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Original Article

Phytoremediation potential of Brazilian mahogany (*Swietenia macrophylla* King) on exposure to nickel: anatomical, biochemical and antioxidant responses

Potencial fitorremediador de mogno brasileiro (*Swietenia macrophylla* King) em exposição ao níquel: respostas anatômicas, bioquímicas e antioxidantes

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Abstract

The advancement and intensification of industrial and mining activities has generated a series of impacts on natural ecosystems, combined with the inappropriate use of agrochemicals and the erroneous disposal of electronic products, contributing to soil contamination with a diversity of chemical elements, including heavy metals. Due to this, this work aimed to evaluate the effect of increasing dosages of nickel on the anatomy, biochemistry and oxidative system of Brazilian mahogany (*Swietenia macrophylla*), a forest species from the Amazon, seeking to indicate the potential use of this species in phytoremediation programs. of soils contaminated with heavy metals. The seeds were grown under a constant temperature of 28°C, relative humidity (RH) of 90% with a 12-hour photoperiod for 43 days. The experimental design used was randomized blocks (DBC), with five treatments (0, 2, 4, 6 and 8 mg.L^{-1} of Nickel), with six replications. Data were subjected to analysis of variance (ANOVA) and means were tested for significant differences using the Tukey test at 5% significance. Changes in the anatomy of the different organs were observed, with differences in the cells in the central region of the leaf, the stem and the root. The concentration of total carbohydrates had no statistical differences with the application of nickel, however changes were observed in photosynthetic pigments, reducing sugars and sucrose as an adaptive form to nickel. The increase in nickel dosages was accompanied by the synthesis of ammonium, amino acids and proline in the root, while the synthesis of glycine was reduced. In the leaf, there was an increase in amino acids with an increase in metal, accompanied by a decrease in glycine. The plant antioxidant defense system was efficient in attenuating the toxic effects of ROS, with significant actions of CAT and SOD enzymes in the root, while the leaf had the main action of APX and CAT. The cultivation of mahogany plants can be advocated to mitigate Ni pollution in these areas, as this forest species has a particular characteristic of resistance to stressful conditions in contact with the heavy metal.

Keywords: mining, phytoremediation, Amazon, ROS, antioxidant defense.

Resumo

O avanço e intensificação de atividades industriais e mineradoras tem gerado uma série de impactos sobre ecossistemas naturais, combinados ao uso inadequados de agroquímicos e o descarte errôneo de produtos eletrônicos, contribuem para contaminação de solos com uma diversidade de elementos químicos, entre eles os metais pesados. Devido a isto, este trabalho teve o objetivo de avaliar a o efeito das dosagens crescentes de níquel na anatomia, bioquímica e sistema oxidativo do mogno brasileiro (*Swietenia macrophylla*), espécie florestal da Amazônia, buscando indicar o potencial de uso desta espécie em programas de fitorremediação de solos contaminados com metais pesados. As sementes foram cultivadas sob temperatura constante de 28°C, umidade relativa do ar (UR) de 90% com fotoperíodo de 12h por 43 dias. O delineamento experimental utilizado foi blocos casualizados (DBC), com cinco tratamentos (0, 2, 4, 6 e 8 mg.L^{-1} de Níquel), com seis repetições. Os dados foram submetidos à análise de variância (ANOVA) e as médias foram testadas para diferenças significativas pelo teste Tukey a 5% de significância. Foi observado mudanças na anatomia dos diferentes órgãos, com diferenças nas células da região central da folha, no caule e na raiz. A concentração de carboidratos totais não teve diferenças estatísticas com a aplicação do níquel, porém foi observado mudanças nos pigmentos fotossintéticos, açúcares redutores e sacarose como forma adaptativa ao níquel. O aumento das dosagens de níquel foi acompanhado pela

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síntese de amônio, aminoácidos e prolina na raiz, em contra partida diminui-se a síntese de glicina. Já na folha, houve o aumento dos aminoácidos com o aumento do metal, acompanhado da diminuição de glicina. O sistema de defesa antioxidante de plantas foi eficiente para atenuar os efeitos tóxicos das EROs, com ações significativas das enzimas CAT e SOD na raiz, enquanto na folha teve ação principal da APX e CAT. O cultivo de plantas de mogno pode ser defendido para mitigar a poluição de Ni nessas áreas, já que esta espécie florestal possui uma característica particular de resistência às condições de estresse em contato com o metal pesado.

Palavras-chave: mineração, fitorremediação, Amazônia, EROs, defesa antioxidante.

1. Introduction

The advancement and intensification of industrial and mining activities has generated a series of impacts on natural ecosystems (Krzemień et al., 2023), combined with the inappropriate use of agrochemicals and the erroneous disposal of electronic products (Datta et al., 2023; Danish et al., 2023), contribute to the contamination of soils and water bodies with a diversity of chemical elements, including heavy metals (Naz et al., 2022).

Heavy metals have caused concern worldwide, as their presence in soil tends to be more persistent when compared to organic pollutants, and can be introduced into the food chain through the consumption of contaminated food (Maftouh et al., 2023; Shaari et al., 2022). Direct or indirect exposure to heavy metal represents a threat to human health, as they cause a series of dysfunctions, such as neurodegenerative and respiratory diseases, DNA degeneration and cancer (Maftouh et al., 2023; Sahoo and Sharma, 2023).

Among the most dangerous heavy metals, nickel (Ni) stands out, present in greater quantities in the environment, has great resistance to corrosion and oxidation, and its main compounds are in the form of oxide, hydroxide, sulfate and nickel chloride (Ahmad and Shah, 2023; Su et al., 2023).

Plants in contact with heavy metals tend to change their metabolism and anatomical structures to adapt to the harmful effects of the metal, in order to guarantee their survival, and these changes may occur in root or leaf organs (Andrade Júnior et al., 2021; Zhao et al., 2022; Sruthi and Puthur, 2019; Yildirim et al., 2019).

Given the generalization and complexity of the problem of ecosystem contamination, ways are being sought to reduce the toxic effects generated by heavy metals, such as nickel. Thus, phytoremediation stands out, a technique that consists of planning plant species capable of removing, transforming or immobilizing contaminating elements in the soil, recovering these contaminated areas (Sonowal et al., 2022).

Phytoremediation is an economically viable practice with less environmental impact. To achieve this, species native to the contaminated region must be selected, preferably species with rapid growth, a widely distributed and branched root system, good biomass production with the capacity to accumulate metals. heavy in the root tissues and transport them to the aerial part (Muthusaravanan et al., 2020; Ma et al., 2022; Yildirim et al., 2019).

Given this, Brazilian mahogany (*Swietenia macrophylla* King), a representative species native to the Amazon, can be associated with phytoremediation strategies. The economically valuable timber tree is known for its use in the creation of furniture, crafts, decorative materials and musical instruments (Telrandhe et al., 2022; Mahendra et al., 2022).

In view of the above, this work aimed to evaluate the effect of increasing dosages of nickel on the anatomy, biochemistry and oxidative system of Brazilian mahogany (*Swietenia macrophylla*), seeking to indicate the potential use of this species in phytoremediation programs for soils contaminated with heavy metals.

