Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Growth and tolerance of *Ilex paraguariensis* A. St.-Hil. inoculated with ectomycorrhizal fungi in copper-contaminated soil

Crescimento e tolerância de *Ilex paraguariensis* A. St.-Hil. inoculados com fungos ectomicorrízicos em solo contaminado com cobre

Djavan Antonio Coinaski[1], *Rodrigo Ferreira Silva*[1], *Clóvis Orlando Da Ros*[1], *Genesio Mário da Rosa*[1], *Hilda Hildebrand Soriani*[1], *Robson Andreazza*[2]

ABSTRACT

Copper (Cu) is one of the main heavy metals contaminating the soil. Plants have different behavior in terms of tolerance and toxicity to metals, being able to grow and produce even in soils contaminated with high concentrations. This study aimed to determine the influence of ectomycorrhizal fungi on the growth and tolerance of yerba mate plants grown in soil contaminated with Cu. The design was completely randomized in a factorial arrangement (4x6), with four possibilities of inoculum: without inoculum (control) and three ectomycorrhizal fungi (UFSC-PT116 — *Pisolithus microcarpus*, UFSC-PT132 — *Pisolithus tinctorius* and UFSC-SU118 — *Suillus cothurnatus*), with six Cu doses amended to the soil (0, 80, 160, 240, 320 and 400 mg kg^{-1} of Cu) in seven replicates. The height of the aerial part, the diameter of the lap, the dry mass of the aerial part and root system, the leaf area, the specific surface area of the roots, the contents of Cu in the aerial and radicular parts, the tolerance index, and mycorrhizal association were assessed. Inoculation of *Ilex paraguariensis* seedlings with ectomycorrhiza fungi UFSC-PT116, UFSC-PT132, and UFSC-SU118 mitigates the toxicity effect caused by the excess of Cu in the soil. The UFSC-PT116 isolate promoted the highest growth and tolerance of *Ilex paraguariensis* seedlings under the treatments. In general, the isolates promoted the reduction of Cu toxicity in *Ilex paraguariensis* plants, being an important alternative to remediate Cu-contaminated areas.

Keywords: yerba mate; symbiose; heavy metal; soil contamination.

RESUMO

O cobre é um dos principais metais pesados contaminantes do solo. As plantas diferenciam-se quanto à sua tolerância e toxicidade, o que lhes permite crescer e produzir até mesmo em solos com altas concentrações. O trabalho objetivou determinar a influência do uso de fungos ectomicorrízicos no crescimento e tolerância de mudas de erva-mate (*Ilex paraguariensis*) cultivadas em solo contaminado com cobre. O delineamento experimental foi inteiramente casualizado em arranjo fatorial (4x6), sendo quatro inóculos: controle (sem inóculo) e três ectomicorrizas (UFSC-PT116 — *Pisolithus microcarpus*, UFSC-PT132 — *Pisolithus tinctorius* e UFSC-SU118 — *Suillus cothurnatus*) e seis doses de cobre adicionadas ao solo (0, 80, 160, 240, 320 e 400 mg kg^{-1}), com sete repetições. Avaliaram-se: altura da parte aérea, diâmetro do colo, massa seca da parte aérea e sistema radicular, área foliar, área superficial específica de raízes, teores de cobre na parte aérea e radicular, índice de tolerância e associação micorrízica. A inoculação de mudas de erva-mate com ectomicorrizas UFSC-PT116, UFSC-PT132 e UFSC-SU118 ameniza o efeito de fitotoxicidade provocado pelo excesso de cobre no solo. O isolado ectomicorrízico UFSC-PT116 promoveu os maiores crescimento e tolerância de mudas de erva-mate cultivadas em solo contaminado com cobre. Em geral, os isolados promoveram a redução da toxicidade do cobre nas plantas de *Ilex paraguariensis*, sendo uma alternativa interessante para a recuperação de áreas contaminadas com cobre.

Palavras-chave: erva-mate; simbiose; metal pesado; contaminação do solo.

[1]Universidade Federal de Santa Maria – Santa Maria (RS), Brazil.
[1]Universidade Federal de Pelotas – Pelotas (RS), Brazil.
Correspondence address: Robson Andreazza – Rua Benjamin Constant, 989 – Porto – CEP: 96010-020 – Pelotas (RS), Brazil.
E-mail: robsonandreazza@yahoo.com.br

Conflicts of interest: the authors declare no conflicts of interest.
Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).
Received on: 09/29/2021. Accepted on: 05/23/2022
https://doi.org/10.5327/Z2176-94781236

Introduction

Plants growing in soils with high Cu contents can develop symptoms of toxicity (Kabata-Pendias, 2011), such as necrosis and reduced root and leaf system, early defoliation, and reduced aerial growth (Grassi Filho, 2005). Such symptoms can reduce growth as a result of morphological (Ambrosini et al., 2015; Guimarães et al., 2016), biochemical, and physiological (Cambrollé et al., 2012; Mateos-Naranjo et al., 2013) changes suffered by the plants. In addition, Cu can be accumulated by the plants and also cause nutritional deficiency (Marastoni et al., 2019). According to CONAMA Resolution No. 420/2009 (Brasil, 2009), the concentration of 200 mg of Cu kg^{-1} of soil indicates the need for intervention in agricultural areas, as it poses a risk to public health and the environment.

Yerba mate (*Ilex paraguariensis* St. Hil.) is an evergreen tree of the Aquifoliaceae family, Bercht & J. Presl, native to South America (Lorenzi, 2009). The culture of yerba mate has great commercial and social value, its leaves and fine branches are processed to originate stimulating drinks made by infusions, such as *chimarrão*, *tereré*, and mate tea (Saidelles et al., 2010), in addition to being used in the food, cosmetics, beverages, and pharmaceutical industries due to the diversity of phytochemical compounds contained in the plant (Jacques et al., 2007). However, there is no information in the scientific literature on the growth of yerba mate in areas contaminated with Cu and its tolerance to this metal.

The use of mycorrhizal fungi may be an alternative to improve plant development in contaminated areas (Dellai et al., 2014). These fungi form symbiotic associations with vascular plants increasing the area explored by the roots through their hyphae in the soil (Brundrett, 2008; Smith and Read, 2008). The literature has shown a beneficial effect of the mycorrhizal association on the growth of tree species of the Fabaceae family with the use of *Pisolithus microcarpus* in bracatinga seedlings (*Mimosa scabrella* Benth.) (Dellai et al., 2014) and canafístula seedlings (*Peltophorum dubium* (spreng.) taub.) (Silva et al., 2010) in soil contaminated with Cu, also demonstrating the efficiency of this association in the tolerance of plants to this metal, as the hyphae and the fungal mantle act by retaining the metal physically and chemically (Smith and Read, 2008), allowing greater plant growth.

