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DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n11e277661 ORIGINAL ARTICLE

Bioindicator potential of *Cedrela fissilis* seedlings in copper-contaminated areas[1]

Potencial bioindicador de mudas de *Cedrela fissilis* em áreas contaminadas por cobre

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HIGHLIGHTS:
Copper had a negative effect on Cedrela fissilis *seedlings' growth.*
Copper promoted oxidative damage in C. fissilis *seedlings.*
Copper was mainly retained in the roots of C. fissilis *seedlings.*

ABSTRACT: Soil contamination with toxic metals brings along severe environmental issues. Among these metals, copper (Cu) is harmful to plant development when it reaches high contamination levels in the soil. Thus, identifying species capable of resisting this contamination type helps these sites' revegetation and decontamination processes. Therefore, the present study aimed to investigate the tolerance of *Cedrela fissilis* plants to excess Cu through morphophysiological and biochemical variables. *C. fissilis* seedlings were subjected to five concentrations of Cu (0, 2, 4, 6, and 8 mg L^{-1}). The experimental design was completely randomized, with five treatments and four repetitions. Morphophysiological (number of leaves, shoot height, root length, dry weight, morphological variables of the root system, and leaf area) and biochemical (antioxidant enzymes, lipid peroxidation, hydrogen peroxide concentration, and photosynthetic pigments) traits and Cu accumulated in roots and shoot were evaluated. High concentrations of Cu had a negative effect on the shoot and root dry weight, photosynthetic rate, stomatal conductance, and transpiration rate. Overall, Cu increased the activity of the antioxidant enzymes and lipid peroxidation. Therefore, Cu incidence in the nutrient solution has negatively influenced the biochemical and physiological traits of *C. fissilis* seedlings to the detriment of their growth. Thus, it was possible to identify sensitive behavior by the investigated species. Because of these features, *C. fissilis* seedlings can be used as markers for copper-contaminated areas.

Key words: antioxidant enzymes, copper toxicity, gas exchange, heavy metals, metal toxicity

RESUMO: A contaminação do solo com metais tóxicos traz consigo graves problemas ambientais. Dentre esses metais, o cobre (Cu) é prejudicial ao desenvolvimento das plantas quando atinge elevados níveis de contaminação no solo. Assim, identificar espécies capazes de resistir a esse tipo de contaminação auxilia nos processos de revegetação e descontaminação desses locais. Portanto, o presente estudo teve como objetivo investigar a tolerância de plantas de *Cedrela fissilis* ao excesso de Cu através de variáveis morfofisiológicas e bioquímicas. Mudas de *C. fissilis* foram submetidas a cinco concentrações de Cu (0, 2, 4, 6 e 8 mg L^{-1}). O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e quatro repetições. Características morfofisiológicas (número de folhas, altura da parte aérea, comprimento da raiz, massa seca, variáveis morfológicas do sistema radicular e área foliar) e bioquímicas (enzimas antioxidantes, peroxidação lipídica, concentração de peróxido de hidrogênio e pigmentos fotossintéticos) e Cu acumulado nas raízes e folhas foram avaliados. Altas concentrações de Cu tiveram efeito negativo no peso seco da parte aérea e das raízes, na taxa fotossintética, na condutância estomática e na taxa transpiratória. No geral, o Cu aumentou a atividade das enzimas antioxidantes e a peroxidação lipídica. Portanto, a incidência de Cu na solução nutritiva influenciou negativamente as características bioquímicas e fisiológicas das mudas de *C. fissilis* em detrimento do seu crescimento. Assim, foi possível identificar o comportamento sensível da espécie investigada. Devido a essas características, mudas de *C. fissilis* podem ser utilizadas como bioindicadoras de áreas contaminadas por cobre.

Palavras-chave: antioxidant enzymes, copper toxicity, gas exchange, heavy metals, metal toxicity

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Introduction

An adversity currently rising and becoming a serious environmental problem is soil pollution with toxic metals through intense agricultural, industrial, and urban activities (Mattos et al., 2023). High concentrations of heavy metals in the biosphere cause significant environmental damage, resulting in several negative effects on humans, animals, and plants (Aguilar et al., 2023). Cu is considered essential for plant growth, being an important micronutrient in enzyme and protein biological and structural functions (Melo et al., 2021). However, plants grown in soil with high Cu availability can accumulate this metal in their tissues and develop toxicity symptoms (Trentin et al., 2022).

Thus, Cu contamination is a major problem for crop yield since Cu is an inhibitor of key nutrient absorption and negatively affects plant development and balance when found in excess in the environment (Feil et al., 2020). Areas contaminated by metals must be recovered to decrease or prevent contaminants from being dispersed to other ecological niches to avoid contaminating living beings (Mattos et al., 2023). One strategy among several technologies that can be used for contaminated site remediation is phytoremediation, which can assist in the depollution or removal of these contaminating elements (Bamagoos et al., 2022).

Among the various tree species with phytoremediation potential, *Cedrela fissilis* Vell stands out, which belongs to the Meliaceae family and is popularly known as "cedro" (Silva et al., 2020). This species presents fast growth, high biomass production, and filtration capacity due to its extensive root system. These features are essential in phytoremediation programs (Covre et al., 2020). Thus, the hypothesis was raised that *C. fissilis* seedlings adopt copper tolerance mechanisms to mitigate the toxic effects caused by excess of this metal without interfering with plant growth, biomass production, and hydrogen peroxide concentration in plant tissues. Thus, the study aimed to investigate the tolerance of *Cedrela fissilis* plants to Cu through the morphophysiological and biochemical variables.