2. Material and Methods

2.1. Local of experiment

The experiment was carried out in the growth room of the Higher Plant Biodiversity Study group (EBPS), located at the Institute of Agricultural Sciences at the Federal University of the Amazon (ICA/UFRA).

2.2. Growing condition and experimental design

Brazilian mahogany seeds (*S. macrophylla*) were collected in the area of the Brazilian Agricultural Research Corporation (Embrapa Amazônia Oriental), located in Belém, State of Pará, Brazil. These seeds were sown in 5L polyethylene pots containing previously washed and autoclaved sand, after which they were kept at a constant temperature of 28°C, relative air humidity (RH) of 90% and a 12-hour photoperiod. The seedlings were grown in a growth room for 43 days, irrigated daily to replace water lost through evapotranspiration and monitored according to the rules and instructions for seed analysis.

To evaluate the toxic effect of nickel, the seeds were exposed to an aqueous solution of nickel chloride (OSO) P.A. ($NiCl_2.6H_2O$), made at concentrations corresponding to 0 mg.L^{-1} (control), 2 mg.L^{-1}, 4 mg.L^{-1}, 6 mg.L^{-1} and 8 mg.L^{-1}. The zero level corresponded to the control, where only deionized water was used. The work involves the three principles of experimentation: repetition, randomization and local control, therefore the experimental design used was randomized blocks, with six replications per treatment, totaling 30 experimental units. Each unit consists of a pot with 7 seeds.

2.3. Anatomical analysis

The semi-permanent slides were prepared with transverse histological sections, made with the aid of steel blades, sectioned into the different organs (root, stem and leaf), according to the usual methods of plant anatomy. Stained with safrablau - a mixture of astra blue and safranin (Bukatsch, 1972; Kraus et al., 1998). Minutes after staining, it was placed on a glass slide with 50% glycerin (Purvis et al.,

1964) to better preserve the botanical material, mounting between the slide and coverslip.

For stem and root anatomy, segments of the stem and root were selected, while for leaf anatomical studies, leaves were collected in the central region for studies of the mesophyll and dissociation of the epidermal faces (adaxial and abaxial). The leaf surface was obtained using the chemical method to separate the abaxial and adaxial sides, with the aid of Jeffrey's mixture (Johansen, 1940) for approximately 24 hours. After dissociating, the material was washed to remove excess acids from the fabric and consequently open the radicals for dye absorption. The leaf mesophyll, obtained through transverse cuts in the central region of the leaf to observe the parenchymatic and conductive tissues of the inner part of the leaf.

2.4. Determination of photosynthetic pigments

100 mg of fresh leaf was used in a mortar, containing 3mL of 80% acetone, followed by maceration and then centrifugation. The supernatant was transferred to a 25 mL volumetric flask and the volume was checked with 80% acetone. The levels of pigments present in the extracts were measured, using absorbance readings, in a spectrophotometer, at wavelengths of 663 and 647 ηm to calculate the levels of chlorophylls a and b. The wavelength of 537 ηm was used to calculate anthocyanin levels and 470 ηm to calculate carotenoid levels. Methodology described by Lichtenthaler (1987) and Sims and Gamon (2002).

2.5. Analysis of carbon and nitrogen metabolism

The concentration of total soluble carbohydrates was determined by the colorimetric method, described by Dubois et al. (1956), using phenol (5%) and concentrated sulfuric acid solutions. Readings were taken on a spectrophotometer at 490 ηm.

The concentration of sucrose was determined using the Van Handel method (Van Handel, 1968), which was divided into three stages: extraction, phase separation by solvents and purification and dosage of sucrose. The extraction was done with MCW solution (methanol, chloroform and water solution). The separation of the phases is done by adding water to the extract, causing the solution to divide, the methanolic part separates from the chloroform. When dosing sucrose, potassium hydroxide was added to the methanolic aqueous phase to break down the non-reducing sugars so that they join the anthrone and form a complex with a shade that varies from orange to green. The reading was on a spectrophotometer at 620 ηm.

To determine reducing sugars, 20 mg of dry matter powder were placed in test tubes with 2.5 mL of 80% ethanol. Afterwards, 500 µL of the extract was used with 500 µL of Nelson AB reagent, heated to 100°C and then cooled in an ice bath, adding 500 µL of the arsenitomolybide solution and vortexing. Add 3.5 mL of deionized H_2O and vortex. Readings were taken on a spectrophotometer at 540 nm, according to the methodology of Rinne et al. (2012).

Free ammonium concentrations were determined by the Phenolate-hypochlorite method, described by Weatherburn (1967) and Felker (1977). This method is based on the formation of a bluish-colored compound, indophenol, after the reaction of ammonia with phenol and hypochlorite at alkaline pH, induced by the addition of NaOH to the reaction solution. Indophenol was quantified in a spectrophotometer with an absorption of 640 ηm.

Total soluble amino acid concentrations were analyzed by the method described by Shriner et al. (1983) and Peoples et al. (1989). This method is based on the formation of a violet-colored compound, after the reaction of ammonium with hydritantin, and was quantified in a spectrophotometer at 570 ηm.

Free proline concentrations were determined using the method described by Bates et al. (1973). This method is based on chromatophoric extraction that forms a pink/red compound through the reaction of toluene with acid ninhydrin and glacial acetic acid, and is carried out using spectrophotometry with a wavelength of 520 ηm.

Glycine-betaine concentrations were measured by the method of Grieve and Grattan (1983). In this method, the glycine-betaine present in the extract is precipitated and determined spectrophotometrically at a wavelength of 365 ηm. Upon addition of $KI\text{-}I_2$, glycine betaine forms the crystallized betaine aldehyde periodide complex. Then, the addition of sulfuric acid ensures complete crystallization of the complex, allowing spectrophotometric determination.

2.6. Antioxidant system analysis

The extracts for determining the activity of the enzymes SOD, APX and CAT were obtained from the maceration in liquid nitrogen of 150 mg of fresh root and leaf tissues with 1.5 mL of 100 mM potassium phosphate buffer pH 7, 0 EDTA 1 mM AsA 1 mM. The homogenate was centrifuged at 14,000 G for 30 min at 4ºC and the supernatant was collected and stored in a -80 ºC freezer until analysis.

In Superoxide Dismutase (SOD EC 1.15.1.1), the analysis was composed of 25 µL of extract + 75 µL of extraction buffer + 1660 µL of reaction medium + 200 µL NBT 750 µM + 40 µL Riboflavin 1 mM. The reaction was carried out in a chamber, illuminated with a 30 W fluorescent lamp (30 µmol photons m^{-2} s^{-1}), for 5 min. SOD activity was estimated through the increase in absorbance at 560 nm, riboflavin is photochemically excited through reduction by methionine to a semiquinone, which donates an electron to oxygen, forming the source of superoxide, and this readily converts the nitroblue tetrazolium (NBT) to a purple formazone product (Jiménez et al., 1997; Foyer and Noctor, 2000, 2005; Alscher et al., 2002).

