









ORIGINAL ARTICLE

Manganese toxicity and stress-induced tolerance responses in *Psidium cattleianum* and *Eugenia involucrata*

Toxicidade e respostas de tolerância induzidas por estresse de manganês em *Psidium cattleianum* e *Eugenia involucrata*

Marcos Vinícius Miranda Aguiar¹ , Caroline Castro Kuinchtner¹ , Gerâne Silva Wertonge¹ ,
Thalia Preussler Birck¹ , Thomas Wink Peixoto¹ , Maristela Machado Araujo¹ ,
Gustavo Brunetto¹ , Luciane Almeri Tabaldi¹ 

¹Universidade Federal de Santa Maria – UFSM, Santa Maria, RS, Brasil

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Abstract

Manganese (Mn) toxicity limits plant growth and productivity, especially in acidic soils in many parts of the world. Thus, it is necessary to use phyto-remediation species in reforestation programs to restore the ecological conditions of the environment. The study aimed to evaluate morpho-physiological and biochemical parameters in addition to Mn translocation in plant tissues of *Psidium cattleianum* and *Eugenia involucrata* under different Mn concentrations to assess the phyto-extraction potential of the species to be cultivated in environments contaminated with Mn. *P. cattleianum* and *E. involucrata* were cultivated in five Mn concentrations: 2 (control), 30, 60, 90 and 120 μM . Each sampling unit consisted of a pot with five plants. Morphological parameters of the shoot and root system, photosynthetic parameters, photosynthetic pigments, antioxidant enzyme activity, concentration of hydrogen peroxide and lipid peroxidation, as well as Mn accumulation in tissues, and Mn translocation factor (TF) were evaluated. Excess Mn promoted the activation of the enzymatic antioxidant system in *Psidium cattleianum* and *Eugenia involucrata*, reducing its negative effects on plant biomass production. Furthermore, high Mn concentrations in plant shoots of *P. cattleianum* and *E. involucrata* are indicative of the species' tolerance to Mn, demonstrating their phyto-extraction potential in soils contaminated by Mn.

Keywords: Antioxidant enzymes; Araçá; Cerejeira-do-mato; Degraded areas; Gas exchange; Environmental toxicology; Heavy metals.

Resumo

A toxicidade do manganês (Mn) limita o crescimento e a produtividade das plantas, especialmente, em solos ácidos em diversas partes do mundo. Assim, é necessário o uso de espécies fitorremediadoras em programas de reflorestamento para restabelecer as condições ecológicas do ambiente. O estudo objetivou avaliar as variáveis morfofisiológicas, bioquímicas e translocação de Mn em tecidos vegetais de *Psidium cattleianum* e *Eugenia involucrata*, cultivadas sob diferentes concentrações de Mn, para definir o potencial de fitoextração de Mn das espécies, a serem cultivadas em ambientes contaminados com Mn. *P. cattleianum* e *E. involucrata* foram cultivadas em cinco concentrações de Mn: 2 (controle), 30, 60, 90 e 120 μM . Cada unidade amostral foi composta por um vaso com cinco plantas. Variáveis morfológicas da parte aérea e do sistema radicular, variáveis fotossintéticas, pigmentos fotossintéticos, atividade de enzimas antioxidantes, concentração de peróxido de hidrogênio e peroxidação lipídica, bem como a concentração de Mn acumulado nos tecidos, e fator de translocação (FT) de Mn foram determinadas. O excesso de Mn promoveu a ativação do sistema antioxidante enzimático nas plantas de *Psidium cattleianum*

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Corresponding author: aguilarmarcos2009@hotmail.com

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e *Eugenia involucrata*, reduzindo os efeitos negativos deste metal sobre a produção de biomassa das plantas. Além disso, as altas concentrações de Mn na parte aérea das plantas de *P. cattleianum* e *E. involucrata* são indicativos da tolerância das espécies ao Mn, demonstrando a potencialidade das mesmas para fitoextração de Mn em solos contaminados com esse metal.

Palavras-chave: Araçá; Áreas degradadas; Cerejeira-do-mato; Enzimas antioxidantes; Metais pesados; Trocas gasosas; Toxicologia ambiental.

