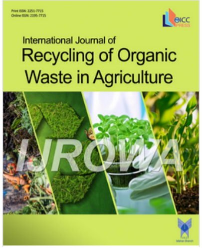

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Cymbopogon citratus biochar as a fertilizing and remediating agent for soils with high copper content

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Original Research

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Abstract:

Purpose: The use of biochar and its products has been increasing in recent years. However, although being studied, most of the potential of biochar to be used as a remediating and fertilizing agent is still unclear. Given this, the present work aimed to assess the effect of different concentrations of *Cymbopogon citratus* (lemongrass) biomass applied to soil contaminated with Cu.

Method: Using natural soil, seven treatments were tested, with three replicates each: soil pH 4.7 (T1), soil pH 6.0 (T2), soil pH 6.0, and adding 5 wt.% biochar (T3), soil with 50 mg·kg^{-1} Cu (T4), soil at pH 6.0 with 50 mg·kg^{-1} Cu (T5), soil with 50 mg·kg^{-1} Cu and addition of 5 wt.% biochar (T6) and soil with 50 mg·kg^{-1} Cu and addition of 10 wt.% biochar (T7). The treatments were incubated for 30 days in 30 cm^3 containers. Afterward, the soil and the leached solution were analyzed. Subsequently, the incubated soil was transferred to recipients, and seedlings of *Catharantus roseus* were transplanted, totaling twelve plants per treatment. After 60 days, the plants were evaluated according to the biometric parameters of plant size and root size, root volume, and fresh and dry mass of the plant and roots. The contents of macronutrients and Cu in plant tissue were also determined.

Results: The data showed that *C. citratus* biochar acted as a soil acidity-neutralizing agent at 5 wt.% and 10 wt.%. Adding biochar increased all biometric parameters of *C. roseus* seedlings. Using the biochar also reduced the Cu levels in the plant tissue, although the treatment with 10 wt.% biochar had the highest copper content in the leachate.

Conclusion: According to this study, *C. citratus* biochar has the potential to be used as soil fertilizer and remediating agent.

Keywords: Heavy metals; Leachate; Micronutrients; Phytotoxicity; Soil contamination

1. Introduction

Soil contamination by copper (Cu^{2+} and Cu^{+} ions) is associated with agricultural and industrial activities and urban and/or agricultural waste (Caires et al., 2011). Soils from sedimentary material tend to have 2 mg·kg^{-1} of copper, while soils from basaltic rocks tend to have contents of more than 150 mg·kg^{-1} of copper. In Brazil, Conama Resolution 420/2009 establishes that the limit of acceptable copper in the soil is 60 mg·kg^{-1}. Above this level, intervention for remediation is necessary to avoid environmental concerns (Gasparin, 2023).

In agricultural production, the most common situations of soil contamination with copper occur in fruit plant cultivations due to using copper-based fungicides (Gasparin, 2023). In Brazil, the state of Rio Grande do Sul concentrates the largest wine-growing region in the country (Casali et al., 2008), accounting for approximately 49.3% of national production in 2021 (Sul, 2023). To obtain a high yield and superior quality in fruit production, the intensive use of fungicides occurs especially to control important phytopathogens in grapevine cultivation (Silva et al., 2010), such as downy mildew caused by the fungus *Plasmopara viticola*, anthracnose whose causative agent is the fungus *Elsinoë ampelina*, and powdery mildew caused by the fungus *Erysiphe necator* var. *necator* Schwein (*Oidium tuckeri* Berk), among others (Nogueira et al., 2017).

Many of these antifungal products contain copper ions in their composition due to the biocidal effect of copper (Casali et al., 2008; Nogueira et al., 2017). With the use of fungicides containing copper, it is estimated that approximately 30 $kg{\cdot}ha^{-1}$ of this element are added to the soil in each vine cycle, exceeding the maximum adsorption capacity of this element (Casali et al., 2008), and its critical content (above 0.4 $mg{\cdot}dm^{-3}$) in the soil (SBCS 2016).

Copper is a micronutrient for plants and animals, being required at microgram doses. However, at high doses (above a critical threshold), this element can cause important physiological disorders (SBCS 2016). Thus, in soils with high clay and/or organic matter levels and high pH (close to or greater than 6.0), there is greater adsorption and immobilization of copper in the soil, either by binding with clay particles, complexation with organic matter, or rendering it unavailable for absorption due to the formation of copper hydroxide at neutral and alkaline pH values. The adsorption and immobilization of copper in the soil help reduce this metal's phytotoxicity and leaching through the soil profile (Melo et al., 2013). Coming from the use of products from non-biodegradable sources, the Cu^{2+} ion (Cárdenas-Aguiar et al., 2017) is often related to the use of cupric fungicides, directly interfering with the availability of this nutrient to plants, also causing different levels of toxicity when in higher contents (Casali et al., 2008).

