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Research article

Phytoremediation potential of *Khaya ivorensis* and *Cedrela fissilis* in copper contaminated soil

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ABSTRACT

Mineral exploration of copper (Cu) in the Amazon has significantly impacted the environment, leading to contamination of large areas that require remediation. Tropical tree species that can immobilize metals and restore plant cover should be selected for phytoremediation programs. The phytoremediation behavior of *Khaya ivorensis* and *Cedrela fissilis* was studied in Cu contaminated soil (60, 200, 400, and 600 mg kg^{-1}). *K. ivorensis* absorbed extremely high amounts of Cu in the roots (329 mg kg^{-1}) and excessive amounts in the shoot (52 mg kg^{-1}), while maintaining similar growth to control plants. *C. fissilis* seedlings presented a higher Dickson quality index. Bioaccumulation (BCF) and translocation (TF) factors were low in both species, indicating that even with the high amounts of copper absorbed, these contents were lower than the soil concentration (BCF < 1) and that most of Cu was compartmentalized in the roots (TF < 1). The tolerance index of *K. ivorensis* (>1) and *C. fissilis* (~ 1) indicate their ability to grow in Cu contaminated soil. These results suggest that these species could potentially be used as phytoremediators.

1. Introduction

Copper (Cu) mining is an important economic activity in the eastern Amazon, where the largest ore deposits in Brazil (Melo et al., 2014) and in the world are located. However, mining is one of the activities with the greatest potential for soil and water pollution by heavy metals in this region (Pereira et al., 2020). Although Cu is essential for plants, in excess this element causes detrimental effects on primary plant production, human health, and plant species growth (Sarwar et al., 2017).

In the state of Pará, the natural soil concentration of Cu is 9.9 mg kg^{-1}, which is based on quality reference values (QRVs) of metals (Fernandes et al., 2018). Worldwide, soil Cu values range from 2 to 250 mg kg^{-1}, with plant toxicity observed at concentration ranges of 60–125 mg kg^{-1} (Kabata-Pendias, 2010). The Brazilian National Environmental Council (CONAMA, 2009) has defined 60 mg kg^{-1} as a prevention value (PV) for Cu (suggesting anthropogenic contamination) and 200 mg kg^{-1} as an investigation value (IV; potential risk to human health and ecosystems). Thus, recovery strategies must be developed to control soil Cu concentrations to levels permitted by Brazilian law.

Phytoremediation is a technique involving vegetation cover restoration in mining areas, which allows the retention or removal of metals and soil erosion reduction. Owing to their high biomass accumulation and very dense root system, arboreal species promote metal immobilization in plant tissues, delaying their return to the soil (Kang et al., 2018), or greater removal due to high biomass production. Moreover, they afford restoration of degraded areas with lower management costs (Shukla et al., 2011), maintaining environmental protection and generating economic return with forest products. However, the excessive content of metals may cause negative effects on the development of tree species, especially in the initial growth phase (Souza et al., 2012).

Some effects of Cu toxicity on plants include restriction of root development, reduced shoot growth, and chlorosis in young leaves, as result of physiological, nutritional and hormonal imbalances (Emamverdian et al., 2015). In order to avoid or detoxify excessive metal

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concentrations, plants present different tolerance mechanisms (Bhargava et al., 2012). Plant stress can be prevented by restricting metal uptake from the soil by immobilization through mycorrhizal association, sequestration or complexation by root exudates (Cambrollé et al., 2015; De Conti et al., 2019). In plant tissues, metals are retained in the roots and immobilized in cell walls, preventing translocation to the aerial part (Emamverdian et al., 2015; Silva et al., 2018).

The tolerance, accumulation and translocation of heavy metals to the aerial part of plants are fundamental aspects to be followed in the selection of species in phytoremediation programs (Fan et al., 2011). Metal tolerance is an essential requirement for metal accumulation and, therefore, successful implementation of phytoremediation (Hussain et al., 2017). Some tree species have been identified as Cu-tolerant (Nirola et al., 2015; Silva et al., 2015; Meyer et al., 2016; Kang et al., 2018). However, studies on the tolerance of tropical tree species in metal-contaminated soils in the Amazon remain limited, particularly considering Cu.

Khaya ivorensis A. Chev. is an exotic tree species in the Amazon which exhibits good adaptation in the edaphoclimatic conditions of the region (Ros et al., 2019), and *Cedrela fissilis* Vell. is native to tropical America (Meyer et al., 2016). Both Meliaceae species demonstrate fast growth, produce high quality wood, and have high biomass and filtering capacity due to their extensive root system (Yavari et al., 2017), important characteristics for phytoremediation programs (Shukla et al., 2011). Exotic tree species can be used for recovery of areas degraded by mining, provided they meet the Brazilian legal criteria, favoring regeneration of native species (Jesus et al., 2016; Reis et al., 2019).