Some studies on phytoremediation, which would be the use of plants in contaminated environments to remove or stabilize these contaminants, advocate that its efficiency depends on several parameters, such as soil properties (soil pH and type), type of heavy metal, nature of the rhizosphere, and characteristics of the rhizosphere microflora (Yaashikaa et al., 2022), where the efficiency of using these fungi to improve the soil-plant-contaminant relationship can be pronounced. In addition, ectomycorrhizal fungi can have several effects, such as acting as a biocide (Volcão et al., 2022), which can improve the growth of host plants.

Some plant species have adaptive mechanisms, such as the accumulation of metals in the vacuole (Taiz and Zeiger, 2009) and bioaccumulation of Cu in the roots, restricting its translocation to the shoots (Silva et al., 2015; Marques et al., 2018; Afonso et al., 2022), which makes them more tolerant to toxicity at high concentrations (Lequeux et al., 2010). In this sense, there is still doubt about the efficiency of the use of ectomycorrhizal fungi in association with yerba mate as an alternative to increase its tolerance and growth in soils contaminated with Cu. Thus, this study aimed to evaluate the influence of the use of ectomycorrhizal fungi on the growth and tolerance of *Ilex paraguariensis* cultivated in soil contaminated with Cu.

Material and Methods

The experiment was carried out in a greenhouse at the Forest Engineering Department at Universidade Federal de Santa Maria (UFSM), Frederico Westphalen Campus. The soil used in the experiment was characterized as Oxisol, sampled in an agricultural area in the 0-20-cm layer, whose chemical and physical attributes, determined according to the methodology described in Silva (2009), are specified in Table 1.

The experimental design was completely randomized in a 4 x 6 factorial arrangement, with four sources of inoculum: control (without inoculum) and three ectomycorrhizal fungi (UFSC-PT116 — *Pisolithus microcarpus*, UFSC-PT132 — *Pisolithus tinctorius*, and UFSC-SU118 — *Suillus cothurnatus*) with six doses of Cu added to the soil [0 (natural soil content), 80, 160, 240, 320, and 400 mg kg^{-1}], in seven replications.

Ectomycorrhizal fungi were obtained from the fungal bank of the Universidade Federal de Santa Maria, Frederico Westphalen campus, state of Rio Grande do Sul (RS), and were multiplied in solid medium (Modified Melin-Norkrans — MNM) (Marx, 1969), in Petri dishes cultured in a BOD incubator at 25°C, 30 days before the experiment was implemented.

The yerba mate seeds used in the experiment were provided by Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), in Chapecó, state of Santa Caterina (SC). The seeds were disinfected with 5% sodium hypochlorite for 20 min and washed in running water for 5 min, individually sown in 120 cm^3 tubes containing Carolina Soil® commercial substrate sterilized in an autoclave at 121°C in three 30-min cycles, being irrigated with distilled water. When the seedlings showed a pair of definitive leaves, they were transplanted to plastic pots with a volumetric capacity of 1,000 cm^3, being considered an experimental unit (EU).

Table 1 – Characterization of the soil used in the experiment with Ilex paraguariensis.

pH water	Ca+Mg	Al	H+Al	P	K	Cu	M.O.	Argila
1:1	$cmol_c\,kg^{-1}$			mg kg^{-1}			%	
5.3	2.23	0.0	3.3	6.5	126.5	12.73	1.1	62.00

$cmol_c\,kg^{-1}$: centimol charge per kg of soil.

Cu doses were prepared and applied 30 days before transplanting the seedlings in the form of copper sulfate solution ($CuSO_4.5H_2O$). At the time of soil amendment, the doses were diluted in 50 mL of distilled water to allow their homogenization in the soil. Immediately before planting the seedlings, soil samples were collected from each treatment to extract the pseudo-total Cu contents, according to methodology 3050b (USEPA, 1996) and determination by atomic absorption spectrophotometry (Miyazawa et al., 2009).

The doses of Cu applied to the soil increased the pseudo-total contents of the metal in a linear profile (Figure 1), achieving values above the limit of 200 mg kg^{-1} established in the Brazilian legislation for investigation in agricultural areas (Brasil, 2009). The quality reference value of 203 mg kg^{-1} was also exceeded for soils from volcanic rocks in the Plateau of the State of Rio Grande do Sul (FEPAM, 2014).

The inoculations of the ectomycorrhizal fungi in the seedlings were carried out through an inoculant produced from the fungal mycelium grown in a Petri dish containing MNM culture medium, using 10 Petri dishes per fungus, totaling 21 colonies for each fungus used, which was crushed in a blender with 500 mL of distilled water for 10 s. For inoculation, 10 mL of this solution was applied, using a graduated syringe, directly on the roots and on the soil adjacent to the root system of each treatment at the time of transplanting the seedlings. The uninoculated control treatment also received 10 mL of a solution containing only the MNM culture medium. The experiment was carried out for 120 days after the seedlings were transplanted. The EUs containing 5 L were weighed and the field capacity was estimated. Subsequently, soil moisture was kept at 70-80% of field capacity. To meet the requirements of the experimental design, the location of the EUs was changed weekly. Natural light was maintained without using a shade screen.

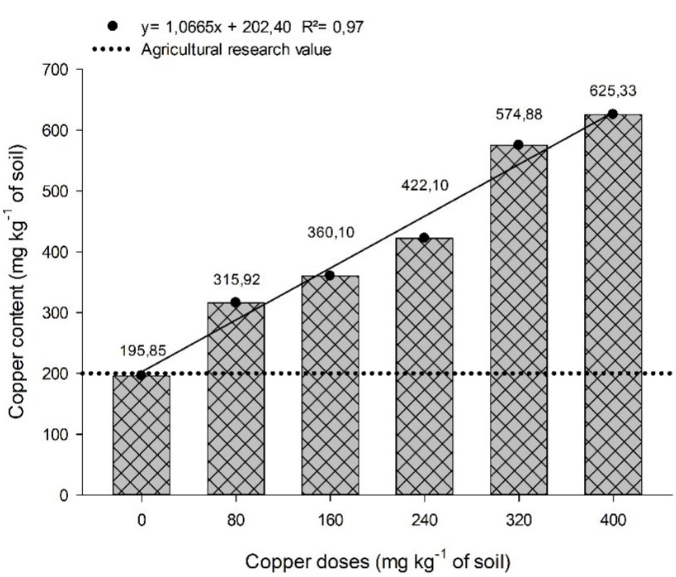

Figure 1 – Pseudo-total copper levels as a function of the doses of copper amendment to the soil.

