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Biochar in copper reduction in black beans and soil decontamination

Biocarvão na redução de cobre no feijão preto e na descontaminação do solo

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ABSTRACT

When present in high concentrations in the soil, copper causes toxicity in plants, requiring the development of studies for the reduction or immobilization of this element. In this sense, biochar could be an alternative to immobilizing copper in the soil, aiming for lower levels of this element in the biomass and grains of black beans (*Phaseolus vulgaris*) used for human consumption. However, there are variations in biochar reactivity due to its source material and pyrolysis time. Therefore, the objective of the present study was to determine the effect of eucalyptus biochar on the availability of copper in the soil and on its contents in beans grown in contaminated soil. The experimental design was completely randomized in a 5 × 2 factorial arrangement, with five doses of biochar (0.0, 0.5, 1.0, 1.5, and 2.0% mm^{-1} of dry soil), soil without and with the addition of copper (1,000 mg kg^{-1} of dry soil), and with eight repetitions. Thecopper content available in the soil, root, aerial part, and bean grain; the chlorophyll index; and the bioconcentration and translocation factors of copper in the plant were evaluated. Biochar derived from eucalyptus residues decreases copper availability in contaminated soil. The copper levels in the roots, aerial part, and grains of *P. vulgaris* are reduced with the application of biochar to the soil, remaining in the grains, from a dose of 1.66% mm^{-1}, below the maximum limit tolerable by Brazilian legislation.

Keywords: *Phaseolus vulgaris*; heavy metal; pyrolyzed biomass; immobilization.

RESUMO

O cobre em elevada concentração no solo, causa toxidez nas plantas, sendo necessário o desenvolvimento de estudos que reduzam, ou imobilizem esse elemento. Nesse sentido, o biocarvão poderia ser uma alternativa para a imobilização de cobre no solo, visando menores teores deste elemento na biomassa e nos grãos do feijão preto (*Phaseolus vulgaris*) utilizado para consumo humano. Contudo, há variações na reatividade do biocarvão em decorrência do seu material de origem e tempo de pirólise. Portanto, o objetivo deste trabalho foi determinar o efeito do biocarvão de eucalipto na disponibilidade de cobre no solo e nos teores em feijão cultivado em solo contaminado. O delineamento experimental foi inteiramente casualizado em arranjo fatorial 5 × 2, sendo cinco doses de biocarvão (0, 0.5, 1, 1.5 e 2% mm^{-1} de solo seco), solo sem e com adição de cobre (1.000 mg kg^{-1} de solo seco), com oito repetições. Avaliou-se o teor de cobre disponível no solo, teor de cobre na raiz, na parte aérea e no grão do feijão; índice de clorofila e fatores de bioconcentração e de translocação de cobre na planta. O biocarvão derivado de resíduos de eucalipto diminui a disponibilidade de cobre em solo contaminado. Os teores de cobre na raiz, parte aérea e nos grãos de *Phaseolus vulgaris* são reduzidos com a aplicação de biocarvão no solo, mantendo-se nos grãos, a partir da dose 1,66% m m^{-1}, abaixo do limite máximo tolerável pela legislação brasileira.

Palavras-chave: *Phaseolus vulgaris*; metal pesado; biomassa pirolisada; imobilização.

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Introduction

When present in high concentrations in soil, copper can cause environmental toxicity and damage to the food chain due to the bioaccumulation process (Ali, H. et al., 2019). The reference value for interventions in agricultural areas, according to resolution n° 420, is 200 mg kg^{-1} of soil (Brazil, 2009). In the roots, the symptoms of toxicity caused by copper include shortening, thickening, and enlargement of the lateral roots (Liu et al., 2021), reduced water and nutrient absorption, and consequently, growth inhibition of the aerial parts (Keller et al., 2015). In addition, it can lead to oxidative stress in plants, due to increased production of free radicals that damage lipids and cell membrane proteins, amino acids, and nucleic acids (Ali, M. et al., 2019).

