Biochar as a mitigator of copper toxicity and phytoremediation potential of Swietenia macrophylla King.

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Biochar as a mitigator of copper toxicity and phytoremediation potential of *Swietenia macrophylla* King.

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Abstract

Heavy metal contamination promotes soil degradation, reduces fertility, and affects crop growth. Therefore, the use of residues from the açaí (*Euterpe oleracea* Mart.) agroindustry for biochar production, associated with the cultivation of plants with potential to phytoremediate contaminated soils, such as Brazilian mahogany (*Swietenia macrophylla* King.), is a promising and sustainable strategy to mitigate the toxic effects of the metal. The study was conducted to evaluate the impact of BCA in mitigating excess Cu, exploring its effects on plant growth and levels, and to investigate the phytoremediation potential of the species exposed to Cu toxicity in the soil. The design was randomized blocks with five blocks, in a 4x3 factorial scheme, with the control treatment (without Cu) and three doses of copper (200, 400, and 600 mg kg^{-1}) and three levels of BCA (0%, 5%, and 10%), totaling sixty experimental units. The application of 5% BCA attenuated Cu toxicity in soils up to 400 mg kg^{-1}, with adequate Cu levels in the aerial part and accumulation in the roots, without compromising the biomass. In addition, Brazilian mahogany has a phytoremediation capacity of Cu up to 200 mg kg^{-1}, and when associated with 5% BCA, it phytostabilized the metal up to a concentration of 400 mg kg^{-1}. The selection of the species and the use of BCA can recover soils contaminated by Cu, being an excellent candidate in recovery programs of areas contaminated by metals in the Amazon.

Keywords: heavy metals, residue, charcoal, phytoremediation, brazilian mahogany

1. Introduction

The presence of heavy metals (HMs) in the environment is a limiting factor for crops, promoting changes in their metabolism and, consequently, affecting plant growth and development. In addition, environmental contamination by toxic metals promotes soil degradation, intensification of erosion processes, decreased natural fertility, changes in soil microbiota and reduced crop productivity (Murtaza et al., 2021). Among the toxic metals capable of causing environmental degradation, we can highlight non-essential chemical elements such as cadmium (Cd), arsenic (As), lead (Pb) and chromium (Cr), and essential for plants such as manganese (Mn), zinc (Zn), nickel (Ni), iron (Fe) and copper (Cu) (Adrees et al., 2015).

Cu is an essential micronutrient that exists in the form of Cu^{1+} and Cu^{2+}, acting in physiological and biochemical processes of plants, such as: mitochondrial respiration, electron transport chain, structural component of proteins, enzymatic activation, etc. (Mir; Pichtel; Hayat, 2021). Despite its essentiality, excess copper in the soil can trigger toxic effects that reduce plant growth, cause nutritional disorders, morphoanatomical changes, reduction in physiological parameters and increase in the activity of antioxidant enzymes to combat the generation of reactive oxygen species (ROS) (Cruz et al., 2022).

In Brazil, the National Environmental Council (CONAMA) establishes that concentrations of Cu in the soil above 60 mg kg^{-1} (prevention value) can cause changes in soil and groundwater quality, and values above 200 mg kg^{-1} (intervention value) present risks to human health (CONAMA, 2009). In addition, Cu in high concentrations causes toxic effects on microorganisms (Dias-Júnior; Siqueira; Silva, 1998), impairing the mineralization of phosphorus (P) and nitrogen (N), and can induce nutritional deficiencies in the soil-plant system through antagonistic interactions, such as reduced availability of P, iron (Fe) and zinc (Zn) in plants, due to the accumulation of Cu in the soil (Sodré et al., 2001). Thus, soil contamination by heavy metals from anthropogenic activities can pose risks to the environment, crop productivity and human health.

Soil contamination by Cu is associated with increased industrial, mining, and agricultural activities. Among the practices responsible for the accumulation of copper in the soil, we can highlight the smelting of ores containing Cu, inadequate disposal of solid waste such as batteries, metal alloys, and paints in the environment (Mir; Pichtel; Hayat, 2021). In agricultural soils, the intensive use of agrochemicals (herbicides and fungicides) containing copper or pig waste are the main sources of Cu input (Adrees et al., 2015; Rehman et al., 2019), in addition to the long-term contribution of the millennial use of Bordeaux mixture (copper-based fungicide) to control diseases in crops, causing a continuous accumulation of this metal in the soil (Brunetto et al., 2016).

In this context, several strategies can be adopted to reduce the toxic levels of an element in the soil, among which the use of biochar as a soil conditioner stands out, helping to reduce stress in plants, and the use of plant species for phytoremediation in areas contaminated by heavy metals.

Biochar is a carbon-rich material, produced in the absence of oxygen from the biomass of discarded organic waste (Hamid et al., 2021), and can be efficient in reducing and immobilizing soil pollutants, reducing their availability to plants, and consequently, promoting better growth and greater crop production (Murtaza et al., 2021). Thus, one of the residues used in the production of biochar is the açaí seeds obtained from the açaí palm.

The açaí palm (*Euterpe oleracea* Mart.) is a species native to the Amazon, occurring in large areas of the Amazon estuary, covering the states of the northern region of Brazil and having as its largest representative the state of Pará. Fruit production is the oldest form of exploitation of the species, where they are processed to acquire a juice known as açaí, consumed by the regional population (Oliveira; Farias Neto; Pena, 2007). In 2022, the number of fruits produced in Pará reached 1,595,455 tons (IBGE, 2022) and considering that only 20% of the fruit is processed, 80% of the production is pits that are discarded daily, generating a high volume of waste without destination, representing risks to the environment (Domingues et al., 2017).