At the end of the experiment, the height of the shoots (H) was quantified using a graduated ruler, measured from the neck of the seedlings to the shoot apex; the diameter of the stem (DC) was measured with a digital caliper, Black Jack Tools® brand; the dry mass of the root system (MSR) and the shoots (MSPA), both separated in the region of the stem of the seedling, were dried in an oven at 60 ± 1°C to constant mass, weighed on an analytical scale, and the total dry mass (MST) was calculated by the sum of the MSR and MSPA (Silva, 2009).

The roots were separated from the soil by washing with running water, using sieves with a mesh size of 0.5 mm in diameter. Subsequently, the roots were digitized using a scanner (HP D110) to determine the root specific surface area (ASE) by processing the images in Sapphire 2.0 (Jorge and Silva, 2010) software for fiber and root analysis. Leaf area (FA) was measured and analyzed using ImageJ software (version 2.0; US National Institutes of Health, Bethesda, Maryland, USA) (Schneider et al., 2012).

The dry mass of the roots and shoots was ground in a Wiley mill with a 10 mesh sieve to determine Cu contents in plant tissue, through nitric-perchloric digestion (3:1) and determination through atomic absorption spectrophotometry (Miyazawa et al., 2009).

Based on the MST of the zero copper dose (d0) and the other doses ranging from 80 to 400 mg kg^{-1} (dn), the tolerance index (Itol) was calculated, according to Equation 1. The Itol estimates the ability of seedlings to grow in environments with high Cu concentrations (Wilkins, 1978).

$$\text{Itol} = \frac{\text{MSTdn}}{\text{MSTdo}} * 100 \quad (1)$$

The roots were separated from the soil by washing with running water, using sieves with a mesh size of 0.5 mm in diameter. Subsequently, the roots were digitized using a scanner (HP D110) to determine the root specific surface area (ASE) by processing the images in Sapphire 2.0 (Jorge and Silva, 2010) software for fiber and root analysis. Leaf area (FA) was measured and analyzed using ImageJ software (version 2.0; US National Institutes of Health, Bethesda, Maryland, USA) (Schneider et al., 2012).

The evaluation of mycorrhizal root colonization was performed using the technique of clarification and staining of the roots with 0.05% Trypan Blue, for visualization of the ectomycorrhizae under a microscope and a magnifying glass (Brundrett, 2008). Ectomycorrhizal colonization was estimated at five replicates per plant by the checkered plate method (Giovannetti and Mosse, 1980).

The results were submitted to analysis of variance and when they showed significant interaction, they were submitted to regression analysis of the quantitative variation factor (doses) within each level of the qualitative factor (mycorrhizal inocula). For the parameters without interaction, the simple effects were split, and the means of the qualitative factor (Factor A) were compared by Tukey's test at 5% error probability and the means of the quantitative factor (Factor D) submitted to polynomial regression analysis by the SISVAR program (Ferreira, 2011).

Results and Discussion

Yerba mate plants showed a significant interaction ($p \leq 0.05$) between the ectomycorrhizal inoculum and Cu doses applied to the soil for plant height, stem diameter, aerial and root dry mass, leaf area, and specific surface area of the roots (Figure 2).

The doses of Cu applied to the soil induced a linear reduction in the height of seedlings without inoculation, while the inoculated treatments were significantly higher with the UFSC-PT116 isolate, promoting a linear increase, being 58% higher than the control at the dose of 400 mg kg^{-1} (Figure 2A). These results corroborate the results obtained by Dellai et al. (2018), in which *Eucalyptus saligna* Sm. produced in soil contaminated with Cu showed greater height when inoculated with the UFSC-PT116 isolate. Ectomycorrhizal fungi, when establishing an association with plants, increase the absorption of water and nutrients from the soil, while the fungal mantle located on the surface of the roots acts as a physical and chemical barrier to heavy metals (Moreira and Siqueira, 2006; Smith and Read, 2008). The results demonstrate that inoculation with these isolates allows for lower Cu toxicity in terms of the height of yerba mate seedlings.

The stem diameter showed a reduction of 31.3% at the highest dose of Cu in plants without inoculation (Figure 2B). In ectomycorrhizal isolates, the response was quadratic positive, with an increase of 6% when the UFSC-PT116 isolate was inoculated at the Cu dose of 211.50 mg kg^{-1} of soil. Dellai et al. (2014) reported that the stem diameter of the *M. scabrella* was significantly reduced when inoculated with the UFSC-PT116 fungus, which indicates that the response of this isolate is influenced by the plant species in association. However, for transplanting the seedlings to the field, a minimum neck diameter of 2 mm is recommended (Wendling and Dutra, 2010) and, in this case, all treatments have adequate values at the Cu doses studied.

The dry mass of the aerial part of the yerba mate seedlings without inoculation was linearly reduced with the Cu doses, while the UFSC-PT116 isolate promoted a linear increase, being 67% higher than the control at the dose of 400 mg of Cu kg^{-1} of soil (Figure 2C). Silva et al. (2010) also found a reduction in shoot dry mass of the seedlings of *Peltophorum dubium* (Spreng.) Fabaceae with up to 450 mg kg^{-1} of Cu added to the soil. Plants submitted to high doses of Cu may show shoot chlorosis (Yruela, 2013), early defoliation, and reduced shoot (Grassi Filho, 2005; Cassanego et al., 2013) and plant growth (Gautam et al., 2016). However, Steffen et al. (2012) showed that the application of both the isolate UFSC-PT116 and eucalyptus essential oil increases the development of the aerial part of *Caesalpinia peltophoroides* Benth. Fabaceae. The results indicate that the UFSC-PT116 isolate promotes an increase in the growth of the aerial part of yerba mate seedlings in soils contaminated with Cu.

The root dry mass was linearly reduced with Cu doses in plants without inoculation and with the UFSC-PT116 isolate, while UFSC-SU118 and UFSC-PT132 had a quadratic response, with a maximum point at doses of 192.5 and 164.5 mg kg^{-1}, respectively (Figure 2D). A high amount of Cu in the soil causes inhibition of root growth and reduced photosynthesis (Yruela, 2009) and, in eucalyptus Citriodora (*Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson) Myrtaceae, Weirich et al. (2018) obtained lower root dry mass when inoculated with the UFSC-PT116 isolate. Despite the reduction in root dry mass of plants inoculated with the UFSC-PT116 isolate with Cu doses, the formation of a mycorrhizal association results in an increase in the absorption area of the root of the plant because of the presence of fungal hyphae (Smith and Read, 2008), modifying the architecture of the roots that become shorter and more branched (Raven et al., 2007).