Forest restoration of areas degraded by mining is fundamental for reducing metal dispersal and consequent contamination of soils and water resources. Identifying plant species that can mitigate mining impacts on biodiversity and restore ecosystem functions by means of rapid growth, high biomass accumulation, and soil protection is a major research challenge. Thus, our objective was to evaluate the behavior of *K. ivorensis* and *C. fissilis* in Cu contaminated soil in order to define their potential for phytoremediation purposes.

2. Material and methods

2.1. Experimental design and growth conditions

K. ivorensis and *C. fissilis* were cultivated in a greenhouse. The soil used in the experiment was collected from the superficial layer (0–0.2 m) of a secondary forest area, classified as Yellow Oxisol, the predominant soil class in the Amazon (Santos et al., 2018). The soil was air dried, processed through a 4 mm diameter sieve, and homogenized. Chemical and particle size characteristics were determined according to Teixeira et al., 2017: pH in water: 4.1; Ca: 0.1 $cmol_c\ dm^{-3}$; Mg: 0.2 $cmol_c\ dm^{-3}$; Al: 2.1 $cmol_c\ dm^{-3}$; H + Al: 9.2 $cmol_c\ dm^{-3}$; K: 0.05 $mg\ dm^{-3}$; P: 0.01 $mg\ dm^{-3}$; cation exchange capacity (CEC): 10.2 $cmol_c\ dm^{-3}$; organic matter (OM): 12.5 $g\ kg^{-1}$; clay content: 120 $g\ kg^{-1}$; silt content: 130 $g\ kg^{-1}$; and sand content: 750 $g\ kg^{-1}$. The available concentration of Cu in the soil was 1.9 $mg\ kg^{-1}$, extracted using Mehlich 1 extraction solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4). The pseudototal concentration of Cu in the soil corresponded to 5.6 $mg\ kg^{-1}$, extracted using the method EPA 3051, as described in topic 2.3.

The experiment consisted of five treatments: control (without Cu addition) and 60, 200, 400, and 600 $mg\ kg^{-1}$ Cu, with two plant species and five replicates (one plant per pot, which was considered one replicate), totaling 50 experimental units in plastic pots containing 5 dm^3 of soil. Doses were established according to the guiding quality values for agricultural soils (CONAMA, 2009): 60 $mg\ kg^{-1}$ (PV), 200 $mg\ kg^{-1}$ (IV), 400 $mg\ kg^{-1}$ (2 × IV), and 600 $mg\ kg^{-1}$ (3 × IV).

Soil acidity correction was performed with dolomitic limestone (30% CaO, 21% MgO, and relative power of total neutralization equal to 72%) to increase base saturation to approximately 55% within a 30-day incubation period (van Raij et al., 1997) and increase the pH above 5.0. Seedlings of forest species, including *K. ivorensis* and *C. fissilis*, have greater development at pH and base saturation above 5.0 and 50% (Silva et al., 2011, 2014; Muniz et al., 2018). The doses of Cu were applied 30 days after acidity correction, using a copper sulfate solution ($CuSO_4.5H_2O$), and the soil was allowed to stabilize for more 20 days as done by Souza et al. (2012). Basic fertilization with macro and micronutrients (150 N, 450 P, 150 K, 2.25 Zn, 0.75 Fe, 0.75 Mn, and 0.45 mg dm^{-3} B) was conducted after the contamination period. Urea, triple superphosphate, KCl, $ZnCl_2$, $FeCl_3.6H_2O$, $MnCl_2.4H_2O$, and $Na_2B_4O_7.10H_2O$ were used according to Souza et al. (2010) and Caires et al. (2011).

Seedlings were acquired from a specialized locale at five months of age. Plants of similar sizes were transferred to the pots with the treatments after 10 days of acclimatization in the greenhouse. During the experiment, the humidity was kept approximately constant at 60% of the total pore volume using deionized water.

2.2. Biometric data

The experiment was conducted during 90 days. This period is longer than the reported by Marques et al. (2000) as enough to plants show reduced growth in function of soil contamination. The stem diameter was measured using a digital pachymeter with 0.01 mm precision and the stem height by a graduated measuring tape from the base to the apical meristem. Leaves, stems, and roots were collected separately. Roots were separated from the soil using a 2 mm mesh to avoid losses. The plant parts were washed in a detergent solution (0.1% v/v), rinsed in deionized water, placed in paper bags, dried in an oven with forced air circulation at 60 °C for 72 h and then weighed to determine dry matter.

2.3. Analytical procedures

After the experiment, soil samples from each treatment were air dried, powdered, and sieved using a 100 mesh and 2 mm mesh to determine pseudototal and available Cu concentrations, respectively. Pseudototal Cu concentrations were obtained by microwave acid digestion (Mars Xpress 6, CEM Corporation) with concentrated HNO_3 (65%) using the EPA 3051 method (USEPA, 1996). Available Cu concentrations were extracted with Mehlich 1 (Teixeira et al., 2017).

Dry matter was powdered using a Willey mill and processed through a 20 mesh sieve. Plant tissue Cu contents were extracted by microwave acid digestion, involving the addition of 2 mL of HNO_3 + 2 mL of H_2O_2 and 5 mL of ultrapure water to 250 mg of sample (MS, 2000; Gehaka) (Araújo et al., 2002).