Material and Methods

The study was conducted in a greenhouse of the Biology Department of the Universidade Federal de Santa Maria (UFSM), Campus of Santa Maria, RS, Brazil (29° 42' 56.35" S, 53° 43' 12.64" W, and altitude of 113 m). The greenhouse has a controlled temperature of approximately 25 °C and an average relative air humidity of 60%. The survey was conducted in the Laboratory of Plant Physiology and Nutrition in the Department of Biology.

Seeds from the UFSM Forest Nursery – Campus of Santa Maria were used to produce seedlings. They were sown in a commercial substrate (Carolina Soil®) comprising *Sphagnum* sp. and vermiculite. Plastic trays (38 × 56 cm) were used as growing containers for germination and initial growth of the *C. fissilis* seedlings.

Fifteen days after sowing (DAS), the seedlings received weekly fertigation with a complete nutrient solution and pH of 5.5 ± 0.1. The nutrient solution comprised (in μM) 6,090.5 of nitrogen, 974.3 of magnesium, 4,986.76 of chlorine, 2,679.2 of potassium, 2,436.2 of calcium, 359.9 of sulfur, 243.592 of phosphorus, 0.47 of copper, 2.00 of manganese, 1.99 of zinc, 0.17 of nickel, 24.97 of boron, 0.52 of molybdenum, and 47.99 of Iron ($FeSO_4$/Na-EDTA) (Hoagland & Arnon, 1950).

When the seedlings were approximately 10 cm in height (approximately 3 months after sowing in the trays), they were carefully removed from the substrate and transferred to the hydroponic system. Then, each seedling was transferred to a 16 L capacity tray containing a complete Hoagland nutrient solution (Hoagland & Arnon, 1950). A Styrofoam sheet with sixteen holes was placed on the surface of each tray to allow the plants to pass through. The Styrofoam sheet allowed the fixing of the plants and the decreasing evaporation of the solution contained in each tray.

The seedlings were distributed in a completely randomized experimental design, with four repetitions and 16 plants per plot. These seedlings were subjected to five concentrations of Cu. The standard concentration of Cu in the Hoagland nutrient solution (Hoagland & Arnon, 1950) of 0.02 mg L^{-1} was used as a control treatment. The other concentrations comprised adding 2, 4, 6, and 8 mg L^{-1} of Cu in the complete nutrient solution.

The seedlings were acclimated for seven days in the Hoagland nutrient solution (Hoagland & Arnon, 1950) with 100% of its original concentration. The solution aeration in each tray was conducted using PVC microtubes connected to an air compressor. The microtubes were inserted into the solution using the Styrofoam sheet in each tray. The nutrient solution contains the following concentrations, in mg L^{-1}: NO_3^- = 196; NH_4 = 14; P = 31; K = 234; Ca = 160; Mg = 48.6; S = 70; Fe-EDTA = 5; Cu = 0.02; Zn = 0.15; Mn = 0.5; B = 0.5; Mo = 0.01.

After acclimation, treatments were applied, and the seedlings remained for 14 days in the Cu concentrations, totaling 21 days in the hydroponic system, when Cu toxicity symptoms were observed, especially at the highest Cu concentrations. The nutrient solution in each tray was replaced weekly, and its pH was adjusted daily to 5.5 ± 0.1, with 1.0 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH.

The evaluation of photosynthetic variables was performed on the third fully expanded leaf, using an infrared radiation gas analyzer (IRGA, Mod. Li-COR® 6400 XT) employing photosynthetic radiation of 1,500 μmol m^{-2} s^{-1} and CO_2 concentration of 400 μmol mol^{-1}. The measurements were taken between 8:00 am and 10:00 am before the plants were collected for growth analysis. On this occasion, the following variables were determined: net photosynthesis (A - μmol CO_2 m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (Ci - μmol mol^{-1} air), intrinsic carboxylation efficiency (iCE - obtained by the ratio between A and Ci), and water use efficiency (WUE - mmol CO_2 mol^{-1} H_2O, - obtained by the ratio between A and E).

Four plants were collected from each experimental unit for the growth evaluations. After collection, the plants were sectioned into shoots and roots. Then, the already separated samples were forwarded for the growth variables' determination.

The shoot height and root length were measured using a millimeter ruler. The measurements and the number of leaves counting were performed before and after the treatments' application, considering growth increments in this period.

In order to determine the shoot dry weight (SDW; g per plant) and root dry weight (RDW; g per plant), the plants were harvested and separated into shoot and root, washed in running water, and dried in an oven with forced air circulation at approximately 65 °C, until constant weight for later determination of the variables mentioned above.

The root morphological characterization was obtained from digitized images using WinRhizo Pro 2013 software and an EPSON Expression 11000 scanner with additional light (TPU) at a 600 DPI resolution. Root surface area (cm^2 per plant), total root length (cm per plant), and root volume (cm^3 per plant) were measured. Leaf area was also measured in the WinRhizo 2013 system. Samples were digitized in a professional scanner (EPSON Expression 11000), and images were saved in TIFF format.