Current studies have focused on remediation alternatives for areas contaminated with heavy metals, especially copper. In this context, biochar can be used for the immobilization or adsorption of heavy metals in the soil (Gholizadeh and Hu, 2021). This material comes from the pyrolysis of organic materials under anoxic conditions and at temperatures ranging from 350 to 900°C (Zhang et al., 2021). It has a high carbon content, it is aromatized, porous, and has a large specific surface area (Yang et al., 2019; Zhang et al., 2019). Its remediation capacity is related to contaminant control through physisorption (Gomez-Eyles and Ghosh, 2018), chemical adsorption (Xia et al., 2019), and increased hydrogen potential (pH), which indirectly reduces the bioavailability of heavy metals (Huang et al., 2018).

Beans (*Phaseolus vulgaris*) are one of the main foods in the Brazilian population's diet and their consumption stands out for their nutritional, social, and economic importance (Landau and Moura, 2020). Agricultural production areas in southern Brazil, in general, receive continuous applications of copper-based fungicides, fertilizers, and wastewater, which causes an excessive increase of this metal in the soil (Poggere et al., 2023). However, the accumulation of copper in *P. vulgaris* is related to the availability of the element in the soil (Grohskopf et al., 2016). Even though beans have good vegetative production capacity when growing in soils with excess copper (Collet et al., 2018), the maximum tolerated limit in grains is 10 mg kg^{-1} (Brazil, 2022).

In this context, research indicates that biochar has the potential to reduce the excess of copper in the soil, decreasing its translocation, and contributing to soil remediation (Rehman et al., 2021). However, the question that persists is whether biochar derived from eucalyptus reduces the presence of copper in the soil, its levels in plants, and grain of black beans to levels below the maximum limit established in Brazilian legislation, when grown in soils contaminated with copper. Therefore, the objective of the present study was to determine the effect of eucalyptus biochar on the availability of copper in the soil, the content of copper in plants, and in bean grains grown in contaminated soil.

Methodology

Experimental design

The experiment was conducted in a greenhouse at the Department of Agronomic and Environmental Sciences of the Federal University of Santa Maria (UFSM), Frederico Westphalen Campus, between October 2020 and March 2021, for 150 days.

The soil used in the experiment was characterized as an oxisol (Santos, 2018), collected from a natural field area, from the 0–20 cm layer. The soil was air-dried and sieved in a 2 mm mesh, and then a sample was taken to determine chemical and physical attributes, according to the methodology described by Brazilian Agricultural Research Corporation — Embrapa (1997) for texture, and by Tedesco et al. (1995) for other attributes (Table 1).

The soil was submitted to acidity correction with dolomitic limestone application (relative power of total neutralization — RPTN: 85%) at a dose equivalent to 4 tons per hectare. Macronutrients nitrogen, phosphorus, potassium, and sulfur were applied through the use of formulated mineral fertilizer NPK(S) 08:20:20:02 at a dose equivalent to 300 kg ha^{-1}, as recommended for bean culture (CQFS RS/SC, 2016). Top dressing with nitrogen (N) was also carried out through the use of urea in the V4 phenological stage, applying the equivalent of 50 kg ha^{-1} of N. The soil was conditioned in plastic bags and distributed in plastic pots of 3.5 liter-capacity, totaling a mass of 4 kg for subsequent application of treatments and sowing of black beans, with each pot considered as an experimental unit.

The experimental design was completely randomized in a 5 × 2 factorial arrangement, with five doses of biochar (0.0, 0.5, 1.0, 1.5, and 2.0% mm^{-1} of dry soil) which represent the percentage of biochar added in relation to the mass of dry soil.

Table 1 – Chemical and physical characterization of the soil.

pH* H_2O	V	SOM	Clay	CEC pH 7.0	Ca	Mg	Al	H+Al
	%				cmolc L^{-1}			
5.0	10.3	1.5	79.0	12.2	1.0	0.2	1.5	10.9

P	K	Cu	Zn	S	B	Mn
mg kg^{-1}						
2.0	21.0	8.4	0.2	15.8	0.18	3.5

*pH: hydrogen potential; H_2O: water; V: base saturation; SOM: soil organic matter; CEC: cation exchange capacity; Ca: calcium; Mg: magnesium; Al: aluminum; H+Al: potential acidity; P: phosphorus; K: potassium; Cu: copper; Zn: Zinc; S: sulfur; B: boron; Mn: manganese.

