biological activity of the plants themselves (respiration, exudates, etc.). In fact, mobilization of metals in the rhizosphere has been demonstrated in several studies (Tao et al., 2003; Martínez-Alcalá et al., 2010) and may represent a risk to ecosystems, because if the plants do not take up the same amount of metal that has been mobilized, the excess metal may be transferred to the environment.

3.2. Copper concentration in plants, enrichment and translocation factors and correlation with bulk/rhizosphere soils properties

The average copper content in leaves and roots are shown in Fig. 3 and the Enrichment (EF) and translocation (TF) factors are summarized in Table 3.

Despite the high copper concentration in bulk and rhizosphere soils, the concentration of metal in leaves was within the usual range of 5.0–30.0 mg kg^{-1} (Kabata-Pendias, 2010), except in *B. pilosa*. By contrast, the mean concentration of Cu in the leaves of *B. pilosa* was 234 mg kg^{-1}, which is almost eight times higher than the usual concentration reported for plant leaves. Contrasting Cu uptakes by plants are probably related with the different changes

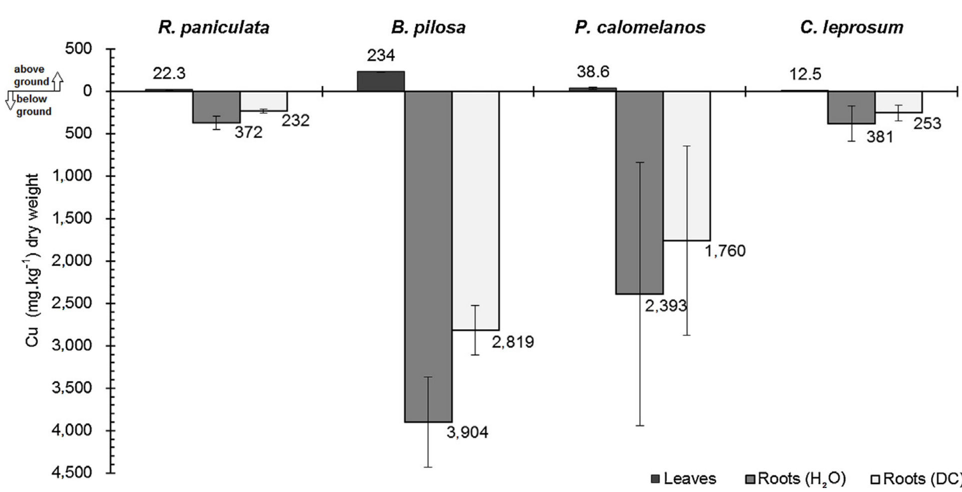

Fig. 3. Distribution and concentration of copper in leaves and roots (roots H_2O: washed only with water; roots DC: roots washed with water and treated with dithionite + citrate).

Table 3
Mean values (±standard deviation) of the enrichment factor (EF) and translocation factor (TF) in the plant species under study.

Plant species	Enrichment factor			Translocation factor
	$Cu–MgCl_2$	Cu–Me	Cu p-total	
R. paniculata	1.17 ± 0.46	0.06 ± 0.01	0.004 ± 0.002	0.096 ± 0.016
B. pilosa	1.57 ± 0.65	0.28 ± 0.02	0.071 ± 0.009	0.084 ± 0.007
P. calomelanos	1.33 ± 0.82	0.14 ± 0.07	0.043 ± 0.031	0.031 ± 0.021
C. leprosum	1.11 ± 0.77	0.04 ± 0.01	0.009 ± 0.001	0.055 ± 0.034

E.F. = Cu shoots/Cu rhizosphere soil; T.F. = shoots/roots.

that occur in the physicochemical characteristics of the soil under their rhizospheres, which may directly influence copper mobility and consequently the amount of metal available to plants. Another point is the physiological characteristics of each plant species and the forms in which they take up and translocate nutrients/metals (e.g. diffusion or mass flow), a point that should be further investigated in future studies.