For the biochemical variables, 12 plants were collected from each treatment, with a total of 240 plants. Then, these plants were sectioned into shoots and roots, washed with distilled water, placed in aluminum foil envelopes, and immediately frozen with liquid nitrogen to prevent sample degradation. They were kept in an ultra-freezer at -80 °C until the samples were pre-prepared for analysis. The samples were prepared through manual maceration with liquid nitrogen, and each sample was macerated into a fine powder.

Chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations in the shoot were extracted based on the Hiscox & Israelstan (1979) method and estimated based on the equation by Lichtenthaler (1987). Superoxide dismutase (SOD) activity was determined through the spectrophotometric method described by Giannopolitis & Ries (1977), and guaiacol peroxidase (GPOX) activity was determined based on Zeraik et al. (2008). The hydrogen peroxide (H_2O_2) concentration was determined based on Loreto & Velikova (2001). H_2O_2 concentration was expressed as μmol g^{-1} fresh weight. The peroxidation degree was estimated through the method by El-Moshaty et al. (1993) for membrane lipid peroxidation. Lipid peroxidation was expressed as nmol MDA mg^{-1} of protein.

Plant tissue was submitted to nitric and perchloric acid digestion (3.0 mL HNO 65% (Reagent Grade) and 1 mL HClO 70% (Reagent Grade)) (EMBRAPA, 2009). The total Cu concentration was determined using an atomic absorption spectrophotometer (AAS, Perkin Elmer Analyst 200, USA). The Cu concentration in tissues was calculated and expressed as mg kg^{-1}.

The data obtained were subjected to the analysis of the normality of errors by the Shapiro-Wilk test and the analysis of homogeneity of variances by Bartlett's test. After meeting the requirements, the data were submitted to the analysis of variance and regression analysis at $p \leq 0.05$ using the Sisvar software (Ferreira, 2019).

Results and Discussion

It was observed that the height increment (HI, Figure 1A), root increment (RI, Figure 1B), increment of leaf number (Figure 1C), and root dry weight (Figure 1F) responded quadratically to the Cu concentrations in the nutrient solution ($p \leq 0.05$). The minimum point for the increase in shoots, roots, and the number of leaves was found at concentrations of 5.55, 5.28, and 5.69 mg L^{-1} Cu, respectively (Figures 1A, B, and C), and for root dry weight at 5.0 mg L^{-1} Cu (Figures 1F).

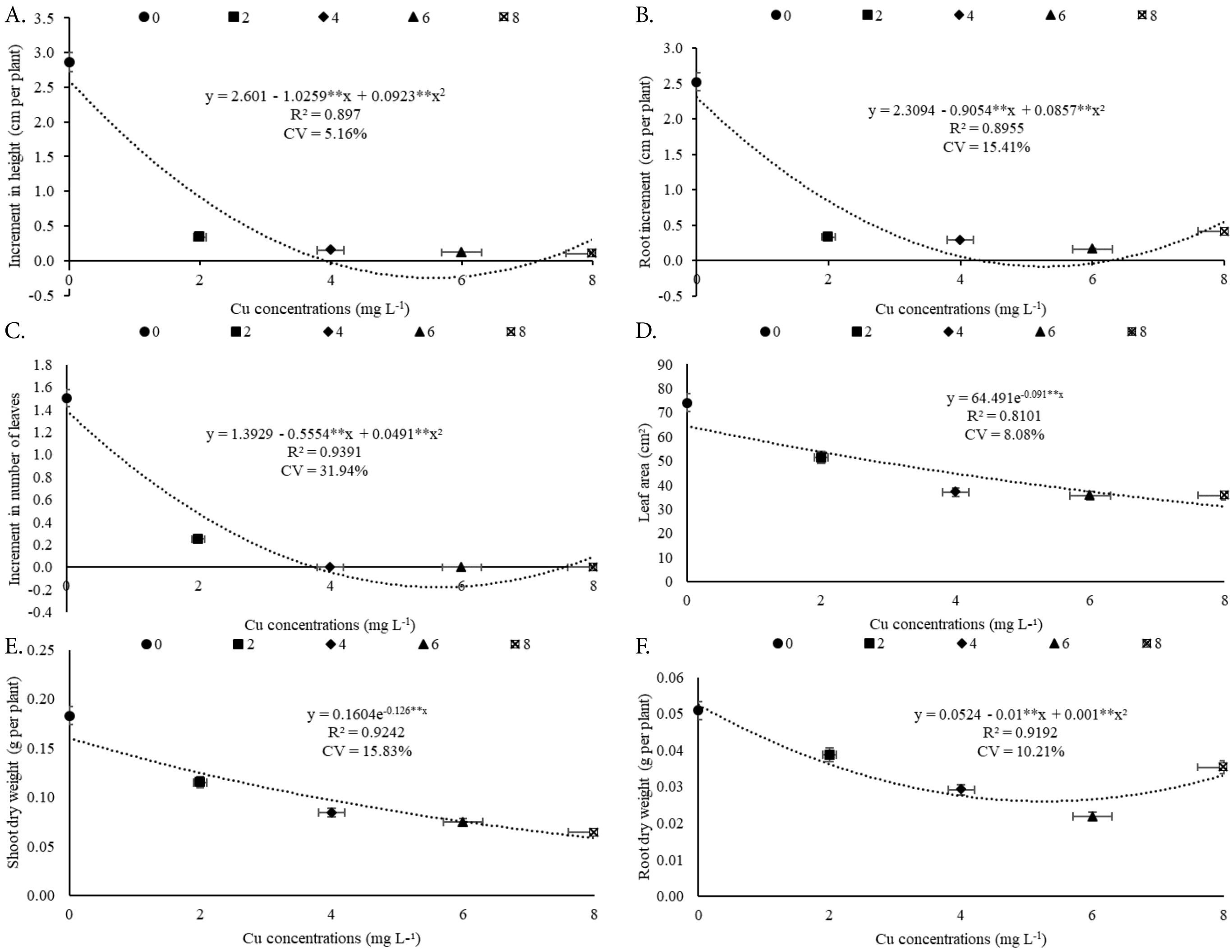
It was observed that the lowest values for the variables mentioned above were evident as there was an increase in Cu concentrations in the nutrient solution (mainly at concentrations 6 and 8 mg L^{-1}). Thus, it is noticed that the different concentrations of Cu promoted a marked decrease in the root increment and root dry weight of *C. fissilis* seedlings (Figures 1B and 1F). This response can be attributed to the increased Cu in the nutrient solution and its direct contact with the roots, implying increased absorption and accumulation in these tissues (Trentin et al., 2022).