The experiment was conducted both without and with the addition of copper (1,000 mg kg^{-1} of dry soil), with eight repetitions. Soil contamination with copper was carried out through a solution of copper sulfate ($CuSO_4$) added and mixed to the soil by stirring in a plastic bag, at a dose of 1,000 mg kg^{-1} (Cu^{2+}) thirty days before sowing the beans, in order to allow sufficient time for stabilization of the chemical reactions.

The biochar was produced using equipment developed at the Federal University of Santa Maria, through the pyrolysis of eucalyptus residues, as thin branches, for two hours, at a temperature of 350°C, and under low oxygen flow conditions, as recommended by Róz et al. (2015). After manufacture, the biochar was standardized by sieving through a 2 mm mesh, and thereafter, samples were taken for physical and chemical characterization according to Tedesco et al. (1995), as shown in Table 2. Biochar was manually applied to the soil surface due to greater similarity in relation to the use in field conditions. The applications were made 15 days before sowing the beans, which corresponds to the minimum time necessary for the stabilization of chemical reactions, according to the recommendation adapted from Marco et al. (2021).

Installation and conduct of the experiment

The seeds of black beans (*P. vulgaris*) were the IPR Tuiuiú provided by the Agronomic Institute of Paraná (IAPAR), which were previously disinfected with 2% sodium hypochlorite solution for 15 minutes and washed under running water for 5 minutes. Sowing was held in December 2020 at a depth of 4 cm, totaling three seeds per pot.

Soil moisture was maintained close to field capacity during the entire period of conducting the experiment — from the conditioning of the soil in the pots to the end of the 90-day cycle of the beans. Irrigation was conducted manually by applying a water depth of 8 mm daily. During the cycle, phytosanitary management was also carried out, with three applications of registered insecticides and fungicides for the black bean crop through aerial spraying. The first application was performed 14 days after emergence (DAE) of plants, using thiamethoxam and lambda-cyhalothrin combined with trifloxystrobin and tebuconazole; the second application was at 28 DAE using the same products and dosages mentioned above; and the third application was at 45 DAE with chlorfenapyr combined with pyraclostrobin, and metconazole. In all steps, a syrup equivalent to 200 L ha^{-1} was used.

Ratings

Regarding the evaluations, the total chlorophyll index of bean leaves was determined by means of a portable chlorophyll meter (ClorofiLOG®, Falker, model CFL 1030) (Falker, 2008). This determination was performed at stage R6 (flowering), which is the recommended period to collect leaves for analysis of foliar nutrient content. Leaves from the lower, middle, and upper third of the plants were used, averaging the results. The results were expressed in the total chlorophyll index.

At the end of the experimental period, soil samples from each experimental unit were collected in order to determine the copper content available in the soil, using the extraction solution Mehlich-1, which acts by acid dissolution in the presence of sulfuric and hydrochloric acids (Sobral et al., 2013).

One plant from each pot was collected at the flowering stage of the bean, cut close to the ground to separate the aerial part and the root, and next the root was washed under running water for untwisting and cleaning. The samples of the materials (root and aerial part) were dried in a forced air circulation oven at 65°C until reaching a constant mass for subsequent analysis of copper content in the plant tissue.

The beans were collected at the maturation stage, one plant per experimental unit, also dried in a forced air circulation oven at 65°C until they reached 14% humidity. The dried root, aerial, and grain masses were ground in a Wiley mill and sieved through a 10-mesh sieve, and then subjected to copper content determinations by nitric perchloric digestion (3:1) and determination in atomic absorption spectrophotometry, as described by Miyazawa et al. (2009).

The results of the copper content quantified in the aerial part and in the roots of black beans were used for the calculations of the translocation factor (TF) and bioconcentration factor (BCF). TF represents the ability to translocate metals from roots to aerial parts by plants (Shi et al., 2011), calculated by the equation TF=CuAP/CuR, with CuAP being copper in the aerial part of plants and CuR being copper in the roots. The BCF represents the relationship between the metal present in the aerial part of the plants and its amount present in the soil, i.e., the ability of the plant to absorb metal from the soil and translocate it to the tissues of the aerial biomass (Liu et al., 2008). The BCF is calculated by the equation BCF=CuPA/CuD, where CuPA is copper in the aerial part of the plants, and CuD is copper content available in the soil.

