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Scientific Article

ZINC TOXICITY AND TOLERANCE-RELATED RESPONSES IN *Inga marginata* AND *Allophylus edulis* SEEDLINGS

Marcos Vinícius Miranda Aguilar[2*], Caroline Castro Kuinchtner[2], Gerâne Silva Wertonge[2], Thomas Wink Peixoto[3], Thalía Preussler Birck[4], Daniel Vinicios Valsoler[3], Fernando Teixeira Nicoloso[5], Gustavo Brunetto[6] and Luciane Almeri Tabaldi[5]

1 Received on 14.04.2023 accepted for publication on 16.11.2023.
2 Universidade Federal de Santa Maria, Programa de Pós-Graduação em Engenharia Florestal, Santa Maria, RS - Brasil. E-mail: <aguilarmarcos2009@hotmail.com>, <ccastrokuinchtner@gmail.com> and <gerane-wertonge@hotmail.com>.
3 Universidade Federal de Santa Maria, Graduando em Ciência Biológicas, Santa Maria, RS - Brasil. E-mail:<thomaswinkpeixoto@gmail.com> and <danielvalsoler15@gmail.com>.
4 Universidade Federal de Santa Maria, Programa de Pós-Graduação em Bioquímica Toxicológica, Santa Maria, RS - Brasil. E-mail:<thaliapbirck@gmail.com>.
5 Universidade Federal de Santa Maria, Departamento de Biologia, Santa Maria, RS - Brasil. E-mail:< ftnicoloso@yahoo.com> and <lutabaldi@yahoo.com.br>.
6 Universidade Federal de Santa Maria, Departamento de Solos, Santa Maria, RS - Brasil. E-mail:<brunetto.gustavo@gmail.com>.
*Corresponding author.

ABSTRACT

Although zinc (Zn) is a micronutrient, excessive amounts in the soil can have toxic effects on plants. Fertilizers, limestone materials, pesticides, and fungicides added with Zn have contributed to increasing the concentration of this element in agricultural soils. Accordingly, it is necessary to find Zn-tolerant plant species to be properly used in degraded soil restoration programs. Thus, the current study aims to investigate the influence of different Zn concentrations on photosynthetic variables, antioxidant activity, and growth of *I. marginata* and *A. edulis* seedlings to determine their potential to be used as phytoremediation species. The experiment was installed in a 2×5 factorial scheme, with the first factor being two species (*Allophylus edulis* and *Inga marginata*), and the second factor: five concentrations of Zn (2, 75, 150, 225, and 300 μM), with three replications per treatment. Each sampling unit consisted of a pot with five plants. Photosynthetic, morphological variables of the shoot and root systems, chlorophyll a fluorescence, photosynthetic pigments, antioxidant enzyme activity, lipid peroxidation, hydrogen peroxide concentration, and Zn accumulated in the roots and shoot were evaluated. Zn stress has activated an efficient antioxidant system, which reduced oxidative damage in the leaves of both species; consequently, it did not decrease shoot biomass production in *Inga marginata* and *Allophylus edulis* seedlings. High Zn accumulation in plant tissues and lack of negative effects on *Inga marginata* and *Allophylus edulis* shoot have suggested that these plant species are tolerant to Zn and may be indicated for Zn-polluted soil phytoremediation purposes.

Keywords: Antioxidant System; Gas exchange; Mineral nutrition; Oxidative stress.

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TOXICIDADE DO ZINCO E RESPOSTAS RELACIONADAS À TOLERÂNCIA EM MUDAS DE *Inga marginata* E *Allophylus edulis*

RESUMO – Embora o zinco (Zn) seja um micronutriente, quantidades excessivas no solo podem causar efeitos tóxicos às plantas. Fertilizantes, materiais calcários, pesticidas e fungicidas adicionados de Zn têm contribuído para aumentar a concentração deste elemento nos solos agrícolas. Dessa forma, é necessário encontrar espécies de plantas tolerantes ao Zn para serem adequadamente utilizadas em programas de restauração de solos degradados. Assim, o presente estudo tem como objetivo investigar a influência de diferentes concentrações de Zn nas variáveis fotossintéticas, na atividade antioxidante e no crescimento de mudas de *I. marginata* e *A. edulis* para determinar seu potencial para serem utilizadas como espécies fitorremediadoras. O experimento foi instalado em esquema fatorial 2×5, sendo o primeiro fator duas espécies (*Allophylus edulis* e *Inga marginata*), e o segundo fator: cinco concentrações de Zn (2, 75, 150, 225 e 300µM), com três repetições por tratamento. Cada unidade amostral foi constituída por um vaso com cinco plantas. Foram avaliadas variáveis fotossintéticas, morfológicas da parte aérea e do sistema radicular, fluorescência da clorofila a, pigmentos fotossintéticos, atividade de enzimas antioxidantes, peroxidação lipídica, concentração de peróxido de hidrogênio e Zn acumulado nas raízes e na parte aérea. O estresse por Zn ativou um eficiente sistema antioxidante, que reduziu o dano oxidativo nas folhas de ambas as espécies; consequentemente, não diminuiu a produção de biomassa aérea em mudas de *Inga marginata* e *Allophylus edulis*. O alto acúmulo de Zn nos tecidos vegetais e a falta de efeitos negativos na parte aérea de *Inga marginata* e *Allophylus edulis* sugeriram que essas espécies de plantas são tolerantes ao Zn e podem ser indicadas para fins de fitorremediação de solos poluídos com Zn.

Palavras-Chave: Estresse oxidativo; Nutrição mineral; Sistema Antioxidante; Troca gasosa.

1. INTRODUCTION

Environmental pollution caused by heavy metals poses a serious threat to living organisms (Aguilar et al., 2023). The long-term deposition of these materials in the soil can lead to their accumulation, transport, and toxicity in plants (Hammerschmit et al., 2020). Activities such as mining and smelting of metallic ores, industrial emissions, insecticide applications, fertilizers, and sewage sludge disposal have contributed to increasing heavy metals levels in the soil (Kuinchtner et al., 2021). In addition, plant micronutrients such as zinc (Zn), copper (Cu), and manganese (Mn) have been added to agricultural systems, a fact that increases the risk of toxicity in plants (Tiecher et al., 2016). Zinc is an essential micronutrient for plants since it acts as a cofactor for several metalloproteins such as superoxide dismutase, carbonic anhydrase, dehydrogenases, proteases, peptidase, and phosphohydrolases (Brunetto et al., 2018; Somavilla et al., 2018). Despite the key role played by Zn in plant growth and development, this micronutrient can easily become toxic when its concentration reaches high levels in the soil and, consequently, in plant tissues (Bernardy et al., 2016).

Zinc accumulation level in plants depends on differences between plant species. Moreover, it can be affected by varied factors, such as plant growth stage and Zn absorption, accumulation, and translocation control in plant tissues (Hammerschmit et al., 2020). Plants grown in soils subjected to Zn excess can significantly accumulate this heavy metal in their tissues and develop toxicity symptoms. In general, changes such as reduced growth, increased mean root diameter, and reduced root length can be observed in plants subjected to high Zn concentrations (Zhang et al., 2019). In addition, plants can present negative biochemical and physiological responses due to such disorders, which can change depending on the plant species and organ affected, on element concentration, and on tissue tolerance to high Zn levels (Tiecher et al., 2018; Aguilar et al., 2023).