1. INTRODUCTION

Soil acidity is a major edaphic problem that affects agricultural systems in many parts of the world (Shetty et al., 2021). In acidic soils, plant productivity may be impaired by induced nutritional deficiencies, decreased P solubility, as well as increased bioavailability of H⁺, aluminum (Al), iron (Fe) and manganese (Mn) (Meng et al., 2021). Mn bioavailability depends on soil acidity (pH), redox potential, temperature and humidity. These factors can increase bioavailable Mn to toxic concentrations, individually or synergistically (Faria et al., 2020).

In addition, human activities such as mining, fertilization and wastewater irrigation further increase excessive accumulation of Mn in the soil, and consequently have several negative effects on the health of living beings (Pan et al., 2019). This is because excess Mn in agricultural soils can harm animals and threaten human health through the food chain. In humans, Mn accumulation affects the central nervous system and causes the development of Parkinson-like disorders (Tang et al., 2021). In plants, Mn toxicity is one of the main threats to the growth of vegetable crops in numerous countries (Faria et al., 2020).

Therefore, excess Mn in plant tissues acts as a toxic heavy metal that limits crop production and quality (Li et al., 2019). Thus, Mn toxicity can negatively affect plant growth and development through various physiological processes, such as inhibition of photosynthesis, accumulation of reactive oxygen species (ROS), interruption of several key enzyme activities, and impairment of uptake and transport of other mineral elements (Tang et al., 2021).

However, plants tolerant to excess Mn can use strategies to reduce its toxic effects, such as Mn accumulation in tissues and/or translocation to shoots (Faria et al., 2020), sequestration and compartmentalization of Mn, Mn detoxifying complexes in the vacuole, trichomes or other vesicles (Alejandro et al., 2020), and increased production and/or activity of antioxidant enzymes capable of detoxifying excess ROS (Pan et al., 2019). The antioxidant enzyme system includes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) which can remove excess ROS and reduce oxidative damage caused by heavy metal stress (Xiao et al., 2020). Thus, understanding morphophysiological and biochemical responses of plants exposed to heavy metals is key for the recovery of contaminated areas (Trentin et al., 2022). With this information not only is it possible to understand the adaptation strategies or mechanisms used by plants in conditions of excess Mn, but it may help to define phyto-remediation strategies in environments with excess Mn (Gautam et al., 2016). In practice, this would make it possible to identify functional traits, capable of effectively contributing to areas undergoing recovery processes (Gann et al., 2019).

An interesting strategy to help recovering areas contaminated with metals lies on using tree species, due to their long life cycle, high biomass production and dense root system. In addition, tree species can concentrate large amounts of metals in their stem and roots, immobilize metals in plant tissues and delay their return to the soil (Yan et al., 2020).

Among the plants of interest for phytoremediation is *Eugenia involucrata* DC. and *Psidium cattleianum* Sabine. *E. involucrata* is popularly known as *Cerejeira-do-Mato*, belonging to the Myrtaceae family (Lorenzi, 2002). This species is classified as late secondary, selective hygrophyte and tolerant to low temperatures. It is used for logging, landscaping and recovery of degraded areas (Tonetto et al., 2013). *P. cattleianum* Sabine is popularly known as *Araçá* and also belongs to the Myrtaceae family (Gomes et al., 2015). This species occurs in secondary vegetation, and it is indicated for afforestation, landscaping, as well as in heterogeneous reforestation plantations intended for the recovery of degraded areas (Ballestreri et al., 2021). However, the adaptive performance and response mechanisms of these species under Mn

stress are not sufficiently known, which limits further application in phyto-remediation. The study aimed to (a) determine growth parameters, Mn accumulation and Mn phytoextraction potential in *P. cattleianum* and *E. involucrata* cultivated under different Mn concentrations; and (b) to evaluate photosynthetic and biochemical responses of *P. cattleianum* and *E. involucrata* to Mn stress. We believe the results of this study will provide a better understanding of plant physiological and biochemical responses to Mn stress and will help to increase the feasibility of using these species in the phyto-remediation of Mn-polluted soils.

2. MATERIAL AND METHODS

2.1 Study location and plant material

The study was carried out in a greenhouse located in the city of Santa Maria, southern Brazil (29°42'56.35"S and 53°43'12.64"W). Inside the greenhouse, the relative humidity was 60%, and the average temperature was 25 °C.