The detection of Catalase activity (CAT EC 1.11.1.6) was determined by the H_2O_2 consumption method, monitored according to the decay in its absorbance at 240 ηm (Beers Junior and Sizer, 1952). The reaction began with the addition of 12.5 µL of extract + 37.5 µL of extraction buffer, after which 2.95 mL of 50 mM potassium phosphate buffer pH 7.0 containing 20 mM H2O2 (Havir and McHale, 1987).

Ascorbate Peroxidase (APX EC 1.11.1.11) was measured using the spectrophotometric method of APX activity, based on monitoring the decrease in ascorbate at 290 ηm. Its reaction medium was composed of 50 µL of the extract + 50 µL extraction buffer, with 2.5 mL of 50 mM potassium phosphate buffer (pH 6.0) and 200 µL of 6.75 mM ascorbate

+ 200 µL of H_2O_2 a 30 mM.. The results were calculated using the molar extinction coefficient of ascorbate (Ɛ = 2.8 mM^{-1}. cm^{-1}) (Foyer and Noctor, 2005; Nakano and Asada, 1981).

2.7. Statistical analysis of data

Statistical analysis of variables and generation of graphs was performed using the R-Studio software (version 2023.06.2+561).

To identify statistical differences between nickel dosages, the ExpDes.pt package and the "dbc" function were used. Subsequently, the data were subjected to Tukey's Post-Hoc test, where a significance level of 5% was considered.

To group and identify the behavior of correlations between the variables of the antioxidant system and osmolytes, a multivariate analysis by principal components (PCA) and factorial was carried out using the factoextra and FactoMineR packages. Bartlett's test of sphericity and the Kaiser-Meyer-Olkin (KMO) test were performed as assumptions to validate the analysis.

3. Results and Discussion

3.1. Plant anatomy

The anatomy of the different organs of the plant is verified under different concentrations of nickel, where a significant difference is initially seen in the central region of the leaf, in the stem and in the root. While the adaxial and abaxial epidermal surfaces, together with the mesophyll, did not suffer metal interference.

At the mesophyll level (Figure 1A), the leaf has an abaxial surface formed by tabular cells with an interspersed presence of stomata at the cell level. As for the adaxial surface, it does not present stomatal intercalations, characterizing a hypostomatic leaf. The mesophyll is heterogeneous dorsiventral, with the cells of the palisade parenchyma facing the upper face being composed of a single layer of cells that represent 1/3 of the mesophyll region, while the spongy (lacunous) parenchyma is facing the lower face, occupying in around 2/3 of the mesophyll. No visual variation in mesophyll thickness was observed between different treatments. The cells on the adaxial surface in frontal view (Figures 1B and 1C) reveal well-wavy cell walls with the absence of stomata (guard cells), while the cells on the abaxial epidermal surface are slightly curved with the presence of anomocytic-type stomata (Figure 1D and 1E). In both cases, there were no major variations between the control and the nickel dosages applied in the experiment. In the phloem, no anatomical difference was evident. While in the central vein, it is observed that dosages above 4 mg.L^{-1} have their vascular bundles in poles in the central cylinder that are more distant and the formation of the xylem is more rudimentary, when compared to dosages 0 and 2 mg.L^{-1} which has vascular bundles at poles very close to each other, with apparently regular development (Figure 1F, 1G, 1H and 1I).

The absorption of heavy metals influences the change in the quantity and size of the leaf's sap-conducting vessels, which implies a lower performance in the conduction of raw sap (mineral salt water) and transport of elaborated sap (photoassimilates), due to interference of metal ions in plant-water relations (Sruthi and Puthur (2019), since nickel can be translocated to the upper parts of the plant (Duarte et al., 2007; Yildirim et al., 2019). According to Appezzato-da-Glória and Carmello-Guerreiro (2022), vessel elements play a fundamental role in hydraulic conductivity and plant development. Setting up an inversely proportional relationship between cells of vessel elements and plant growth with heavy metal concentrations, as the higher the metal dosage, the smaller the xylem vessel, and consequently the lower the plant growth due to the little receipt of raw sap. for the production of photoassimilates, affecting development as a whole (Taiz et al., 2017), this fact explains the biochemical results of sucrose and reducing sugars (Figure 3G and 3H).

At dosage 0 to 6 mg.L^{-1} (Figure 1K) there is greater regularity in the anatomical formation of the cortical and central parenchyma of the stem when compared to this same tissue at dosage 8 mg.L^{-1} (Figure 1L, 1M and 1N) , where it presents with several ruptured cells, forming large intercellular spaces in the fundamental parenchyma, both cortical and central. The action of the metal was at the primary stage in cell wall development, where cells have the thinnest cell walls. Regarding the conduction system, no apparent variations were observed with the application of the metal (Figure 1J), with a very continuous vascular cambium with 8 layers of cells, maintaining a certain similarity in thickness between treatments.

Heavy metals can also accumulate in the xylem regions of the stem, therefore, they can block the vessels, affecting water absorption (Sruthi and Puthur, 2019). However, in this study, damage was identified only in the cortical and central parenchyma of the stem, while the vessels remained intact.

In the root, the vascular system presents clear differentiation in relation to its development, with dosages 0 and 2 mg.L^{-1} (Figure 1O and 1P) showing its secondary growth well characterized when compared to the dosages above (Figure 1Q and 1R), whose beams are still polarized, where a discontinuity and interruption of the vascular exchange is evident, characterizing primary growth. Furthermore, at dosages of 0, 2, 4 and 6 mg.L^{-1} the phloem is well expanded with a greater range of cells when compared to the dosage of 8 mg.L^{-1} (Supplementary table).

The absorption of heavy metal in the root epidermis layer can occur in two ways: via the apoplast via adsorption processes, being diffused or transported passively by the water flow from the root cortex; or via the symplast, through appropriate ion channels (Song et al., 2017; Sterckeman and Thomine, 2020), such as Ca^{2+} channels in the plasma membranes of root cells, which are permeable to a wide range of monovalent and divalent cations (Chen et al., 2018), which is the case of nickel, which normally has a valence of $^{+2}$, but, in specific cases, may have a valence of $^{+1}$ or $^{+4}$ (Memlak, 2021). The Caspary stripe of the root endodermis may have considerably reduced the most intense deleterious effects of nickel on the plant due to its protection by the impermeable belts composed of its cell wall impermeable by lignin and suberin (Sruthi and Puthur, 2019; Negreiros and Lichston, 2022) , this acts

