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Cu Dynamics in the Rhizosphere of Native Tropical Species: Assessing the Potential for Phytostabilization in Mining-Impacted Soils

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Abstract: The use of native plants for reforestation and/or remediation in areas contaminated by mining is a technique with low implantation and maintenance costs. The success of this practice depends on the plant species and geochemical processes at the soil–plant interface (e.g., rhizosphere). This study evaluated the potential of spontaneous species for mobilizing and altering mineral and metal dynamics in the rhizosphere of Cu-rich soils resulting from the abandoned Pedra Verde mine in NE Brazil. Rhizosphere and bulk soil samples were collected from five shrubby/arboreal species. The pH, organic matter content, Cu fractionation, mineralogical characterization, and Cu content in the leaves and roots of all studied species were determined. In addition, the bioaccumulation factor (BCF) and translocation factor (TF) were used to evaluate the potential of these species for Cu hyperaccumulation. The Cu concentration in leaf plant tissues varied from 18 to 34 mg kg^{-1}, and all plants presented TF and BCF < 1, indicating that the species were not Cu hyperaccumulators. However, the root exudates induce mineral dissolution, indicating potential Cu accumulation in the roots (from 36 to 249 mg kg^{-1}). *Combretum aff. pisoniodes* Taub was the species with the greatest potential for decreasing Cu bioavailability and phytostabilization. Our findings indicate the potential of native Brazilian plants for growth in Cu-contaminated soil. These findings may be used for reforestation programs.

Keywords: Cu biogeochemistry; abandoned mine soils; phytoremediation

1. Introduction

Soils within abandoned mining areas are commonly associated with acute impacts on their physical and chemical characteristics. The loss of soil structure, a decrease in soil organic matter content, extreme pH (alkaline or acidic) values, and high metal contents are the most commonly reported impacts [1–4]. These impacts on soils limit the establishment of successional vegetation or reclamation practices within abandoned mining areas [5] and expose adjacent compartments (e.g., surrounding soils, streams, rivers, and groundwater) to contamination risks [6,7].

In this sense, cost-effective and environmentally friendly reclamation technologies such as plant-based approaches are some of the most successful techniques for remediating

polluted soil from abandoned mining sites [8,9]. Phytoremediation is a technique based on the extraction, immobilization, or volatilization of metals using plants [10,11]. The principle of phytoremediation results from the natural ability of plant species to grow in contaminated areas and accumulate high concentrations of metals in their shoots or restrict high metal contents into the roots and rhizosphere zone [12–14]. Accordingly, phytoextraction and phytostabilization are two promising techniques that are widely applied within the field of phytoremediation [15]. Phytoextraction emerges as a technology for the removal of metals from the environment using metal-accumulating plants [16]. By contrast, phytostabilization involves the use of plants to reduce the mobility of contaminants (e.g., metals) in contaminated environments via pollutant accumulation or immobilization in their roots or rhizosphere [17].

Although several studies have reported phytoremediation efficiency, few studies have been conducted using native species from tropical and subtropical regions, such as those found in Brazil [4,18,19]. Moreover, most plant species classified as phytoremediators are from temperate regions. Despite growing in tropical regions, the use of exotic species can cause ecological problems, especially if the objective is to restore ecological processes [20,21]. Globally, Brazil is one of the nations with the most significant mining activities and a tropical hotspot of sites polluted by metals from such activities [22–25]. Additionally, most of these polluted sites present a great diversity of species growing on them spontaneously, which offers the possibility of finding several new phytoremediator plants [26].

Therefore, the objective of this study was to identify native Brazilian species (e.g., shrubby and arboreal) with the potential for use in Cu phytoremediation initiatives. To achieve our objective, we assessed both the Cu content in five native species from the *Caatinga* biome and the biogeochemical characteristics of its rhizospheres.

2. Materials and Methods

2.1. Site Description

The study area is near the Pedra Verde mine, located near the municipality of Viçosa do Ceará, located in the Brazilian state of Ceará, within the NE Brazilian district (Figure 1). The region is characterized by a tropical, hot, semi-arid climate, with a rainy (January and May) and dry season (June to December), average annual precipitation of 981 mm, and annual temperatures ranging from 21 °C to 32 °C [27,28]. In addition, the region is characterized by seasonal deciduous forests, which represent transition vegetation between the ombrophilous forest and *Caatinga* [4,28].