Seeds were collected from mother trees located in forest fragments in southern Brazil. Seeds were sown directly in substrate packed in polypropylene tubes of 180 cm³. We used Carolina Soil® substrate composed of *Sphagnum* sp. and vermiculite, plus 30% of carbonized rice husk for seedling production. Base fertilization was carried out with Osmocote® controlled-release fertilizer (CRF) with a nutrient release time of six months, containing (in %) 15 N, 12 K₂O, 9 P₂O₅, 2.3 S, 1 Mg, 0.45 Fe, 0.2 Mo, 0.05 Cu and 0.06 Mn.

When seedlings were 120 days old, approximately 25 cm in height and 2 mm in root collar diameter, they were sent to the greenhouse for acclimatization, until the time of the experiment. During this period, the seedlings were irrigated daily until the experiment was set up.

2.2 The experimental design

A completely randomized design was used with three replications, each containing five plants. These seedlings of *P. cattleianum* and *E. involucrata* were subjected to different concentrations of Mn. Two (2.0) µM is the standard concentration of Mn in the nutrient solution of Hoagland & Arnon (1950), which was used as a control treatment, and for the other concentrations 30, 60, 90 and 120 µM Mn were added in the complete nutrient solution. Thus, a total of fifteen plants were managed per treatment.

At the time of implantation, the seedlings of *P. cattleianum* and *E. involucrata* were removed from the tubes and the roots were carefully washed to remove the substrate from their surroundings. Then, the plants were transferred to the hydroponic system for acclimatization in which seedling roots were immersed in a 6L pot filled with Hoagland & Arnon's (1950) solution. A Styrofoam sheet was added to the surface of each pot, with five holes in the center to allow the plants to pass through. The sheet allowed the plants to be fixed and reduced evaporation of the solution contained in each pot.

The seedlings were acclimated for 15 days in full-strength Hoagland & Arnon's (1950) nutrient solution. The aeration of the solution in each container was done via PVC microtubes connected to an air compressor. The nutrient solution, in its original form, contained the following concentrations (in mg L⁻¹): NO₃⁻ = 196; NH₄ = 14; P = 31; K = 234; Ca = 160; S = 70; Mg = 48.6; Fe-EDTA = 5; Zn = 0.15; Mn = 0.5; B = 0.5; Cu = 0.02 and Mo = 0.01.

The treatments were applied following the acclimatization period. The seedlings remained in the different concentrations of Mn for 28 days, totaling 43 days in the hydroponic system. The nutrient solution in each pot was replaced twice a week and the pH was adjusted daily to 5.5±0.1 with 1.0 mol L⁻¹ HCl or 1.0 mol L⁻¹ NaOH.

2.3 Morphological parameters

Two plants from each experimental unit were collected to analyze growth, totaling 60 plants. Prior to installing the experiment and at its end, shoot height and main root length were

measured with a ruler. With the results, the values of increased taproot length and shoot height were calculated.

Leaf area was measured using WinRhizo 2013 software, which uses the methodology established by Tennant (1975) as a principle. The samples were digitized in a professional scanner (EPSON Expression 11000), and TIFF format images were analyzed.

Root morphology was assessed from digitized images, using WinRhizo Pro 2013, coupled to an EPSON Expression 11000 scanner equipped with additional light (TPU) and a resolution of 600 DPI. Root surface area ($\text{cm}^2 \text{ plant}^{-1}$), total root length (cm plant^{-1}), average root diameter (mm) and root volume ($\text{cm}^3 \text{ plant}^{-1}$) were determined.

The plants were separated into shoots and roots, washed in running water and then in distilled water. Later, the shoots and roots were dried in a forced air circulation oven at 65°C and finally weighed on a precision scale (0.0001g) until constant weight was reached.

2.4 Photosynthetic parameters

On the twenty-seventh day of exposure to Mn concentrations, the physiological parameters related to the photosynthetic apparatus were assessed in each plant. The analysis took place from 8 am to 10:30 am, using a Li-COR® 6400-XT infrared gas analyzer (IRGA)]. On that occasion net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), Rubisco carboxylation efficiency (A/Ci) and water use efficiency (WUE) at an ambient CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ at $20\text{-}25^\circ\text{C}$, $50 \pm 5\%$ relative humidity and photon flux density of $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ were determined¹.