However, plants may adopt different strategies to deal with high levels of metals, such as Zn, in their growth environment. Tolerance mechanisms mainly comprise detoxification processes based on complexation by organic chelators, Zn sequestration in vacuoles (Zalamena et al., 2015), and antioxidant system activation. These strategies help protect the metabolism of plants subjected to Zn excess.

Despite the great interest of the scientific community in ecological and environmental issues caused by excess heavy metals in the soil-plant system, little emphasis has been given to the impact of these metals on tree species (Marques et al., 2018). Therefore, it is necessary to improve knowledge about forest species with phytoremediation potential, mainly those presenting a fast growth rate, extensive geographic distribution, and phenotypic plasticity that can be used to reforest contaminated areas (Aguilar et al., 2023).

The *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Radlk species belongs to the Sapindaceae family (Pereira et al., 2016); it was described as an early secondary species (Ballestreri et al., 2021). It is popularly known as “chal-chal” or “cocu”; moreover, it can be found in different South American countries such as Brazil, Bolivia, Paraguay, Argentina, and Uruguay. *A. edulis* is a medium-sized tree species capable of growing in soils presenting medium-to-poor fertility, so it is widely used to help to recover degraded ecosystems (Pereira et al., 2016).

Inga marginata is a pioneer species belonging to the Fabaceae family. It is known as “ingazinho”, “inga”, and “ingá-feijão”, and is widely distributed in South America (Turchetto et al., 2020). Since *I. marginata* is a leguminous species, it can establish symbiotic associations with nitrogen-fixing bacteria, making it an ideal species for nutrient-poor environments and regeneration areas. In addition, *I. marginata* has the potential to be used for phytoremediation purposes; therefore, it is useful for ecological restoration (Cabral et al., 2017).

Thus, the present study aimed to investigate the influence of different Zn concentrations on the photosynthetic variables, antioxidant activity, and growth of *I. marginata* and *A. edulis* seedlings to determine their potential to be used as Zn phytoremediation species. The current hypothesis is that the investigated species can accumulate and tolerate high Zn concentrations by maintaining biomass production and activating antioxidant mechanisms.

2. MATERIAL AND METHODS

2.1 Study site and plant material

The study was conducted in a greenhouse at the Biology Department of the Federal University of Santa Maria (UFSM) - Santa Maria Campus, Rio Grande do Sul State (RS), Brazil. The average temperature inside the greenhouse was 25ºC, and relative air humidity was 60%.

I. marginata and *A. edulis* seedlings were produced at UFSM Forest Nursery - Santa Maria Campus. Seeds were collected from parent trees grown in forest fragments in the investigated region, and sowing was carried out right in non-toxic polypropylene plastic tubes (180 cm^3 in volume) filled with substrate. The substrate used for seedlings’ production was Carolina Soil®, composed of Sphagnum sp., vermiculite, and added with 30% carbonized rice husk. Sowing fertilization was carried out with controlled-release fertilizer of the Osmocote® type (CRF), which comprised 15% of N, 9% P_2O_5, 12% KCl, 1% Mg, 2.3% S, 0.05% Cu, 0.06% Mn, 0.45% Fe, and 0.2% Mo, and six-month nutrient release.

Seedlings at the age of 120 days and approximately 25 cm in height and 2.0±0.2 mm in stem diameter were sent to the greenhouse of the Biology Department at UFSM – Campus of Santa Maria for acclimation purposes until the experiment was carried out.

2.2 Conducting the experiment

The experiment was carried out in a completely randomized design arranged in a 2×5 factorial scheme with three replications. The first factor was composed of two tree species (*Allophylus edulis* and *Inga marginata*). The second factor was composed of five Zn concentrations (2, 75, 150, 225, and 300 μM). Each replication consisted of a pot containing five plants, totaling fifteen plants per treatment. The standard Zn concentration in the nutrient solution of Hoagland and Arnon (1950) is 2 μM, which was used as a control treatment, and for the other concentrations, 75, 150, 225, and 300 μM of Zn were added into the nutrient solution complete.

I. marginata and *A. edulis* seedlings were removed from the tubes and had their roots carefully washed with tap water to remove the substrate from their surroundings. Each seedling was placed in a 6 L pot filled with a complete nutrient solution, based on Hoagland and Arnon (1950). A Styrofoam sheet was added to the surface of each pot, with five holes to allow the plant to pass through. Besides allowing plants to be fixed, the Styrofoam sheet has also contributed to reducing nutrient solution evaporation in each pot.

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Seedlings were left to acclimatize in Hoagland and Arnon's (1950) nutrient solution for 15 days, with 100% of their full strength. Solution aeration in each pot was carried out through PVC microtubes connected to an air compressor to enable root aeration. The nutrient solution, in its original form, comprised 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.

Treatments were applied after the acclimation period was over. Seedlings were subjected to different Zn concentrations for 21 days, which totaled 36 days in the hydroponic system. The nutrient solution in each pot was replaced twice a week, and its pH was adjusted to 5.5±0.2 daily by using 1.0 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH.

2.3 Evaluated morphological variables and Tissue Zn analysis

Shoot height, and the length of the main root of the investigated plants were measured with a millimeter ruler before the experiment installation and at the end of it. Shoot height (SHI) and main root (RI) increase values were calculated based on measurement results.

The morphological featuring of the roots was conducted based on digitized images in the WinRhizo Pro 2013 software, coupled with EPSON Expression 11000 scanner, equipped with additional light (TPU), at 600-DPI resolution. After the images' scanning procedure was over, root surface area (cm^2 $plant^{-1}$), total root length (cm $plant^{-1}$), root volume (cm^3 $plant^{-1}$), and average root diameter (mm) were determined.

Shoot and roots were washed in running water and, subsequently, in distilled water. They were dried in an air-forced circulation oven at 65 ºC until they reached constant weight. Shoot (SDW) and root (RDW) dry weight were calculated based on these results. Afterward, shoot and root dry weight samples were ground in a Wiley mill and sieved in 2 mm mesh. Plant tissues (roots and shoot) were subjected to nitric-perchloric digestion (3.0 mL of HNO 65% PA and 1 mL of HClO 70% PA) (Embrapa, 2009). Total Zn concentration in tissues was analyzed in an atomic absorption spectrophotometer (AAS, Perkin Elmer Analyst 200, USA).

2.4 Photosynthetic variables

The net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (Gs), and Rubisco instantaneous carboxylation efficiency (A/Ci - obtained based on net CO_2 assimilation: intercellular CO_2 concentration ratio were evaluated in the third fully expanded leaf by using the infrared gas analyzer [(IRGA), Mod. Li-COR® 6400 XT], at photosynthetic radiation of 1,500 µmol m^{-2} s^{-1} and CO_2 concentration of 400 µmol mol^{-1}. Measurements were taken in the morning, between 8:00 a.m. and 10:00 a.m., 20 days after the beginning exposure of plants to the treatments, before plants were collected for growth analysis.