The phytoremediation process is characterized by the adoption of plant species with the potential to extract toxic levels of chemical elements from the soil and accumulate them in their tissues (Ashraf et al., 2019). This technique is low-cost, promotes the maintenance of soil fertility, and uses solar energy for plant growth through the photosynthetic process (Robinson et al., 2003).

In the search for forest species that have this potential for soil recovery, Brazilian mahogany (*Swietenia macrophylla* King.) stands out, exploited for its high economic value, in addition to presenting characteristics of moderately fast growth and high production of plant biomass (Souza et al., 2010). Being a species native to the Amazon, its wood is used in the furniture industry, crafts, civil construction, decorative pieces and in the production of musical instruments (Costa et al., 2013).

In view of the above, it is important to test two hypotheses: (i) the use of residue from the açaí production chain is a sustainable and efficient alternative for the production of biochar in the Amazon under conditions of excess copper, making the metal unavailable to plants and improving their growth; and (ii) Brazilian mahogany is capable of absorbing Cu from the soil and accumulating it in its organs, presenting a phytoremediation capacity for copper.

Therefore, the objective of the study was to evaluate the impact of BCA in mitigating excess copper, exploring its effects on plant growth and levels, and to investigate the phytoremediation potential of Brazilian mahogany exposed to copper toxicity in the soil.

2. Material and methods

2.1 Experimental design, sampling and soil characterization

The experiment was conducted in a greenhouse located in the experimental area of the Federal Rural University of Amazônia (UFRA), Belém Campus, Pará, Brazil (1°27'17.3"S 48°26'18.0"W). The soil used was collected in a native forest area of UFRA, in the 0-20 cm depth layer. The experimental design used was a randomized block design with five blocks, in a 4x3 factorial scheme, corresponding to the control treatment (without Cu) and three doses of copper (200, 400 and 600 mg kg^{-1}) in the form of copper sulfate ($CuSO_4.5H_2O$) and three levels of BCA (0%, 5% and 10%,) proportional to 0, 100 and 200 t of biochar ha^{-1}).

The collected soil was air-dried, crushed, homogenized and passed through 2 mm sieves, and a composite sample was taken for chemical characterization (Brasil et al., 2020) and granulometric analysis (Donagemma et al., 2017). The results of the chemical and granulometric analysis are described in Tables 1 and 2, respectively.

Table 1

Table 2

After chemical analysis of the soil (Table 1), acidity was corrected using the base saturation method (V%) using 9.7 g of dolomitic limestone per pot (PRNT 97.4%), with the aim of achieving 50% base saturation and maintaining soil pH between 5.5 and 6.5. Throughout the experiment, soil moisture was maintained at field capacity at 60% of the total pore volume, through daily watering in the late afternoon, starting at 5 p.m. After a 30-day period for the limestone to react, the soil was contaminated with copper (Cu) in 3 dm^3 pots at concentrations of 200, 400, and 600 mg Cu kg^{-1}. The Cu doses used in the experiment are in accordance with the values established for agricultural, residential, and mining areas, respectively (CONAMA, 2009).

Immediately after soil contamination, the pots were moistened and incubated for 40 days in hermetically sealed plastic bags, a period sufficient for the metal to react with the substrate and stabilize (Lunkes et al., 2022). In addition, during this period, the soil was turned over every two days so that the reaction between them was homogeneous.

2.2 Production, characterization and biochar application

To produce açaí seed biochar (BCA), açaí agroindustrial waste (seeds with fibers) was collected from commercial establishments in the metropolitan region of Belém, Pará. The collected waste was washed in running water and dried in an oven at 70 °C for 24 hours, only to dry the material before being subjected to high temperatures.

After drying, the material was wrapped in aluminum foil and placed in a muffle furnace with a heating time of 10 °C min^{-1}. After reaching the ideal temperature for heat treatment under pyrolysis conditions, the carbonization of the material followed the methodology proposed by (Sato et al., 2019), being one hour at 600 °C. With the BCA produced, the material was ground and passed through a 2 mm sieve.

To characterize the biochar, all analyses were performed in triplicate, and the mean and standard deviation of the results obtained were calculated. The hydrogen potential (pH), specific surface area (SSA) and water retention capacity (WRC) followed the Embrapa methodology (2017).

From the immediate analysis of the biochar produced, the following material characteristics were determined: moisture (U), volatile material (MV), ash (Cz) and fixed carbon (CF), following the methodology proposed in the ASTM D1762-84 standard (ASTM, 2001).

Table 3

The application of BCA (150 and 300 g) corresponds to treatments of 5 and 10% of the total amount of soil in the pot (3 kg), respectively, remaining incubated for 60 days for complete stabilization with the soil. At this stage, the turning was disregarded, because the improvements in soil quality resulting from biochar are affected by the soil disintegration process (Zahra et al., 2021).

Fertilization with macro and micronutrients was carried out after the BCA incubation period. Fertilization with macronutrients (0.075 g of N, 0.053 g of P and 0.116 g of K pot^{-1}) occurred in the form of urea, triple superphosphate and potassium chloride; for micronutrients, fertilization was carried out for copper and manganese (0.008 g of Cu and 0.046 g of Mn), as they presented low levels in the soil analysis, in the form of copper sulfate and manganese sulfate, respectively, according to the recommendation proposed in the fertilization manual (Falesi; Bittencourt, 2020).