2.5 Mn concentration in tissue

Samples of shoot and roots dry mass were ground in a Wiley mill after drying, and then passed through a 2 mm mesh sieve. Plant tissue was submitted to nitro-perchloric digestion (3.0 mL of HNO_3 65% PA and 1 mL of HClO_4 70% PA) (Empresa Brasileira de Pesquisa Agropecuária, 2009). Total Mn concentration was analyzed in an atomic absorption spectrophotometer (AAS, Perkin Elmer Analyst 200, USA). Subsequently, Mn accumulation in plant shoots and roots was calculated. The translocation factor (TF) was calculated according to Equation 1 (Raj et al., 2020):

$$\text{TF} = (\text{shoot Mn concentration}) / (\text{root Mn concentration}) \quad (1)$$

2.6 Biochemical parameters

Fresh leaf and root samples were frozen (-80°C), and then macerated in liquid N to determine the biochemical parameters. Chlorophylls *a* and *b* and carotenoids were extracted according to the method described by Hiscox & Israelstam (1979) and estimated using the equation proposed by Lichtenthaler (1987). Hydrogen peroxide content was determined according to Loreto & Velikova (2001), and H_2O_2 concentration was expressed as $\mu\text{mol g}^{-1}$ fresh weight. Lipid peroxidation was determined by malondialdehyde (MDA) concentration, following the method of El-Moshaty et al. (1993) and expressed as nmol of MDA mg^{-1} of protein. Guaiacol peroxidase activity was determined according to Zeraik et al. (2008), using guaiacol as a substrate. Results were expressed in units of enzyme per mg of protein (U mg^{-1} protein). Superoxide dismutase (SOD) activity was determined based on the spectrophotometric method described by Giannopolitis & Ries (1977). The enzyme unit of SOD was defined as the number of enzymes that inhibit NBT photoreduction by 50% (Beauchamp & Fridovich, 1971).

2.7 Statistical analysis

Normality of error distribution and homogeneity of error variance were analyzed using the Shapiro-Wilk and Bartlett tests, respectively, for all the variables (Storck et al., 2016). When these assumptions were met, analysis of variance and the Scott-Knott test were performed for the treatments at 5% probability of error, using Sisvar statistical software (Ferreira, 2019).

3. RESULTS AND DISCUSSION

According to the results of analysis of variance, there was a significant effect ($p \leq 0.05$) of the factors evaluated (different species and Mn concentrations) on the morphological growth parameters.

The smallest increases in taproot length, root length and root surface area were observed in *P. cattleianum* and *E. involucrata* treated with Mn (Figures 1a and 2a-2b). This may be because Mn toxicity inhibits primary root growth, reducing auxin biosynthesis and repressing the expression of auxin efflux transporters such as PIN4 and PIN7, which causes decreased meristematic cell division and elongation of the root, promoting changes throughout the root axis (Zhao et al., 2017).

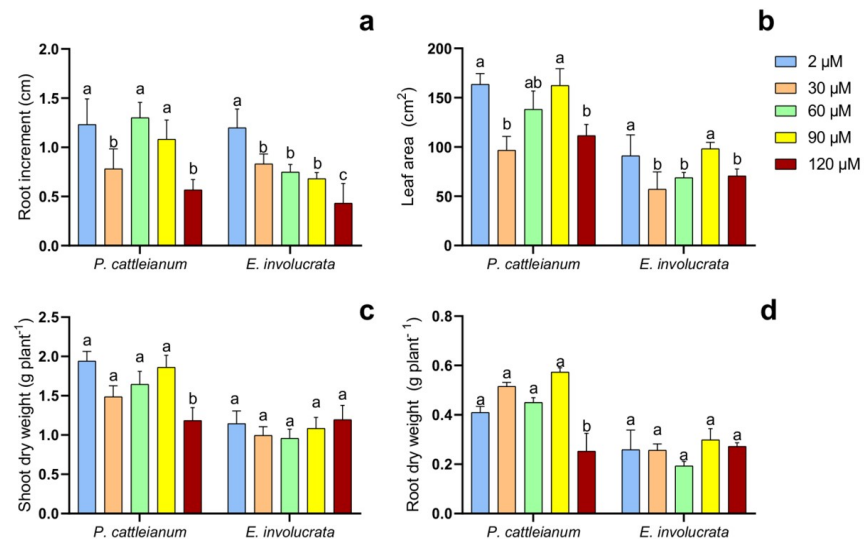


Figure 1. Mean values recorded for increase in taproot length (a), leaf area (b) shoot dry weight (c) and root dry weight (d) in *Psidium cattleianum* (*P. cattleianum*) and *Eugenia involucrata* (*E. involucrata*) seedlings grown in different Mn concentrations. Different letters between treatments represent statistically significant differences by the Scott-Knott test. Bars represent the mean ± standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