In grapevines, excess copper can lead to iron deficiency because it hinders plants' iron absorption. In high concentrations in the soil, copper also causes a reduction in the absorption of molybdenum and zinc, causing chlorosis, white veins, deformations, and necrosis of the margins of the leaves due to the excess nitrate. This is because molybdenum is a precursor of nitrate and nitrogenase reductase enzymes (Albuquerque, 2002). Excess copper in the crop may also be responsible for low pollen germination, resulting in low flower fertilization and berry drop (Silva et al., 2010). Phytotoxicity caused by excess copper in the soil leads to delays in seedlings' development and reduced biomass in cover crops. In plants such as *Avena sativa* (oat), excess copper hinders photosynthetic processes and disrupts the root morphology of plants (Scheibe et al., 2022).

Using adsorbent materials, which have a certain degree of porosity and a greater specific surface area, such as clay minerals and activated carbon, facilitates the adsorption of heavy metals in the soil. Activated carbon is a carbonaceous material with a non-graphitic microcrystalline structure, whose internal porosity is compared to a network of tunnels that allow its use in the removal of organic micropollutants, such as pesticides, pharmaceuticals, and heavy metals, among others (Fernandes, 2021; Liang et al., 2021). In this way, biochar can be an alternative to soil remediation, as it has been used as a carbon-based adsorbent and fertilizer, with direct addition to the soil (Gascó et al., 2012; Lei et al., 2009; Liang et al., 2021). Biochar has a porous and stable structure composed chiefly of carbon and, to a lesser extent, of other elements such as nitrogen, potassium, and magnesium, depending on the source material (Kavitha et al., 2018). Its amorphous and graphitic structure is markedly characterized by the presence of aromatic carbon (Kocsis et al., 2022), and with increasing pyrolysis temperature, the biomass structure changes from an amorphous state to a more crystalline structure, tending to graphitization (Lehmann and Joseph, 2009). Through pyrolysis, it is possible to manage residues from different sources, such as agroindustry, forestry, sewage sludge, and biomass residues from the essential oil industry, obtaining products such as biochar (solid fraction), bio-oil (liquid fraction), and non-condensable gases (gaseous fraction) (Gascó et al., 2012; Liang et al., 2021; Qiu et al., 2022).

Cymbopogon citratus, commonly known as 'lemongrass', is a plant native to India and belonging to the *Poaceae* family. It is characterized by developing plants with long, narrow, light green leaves, forming large clumps, and presenting citral as the predominant compound of its essential oil (Oliveira and Santos, 2021). In the essential oil extraction industry from *C. citratus*, it is possible to obtain a minimum essential oil yield of 0.5% v/w from the dry mass, containing approximately 60 wt.% citral (Anvisa, 2019), with the residual *C. citratus* biomass an agroindustrial residue from this extraction. Since this waste is primarily underused, employing this biomass as a feedstock for pyrolysis may add value to this material, also helping mitigate the generation and disposal of agroindustrial wastes.

Applying biochar in soil can be a sustainable alternative for removing or immobilizing contaminating copper and other heavy metals (Cárdenas-Aguiar et al., 2017). The contaminant removal effectiveness depends on the porosity and cation exchange capacity of the biochar, properties that are influenced by the composition of the biomass, reactor kind, pyrolysis temperature, and the residence time of the pyrolyzing material during the process (Lahori et al., 2017).

Vithanage et al. (2017) observed the effects of soybean straw biochar using concentrations of 0.5 wt.%, 1.0 wt.%, and 2.5 wt.%. The authors verified that in soils contaminated with copper, the immobilization of this metal in the soil obtained an efficiency of up to 16.2%. Precipitation and adsorption were the interactions indicated as involved between copper ions in the soil and the surface of the biochar. In addition, the study pointed out the potential of biochar from soybean straw as a low-cost technology for the remediation of soils contaminated with copper.

Cárdenas-Aguiar et al. (2017) studied the effects of biochar and biochar associated with organic compost applied to the soil to verify copper mobility in remediated soil with 1,000 mg/kg of copper. Treatments were performed with 10 wt.% biochar and 10 wt.% biochar and compost. For four weeks, the soils were cultivated with plants of different species (mustard, watercress, and ryegrass). Afterward, the authors observed reduced soil copper mobility in both treatments and greater root length in remediated soil than in those contaminated.