The greater Cu retention in the roots may be a survival strategy developed by some plants, aiming to avoid greater metal translocation to the more sensitive and metabolically active photosynthetic organs of the plants, such as the leaves. Therefore, plants store most excess metal in less sensitive organs, such as the roots (Tiecher et al., 2017). Despite this, there was a reduction in leaf area (Figure 1D) and shoot dry weight (Figure 1E) with increasing copper concentrations.

The application of different concentrations of Cu also resulted in a quadratic response for root length (Figure 2A), root area (Figure 2B), and root volume (Figure 2C), with minimum points at concentrations of 5.06, 5.20, and 5.35 mg L^{-1} Cu, respectively. It may have occurred because excess Cu interferes mainly in the cell division region, promoting changes over the rest of the root axis. Thereby, Cu interacts with hard ligands, low polarity, and high electronegativity compounds in the cell division region (Trentin et al., 2022), promoting changes in the root cap constituent cells and also in the cells that form the rest of the root axis.

Although *C. fissilis* seedlings may present mechanisms in their root systems to prevent or reduce translocation of excess absorbed Cu, the shoot was harmed, as there was a reduction in the height increment (Figure 1A) and shoot dry weight (Figure 1E). It may have occurred due to the severe damage caused by Cu on the morphological characteristics of roots, interfering with the ability to absorb water and nutrients. Such behavior can lead to nutritional imbalances and reduce photosynthetic activity, contributing to less carbon assimilation and consequently reducing dry matter production (Hammerschmitt et al., 2020). Thus, there was also less leaf area per unit of leaf dry weight since Cu concentrations may have inhibited energy metabolism, cell division, and expansion, reflected in the reduction of the leaf area of the seedlings (Figure 1D).

Plants adjust their allocation and distribution of relative biomass in their organs when subjected to stress conditions, referred to as allocation plasticity. As stress effects caused by heavy metals affect growth, consequently, they also alter basic processes such as photosynthesis and respiration (Bamagoos et al., 2022). Therefore, changes in plant growth under heavy metal contamination can be attributed to damage in net photosynthesis (A, Figure 3A). The values of net photosynthesis



** Significant at $p \leq 0.05$ by the F test

Figure 1. Height increment (A), root increment (B), leaf number increment (C), leaf area (D), shoot dry weight (E), and root dry weight (RDW) (F) of *Cedrela fissilis* seedlings in response to Cu concentrations