2.3 Growth conditions

In parallel with the previous steps, Brazilian mahogany seedlings aged 30 days after sowing (DAS) were provided by Embrapa Amazônia Oriental and were transferred to polystyrene bags with soil and plant residues, where they remained for 3 months until seedlings formed (120 DAS). Subsequently, 60 healthy seedlings with uniform height were visually selected and then definitively planted in 3 kg pots to compose the experimental units.

The Brazilian mahogany plants were cultivated for 90 days after definitive planting, a period sufficient for changes in metabolism to result in reductions in the growth of forest species in soils contaminated by metals (Marques; Moreira; Siqueira, 2000).

2.4 Dry mass production and Dickson quality index

After the end of the experiment (210 DAS), the plant material was separated into leaves, stem and root, placed in paper bags and dried in a forced air circulation oven at 60 °C for 48 hours. After this period, the leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and total biomass were determined using an analytical balance expressed in g $plant^{-1}$.

The Dickson quality index - IQD (Dickson; Leaf; Hosner, 1960) was determined based on height (H), diameter (D), total dry biomass, dry mass of the aerial part (SDM - leaves and stem) and root (RDM), according to equation 1:

Equation (1)

2.5 Copper content

To determine Cu in the soil, soil samples were collected from the experimental units after the collection of the plants, which were subjected to the DTPA extractor, at pH 7.3 and atomic absorption spectrophotometry, while, for the plant tissues, after weighing the dry matter, they continued to maceration in a mill, and then subjected to nitric-perchloric digestion and atomic absorption spectrophotometry, as Embrapa (2017).

2.6 Phytoremediation indices

The tolerance index (TI) (Wilkins, 1978) of plants to Cu was calculated by the ratio between the total biomass of plants exposed to Cu concentrations and the total biomass of control plants (without Cu), according to equation (2):

Equation (2)

The translocation factor (TF) (Zayed et al., 1998) and the bioconcentration factor (BCF) (Caille; Zhao; McGrath, 2005) were determined according to equations 3 and 4, respectively, expressed below:

Equation (3)

Equation (4)

2.7 Statistical analysis

The data were subjected to analysis of variance using the F test, and when significant, the Tukey test was applied to compare the means of the treatments at a 5% probability level. The collected data were analyzed in the statistical program AgroEstat (Barbosa; Maldonado Júnior, 2014).

3. Results

3.1 Biomass production

According to the analysis of variance (Table 4), there was a significant interaction between the copper (Cu) doses and the açaí seed biochar (BCA) concentrations for the variables leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and Dickon quality index (IQD).

For the total biomass variable, there was a significant isolated effect for Cu doses and BCA concentrations.

Table 4

For leaf dry mass (LDM) (figure 1A), the use of açaí seed biochar (BCA) at 5% obtained a higher amount of LDM at a dose of 400 mg kg^{-1} Cu (18.63 g $plant^{-1}$), with an increase of 36.7% compared to control plants (13.18 g $plant^{-1}$). Plants with 10% BCA showed an increase of 48.27% of LDM at a concentration of 200 mg kg^{-1} Cu in the soil (19.32 g $plant^{-1}$) compared to plants that did not receive Cu (13.03 g $plant^{-1}$). For the stem dry mass (SDM) variable (Figure 1.B), plants with 5% BCA, regardless of the concentration of 200 (11.98 g $plant^{-1}$), 400 (12.22 g plant-1) or 600 (10.55 g $plant^{-1}$) mg kg^{-1} Cu, all showed an increase in SDM of 75.6%, 82.9% and 54.7%, respectively, in relation to treatments without Cu (6.82 g $plant^{-1}$). In plants with 10% BCA, only the concentration of 400 mg kg^{-1} Cu (9.41 g $plant^{-1}$) differed from plants without Cu (5.95 g $plant^{-1}$), with an increase of 58.1%.

For root biomass (figure 1C), there was an increase of 147.4% and 142.1% at concentrations of 200 (0.47 g $plant^{-1}$) and 400 (0.46 g $plant^{-1}$) mg kg^{-1} Cu when biochar was used at a proportion of 5%, respectively, compared to plants without Cu in the soil (0.19 g $plant^{-1}$).

In all treatments evaluated, the Dickson quality index (DQI) (figure 1.D) presented values equal to and/or above the upper limit (UL), demonstrating the good quality of the seedlings for the field, with emphasis on the concentrations of 200 (0.43) and 400 (0.42) mg kg^{-1} Cu with 5% BCA, with increases of 138.8% and 133.3%, respectively, compared to the control treatment (0.18).

Figure 1

Considering the isolated effect of copper concentrations on total biomass (Figure 2.A), plants with 200, 400 and 600 mg kg^{-1} of Cu showed an increase of 40.32% (28.64 g $plant^{-1}$), 35.81% (27.72 g $plant^{-1}$) and 22.09% (24.92 g $plant^{-1}$), respectively, compared to the control treatment (21.41 g $plant^{-1}$).

When evaluating the isolated effect of açaí biochar (BCA) proportions on total biomass (Figure 2.B), there were no differences for 5% BCA (26.58 g $plant^{-1}$) in relation to plants that did not receive BCA (26.6 g $plant^{-1}$), however, the addition of 10% BCA (23.09 g $plant^{-1}$) reduced 13.19% of total biomass.

Figure 2

3.2 Copper contente and phytoremediation indices

The distribution of copper (Cu) in plant organs (figure 3) followed the order root > stem > leaves. Despite the preference of Cu to concentrate in the root, in control plants the distribution occurred up to 50%; however, in treatments that suggest soil contamination by Cu through anthropic activities (200, 400 and 600 mg kg^{-1}), with or without the addition of BCA, more than 68% of the absorbed metal remained in the root system.