On the other hand, Barbosa (2020) reported the phytoremediation effects of copper by *Catharantus roseus* on substrates based on sewage sludge. *C. roseus* is native to Madagascar, is popularly known as bright eyes, Madagascar periwinkle, and old maid, among other common names (Barbosa, 2020), and is commonly used as an ornamental plant. This species belongs to the Apocynaceae family and has branched foliage, simple pink flowers, and oval leaves (Reis and Henrique, 2007).

Although there is an increasing interest in the use of biochar in agriculture, with several studies assessing its fitness for such uses, much of the real potential for this type of material is unexplored, especially when considering its effect as a remediating agent for areas contaminated with heavy metals (Kuppusamy et al., 2016; Luo et al., 2023; Masud et al., 2023).

The need to contain the harmful effects of high concentrations of copper in vineyard soils, reducing the impacts of anthropic actions arising from phytosanitary management, is a growing concern. Considering the unexplored potential of using *Cymbopogon citratus* biochar as a remediating and fertilizing agent, the present work aimed to evaluate the effects of applying different concentrations of biochar obtained through slow pyrolysis of *C. citratus* biomass in contaminated soil, seeking to assess the immobilization of this metal in the biochar-treated soil and its accumulation in *C. roseus* plants.

2. Material and methods

The experiment was carried out in two stages at the University of Caxias do Sul (UCS) during September 2022 and February 2023. The first stage was conducted with the application of biochar and evaluation of the soil and leachate, and the second stage was carried out with the planting of *C. roseus* seedlings to verify the effects of biochar treatments on plant development.

A non-treated soil sample was collected in the experimental area of the UCS in the district of Fazenda Souza, Caxias do Sul, at a depth down to 20 cm, with no previous history of agricultural use. The collected soil was dried in an oven with forced air circulation at 45 – 50 °C. A hammer mill (De Leo, Brazil) was used to grind the soil, which was homogenized through a 2.0 mm mesh sieve. Soil fertility was assessed according to the methods described by Tedesco et al. (1995). The soil used in the experiment was classified as cambisol (Streck et al., 2008), presented a clayey textural class, and was composed of 50 wt.% clay, 22 wt.% sand, and 28 wt.% silt. The soil had the following fertility parameters: pH-H_2O 4.7, pH-SMP 4.3, clay 49% w/v, organic matter 4.1% w/v, P < 0.5 mg/dm^3, K 63.4 mg/dm^3, Ca $<$ 0.4 cmol$_c$/dm^3, Mg 0.3 cmol$_c$/dm^3, Al > 7.0 cmol$_c$/dm^3, H + Al 30.4 cmol$_c$/dm^3, S < 4.1 mg/dm^3, Cu 2.7 mg/dm^3, Zn < 0.2 mg/dm^3, B 0.5 mg/dm^3, Mn 10.6 mg/dm^3, effective exchange cation capacity (CECeff) 7.9 cmol$_c$/dm^3, CEC at pH 7 31. cmol$_c$/dm^3, base saturation 9.6%, Al saturation 90.4%.

The biochar was obtained by the slow pyrolysis of lemongrass biomass (shoot, stems, and leaves) in an Auger-type reactor at a final pyrolysis temperature of 450 °C, heating rate of 10 °C/min and residence time of the material in the reactor of approximately 90 min. The biochar obtained was previously ground and sieved (particle size of 2.0 mm), following the same procedure and parameters used for the soil.

According to physical-chemical characterization, the biochar used had the following composition: pH 10.04, electrical conductivity 5.80 dS/m, moisture 9.6 wt.%, volatile matter 27.4 wt.%, ash 20.0 wt.%, fixed carbon 43.1 wt.%, average density 160 kg/m^3, B 14.5 mg/kg, Ca 12.1 g/kg, Cu 24.7 mg/kg, S 1.7 g/kg, Fe 3.8 g/kg, P 5.2 g/kg, Mg 7.3 g/kg, Mn 120.6 mg/kg, N 11.9 g/kg, K 44.5 g/kg, Zn 114.4 mg/kg, Mo 1.7 mg/kg, Cr^{+6} 2.7 mg/kg, Cr^{+3} 19.3 mg/kg, As < 0.5 mg/kg, Cd 1.2 mg/kg, Pb 22.3 mg/kg, Hg < 0.2 mg/kg, Al 8.6 g/kg, Co 3.7 mg/kg, Sn < 0.3 mg/kg, Sr 167.5 mg/kg, Ni 200.5 mg/kg.