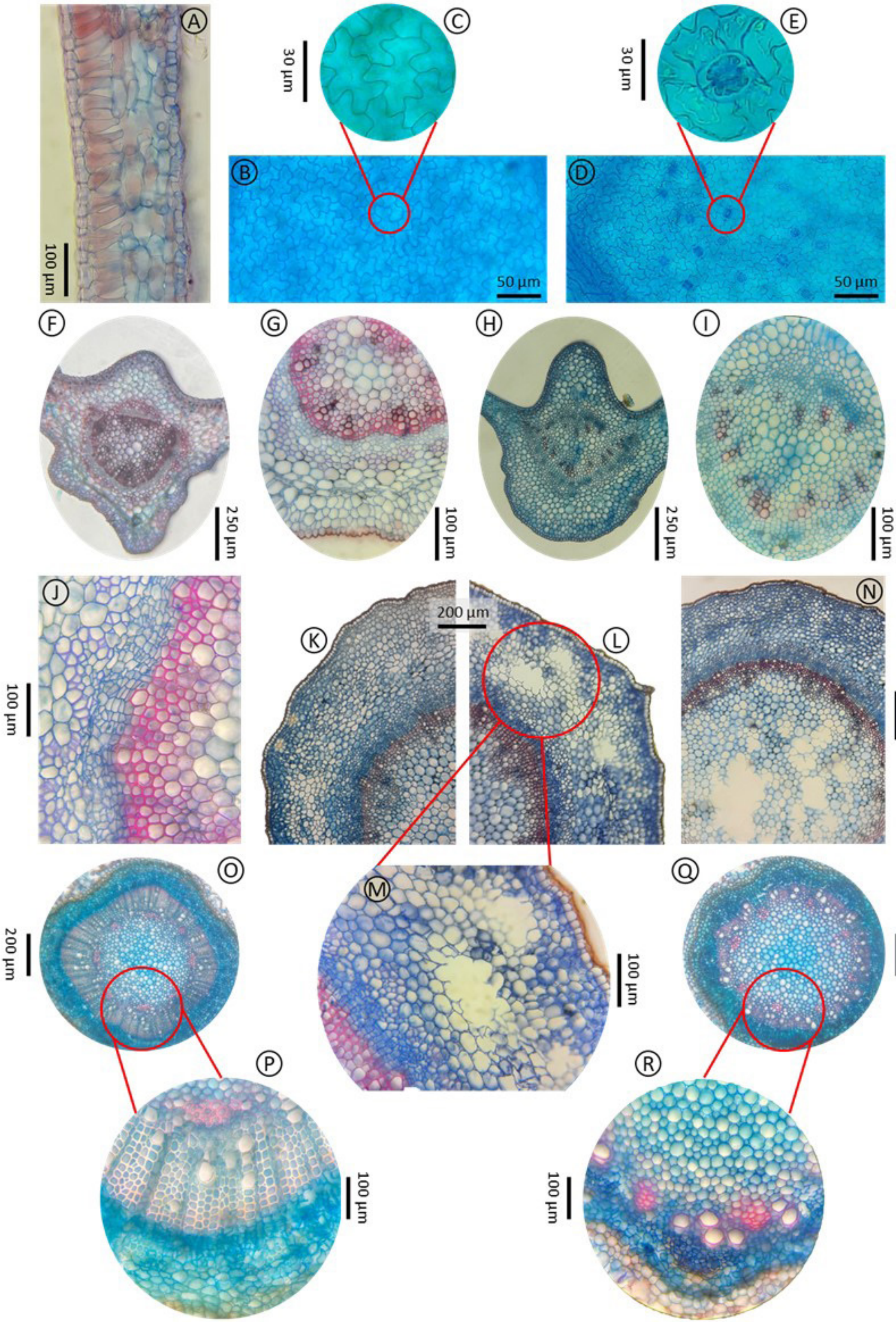

Figure 1. Anatomical plank of Brazilian mahogany (*Swietenia macrophylla*) seedlings exposed to nickel: A – leaf mesophyll (0 mg.L^{-1}); B – adaxial surface (0 mg.L^{-1}); C – attached cells on the adaxial surface (0 mg.L-^{1}); D – abaxial surface (0 mg.L^{-1}); E – guard and attached cells of the abaxial surface (0 mg.L^{-1}); F – central region of the leaf (0 mg.L^{-1}); G – leaf vascular system (0 mg.L^{-1}); H – central region of the leaf (8 mg.L^{-1}); I – leaf vascular system (8 mg.L^{-1}); J – Stem vascular bundles (0 mg.L^{-1}); K – Stem (0 mg.L^{-1}); L – Stem (8 mg.L^{-1}); M – Cortical parenchyma (8 mg.L^{-1}); N – Central parenchyma (8 mg.L^{-1}); O – Root (0 mg.L^{-1}); P – Root xylem and phloem (0 mg.L^{-1}); Q – Root (8 mg.L^{-1}); R – Root xylem and phloem (8 mg.L^{-1}).

as an apoplastic barrier, blocking periplasmic spaces, diverting the apoplastic flow to the symplastic flow (Plasma Membrane), providing ionic selectivity, which can prevent excessive entry of ions and metals into xylem vessels (Appezzato-da-Glória and Carmello-Guerreiro, 2022).

Studies show that heavy metal content in roots is normally much higher than in leaves (Zhao et al., 2022), since root hairs and the epidermis are the most active regions for absorbing water and ions from the soil (Marques, 2020), a fact that possibly enabled the absorption of most of the nickel from the substrate. The layers of epidermal cells in plants are considered absorbent tissue because they contain a more active system of membrane transporters (Taiz et al., 2017; Appezzato-da-Glória and Carmello-Guerreiro, 2022), so it is assumed that the epidermal cells of mahogany roots have the potential to accumulate nickel and resources for ion absorption.

This also explains the delay in root development, as plants that possibly accumulated nickel in their roots still had their xylem and phloem in the primary stage, compared to plants without metal or in low doses that had their secondary xylem and phloem already developed or at least less in development, both evaluated in the same period of time (43 days). Therefore, these facts are associated with less absorption of raw sap, and less arrival of elaborated sap from the leaf, limiting the height and biomass of the root.

3.2. Pigments and photosynthetic metabolism

In the leaf pigments of *S. macrophylla*, degradation of anthocyanins and carotenoids occurred by 39.5 and 32.7%, respectively, in plants treated with 4 mg L^{-1} Ni when compared to the control treatment (Figure 2A and 2B). While the contents of chlorophyll a, chlorophyll b and total chlorophylls (a + b) increased respectively by around 40, 37 and 39% when exposed to 4 mg L^{-1} Ni compared to the control (Figure 2C, 2D and 2F).

The greater production of chlorophylls causes an increase in the photosynthetic rate, considered as characteristic symptoms of nickel stress (Kumar et al., 2007). In contrast, studies reported by Natasha et al. (2019) attributed changes in chlorophyll content induced by Ni, as heavy metals can replace Mg in the chlorophyll present in both complex antennas and reaction centers, which can cause a decrease in the photosynthetic rate. (Sruthi and Puthur, 2019). One of the metabolic effects associated with anthocyanins and carotenoids is the protection of the photosynthetic system (Geng et al., 2020), as they can be produced in response to heavy metal stress conditions (Sruthi and Puthur, 2019). These pigments can participate in the antioxidant system of reactive oxygen species generated under stress conditions (Kumar et al., 2007, Naing and Kim, 2021). Furthermore, anthocyanins contribute to the sequestration of metals in the cellular vacuole (Naing and Kim, 2021).

Leaf total soluble carbohydrates had no significant effect on nickel (Figure 2F). Sucrose concentrations at dosages of 2 and 6 mg/L Ni reduced the plant in a harmful way, with significant decreases of 2.7 mg of sucrose per g of DM, while at dosage of 8 mg/L Ni they reduced 3.7 mg /g MS, all values determined when compared to control plants (Figure 2G). The concentrations of reducing sugars decreased significantly in the leaves of mahogany plants subjected to the presence of nickel (Figure 2H), values of 2.73 and 0.89 µmol carb g^{-1} DM were obtained, representing a reduction of 67.4% in dose of 8 mg L^{-1} of Ni when compared to the control treatment.

