Sequential extraction of copper in bulk and rhizosphere soils of *Combretum Leprosum M.* in an abandoned mine site in NE Brazil

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ABSTRACT: We evaluated the biogeochemical processes occurring in the rhizosphere of *Combretum leprosum* (Mufumbo); a plant native from the Brazilian semiarid; growing spontaneously in a highly Cu contaminated soil in an abandoned mine site in NE Brazil. The effect of these processes on copper mobility, toxicity, and the ability of this plant to be used as pioneer specie on restoration programmes were also assessed. Chemical properties (pH, % of TOC and % of TIC), and Cu sequential extraction were determined in both rhizosphere and bulk soil. The study findings show that the plant have greatly altered the physicochemical characteristics of the soil under the influence of their roots, by altering various soil components, affecting the biogeodynamic cycle of copper. The changes that occur in the rhizosphere affected copper dynamics mainly by decreasing the concentration of potentially bioavailable Cu evidenced by a significant reduction in the exchangeable and carbonate-associated Cu. Results also showed an increase in the Cu associated with amorphous Fe oxides, enhancing Cu immobilization and probably minimizing the risks of Cu toxicity and mobility in the rhizosphere. The biogeochemical processes observed in the rhizosphere of the studied specie seem to indicate that this plant may promote phytostabilization of Cu in their rhizosphere zone, and thus show desirable characteristics for use in phytoremediation programmes.
Key words: bioavailability, phytoremediation, ecological restoration.

INTRODUTION

The interactions that occur at the soil-plant interface play a vital role in the functioning of terrestrial ecosystems. Such interactions occur in a thin layer of soil, called the rhizosphere, which is directly influenced by plant roots. In this unique soil zone, the biological activity of roots and the associated microorganisms can significantly alter the physical and chemical characteristics of soil (Hisinger et al., 2003; Ahmad et al., 2013; Houben et al., 2012), which may increase or decrease the mobility of essential nutrients and soil contaminants.

The presence of plants in soils containing high amounts of metals, such as copper, may produce antagonistic results. Plants can immobilize metals in the root zone, via the formation of stable organometallic complexes (Strawn and Baker, 2009) or by precipitation with other soil components (Austruy et al., 2014). By contrast, plants can also increase metal mobility, through acidification (Chaignon et al., 2009), increasing the risk of dispersion of the metal to adjacent ecosystems.

There is growing interest in using plants to remediate contaminated soils, and many studies have been carried out to identify the suitability of different plants in phytoremediation programs (Dickinson et al., 2009). Investiganting the biogeochemical interactions involved in the metal-soil-plant system, can hellp provide more precise information about the ability of plants to survive in contaminated environments, as well as the consequences of their use in restoration programs (Hisinger et al., 2009).

This study aimed to investigate the bulk and rhizosphere soils from a native plant specie, growing in a highly Cu contaminated soil from an abandoned mine site (NE Brazil). Additionally, this study aimed to identify the main changes in rhizosphere biogeochemical process and their possible effects on copper mobility.

MATERIAL E METHODS

The study was conducted in an abandoned copper mine located in Viçosa do Ceará – CE, at NE-Brazil. The mine was exploited in the 1980s for the extraction and processing of Cu from sulfide (chalcopyrite and chalcocite) and carbonate (malachite) minerals. After the mine's closure, in 1987, the processed minerals were dumped into piles on terraces on the slope of the plateau. The terraces eroded spreading waste rock over the slope, leading to a high soil contamination with copper. At some sites, the total Cu content in the soil exceeded 11,000 mg.kg^{-1} (Perlatti et al., 2014). This level of contamination caused a substantial

limitation to plant growth and, consequently, to the natural restoration of the area.

There were few individuals of *C. leprosum* growing on the mine wastes. One bulk and two rhizosphere (rhizo I and rhizo II, as described below) soil samples were collected. The soil around plants (bulk soil) was collected, at least 3 cm from the roots up to approximately 30 cm from the plants and at a depth of 0 to 20 cm. Bulk soil samples were collected in triplicate in order to obtain a composite sample. The rhizosphere samples were obtained by the method described in Otero et al. (2012), where two kinds of the rhizosphere are considered, according to the distance from roots.

Rhizo II comprises the soil material that was separated by shaking the roots previously parted from the bulk soil and represents the soil loosely adhered to roots (approximately 2.0 to 30.0 mm from the root surface). Rhizo I comprises the soil material that remains adhered to the roots after rhizo II extraction, and represents the soil that is firmly adhered to roots, at approximately 0.0 to 2.0 mm from the root surface. The samples were collected from three individuals in triplicate.