2.5 Chlorophyll a fluorescence

Analyses were performed on fully expanded leaves grown in the middle third of plants in the morning of a sunny day, between 8:00 a.m. and 11:30 a.m., with the aid of a portable light-modulated fluorometer (Junior-Pam Chlorophyll Fluorometer WalzMess-und-Regeltechnik, Germany). Leaves were preadapted to the dark for 30 min before measurements to determine initial fluorescence (Fo). Subsequently, samples were subjected to a saturating light pulse (10,000 µmol m^{-2} s^{-1}) for 0.6 s to determine maximum fluorescence (Fm) and electron transport rate (ETRm). PSII (Fv/Fm) maximum potential quantum yield was obtained based on the ratio between variable fluorescence and maximum fluorescence (Fv = Fm-Fo).

2.6 Biochemical variables

Three plants from each experimental unit were collected for biochemical analysis, totaling 90 plants. They were evaluated, sectioned, and washed their leaves and roots in distilled water, added to aluminum foil envelopes, and frozen in liquid nitrogen (N) immediately to avoid sample degradation. These samples were kept in an ultra-freezer at -80°C until analysis time, when they were macerated in liquid N, homogenized in a specific buffer, and analyzed later.

Chlorophyll a, chlorophyll b, and carotenoid concentrations in the shoot were extracted based on the method by Hiscox and Israelstan (1979) and estimated based on the equation by Lichtenthaler (1987). The hydrogen peroxide (H_2O_2) concentration was determined based on Loreto and Velikova (2001), and results were expressed as µmol g^{-1} fresh weight.

Superoxide dismutase (SOD) activity was determined based on the spectrophotometric method described by Giannopolitis and Ries (1977), whereas guaiacol peroxidase activity was determined based on Zeraik et al. (2008). The membrane lipid peroxidation degree was estimated based on the method by El-Moshaty et al. (1993). Lipid peroxidation results were expressed as nmol MDA mg^{-1} of protein.

2.7 Statistical analysis

Error distribution normality was checked through the Shapiro-Wilk test, whereas error variance homogeneity was checked through the Bartlett test; these tests were applied to all experimental variables. Whenever these assumptions were met, the data were subjected to analysis of variance and the Scott-Knott test ($p < 0.05$) in the Sisvar statistical software (Ferreira, 2019).

3. RESULTS

3.1 Morphological variables

Based on the results of the analysis of variance, the factors evaluated (two plant species and Zn concentrations) had a significant effect ($p \leq 0.05$) on the morphological growth variables. Thus, the highest mean values of taproot increase were observed for the control and 75 µM Zn in *A. edulis* and only for the control in *I. marginata* (Figure 1A). When comparing species, it was observed that *I. marginata* seedlings grown in the control showed the highest increment

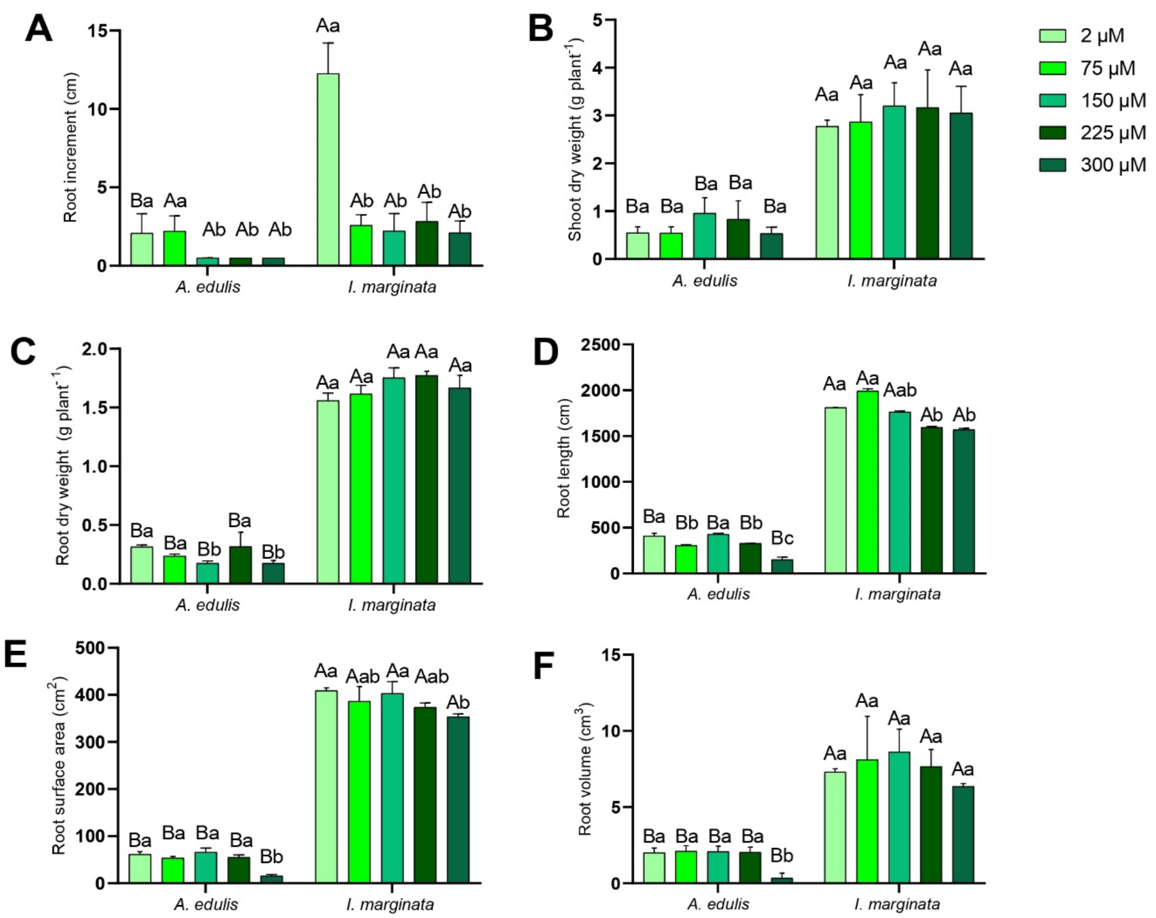

Figure 1 – Mean values recorded for root increase (RI) (A), shoot dry weight (B), root dry weight (C) total root length (D), root surface area (E) and root volume (F) in *Allophylus edulis* (*A. edulis*) and *Inga marginata* (*I. marginata*) seedlings grown under different Zn concentrations. Different letters between treatments represent statistically significant difference in the Scott-Knott test. Bars represent the mean ± standard deviation.

Figura 1 – Valores médios registrados para aumento de raízes (IR) (A), peso seco da parte aérea (B), peso seco da raiz (C), comprimento total da raiz (D), área superficial da raiz (E) e volume da raiz (F) em mudas de *Allophylus edulis* (*A. edulis*) e de *Inga marginata* (*I. marginata*) cultivadas sob diferentes concentrações de Zn. Letras diferentes entre os tratamentos representam diferença estatisticamente significativa no teste de Scott-Knott. As barras representam a média ± desvio padrão.

values in the main root, significantly differing from *A. edulis* (Figure 1A).

Zn concentrations did not negatively affect the shoot dry weight of both species, compared to the control, and similar behavior was observed for the root dry weight of *I. marginata* (Figures 1B and 1C). Furthermore, the lowest value recorded for the root dry weight of *A. edulis* seedlings was only evident at the highest Zn concentration (300 μM) (Figure 1C). Root length in both species significantly reduced as Zn concentrations in the nutrient solution increased (Figure 1D). However, the lowest average seedling root volume for *A. edulis* and the lowest root surface area for both species were observed at 300 μM Zn (Figures 1F and 1E). However, it was observed that higher average shoot and root dry weight, root length, root surface area, and root volume were evident in *I. marginata* seedlings compared to *A. edulis* species at all Zn concentrations (Figure 1).