Figure 3

According to the analysis of variance (table 5), there was a significant interaction between the doses of copper (Cu) and the concentrations of açaí seed biochar (BCA) for the Cu contents in the aerial part (Shoots), in the root (Roots), tolerance index (TI), translocation factor (TF) and bioconcentration factor (BCF).

Table 5

For the Cu content in the shoots (Figure 3.A), plants without BCA and with 5% had an increase in Cu content up to the maximum concentration of the metal in the soil (600 mg kg^{-1}), representing an increase of 13.9% without BCA (17.62 mg kg^{-1}) and 42.3% with the use of biochar at 5% (16.88 mg kg^{-1}) in relation to plants without Cu (15.47 and 11.86 mg kg^{-1}, respectively). Plants with 10% BCA had an increase of 97.6% in Cu content at the concentration of 400 mg kg^{-1} (25.25 mg kg^{-1}) in comparison to the control (12.78 mg kg^{-1}). Observing the different proportions of biochar, the use of 10% BCA in soil contaminated with 400 (25.25 mg kg^{-1}) and 600 mg kg^{-1} of the metal (22.11 mg kg^{-1}), promoted an increase of 71.8% and 25.5% in the aerial part, in relation to plants without incorporation of BCA (14.69 and 17.62 mg kg^{-1}, respectively).

Regardless of the use of BCA, the increase in copper content in root tissues (figure 3.B) followed the increase up to the maximum concentration of Cu in the soil. In treatments without BCA and different concentrations of Cu, Brazilian mahogany obtained a 266% increase in Cu in the root up to 600 mg kg^{-1} (59.3 mg kg^{-1}) compared to the control (16.2 mg kg^{-1}).

In soils contaminated with 600 mg kg^{-1} Cu, the addition of 5% BCA (146.15 mg kg^{-1}) increased the Cu content in the root by 490%, compared to plants without Cu (24.77 mg kg^{-1}). The use of 10% BCA (129.4 mg kg^{-1}), the same dose of the metal, obtained an increase of 304% compared to the control plants (32 mg kg^{-1}).

For different proportions of BCA at the dose of 400 mg kg-1 Cu, the use of 5% (65.85 mg kg^{-1}) and 10% (74 mg kg^{-1}) of BCA increased the Cu content in the root by 19.3% and 34.2%, respectively, compared to the control plants (55.18 mg $kg^{-1).}$ At the dose of 600 mg kg^{-1} Cu, the addition of BCA 5% (146.15 mg kg^{-1}) and 10% (129.42 mg kg^{-1}) contributed to an increase of 146% and 118% of Cu in the root system, respectively, in contrast to the treatment without application of biochar in the soil (59.3 mg kg^{-1}).

Figure 4

According to the phytoremediation indices (Table 6), Brazilian mahogany showed a high tolerance index (TI) (Gautam; Pandey; Agrawal, 2017) at all applied Cu concentrations. The translocation factor (TF) was lower than the reference parameter (<1) (Deng; Ye; Wong, 2004) in all evaluated treatments.

For the bioconcentration factor (BCF), the species presented results >1, which suggests that the amount of metal extracted from the soil and concentrated in the plant was satisfactory (Yanqun et al., 2005). In the experiment, plants that did not receive BCA (5% or 10%) were only able to bioconcentrate the metal up to 200 mg kg^{-1}, compared to treatments with BCA which demonstrated efficiency at all metal concentrations (200, 400 and 600 mg kg^{-1}).

Table 6

4. Discussion

4.1 Biomass production

In the study, the growth of Brazilian mahogany was influenced by soil copper concentrations and the addition of açaí biochar (BCA). Leaf dry mass (LDM) (figure 1a) increased with the use of 5% BCA up to intermediate concentrations of the metal (200 and 400 mg kg^{-1}). Studies indicate that the ability of biochar to absorb excess Cu in its porous structures (Murtaza et al., 2021) and its high ash content (table 3) favor the availability of nutrients to plants, with no reduction in LDM even under toxic conditions.

For stem dry mass (SDM) (figure 1b), plants subjected to 5% BCA at the same Cu concentrations described above showed biomass gain. This behavior is associated with mechanisms of the species under stress conditions where the increase in stem diameter improves its resistance to lodging and toppling (Griffing, 2017), promoting an increase in SDM. Furthermore, the characteristics of biochar may have favored Cu mitigation, allowing better absorption of essential elements, allowing a gain in stem mass.

In the root dry mass (RDM) (figure 1C) up to a concentration of 400 mg kg^{-1} of Cu, the use of 5% BCA was efficient in promoting root biomass gain. However, the excess of Cu promoted by high concentrations, depending on the sensitivity of the species, can alter its morphology and prevent the growth of roots and shoots (Adrees et al., 2015), a fact evidenced by RDM only at the highest concentration of the metal (600 mg kg^{-1}).

When we observe the total biomass in isolation, Brazilian mahogany plants up to 400 mg kg^{-1} of Cu (figure 2a) and the addition of 5% BCA (figure 2b) promoted plant biomass gains. After this concentration, the biochar was not able to mitigate the stress, resulting in a decrease in the dry biomass of the plants.

In studies with *Ilex paraguariensis* (Lunkes et al., 2022) and *Eucalyptus grandis* (Marco et al., 2017), high concentrations of copper (200 and 300 mg kg^{-1}, respectively) negatively affected height, stem diameter, leaf area, dry mass of the aerial part and roots, decreasing the total biomass of the plants and affecting their growth.