According to Sanches et al. (2017), the production or degradation of these compounds is due to a defense mechanism called osmoregulation, which plants present when subjected to adverse conditions, such as exposure to heavy metals, providing an increase in energy gain from the leaf-atmosphere ratio (Xue et al., 2018). After the degradation of these compounds occurs, their accumulation contributes to the preservation of the integrity of proteins, enzymes and cell membranes (Oliveira et al., 2017). In the case of sucrose, the main transport sugar, its hydrolysis releases hexoses, and this action is mediated by specific enzymes that regulate the entry of sucrose in different directions of use, improving plant stress tolerance (Yildirim et al., 2019). The lower concentration of reducing sugars in plants under stress caused by Ni is supposed to be energy saving by the plants, indicating that Ni may have affected the assimilated transport system and leaf cellular respiration (Mizushima et al., 2019; Andrade Júnior et al., 2021).

3.3. Nitrogen metabolism

Nickel concentrations did not significantly interfere with free ammonium concentrations in the leaves, but on the other hand, they interfered with the roots, where they had an increase of 150% when compared to the control treatment (Figure 3A). Accordingly, total soluble amino acids had significant metal effects in the root and leaf, with an increase of 102% and 42%, respectively (Figure 3B). Along with the increase in amino acids, the roots also increase the synthesis of proline, 143% higher than the control, while in the leaf its production does not differ statistically (Figure 3C). However, glycine-betaine concentrations act antagonistically to proline, with a decrease of 53% in roots and 44% in leaves (Figure 3D).

In this stress condition, these plants may be absorbing more ammonium in the proplastid structures of the roots and accumulating in them, this can be considered an alternative route triggered by NADH-dependent glutamate dehydrogenase (Ristova et al., 2016). The possible low activity of the enzyme glutamine synthetase (GS) may be contributing to the increase in ammonium concentration because this enzyme is a precursor for the transformation of ammonium into amino acids (Wu et al., 2016; Razgallah et al., 2016). Furthermore, the absorption of ammonium by the plant can also occur passively through the low-affinity system, through aquaporins and cation channels, as well as through high-affinity transporters, which ends up being an alternative source of nitrogen for the plant roots, in addition to nitrate (Ataíde et al., 2020).

The amino acids produced by the plant are used to mitigate the harmful effect of nickel, as this increase is a mechanism to avoid damage to the enzymatic systems (Munns and Tester, 2008). Furthermore, they are vital in the

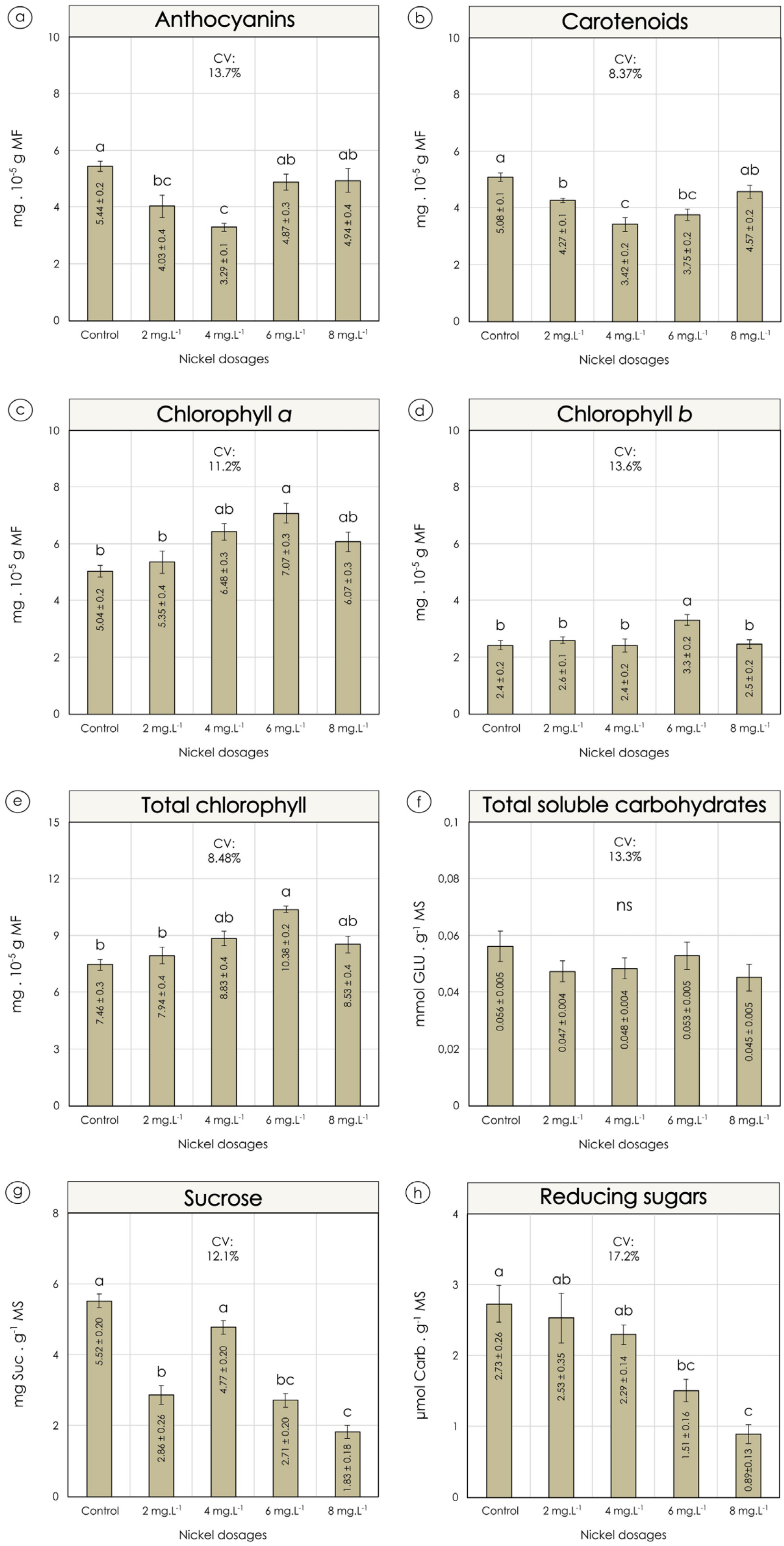

Figure 2. a Anthocyanins, b Carotenoids, c Chlorophyll a, d Chlorophyll b, e Total chlorophylls, f Total soluble carbohydrates, g Sucrose, h Reducing carbohydrates from Brazilian mahogany leaves (*Swietenia macrophylla*) exposed to five concentrations of nickel (0, 2, 4 , 6 and 8 mg.L^{-1}). Different letters indicate significant differences in the Tukey test ($P < 0.05$). Mean ± SE, n = 6. ns: not significant.