The studied site is located within an abandoned mine site that operated until 1987. Cu from sulfides (chalcopyrite and chalcocite) and carbonates were excavated, which produced a large volume of tailings waste [29]. After the mine activity, the tailings eroded, spreading waste rocks containing Cu-enriched wastes (Cu concentration: ~49,000 mg kg^{-1}) to soil and rivers within a 1.5 km distance and representing a severe risk to the ecosystem and public health [4,29,30].

Figure 1. The Pedra Verde mine near the municpliatity of Viçosa do Ceará, within NE-Brazil (**a**,**b**). Sampled sites containing the five most abundant shrub-by/arboreal species, which were observed to randomly grow on the Cu-rich eroded piles (**c**). The five studied randomly growing species included: *Bauhinia ungulate* L. (**d**), *Combretum aff. pisoniodes* Taub (**e**), *Combretum leprosum* Mart (**f**), *Croton blanchetianus* Baill (**g**), *Hymenaeae courbaril* L. (**h**), and the Cu-rich eroded piles (**i**).

2.2. Plant and Soil Sampling

To identify potential hyperaccumulator plant species, the five most abundant shrubby/ arboreal species (Figure 1d–h) observed to randomly grow on the eroded tailings were selected: *Bauhinia ungulate* L., *Combretum aff. pisoniodes* Taub, *Combretum leprosum* Mart, *Croton blanchetianus* Baill, and *Hymenaeae courbaril* L. For each species, three juvenile specimens were collected from the mining area. Twenty leaves and whole-root biomass were collected from each plant area and transported to the laboratory. In the laboratory, the leaves and roots were carefully rinsed three times with distilled water, dried in a fan-forced oven (at 65 °C for 72 h), ground, and stored in plastic bags for posterior analysis.

Soil samples were collected from the specimen bulk and rhizosphere soil. The rhizo-spheric soil represents the portion of soils directly influenced by root plants; therefore, it is chemically distinct from the bulk soil [31]. The rhizosphere samples of each plant species

were collected in four steps, as follows: (i) the collected roots were shaken to remove the weakly adhered soil, (ii) soil still attached to the roots was manually removed, (iii) roots were vigorously shaken within plastic bags, and (iv) soil particles still adhering to the roots were removed with a brush [32]. The bulk, which represents the soil mass without direct root influence, was collected using a Dutch auger, close (~1.5 m) to plant specimens up to a depth of 0–20 cm. At the laboratory, the soil samples (rhizosphere and bulk) were dried at room temperature, sieved to 2 mm, and characterized for organic matter content, pH, Cu solid-phase fractionation, and mineralogical characterization.

2.3. Chemical Analyses: pH, Organic Matter Content, and Cu Solid-Phase Fractionation

The pH was measured in a 1:2.5 suspension soil/water in bulk and rhizosphere soils using a previously calibrated (pH 4.0 and 7.0) glass electrode [33]. The pH values were determined after the suspension was decanted for 30 min by submerging the electrode in the clear portion of the suspension, and the pH readings were determined after stabilization [32].

The organic matter (OM) contents were determined via loss of ignition after heating at 450 °C for 2 h. Prior to drying at 105 °C, the samples were re-weighed after heating at 450 °C [34].

Cu fractionation in the samples was determined using a combined method of sequential chemical extraction [29,33,35,36]. This procedure allows for a better understanding of the dynamics of metals in soil. The following operationally distinct fractions were determined:

1. Exchangeable or soluble (CuEX)–extracted using a $MgCl_2$ 1 mol L^{-1} solution at a pH 7.0.
2. Associated with carbonates (CuCAR)–extracted with a 1 mol L^{-1} NaOAC solution at a pH 5.0.
3. Associated with organic matter (CuOM) extracted with 6% NaOCl at a pH 8.0;
4. Associated with amorphous iron oxides (CuAM), extracted with an oxalic acid solution (0.2 mol L^{-1}) + ammonium oxalate (0.2 mol L^{-1}) at a pH 3.0;
5. mol L oxides (CuOX), extracted with sodium bicarbonate (0.25 mol L^{-1}) + sodium bicarbonate (0.11 mol L^{-1}) + 3 g sodium dithionite;
6. Associated with sulfides (CuS), extracted with a 4 mol L^{-1} HNO_3 solution;
7. Residual Cu (CuRES) was extracted by triacid digestion (HCl + HNO_3 and HF) during microwave-assisted digestion.