The Dickson Quality Index (DQI) in Brazilian mahogany demonstrated good adaptability of the seedlings to the treatments evaluated, with results equal to or higher than the minimum reference value (0.20) (Hunt, 1990). Because the DQI is an index that integrates the robustness of seedlings (height/stem diameter) and the balanced distribution of biomass in the plant (aerial part/root) (Fonseca et al., 2002), it has been recommended as a good indicator of seedlings for definitive planting in forestry (Eloy et al., 2013). Furthermore, the incorporation of 5% BCA enhanced the DQI in the species at intermediate concentrations of the metal.

4.2 Copper content in plant tissue

In all treatments evaluated, 70% of the copper (Cu) distributed in the plant organs is present in the root (figure 3). This ease of accumulation in the root system demonstrates the rapid incorporation of the metal by proteins and enzymatic groups present in the cell wall and apoplast of root cells, managing to retain most of the copper absorbed by the plants while still in the root system (Girotto et al., 2016).

In addition, the production of root exudates, metal chelation and storage in the cell vacuole (Kumar et al., 2021) are strategies used by plants to limit Cu in the roots, preventing its translocation in high levels to the aerial part, preventing possible damage to the photosynthetic apparatus (Marques et al., 2018).

In this study, copper levels in the shoots and roots increased proportionally to the Cu concentrations in the soil (figure 4), however, high levels of the metal were observed in the root system.

The process of absorption of heavy metals in plants occurs through membrane transporters, these transporters are important for maintaining homeostasis and tolerance conditions in the plant (Grotz; Guerinot, 2006), being present in the plasma membrane of the cells of the epidermis and cortex of the roots, in addition to the root hairs, and membranes of cellular organelles, such as the vacuole (Marschner; Rengel, 2011).

Copper absorption in root cells is mediated by copper transport proteins (*COPT*) in a manner dependent on *SPL7* gene expression, with *COPT1* (Mir; Pichtel; Hayat, 2021) as the largest representative of the family, a high-affinity transporter responsible for the entry of Cu into the cell, frequently occurring in plants that have low copper availability in the soil and having little expression when there is high accumulation of this metal (Yruela, 2009). However, with increasing soil Cu concentrations, other membrane transport proteins not regulated by *SPL7* gene expression may continue to transport the metal.

Copper is also mediated by heavy metal *ATPases*, proteins that act as membrane transporters. Heavy metal *ATPases* are not exclusive to copper, as they enable the transport of several metals by using *ATP* to pump ions through cell membranes (Grotz; Guerinot, 2006). In addition, proteins that transport zinc (*ZIP2* or *ZIP4*) can absorb Cu, in addition to non-selective cation channels (*NSCC*), which, as an ion channel, stimulates the entry of Cu^{2+} into the root and influences the absorption of other minerals (Mir; Pichtel; Hayat, 2021). Thus, the entry of Cu is not conditioned solely by an absorption mechanism, promoting the accumulation of Cu in plant tissues.

High concentrations of Cu were determined in the roots compared to the aerial part (figure 4). Treatments with BCA (5% and 10%) in the root presented higher Cu contents. However, when compared with studies that did not use any stress mitigator, the Cu contents obtained in the study are below those found in the literature.

Studies conducted with African mahogany in soils contaminated with Cu (60, 200, 400 and 600 mg kg^{-1}) presented Cu contents in the aerial part and root above 40 mg kg^{-1} and 80 mg kg^{-1}, respectively, in the treatment with 200 mg kg^{-1}, reaching contents above 50 mg kg^{-1} in the aerial part and 325 mg kg^{-1} in the root at the highest dose of Cu applied (600 mg kg^{-1}) (Covre et al., 2020).

In the present study, the application of BCA (5% and 10%) was efficient in immobilizing excess Cu in the soil and controlling the availability of the metal to be absorbed by the roots. Due to the specific surface area (SSA), biochar is considered an efficient solvent for organic and inorganic contaminants, since it remains in the soil and immobilizes these contaminants, increasing the protection of plants against metal toxicity (Almeida, 2008).

Studies demonstrate the effectiveness of biochar in reducing the mobility and availability of heavy metals in contaminated soils, reducing the amount absorbed by the plant and immobilizing them in the soil through mechanisms such as electrostatic attraction, ion exchange, complexation and precipitation of metals (Meng et al., 2018; Yang et al., 2017).

Furthermore, the benefits evidenced by the characterization of biochar (Table 3), such as: high SSA, greater water retention capacity (WRC) and high ash content (Ash), contribute to improvements in soil porosity, greater water quantity and nutrient availability (Hossain et al., 2020; Laghari et al., 2016), respectively, creating a favorable environment for greater root growth without being affected by excess copper, a fact confirmed in this study with 5% BCA up to 400 mg kg^{-1} of Cu (Figure 1C). This favorable root environment, associated with voluminous, cylindrical roots and soil exploration up to five meters (Costa et al., 2013), contributed to Brazilian mahogany continuing to absorb Cu without compromising growth.

The copper content in the aerial part (figure 4A) increased in the treatments with 0% and 5% BCA up to the highest concentration of Cu applied (600 mg kg^{-1}); however, with 10% BCA, the highest content in the plant occurred at 400 mg kg^{-1} of the metal in the soil.

Copper is an essential micronutrient and is associated with several functions in plant metabolism. However, when Cu is found in high concentrations, it can cause toxicity to the plant, resulting in metabolic changes that promote reduced growth and nutritional, biochemical or physiological disorders (Adrees et al., 2015).