To ensure analytical accuracy, blank samples and certified reference materials (ERM-CD281 and ERM-CC141) were included. Recovery rates were 97% from soil and 95% from plants. Concentrations of Cu in the extracts were determined by flame atomic absorption spectrometry (FAAS; iCE 3000 Series; Thermo Scientific).

2.4. Phytoremediation efficiency parameters

The plant tolerance index (TI) to Cu was calculated based on the total dry matter (TDM) ratio of plants exposed to Cu doses and the total dry matter of control treatment plants (TDMc), according to equation (1) (Souza et al., 2012):

$$TI = TDM\ (g)/TDMc\ (g) \tag{1}$$

The Dickson quality index (DQI) was used to evaluate the quality of seedlings. It is known as an integrated morphological index which considers the robustness and balance of plant biomass (Eloy et al., 2013). DQI was determined as a function of height (H), stem diameter (SD), shoot dry matter (SDM), root dry matter (RDM), and TDM, using the formula described in equation (2) (Dickson et al., 1960).

$$DQI = \{TDM\ (g)\} / \{[(H\ (cm) / SD\ (mm)] + [SDM\ (g) / RDM\ (g)]\} \tag{2}$$

Accumulation of Cu (μg plant^{-1}) in the roots and shoots was calculated based on the element content and the RDM and SDM, following equation (3) (Nardis et al., 2018).

$$\text{Accumulation} = \text{RDM or SDM (mg)} \times [\text{Cu}]\ \text{RDM or SDM}\ \left(\text{mg kg}^{-1}\right)/1000 \quad (3)$$

Root-to-shoot translocation (TF) and bioconcentration factor (BCF) were calculated according to equations (4) and (5), as described by (Hussain et al., 2017), and the biotransfer factor (BTF) was calculated using equation (6), adapted from (Rodríguez-Vila et al., 2014):

$$\text{TF} = [\text{Cu}]\ \text{shoot}/[\text{Cu}]\ \text{root} \quad (4)$$

$$\text{BCF} = [\text{Cu}]\ \text{root}/[\text{Cu}]\ \text{total soil} \quad (5)$$

$$\text{BTF} = [\text{Cu}]\ \text{root}/[\text{Cu}]\ \text{available soil} \quad (6)$$

2.5. Statistical analysis

Results were subjected to analysis of variance using the Sisvar 5.3 program; when significant according to the F test, the means were compared by Tukey's test ($p < 0.05$). The effect of Cu doses was evaluated by regression study. In addition, a correlation was calculated between pseudototal and available concentrations in soil and plants (Ferreira, 2011).

3. Results

3.1. Concentrations of Cu in soil and plant

Pseudototal and available concentrations of Cu in the soil increased with the applied doses, with no differences between the cultivated species (Fig. 1A and B). The pseudototal concentrations of Cu in the soils of control treatments cultivated with *C. fissilis* (4.5 mg kg^{-1}) and *K. ivorensis* (3.9 mg kg^{-1}) were lower than the content observed before cultivation (5.6 mg kg^{-1}). The available concentrations increased for *C. fissilis* (2.02. mg kg^{-1}) and decreased for *K. ivorensis* (1.82 mg kg^{-1}) when compared to the concentration before planting (1.9 mg kg^{-1}). A linear increase in the pseudototal concentration of Cu in the soil was observed from the dose 60 mg kg^{-1}, with 92–100% of variation of the applied doses, indicating the effectiveness of the contamination. The available concentration of Cu corresponded to approximately 50% of the pseudototal concentration. At the dose equivalent to 200 mg kg^{-1} (IV), 83 and 110 mg kg^{-1} of Cu were available in soils with *C. fissilis* and *K. ivorensis*, respectively, reaching 278 mg kg^{-1} of available Cu in the highest dose in both crops. The soil pH after the experimental period decreased from 5.9 to 5.5 according the increase of the Cu levels applied to the soil (data not shown).

Distribution of Cu in plants followed the pattern root > leaf > stem, with increasing root distribution with Cu dose (Fig. 2). At normal soil Cu concentrations (control) and those suggesting anthropogenic contamination (60 mg kg^{-1}), Cu was evenly distributed in *K. ivorensis* plant parts, whereas in *C. fissilis* it was preferentially distributed in the roots, regardless of Cu level.

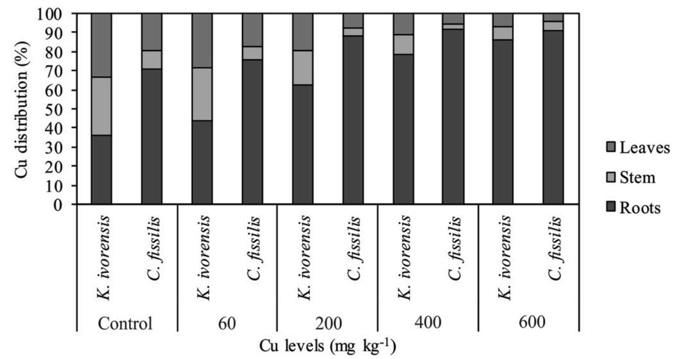

Fig. 2. Percentage distribution of Cu in *K. ivorensis* and *C. fissilis* tissue exposed to various Cu levels.