Thus, the sum of the seven fractions was considered to constitute the pseudo-total content.

2.4. Plant Tissues Digestion and Factors Calculation

The Cu content in the leaves and roots was determined after hot digestion (250 °C) using a combination of nitric acid (65%) and perchloric acid (37%) in a 3:1 solutoin ratio [37].

The bioconcentration factor (BCF) and translocation factor (TF) were calculated considering the Cu soil content (pseudo-total Cu), roots, and leaves. The *BCF* was calculated to evaluate plant capacity for Cu phytoextraction from the soil, as the ratio of Cl or Cs (Cu concentration in the plant tissue, i.e., Cl- leaves or Cr- roots), denoted as C_p, and C_{soil} (Cu concentration in soil).

$$BCF = \frac{C_p}{C_{Soil}}$$

When the *BCF* for leaves was < 1, the species were categorized as excluders. The plants were considered accumulators when the coefficient values were between 1 and 10, and hyperaccumulators had a coefficient *BCF* > 10 [12,38].

The translocation factor was calculated to evaluate the hyperaccumulation potential through root-to-shoot Cu transfer, as the ratio between C_l (Cu concentration in the leaves) and C_R (Cu concentration in the roots).

$$TF = \frac{C_l}{C_R}$$

2.5. Determination of Cu Concentrations and Quality Procedures

The Cu concentrations from all studied matrices (Cu fractionation in the solid phase, roots, and leaves) were determined by atomic absorption spectroscopy (AANALYST 400 AA, PerkinElmer, Waltham, MA, USA). The calibration solutions were prepared by diluting certified standard solutions, and certified reference materials from NIST (SRM 1547 and 2709a) were used in triplicate to guarantee quality control procedures. The Cu recovery values were, on average, 97% ± 11%.

2.6. Mineralogical Characterization

The mineralogical characterization used to observe possible shifts in the mineral phases resulting from plant activities was also assessed by X-ray diffraction (XRD, (Rigaku MiniFlex benchtop, Tokyo, Japan)). Thus, XRD analysis was performed using bulk and rhizosphere soil samples. Subsamples were ground in an agate mill for XRD analysis and sieved through a mesh size of 0.5 mm. These samples did not receive chemical treatment to avoid the dissolution of some mineral phases, for example, carbonates. The diffractograms were obtained using a Rigaku Miniflex II device with CuKα radiation from randomly oriented samples, at intervals of 3–50°, a 2θ step size of 0.02°, and counting time of 1 s step^{-1}.

2.7. Statistical Analysis

The differences between rhizosphere and bulk soils, as well as among the plant species, were tested using analysis of variance (ANOVA) with two factors (factor 1: bulk vs. rhizosphere; factor 2: plant species), followed by Tukey's test ($p < 0.05$), to distinguish differences among mean values.

3. Results and Discussions

3.1. Plant Species Effects on Cooper Geochemistry in the Bulk and Rhizospheric Soils

The pH values differed significantly between plant species (Figure 2a). However, except *for Hymenaeae courbaril* L., no significant differences were observed between the rhizosphere and bulk soils (Figure 2a). In the rhizosphere, the greatest pH value was observed for *Combretum aff. Pisoniodes* Taub (5.3 ± 0.3), and the lowest was observed for *Hymenaeae courbaril* L. (4.9 ± 0.1). No significant differences in the pH values of the rhizosphere were observed between *Croton blanchetianus* Baill (5.2 ± 0.1), *Bauhinia ungulate* L. (5.0 ± 0.2), and *Combretum leprosum* Mart (4.9 ± 0.3; Figure 2a).

In contrast to the pH, the OM content differed significantly between the bulk soil and rhizosphere (Figure 2b). The OM content in the rhizosphere was significantly greater in all plant species. Regarding the OM contents in the rhizosphere between plant species, greater abundances were observed in the *Croton blanchetianus* Baill (19.5 ± 2.4%), followed by *Combretum leprosum* Mart (18.3 ± 6.7%), *Bauhinia ungulate* L. (14.5 ± 5.4%), *Hymenaeae courbaril* L. (12.1 ± 0.7%), and the lower contents were observed in the *Combretum aff. pisoniodes* Taub (6.4 ± 1.3%; Figure 2b).