Table 2 – Chemical characterization of biochar, according to Tedesco et al. (1995).

pH* H_2O	C-Organic	Ca	Mg	P	K	S	Cu	Zn	Fe	Mn
	%	g kg^{-1}					mg kg^{-1}			
8.0	42.53	1.3	1.5	1.0	4.5	17.1	12.0	23.0	41.3	150.0

*pH: hydrogen potential; H_2O: water; C-organic: organic carbon; Ca: calcium; Mg: magnesium; P: phosphorus; K: potassium; S: sulfur; Cu: copper; Zn: zinc; Fe: iron; Mn: manganese.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) and if significant interaction was found, they were submitted to regression analysis of the quantitative variation factor within each level of the qualitative factor. For the parameters without interaction, the simple effects were unfolded. The means of the qualitative factor were compared by the Tukey test at a 5% probability of error, and the means of the quantitative factor were subjected to regression analysis through Sisvar software, version 5.6 (Ferreira, 2019). In all cases, a 5% probability of error was used.

Results and Discussion

The high carbon content in biochar (Table 2) combined with high pH results in negative electrical charges of the functional groups responsible for cation retention, which is a fundamental mechanism for the immobilization of metals in the soil (Gholizadeh and Hu, 2021). Thus, the presence of copper in biochar with high pH does not directly imply its reactivity in the soil.

The chemical analysis of biochar (Table 2) also showed the presence of the macronutrients calcium, magnesium, phosphorus, potassium, and sulfur, all of which are of great importance for plant development (Osório et al., 2020). In this sense, in addition to the desired effect of retaining soil contaminants, this biochar also served to supply essential nutrients for plant development.

Figure 1 shows an image of the experimental units, with black beans in phenological stage V2, revealing a visible difference between the treatment without biochar (left) and with biochar (right).

Figure 1 – Experimental units of black beans in phenological stage V2.

The availability of copper significantly reduced linearly according to the increase of biochar doses in the treatment without adding the metal, being 44.5% lower at the dose of 2.0% mm^{-1} of biochar than at the dose 0.0% mm^{-1} (Figure 2). Studies point out that biochar promotes the immobilization of copper and other heavy metals in the soil (Wang et al., 2021), reducing its availability (Munir et al., 2020) due to surface adsorption and increased soil pH (Cárdenas-Aguiar et al., 2017) — effects that clarify the lower availability of copper in the soil.

In the treatment with copper addition, the availability of the metal in the soil reduced significantly and linearly by 53.5% at a dose of 2.0% mm^{-1} of biochar in relation to a dose of 0.0% mm^{-1} of biochar (Figure 2). In this sense, the availability of copper increases in contaminated soils (Turchetto et al., 2022). Thus, making a comparison, when copper was not applied there was a reduction of 1.1 mg of copper for each 1.0% of biochar and when copper was applied there was a reduction of 243.01 mg of the metal for each 1.0% of biochar added. Results similar to the present study were described for the availability of copper in the soil with the addition of biochar, reaching a 96.0% reduction in the concentration of available copper, up to a dose of 10.0% mm^{-1} (Rehman et al., 2019), and 18.6% in soil contaminated with copper (Jia et al., 2017). Biochar has the capacity to retain a certain amount of copper, decreasing its availability to the plant (Moore et al., 2018). In addition, different pyrolysis temperatures influence the adsorption of metals: at temperatures from 300 to 500°C, biochar has a higher affinity with copper in the soil (Guo et al., 2022).

The results indicated a significant interaction between the factors for the total chlorophyll of *P. vulgaris* plants, with a quadratic adjustment for the doses of biochar in the soil, being higher in the soil without copper, and with a maximum point estimated at a dose of 1.68%.