The experimental design was completely randomized, with seven treatments and three replications containing seven containers (tubes), totaling 21 tubes for each treatment. Tubes of 30 cm^3 were used to carry out the tests. Soil samples were homogenized with different proportions of biochar according to each treatment. Limestone (dolomitic limestone, DB, Brazil) was also used in some treatments to adjust the soil pH close to 6.0 (SBCS 2016). The presence of copper ions in the soil was increased by adding an aqueous solution (4.91 mg/L) of cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99.9% purity, Labsynth, Brazil) to some treatments so that the theoretical final concentration of available copper in the samples was approx. 50 mg/kg. The codification and details of the treatments assessed in the study are compiled in Table 1.

After placing the material, 10 mL of distilled water was added to each tube, keeping it close to the capacity of the

Table 1. Codification and description of the treatments used in the experiment.

Codification	Treatment
T1	Soil (pH 4.7)
T2	Soil + limestone (pH 6.0)
T3	Soil + limestone (pH 6.0) + 5 wt.% biochar
T4	Soil with 50 mg·kg^{-1} Cu
T5	Soil with 50 mg·kg^{-1} Cu + limestone (pH 6.0)
T6	Soil with 50 mg·kg^{-1} Cu + 5 wt.% biochar
T7	Soil with 50 mg·kg^{-1} Cu + 10 wt.% biochar

Source: Authors (2023).

container to moisten the treatments. Samples were incubated for 30 days under laboratory conditions and covered by plastic to prevent evaporation. Fig. 1 shows the biochar incubation phase.

After, the first evaluation of the experiment was carried out. In each repetition, 60 mL of soil was collected for soil analysis. Another 30 mL of each sample was rinsed with distilled water until 25 mL of leachate was obtained. *C. roseus* seedlings were planted in the tubes and conducted in three repetitions of four tubes, totaling twelve tubes per treatment. Fig. 2 shows the aspect of the plants during the experiment.

Sixty days after transplanting the seedlings, the plants were collected, and the biometric parameters of plant and root size (cm), root volume (cm^3), and fresh and dry mass of plant and root (g) were assessed.

The plants and roots were dried in an oven (De Leo, Brazil) with forced air circulation for 48 h at 35 – 40 °C to determine the dry mass. Soil analyses were carried out according to the procedures proposed by Tedesco et al. (1995). The macronutrients (N, P, K, Ca, Mg, and S) and Cu contents present in biochar, leachate, and plant material were determined according to the methods described by Malavolta et al. (1997).

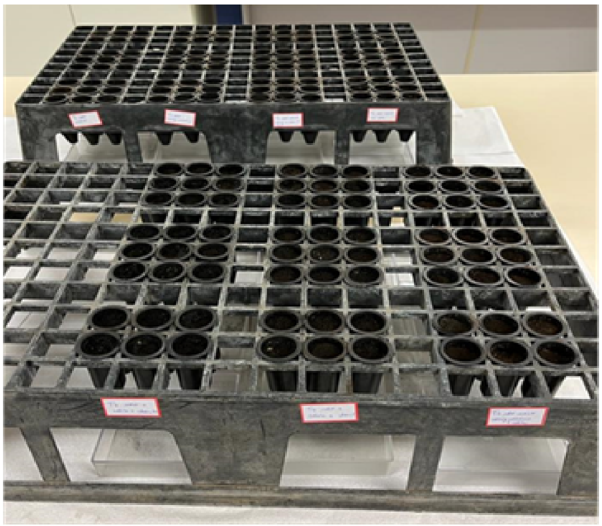

Figure 1. Incubation of biochar in soil before the experiments with *C. roseus*.
Source: Authors (2023).

Figure 2. Growth of *C. roseus* plants in a greenhouse during the second phase of the experiment.
Source: Authors (2023).

The results were assessed for homoscedasticity (Levene's test) and normality of residuals (Shapiro-Wilk test) and underwent analysis of variance (ANOVA). Parameters that showed statistical significance were analyzed using Tukey's multiple comparison test with a 95% confidence interval ($\alpha = 0.05$). Statistical analyses were performed using the AgroEstat® software (Brazil).

3. Results and discussion

Table 2 presents the pH, organic matter, and copper content results relative to the first soil evaluation carried out 30 days after biochar incubation in the soil samples.