Several studies have reported acidification in the rhizosphere compared to bulk soils due to organic acid exudation from roots [39–41]. In this sense, the organic acid exudation (e.g., acetic, citric, malic, and oxalic) from roots can be corroborated by the significantly greater OM content (Figure 2b) in the rhizosphere for all studied plant species [42,43]. Indeed, previous studies have reported higher OM contents within the rhizosphere than in bulk soils due to root exudates, higher microbial activity, and decaying roots [32,44].
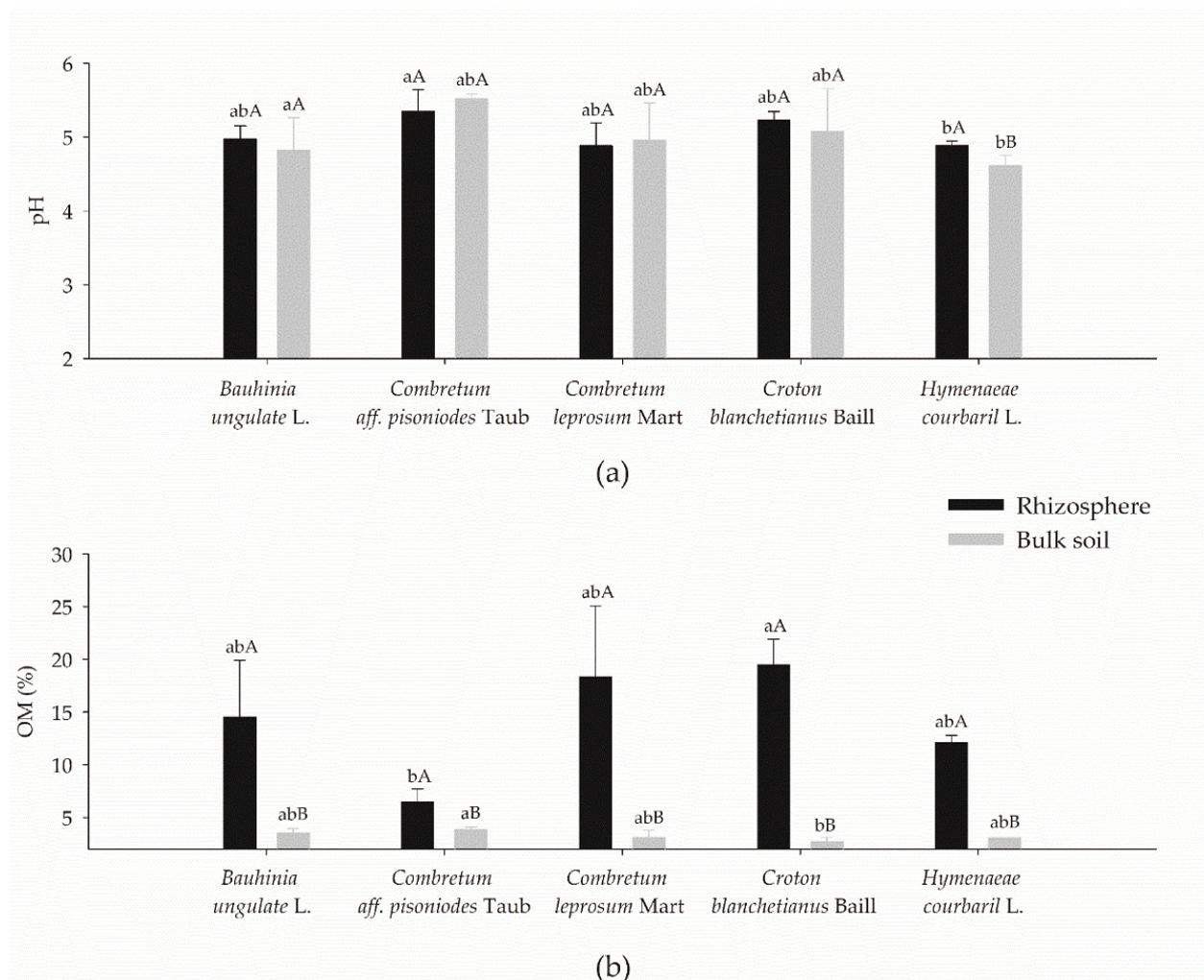
Figure 2. Observed soil pH values (**a**) and content of organic matter (**b**) in the rhizosphere and bulk soil, under the influence of the growth in the five spontaneous plant species in abandoned mine soil. Means followed by the lowercase letters differ between plant species, while means followed by uppercase letters differ between rhizosphere and bulk soils. Test significant at the level of 5% (Tukey).