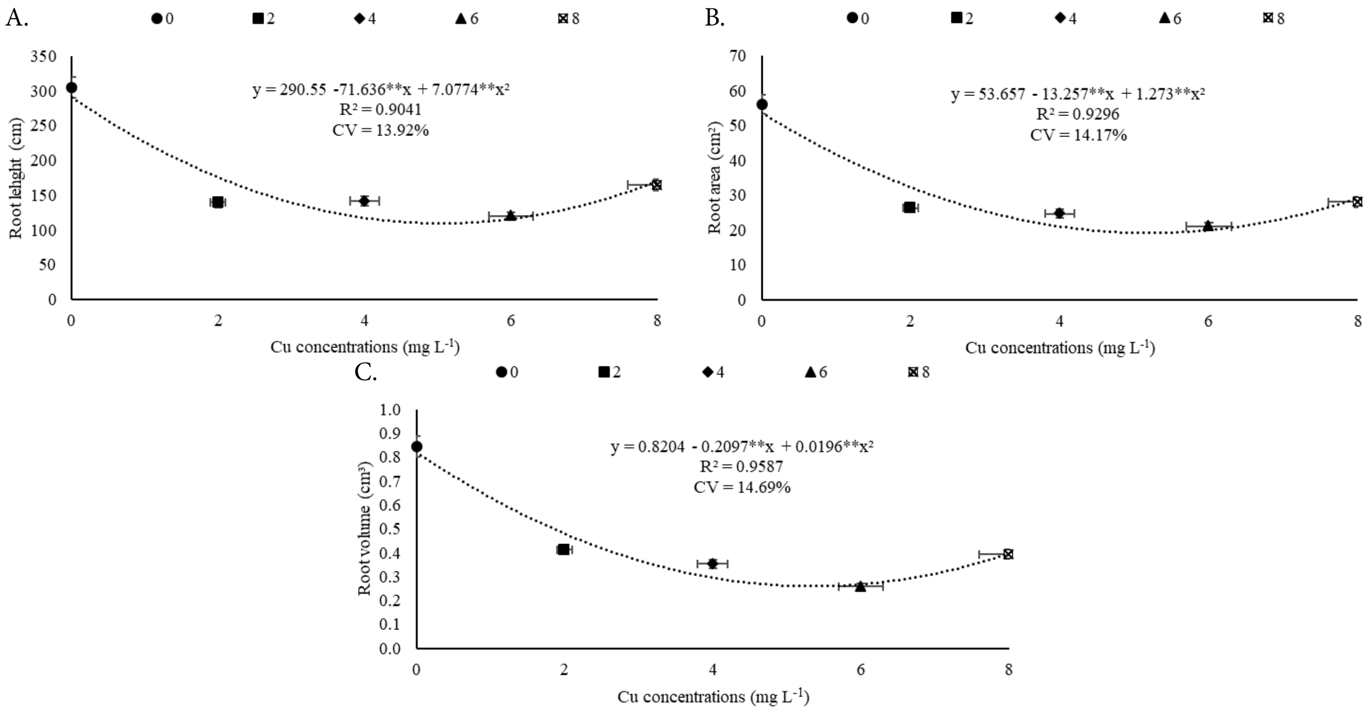


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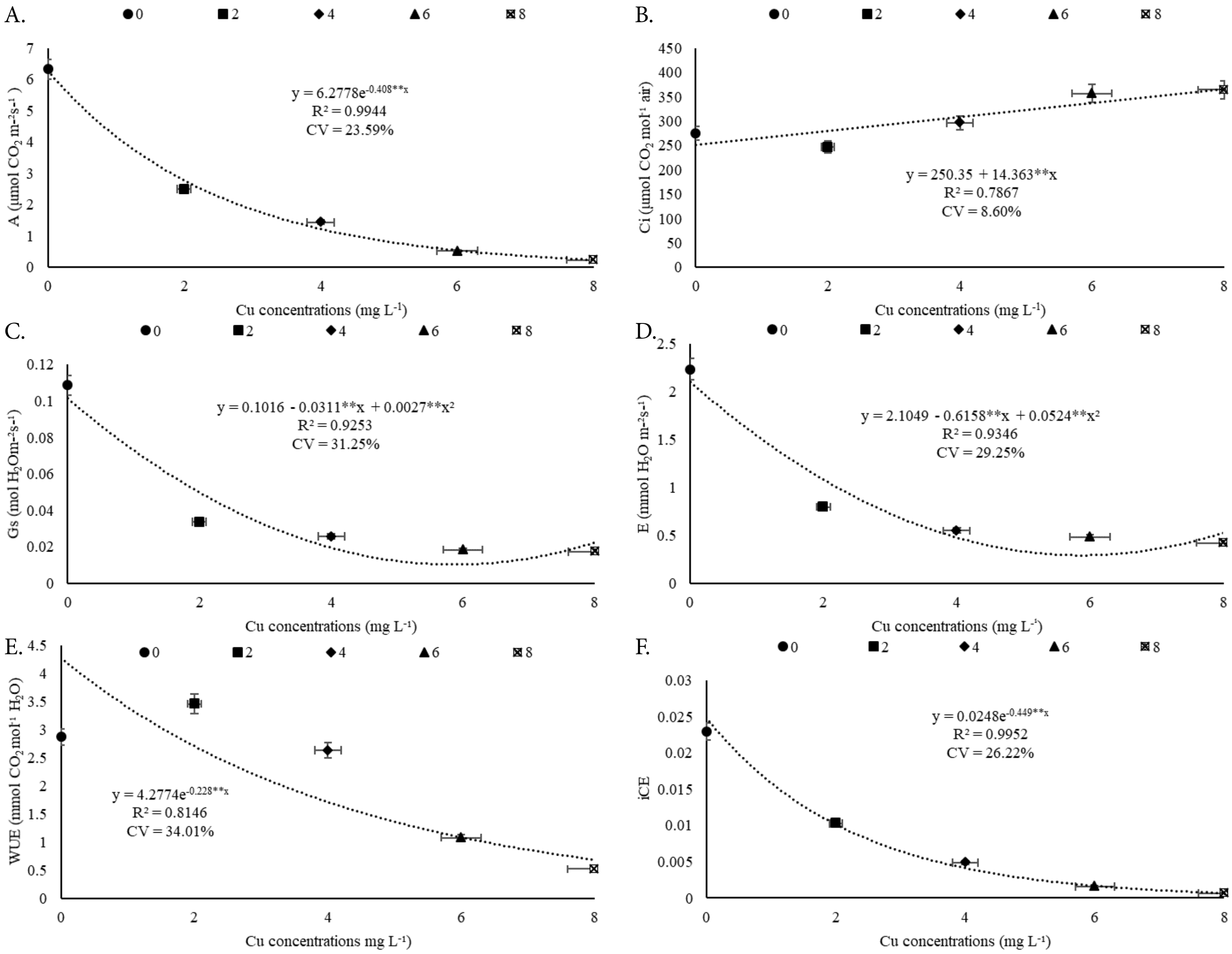
Figure 2. Root length (A), root surface area (B), and root volume (C) of *Cedrela fissilis* seedlings in response to Cu concentrations



**Significant at p≤ 0.05 by the F test

Figure 3. Net photosynthesis (A, A), intercellular CO_2 concentration (Ci, B), stomatal conductance (Gs, C), transpiration rate (E, D), water use efficiency (WUE, E), and intrinsic carboxylation efficiency (iCi, F) of *Cedrela fissilis* seedlings in response to Cu concentrations

(A, Figure 3A), water use efficiency (WUE, Figure 3E), and intrinsic carboxylation efficiency (iCi, Figure 3F) decreased with increasing Cu concentrations ($p \leq 0.05$).