Biochar application into the soil at 5 wt.% and 10 wt.% raised the pH, as observed in T6 and T7 (5.7 and 6.4), although to a lesser extent than in the treatments with the addition of limestone (T2, T3, and T5). The organic mat-

Table 2. Results of pH, organic matter, and copper content in the soil for the different treatments assessed after 30 days of incubation of the biochar in the soil.

Codification	Treatment	pH	Organic matter (% w/v)	Available copper ($mg \cdot dm^{-3}$)
T1	Soil (pH 4.7)	4.7 d	3.2 ab	2.7 b
T2	Soil + limestone (pH 6.0)	6.8 a	3.2 ab	1.1 b
T3	Soil + limestone (pH 6.0) + 5 wt.% biochar	6.9 a	3.3 ab	1.0 b
T4	Soil with 50 $mg \cdot kg^{-1}$ Cu	4.7 d	3.3 ab	26.8 a
T5	Soil with 50 $mg \cdot kg^{-1}$ Cu + limestone (pH 6.0)	6.7 a	3.0 b	17.0 a
T6	Soil with 50 $mg \cdot kg^{-1}$ Cu + 5 wt.% biochar	5.7 c	3.3 ab	25.7 a
T7	Soil with 50 $mg \cdot kg^{-1}$ Cu + 10 wt.% biochar	6.4 b	3.4 a	21.5 a
	Coefficient of variation (%)	1.5	3.8	33.4

Means in columns followed by the same letter do not differ statistically by Tukey's multiple range test at a 5% error probability ($\alpha = 0.05$). Source: authors (2023).

ter contents showed a statistically significant difference in T5 and T7 (3.0% and 3.4% w/v). However, no difference was observed regarding the control (T1) and the other treatments.

The results for the copper contents obtained from T1 to T3 allowed observing the effect of adding the aqueous solution of $CuSO_4$ since the contents of available copper in the soil did not differ statistically between the treatments that had the addition of Cu to the soil (T4 to T7, 17.0 – 26.8 mg/dm^3). However, the final available amount observed in soils that received copper was around 50% of the total added, considering that the amount of available copper added to the samples by the biochar was negligible (< 0.01 mg/L). It is also essential to notice that T2, T3, and T5 had higher pH values mainly because of the addition of limestone, the most used material to increase soil pH in agricultural practices.

Ippolito et al. (2017) verified the effects of different biochars, pyrolyzed in the range of 500 – 700 °C, on the remediation of metals in mine soils. However, Silvestre et al. (2018), observing the effects of different biochars, emphasized that biochar can be used as an auxiliary agent in liming, as it raises soil pH when applied. It should be noted that, although lower than the pH obtained by applying limestone, the biochar dose of 10 wt.% increased the soil pH to 6.4, within the ideal pH range for several crops (5.5 – 6.5), as SCBS (2016) recommended. Melo et al. (2013), evaluating the effects of applying limestone in the soil to reduce copper toxicity in white oats, emphasized the importance of raising soil pH to control the toxicity levels of this metal and other micronutrients to plants.

Martins et al. (2011) highlighted that among the properties that interfere in the chemical distribution, mobility, and availability of metals such as Cu is the pH, as it increases the cation exchange capacity (CEC) of the soil by deprotonation of the acidic groups of the organic matter and of the surface of the oxides, reducing the availability of copper in the soil. As commented by Yang et al. (2020), the application of biochar has a positive effect on the soil organic matter, increasing the activity of microorganisms and the contents of total carbon in the soil. Silvestre et al. (2018) pointed out that high soil pH can make harmful elements such as aluminum and micronutrients such as copper, zinc, manganese, and iron insoluble due to the formation of their respective hydroxides, which are insoluble, and the chelating effect of biochar.

Ferreira et al. (2018) observed that the presence of microorganisms in the soil can assimilate the carbon present in biochar, increasing the levels of organic matter. Qiu et al. (2022) and Martins et al. (2011) related the presence of functional groups, such as phenols and carboxylic acids in organic matter and biochar as a factor that favors complexation with metals such as copper in the soil, reducing the toxicity of this metal. In addition, Ferreira et al. (2018) also pointed out that the metabolism of these organic chains by microorganisms helps increase the levels of humic and fulvic acids, components of soil organic matter that help provide sources of organic nitrogen.

Qiu et al. (2022), evaluating the effects of biochar in the removal and complexation of soil contaminants, also highlighted the importance of clay as an auxiliary material in soil decontamination. Thus, adding biochar to the soil of the experiment containing 50 wt.% clay, there is the possibility of obtaining an auxiliary agent for soil remediation together with its clay. Melo et al. (2013) pointed out that soils with high clay content allow for greater adsorption and immobilization of copper and other heavy metals through complexation, interaction with the clay fraction, and/or formation of hydroxides.