However, the absence of significant differences between the pH values of the rhizosphere and bulk soils can be explained by the presence of carbonates from the weathering of malachite that is present on mining wastes mixed with the soil [29]. The weathering of carbonates (e.g., malachite) can act as a buffer for acidification promoted by organic acid exudation from roots in the rhizosphere [45]. However, the pH values from the rhizosphere indicate strongly acidic (5.1–5.5) to very strongly acidic (4.5–5.0; Figure 2a) conditions for the studied plant species [46]. In acidified soils, Cu solubility, mobility, and bioavailability are enhanced because they favor desorption [47,48].

In contrast, the higher OM content within the rhizosphere may play a key role in Cu bioavailability. OM compounds increased the cation exchange capacity and Cu adsorption. In addition, organic compounds may establish strong interactions with metals by forming inner-sphere complexes, which decrease their mobility, toxicity, and bioavailability [49,50].

The Cu solid-phase fractionation showed that, on average, Cu in the rhizosphere was principally associated with OM fractions (i.e., CuOM: 39%) for all studied plant species, followed by Cu associated with residual fractions (i.e., CuRES: 29%), carbonates (i.e., CuCAR: 17%), pyrite (CuS: 7%), and Cu associated with amorphous iron oxides (CuAM: 3%; Figure 3). Additionally, on average, the CuCAR, CuAM, and CuEX (i.e., exchangeable

or soluble) contents in the rhizosphere were significantly lower, representing 17%, 3%, and 3% of the pseudo total Cu in comparison with bulk soils, where CuCAR, CuAM, and CuEX represented 25%, 9%, and 11% of the pseudo total Cu, respectively (Figure 3).
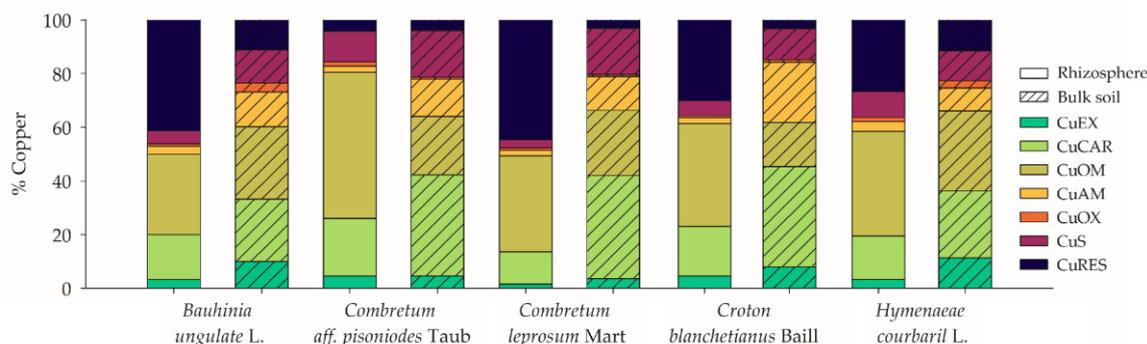
Figure 3. Cu solid-phase fractionation of rhizosphere (**solid bars**) and bulk soils (**dashed bars**) for all studied plant species. CuEX: exchangeable or soluble, CuCAR: Cu associated with carbonates, CuOM: Cu associated with organic matter, CuAM: Cu associated with amorphous iron oxides, CuOX: Cu associated with crystalline iron oxides, CuS: Cu associated with sulfides, and CuRES: residual Cu.