3.2 Physiological variables

There was a significant effect ($p \leq 0.05$) of species and Zn concentrations on the physiological variables observed in the present study. The lowest means of net CO2 assimilation rate (A), stomatal conductance

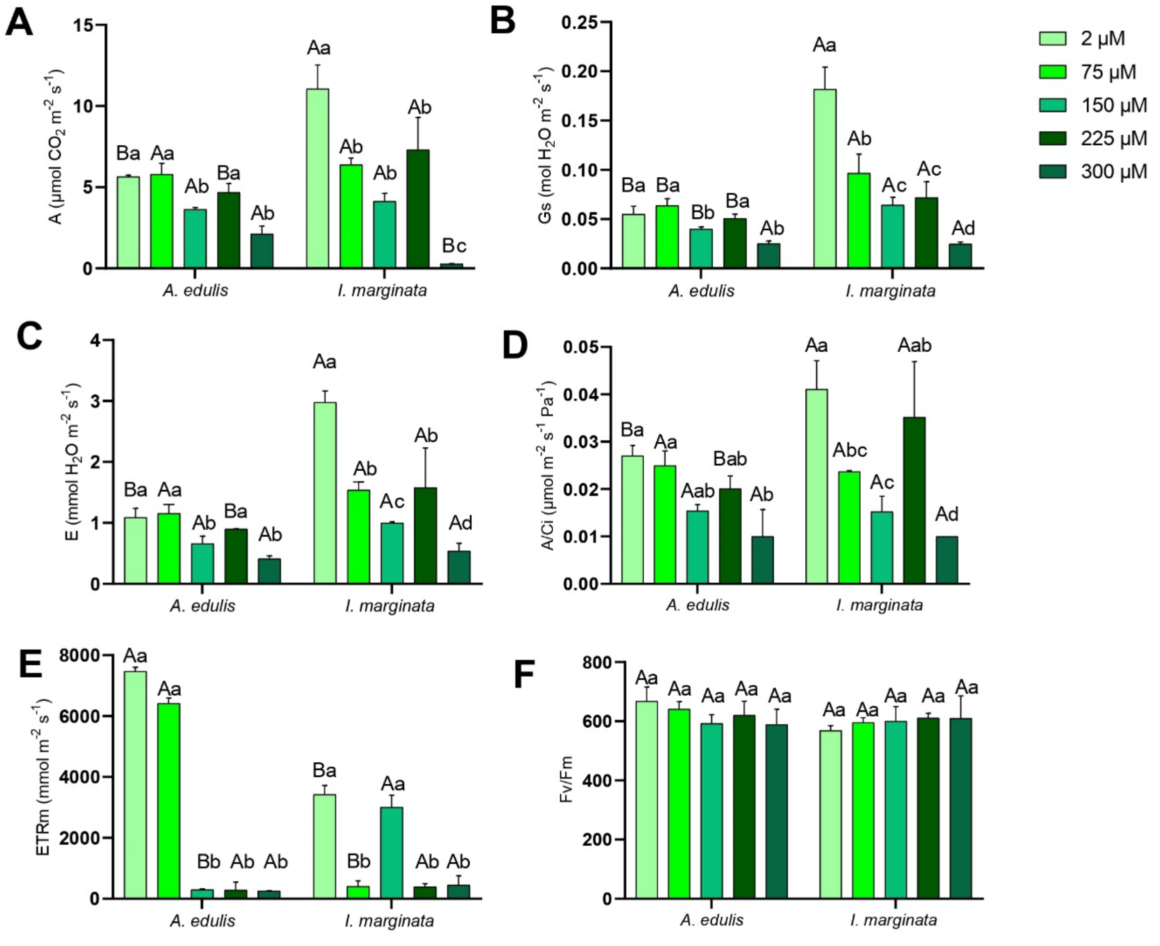

Figure 2 – Mean values recorded for net CO_2 assimilation rate - A (A), stomatal conductance (Gs) (B), transpiration rate (C), instantaneous carboxylation efficiency (by Rubisco) (A/Ci) (D), electron transport rate (ETRm) (E) and maximum quantum yield of PSII (Fv/Fm) (F) in *Allophylus edulis* and *Inga marginata* seedlings grown under different Zn concentrations. Different letters between treatments represent statistically significant difference in the Scott-Knott test. Bars represent the mean ± standard deviation.

Figura 2 – Valores médios registrados para taxa líquida de assimilação de CO_2 - A (A), condutância estomática (Gs) (B), taxa de transpiração (C), eficiência de carboxilação instantânea (pela Rubisco) (A/Ci) (D), taxa de transporte de elétrons (ETRm) (E) e rendimento quântico máximo do PSII (Fv/Fm) (F) em mudas de *Allophylus edulis* (*A. edulis*) e de *Inga marginata* (*I. marginata*) cultivadas sob diferentes concentrações de Zn. Letras diferentes entre os tratamentos representam diferença estatisticamente significativa no teste de Scott-Knott. As barras representam a média ± desvio padrão.

(Gs), and transpiration rate (E) in *A. edulis* seedlings were found at Zn concentrations of 150 and 300 µM (Figure 2). However, *I. marginata* seedlings significantly decreased in the abovementioned variables as the addition of Zn to the nutrient solution increased. The lowest values recorded for instantaneous carboxylation efficiency (A/Ci) of Rubisco in both species were observed at the highest concentration of Zn (300 µM) (Figure 2D).

When comparing species, it was observed that concentrations of 2 µM Zn (control) and 225 µM Zn promoted lower values of photosynthetic rate, transpiration rate, and A/Ci in *A. edulis* seedlings than in *I. marginata* (Figure 2). On the other hand, the photosynthetic rate was higher in *A. edulis* at the highest Zn concentration compared to *I. marginata*. Furthermore, the highest stomatal conductance values were observed in *I. marginata* seedlings compared to the *A. edulis*, except at 300 µM Zn, where no significant difference was observed between the species (Figure 2B).

The highest electron transport rates (ETRm) were observed for *A. edulis* seedlings in the control group and those subjected to 75 µM Zn, differing significantly from *I. marginata* (Figure 2E). At the same time, the highest ETRm values recorded for *I. marginata* seedlings were observed in plants subjected to 150 µM Zn (Figure 2E). However, the significant effect ($p \leq 0.05$) of different Zn concentrations was not observed for Fv/Fm, and there was no significant difference between species (Figure 2F).