The lowest values of shoot and root dry weight in *P. cattleianum* were observed only at the highest Mn concentration (120 µM) (Figure 2c-2d). However, shoot and root dry matter of *E. involucrata* showed no difference regardless of the concentration of Mn (Figure 1c-1d). This response may have occurred because excess Mn did not overload the mechanisms of attachment and compartmentalization of the cell wall and the root vacuole and, therefore, did not affect cell metabolism (Pan et al., 2019). Furthermore, *E. involucrata* may have developed or constitutively presented tolerance mechanisms to Mn, such as Mn accumulation in tissues, translocation to shoots and consequently reduced the toxicity effects of this metal. These factors may have contributed to the maintenance of plant biomass production in the presence of Mn (Shao et al., 2017). Thus, the application of increasing concentrations of Mn did not reduce nutrient and water uptake by the roots to cause a decrease in dry biomass production in plants evaluated in this study, although it did cause some negative effects on root morphology.

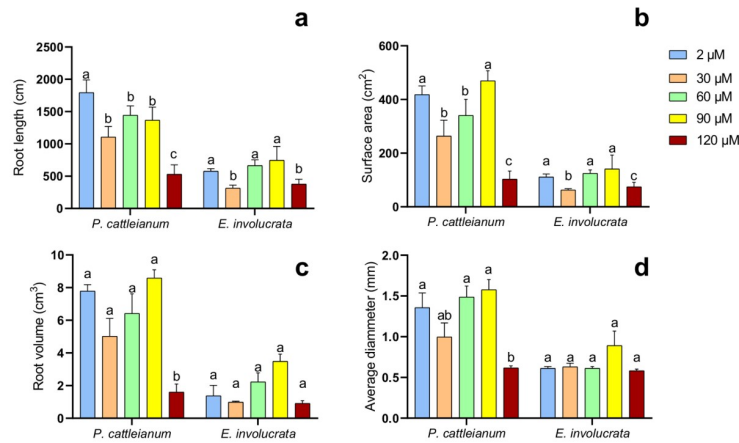


Figure 2. Mean values recorded for total root length (a), root surface area (b) and root volume (c) and root diameter (d) in *Psidium cattleianum* (*P. cattleianum*) and *Eugenia involucrata* (*E. involucrata*) seedlings grown in different Mn concentrations. Different letters between treatments represent statistically significant difference by the Scott-Knott test. Bars represent the mean \pm standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

There was a significant effect ($p \leq 0.05$) of the different species and Mn concentrations on the photosynthetic parameters evaluated in this study, except for water use efficiency (WUE) and Rubisco carboxylation efficiency (A/Ci) with addition of Mn (Figure 3e-3f).

The lowest values for photosynthetic parameters in both species were also observed only in a Mn concentration of 120 µM (Figure 3). This reduction was probably due to stomatal closure, resulting in lower values of G_s and C_i under Mn stress, which results in a lower net CO_2 assimilation rate. Furthermore, the negative effect of Mn on photosynthetic parameters may be related to leaf area, as it was reduced with the addition of 120 µM Mn (Figure 1b). The lowest values for transpiration rate were also observed in *P. cattleianum* with 120 µM Mn (Figure 3b). This may have occurred because stomatal closure prevents H_2O diffusion through transpiration and causes a decrease in transpiration rate (Pan et al., 2018). The lower values observed in photosynthetic parameters may also have occurred because the stress caused by Mn in tissues can cause changes in the composition of the thylakoid membranes and photosynthetic pigments, or by preventing electron flow in the photochemical phase of photosynthesis (Liu et al., 2019).