These results indicate the potential for root activity to modify Cu dynamics in abandoned mine soils. For example, the lower CuCAR values in the rhizosphere result from root respiration, which favors an increase in pCO_2 in the rhizosphere soil, and thus promotes the formation of H_2CO_3, which induces the dissolution of carbonates [45]. Similarly, the lower Cu values associated with amorphous oxides (CuAM) in the rhizosphere can be associated with organic–ligand complexes, which favor the dissolution of amorphous iron oxides [51]. Previous studies have reported that organic acids (e.g., acetic, citric, malic, and oxalic) exudates by roots are efficient in forming organic–ligand complexes with Fe^{3+} present in the structure of amorphous iron oxides, favoring its solubilization and, consequently, mineral dissolution [52–54]. Metal–organic ligand complexes may also react with Cu-forming complexes and increase their solubility in the rhizosphere [55,56]. This mechanism is supported by the higher values of Cu associated with organic phases (i.e., CuOM) in the rhizosphere, which represented 39% of the pseudo total Cu, whereas in bulk soil it represented 24% (Figure 3). Indeed, the lower CuEX value in the rhizospheric soil, representing 3% of the pseudo total Cu compared to bulk soil (11%; Figure 3), indicates a potential Cu control throughout plant uptake.

The XRD diffractograms support the effect of root exudates, promoting the dissolution of minerals (Figure 4). The mineralogical assemblage of bulk soils is composed of mica, malachite, pseudo-malachite, quartz, and orthoclase (Figure 4). The mineralogical composition of the rhizosphere of all the studied plant species was similar to that of bulk soils (Figure 4). However, there was a substantial decrease in the intensity of mineral peaks in the rhizosphere soils, which is associated with a loss of crystallinity or mineral dissolution, except quartz, which presents a high resistance to weathering processes [57–59]. Additionally, our results indicate that the intensity of mineral-phase alterations in the rhizosphere varied between species (Figure 4). Indeed, several studies have reported that root-induced mineral dissolution varies between plant species due to the intensity of acidification, exudate release, interaction with microorganisms, cations, and anion uptake [60–63].
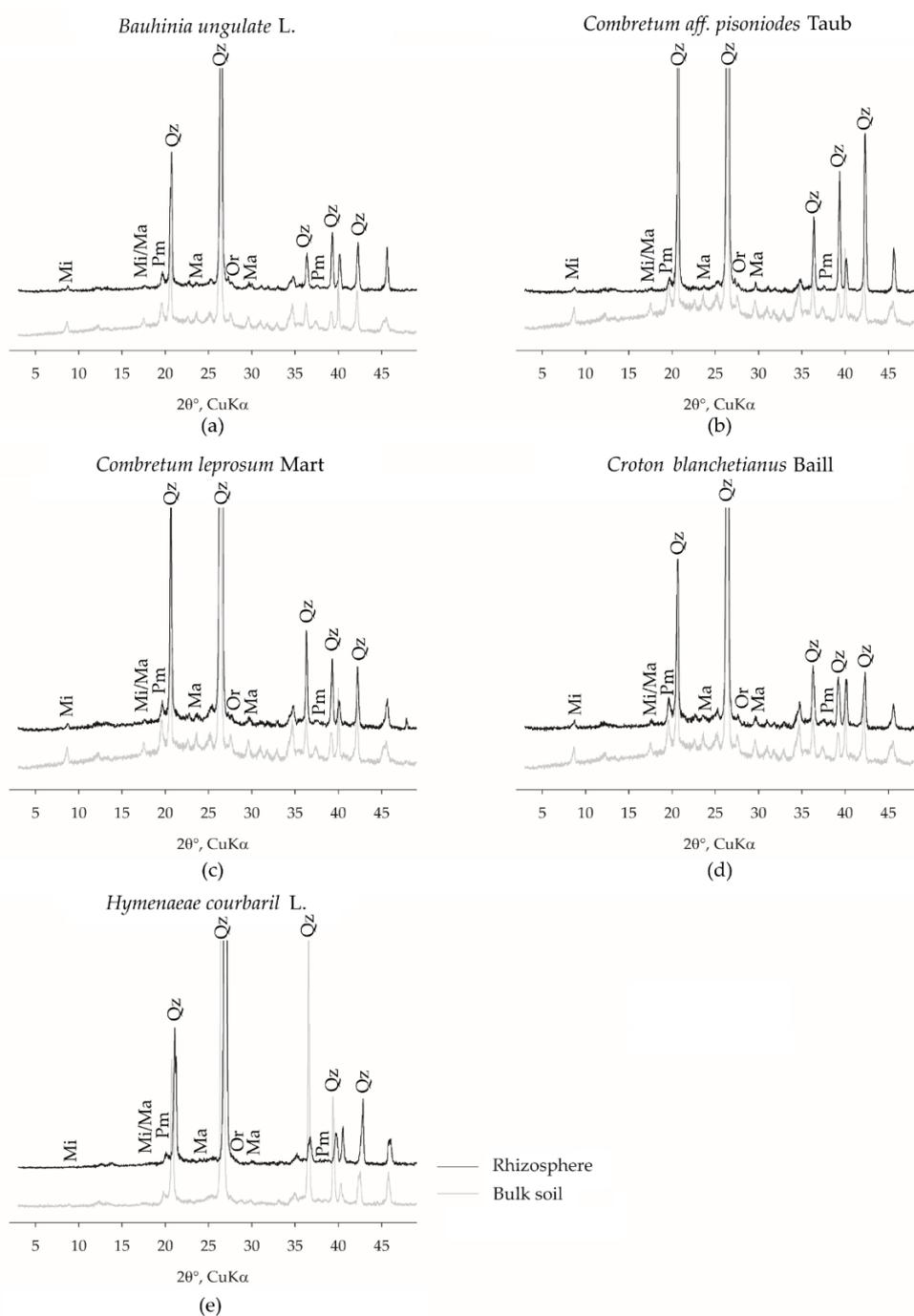
Figure 4. XRD diffractograms of the rhizosphere and bulk soils for *Bauhinia ungulate* L. (**a**), *Combretum aff. pisoniodes* Taub (**b**), *Combretum leprosum* Mart (**c**), *Croton blanchetianus* Baill (**d**), and *Hymenaeae courbaril* L. (**e**). Mi: Mica, Ma: Malachite, Pm: Pseudomalachite, Qz: Quartz, and Or: Orthoclase.