High concentrations of copper in plants affect net photosynthesis, stomatal conductance and transpiration, reducing the efficiency of gas exchange with the atmosphere (Ferreira et al., 2024). Excess Cu compromises the photosynthetic apparatus by altering the composition of pigments and the structure of chloroplasts, decreasing the photosynthetic rate and inhibiting the electron transport chain (Mir; Pichtel; Hayat, 2021), resulting in a decrease in physiological responses.

The adequate Cu contents in plant tissues range from 5-20 mg kg^{-1} of dry mass (Kabata-Pendias; Szteke, 2010). In the present study, the copper content in the aerial part of plants with 0% and 5% BCA was not affected up to the maximum metal concentration in the soil (600 mg kg^{-1}), and with 10% BCA up to 400 mg kg^{-1} of copper, demonstrating the efficiency of BCA in retaining excess copper in its structure and making it unavailable to plants, controlling the absorption and transport of the metal in Brazilian mahogany under conditions of high concentrations in the soil, avoiding toxicity and maintaining the functions performed by Cu in plant metabolism.

4.3 Phytoremediation indices

Brazilian mahogany responded well in soils contaminated with copper, presenting a species tolerance index >0.9, being classified as tolerant (Gautam; Pandey; Agrawal, 2017) in all treatments evaluated. Furthermore, the use of BCA in the proportion of 5% enhanced the species' results. A study that evaluated the tolerance of three forest species, namely: ‘timbaúva’ (*Enterolobium contortisiliquum* (Vell.) Morong), pata-de-vaca’ (*Bauhinia forficata* Link) and ‘apendoim-bravo’ (*Pterogyne nitens* Tul), in soils contaminated with Cu (60, 120, 180, 240 and 300 mg kg^{-1}) were able to tolerate metal toxicity up to the prevention value of 60 mg kg^{-1} (CONAMA, 2009). However, with the increase in Cu treatments, only timbaúva was able to withstand up to the maximum dose (300 mg kg^{-1}) (da Silva et al., 2015).

The translocation factor (TF) measures how much of the metal absorbed by the roots was translocated to the shoot. In this index, the values were <1, indicating restriction in the movement in the root-shoot direction (Deng; Ye; Wong, 2004), demonstrating that there was an accumulation of Cu in the root and immobilization of the metal in the root system (phytostabilization) (Yoon et al., 2006). These results were confirmed by the higher Cu content found in the root in relation to the shoot (figure 4), and by the higher percentage of copper in the root in comparison to the other organs of the plant (leaf and stem) (figure 3). Contrary to the results obtained, a study conducted to select forest species for copper phytoremediation purposes demonstrated that *Myracrodruon urundeuva*, *Cecropia* sp. and *Handroanthus serratifolius*, are capable of phytoremediating Cu up to 100 mg kg^{-1}, characterizing themselves as phytoextractors (accumulation of the metal in the aerial part) (Asensio et al., 2018), which demonstrates that the phytoremediation technique varies with the species evaluated.

The bioconcentration factor (BCF) is an excellent indicator of the capacity to accumulate heavy metals, as it considers the capacity of plants to extract metal from the soil and accumulate it in their organs (McGrath; Zhao, 2003), and can be classified into plants that are efficient in absorbing and accumulating the metal from the soil matrix (>1) or excluders (<1), inefficient in the absorption and accumulation process (Yanqun et al., 2005). In the present study, Brazilian mahogany, when grown without BCA, obtained BCF>1 up to 200 mg kg^{-1}; however, with the addition of BCA (5 or 10%), the species was able to accumulate the metal up to the maximum concentration in the soil (600 mg kg^{-1}).

Studies carried out with African mahogany (*Khaya ivorensis* A. Chev.) and cedar (*Cedrela fissilis* Vell.) grown in soils contaminated with Cu (60, 200, 400 and 600 mg kg^{-1}) had results similar to those of the present study, where the species had satisfactory TI and low translocation (TF<1) of Cu, indicating characteristics of phytostabilizing plants (Covre et al., 2020).

5. Conclusion

The application of 5% BCA attenuated Cu toxicity in soils contaminated with up to 400 mg kg-1, with adequate Cu levels in the aerial part and accumulation in the roots without compromising the biomass of Brazilian mahogany (LDM, SDM, RDM and total).

The species has phytoremediation potential of the phytostabilizing type (immobilization of the metal in the root) in soils contaminated with Cu up to 200 mg kg^{-1}, and when associated with the use of 5% BCA, they are capable of phytostabilizing the metal up to a concentration of 400 mg kg^{-1}, maintaining its growth.

The pre-selection of the species associated with the use of BCA can recover soils contaminated with copper and maintain crop production in the area, being an excellent candidate in recovery programs of areas contaminated by heavy metals in the Amazon.

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Equations

QDI = {Biomass total / [(H/D) + (SDM/RDM)]}   (1)

TI = Biomass total (Pot with Cu) / Biomass total (Pot without Cu)   (2)

TF = [Cu] shoot / [Cu] root   (3)

BCF = [Cu] root / [Cu] soil   (4)

Tables

Table 1. Soil chemical analysis of the 0-20 cm deep layer.

Layer	pH	SOC	P	K^+	Ca^{2+}+Mg^{2+}	Ca^{2+}	Al^{3+}	H^++ Al^{3+}	SB	CTC (pH7)	V*	m**
	$CaCl_2$	g kg^{-1}	mg dm^{-3}	mmol$_c$ dm^{-3}							%	
0-20	3.5	16.8	6	0.6	6	4	18	133	7	140	5	72

pH = hydrogen potential; SOC = Soil organic carbon; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Al = aluminum; H = hydrogen; CTC = cation exchange capacity; SB = sum of bases; *base saturation, **aluminum saturation.