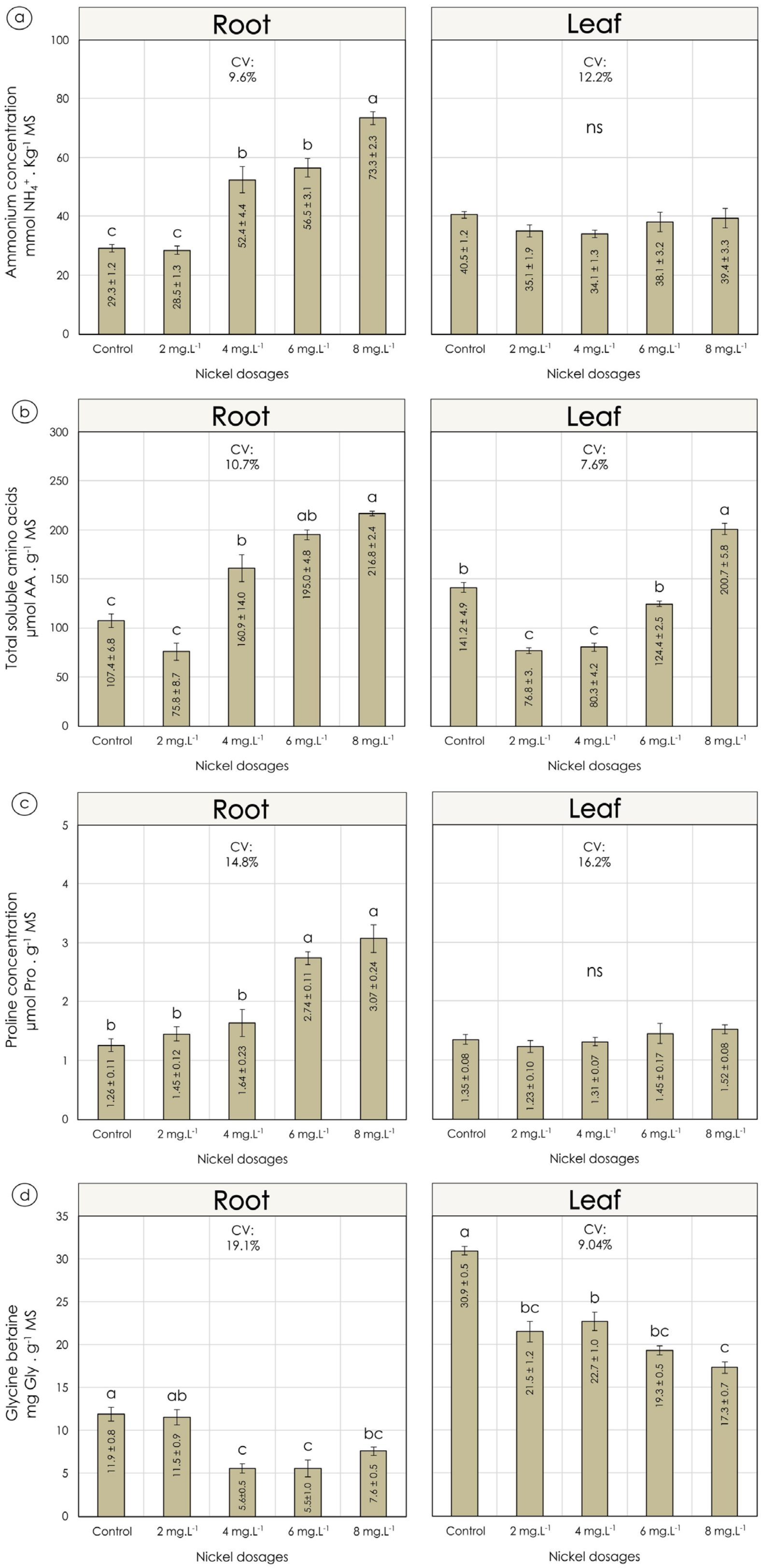

Figure 3. a Free ammonium, b Total soluble amino acids, c Proline, d Glycine-betaine from Brazilian mahogany (*Swietenia macrophylla*) seedlings exposed to five concentrations of nickel (0, 2, 4, 6 and 8 mg.L^{-1}). Different letters indicate significant differences in the Tukey test ($P < 0.05$). Mean ± SE, n = 6. ns: not significant.

absorption and transport of mineral nutrients across the cell membrane (Dörr et al., 2020). According to Zang et al. (2016) and Munns and Tester (2008), the increase in the concentration of organic solutes such as amino acids, in the cytosol or in the vacuole, occurs to reduce the osmotic potential and, consequently, maintain the water potential and the cell turgor close to the optimal level, as the metal affected the root structures, potentially reducing water absorption, as seen in this work (Figure 1Q).

Along with the increase in amino acids, there is an increase in proline, accumulated after periods of metal stress, which can be used as an energy source, redistributing carbon and nitrogen (Teh et al., 2016), to protect the plasma membrane of plants and recover activities. plant physiological and biochemical processes in stressful situations (Nascimento et al., 2019). Another response to the increase in proline concentrations was for a possible osmotic adjustment, which plants resort to as protection when there is low water content, which could be due to low absorption due to the deleterious effect of nickel on the xylem structures of the root (Figure 1R; Blum, 2016). Osmotic adjustment is an adaptive mechanism that provides the maintenance of turgidity and related processes, such as stomatal opening, growth, cell elongation and the production of photoassimilates, thus allowing it to operate even in conditions of low water potential (Fang and Xiong, 2014; Zhang and Becker, 2015). This phenomenon occurs with the aim of maintaining the plant's physiological and metabolic functions for a certain time while waiting for environmental conditions to return to the appropriate level (Per et al., 2017). However, this adjustment process is only effective when the stress conditions do not last long (Álvarez et al., 2018).

The quantification of glycine is a critical determinant of indicating tolerance or not to stress (Nawaz and Wang, 2020). Under stress conditions, it acts on the osmotic adjustment of cells since in large quantities glycine does not influence cellular metabolism and can help control the entry and exit of water in the cell, in the cytoplasm and vacuoles (Hasanuzzaman et al., 2014). In addition to these functions, this amino acid provides reserves of nitrogen (N) and carbon (C), cellular integrity and maintenance of turgor (Cabello et al., 2014; Gupta and Huang, 2014). However, the degradation of glycine is normally observed when the plant begins to recover catabolism (Szabados et al., 2011), but in this study the degradation occurred concomitantly with the increase in the metal. Several studies have demonstrated that the accumulation of compatible solutes, such as proline and glycine, is related to resistance to abiotic stresses (Silva et al., 2009), however, among both, proline had a unique and important role in combating the effects of nickel in the Mahogany root system.

3.4. Antioxidant enzymes

The activity of the enzyme ascorbate peroxidase (APX) in the roots was not significantly affected by Ni (Figure 4A), presenting an average value of 35.2 μM H_2O_2. min^{-1}. g^{-1}. While in leaves the enzyme activity was significantly affected by Ni, with values of 36.4 and 32.9 μM H_2O_2. min^{-1}. g^{-1}, obtained in control plants (0 mg.L^{-1} of Ni) and in the highest dose of Ni (8 mg.L^{-1}), respectively, characterizing a loss of 9.6% in the 8 mg.L^{-1} treatment of Ni when compared to the control treatment.