3.3 Biochemical variables and Zn in tissues

Different plant species and Zn concentrations had a significant effect (p ≤

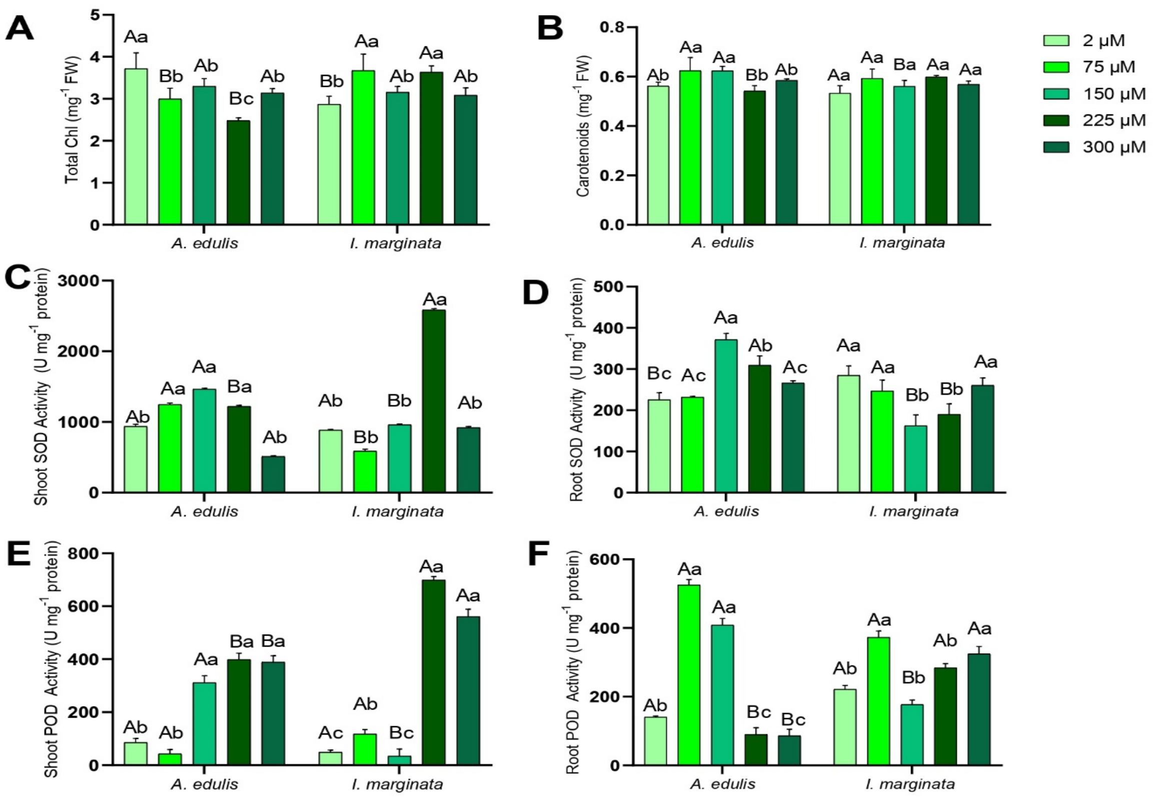

Figure 3 – Mean values recorded for total chlorophyll (A), carotenoids (B), superoxide dismutase (SOD) enzyme activity in shoots (C) and roots (D) and guaiacol peroxidase enzyme (POD) in shoots (E) and roots (F) of *Allophylus edulis* and *Inga marginata* seedlings grown under different Zn concentrations. Different letters between treatments represent statistically significant difference in the Scott-Knott test. Bars represent the mean ± standard deviation.

Figura 3 – Valores médios registrados para clorofila total (A), carotenoides (B), atividade da enzima superóxido dismutase (SOD) na parte aérea (C) e raízes (D) e enzima guaiacol peroxidase (POD) na parte aérea (E) e raízes (F) em mudas de *Allophylus edulis* (*A. edulis*) e de *Inga marginata* (*I. marginata*) cultivadas sob diferentes concentrações de Zn. Letras diferentes entre os tratamentos representam diferença estatisticamente significativa no teste de Scott-Knott. As barras representam a média ± desvio padrão.

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0.05) on the biochemical variables investigated in the present study. The lower levels of total chlorophyll in *A. edulis* seedlings were related to increasing Zn concentration in the nutrient solution (Figure 3A). There was no significant difference in the concentration of carotenoids in *I. marginata* seedlings, regardless of Zn concentrations. In contrast, the highest carotenoid concentration in *A. edulis* seedlings was observed at 75 and 150 µM Zn (Figure 3B). When comparing species, it was observed that concentrations of 75 and 225 µM Zn promoted the lowest total Chl values for *A. edulis*, differing significantly from *I. marginata* (Figure 3A), while in control, *A. edulis* presented higher total Chl content, compared to *I. marginata*. Furthermore, it was noted that concentrations of 150 and 225 µM Zn resulted in the lowest carotenoid content values for *I. marginata* and *A. edulis*, respectively (Figure 3B).

The highest values of superoxide dismutase (SOD) activity in the shoot and roots of *A. edulis* were observed at 150 µM Zn. However, they were statistically equal at Zn concentrations of 75 and 225 µM in the shoot (Figures 3C and 3D). However, the highest values observed for SOD activity in the shoot of *I. marginata* seedlings were found at 225 µM Zn and differed from those recorded for other Zn concentrations (Figure 3C), while Zn reduced SOD activity at roots of *I. marginata* seedlings at concentrations of 150 and 225 µM (Figure 3D).

However, when comparing species, it was observed that concentrations of 75 and 150 µM Zn resulted in the lowest values for SOD activity in the shoot of *I. marginata*. In contrast, the concentration of 225 µM Zn resulted in the highest values for SOD in the shoot of *I. marginata*, differing from *A. edulis* (Figure 3C). Furthermore, it was noted that the lowest SOD values in the roots were observed in *I. marginata* seedlings at 150 and 225 µM Zn (Figure 3D) and in *A. edulis* in the control treatment.

The highest values recorded for POD activity in the shoot of seedlings were evident from 150 µM Zn and at 75, 225, and 300 µM Zn in *I. marginata* seedlings (Figure 3E). POD activity in the roots of *A. edulis* seedlings had the highest values at 75 and 150 µM Zn, while POD activity in the roots of *I. marginata* seedlings had the highest values at 75 and 300 µM Zn (Figure 3F). In the comparison between species, it was possible to observe that the concentration of 150 µM Zn resulted in the lowest POD activity in the shoot and roots in *I. marginata*, while the concentrations of 225 and 300 µM Zn resulted in the lowest POD activity in the roots and shoot of *A. edulis* seedlings (Figures 3E and 3F).

The significant effect ($p \leq 0.05$) of different Zn concentrations was not observed for the H_2O_2 content of the roots, and between species, there was also no significant difference (Figure 4B). The highest MDA concentrations in the shoot and roots of *A. edulis* seedlings were observed at 225 and 300 µM Zn, and similar behavior was observed for MDA levels in the shoot of *I. marginata* (Figures 4C and 4D). In the comparison between the species, it was observed that the lowest averages of H_2O_2 content in the shoot and MDA contents in roots were evident in the seedlings of *I. marginata* compared to *A. edulis* at all Zn concentrations, and similar behavior was observed in *A. edulis* for MDA contents in the shoot (Figure 4).

The zinc concentration in the shoot and roots tissues of seedlings belonging to both species recorded the lowest averages in the control group (Figures 4E and 4F). The significant effect between species was not observed for Zn content in shoot tissues. For Zn content in roots tissues, in general, the species *I. marginata* accumulated lower concentrations of Zn (Figures 4E and 4F).

4. DISCUSSION

The lowest values recorded for taproot increase in *I. marginata* and *A. edulis* seedlings were observed after Zn addition to the nutrient solution (Figure 1A). It may have happened because root elongation inhibition is plants' most evident physiological response to heavy metal-related stress since roots have direct contact with contaminants (Zhang et al., 2019).