Photosynthetic plant capacity is primarily regulated through photochemical reactions that facilitate energy production, gas exchange, and CO_2 fixation and assimilation (Kuinchtner et al., 2021). However, excess Cu can cause disturbances in photosynthetic processes by impeding electron flow in the photosynthesis photochemical phase, altering the composition of the thylakoid membranes and photosynthetic pigments, and inhibiting the synthesis or activity of Calvin cycle enzymes (Schwalbert et al., 2019).

Thus, it was observed a reduction in net photosynthesis. The decrease in net photosynthesis can be attributed to the different Cu effects on the integrity or function of the photochemical apparatus and its impact on photosynthetic pigment concentration (Aguilar et al., 2023). Furthermore, the negative Cu effects on photosynthetic rates may be indirect since the reduction in root growth negatively affects the absorption of water and nutrients, which are essential for photosynthesis.

However, quadratic behavior was evident for stomatal conductance (Figure 3C) and transpiration rate (Figure 3D), with a significant reduction in these variables as there was an increase in Cu concentrations in the growth medium. The minimum points for stomatal conductance and transpiration rate were evident at 5.76 and 5.88 mg L^{-1} Cu, respectively (Figures 3C and D). Toxicity caused by heavy metals can promote stomatal closure and reduced stomatal conductance. Therefore, the toxicity caused by Cu may have induced stomatal closure, resulting in lower values for stomatal conductance (Figure 3C). This stomatal conductance result is related to the internal CO_2 concentration and the photosynthetic rate since the increase in the internal CO_2 concentration can induce stomatal closure and, consequently, a lower photosynthetic rate. Moreover, since Cu negatively affects the photosynthetic apparatus, promoting a reduction in photosynthetic rates, the intercellular CO_2 concentration tends to increase, as was observed in this study (Figure 3B). This process leads to reduced transpiration (Figure 3D) and decreased water loss to the atmosphere in plants subjected to high metal dose application due to decreased stomatal conductance (Paula et al., 2015).

Determining pigments involved in photosynthesis also allows for observing plants' photosynthetic performance since pigments capture the light energy needed for photosynthesis (Kuinchtner et al., 2021).

Total chlorophyll values decreased linearly with adding Cu to the nutrient solution (Figure 4A). The toxic effect of Cu on photosynthetic pigment concentration can be attributed to changes in the composition of thylakoids and changes in the formation of metal-pigment complexes (Aguilar et al., 2023). Substitution of the central magnesium (Mg) ion for Cu in the chlorophyll molecule can also occur, impairing chlorophyll synthesis (Tiecher et al., 2017).

The capacity for photosynthesis in plants under excess Cu may result in a greater accumulation of photon energy under high irradiance. Excess photons cause increased production of reactive oxygen species (ROS) as by products of photosynthesis, including superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet -}$), and singlet oxygen ($^1O_2$) (Schwalbert et al., 2019). When increased ROS formation occurs, one of the possible strategies used by plants is the activation of the antioxidant enzyme system, such as superoxide dismutase (SOD) and guaiacol peroxidase (POD).

SOD and POD are considered the main antioxidant enzymes involved in ROS elimination and act in maintaining homeostasis in plant cells (Zhang et al., 2019). Thus, antioxidant enzymes promote the dismutation of ROS produced by excess light when the carotenoids fail to dissipate it.