Table 2. Particle size analysis of the soil in the 0-20 cm deep layer.

Layer	clay	sand	silt	Textural classification
cm	g kg^{-1}			BSSC*
0-20	212	693	95	Sandy Clay Loam

* Brazilian System of Soil Classification.

Table 3. Characterization of biochar (BCA) from açaí seeds.

Material	pH	SSA	WRC	M	VM	Ash	FC
-	H_2O	m^2 g^{-1}	g g^{-1}	%			
BCA	11	340	2.5	9.2	0.5	98.4	1.1

pH = hydrogen potential; SSA = specific surface area; WRC = water retention capacity; M = moisture; VM = volatile material; Ash = ashes; FC = fixed carbon.

Table 4. Analysis of variance for leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), Dickon quality index (DQI) and total biomass in Brazilian mahogany plants (*Swietenia macrophylla* King) as a function of different concentrations of copper (Cu) and biochar (BCA).

Source of variation	L.D	Mean square				
		LDM	SDM	RDM	QDI	Biomass
Copper (Cu)	3	63.84**	40**	0.084**	0.069**	204.7**
Biochar (BCA)	2	1.17^{ns}	59.12**	0.08**	0.064**	81.61**
Cu x BCA	6	20*	5.51**	0.032**	0.025**	2.17^{ns}
CV (%)	-	16.8	13.6	29.4	27.4	13.7

CV = coefficient of variation; ns = not significant; * = significant (p<0.05); ** = significant (p<0.01) by F test.

Table 5. Analysis of variance for copper concentrations in leaf (L), stem (S) and root (R), tolerance index (TI), translocation factor (TF), bioconcentration factor (BCF) and bioaccumulation factor (BF) in Brazilian mahogany plants (*Swietenia macrophylla* King.) as a function of different concentrations of copper (Cu) and biochar (BCA).

Source of variation	L.D	Mean square				
		[Cu] Shoots	[Cu] Roots	TI	TF	BCF
Copper (Cu)	3	119.1**	20720.3**	0.11*	0.06**	1.16**
Biochar (BCA)	2	111.3**	6344.5**	0.16**	0.04**	2.84**
Cu x BCA	6	42.5**	1888.4**	0.07*	0.01**	0.56**
CV (%)	-	6.8	5.4	12.3	7.7	26.8

CV = coefficient of variation; ns = not significant; * = significant (p<0.05); ** = significant (p<0.01) by F test.

Table 6. Tolerance index (TI), translocation factor (TF) and bioconcentration factor (BCF) in Brazilian mahogany plants (*Swietenia macrophylla* King.) with different copper (Cu) concentrations, considering the biochar proportions (BCA).

Cu (mg kg^{-1})	0% BCA	5% BCA	10% BCA
		Tolerance index (TI)	
200	1,37 aA	1,32 abA	1,22 aA
400	1,17 aB	1,43 aA	1,19 aB
600	1,31 aA	1,17 bA	0,93 bB
		Translocation fator (TF)	
200	0,38 aA	0,25 aC	0,33 aB
400	0,26 bB	0,23 aB	0,34 aA
600	0,29 bA	0,11 bC	0,17 bB
		Bioconcentration fator (BCF)	
200	1,11aB	1,68 aA	1,67 aA
400	0,65 abA	1,0 bA	1,15 bA
600	0,54 bB	1,05 bB	2,09 aA

Averages followed by the same letter (lowercase in the column and uppercase in the row) do not differ at the 5% probability level using the Tukey test.

Legends

Figure 1. Leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and Dickon quality index (DQI) of Brazilian mahogany plants (*Swietenia macrophylla* King.) as a function of different concentrations of copper (Cu)

and biochar (BCA). Means followed by the same lowercase letter do not differ by the Tukey test ($p < 0.05$) between the same Cu doses.

Figure 2. Total biomass in Brazilian mahogany (*Swietenia macrophylla* King.) plants as a function of different copper (Cu) concentrations (A) and biochar (BCA) proportions (B) in the soil. Means followed by the same letter do not differ by Tukey test at 5% probability level ($p < 0.05$). Bars indicate the standard error of the mean (n = 5).

Figure 3. Percentage distribution of copper (Cu) in plant organs of Brazilian mahogany (*Swietenia macrophylla* King.) as a function of different Cu concentrations and biochar proportions (BCA).

Figure 4. Copper (Cu) content in shoots (A) and roots (B) of Brazilian mahogany (*Swietenia macrophylla* King.) plants as a function of different concentrations of copper (Cu) and biochar (BCA). Means followed by the same lowercase letter do not differ by Tukey test ($p < 0.05$) between Cu doses at the same BCA concentration, and means followed by the same uppercase letter do not differ by Tukey test ($p < 0.05$) between BCA concentrations at the same Cu dose. Bars indicate the standard error of the mean ($n = 5$).