The RDM and SDM Cu contents of the species increased as a function of the applied doses and were higher ($p < 0.01$) in *K. ivorensis* (Fig. 3A and B). The RDM Cu content in *K. ivorensis* reached 329 mg kg^{-1} at the highest dose, equivalent to 86% of the total Cu in the plant. For *C. fissilis*, the highest RDM levels were 216 and 207 mg kg^{-1}, corresponding to 92 and 91% of the total Cu in the plant, at doses of 400 and 600 mg kg^{-1} Cu, respectively. The *K. ivorensis* SDM Cu content was approximately 2-fold higher than in *C. fissilis*, reaching 52 mg kg^{-1} at the highest contamination level.

The species presented different behavior in relation to root and shoot accumulation as a function of Cu dose (Fig. 3C and D). *K. ivorensis* accumulated more Cu in the shoot than in the roots under levels up to 400 mg kg^{-1}. In contrast, at a dose of 600 mg kg^{-1}, the species began to accumulate 50% more in the roots (1924 μg plant^{-1}) than in the shoot (1310 μg plant^{-1}) and 92% of Cu accumulated in the roots of control plants. *C. fissilis* demonstrated greater root accumulation relative to the shoot at all doses above 200 mg kg^{-1}. Maximum Cu accumulation in both the roots and shoot occurred at a dose of 400 mg kg^{-1} (2411 and 773 μg plant^{-1}, respectively).

3.2. Plant growth

C. fissilis presented higher height, RDM, and SDM than *K. ivorensis*, with no difference ($p < 0.01$) for RDM at a dose of 600 mg kg^{-1} (Table 1). Both species increased in height up to 200 mg kg^{-1} Cu; at the highest levels of contamination there was no reduction ($p < 0.05$) compared with the control plants. The RDM and SDM of the species were not influenced by Cu levels, except *C. fissilis*, in which the RDM and SDM were reduced 40 and 60%, respectively, at the highest dose (600 mg kg^{-1}) compared with the control. *K. ivorensis* SDM increased up to 200 mg kg^{-1}, while *C. fissilis* showed no variation up to 400 mg kg^{-1} Cu.

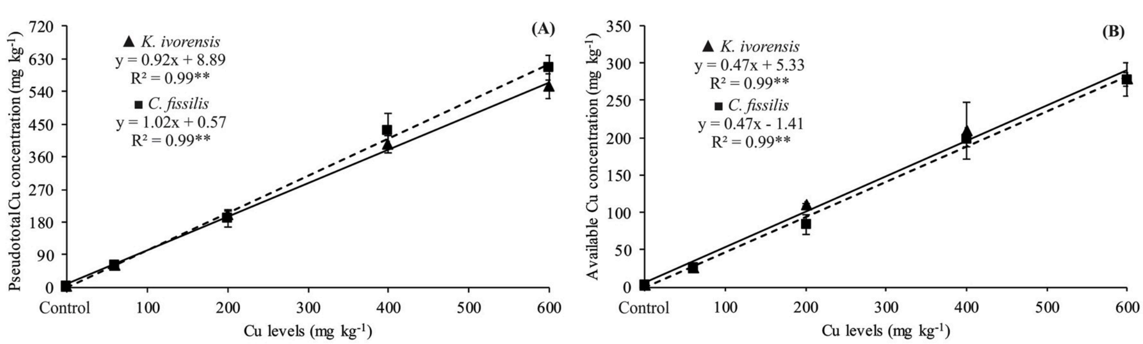

Fig. 1. Regression analysis between doses of Cu and pseudototal (A) and available (B) Cu concentrations in soil following the experiment (**$p < 0.01$).