The significant effect ($p \leq 0.05$) of the different species and Mn concentrations were found for the biochemical parameters and tissue Mn contents in this study. Total chlorophyll production in *E. involucrata* and carotenoid content in *P. cattleianum* were stimulated with the addition of Mn in the nutrient solution (Figure 4a-4b). However, the lowest values for the parameters total Chl content in *P. cattleianum* were observed in the presence of Mn (Figure 4a). This was because the low concentrations of Mn may have promoted an increase in photosynthetic pigment synthesis and photosynthesis because Mn improves the structural integrity of the oxygen-evolving complex, which can trigger an increase in energy transfer and electron transport (Wang & Jiang, 2018). However, high Mn concentrations caused a decrease in pigment biosynthesis or accelerated pigment degradation (Xiao et al., 2020). This decrease in pigment content may also have occurred because Mn replaces magnesium in chlorophyll molecules or binds to ferredoxin in the thylakoid matrix, eventually altering chlorophyll synthesis and the correct assembly of photosystem I (PSI) (Alejandro et al., 2020).

As a toxic metal, excess Mn can generate reactive oxygen species (ROS) and trigger oxidative stress in plants, causing lipid peroxidation and damaging photosynthetic pigments and proteins if ROS are not properly eliminated (Huang et al., 2019). Thus, one of the adaptive changes which mitigates the toxic effects of high Mn in plants involves the activation of the antioxidant system via antioxidant enzymes, such as superoxide dismutase (SOD) and guaiacol peroxidase (POD) (Li et al., 2019). SOD plays a key role in the conversion of superoxide radicals ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) (Pan et al., 2018). POD is responsible for converting H_2O_2 into H_2O and O_2 by dissociating H_2O_2 and, therefore, plays an essential role in providing plant tolerance to unfavorable conditions.

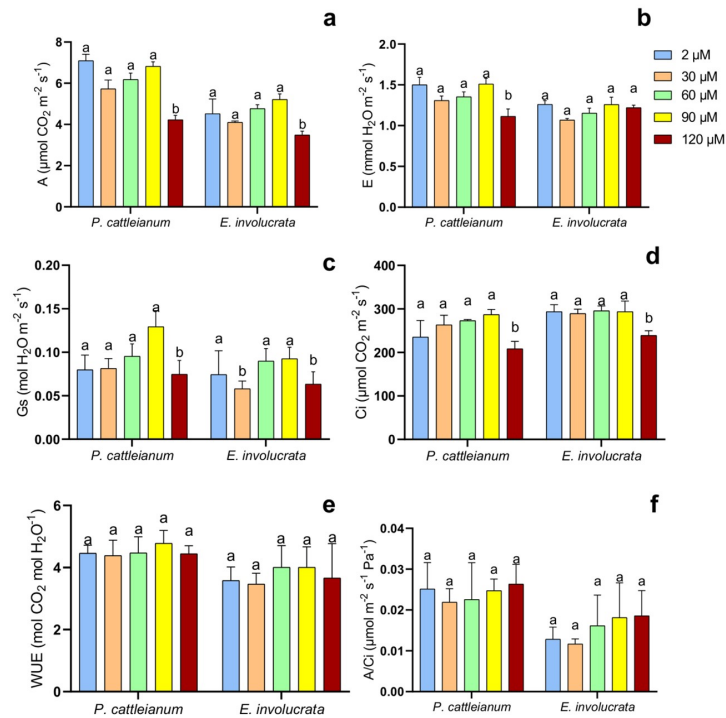


Figure 3. Mean values recorded for net CO₂ assimilation rate (A) (a), transpiration rate (b), stomatal conductance (Gs) (c), intercellular CO₂ concentration (Ci) (d), water use efficiency (WUE) (e) and instantaneous carboxylation efficiency (by Rubisco) (A/Ci) (f) in *Psidium cattleianum* (*P. cattleianum*) and *Eugenia involucrata* (*E. involucrata*) seedlings grown in different Mn concentrations. Different letters between treatments represent statistically significant differences in the Scott-Knott test. Bars represent the mean ± standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