The use of ectomycorrhiza allowed greater leaf area in the yerba mate seedlings with the UFSC-PT116 isolate, promoting a linear increase and being superior to the other treatments at the dose of 400 mg kg^{-1} of soil (Figure 2E). In the seedlings without inoculation, the reduction of the leaf area was linear, 44.5%, with increased doses of Cu in the soil. The *Jatropha curcas* L. of the Euphorbiaceae family had a linearly reduced leaf area with increasing Cu doses and, when submitted to 100 mg kg^{-1}, it was 69% lower compared to the control (Chaves et al., 2010). Although the green stems of plants in general and even the roots of epiphytic species can carry out photosynthesis, the leaves are the main organs responsible for the production of photoassimilates, and a larger leaf area allows plants to have a greater capacity to carry out photosynthesis due to the greater exposure to sunlight, the leaf area being responsible for the production of photoassimilates necessary for the metabolic demand and formation of new plant structures (Silva et al., 2018). Cu had a depletion effect on the assimilatory apparatus of plants without inoculation; however, there was an increase in the leaf area of yerba mate with the UFSC-PT116 isolate.

The specific surface area of roots was reduced with Cu doses in plants without inoculation and with the isolate UFSC-PT132 (Figure 2F). The UFSC-PT116 isolate showed a quadratic effect, showing a higher specific area compared to the other treatments at the dose of 400 mg kg^{-1} of Cu. Increased Cu doses in the soil in species of the Fabaceae family induced a linear reduction in the surface area of the roots of *Bauhinia forficata* Link and *Pterogyne nitens* Tul seedlings and a quadratic increase in *Enterolobium contortisiliquum* Vell. up to a dose of 81 mg kg^{-1} (Silva et al., 2016). The increase in the surface area of absorption of the roots represents an increase in the volume of exploited soil, and according to Harley (1969), the surface area exploited by ectomycorrhizal fungi is estimated to be 1,000 times greater than that of roots without the presence of ectomycorrhiza. Mycorrhizal plants could have a better nutritional status compared to plants without inoculation, which could be a conditioning factor for the survival rate of the species (Silva et al., 2003). These results show a positive effect of the UFSC-PT116 isolate on the surface area of the roots of yerba mate seedlings subjected to Cu contamination.

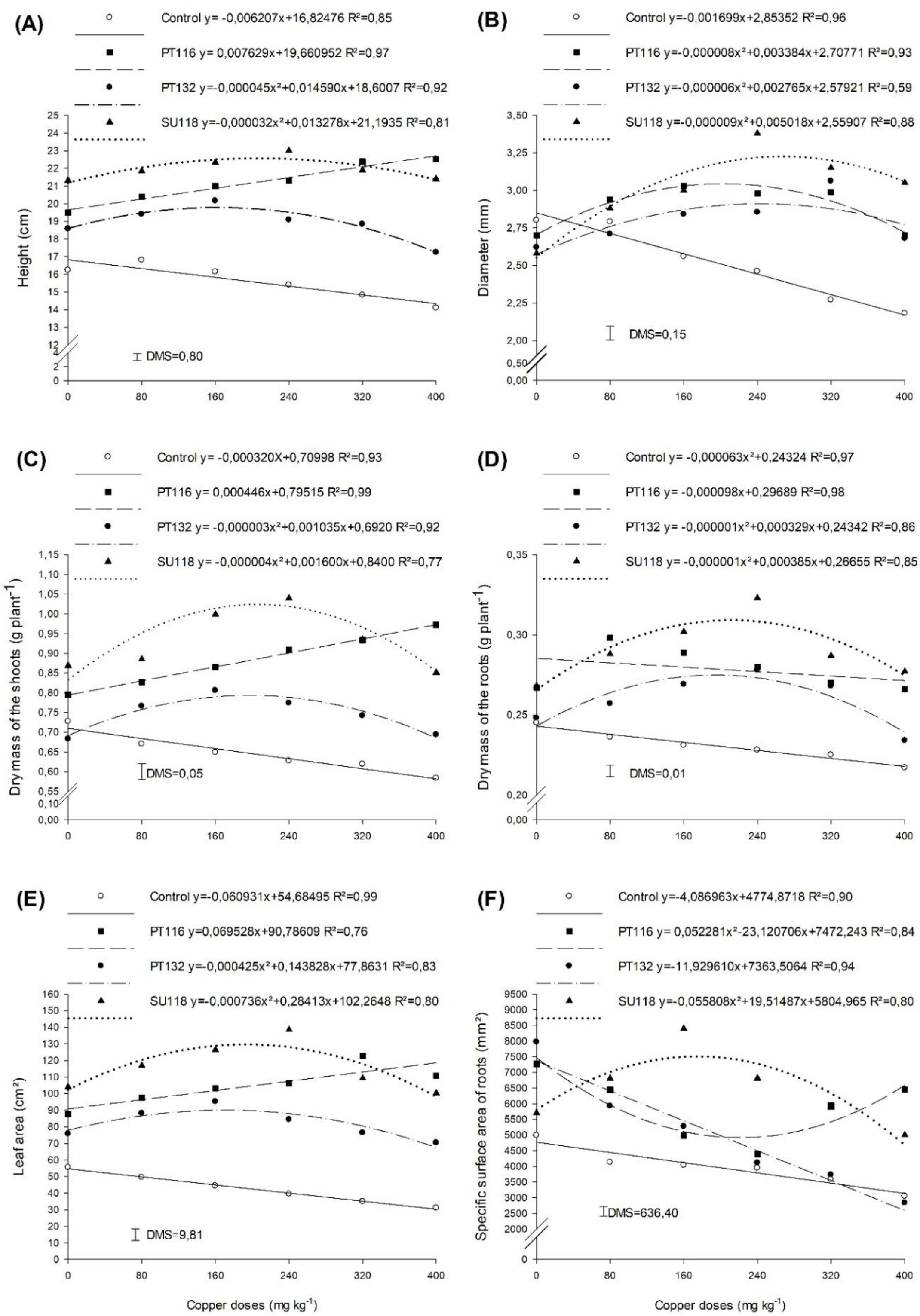

Figure 2 – (A) Ratio of height, (B) stem diameter — Diameter, (C) dry mass of the shoots — MSPA, (D) dry mass of the roots — MSR, (E) leaf area, and (F) specific surface area of the roots — ASE of *Ilex paraguariensis* seedlings with copper doses applied in the soil, without (control — test) and with the addition of three inocula of ectomycorrhizal fungi such as *Pisolithus microcarpus* (PT116), *Pisolithus tinctorius* (PT132), and *Suillus cothurnatus* (SU118).
DMS: minimum significant difference by Tukey's test ($p \leq 0.05$).