Nonetheless, the levels of Cu in above-ground tissue of studied species was not sufficient for the plants be considered as a hyperaccumulator, as it does not reach the minimum requirement of accumulating at least 1.000 mg kg^{-1} of Cu in dry leaf tissue (Baker and Brooks, 1989). Another requirement usually applied in the identification of hyperaccumulator species is the ratio of metal in the plant tissue and the soil, also referred to as the enrichment factor (EF), which must be greater than 1 to indicate that there is more metal in the plant than in the soil (Branquinho et al., 2007). In this case, if we consider the readily bioavailable Cu in soils, the EF values for all species are higher than 1, while for potentially bioavailable and pseudo-total Cu, the EF values are below 1 (Table 3).

Use of pseudo-total concentrations of metal in soils to calculate the EF may not be the best approach. The pseudo-total contents, which are extracted by strong acids such as HNO_3 and $HClO_4$, do not represent metal that can be accessed by plants, as these techniques may extract Cu that is scarcely available to plants, such as those occluded in crystalline forms of primary minerals or forming stable organometallic complexes (Adriano et al., 2004; Fernandez-Calvino et al., 2009).

As shown in Table 4, the concentration of Cu in leaves was significantly correlated with the concentration of $Cu–MgCl_2$ ($r = 0.875$, $p > 0.01$) and Cu–Me ($r = 0.962$, $p > 0.01$) in the rhizosphere of the plants studied, whereas for Cu p-total no significant correlation was observed. The study findings suggest that the readily bioavailable ($MgCl_2$) or potentially bioavailable (Mehlich3) fractions of Cu in soil are more appropriate for calculating the EF; this represents a more realistic approach as the plants can access these forms of Cu (Adriano et al., 2004).

We also considered the use of the bulk soil for calculating the EF. As shown in Table 4, there was no correlation between the concentrations of Cu in bulk soils and plants. However, significant and negative correlations were found between the concentrations of both Cu–Me and Cu p-total in bulk soils and plants (Cu–Me = −0.792 to −0.838, $p < 0.01$; Cu p-total = −0.627 to −0.637, $p < 0.05$). These results clearly showed that the use of Cu levels in bulk soils should not be used as a parameter to assess the ability of a plant to accumulate metals, as it can lead to misinterpretation.

In addition, the concentration of Cu in leaves was more similar to $Cu–MgCl_2$ in rhizosphere than to $Cu–MgCl_2$ in bulk soil. Fig. 4 shows a 1:1 ratio between Cu-leaves and $Cu–MgCl_2$ in the rhizosphere soil. This may indicate that measurement of $Cu–MgCl_2$ in rhizosphere is a more realistic approach to measuring Cu accumulation and concentration in aerial parts of plants.

Most of the copper absorbed by the plants under study was accumulated in the roots (Fig. 3). Treatment with sodium citrate and sodium dithionite revealed that even after repeated washing with water, soil particles may still remain attached to the root surface, and thus the metal concentration in that part of the plants may be overestimated (to some extent). In fact, Cook et al. (2009) showed that is very difficult to remove all of the soil adhered to roots.

In comparison with roots washed only with water, treatment with dithionite + citrate (DC) removed, on average, 37% of Cu from *R. paniculata* roots, 27% from *B. pilosa*, 23% from *P. calomelanos* and 29% from *C. leprosum*. The values obtained for roots treated with DC were used to calculate the TF. This approach appears to be better because the treatment removes strongly adhered soil particles from the roots, thus minimizing overestimation of the Cu content, which would affect the values of the translocation factor.

As observed for leaves, the concentrations of bioavailable and potentially bioavailable Cu in rhizosphere correlated significantly with the concentration of Cu in roots ($r = 0.712$, $p = 0.05$ for $MgCl_2$ and $r = 0.758$, $p = 0.01$ for Cu–Me) (Table 4). However, the concentration of Cu was higher in *B. pilosa* and *P. calomelanos* roots than the concentration of potentially bioavailable Cu in the rhizosphere soils (Fig. 5). This may indicate that these species can absorb greater amounts of Cu, even by extracting more stable forms of Cu from the soil (i.e. Cu associated with oxides and sulphides), which are not readily extracted with Mehlich3 solution.

Because of the high concentration of Cu in the roots of all species, the values of the translocation factor were lower than the value indicating hyperaccumulator species, i.e. >1 (Table 3).