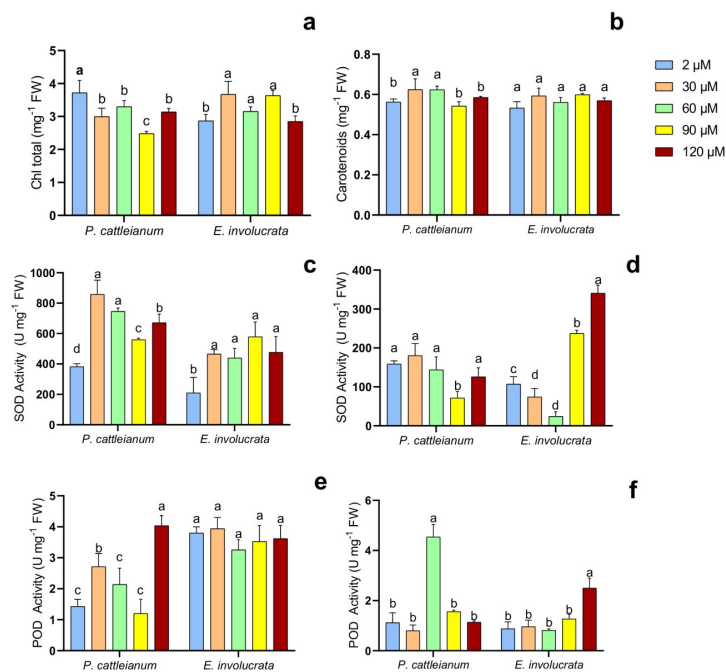


Figure 4. Mean values recorded for total chlorophyll (a), carotenoids (b), superoxide dismutase (SOD) activity in shoots (c) and roots (d) and guaiacol peroxidase (POD) activity in shoots (e) and roots (f) in *Psidium cattleianum* (*P. cattleianum*) and *Eugenia involucrata* (*E. involucrata*) seedlings grown in different Mn concentrations. Different letters between treatments represent statistically significant difference by the Scott-Knott test. Bars represent the mean ± standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

The highest SOD activity in the shoots of *E. involucrata* and the highest POD activity in the leaves of *P. cattleianum* and in the roots of *E. involucrata* with the addition of Mn suggest greater tolerance of these species to Mn (Figure 4). This is because plants exposed to metal toxicity increase antioxidant enzyme activity to counteract increased production and accumulation of ROS resulting from the displacement of metal ions in active enzyme centers (e.g. redox reactions in electron transport chains) or binding to functional groups (Faria et al., 2020).

However, we found an increase in POD activity in roots and SOD in shoots of *P. cattleianum*, followed by a decrease in both enzymes for this species (Figure 4f). This is most likely because high Mn concentrations can cause $O_2^{\cdot-}$ production and possibly overwhelm the scavenging capacity of antioxidant enzymes and break down physical barriers, causing oxidative damage (Pan et al., 2019). This response may also have occurred because mild and moderate Mn stress triggers the plant antioxidant system to eliminate ROS (Li et al., 2019). However, under high Mn concentrations, plant ROS production exceeds the ability to scavenge antioxidant enzymes, which can cause long-term damage to the functional membranes of plant cells and enzyme systems (Xiao et al., 2020).

The increase in H_2O_2 content was observed only in the roots of *P. cattleianum* seedlings in a Mn concentration of 90 μM (Figure 5a). However, this did not negatively affect membrane lipids and, consequently, allowed seedling growth (Figure 5b-5c). H_2O_2 plays a dual role in vascular plants, inducing oxidative damage or acting as a signaling molecule in various physiological processes, including senescence, photorespiration, photosynthesis, growth and development (Pontigo et al., 2017). However, excessive accumulation of H_2O_2 can be an extremely harmful factor, causing increased levels of MDA (lipid peroxidation) and increased permeability of the plasma membrane (Kuinchtner et al., 2021).