The Cu content in the shoots and roots system of yerba mate showed a significant interaction between the inoculum and Cu doses (Figure 3). The Cu content in the shoots increased linearly by 419% in the treatment without inoculation between the baseline and final period, while both isolates UFSC-PT116 and UFSC-SU118 showed a maximum point of 198.9 and 162.3 mg kg^{-1}, respectively. At the highest dose studied, there was a reduction in the content of this metal in 83 and 86% for both UFSC-PT116 and UFSC-SU118 isolates, respectively, compared to the plants without inoculation (Figure 3A). In the root system, the treatment without inoculation showed an increase of 847% in the Cu content at the highest dose, being significantly higher than the other treatments, and the UFSC-PT116 and UFSC-SU118 isolates showed a maximum point at the estimated doses of 179.6 and 296.13 mg kg^{-1}, respectively; and also resulting in lower Cu content in the root at the highest dose applied to the soil (Figure 3B). It can be seen that at certain doses, in the presence of ectomycorrhizal fungi, the Cu content in the tissue of the roots and shoots of the plants was lower, as observed by Gadd (1993) and Fogarty and Tobin (1996), who showed a close relationship between the production of extracellular pigments by ectomycorrhizal fungi and the bioadsorption of metals. Thus, as shown by Bertolazzi et al. (2010), ectomycorrhizal isolates can retain Cu in their mycelium and adsorb the metal, preventing it from being absorbed by the yerba mate roots.

The Itol showed a significant interaction between the inoculum and Cu doses applied to the soil, being reduced in seedlings without inoculation by 14% at the highest dose (Figure 4). In treatments with inoculation, the UFSC-PT116 isolate showed a linear increase of 106%, at the dose of 400 mg kg^{-1} of Cu in the soil, being significantly higher than the other treatments. Cu doses also caused a reduction in the Itol of *Senna multijuga* ((Rich.) H.S. Irwin & Barneby) Fabaceae (De Marco et al., 2017), corroborating with the results found in this study for plants without inoculation. Mycorrhizas play a key role in reducing the toxic effect of Cu on the plant. In the plasma membrane, they play a role in the homeostasis of contaminants, controlling or preventing the entry of the metal into the cell (Ali et al., 2013).

The results showed a significant interaction between the ectomycorrhizal isolates and the Cu doses for the percentage of ectomycorrhizal colonization, being significantly higher with the inoculations compared to the control (without inoculation) that did not show ectomycorrhiza formation, in all Cu doses used (Figure 5). The UFSC-PT116 isolate showed a significantly higher percentage of mycorrhizal colonization than the other inocula from the 160 mg kg^{-1} dose, ranging from 14 to 19%. In a study with the UFSC-PT116 isolate, Souza et al. (2012) obtained values close to 20% of colonization in the species *Eucalyptus grandis* (Hill ex Maiden), family Myrtaceae. These authors emphasize the need for selecting fungal isolates that are efficient in establishing ectomycorrhiza with certain plant species. According to Moreira and Siqueira (2006), ideal fungi for large-scale inoculation need to widely colonize the root system and promote a beneficial response in the root system of the plants.

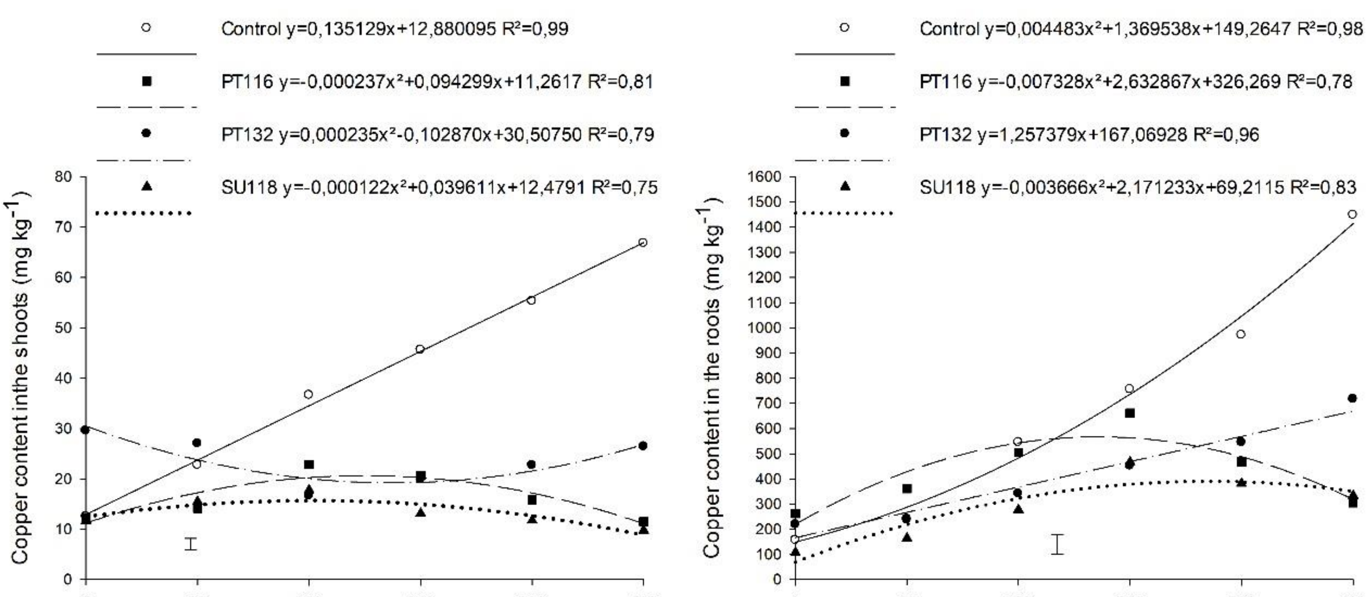

Figure 3 – (A) Copper content in the shoots — CuPA and (B) roots — CuR of *Ilex paraguariensis* seedlings with doses of copper applied in the soil without (Control) and with the addition of three inocula of ectomycorrhizal fungi such as *Pisolithus microcarpus* (PT116), *Pisolithus tinctorius* (PT132), and *Suillus cothurnatus* (SU118).
DMS: minimum significant difference by Tukey's test ($p \leq 0.05$).