3.3. Balance between Cu mobilized in the rhizosphere and uptake by plants: consequences for mine site restoration

For a practical demonstration of how the changes in physicochemical properties that occur in the rhizosphere alter the Cu dynamics; and also to assess the consequences that this may have for the restoration of the affected areas, the balance between Cu

Table 4
Pearson correlation coefficient for copper in plants and soil properties.

	Rhizosphere							Bulk						
	$Cu–MgCl_2$	Cu–Me	Cu p-total	pH	TOC	TIC	TS	$Cu–MgCl_2$	Cu–Me	Cu p-total	pH	TOC	TIC	TS
Cu leaves	0.875**	0.962**	−0.076	−0.873**	−0.566	−0541	−0.702*	0.217	−0.838**	−0.498	−0.349	−0.154	−0.849**	−0.849**
Cu roots (H_2O)	0.720*	0.765**	−0.119	−0.714*	−0.733**	−0.711*	−0.486	0.444	−0.792**	−0.627*	−0.533	−0.399	−0.500	−0.500
Cu roots (DC)	0.712*	0.758**	−0.171	−0.707*	−0.739**	−0.727*	−0.528	0.456	−0.797**	−0.637*	−0.545	−0.412	−0.490	−0.490

* $p < 0.05$.
** $p < 0.01$.

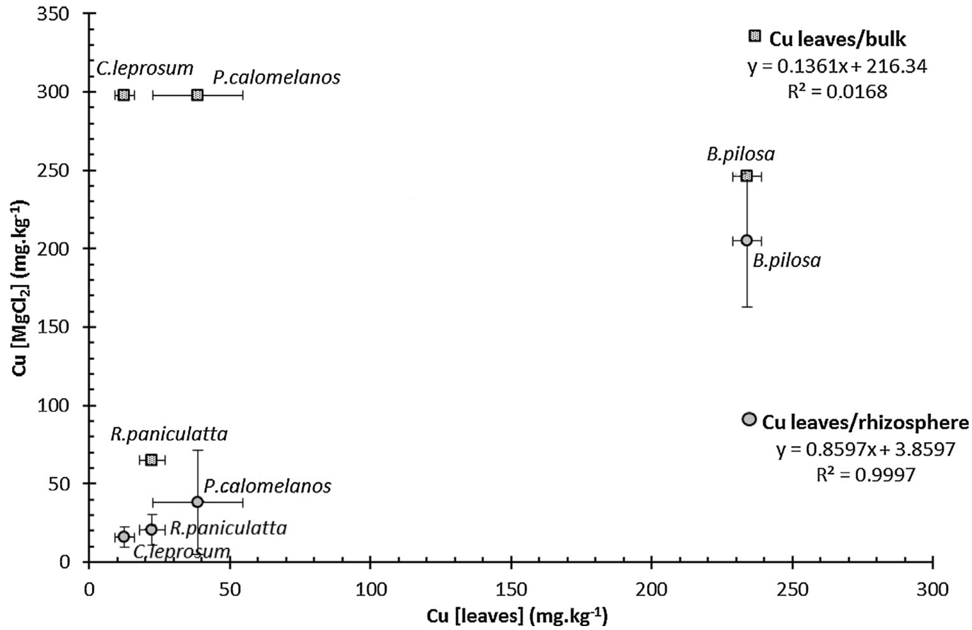

Fig. 4. Correlation coefficients for Cu concentrations in leaves and readily bioavailable Cu in bulk and rhizosphere soils.

mobilized in the rhizosphere, Cu uptake by plants and the amount of Cu that is "lost" in these process was calculated, by applying the same principles used to calculate the enrichment and translocation factors.

The balance between mobilized copper in the rhizosphere and copper absorbed by plants is very important, because if plants mobilize more copper than they are able to take up, part of the copper maybe lost by leaching, thus increasing the risk of contamination of soils and water bodies and transfer to the food chain. In this case, soil constituents are also very important as the mobilized copper may be re-adsorbed (i.e. by carbonates, oxides and organic matter).