3.2. Cooper Content in Plants Tissues and their Potential to Phytoremediation

This variability between plant species regarding mineral dissolution and Cu dynamics reflects the Cu content in the roots and leaves (Figure 5). *Combretum leprosum* Mart was the species with the highest Cu content in leaves (34 ± 16 mg kg^{-1}), whereas *Combretum aff. pisoniodes* Taub had the lowest content (18 ± 5 mg kg^{-1}), followed by *Croton blanchetianus* Bail (19 ± 5 mg kg^{-1}), and *Bauhinia ungulate* L. (19 ± 6 mg kg^{-1}; Figure 5). The mean Cu content in the *Hymenaeae courbaril* L. leaves was 21 ± 6 mg kg^{-1} (Figure 5). In general, the Cu concentrations in the leaves of the studied species were lower than the concentration

generally observed in Cu hyperaccumulator plants (>300 mg kg^{-1}) [64]. However, on average, the Cu concentrations in the leaves are within the expected range for plants that grow in non-contaminated soils [65] and are directly reflected in the low BCF values (Table 1). Consequently, all studied plants may be classified as excluder species, as phytoextractor species require BCF values greater than 1 [14,66].
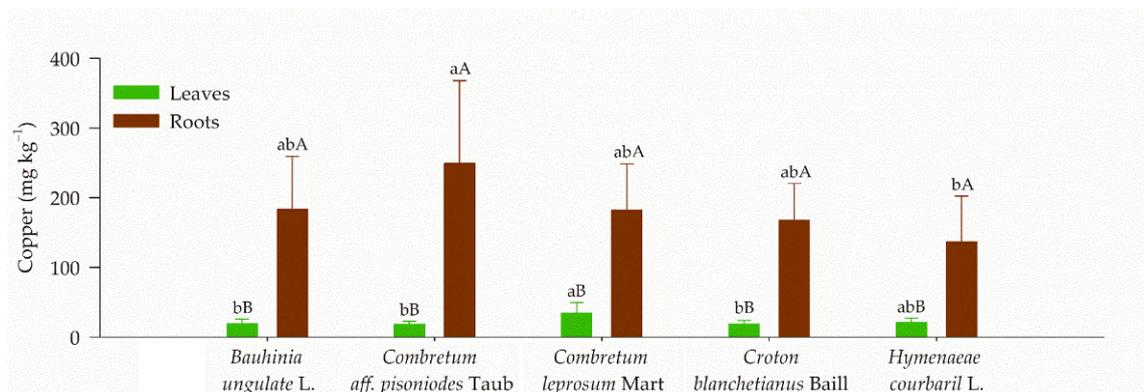
Figure 5. Total Cu contents for the leaves and roots of all studied plant species. Means followed by the same lowercase letters differ between plant species. Means followed by uppercase letters differ between rhizosphere and bulk soils. Test significance was defined as 5% (Tukey).