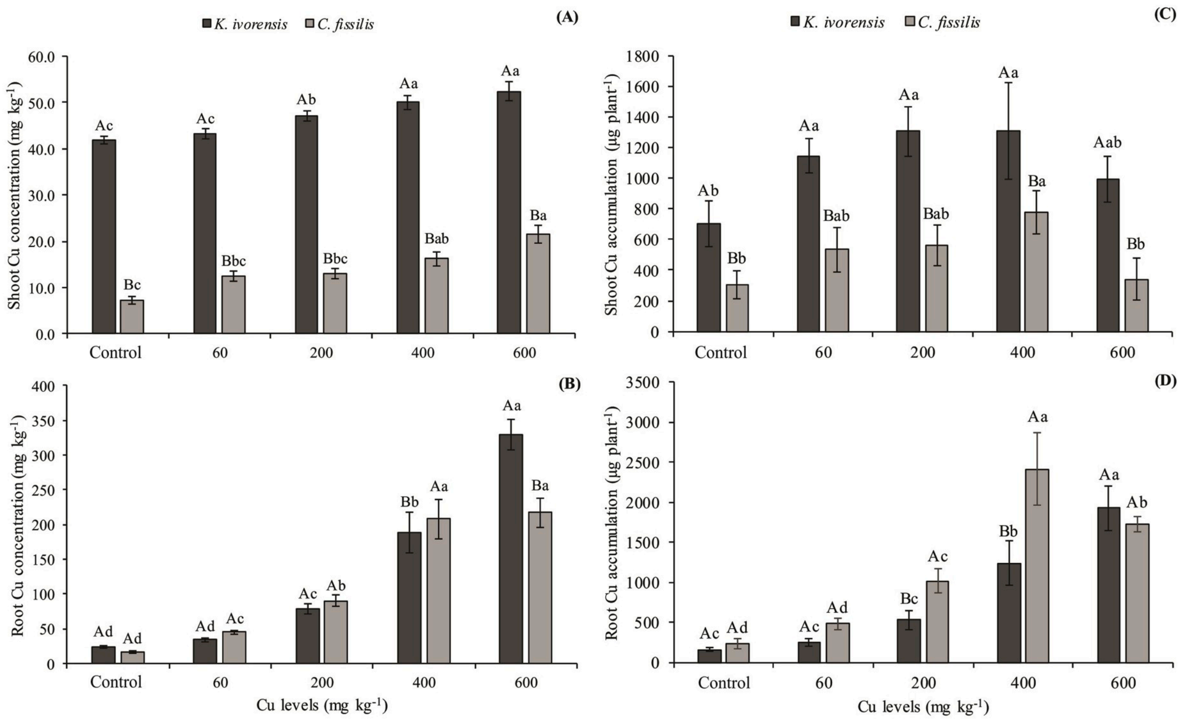

Fig. 3. Concentration of Cu in the shoots (A) and roots (B) and accumulation of Cu in the roots (C) and shoots (D) of *K. ivorensis* and *C. fissilis* exposed to different Cu levels. Uppercase letters designate a comparison between species and lowercase letters designate a comparison between Cu levels. Common letters indicate a statistically non-significant difference based on Tukey's test ($p < 0.05$).

Table 1
Plant height (H), roots dry matter (RDM), shoot dry matter (SDM), and Dickson quality index (DQI) of *K. ivorensis* and *C. fissilis* exposed to different Cu levels.

Cu levels	H	SDM	RDM	DQI
(mg kg^{-1})	(cm)	(g plant^{-1})		–
K. ivorensis				
Control	36.44 ± 5.4 Bb	16.80 ± 3.5 Bc	6.62 ± 1.0 Ba	3.82 ± 2.0 Ba
60	42.78 ± 5.0 Bab	26.45 ± 2.2 Bab	7.42 ± 1.7 Ba	4.46 ± 0.3 Ba
200	51.04 ± 4.1 Ba	27.67 ± 2.9 Ba	6.66 ± 1.0 Ba	3.82 ± 1.1 Ba
400	39.64 ± 5.5 Bb	26.02 ± 5.8 Bab	6.49 ± 0.9 Ba	3.92 ± 0.7 Ba
600	34.34 ± 6.0 Bb	18.96 ± 3.1 Aab	5.82 ± 0.6 Ba	3.69 ± 0.7 Aa
C. fissilis				
Control	66.24 ± 20.5 Aab	43.19 ± 8.4 Aa	13.91 ± 2.8 Aa	7.50 ± 0.5 Aa
60	72.28 ± 10.6 Aab	42.32 ± 4.6 Aa	10.50 ± 0.5 Abc	5.78 ± 1.2 Aab
200	82.22 ± 6.5 Aa	45.21 ± 6.4 Aa	11.18 ± 1.3 Ab	5.47 ± 0.3 Ab
400	73.86 ± 14.1 Aab	42.26 ± 6.6 Aa	11.03 ± 1.2 Ab	5.61 ± 0.3 Ab
600	44.56 ± 16.0 Ab	17.21 ± 5.9 Ab	8.30 ± 0.5 Ac	3.99 ± 0.3 Ab

Uppercase letters designate a comparison between species and lowercase letters designate a comparison between Cu levels. Common letters indicate a statistically non-significant difference based on Tukey's test ($p < 0.05$).

The DQI of *C. fissilis* was higher than *K. ivorensis* up to 400 mg kg^{-1} Cu (Table 1). The DQI of the species was not affected ($p < 0.05$) by Cu dose, except in *C. fissilis* at the highest contamination level, with an index 53% lower than the control.

3.3. Phytoremediation potential

K. ivorensis plants presented TI > 1, significantly higher than *C. fissilis* (TI < 1) (Table 2). There were no significant differences between the species at different Cu doses, except at the highest contamination level in *C. fissilis*. The Cu TF was higher in *K. ivorensis* and decreased with increasing soil metal concentration in both species, < 1 in all treatments except for *K. ivorensis* at 60 mg kg^{-1}. At this concentration, *C. fissilis* also

Table 2
Tolerance index (TI), translocation factor (TF), bioconcentration factor (BCF), and biotransfer factor (BTF) of *K. ivorensis* and *C. fissilis* exposed to various Cu levels.