The lowest values found for total root length were also observed as Zn concentration in the nutrient solution increased, and the lowest values recorded for root surface area were observed at 300 µM of Zn in both species, compared to control (Figures 1D and 1E). Zn excess can lead to changes in root morphological parameters (e.g., lateral root formation inhibition and reduced root length), as well as to anatomical changes

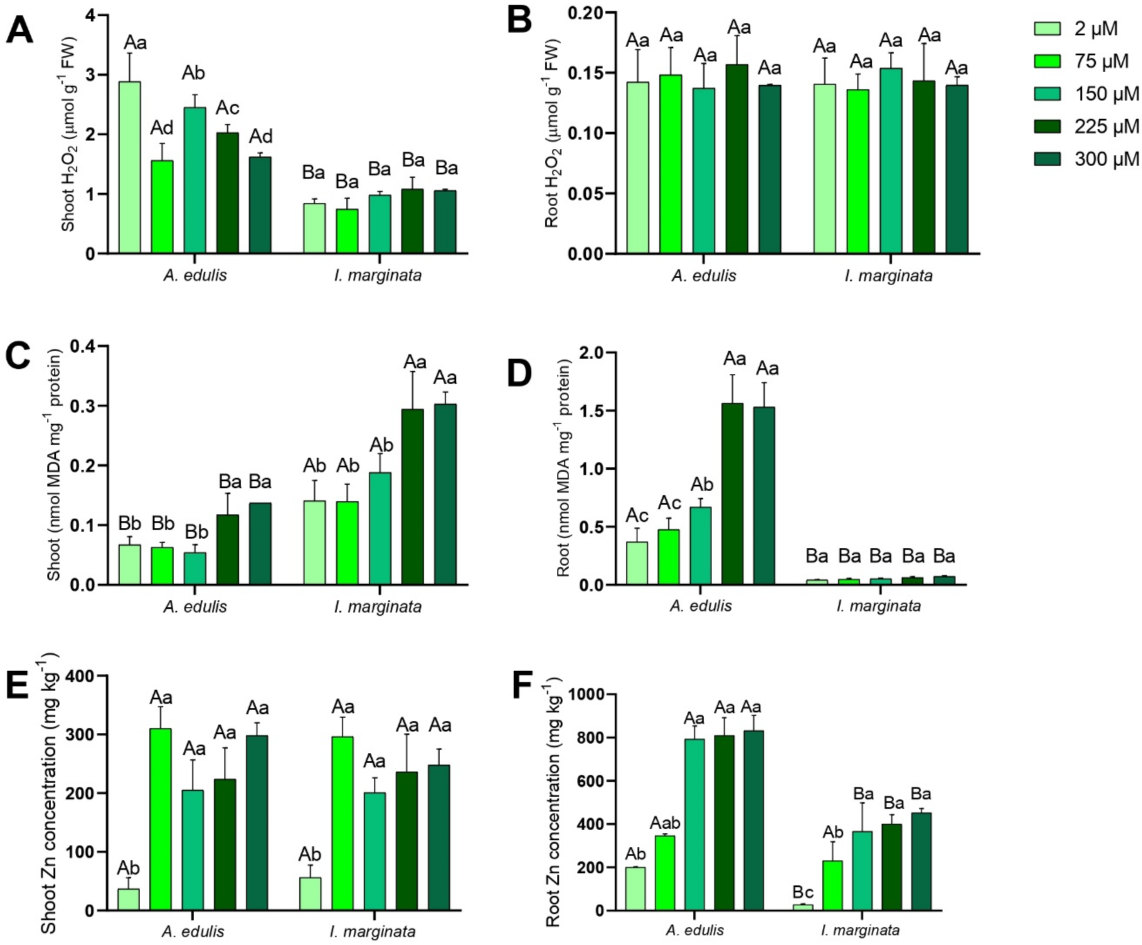

Figure 4 – Mean values recorded for hydrogen peroxide (H_2O_2) concentration in shoot (A) and roots (B), and membrane lipid peroxidation in shoot (C) and roots (D), Zn concentration in shoot (E) and roots (F) of *Allophylus edulis* and *Inga marginata* seedlings grown under different Zn concentrations. Different letters between treatments represent statistically significant difference in the Scott-Knott test. Bars represent the mean ± standard deviation.

Figura 4 – Valores médios registrados para concentração de peróxido de hidrogênio (H_2O_2) na parte aérea (A) e raízes (B), e peroxidação lipídica da membrana na parte aérea (C) e raízes (D), concentração de Zn na parte aérea (E) e raízes (F) em mudas de *Allophylus edulis* (*A. edulis*) e de *Inga marginata* (*I. marginata*) cultivadas sob diferentes concentrações de Zn. Letras diferentes entre os tratamentos representam diferença estatisticamente significativa no teste de Scott-Knott. As barras representam a média ± desvio padrão.

(increased cortex area, vascular cylinder, and number of cortical layers), which result in roots deformations (Ambrosini et al., 2015). However, it is important to notice that, despite differences observed in roots morphological variables, there was no significant difference in roots dry weight in *I. marginata* seedlings (Figure 1C). In other words, negative Zn effects on roots morphological variables did not affect final biomass production in *I. marginata* plants; this outcome may indicate species' tolerance to Zn.

In the comparison between species, it was observed that the highest shoot and roots dry weight, root length, root surface area, and root volume were evident in seedlings of *I. marginata* compared to *A. edulis* at all Zn concentrations (Figure 1). This may have occurred because *I. marginata* is a pioneer species, which presents rapid growth and consequently generates greater biomass production in a shorter period compared to *A. edulis*, which is considered an initial secondary and presents a slower growing rate.

The negative Zn effect on roots morphological variables has negatively affected roots biomass production in species *A. edulis*, mainly at the highest Zn concentration

(300 μM) (Figure 1C). This response was due to higher Zn availability in the nutrient solution and, consequently, higher Zn uptake rate by plants can cause cell division and elongation inhibition, a process capable of explaining the observed roots biomass production reduction (Tiecher et al., 2016).

On the other hand, Zn concentrations did not negatively affect the shoot dry weight of both species compared to the control (Figure 1B). This response was evidenced because Zn toxicity often leads to significant roots growth decrease, mainly of lateral roots, although it has little influence on shoot growth (Zalamena et al., 2015). Thus, it is likely that Zn application and its increasing concentration did not reduce nutrient absorption to the point of decreasing shoot dry weight production in the plants evaluated, although these variables had some negative effects on roots morphological variables. Results in the current study meet those reported by Stoláriková-Vaculíková et al. (2015), who observed that high Zn levels did not reduce leaf dry weight production in Populus deltoides. Similarly, Bernardy et al. (2016) reported that the BRA accession of Pfaffia glomerata (Spreng.), grown in a hydroponic system presenting high Zn concentration, did not show reduced total and shoot dry weight production.