Ni significantly affected the activity of the catalase enzyme (CAT), both in roots and leaves (Figure 4B). In the roots, values of 1.1 μM H_2O_2 were obtained. min-1 . g^{-1} (0 mg.L^{-1} of Ni) and 2.4 μM H_2O_2. min^{-1}. g^{-1} (8 mg.L^{-1} Ni), representing an increase of 118.2% in the highest dose of Ni when compared to the control treatment. In leaves, the effect of Ni promoted a 60.9% increase in enzyme activity at a dose of 8 mg.L^{-1} of Ni (3.7 μM H_2O_2.min^{-1}.g^{-1}) when compared to the control treatment (2.3 μM H_2O_2. min^{-1}.g^{-1}).

The activity of the enzyme superoxide dismutase (SOD) in roots was significantly affected by Ni, with values of 0.8 and 1.6 AU. mg^{-1}. DM, obtained in control plants (0 mg.L^{-1} Ni) and at a dose of 8 mg.L^{-1} Ni, respectively, representing a 100% increase in the 8 mg.L^{-1} Ni treatment when compared to control treatment. While in leaves the enzyme activity was not significantly affected by Ni (Figure 4C), presenting an average value of 1.56 AU. mg^{-1}. DM. As mahogany plants suffer stress from the heavy metal nickel, they develop defense mechanisms through antioxidant enzymes, which in turn catalyze redox reactions (Natasha et al., 2019).

SOD constitutes the first line of defense against Reactive Oxygen Species (ROS) accumulated by oxidative stress, being part of the first adjustment of plant tolerance to stress (Berwal and Ram, 2018). Its catalyzation in superoxide anion dismutase (O2•$^{-}$), has hydrogen peroxide (H_2O_2) and oxygen (O_2) as products, being the only enzyme whose activity can affect the cellular concentration of O_2 and H_2O_2 (Xie et al., 2019; Raza et al., 2022). The increase in H_2O_2 content is closely linked to the activity of CAT and APX, which eliminate H_2O_2, thus maintaining a highly optimized balance of antioxidant enzymes, in order to reduce the risk of oxidative damage generated by stress, and can present different responses according to the organ, species and metal causing the stress (Xie et al., 2019).

APX is the main enzyme responsible for the elimination of hydrogen peroxide, where it acts strongly in the elimination of this compound using reduced ascorbate (Sruthi and Puthur, 2019). This enzyme highlights the plant's defense in the destruction of free radicals as a way of preventing more intense damage, this corroborates the fact that this enzyme acts to combat various abiotic stresses due to metal toxicity, being expressed by increased antioxidant activity (Kumar et al., 2007). Furthermore, it presents a differential response pattern between tissues (Xie et al., 2019).

The ability to maintain CAT activity at high levels under stress conditions is essential for the balance between the formation and removal of H_2O_2 from the intracellular environment, as this enzyme catalyzes the reduction of H_2O_2 to water (H_2O) and O_2, protecting the cell oxidative damage arising from the excessive accumulation of this compound (Natasha et al., 2019; Xie et al., 2019). The increase in catalase is assumed to be an adaptive strategy against damage caused by oxidative stress through high concentrations of heavy metals (Raza et al., 2022). Thus, the need to stimulate catalase was found to reduce H_2O_2 generated by oxidative stress, in order to form other

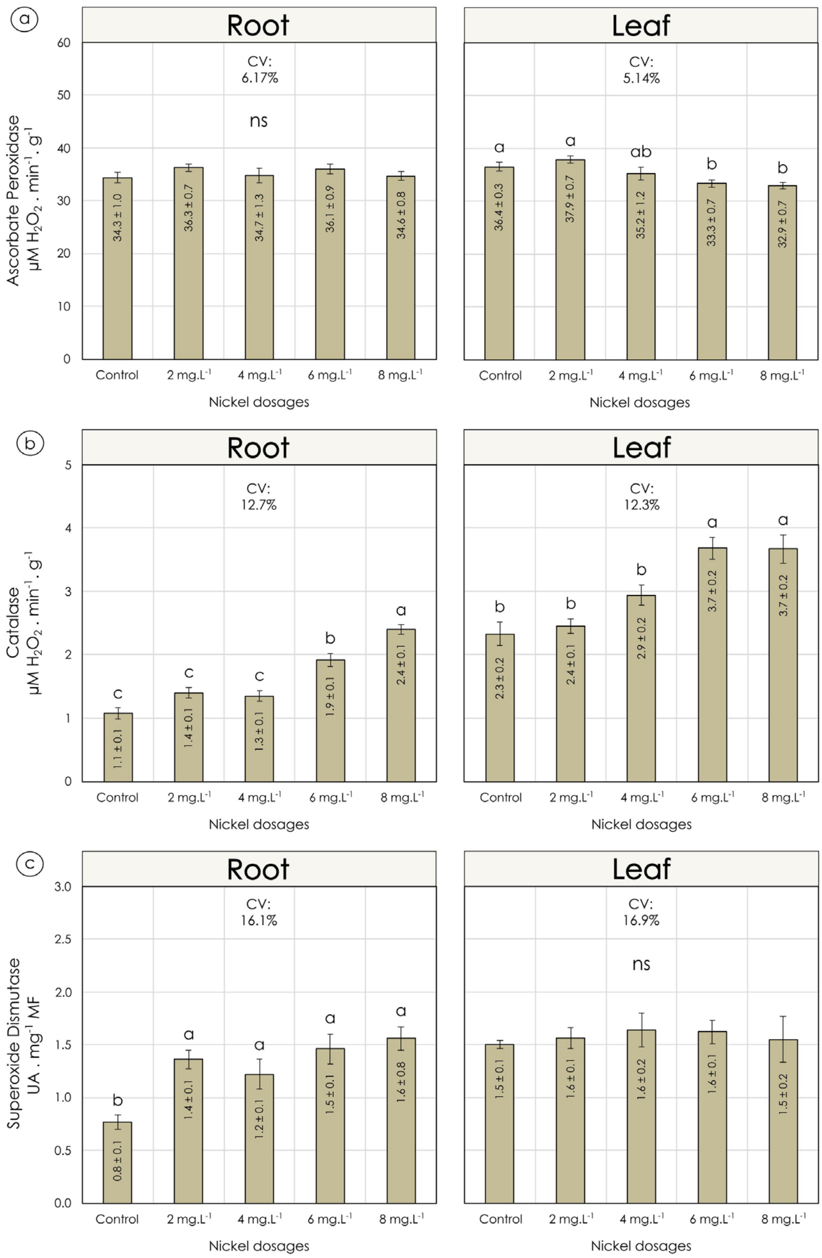

Figure 4. a Ascorbate Peroxidase, b Catalase, c Superoxide Dismutase from Brazilian mahogany (*Swietenia macrophylla*) seedlings exposed to five concentrations of nickel (0, 2, 4, 6 and 8 mg.L-1). Different letters indicate significant differences in the Tukey test (P < 0.05). Mean ± SE, n = 6. ns: not significant.

products that are not toxic to the plant cell and thus promote the maintenance of cellular homeostasis (Raza et al., 2022; Smith et al., 2023).