The behavior observed for copper contents in the treatments may be related to soil clay content. Silvestre et al. (2018) observed the chelating effect of fodder radish (*Raphanus sativus*) biochar, associated with the increase in pH, to reduce copper availability for plants, preventing contamination and exposition to toxic levels of this metal. According to Yang et al. (2022), the fate of biochar in the soil and its efficacy is associated with biochar processing and aging and also with the clay content of the soil, pointing out the interaction between the soil and the biochar for proper incorporation and action of the latter on the former.

According to Ferreira et al. (2018), biochars can potentially retain (adsorb) nutrients for plants, reducing groundwater contamination as it minimizes the effects of metal leaching into the soil. Meier et al. (2021) emphasized that biochar can activate soil microbiota by providing sources of organic carbon and, thus, making copper unavailable in the soil solution. Microorganisms can use this carbon made available by biochar and, in this way, there is an increase in microbial proliferation, with a consequent rise in copper immobilization by the growing microbial biomass. However, it is important to observe that biochar addition may change the physical-chemical parameters of the soil. This can affect the relationship between macro and microporosity, which interferes with the adsorption, percolation, and lixiviation processes within the soil profile (Silvestre et al., 2018).

Table 3 presents the results obtained from evaluating copper contents in the leachate of the different treatments studied. The results of the leachate evaluation demonstrated that the treatment with the addition of 10 wt.% biochar (T7) had the highest Cu concentration available (0.107 mg/L) in the leachate (aqueous medium). It is possible to observe that the treatments with limestone and without biochar (T2 and T5; 0.014 mg/L both) did not differ from the control (T1; 0.003 mg/L). In contrast, all treatments with biochar (T3, T6, and T7) were statistically higher than the copper content observed in the leachate of the natural soil (T1).

However, the treatments with biochar showed the highest concentrations of copper in the leachate (T3, T6, and T7), which may be associated with the need for a longer interaction time between the biochar and the soil to adsorb the copper and avoid the leaching of this element, since in the experiment carried out the biochar and soil mixture was incubated for 30 days. Puga (2015), evaluating the adsorption of Cd, Pb, and Zn in soil by sugarcane straw biochar, reported an incubation period of 120 days for the mixture of biochar and soil. Sousa (2015), evaluating dry mass and length of radish plants, also observed an increase in these parameters in the plants treated with 20 kg/ha and 60 kg/ha of sewage sludge biochar.

Table 3. Copper concentration observed for the leachate of the different treatments evaluated in the present study.

Codification	Treatment	Copper concentration (mg·L^{-1})
T1	Soil (pH 4.7)	0.003 c
T2	Soil + limestone (pH 6.0)	0.014 bc
T3	Soil + limestone (pH 6.0) + 5 wt.% biochar	0.029 b
T4	Soil with 50 mg·kg^{-1} Cu	0.012 bc
T5	Soil with 50 mg·kg^{-1} Cu + limestone (pH 6.0)	0.014 bc
T6	Soil with 50 mg·kg^{-1} Cu + 5 wt.% biochar	0.024 b
T7	Soil with 50 mg·kg^{-1} Cu + 10 wt.% biochar	0.107 a
	Coefficient of variation (%)	25.2

Means in columns followed by the same letter do not differ statistically by Tukey's multiple range test at a 5% error probability ($\alpha = 0.05$). Source: authors (2023).

The increase in copper concentration in T6 and T7 relative to T4 (Table 3) may result from an adsorptive interaction between the biochar particles and the copper ions, mobilizing them and making them more easily leachable. Although Gonzaga et al. (2020) commented that using biochar tends to reduce the most available form of Cu in the soil after long incubation periods (24 months), this material also increased the Cu associated with OM. Since the soil used in the experiment had a high OM content (4.1% w/v), the applied biochar may have rendered the most available Cu forms to OM-associated Cu, which was extracted by water during the leaching tests. On the other hand, the same authors observed that short incubation periods (about one week) increased the content of available and extractable copper in soil solution. Tang et al. (2023) reported a different behavior, in which applying 14 different biochars in a chromated copper arsenate-contaminated soil was ineffective or had dubious results in reducing the content of water-extractable Cu.