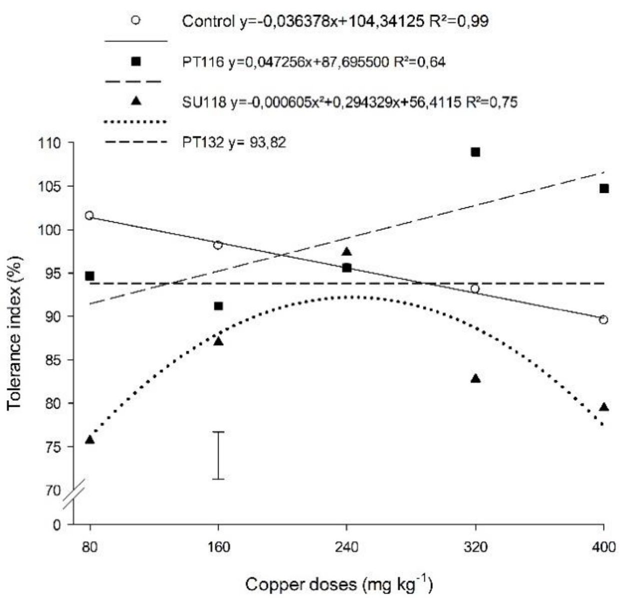

Figure 4 – Relationship between the Tolerance Index (ITOL) of *Ilex paraguariensis* seedlings with the doses of copper applied in the soil, control treatments without inoculation (Control) and with the addition of three inocula of ectomycorrhizal fungi *Pisolithus microcarpus* (PT116), *Pisolithus tinctorius* (PT132), and *Suillus cothurnatus* (SU118).
DMS: minimum significant difference by Tukey's test ($p \leq 0.05$).

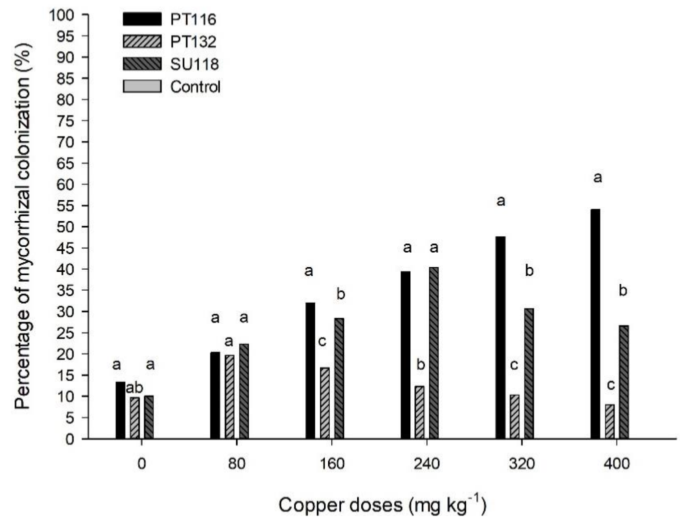

Figure 5 – Percentage of mycorrhizal colonization in *Ilex paraguariensis* in the control treatments, without inoculation (Control) and with the addition of three inocula of ectomycorrhizal fungi *Pisolithus microcarpus* (PT116), *Pisolithus tinctorius* (PT132), and *Suillus cothurnatus* (SU118) in a soil contaminated with doses of copper.
*Means followed by the same letter do not differ from each other at the same dose by the Tukey's test ($P < 0.05$) of the probability of error.

Other studies on excess Cu in other plants, such as *Scrophularia striata*, showed that there is a toxicological effect in increasing the concentration of metals, not only with Cu (Mousavi et al., 2022). However, as this is a pioneering study with yerba mate, it was not possible to discuss the effect of the ectomycorrhizal fungi, yerba mate, and Cu tolerance in conjunction with the literature.

Conclusions

Environmental contamination has been constant in the soil system, causing several environmental problems. The application of clean biotechnologies in contaminated environments is a sustainable, cheap, and viable way to reduce or control pollution and recover these degraded environments. The results of this study, with the application of ectomycorrhizal fungi in *Ilex paraguariensis* plants to reduce the effect of contamination, showed that the use of these fungi contributes to reducing Cu bioaccumulation and mitigates the possible phytotoxic effects on the plants, which may contribute to decreasing high concentrations of this metal in the soil. The inoculation of *Ilex paraguariensis* seedlings with the ectomycorrhizal fungi UFSC-PT116, UFSC-PT132, and UFSC-SU118 mitigates the phytotoxicity effect caused by the excess of Cu in the soil. Although the results shown in this work demonstrate high applicability, long-term studies and the use of new isolates and different plants are needed to increase the use of the technique.

Contribution of authors:

COINASKI, D. A.: Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Validation; Visualization; Writing — original draft; Writing — review & editing. SILVA, R. F.: Conceptualization; Investigation; Methodology; Supervision; Validation; Visualization; Writing — original draft; Writing — review & editing. ROS, C. O.: Investigation; Methodology; Supervision; Validation; Visualization; Writing — original draft; Writing — review & editing. ROSA, G. M.: Investigation; Methodology; Supervision; Validation; Visualization; Writing — original draft; Writing — review & editing. SORIANI, H. H.: Investigation; Methodology; Supervision; Validation; Visualization; Writing — original draft; Writing — review & editing. ANDREAZZA, R.: Investigation; Supervision; Validation; Visualization; Writing — original draft; Writing — review & editing.

References

Afonso, T.F.; Demarco, C.F.; Pieniz, S.; Quadro, M.S.; Camargo, F.A.O.; Andreazza, R., 2022. Analysis of Baccharis dracunculifolia and Baccharis trimera for phytoremediation of heavy metals in copper mining tailings area in southern brazil. applied biochemistry and biotechnology, v. 194, 694-708. https://doi.org/10.1007/s12010-021-03652-3.

Ali, H.; Khan, E.; Anwar, M., 2013. Chemosphere phytoremediation of heavy metals – concepts and applications. Chemosphere, v. 91, (7), 869-881. https://doi.org/10.1016/j.chemosphere.2013.01.075.

Ambrosini, V.G.; Rosa, D.J.; Prado, J.P.C.; Borghezan, M.; Melo, G.W.B.; Soares, C.R.F.S.; Comin, J.J.; Simão, D.G.; Brunetto, G., 2015. Reduction of copper phytotoxicity by liming: a study of the root anatomy of young vines (Vitis labrusca L.). Plant Physiology and Biochemistry, 96, 270-280. https://doi.org/10.1016/j.plaphy.2015.08.012.

Bertolazzi, A.A.; Canton, G.C.; Azevedo, I.G.; Cruz, Z.M.A.; Soares, D.N.E.S.; Conceição, J.M.; Santos, W.O.; Ramos, A.C., 2010. O papel das ectomicorrizas na biorremediação de metais pesados no solo. Natureza Online, v. 8, (1), 24-31.

Brasil. Conselho Nacional do Meio Ambiente (Conama), 2009. Resolução nº 420, de 28 de dezembro de 2009. Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades antrópicas. Diário Oficial da República Federativa do Brasil, Poder Executivo, Brasília, Seção 1, 20 p.