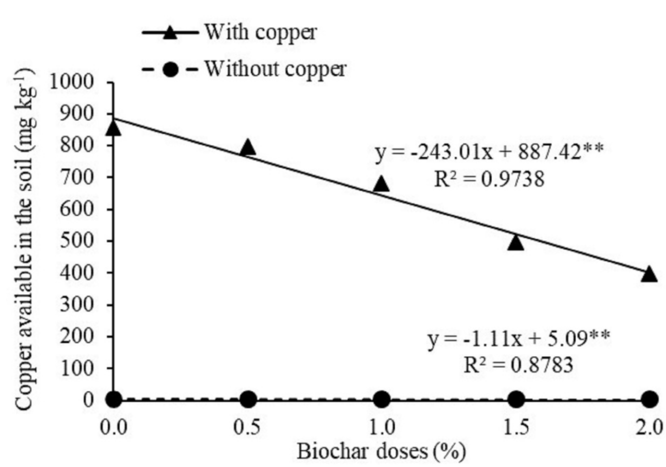

Figure 2 – Copper content available in soil without added copper (-●-), and with added copper (—▲— 1,000 mg kg^{-1}) subjected to doses of biochar (0.0% mm^{-1}, 0.5% mm^{-1}, 1.0% mm^{-1}, 1.5% mm^{-1}, and 2.0% mm^{-1}).

**significant at 5%; y: dependent variable; x: independent variable; R^2: coefficient of determination.

In soil with copper, the maximum point was observed at a dose of 1.78% (Figure 3), varying from 32.26 g g^{-1} at a dose of 0.0% to 46.10 g g^{-1} at a dose of 2.0% mm^{-1} of added biochar. Studies on the effects of excess copper in plants have shown that the metal causes a reduction in photosynthesis due to changes in photochemical reactions of photosystem II (Cambrollé et al., 2013), as it causes inhibition of the electron flow, changes in the composition of chloroplasts and in the structure of chlorophylls (Mendoza et al., 2013). The significant increase in the total chlorophyll index in relation to treatment without biochar has been attributed to the immobilization of the metal and consequent reduction of copper absorption by the plant (Rehman et al., 2019). Research indicated that the application of biochar to the soil makes it possible to increase the total chlorophyll of plants by 85% (Kamran et al., 2020). Thus, the addition of biochar to the soil up to a dose of 1.78% mm^{-1} contributed to the lower availability of copper in the soil, and consequently, to a higher total chlorophyll index of black beans.

The results showed a significant interaction between the variation factors for the copper content in the roots, aerial parts, and grains (Figure 4). There was a linear reduction in the copper content in bean roots of 43.0% in the highest dose in soil with copper and of 47.5% in soil without copper — significantly higher in soil with copper (Figure 4A). The copper content in the roots ranged from 307.71 mg kg^{-1} at a dose of 0.0% up to 71.40 mg kg^{-1} at a dose of 2.0% biochar added to the soil with copper. Research results demonstrate that biochar derived from bamboo, rice straw, and Chinese walnut shell reduces copper accumulation in the roots of moso bamboo (*Phyllostachy pubescens*) by 15.0, 35.0, and 26.0%, respectively. This is attributed to biochar having a porous structure and oxygen-containing functional groups on the surface that can immobilize pollutants in soils (Wang et al., 2019).

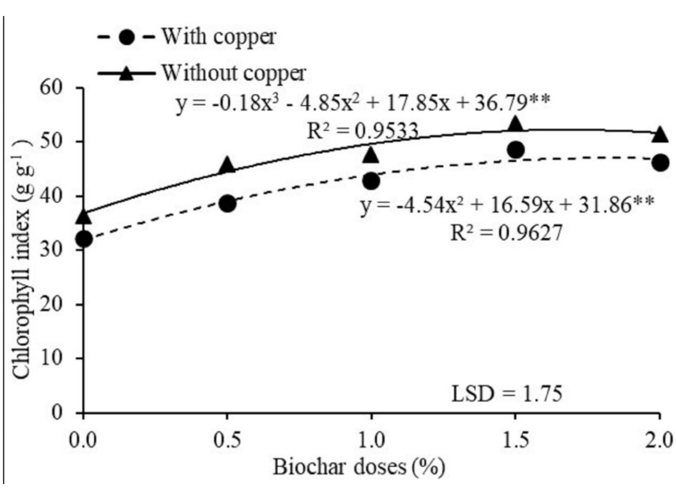

Figure 3 – Total chlorophyll index of black beans grown in soil without added copper (—▲—) and with added copper (- ● - 1,000 mg kg^{-1}) subjected to doses of biochar (0.0% mm^{-1}, 0.5% mm^{-1}, 1.0% mm^{-1}, 1.5% mm^{-1}, and 2.0% mm^{-1}).