Table 1. Total mean Cu content, rhizosphere pseudo total Cu content, TF, and BCF values in within leaf and root tissue.

Plant Specie	Total Cu Content (mg kg^{-1})		Pseudo Total Cu * (mg kg^{-1})	BCF Leaves	BCF Roots	TF
	Leaves	Roots				
Bauhinia ungulate L.	19	183	642	0.030	0.285	0.104
Combretum aff. pisoniodes Taub	18	249	401	0.045	0.621	0.072
Combretum leprosum Mart	34	182	877	0.039	0.208	0.187
Croton blanchetianus Bail	19	168	868	0.021	0.193	0.113
Hymenaeae courbaril L.	21	136	355	0.059	0.384	0.154

* Pseudo-total Cu content in the rhizosphere. BFC: bioaccumulation factor; TF: translocation factor.

In contrast, the mean Cu content in the root system was significantly greater than that in the leaves (Figure 5). In the root system, a higher Cu content was observed in the *Combretum aff. pisoniodes* Taub (249 ± 119 mg kg^{-1}) and lower contents in the *Hymenaeae courbaril* L. (36 ± 66 mg kg^{-1}; Figure 5). No significant differences were observed between *Bauhinia ungulate L.* (183 ± 76 mg kg^{-1}), *Combretum leprosum* Mart (182 ± 66 mg kg^{-1}), and *Croton blanchetianus* Bail (168 ± 52 mg kg^{-1}; Figure 5).

The BCF values in the roots of all the studied plants (Table 1) were also lower than the BCF threshold for Cu hyperaccumulators [64]. The TF values were lower than 1, indicating a lower potential for Cu hyperaccumulation in the harvestable tissues. However, the higher Cu concentration in its roots compared to Cu content in the leaves indicates a low root-to-shoot translocation (i.e., low TF) and a potential for controlling Cu bioavailability through Cu immobilization in the roots [67]. In this sense, Cu exclusion in the roots (Table 1) and the lower Cu bioavailability (i.e., CuEX, CuCAR, and CuAM) in the rhizosphere (Figure 3) compared to the bulk soils characterize these plants as possible species for Cu phytostabilization in abandoned mine soils [68]. Previous studies have reported that an ideal plant, capable of controlling metal availability, should present fast root growth with the ability to remove and potentially toxic metals from bulk soils and accumulate high concentrations within its roots [69,70]. Accordingly, *Combretum aff. pisoniodes* Taub is the studied species with a higher potential for controlling Cu bioavailability due to its significantly higher Cu content in the roots (Figure 5).

Finally, the low Cu translocation from root to shoot (TF; Table 1) can be a plant strategy to preserve younger and physiologically more active tissues (i.e., leaves), avoid Cu toxicity [71], and allow random plant growth on Cu-rich soils of an abandoned mine. This exclusion strategy combined with the plant capacity to decrease Cu availability in the rhizosphere compared to bulk soils reveals their potential for reforestation programs.

4. Conclusions

The studied plants from the *Caatinga* biome were able to promote significant mineral phase and Cu dynamic changes in their rhizospheres. Furthermore, the ability to induce mineral dissolution, mainly mediated by root exudates, indicates that the root system presents the potential to accumulate Cu. This accumulation demonstrates potential control in the rhizosphere, decreasing the exchangeable/soluble Cu in the rhizosphere compared to that of bulk soils. Consequently, *Combretum aff. pisoniodes* Taub is a species with great potential for phytoremediation programs to decrease Cu bioavailability (e.g., phytostabilization). In contrast, the low Cu content in the leaves of all studied plants indicates a low potential for phytoextraction. Therefore, our findings indicate potential plants from the *Caatinga* biome that is capable of growth in the Cu-contaminated soil of abandoned mines, which may be used for reforestation programs.

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