Brundrett, M. C. Mycorrhizal associations: the web resource. 2008 (Accessed Feb 20, 2019) at:. mycorrhizas.info.

Cambrollé, J.; Mancilla-Leytón, J.M.; Muñoz-Vallés, S.; Luque, T., Figueroa, M.E., 2012. Zinc tolerance and accumulation in the salt-marsh shrub Halimione portulacoides. Chemosphere, 86, (9), 867-874. https://doi.org/10.1016/j.chemosphere.2011.10.039.

Cassanego, M.B.; Goldoni, A.; Heldt, F.H.; Migliavacca, D.M.; Windisch, O.P.G.; Droste, A., 2013. Germination and sporophytic development of Regnellidium diphyllum Lindm. (Marsileaceae) in the presence of copper. Acta Botanica Brasilica, v. 27, (1), 26-30. https://doi.org/10.1590/S0102-33062013000100004.

Chaves, L.H.G.; Mesquita, E.F.; Araujo, D.L.; França, C.P., 2010. Crescimento, distribuição e acúmulo de cobre e zinco em plantas de pinhão-manso. Ciência Agronômica, v. 41, (2), 167-176. https://doi.org/10.1590/S1806-66902010000200001.

De Marco, R.; Silva, R.F.; Ros, C.O.; Vanzam, M.; Boeno, D., 2017. Senna multijuga and peat in phytostabilization of copper in contaminated soil. Revista Brasileira de Engenharia Agrícola e Ambiente, v. 21, (6), 421-426. https://doi.org/10.1590/1807-1929/agriambi.v21n6p421-426.

Dellai, A.; Silva, R.F.; Andreazza, R., 2018. Ectomicorriza no crescimento de Eucalyptus salignaem solo contaminado com cobre. Ciência Florestal, v. 28, (2), 624-631. https://doi.org/10.5902/1980509832052.

Dellai, A.; Silva, R.F.; Perrando, E.R.; Jacques, R.J.S.; Grolli, A.L.; Marco, R.D., 2014. Óleo de eucalipto e Pisolithusmicrocarpus no crescimento de bracatinga em solo contaminado por cobre. Revista Brasileira de Engenharia Agrícola e Ambiental, v. 18, (9), 927-933. https://doi.org/10.1590/1807-1929/agriambi.v18n09p927-933.

Ferreira, D.F., 2011. Sisvar: sistema de análise de variância. Versão 5.3. UFLA, Lavras.

Fogarty, V.; Tobin, M., 1996. Fungal melanins and their interactions with metals. Enzyme and Microbial Technology, v. 19, (4), 311-317. https://doi.org/10.1016/0141-0229(96)00002-6.

Fundação Estadual de Meio Ambiente do Rio Grande do Sul (FEPAM), 2014. Portaria nº 85/2014. Dispõe sobre o estabelecimento de Valores de Referência de Qualidade (VQR) dos solos para nove elementos químicos naturalmente presentes nas diferentes províncias geomorfológicas/geológicas do estado do Rio Grande do Sul. FEPAM, Rio Grande do Sul.

Gadd, G.M., 1993. Interactions of fungi with toxic metals. New Phytologist, v. 124, (1), 25-60. https://doi.org/10.1111/j.1469-8137.1993.tb03796.x

Gautam, S.; Anjani, K.; Srivastava, N., 2016. In vitro evaluation of excess copper affecting seedlings and their biochemical characteristics in Carthamus tinctorius L. (variety PBNS-12). Physiology and Molecular Biology of Plants, v. 22, (1), 121-129. https://doi.org/10.1007/s12298-016-0339-1.

Giovannetti, M.; Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist, v. 84, (3), 489-500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x.

Grassi Filho, H., 2005. Cobre na planta. Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agronômicas, Departamento de Recursos Naturais, Ciência do Solo.

Guimarães, P.R.; Ambrosini, V.G.; Miotto, A.; Ceretta, C.A.; Simão, D.G.; Brunetto, G., 2016. Black Oat (Avena strigosa Schreb.) Growth and root anatomical changes in sandy soil with different copper and phosphorus concentrations. Water Air and Soil Pollution, v. 227, 192-201. https://doi.org/10.1007/s11270-016-2900-5.

Harley, J.L., 1969. The biology of mycorrhiza. Leonard Hill, London, 230 pp.

Jacques, R.A.; Freitas, L.S.; Péres, V.F.; Caramão, E.B., 2007. The use of ultrasound in the extraction of Ilex paraguariensis leaves: A comparison with maceration. Ultrasonics Sonochemistry, v. 14, (1), 6-12. https://doi.org/10.1016/j.ultsonch.2005.11.007.

Jorge, L.A.C.; Silva, D.J.C.B., 2010. Safira: manual de utilização. Embrapa Instrumentação Agropecuária, São Carlos.

Kabata-Pendias, A., 2011. Trace elements in soils and plants. 4. ed. CRC Press, Boca Raton.

Lequeux, H.; Hermans, C.; Lutts, S.; Verbruggen, N., 2010. Response to copper excess in Arabidopsis thaliana: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiology and Biochemistry, v. 48, (8), 673-682. https://doi.org/10.1016/j.plaphy.2010.05.005.

Lorenzi, H., 2009. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Plantarum, Nova Odessa, v. 3.

Marastoni, L.; Sandri, M.; Pii, Y.; Valentinuzzi, F.; Brunetto, G.; Cesco, S.; Mimmo T., 2019. Synergism and antagonisms between nutrients induced by copper toxicity in grape vine root stocks: Monocropping vs. intercropping. Chemosphere, v. 214, 563-578. https://doi.org/10.1016/j.chemosphere.2018.09.127.

Marques, D.M.; Silva, A.B.; Mantovani, J.R.; Pereira, D.S.; Souza, T.C., 2018. Growth and physiological responses of tree species (Hymenaeacour baril L., Peltophorum dubium (Spreng.) Taub. and Myroxylon peruiferum L. F.) exposed to different copper concentrations in the soil. Viçosa: Revista Árvore, v. 42, 2, e420202. https://doi.org/10.1590/1806-90882018000200002.

Marx, D.H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathologist, v. 59, (2), 153-163.

Mateos-Naranjo, E.; Andrades-Moreno, L.; Cambrollé, J.; Perez-Martin, A., 2013. Assessing the effect of copper on growth, copper accumulation and physiological responses of grazing species Atriplex halimus: ecotoxicological implications. Ecotoxicology Environmental Safety, v. 90, 136-142. https://doi.org/10.1016/j.ecoenv.2012.12.020.