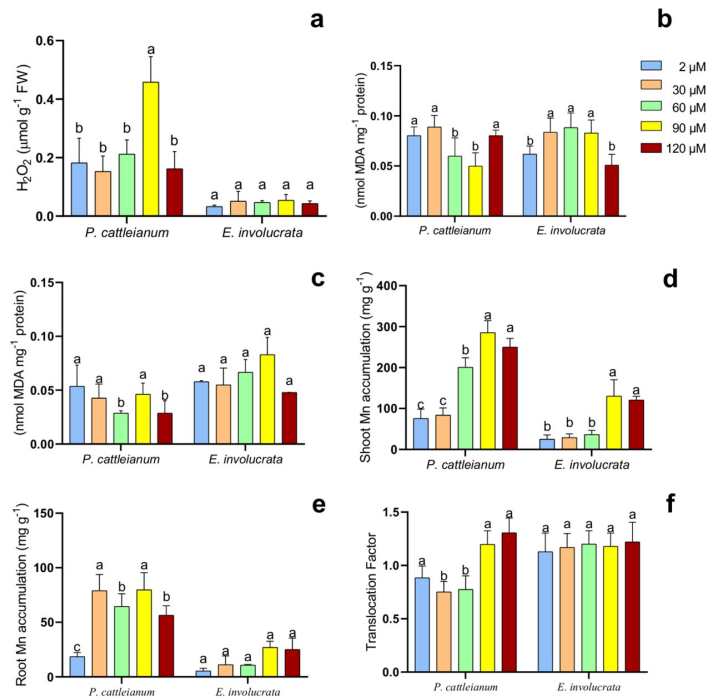


Figure 5. Mean values recorded for hydrogen peroxide (H_2O_2) concentration in roots (a), and membrane lipid peroxidation in shoots (b) and roots (c) Mn accumulation in shoots (c) and roots (d) and Mn translocation factor (e) in *Psidium cattleianum* (*P. cattleianum*) and *Eugenia involucrata* (*E. involucrata*) seedlings grown in different Mn concentrations. Different letters between treatments represent statistically significant difference in the Scott-Knott test. Bars represent the mean \pm standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

The lowest MDA values were observed in *P. cattleianum* with Mn addition in the nutrient solution (Figure 5b-5c). This was possibly because the rate of ROS elimination was faster than its accumulation in plants exposed to Mn, preventing membrane lipid peroxidation, in addition

to preventing cell membrane damage and osmotic pressure imbalance. Thus, antioxidant enzyme activity was consistent with changing MDA levels, suggesting that SOD and POD played an important regulatory role at low Mn concentrations (Huang et al., 2019).

Furthermore, it was observed that the translocation factor of *P. cattleianum* and *E. involucrata* under exposure to Mn was greater than 1.0 (Figure 5e). This response indicates a high capacity of these species to translocate Mn from the roots to the shoots. The translocation factor is a criterion used to estimate the phyto-remediation potential of plant species, as it calculates the ability of plants to transfer metals from the root to the shoot (De Conti et al., 2021). It is given by the ratio of metal concentration in shoots and roots. One of the criteria used to identify metal hyperaccumulating species is to have coefficients greater than 1 (Raj et al., 2020). Plants with a translocation factor > 1 will transfer heavy metals to the shoots, while those with a translocation factor < 1 have a lower capacity to transfer heavy metals from the roots to the shoots (Mahdavian, 2021). Root to shoot translocation can be used as a plant defense mechanism to Mn toxicity, directing excess Mn to shoot detoxification sites, such as the vacuole or shoot cell walls (Faria et al., 2020).

Thus, greater Mn accumulation in the shoots may have contributed to reducing adverse effects on seedling growth in this study (Figure 5d). This is possibly because excess Mn may have been stored in vacuoles, cell walls or distributed to different leaf tissues (Li et al., 2019). By accumulating most Mn in the leaves, the cell wall may have decreased the amount of Mn entering chloroplasts, cell nuclei, and mitochondria, helping to reduce toxicity and preserve normal metabolic activities in the cells (Pan et al., 2019). Furthermore, Mn may have been chelated and compartmentalized in the endoplasmic reticulum or in the Golgi apparatus and also complexed by organic acids in leaves, playing crucial roles in Mn tolerance (Shao et al., 2017). Thus, the high accumulation of Mn in the shoots of *P. cattleianum* and *E. involucrata* indicates that these species can be used in phyto-extraction of Mn in soils contaminated with this metal.

4. CONCLUSION

Excess manganese promoted the activation of the enzymatic antioxidant system in *Psidium cattleianum* and *Eugenia involucrata*, reducing its negative effects on plant biomass production. Furthermore, high Mn concentrations in the shoots of *P. cattleianum* and *E. involucrata* indicated that these species are tolerant to Mn and can be used in the phytoextraction of Mn in contaminated soils.

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