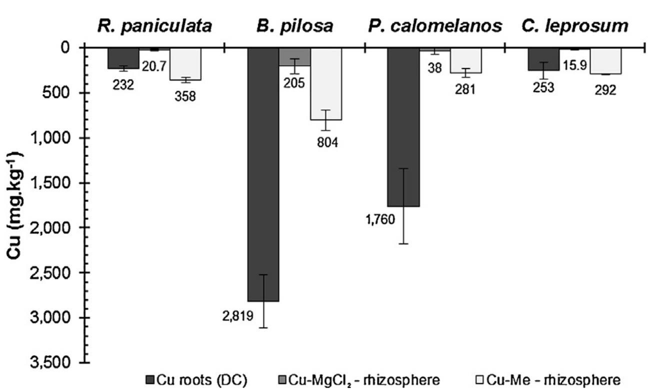

Fig. 5. Concentration of copper accumulated in roots and the readily bioavailable (Cu–$MgCl_2$)/potentially bioavailable (Cu–Me) copper in the rhizosphere soil.

However, plants that can accumulate more copper than mobilized by their roots are of interest for remediation purposes; as the copper may be immobilized in their tissues and be unavailable to other organisms, thus minimizing the risk of environmental contamination if removed from the area before decompose.

Table 5 highlights the case of the species from Pedra Verde mine. Both *R. paniculata* and *C. leprosum* display a positive balance between Cu mobilized in the rhizosphere (C) and Cu absorbed by plants (D), indicating that the plants add labile copper to the system; if the excess is not re-adsorbed by other soil constituents, it may be lost by leaching, increasing the risk of the contamination spreading to adjacent areas. By contrast, *B. pilosa* and *P. calomelanos* display a negative balance (E) between mobilized and absorbed Cu, indicating that they can take up and accumulate all labile Cu forms. These species are therefore potentially valuable for phytoremediation programs, as they will concentrate the metal in their biomass, thus making it less available to other organisms.

Table 5
Balance between Cu in bulk and rhizosphere soil and that taken up by plants (leaves + roots) in mg kg^{-1}.

Plant species	Total Cu in bulk soil [A] (mg kg^{-1})	Total Cu in rhizosphere soil [B] (mg kg^{-1})	Mobilized Cu[a] [C] (A–B) (mg kg^{-1})	Cu in plants[b] [D] (mg kg^{-1})	Balance [E] (C–D) (mg kg^{-1})
R. paniculata	9601	6310	3291	254	+3037
B. pilosa	3601	3143	458	3053	−595
P. calomelanos	4302	3701	601	2431	−1830
C. leprosum	4302	1180	3122	266	+2857

[a] Copper in bulk soil less copper in rhizosphere soil.
[b] Leaves + roots (dry weight).

4. Conclusions

Given the complex geochemical interaction between Cu ions and different soil components, the use of total (or pseudo-total) metal concentrations in soils should be used with caution especially when used as a parameter for calculating the capacity of plants to accumulate metal. On the basis of our findings, the levels of the readily ($MgCl_2$-extracted) or potentially (Mehlich3-extracted) bioavailable metal in soil appears to be a more realistic approach. Given the significant differences between the concentrations of Cu in the bulk and the rhizosphere soils, the use of the latter proved to be more suitable for such assessments, since the rhizospheric soil reflects the specific biogeochemical environment where plants grow. Changes caused by the activity of roots may alter many soil properties, such as pH and organic carbon contents, which in turn, will directly affect Cu mobility.

None of the studied plant species fulfilled the requirements for a classification as hyperaccumulator species (i.e. >1000 mg kg^{-1} Cu in shoots or ratio between Cu in leaves and soil >1). Both *R. paniculata* and *C. leprosum* showed the characteristics of excluder species, because although growing in highly Cu contaminated soils, they did not contain high levels of metal in their tissues. However, these species showed some undesirable characteristics, as they were not able to absorb the Cu that was mobilized in their rhizospheres, which may extend the risk of metal release to adjacent areas and/or watercourses, as indicated by the positive balance between the amount of copper mobilized in their rhizospheres and the amount of Cu accumulated in their tissues.

On the other hand, *P. calomelanos* and *B. pilosa* showed the characteristics of immobilizing species, since the ratio between the mobilized Cu in the rhizosphere and the Cu accumulated in their tissues was negative, indicating that these species can uptake more Cu than that is mobilized by their rhizospheric processes, decreasing the amount of bioavailable or potentially bioavailable copper in the environment. These plants may, therefore, be suitable for remediation and/or revegetation of Cu-contaminated areas.