Miyazawa, M.; Pavan, M.A.; Muraoka, T.; Carmo, C.A.F.S.; Melo, W.J., 2009. Análise química de tecido vegetal. In: Silva, F.C. (Ed.). Manual de análises químicas de solos, plantas e fertilizantes. 2. ed. Embrapa Informação Tecnológica, Brasília, pp. 59-85.

Moreira, F.M.S.; Siqueira, J.O.S., 2006. Microbiologia e bioquímica do solo. UFLA, Lavras, 729 pp.

Mousavi, S.S.; Karami, A.; Haghighi, T.M.; Tahmasebi, A., 2022. Lead, copper, zinc and aluminum tolerance in contrasting ecotypes of Scrophularia striata. Acta Ecologica Sinica. https://doi.org/10.1016/j.chnaes.2022.01.005.

Raven, P.H.; Evert, R.F.; Eichhorn, S.E., 2007. Biologia vegetal. Guanabara Koogan, Rio de Janeiro, 906 pp.

Saidelles, A.P.F.; Kiechner, R.M.; Santos, N.R.Z.; Flores, E.M.M.; Bartz, F.B., 2010. Análise de metais em amostras comerciais de erva-mate no sul do Brasil. Alimentos e Nutrição, v. 21, (2), 259-265.

Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods, v. 9, 671-675. https://doi.org/10.1038/nmeth.2089.

Silva, F.C., 2009. Manual de análises químicas de solos, plantas e fertilizantes. 2. ed. Embrapa Informação Tecnológica, Brasília, 627 pp.

Silva, I.C.; Rocha, C.; Rocha, M.C.; Sousa, C.M., 2018. Growth of Brachiaria decumbens in Latosol contaminated with copper. Ciência e Agrotecnologia, v. 42, (2), 168-175. https://doi.org/10.1590/1413-70542018422030317.

Silva, R.F.; Antoniolli, Z.I.; Andreazza, R., 2003. Produção de mudas de Pinus elliottii Engelm. micorrizadas em solo arenoso. Ciência Florestal, v. 13, (2), 57-65. https://doi.org/10.5902/198050981742.

Silva, R.F.; Antoniolli, Z.I.; Lupatini, M.; Trindade, L.L.; Silva, A.S., 2010. Tolerância de mudas de canafístula (Peltophorum dubium (spreng.) taub.) inoculadas com Pisolithus microcarpus a solo com excesso de cobre. Ciência Florestal, v. 20, (1), 147-156. https://doi.org/10.5902/198050981768.

Silva, R.F.; Ros, C.O.; Dellai, A.; Grolli, A.L.; Scheid, D.L.; Viel, P., 2016. Interferência de doses de cobre no crescimento e na qualidade de mudas de Bauhinia forficata Link, Pterogyne nitens Tul e Enterolobium contortisiliquum Vell. Ciência Florestal, v. 26, (2), 647-655. https://doi.org/10.5902/1980509822764.

Silva, R.F.; Ros, C.O.; Scheid, D.A.; Grolli, A.L.; De Marco, R.; Missio, E.L., 2015. Copper translocation and tolerance in seedlings of tree species grown in contaminated soil. Revista Brasileira de Engenharia Agrícola e Ambiente, v. 19, (11), 1093-1099. https://doi.org/10.1590/1807-1929/agriambi.v19n11p1093-1099.

Smith, S.E.; Read, D.J., 2008. Mycorrhizalsymbiosis. 3. ed. Academic Press, San Diego, 787 pp.

Souza, E.L.; Antoniolli, Z.I.; Machado, R.G.; Eckhardt, D.P.; Dahmer, S.F.B.; Schirmer, G.K., 2012. Efeito da inoculação com isolados de fungos ectomicorrízicos sobre o desenvolvimento de mudas de Eucalyptus grandis Hillex Maiden. Ciência Florestal, v. 22, (2), 251-261. https://doi.org/10.5902/198050985732.

Steffen, R.B.; Antoniolli, Z.I.; Steffen, G.P.K.; Silva, R.F., 2012. Óleo essencial de Eucalyptus grandis Hillex Maiden no estímulo à micorrização de mudas de sibipiruna (Caesalpinia peltophoroides Benth.). Ciência Florestal, v. 22, (1), 69-78. https://doi.org/10.5902/198050985080.

Taiz, L.; Zeiger, E., 2009. Fisiologia vegetal. 4ª ed. Artmed, Porto Alegre, 848 pp.

United States Environmental Protection Agency (USEPA), 1996. Method 3050 B: acid digestion of sediments, sludges, and soils. USEPA, Washington, D.C., 12 pp.

Volcão, L.M.; Halicki, P.C.B.; Christ-Ribeiro, A.; Ramos, D.F.; Badiale-Furlong, E.; Andreazza, R.; Bernardi, E.; Da Silva Júnior, F.M.R., 2022. Mushroom extract of Lactarius deliciosus (L.) Sf. Gray as biopesticide: Antifungal activity and toxicological analysis. Journal of Toxicology and Environmental Health, Part A, v. 85, (2), 43-55. https://doi.org/10.1080/15287394.2021.1970065.

Weirich, S.W.; Silva, R.F.; Perrando, E.R.; Ros, C.O.; Dellai, A.; Scheid, D.L.; Trombeta, H.W., 2018. Influência de ectomicorrizas no crescimento de mudas de Eucalyptus grandis, Corymbia citriodora, Eucalyptus saligna e Eucalyptus dunnii. Ciência Florestal, v. 28, (2), 765-765. https://doi.org/10.5902/1980509832129.

Wendling, I.; Dutra, L.F., 2010. Produção de mudas de eucalipto por sementes. In: Wendling, I.; Dutra, L.F. Produção de mudas de eucalipto. Embrapa Florestas, Colombo.

Wilkins, D.A., 1978. The measurement of tolerance to edaphic factors by means of root grown. The New Phytologist, v. 80, (3), 623-633. https://doi.org/10.1111/j.1469-8137.1978.tb01595.x.

Yaashikaa, P.R.; Kumar, P.S.; Jeevanantham, S.; Saravanan, R., 2022. A review on bioremediation approach for heavy metal detoxification and accumulation in plants. Environmental Pollution, v. 301, 119035. https://doi.org/10.1016/j.envpol.2022.119035.

Yruela, I., 2009. Copper in plants: acquisition, transport and interactions. Functional Plant Biology, v. 36, (5), 409-430. https://doi.org/10.1071/FP08288.

Yruela, I., 2013. Transition metals in plant photosynthesis. Metallomics, v. 5, (9), 1090-1109. https://doi.org/10.1039/c3mt00086a.