Cu levels (mg kg^{-1})	TI	TF	BCF	BTF
K. ivorensis				
60	1.48 ± 0.25 Aa	1.29 ± 0.11 Aa	0.56 ± 0.09 Bab	1.35 ± 0.21 Ba
200	1.48 ± 0.11 Aa	0.61 ± 0.05 Ab	0.39 ± 0.03 Bc	0.71 ± 0.06 Bc
400	1.40 ± 0.28 Aa	0.27 ± 0.04 Ac	0.48 ± 0.07 Abc	1.05 ± 0.17 Ab
600	1.08 ± 0.21 Aa	0.16 ± 0.01 Ad	0.60 ± 0.34 Aa	1.18 ± 0.06 Aab
C. fissilis				
60	0.93 ± 0.09 Ba	0.28 ± 0.08 Ba	0.74 ± 0.08 Aa	1.74 ± 0.13 Aa
200	0.99 ± 0.07 Ba	0.14 ± 0.32 Bb	0.49 ± 0.07 Ab	1.10 ± 0.16 Ab
400	0.94 ± 0.06 Ba	0.08 ± 0.02 Bb	0.51 ± 0.06 Ab	1.10 ± 0.14 Ab
600	0.45 ± 0.11 Bb	0.10 ± 0.01 Ab	0.35 ± 0.02 Bc	0.75 ± 0.05 Bc

Uppercase letters designate a comparison between species and lowercase letters designate a comparison between Cu levels. Common letters indicate a statistically non-significant difference based on Tukey's test ($p < 0.05$).

presented the highest TF (0.28). Cu BCF was low in both species (<1). *K. ivorensis* showed higher BCF ($p < 0.05$) at 600 mg kg^{-1}, while at 60 and 200 mg kg^{-1} it was higher in *C. fissilis*. The species presented BTF >1 in most treatments. The highest Cu BTF for the species was observed at 60 mg kg^{-1} of Cu in the soil, with significant reduction with increasing doses, except for *K. ivorensis* at 600 mg kg^{-1}.

4. Discussion

4.1. Concentrations of Cu in soil

The concentration of Cu in the soil is affected by pH, OM content, Fe and Al oxides, and CEC (Alleoni et al., 2005; McGrath et al., 2014; Gonçalves et al., 2016; Chileshe et al., 2019). The lower pseudototal concentration after cultivation may be related to plant exudation of organic acids and flavonoids, which solubilize Fe oxides (Colombo et al., 2014) and, therefore, associated metals (Asensio et al., 2018) in order to improve nutrient absorption. Plants also mobilize Cu linked to OM and carbonates, resulting in lower available concentration after planting, as a consequence of plant absorption (Asensio et al., 2018). In this study, the reduction in soil pH after cultivation, which increases protonation, may also have contributed to increase the mobility of Cu (Silva et al., 2018), leading to absorption of considerable amounts of this metal, even in low concentration in the soil before cultivation (1.9 mg kg^{-1}).

The pseudototal and available concentrations of Cu in the control soils before and after the cultivation of the species were low, characteristic of the soils of the Amazon region, except in those areas with mineral deposits, which have a background of 9.9 mg kg-1, according to Fernandes et al. (2018). Likewise, the low available Cu concentration (0.67 mg kg^{-1}) in Oxisols in this region was reported by Birani et al. (2015). However, the concentrations of Cu in the soil in response to a dose of 60 mg kg^{-1} after the experiment warns of its vulnerability to anthropic contamination, as found from 200 mg kg^{-1}. Therefore, pseudototal concentrations of Cu in the soil indicated that it was highly polluted, with values similar to the amounts applied to the soil. This demonstrates that the doses applied in this work were efficient for contamination and that as suggested by Brazilian law, at concentrations >200 mg kg^{-1}, there is need of intervention and remediation to avoid risks to human health and the ecosystem (CONAMA, 2009). The level of increase in the total and available concentrations of Cu, depending on the doses, is related to the content of clay, OM and oxides, CEC and pH, that is, it may vary with the type of soil. In study performed by Asensio et al. (2018), the pseudototal and bioavailable concentrations of Cu in the soil increased with the added concentrations, which was attributed to the low content of OM, clay and CEC.

Under contamination levels above IV, the available concentrations of Cu exceeded the phytotoxic limit (between 40 and 60 mg kg-1) reported by Monterroso et al. (1999) and were higher than those found in soils from area of Cu mining in northeastern Brazil (Perlatti et al., 2015), which were considered contaminated. The decrease in pH after cultivation with *K. ivorensis* and *C. fissilis*, associated with the increased availability of Cu and sulfate concentration in the substrate according the applied doses, may have contributed to the release of Cu in the system (Chileshe et al., 2019).