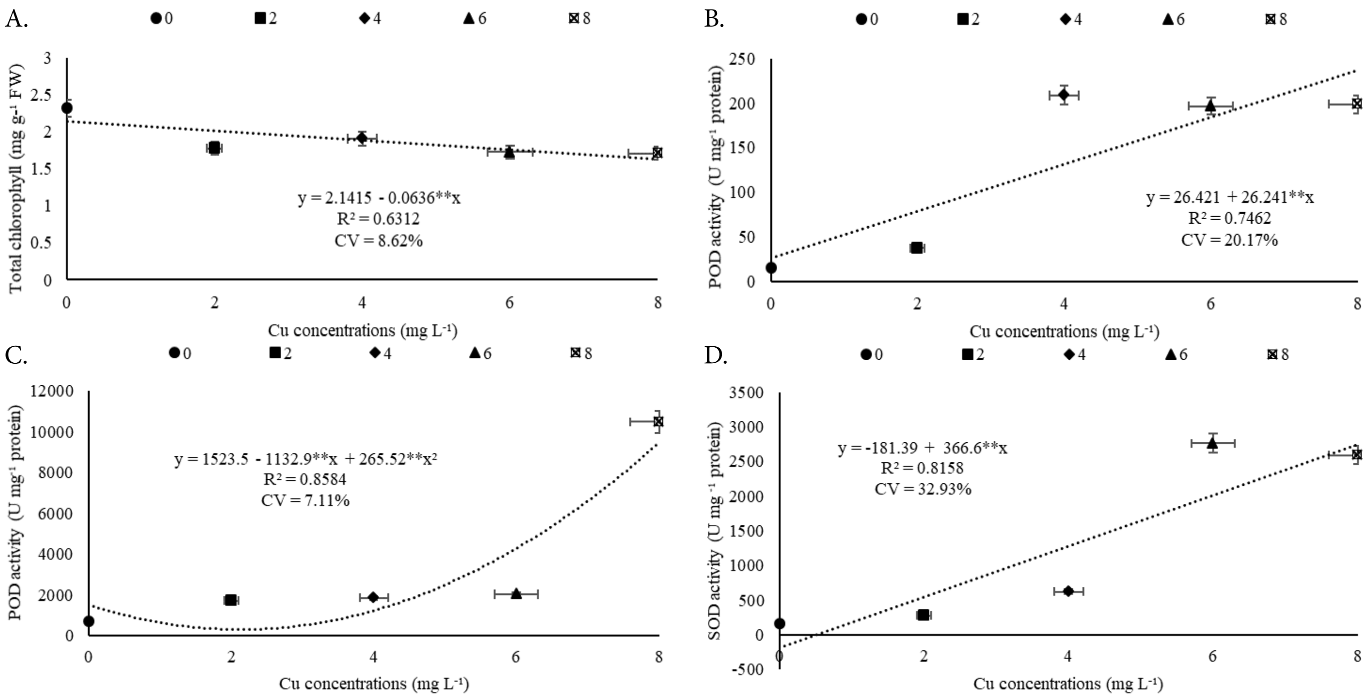
SOD is an essential component of the antioxidant stress defense system in plants, catalyzing the dismutation of the superoxide radical into oxygen and hydrogen peroxide (Schmitt et al., 2020). On the other hand, POD acts in converting H_2O_2 to water and oxygen by H_2O_2 dissociation, playing an essential role in providing tolerance to unfavorable conditions in plants (Bamagoos et al., 2022).

The SOD activity in the shoot was not influenced by the Cu concentrations applied to the nutrient solution (data not shown). However, Cu promoted an increasing linear response in the activity of POD in the shoot and SOD in the root (Figures 4B and D). In contrast, for roots, the POD activity increased quadratically, with the minimum point for this variable being found at a concentration of 2.13 mg L^{-1} Cu (Figure 4C).

The increase in antioxidant enzyme activity in this study indicates that Cu caused oxidative stress, leading to excessive ROS production, which may have increased these enzymes' activity. This increase in SOD activity may have occurred due to the increase in superoxide radical concentration in roots, leading to the synthesis of new enzyme units (Schmitt et al., 2020). Furthermore, SOD activity in the root (Figure 4D) had a positive correlation with Cu concentration in the root (Figure 5E) because SOD and other antioxidant enzymes often show increased activity at high Cu concentrations.

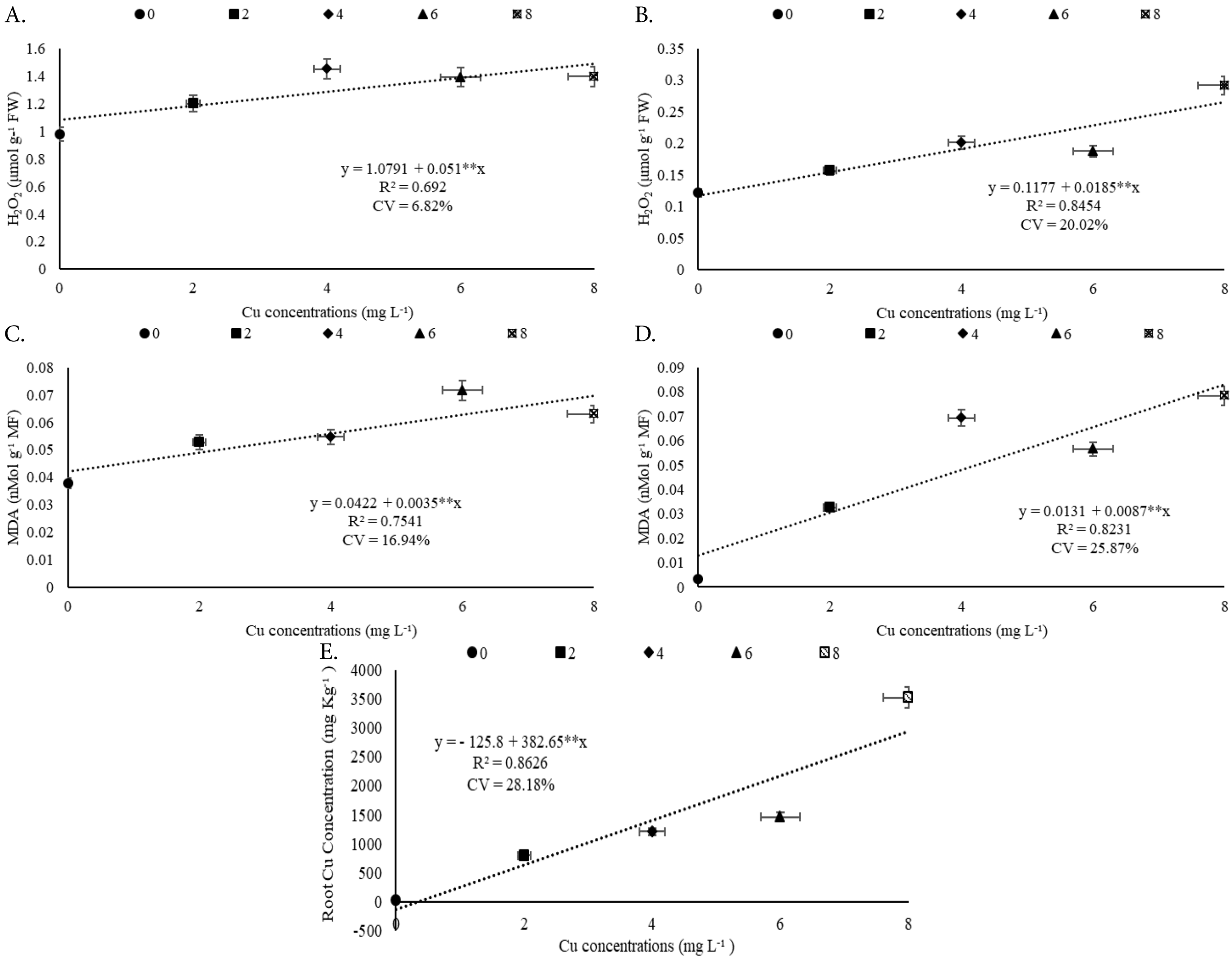
The increased POD activity in plants indicates the activation of an enzymatic response to prevent oxidative damage caused by Cu toxicity (Aguilar et al., 2023). However, the increase in POD activity in the shoot and roots (Figures 4B e C) was not enough to prevent higher H_2O_2 production in *C. fissilis* seedlings, as the H_2O_2 concentration of both shoot and roots increased linearly with Cu application in nutrient solution (Figures 5A and 5B). Therefore, the antioxidant enzymes failed to protect the plants sufficiently, and increased concentrations of H_2O_2 and possibly other ROS occurred, potentiating oxidative damage.