Once the bulk, rhizo I and rhizo II soil samples were separated, the pH was determined in water (soil/solution ratio, 1:2.5). Total S was measured in a Leco SC-144DR, and total N and C contents were measured in a Leco TruSpec CHN analyzer. The contents of total organic (TOC) and inorganic (TIC) carbon were determined following the methodology described by Cambardella et al. (2001). Potentially bioavailable Cu were extracted by the Mehlich-3 solution. The sequential extraction was performed following the methodology described in Perlatti et al., 2014, that allows the differentiation of seven operational Cu fractions, defined as: F1-Exchangeable, F2-associated with carbonates, F3-associated with organic matter, F4-associated with amorphous Fe oxides, F5-associated with crystalline Fe oxides, F6-associated with sulfides and F7-residual Cu.

The differences between samples were established by one-way ANOVA followed by a Tukey test or Kruskal–Wallis One Way Analysis of Variance on Ranks when the normality test failed.

RESULTS AND DISCUSSION

The results showed that there were no significant differences in the general characteristics between the rhizo-I and rhizo-II, however, significant differences were observed between the rhizosphere and bulk soil (figure 1).

The pH values in the rhizo-I and rhizo-II (5.5 ±0.1 and 5.8 ±0.1), were higher than those observed in the bulk soils (4.9), indicating a alkalization of the rhizosphere. The absence of acidification in the rhizosphere of the studied species can be related to the presence of Cu and Ca carbonates in the area. The former was explored as a metal ore (malachite (Cu_2 (CO_3) (OH) $_2$)). The latter is reported to be one of the main gangue materials (calcite; $CaCO_3$) (Collins and Loureiro, 1971). These minerals, when exposed to the action of microorganisms and root exudates, and thus, to more acidic conditions, tend to have their dissolution increased, which may buffer the acidification commonly observed in the rhizosphere (Uroz et al., 2009).

The results for total inorganic carbon (TIC) did not show significant differences (p>0.05) between rhizo I, rhizo-II and bulk (0.35%, 0.33% and 0.37, respectively). The TOC contents were significantly higher (p<0.05) in the rhizospheres (rhizo I 15.7% and rhizo II 11.0%) when compared to the bulk soil (0.11%) and TN contents showed similar results. The TS were significantly higher (p<0.05) in the rhizosphere than those registered in the bulk soils (rhizo I 0.13%, rhizo II 0.11% and bulk 0.03%). The highest total organic carbon (TOC) contents in the studied rhizospheres corroborate other studies that demonstrated an increase in organic C contents in this soil zone. Higher TOC contents are usually caused by the rhizodeposition of organic compounds and the greater presence of microorganisms (Martínez-Alcalá et al., 2010). Also in response to these factors, the increase in the concentration of total nitrogen (TN) in the rhizospheric soil was related to the increase in TOC.

The results showed clear differences in the distribution of copper between the rhizosphere and bulk soils (figure 2). In general, bulk soil showed a significant higher concentration of total Cu (sum of the seven fractions) than the rhizosphere soils, with 1,547; 1,320 and 4,747 mg.kg^{-1} of Cu in rhizo I, rhizo II and bulk soil respectively. In the rhizosphere, the Cu were mainly associated with amorphous iron oxides, while in bulk soil, the metal was distributed almost equally between carbonates, amorphous oxides and sulfides.

In the bulk soil the exchangeable Cu (F1) reached 298.0 mg.kg^{-1}. In the rhizo I and rhizo II the values were 18.5 and 13.3 mg.kg^{-1}. Regarding the Cu associated with carbonates (F2), the concentration in the bulk soil reach 1,290 mg.kg^{-1}, decreasing to 63,0 mg.kg^{-1} in rhizo I and 65.3 mg.kg^{-1} in rhizo II. A proportional increase in the Cu associated with organic matter (F3) and especially amorphous iron oxides (F4) were observed in the rhizosphere. The Cu associated with amorphous iron oxides reached 1,425 mg.kg^{-1} in the bulk soils. In rhizo I and rhizo II the

concentrations were 1,132 mg.kg^{-1} and 951.0 mg.kg^{-1}, respectively. A significant decrease in the Cu associated with sulfides (F6) was found in the rhizosphere soils, while the Cu associated with the residual fraction (F7) was low, with no significant differences between the rhizosphere and bulk soils.

The main response regarding Cu dynamics in the rhizosphere was a decrease in exchangeable and carbonate associated Cu, followed by an increase in the concentration of Cu associated with amorphous iron oxides (a more stable Cu form in soils) (figure 2).