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References

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risk of Metals, 2nd ed. Springer-Verlag, New York.
Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004. Role of assisted natural remediation in environmental cleanup. Geoderma 122, 121–142.
Anawar, H.M., Canha, N., Santa-Regina, I., Freitas, M.C., 2013. Adaptation, tolerance, and evolution of plant species in a pyrite mine in response to contamination level and properties of mine tailings: sustainable rehabilitation. J. Soils Sediments 13, 730–741.
Aykol, A., Budakoglu, M., Kumral, M., Gultekin, A.H., Turhan, M., Esenli, V., Yavuz, F., Orgun, Y., 2003. Heavy metal pollution and acid drainage from the abandoned Balya Pb–Zn sulfide Mine, NW Anatolia, Turkey. Environ. Geol. 45, 198–208.
Baker, A.J.M., Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81–126.
Bell, L.C., 2001. Establishment of native ecosystems after mining – Australian experience across diverse biogeographic zones. Ecol. Eng. 17, 179–186.
Boojar, M.M., Goodarzi, F., 2007. The copper tolerance strategies and the role of antioxidative enzymes in three plant species grown on copper mine. Chemosphere 67, 2138–2147.
Boyd, R., 2004. Ecology of metal hyperaccumulation. New Phytol. 162, 563–567.
Bradshaw, A.D., 1997. Restoration of mined lands – using natural processes. Ecol. Eng. 8, 255–269.
Branquinho, C., Serrano, H.C., Pinto, M.J., Martins-Loução, M.A., 2007. Revisiting the plant hyperaccumulation criteria to rare plants and earth abundant elements. Environ. Pollut. 146, 437–443.
Bravin, M.N., Garnier, C., Lenoble, V., Gérard, F., Dudal, Y., Hinsinger, P., 2012. Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. Geochim. Cosmochim. Acta 84, 256–268.
Bravin, M.N., Martí, A.L., Clairotte, M., Hinsinger, P., 2009. Rhizosphere alkalisation – a major driver of copper bioavailability over a broad pH range in an acidic, copper-contaminated soil. Plant Soil 318, 257–268.
Brun, L.A., Maillet, J., Richarte, J., Herrmann, P., Remy, J.C., 1998. Relationships between extractable copper: soil properties and copper uptake by wild plants in vineyard soils. Environ. Pollut. 102, 151–161.
Cambardella, C.A., Gajda, A.M., Doran, J.W., Wienhold, B.J., Kettler, T.A., 2001. Estimation of particulate and total organic matter by weight loss-on-ignition. In: Lal, R., Kimble, M., Follett, R.F., Stewart, B.A. (Eds.), Assessment Methods for Soil Carbon. Lewis, BocaRaton, pp. 349–359.
Collins, J.J., Loureiro, R., 1971. A metamorphosed deposit of Precambrian supergene copper. Econ. Geol. 66, 192–199.
CEC – Commission of the European Communities, 1986. Council directive on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. J. Eur. Commun. L181 (Annex 1A).
Chung, J.B., Zazoski, R.J., 1994. Ammonium–potassium and ammonium–calcium exchange equilibrium in bulk and rhizosphere soil. Soil Sci. Soc. Am. J 58, 1368–1375.
CONAMA – Conselho Nacional do Meio Ambiente/Brasil. 2009. Resolução N°420 – Dispõe sobre critérios e valores orientadores de qualidade do solo. Ministério do Meio Ambiente, Brasília/DF.
Cook, L., McGonigle, T.P., Inouye, R.S., 2009. Titanium as an indicator of residual soil on arid-land plants. J. Environ. Qual. 38, 188–199.
Dzantor, E.K., Beauchamp, R.G., 2002. Phytoremediation, part I: fundamental basis for the use of plants in remediation of organic and metal contamination. Environ. Pract. 4, 77–87.