At low concentrations, H_2O_2 is important for plant growth and development because it regulates physiological processes such as stomatal opening, photosynthesis, cell wall strengthening, and protection from abiotic stress. On the other hand, high concentrations of H_2O_2 in tissues can have detrimental effects (Mattos et al., 2023). The observed increases in H_2O_2 under Cu stress are likely responsible for lipid peroxidation, which is indicated by excessive malondialdehyde



**Significant at $p \leq 0.05$ by the F test

Figure 4. Total chlorophyll (A), guaiacol peroxidase enzyme activity (POD) in the shoot (B) and roots (C), and superoxide dismutase enzyme activity (SOD) in the roots (D) of *Cedrela fissilis* seedlings in response to Cu concentrations



**Significant at p≤ 0.05 by F test

Figure 5. Hydrogen peroxide (H_2O_2) concentration in the shoot (A) and roots (B), membrane lipid peroxidation in the shoot (C) and roots (D), and Cu concentration in the root tissues (E) of *Cedrela fissilis* seedlings in response to Cu concentrations

(MDA) accumulation. Thus, there was a linear increase in MDA levels in the shoot and roots (Figures 5C and D). This increase in MDA concentration may directly affect Cu toxicity, indicating oxidative stress in *C. fissilis* seedlings, and as a result, may cause irreversible damage to long-term plant tissue development and function.

Hence, high concentrations of these elements in plant tissues induce changes in membrane properties, which affect the function of membrane transporters and ion channels. These effects cause increased membrane permeability, which may be responsible for the imbalance in nutrient concentration in plants grown in environments with elevated levels of heavy metals (Trentin et al., 2022).

Moreover, ROS generation may have been responsible for the decrease in the plants' dry biomass due to the negative correlation between H_2O_2 concentration (Figures 5A and B) and dry weight production at different Cu concentrations (Figures 1E and F), revoking our initial hypothesis. This process is based on the principle that organisms can mobilize their energy reserves to withstand stress conditions, such as the detoxification process influencing costs with biological functions such as growth (Schwalbert et al., 2019). Therefore, it is possible to infer that the *C. fissilis* species had its photosynthetic variables negatively affected, along with root system morphology, increment in growth, biomass, reduced pigment concentration, and an increase in lipid peroxidation and oxidative stress even at low Cu concentrations.

Conclusions

1. The presence of Cu in the nutrient solution impaired the biochemical and physiological variables of *Cedrela fissilis* seedlings, and thus, the species is considered sensitive.
2. *Cedrela fissilis* seedlings can be indicated as a marker for copper-contaminated areas.

Contribution of authors: Caroline Castro Kuinchtner: Conceptualization, project administration, writing-revision, carrying out the experiments, biochemical and physiological analysis. Marcos Vinícius Miranda Aguilar: Supervision, carrying out experiments, biochemical and physiological analyses, writing-revision, software and formal analysis. Daiane Franchesca Senhor: Formal analysis and performance of the experiment, validation, and biochemical and physiological analysis. Gerâne Silva Wertonge de Oliveira: Software, formal analysis, performance of experiments, and biochemical and

physiological analysis. Luana Da Rosa Lovato: Visualization, project administration, and biochemical and physiological analysis. Flaiane Catarine Rosa Da Rosa: Validation, project administration, and biochemical and physiological analysis. Luciane Almeri Tabaldi: Orientation, conceptualization, project fundraising, biochemical and physiological analysis and writing-revision.

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