**significant at 5%; y: dependent variable; x: independent variable; R^2: coefficient of determination; LSD: least significant difference.

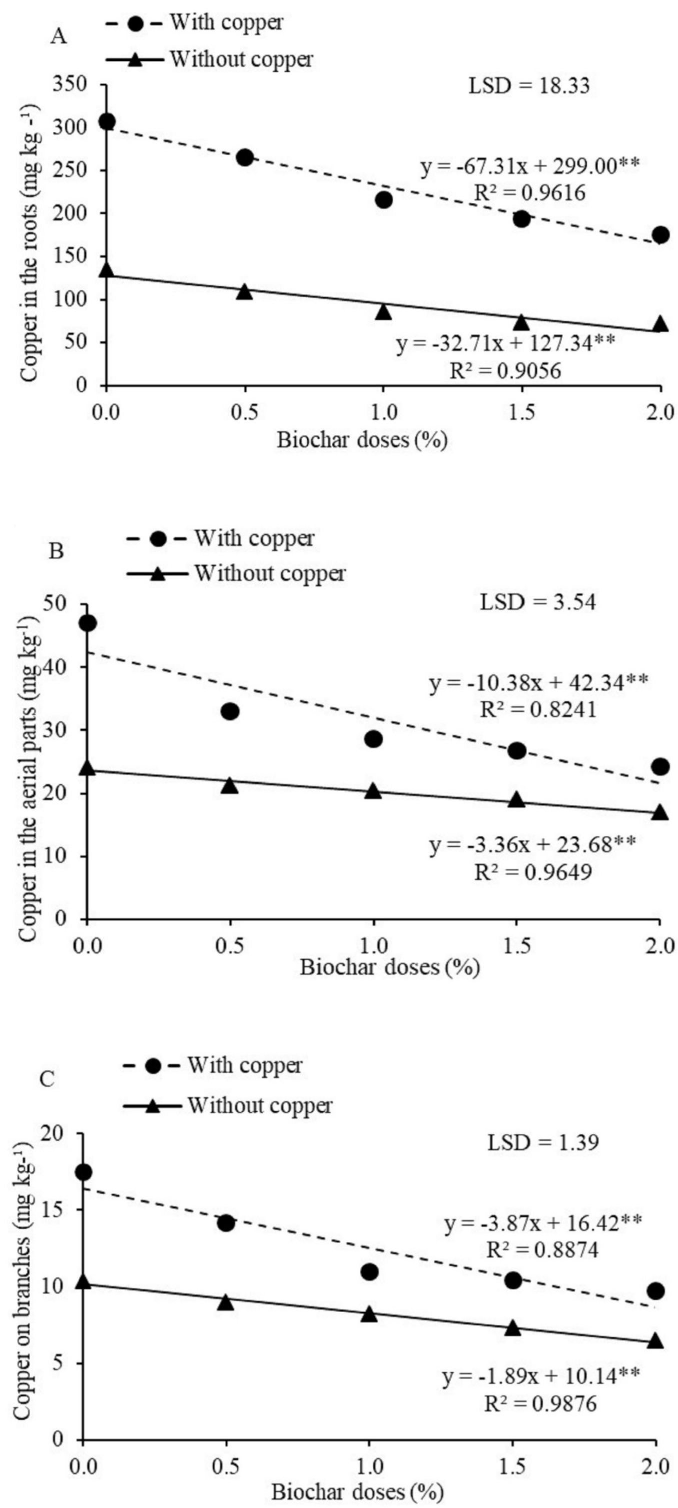

Figure 4 – Copper content in roots (A), aerial parts (B), and grains (C) of black beans grown in soil without added copper (—▲—) and with the addition of copper (- ● - 1,000 mg kg^{-1}) subjected to doses of biochar (0.0% mm^{-1}, 0.5% mm^{-1}, 1.0% mm^{-1}, 1.5% mm^{-1}, and 2.0% mm^{-1}).