Although the available Cu concentration is high, there was high soil adsorption, even with low clay content, as well as the high plant absorption. High Cu adsorption may be related to the OM content, mainly carboxylic and phenolic groups (McGrath et al., 2014), with which the metal forms complexes and chelates, reducing the availability. In addition, high levels of Fe and Al oxides, common in very weathered tropical soils, may have contributed to Cu adsorption owing to the strong affinity for reactive oxide surfaces (Alleoni et al., 2005). In oxisols from the Amazon region, Cu adsorption increased as a function of metal addition rates and OM was one of the most important contributing factors (Gonçalves et al., 2016).

4.2. Content of Cu and plant growth

K. ivorensis and *C. fissilis* absorb and accumulate Cu by different forms (Figs. 2 and 3). Interspecific contrast was also observed in *Bauhinia forficata*, *Pterogyne nitens*, and *Enterolobium contortisiliquum*, belonging to Fabaceae family, which presented Cu levels of 316, 274, and 91 mg kg^{-1}, respectively, when exposed to a dose of 300 mg kg^{-1} (Silva et al., 2015). Other Fabaceae species behaved differently regarding the distribution of Pb in the tissues (Souza et al., 2012). Even so, Cu was concentrated in greater content in the roots, which is common among tree species for most heavy metals (Pulford and Watson, 2003).

Correlations were higher between Cu concentrations in soil and roots than in shoots (Table 3), indicating that the higher accumulation in the roots at higher doses represents a growth protection mechanism in contaminated environments. The higher metal concentration in the roots is a desirable characteristic for plants used as phytoremediators, as it suggests tolerance (Kabata-Pendias, 2010). Plants activate different shoot metal translocation regulatory mechanisms in order to avoid damage to metabolic systems and maintaining survival and development (Bhargava et al., 2012). Species that accumulate high amounts of metals with minimal effects on metabolism and growth have potential for use in phytoremediation processes (Sarwar et al., 2017).

The concentration considered toxic to many plants and the highest Cu accumulation in *K. ivorensis* SDM characterize its greater potential as a phytoextractor (Yu et al., 2019). On the other hand, *C. fissilis* presented high concentrations and accumulation in the roots, demonstrating potential as a phytostabilizing species. Levels of Cu < 20 mg kg^{-1} in the shoot of *Peltophorum dubium* and *Enterolobium contortisiliquum*, even at high Cu doses (256 mg kg^{-1}), have been attributed to a plant defense mechanism, regulating translocation (Silva et al., 2011). Similar to our results, De Conti et al. (2019) observed lower content in the shoots of vines (*Vitis vinifera* cv.) cultivated in Cu-contaminated soil. Other authors have also suggested this is a plant defense mechanism, reducing the translocation of excess Cu from the roots to the shoots (Cambrollé et al., 2015; Oustriere et al., 2016).

Dry matter concentrations of Cu between 20 and 100 mg kg^{-1} are considered excessive for plants (Kabata-Pendias, 2010). The high Cu content in both species (>100 mg kg^{-1}) indicates that they are Cu accumulators (Boyd, 2007). *Swietenia macrophylla* (Brazilian mahogany) seedlings were grown in hydroponic solutions with Cd tolerated and accumulated high levels (Fan et al., 2011), which may be a characteristic of the woody species of Meliaceae. Given the difficulties in cultivating Brazilian mahogany (Corcioli et al., 2016), *K. ivorensis* and *C. fissilis* could provide alternatives for the restoration of areas degraded by Cu mining, producing wood of excellent quality.

The high levels of Cu in the plant parts did not cause visible toxicity symptoms in either species or reduced height and dry matter (Table 1), suggesting that biochemical and physiological processes, such as

Table 3
Pearson correlation between soil pseudototal concentrations, soil available concentrations, roots concentrations, and shoot concentrations of Cu in *K. ivorensis* and *C. fissilis* plants exposed to Cu levels.

	Cu roots	Cu shoot	Available Cu
K. ivorensis			
Pseudototal Cu	0.91**	0.66**	0.98**
Available Cu	0.90**	0.65**	
Cu shoot	0.54**		
C. fissilis			
Pseudototal Cu	0.94**	0.88**	0.96**
Available Cu	0.87**	0.81**	
Cu shoot	0.79**		

**Highly significant correlation ($p < 0.01$).

oxidative stress, decreased photosynthetic rate and chlorophyll concentration, have not been compromised (Sarwar et al., 2017; Silva et al., 2018). The reduction of *C. fissilis* SDM at the highest Cu dose applied to the soil may be due to the higher sensitivity of tree seedlings to metal stress than adult plants (Souza et al., 2012). Asensio et al. (2018) reported the death of seedlings of tropical tree species and severe visual symptoms in *C. fissilis* exposed to 500 mg kg^{-1} of Cu in the soil for 60 days. A study by Pulford and Watson (2003) showed that the heavy metal tolerance of *Salix* sp. clones in contaminated soils increased owing to the gradual exposure and acclimation of the plants. The plants under high Cu levels showed a growth pattern similar to that of control plants, indicating that the species may have good adaptability in contaminated sites in the first months of exposure. Sensitive plants manifest the toxic effect in a short period when cultivated in contaminated substrate (Baker and Walker, 1989), as observed by Asensio et al. (2018) and Marques et al. (2000), which highlights the potential of the tested species.