Ernst, W.H.O., 2005. Phytoextraction of mine wastes – options and impossibilities. Chem. Erde – Geochem. 65, 29–42.
Fernandez-Calvino, D., Perez-Novo, C., Novoa-Munoz, J.C., Arias-Estevez, M., 2009. Copper fractionation and release from soils devoted to different crops. J. Hazard. Mater. 167, 797–802.
Filgueiras, A.V., Lavilla, I., Bendicho, C., 2002. Chemical sequential extraction for metal partitioning in environmental solid samples. J. Environ. Monit. 4, 823–857.
FUNCEME – Fundação Cearense de Meteorologia e Recursos Hídricos, 2014. http://www.funceme.br/index.php/areas/tempo/chuvas-mensais-municipios (accessed on 15.09.14).
García-Lorenzo, M.L., Pérez-Sirvent, C., Martínez-Sánchez, M.J., Molina-Ruiz, J., 2012. Trace elements contamination in an abandoned mining site in a semiarid zone. J. Geochem. Explor. 113, 23–35.
Hernández, A.J., Pastor, J., 2008. Relationship between plant biodiversity and heavy metal bioavailability in grasslands overlying an abandoned mine. Environ. Geochem. Health 30, 127–133.
Hisinger, P., Bangough, A.G., Vetterlein, D., Young, I.A., 2009. Rhizosphere: biophysics: biogeochemistry and ecological relevance. Plant Soil 321, 117–152.
IBGE – Brazilian Institute of Geography and Statistics. 2012. Technical Handbook of the Brazilian Vegetation. 2nd ed. Rio de Janeiro. ISSN 0103-9598.
Jurjovec, J., Ptacek, C.J., Blowes, D.W., 2002. Acid neutralization mechanisms and metal release in mine tailings: a laboratory column experiment. Geochim. Cosmochim. Acta 66, 1511–1523.
Kabata-Pendias, A., 2004. Soil–plant transfer of trace elements – an environmental issue. Geoderma 122, 143–149.
Kabata-Pendias, A., 2010. Trace Elements in Soils and Plants, 4th ed. CRC Press, Boca Raton.
Leblanc, M., Borrego, J.A.M., Elbaz-Poulichet, F., 2000. 4500-year-old mining pollution in southwestern spain: long-term implications for modern mining pollution. Econ. Geo. 95, 655–662.
Liu, W.J., Zhu, Y.G., Smith, F.A., Smith, S.E., 2004. Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? J. Exp. Bot. 55, 1707–1713.
Liu, W.J., Zhu, Y.G., Hu, Y., Willian, P.N., Gault, A.G., Meharg, A.A., Charnock, J.M., Smith, F. A., 2006. Arsenic sequestration in iron plaque: its accumulation and speciation in mature rice plants (*Oryza sativa* L.). Environ. Sci. Technol. 40, 5730–5736.
Lottermoser, B.G., 2007. Mine Wastes: Characterization, Treatment Environmental Impacts, second ed. Springer, Heidelberg.
Manara, A., 2012. Plant responses to heavy metal toxicity. In: Furini, A. (Ed.), Plants and Heavy Metals. Springer Briefs in Biometals, pp. 27–53. doi:http://dx.doi.org/10.1007/978-94-007-4441-7-2.
Martínez-Alcalá, I., Walker, D.J., Bernal, M.P., 2010. Chemical and biological properties in the rhizosphere of Lupinus albus alter soil heavy metal fractionation. Ecotoxicol. Environ. Saf. 73, 595–602.
Martínez-Sanchez, M., García-Lorenzo, M.L., Pérez-Sirvent, C., Bech, J., 2012. Trace element accumulation in plants from an aridic area affected by mining activities. J. Geochem. Explor. 123, 8–12.
McLaughlin, M.J., 2001. Bioavailability of metals to terrestrial plants. In: Allen, H.E. (Ed.), Bioavailability of Metals in Terrestrial Ecosystems Importance of Partitioning for Bioavailability to Invertebrates Microbes and Plants. SETAC Press, Pensacola, FL, pp. 39–68.