**significant at 5%; y: dependent variable; x: independent variable; R^2: coefficient of determination; LSD: least significant difference.

There was a linear significant reduction in copper content in the dry mass of the aerial part of the beans with the increase in doses of biochar in the soil, being 29.95% in soil without the addition of the metal and 48.58% in soil with the addition of copper — higher in soil with copper (Figure 4B). Therefore, the contents ranged from 47.09 mg kg^{-1} at a dose of 0.0% up to 24.20 mg kg^{-1} at a dose of 2.0% biochar added to the soil with copper. These results corroborate those in the scientific literature, which showed that with the use of biochar in the remediation of sandy soil contaminated with metals and cultivated with berseem clover *(Trifolium alexandrinum*, L.), the concentration of copper in the aerial part of the plants was significantly reduced (Pescatore et al., 2022). Furthermore, the metal contents in bean leaves and the activity of anti-oxidative enzymes reduced, while the soluble protein contents increased with the application of biochar — the result being attributed to the reduction in the availability of metals in the soil and consequent lower absorption by plants (Hmid et al., 2015).

The copper content in black bean grains decreased linearly by 80.30% in soil contaminated with copper and by 60.89% in soil without copper — lower in soil without copper (Figure 4C). A study with other crops also showed a significant reduction in the accumulated copper content in the grains (Zong et al., 2021). When analyzing the regression equations, it is evident that doses of biochar greater than 1.66% mm^{-1} applied to the soil with copper (1,000 mg kg^{-1}) allow the copper content in bean grains to be below the maximum tolerated limit of 10 mg kg^{-1} established in Brazilian legislation (Brazil, 2022), and, in soil without added copper, by applying doses starting from 0.07% mm^{-1} of biochar. These results demonstrate the importance of biochar to mitigate copper contamination, enabling the production of beans in soil that receives inputs containing copper in its formulation, therefore allowing its use for human consumption.

The results showed significant interaction between the variation factors for TF and BCF with the doses of biochar added to soil with and without copper addition (1,000 mg kg^{-1}) (Figure 5). The TF in the soil without copper increased linearly reaching 0.24 in the highest dose of 2.0% biochar, being significantly higher than the treatment with copper, in which a polygonal adjustment was not possible, resulting in a mean of 0.137 (Figure 5A). According to research results, the closer to zero the translocation factor, the higher the probability of plant survival and growth in contaminated environments (Scheid et al., 2018), which indicates that the culture has low translocation of copper to the above-ground plant tissue, being even lower with the use of biochar. Studies indicate that the application of biochar derived from pig manure also reduced the translocation of copper to the aerial part of common beans (Vandionant et al., 2019).

The mean BCF was 0.053 in beans grown in soil with copper, with no adjustment to any degree of polynomials, while in soil without copper addition, there was a linear increase in BCF with the doses of biochar in the soil, being statistically superior to copper treatments (Figure 5B).