The lowest values recorded for stomatal conductance, photosynthetic, transpiration, and electron transport (ETRm) rates were observed in both species after Zn application, compared to control (Figure 2). Such a photosynthetic rate reduction occurred because heavy metals can decrease Rubisco enzymatic activity and reduce transpiration and stomatal conductance in the mesophyll (Tiecher et al., 2018). The ETRm value may have decreased due to increased non-photochemical dissipation, indicating that plants have dissipated light in the form of heat to protect leaves from light-induced damage (Singh and Reddy, 2015). However, despite the decrease in the photosynthetic rate, there was no significant difference in the Fv/Fm value recorded for *A. edulis* and *I. marginata* seedlings (Figure 2F). This outcome has indicated a lack of shoot dry weight decrease in plants belonging to both species, which showed high tolerance to Zn.

Concentrations of 75 and 225 μM Zn promoted the lowest total Chl values for *A. edulis*, differing significantly from *I. marginata* (Figure 3A). Such a decrease in chlorophyll synthesis or an increase in its degradation associated with Zn excess can cause negative effects on the electron transport rate during photosynthesis (Figure 2E) (Tiecher et al., 2018). Carotenoid concentration in *I. marginata* seedlings did not show a significant difference with the application of Zn (Figure 3B). However, compared to the control, the highest carotenoid concentration was observed in *A. edulis* at 75 and 150 μM Zn (Figure 3B). Such an increase in carotenoid concentration may have happened due to plants' response to oxidative stress since carotenoids play a key role in controlling free radicals and protecting thylakoid membranes (Kuinchtner et al., 2021).

Although Zn cannot directly generate reactive oxygen species (ROS) through the Fenton reaction, high concentrations can generate oxidative stress and interfere with plants' antioxidant defense system (Bernardy et al., 2016). Thus, excessive Zn uptake by plants can lead to oxidative stress due to an imbalance between antioxidant responses and increased ROS production (Brunetto et al., 2018). ROS can damage plants' membrane lipids, proteins, pigments, and nucleic acids and in extreme cases, it can lead to plant death. SOD is one of the main antioxidant enzymes involved in ROS elimination processes, and it also helps maintain homeostasis in plant cells (Zhang et al., 2019). Thus, increased SOD activity was observed in the shoot of both investigated species, as well as in *A. edulis* roots subjected to Zn application, compared to *I. marginata* (Figure 3C).

This response may have occurred because SOD plays a crucial role in removing O_2-• from compartments where radicals are formed. The O_2-• decomposition is always followed by H_2O_2 production, which acts as an oxidant and reductant. H_2O_2 is less harmful and reactive than superoxide anion when it accumulates in plant tissues, and it can be eliminated by catalases and peroxidases (Aguilar et al., 2023).

Zinc, in general, triggered an increase in POD enzyme activity in the shoot in both species compared to the control (Figure 3E). The highest values of guaiacol peroxidase (POD) enzyme activity and the lowest values of H_2O_2 concentration were observed after Zn application in both species, compared to the control (Figures 3 and 4). Increased POD enzyme activity has suggested that H_2O_2 was mainly degraded in the cells. POD plays an important role in cellular detoxification processes since it can catalyze many oxidative reactions in plants by using H_2O_2 as a substrate (Bernardy et al., 2016).

Despite the reduction in H_2O_2 concentration, malondialdehyde (MDA) increase was observed in the shoot of both investigated species, as well as in the roots of *A. edulis* seedlings at the highest Zn concentration, compared to the control (Figures 4c and 4d). Such a membrane lipid peroxidation increase is likely associated with an increase in other ROS produced from Zn excess, which contributed to increased MDA values. MDA is an oxidized membrane lipid product that accumulates in plants exposed to oxidative stress. Increased MDA concentration may be associated with Zn toxicity, indicating oxidative stress in the seedlings. Consequently, it may cause irreversible damage to long-term plant tissue development and function. However, the greatest damage to membrane lipids caused by Zn was not manifested by the shoot biomass reduction of I. margina and *A. edulis* plants. This response emphasizes these species' high tolerance to Zn excess.

The highest Zn concentration in tissues was mainly observed in the roots of both species (Figure 4F). Such higher Zn accumulation in the root may have been a strategy adopted by plants to mitigate the impact of this micronutrient on their growth since Zn can cause more severe damage when it is translocated to the shoot (Somavilla et al., 2018) - which is the most metabolically active tissue in the plant. This result may also have been attributed to high Zn levels added to the solution, as well as to the similarity between ionic radii of divalent cations, such as copper (Cu), manganese (Mn), and iron (Fe) (Hammerschmit et al., 2020). Thus, Zn ions can replace any of these divalent cations and be absorbed by roots. Therefore, when these ions enter plants, Zn excess can change their physiological balance due to competition with other cations at primary absorption sites or in nutrient transport zones in the roots (Tiecher et al., 2018). High Zn concentrations in the root system of *I. marginata* and *A. edulis* plants have indicated that these species can be used for Zn phytostabilization in soils contaminated with this metal.

Thus, high metal concentrations lead to increased nonspecific membrane permeability, and they may cause nutritional imbalance in plants grown in soils subjected to high concentrations of heavy metals, such as Zn. However, despite the high Zn concentrations found in roots, they may not have been high enough to impair plants' biochemical and physiological processes; consequently, they did not affect shoot dry weight production, as observed for I. margina and *A. edulis* seedlings, compared to the control, a fact that confirms our initial hypothesis. It may have happened because plants' tolerance to heavy metals results from the combination of biochemical, physiological, and anatomical responses, as well as depends on the chemical element plants are exposed to, their ability to translocate it, and the time they remained exposed to it (Ambrosini et al., 2015). Furthermore, other tolerance mechanisms may also be operating in the species, such as, for example, Zn compartmentalization in the cellular vacuole, Zn complexation/chelation, and accumulation in less sensitive plant tissues, such as roots, decreasing Zn bioavailability (Zalamena et al., 2015).

In general, the seedlings of *I. marginata* and *A. edulis* showed high Zn accumulation in the roots tissues, and the dry mass production of the shoot of both species was not affected by Zn, being considered suitable for phytoremediation of contaminated soils by zinc. However, *I. marginata* is a pioneer species, which presents fast growth and tolerance to light and is therefore usually cultivated under full sun conditions, as the species grows very well under these conditions. On the other hand, *A. edulis* is a non-pioneer species with a slower growth rate, requiring a shadier environment, and does not adapt very well under full sun conditions, as the species grows better in a subtropical environment forest. Therefore, when choosing species for phytoremediation, these factors must be considered.

5. CONCLUSION

Toxic effects caused by Zn excess were significant in both plant species investigated in the current study, and these effects were mainly evidenced by changes in chlorophyll concentration and decreased photosynthetic rates. On the other hand, Zn stress has activated an efficient antioxidant system, reducing H_2O_2 levels. Consequently, there was no reduction in biomass production in the shoot of *Inga marginata* and *Allophylus edulis*, even with the increase in MDA content. High Zn accumulation in plant tissues and lack of negative effects on shoot of *Inga marginata* and *Allophylus edulis* have suggested that these plants are tolerant to Zn and can be used for the phytoremediation of Zn-polluted soils.