Mehlich, A., 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Comm. Soil Sci. Plant Anal. 15, 1409–1416.
Mendez, M.O., Maier, R.M., 2008. Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology. Environ. Health Perspect. 116, 278–283.
Monterroso, C., Alvarez, E., Marcos, L.F., 1999. Evaluation of Mehlich 3 reagent as a multielement extractant in mine soils. Land Degrad. Dev. 10, 35–47.
Närhi, P., Räisänen, M.L., Sutinen, M.L., Sutinen, R., 2012. Effect of tailings on wetland vegetation in Rautuvaara, a former iron–copper mining area in northern Finland. J. Geochem. Explor. 116–117, 60–65.
Nikolic, N., Kostic, L., Djordjevic, A., Nikolic, M., 2010. Phosphorus deficiency is the major limiting factor for wheat on alluvium polluted by the copper mine pyrite tailings: a black box approach. Plant Soil 339, 485–498.
Otero, X.L., Álvarez, E., Fernandez-Sanjurjo, M.J., Macias, F., 2012. Micronutrients and toxic trace metals in the bulk and rhizospheric soil of the spontaneous vegetation at an abandoned copper mine in Galicia (NW spain). J. Geochem. Explor. 112, 84–92.
Peplow, D., Edmonds, R., 2005. The effects of mine waste contamination at multiple levels of biological organization. Ecol. Eng. 24, 101–119.
Perlatti, F., Otero, X.L., Macias, F., Ferreira, T.O., 2014. Geochemical speciation and dynamics of copper in tropical semi-arid soils exposed to metal-bearing mine wastes. Sci. Total Environ. 500–501, 91–102.
Pilon-Smits, E.A.H., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15–39.
Pilon-Smits, E.A.H., Freeman, J.L., 2006. Environmental cleanup using plants: biotechnological advances and ecological considerations. Front. Ecol. Environ. 4, 203–210.
Reeves, R.D., Baker, A.J.M., 2000. Metal-accumulating plants. In: Raskin, I., Ensley, B. D. (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. Wiley, New York.
Reeves, R.D., 2006. Hyperaccumulation of trace elements by plants p. 25-52. In: Morel, J.L., Echevarria, G., Goncharova, N. (Eds.), Phytoremediation of Metal-Contaminated Soils. Springer, Dordrecht, Netherlands, pp. 346 p.
Schulz, F., Wiegleb, G., 2000. Development options of natural habitats in a post-mining landscape. Land Degrad. Dev. 11, 99–110.
Shrestha, R.K., Lal, R., 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161, 168–176.
Song, J., Zhao, F.J., Luo, Y.M., McGrath, S.P., Zhang, H., 2004. Copper uptake by Elsholtzia splendens and Silene vulgaris and assessment of copper phytoavailability in contaminated soils. Environ. Pollut. 128, 307–315.
Tao, S., Chen, Y.J., Xu, F.L., Cao, J., Li, B.G., 2003. Changes of copper speciation in maize rhizosphere soil. Environ. Pollut. 122, 447–454.
Taylor, G.J., Crowder, A.A., 1983. Use of the DCB technique for extraction of hydrous iron oxides from roots of wetland plants. Amer. J. Bot. 70, 1254–1257.
Wu, F., Liu, Y., Xia, Y., Shen, Z., Chen, Y., 2011. Copper contamination of soils and vegetables in the vicinity of Jiuhuashan copper mine. China Environ. Earth Sci. 64, 761–769.
Whiting, S.N., Reeves, R.D., Richards, D., Johnson, M.S., Cooke, J.A., Malaisse, F., Paton, A., Smith, J.A.C., Angle, J.S., Chane, E.L., Ginocchio, R., Jaffré, T., Johns, R., McIntyre, T., Purvis, O.W., Salt, D.E., Schat, H., Zhao, F.J., Baker, A.J.M., 2004. Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. Restor. Ecol. 12, 106–116.
Yang, X.E., Long, X.X., Ni, W.Z., Ye, Z.Q., He, Z.L., Stoffella, P.J., Calver, D.V., 2002. Assessing copper thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. J. Environ. Sci. Health B 37, 625–635.
Zornoza, R., Carmona, D.M., Acosta, J.A., Martínez-Martínez, S., Weiss, N., Faz, A., 2012. The effect of former mining activities on contamination dynamics in sediments, surface water and vegetation in El Avenque Stream, SE Spain. Water Air Soil Pollut. 223, 519–532.