The rate of carbonates dissolution seemed to increase in the rhizosphere, which may reflected in lower concentrations of Cu associated with carbonates (F2). In this case, the Cu dissolved from carbonates (malachite) would be mobilized to the soil solution and adsorbed onto iron oxides. We hypothesize that, given the action of microorganisms and organic acids in the rhizosphere, the dissolution of Cu carbonate, may have mobilized copper ions (Cu^{2+}) to the soil solution. The released Cu would either be uptake by plants or adsorbed onto iron oxides (Cerqueira et al., 2011). Both mechanisms would reduce the metal mobility, in response to the higher stability of iron oxides to that of carbonates. Additionally, the increase in the proportion of Cu associated with organic matter in the rhizosphere could also reduce the Cu mobility given the well-know affinity between Cu ions and organic matter, forming high stable organometallic complexes. In this case, the studied plants seemed to provide some phytostabilization of Cu since they decreased the concentration of the most toxic copper forms.

Cu associated with sulfides (F6) showed a drastic decrease in the rhizosphere, probably due to more oxidizing conditions. In this case, the oxidation of Cu sulfides would lead to the formation of amorphous iron oxides with the release of Cu^{2+} and sulfate (Faure, 1991). These reactions reinforce the increase in Cu associated with amorphous oxides in the rhizosphere since the Cu mobilized from the sulfides could also be re-adsorbed onto iron oxides. The residual Cu fraction (F7) seemed to have been little affected by rhizosphere biogeochemical processes since no significant differences between bulk, and rhizospheric soils were observed for this fraction.

CONCLUSIONS

Plants have altered the physicochemical conditions of soil under the influence of their roots. These changes observed in the rhizosphere have influenced the geochemical dynamics of copper.

The most significant pattern observed was the greater reduction in the proportion of the more labile (and toxic) forms of copper, such as the exchangeable and Cu associated with the carbonates (malachite) in the rhizosphere.This response is mainly associated with the immobilization of Cu via adsorption, mostly onto the amorphous iron oxides, resulting in a decrease in the concentration of potentially bioavailable Cu in the rhizosphere and a consequent decrease in its phytotoxicity.

This results indicate that *C.leprosum* were able to promote the phytostabilization of Cu in their rhizosphere, and should be suitable as pioneer species for use in the restoration of Cu contaminated areas.

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Figure 1. General characteristics of rhizosphere and bulk soil of *C. leprosum*. Values are mean ± s.d and * represents significance differences (p<0.05; n=3).
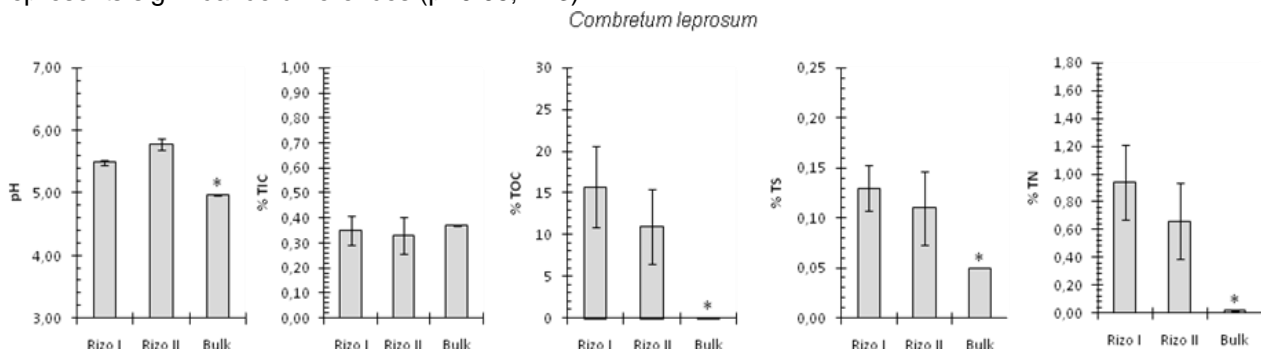

Figure 2. (a) Distribution of copper in soil fractions (in mg.kg^{-1}), (b) levels of potentially bioavailable copper extracted by Mehlich 3 and (c) percentage of copper in each fraction in rhizosphere and bulk soil.

	F1	F2	F3	F4	F5	F6	F7
rhizo I	1.2%	4.1%	4.1%	73.2%	3.7%	11.3%	2.3%
rhizo II	1.0%	4.9%	5.4%	72.0%	3.5%	11.0%	2.1%
bulk	6.3%	27.2%	2.0%	30.0%	1.0%	32.0%	1.6%