C. fissilis and *K. ivorensis*, which presented high DQI in highly contaminated soils, could potentially be used to revegetate areas degraded by high Cu levels, considering that the DQI is a good quality indicator for forest species seedlings (Eloy et al., 2013). When seedlings of forest species were subjected to different Cu levels (0–400 mg kg^{-1}), the DQI of *Stryphnodendron polyphyllum* decreased with increasing metal levels, while there was no influence on *Cassia multijuga* seedling quality (Silva et al., 2014); this was attributed to the greater potential of *C. multijuga* for revegetation of Cu contaminated areas.

4.3. Phytoremediation potential

The tolerance of species to Cu has been well characterized as the absence of any effect on growth variables, even with high accumulation. The TI presented by both species corroborates this statement. The higher *K. ivorensis* TI at all contamination levels suggests greater tolerance than *C. fissilis* and that it would be more useful for phytostabilization (Souza et al., 2012). On the other hand, a TI close to 1 shows low loss of dry matter by *C. fissilis*, indicating tolerance to Cu.

Low heavy metal translocation is characteristic of several tropical tree species (Souza et al., 2012; Meyer et al., 2016; Asensio et al., 2018), which have potential for phytoremediation of metal contaminated areas. The low TF and BCF (<1) values of the species at all contamination levels, except the *K. ivorensis* TF at 60 mg kg^{-1}, demonstrate that they are suitable for soil Cu phytostabilization (Ali et al., 2013).

BCF and BTF can be calculated for different plant parts (Rodríguez-Vila et al., 2014; Nirola et al., 2015; Yousaf et al., 2016; Hussain et al., 2017); in this study we used root concentration. The adoption of BCF to analyze soil concentrations enables better interpretation of plant potential (Asensio et al., 2018), as high concentrations of soil metals can result in BCF < 1, even if the plant absorbs large amounts (Ali et al., 2013). For example, the Cu content in *K. ivorensis* roots (Fig. 2B) at the highest dose corresponded to an accumulator species (Boyd, 2007), whereas pseudototal Cu concentration in the soil (Fig. 1A) at this same dose represents at least four-fold the concentration that causes toxicity to plants (125 mg kg^{-1}) (Kabata-Pendias, 2010). In contrast, the BCF obtained by the species, regardless of the metal concentration in the soil, fits the bioaccumulation scale described by Hussain et al. (2017), considering an average bioaccumulation, with values varying from 0.1 to 1.0.

BTF allows an evaluation of the efficiency of plant metal absorption from the soil, with reference to the phytoavailable forms (Rodríguez-Vila et al., 2014). Under soil conditions that restrict the availability of metals, their transfer to plant roots is reduced (Yousaf et al., 2016). In this study, Cu addition to the soil promoted metal availability independent of the cultivated species; the absorption capacity by *K. ivorensis* and *C. fissilis* determined the distinct responses to BTF at the highest stress level. Despite tolerance at high concentrations, some plants reduce Cu absorption as exposure time increases. This process is governed by the reduction of sweating by structural changes in the leaves (Fu et al., 2015), behavior that may have been adopted by *C. fissilis* throughout the experiment when exposed to excess Cu, reducing biotransference. However, the Cu BTF of *K. ivorensis* and *C. fissilis* demonstrates their ability to absorb and compartmentalize the metal, primarily in the root, which reinforces the potential of these Meliaceae species for Cu phytostabilization in contaminated soils.

5. Conclusions

The results indicated that the tested tropical species have potential to phytoremediate Cu-contaminated soils. *K. ivorensis* and *C. fissilis* showed Cu tolerance due to good dry matter production and high seedling quality standard. The species present low translocation (TF < 1) of Cu, immobilizing and accumulating this metal in the roots, which indicates their phytostabilization potential. The high biotransference factor confirms the efficiency of these woody Meliaceae species in reducing soil Cu availability through root absorption and compartmentalization, results that support the use of *K. ivorensis* and *C. fissilis* for Cu phytoremediation in contaminated soils. *K. ivorensis*, owing to its higher TI compared with *C. fissilis*, has greater potential for use in revegetation programs in Cu-contaminated areas. It is emphasized that field studies should be carried out to enhance these results. The adoption of these species in the revegetation of Cu mining areas may enable the ecological recovery, associated with the production of high-quality wood, as well as reducing the negative impact on the Amazon forest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Watilla Pereira Covre: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Wendel Valter da Silveira Pereira:** Conceptualization, Formal analysis, Writing - review & editing, Visualization. **Deyvison Andrey Medrado Gonçalves:** Methodology, Writing - review & editing. **Orivan Maria Marques Teixeira:** Resources. **Cristine Bastos do Amarante:** Resources. **Antonio Rodrigues Fernandes:** Conceptualization, Methodology, Supervision, Writing - review & editing, Project administration, Funding acquisition.

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