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AUTHOR CONTRIBUTIONS

Marcos Vinícius Miranda Aguilar: Writing-Revision, carrying out the experiments conceptualization, project administration, biochemical and physiological analysis and data analysis. Caroline Castro Kuinchtner: Supervision, research, programming, carrying out experiments and biochemical and physiological analyses. Gerâne Silva Wertonge: Formal analysis, software, supervision and performance of experiments and biochemical and physiological analysis. Thomas Wink Peixoto: Formal analysis and performance of the experiment, validation, and biochemical and physiological analysis. Thalia Preussler Birck: Project administration, visualization, validation, conducting experiments, and biochemical and physiological analysis. Daniel Vinicios Valsoler: Visualization, validation, project administration, conducting experiments, and biochemical and physiological analysis. Fernando Teixeira Nicoloso: Supervision, visualization, co-supervision of the project. Gustavo Brunetto: Project Co-supervision, Supervision and Fundraising. Luciane Almeri Tabaldi: Project Fundraising, orientation, conceptualization, biochemical and physiological analysis and writing-revision.

7. REFERENCES

Aguilar, M. V. M., Sasso, V. M., Tabaldi, L. M. Handroanthus heptaphyllus as bioindicator of chromium-contaminated environments. South African Journal of Botany, v.155, p. 35-44, 2023.

Ambrosini, V. G., Rosa, D. J., Prado, J. P. C., Borghezan, M., Melo, G. W. B., Soares, C. R. F. S., et al. 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, 2015.

Ballestreri, A. A., Araújo, M. M., Aimi, S. C., Nascimento, N. F., Berghetti, Á. L. P., Gasparin, E. et al. Morphophysiological responses of forest tree species conducted under different levels of shading in the enrichment of degraded ecosystem. Forest Ecology and Management, 488, 01-12, 2021.

Bernardy, K., Farias, J. G., Dorneles, A. O. S., Pereira, A. S., Schorr, M. R. W., Thewes, F. R., et al. Changes in root morphology and dry matter production in Pfaffia glomerata (Spreng.) Pedersen accessions in response to excessive zinc. Revista Brasileira de Plantas Medicinais, 18, 613-620, 2016.

Brunetto, G., Tiecher, T. L., Hammerschmitt, R. K., Basso, F. D., Ferreira, P. A. A., De Conti, L. et al. Peach rootstock tolerance to excess zinc in Sandy acidic soil. Acta Horticulturae, 75-82, 2018.

Cabral, C. M., Santos, J. B., Ferreira, E. A., Machado, V. M., Pereira, I. M., Silva, D. V. et al. Tolerance to the herbicide clomazone and potential for changes of forest species. Bioscience Journal, 33, 897-904, 2017.

El-Moshaty, F. I. B., Pike, S. M., Novacky, A. J., Sehgal, O. P. Lipid peroxidation and superoxide productions in cowpea (Vigna unguiculata) leaves infected with tobacco rings virus or southern bean mosaic virus. Journal Physiological and Molecular Plant Pathology, 43, 109-119, 1993.

EMBRAPA - EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. 2009. Manual de análises químicas de solos plantas e fertilizantes. 2a ed., Brasília, 627 p.

Ferreira, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. Revista Brasileira de Biometria, 37, 529-535, 2019.

Giannopolitis, C. N., Ries, S. K. (1977). Purification and quantitative relationship with water-soluble protein in seedlings. Journal of Plant Physiology, 48, 315-318.

Hammerschmit, R. K., Tiecher, T. L., Facco, D. B., Silva, L. O. S., Schwalbert, R., Drescher, G. L. et al. Copper and zinc distribution and toxicity in 'Jade' / 'Genovesa' young peach tree. Scientia Horticulturae, 259, 01-09, 2020.

Hiscox, J. D., Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany, 57, 1132-1334.

Hoagland, D. R., Arnon, D. I. The waterculture method for growing plants without soil. Berkeley, CA: Agric. Exp. Stn., Univ. Of California. (Circ. 347). 1950.

Kuinchtner CC, Oliveira GSW, Aguilar MVM, Bernardy D, Berger M, Tabaldi LA. Can species Cedrela fissilis Vell. be used in sites contaminated with toxic aluminum and cadmium metals? iForest - Biogeosciences and Forestry, 14: 508-516, 2021.

Lichtenthaler, H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer L, Douce R (eds). Methods in Enzimology, London: Academic Press. v. 148, p. 350-381, 1987.

Loreto, F., Velikova, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology, 12, 1781-1787, 2001.

Marques, D. M., Júnior, V., Silva, A. B., Mantovani, J. R., Magalhães, P. C., Souza, T. C. Copper toxicity on photosynthetic responses and root morphology of Hymenaea courbaril L. (Caesalpinioideae). Water, Air, & Soil Pollution, 229, 01-14, 2018.

Pereira, I. M., Botelho, S., Araújo, F., Morandi, D., Paschoal, E. Espécies potenciais para recomposição de matas ciliares na região do Alto Rio Grande, Minas Gerais. Enciclopédia Biosfera, 13, 571-587, 2016.

Singh, S. K., Reddy, V. R. Response of carbon assimilation and chlorophyll fluorescence to soybean leaf phosphorus across CO2: Alternative electron sink, nutrient efficiency and critical concentration. Journal of Photochemistry and Photobiology B: Biology, 151, 276-284, 2015.

Somavilla, L. M., Simão, D. G., Tiecher, T. L., Hammerschimitt, R. K., Oliveira, J. M. S., Mayer, N. A. et al. Structural changes in roots of peach rootstock cultivars grown in soil with high zinc contente. Scientia Horticulturae, 237, 01-10, 2018.

Stoláriková-Vaculíková, M., Romeo, S., Minnocci, A., Luxová, M., Vaculík, M., Lux, A. Anatomical, biochemical and morphological responses of poplar Populus deltoides clone Lux to Zn excess. Environmental and Experimental Botany, 109, 235-243, 2015.

Tiecher, T. L., Cereta, C. A., Tiecher, T., Ferreira, P. A. A., Nicoloso, F. T., Soriani, H. H. et al. Effects of zinc addition to a copper-contaminated vineyard soil on sorption of Zn by soil and plant physiological responses. Ecotoxicology and Environmental Safety, 129, 109-119, 2016.

Tiecher, T. L., Soriani, H. H., Tiecher, T., Ceretta, C. A., Nicoloso, F. T., Tarouco, C. P. et al. The interaction of high copper and zinc doses in acid soil changes the physiological state and development of the root system in young grapevines (Vitis vinifera). Ecotoxicology and Environmental Safety, 148, 985-994, 2018.

Turchetto, F., Araújo, M. M., Griebeler, A. M., Rorato, D. G., Berghetti, Á. L. P., Barbosa, F. M. Can intensive silvicultural management minimize the effects of frost on restoration plantations in subtropical regions? Journal of Environmental Management, 269, 01-09, 2020.

Zalamena, J., Melo, G. W., Santos, H. P., Silva, L. S., Fialho, F. B. et al. Physiological characterization of grapevine rootstocks grown in soil with increasing zinc doses. Revista Brasileira de Engenharia Agrícola e Ambiental, 19, 973-980, 2015.

Zeraik, A. E., Souza, F. S., Fatibello-Filho, O. Desenvolvimento de um spot test para o monitoramento da atividade da peroxidase em um procedimento de purificação. Química Nova, 31, 731-734, 2008.

Zhang, H. H., Xu, N., Teng, Z., Wang, J., Ma, S., Wu, X. et al. 2-Cys Prx plays a critical role in scavenging H2O2 and protecting photosynthetic function in leaves of tobacco seedlings under drought stress. Journal of Plant Interactions, 14, 119-128, 2019.