3.5. Multivariate analysis

A Principal Component Analysis (PCA) was carried out for osmoregulators (amino acids, proline, glycine, sucrose, reducing and total carbohydrates), along with antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase), encompassing the main sources of defense of the plant under stress (Figure 5), similar analysis carried out by Natasha et al. (2019).

The dimension chart was evaluated by the square cosine (Cos^2), as the quality of the representation of the osmolyte variables and antioxidant enzymes was estimated, this quality called commonality, which is the sum of the squares of the correlations between each variable "i" and the principal component "j". The larger the variable vector, the better its representation in the factorial map, consequently the more important the interpretation of these components,

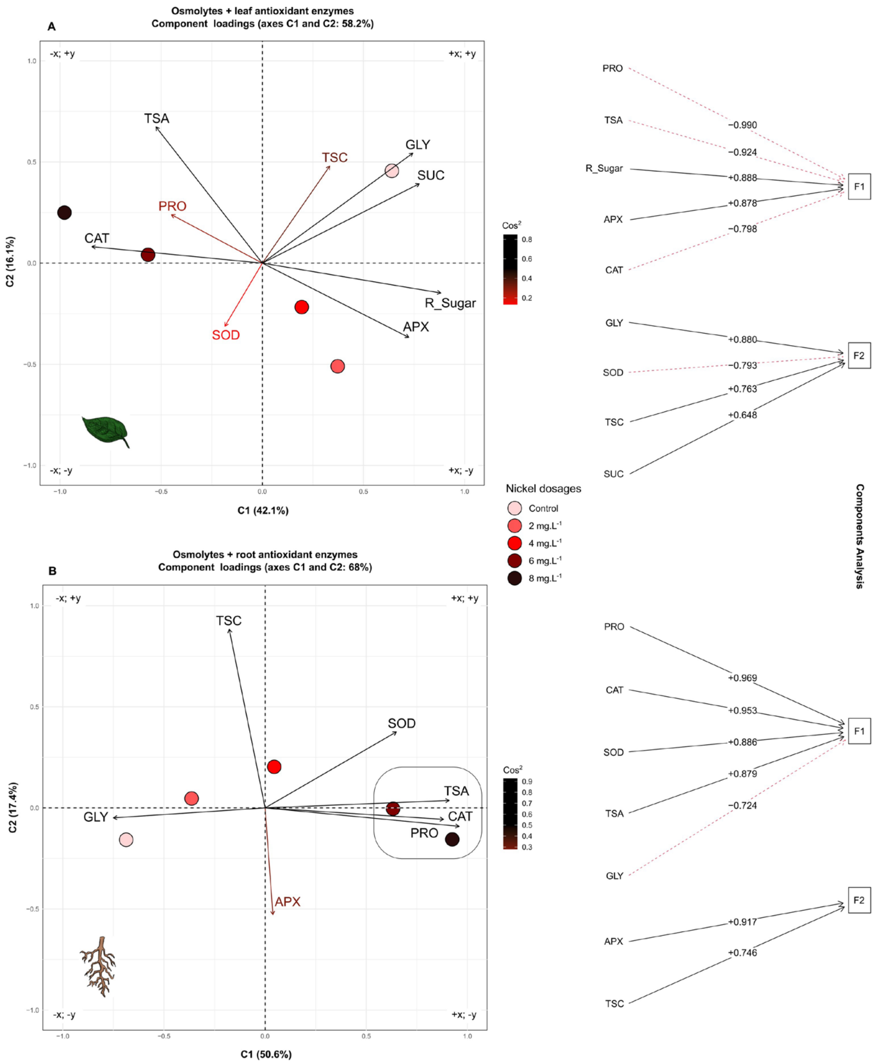

Figure 5. Principal Component Analysis (PCA) C1 x C2, indicating the contribution of variables in the dimensions and weight of the contribution of osmolytes and antioxidant enzymes from leaf (A) and root (B) of Brazilian mahogany (*Swietenia macrophylla*) subjected to different dosages of nickel.

being CAT, TSA, GLY, SUC, APX and R_Sugar for the leaves while for the roots they were GLY, TSC, SOD, TSA, CAT and PRO. In contrast, the variables close to the center of the graph were the least important for the first components, resulting in less representation in $\cos^2$ (Wold et al., 1987; Valladares et al., 2008; Liu et al., 2023).

The results obtained by the PCs technique have respective eigenvalues and percentages of variance. At the root, it appears that the first two PCs were responsible for 68% of the total variation in the mahogany defense system, with main component 1 (C1) being responsible for 50.6% and main component 2 (C2) for 17.4%. % of data variance. The results in the root showed a strong positive correlation of amino acids (+0.879), proline (+0.969) and catalase (+0.953), revealing that these three variables showed a similar trend under different heavy metal treatments, more significant at the highest dosages. (figure 5B). However, the other parameters appeared scattered in the PCA graph, showing their different behaviors under strong metallic stress. There is also an inversely proportional relationship between the variables amino acids (+0.879) and glycine (-0.724), possibly due to transamination relationships acting as osmotic protection during periods of stress (Fuertes-Mendizábal et al., 2020).

While on the sheet, data variability was explained by 42.1% in dimension 1 and 16.1% in dimension 2, totaling 58.2% of the total data variability. And according to the analysis of the contribution of the sheet variables to the dimensions and their respective weights, it shows that the variables that contributed most to dimension 1 were PRO (-0.990) and TSA (−0.924), while the variables that contributed most to dimension 2 were GLY (+0.880) and SOD (-0.793), indicating the main forms of plant defense in combating the harmful effects of nickel (Figure 5A).

In addition, there is an inversely proportional relationship with the variables CAT (-0.798) and APX (+0.878), indicating that nickel can influence the response pattern of different enzymes in different tissues, an assumption also verified by Sruthi and Puthur (2019) in the MDA compound.

4. Conclusion

Anatomical changes were observed in the different organs, where initially a difference was seen in the cells in the central region of the leaf, in the stem and in the root. While the adaxial and abaxial epidermal surfaces, together with the mesophyll, apparently did not suffer interference from the metal. As the dosage of nickel increases, the synthesis of ammonium, amino acids and proline in the root also tends to increase. In the leaf, the increase in metal tends to only increase the concentration of amino acids and decrease the concentration of glycine. The plant antioxidant defense system was efficient in attenuating the toxic effects of ROS at different concentrations of nickel, with significant actions of CAT and SOD enzymes in the root, while the leaf had the main action of APX and CAT.

Changes in such parameters are forms of resistance and suitability to survive environments contaminated by nickel, being very useful for alleviating soils contaminated with this metal and designing a remediation process for a contaminated site. The cultivation of Brazilian mahogany plants in areas contaminated by Ni indicates possible mitigation, as this forest species has a particular characteristic of resistance to stress conditions in contact with the heavy metal. Therefore, carrying out additional studies using mitigants on this species is essential, as it has great potential to be the target of phytoremediation programs.

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Supplementary Material

Supplementary material accompanies this paper.

Supplementary table. Result of the analysis of variance in a randomized block design (DBC) for the response variables of Brazilian mahogany (Swietenia macrophylla) seedlings exposed to nickel.

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