According to Liu et al. (2022), there is a vast and largely unexplored potential for biochar to be used as a remediating agent in soils of contaminated areas. However, specific properties of both the soil and the biochar must be matched for improved remediation efficiency. In addition, the remediation can be direct, by interactions between the biochar and the heavy metal ions, or through indirect means, i.e., the biochar helping amend the environment and, therefore, affecting the heavy metal absorption and transportation through the food chains. Both situations should be considered when assessing the biochar's fitness as a remediating and fertilizer agent.

The visual assessment of the *C. roseus* plants grown for 60 days in soil treated with different doses of lemongrass biochar is presented in Fig. 3.

Table 4 presents the results relative to the biometric parameters of *C. roseus* plants grown with different treatments. Regarding plant and root lengths, a greater increase can be observed in T3, T6, and T7 treatments (plant lengths of 8.64

Figure 3. Visual aspect of *C. roseus* plants grown in biochar-treated soil 60 days after planting. T1 – soil (pH 4.7); T2 – soil + limestone (pH 6.0); T3 – soil + limestone (pH 6.0) + 5 wt.% biochar; T4 – soil with 50 mg·kg^{-1} Cu; T5 – soil with 50 mg·kg^{-1} Cu + limestone (pH 6.0); T6 – soil with 50 mg·kg^{-1} Cu + 5 wt.% biochar; T7 – soil with 50 mg·kg^{-1} Cu + 10 wt.% biochar. Source: Authors (2023).

Table 4. Results of biometric parameters of *Catharantus roseus* plants grown in soil with different doses of lemongrass biochar.

Cod.	Treatment	Plant length (cm)	Root length (cm)	Plant dry mass (g)	Root dry mass (g)	Root volume (mL)
T1	Soil (pH 4.7)	5.51 c	4.46 c	0.18 bc	0.04 c	0.50 d
T2	Soil + limestone (pH 6.0)	5.04 c	6.29 b	0.14 bc	0.04 c	0.79 cd
T3	Soil + limestone (pH 6.0) + 5 wt.% biochar	8.64 ab	7.67 a	0.39 a	0.12 a	1.92 a
T4	Soil with 50 mg·kg^{-1} Cu	4.34 c	3.08 d	0.11 c	0.06 bc	0.50 d
T5	Soil with 50 mg·kg^{-1} Cu + limestone (pH 6.0)	7.24 b	5.33 bc	0.20 b	0.06 bc	0.92 c
T6	Soil with 50 mg·kg^{-1} Cu + 5 wt.% biochar	9.40 a	7.79 a	0.34 a	0.11 ab	1.50 b
T7	Soil with 50 mg·kg^{-1} Cu + 10 wt.% biochar	9.82 a	7.79 a	0.36 a	0.11 ab	1.58 ab
	Coefficient of variation (%)	8.07	7.82	11.53	22.71	12.39

Means in columns followed by the same letter do not differ statistically by Tukey's multiple range test at a 5% error probability ($\alpha = 0.05$). Source: authors (2023).

Table 5. Macronutrients and copper contents in the plant tissue of *Catharantus roseus* grown in soil with different doses of lemongrass biochar.

Cod.	Treatment	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})	Cu (mg·kg^{-1})
T1	Soil (pH 4.7)	14.3 b	36.7 bc	18.3 b	4,6 e	1.0 e	9.0 b
T2	Soil + limestone (pH 6.0)	16.3 a	39.5 a	12.5 e	10.9 a	4.8 a	5.2 d
T3	Soil + limestone (pH 6.0) + 5 wt.% biochar	8.7 c	36.7 bc	14.8 d	7.1 c	3.3 b	4.5 e
T4	Soil with 50 mg·kg^{-1} Cu	16.7 a	35.4 c	16.0 c	4.8 d	1.0 e	10.7 a
T5	Soil with 50 mg·kg^{-1} Cu + limestone (pH 6.0)	14.5 b	36.6 bc	12.8 e	9.7 b	4.8 a	5.8 c
T6	Soil with 50 mg·kg^{-1} Cu + 5 wt.% biochar	8.8 c	39.6 a	18.3 b	4.3 f	1.4 c	4.9 d
T7	Soil with 50 mg·kg^{-1} Cu + 10 wt.% biochar	9.3 c	37.6 b	22.8 a	3.4 g	1,3 d	5.0 d
	Coefficient of variation (%)	3.8	1.5	2.2	0.5	1.2	1.8

Means in columns followed by the same letter do not differ statistically by Tukey's multiple range test at a 5% error probability ($\alpha = 0.05$). Source: authors (2023).

cm, 9.40 cm, and 9.82 cm, and root lengths of 7.67 cm and 7.79 cm, respectively) in which lemongrass biochar was applied in the soil. This same increment tendency can be noticed when observing the plant and root dry mass results in these three treatments.