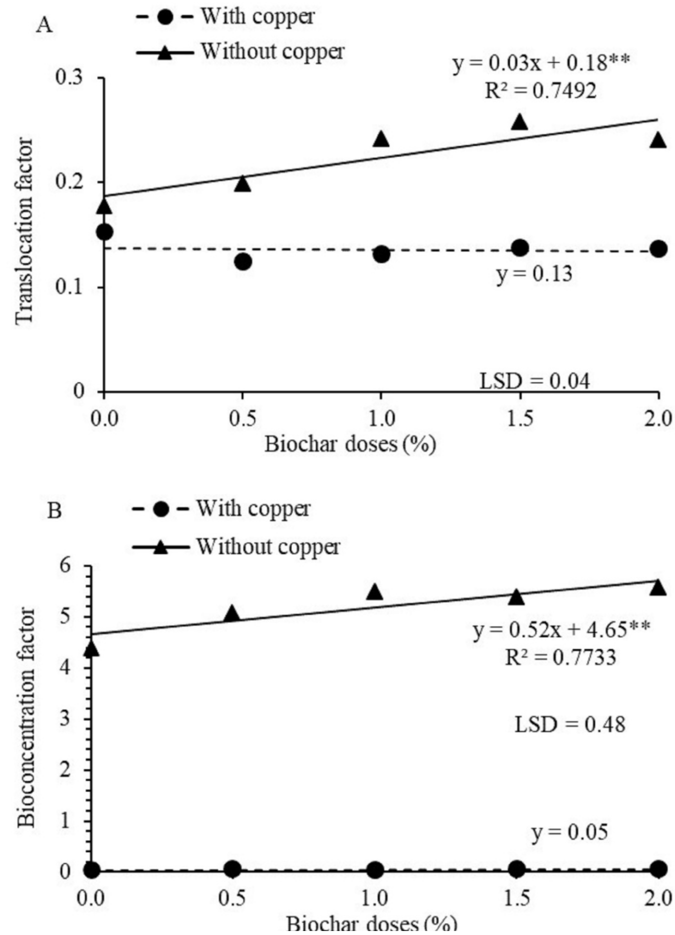

Figure 5 – Translocation factor (A) and bioconcentration factor (B) of black beans grown in soil without the addition of copper (—▲—) and with the addition of copper (- ● - 1,000 mg kg^{-1}) subjected to doses of biochar (0.0% mm^{-1}, 0.5% mm^{-1}, 1.0% mm^{-1}, 1.5% mm^{-1}, and 2.0% mm^{-1}).

**significant at 5%; y: dependent variable; x: independent variable; R^2: coefficient of determination; LSD: least significant difference.

When BCF is less than 1.000, it indicates that the concentration of metals was restricted to the roots and the plant has low translocation to the aerial part (Rashid et al., 2023), which demonstrates the importance of applying biochar in the cultivation of plants in soil contaminated with heavy metals.

However, the doses of biochar in soil without copper addition increased the bioconcentration and translocation of copper in *P. vulgaris*. Research results indicate that, in uncontaminated soil, there is an improvement in the nutrient absorption capacity of cultivated plants with the application of biochar (Uzoma et al., 2011). In this case, since it is a plant grown for human food, the biochar would act as an input for grain production once the copper content in the grains remained below the maximum limit established in Brazilian legislation, as shown in Figure 3C.

Plants that are tolerant to high concentrations of heavy metals are generally able to compartmentalize metal ions, sequestering them in vacuoles and thus excluding them from cell sites where processes such as cell division and respiration occur (Singh et al., 2012). The process of translocation of metals in plants is a crucial factor in determining their distribution in plant tissues (Tauqeer et al., 2016). In the present study, biochar allowed lower copper contents in the plants and grains of beans, even when exposed to a high concentration of the element in the soil, and demonstrated the potential for remediation of contaminated areas, reducing the availability of copper in the soil.

Conclusions

Biochar derived from eucalyptus residues decreases copper availability in contaminated soil.

The copper levels in the roots, aerial parts, and grains of *P. vulgaris* are reduced with the application of biochar to the soil, remaining in the grains at a dose of 1.66% mm^{-1} — below the maximum limit tolerable by Brazilian legislation.

Contribution of authors

FONTANIVE, D.E.: conceptualization; data curation; formal analysis; research; methodology; validation; visualization; writing — original draft; writing — review and editing. RAFAELE, D.M.: conceptualization; data curation; research; methodology; visualization; writing — original draft. ANDREOLA, D.S.: conceptualization; data curation; research; methodology; visualization, writing — original draft. STUMM, J.O.: conceptualization; data curation; formal analysis; research; methodology; visualization, writing — original draft. SERAFINI, R.F.: conceptualization; data curation; formal analysis; research; methodology; visualization, writing — original draft. SILVA, D.M.: conceptualization; investigation; supervision; validation; visualization; writing — review and editing. LUZ, F.B.: conceptualization; investigation; supervision; validation; visualization; writing — review and editing. ROS, C.O.: conceptualization; investigation; resources; supervision; validation; visualization; writing — review and editing. SILVA, R.F.: conceptualization; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing-original draft; writing — review and editing.

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