Figures

Figure 1

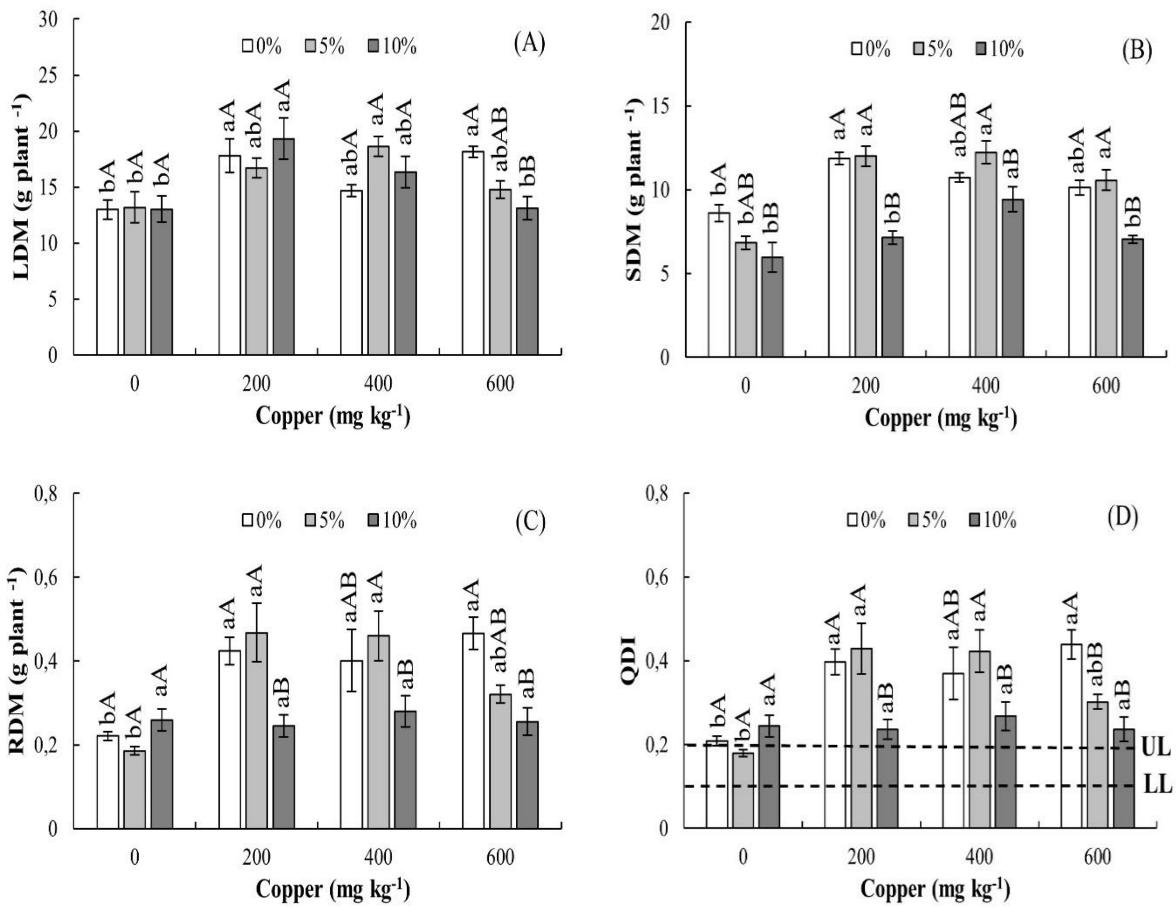

Figure 2

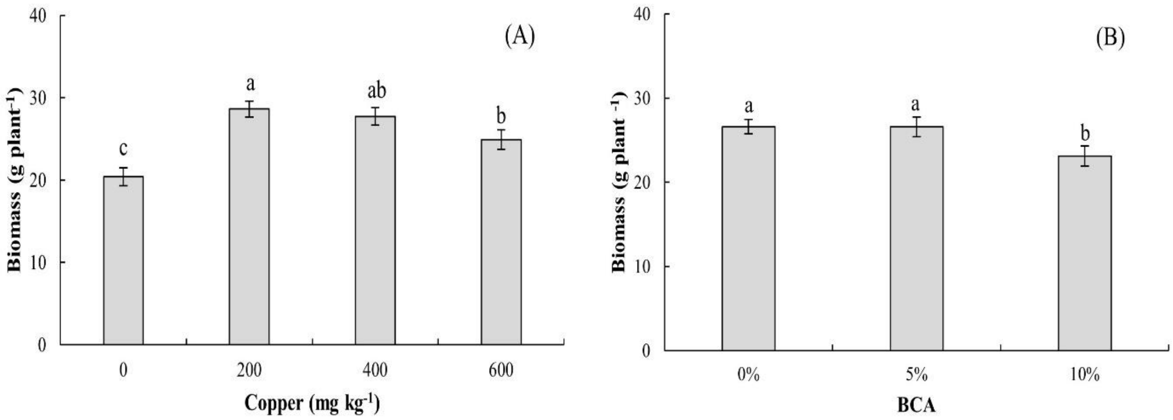

Figure 3

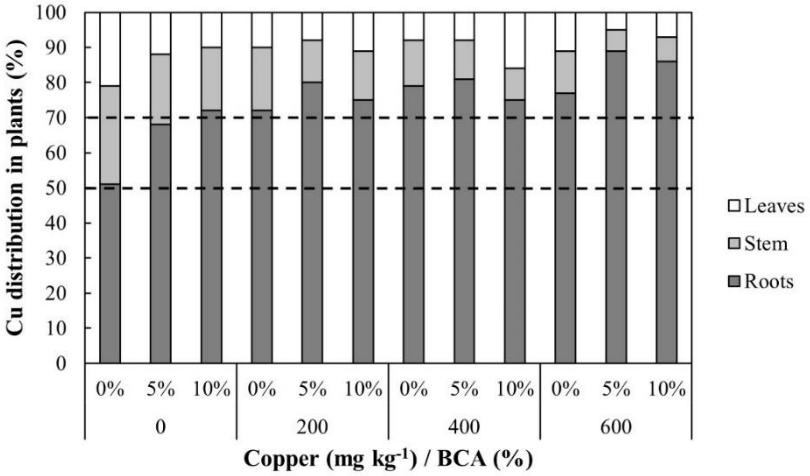

Figure 4