The evaluation of the results obtained in T2 and T5 for the biometric parameters demonstrates that the sole application of limestone is not enough to enhance plant growth, unlike the treatments using lemongrass biochar. Such a phenomenon may be attributed to a higher nutrient availability for plant absorption. This indicates that besides the soil acidity-neutralizing properties, the biochar probably supplied the soil with additional nutrients, showing fertilizing properties. Silvestre et al. (2018) and Ferreira et al. (2018), assessing the effect of applying biochar on soil fertility parameters, observed that adding biochar increased some fertility parameters of the soil, such as pH, P, K, and Ca contents, and soil porosity.

Meier et al. (2021) observed that three different biochars obtained from chicken manure, oat husk, and pine bark reduced the available copper content in the soil, allowing for the increase in dry mass of *Lolium perenne* plants, probably because of reduced copper absorption. Ohland et al. (2019) evaluated the effects of different limestone concentrations on jatropha and observed that the copper contents in the plant tissue were not influenced by increasing limestone dosages.

Table 5 presents the results obtained for the contents of macronutrients and copper in the plant tissue of *C. roseus* relative to the different treatments assessed.

Regarding the copper content in the plant material, the treatments with limestone (T2 and T5) and biochar (T6 and T7) presented lower copper content ($4.9-5.0$ mg·kg^{-1}), possibly caused by a lower availability for the plants. The concurrent application of limestone and biochar incorporation (T3) yielded the lowest copper concentration in the plant material (4.5 mg·kg^{-1}).

Abdullah et al. (2021) found that different types of biochar from rice husks, empty fruit bunches, and palm kernels reduced the bioavailability of copper in the soil for corn plants and reduced the levels of available copper in the soil, preventing the absorption of this metal by the plants at harmful and/or toxic levels. The physiological stress caused to seedlings of *C. roseus* in the treatments with Cu was verified by the lower development of T4 regarding the biometric parameters of root length and volume. It was possible to observe the incorporation of biochar in both concentrations of 5 wt.% and 10 wt.% (T3, T6, and T7) allowed for a reduction of Cu effects in the soil. Moreover, biochar can also supply nutrients such as P (T6) and K (T7), helping plant development. However, N, Ca, and Mg contents of the plant material were lower in the treatments with the addition of biochar.

It was possible to observe an increase in Cu content in the leachate by applying 10 wt.% biochar (Table 3). Nevertheless, using biochar at 5 wt.% and 10 wt.% in the soil favored the development of *C. roseus* plants regarding the biometric parameters and reduced Cu content in plant tissue. Incorporating biochar in the soil and its combination with limestone helped reduce plants' Cu uptake. From this, it is possible to point out the potential use of biochar as a tool in soil remediation and correction of soil pH. It was also possible to verify the effect of this material as a nutrient source.

Based on the results obtained, the possibility of new studies in areas with widespread use of copper-based products is indicated to evaluate situations *in loco* of the effects of biochar as a remediating, corrective, and fertilizer agent in areas contaminated by Cu and other heavy metals.

4. Conclusion

The lemongrass biochar raises the soil pH and OM when applied at 5 wt.% and 10 wt.% and can be considered a promising material as a soil corrective. Despite the higher copper content in the leachate of the soil with 10 wt.% biochar, the biometric parameters were superior, with a better performance with biochar, regardless of the use of limestone. The Cu content in plant tissue was smaller when biochar was added; the same behavior was observed for the macronutrients, except for K, which was the highest with the addition of 10 wt.% biochar. Thus, biochar application at 5 wt.% to 10 wt.% reduced the Cu content in the plant material of *C. roseus* and potentially acted as a nutrient source. Given this, *C. citratus* biochar has the potential to be used as a remediating and fertilizing agent in the soil.

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Authors contributions
The authors confirm the study conception and design: V. S. R. Bisi, G. F. Pauletti; data collection: Y V. S. R. Bisi, M. H. Tramontin; analysis and interpretation of results: V. S. R. Bisi, W. P. Silvestre, E. D. Conte, M. Godinho, G. F. Pauletti; draft manuscript preparation: V. S. R. Bisi, W. P. Silvestre, E. D. Conte, M. Godinho, G. F. Pauletti. The results were evaluated by all authors, and the final version of the manuscript was approved.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interests
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.

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