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Phytoremediation potential in soils contaminated with zinc

Potencial de fitorremediação em solos contaminados com zinco

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ABSTRACT

The accumulation of heavy metals in soils and waters represents a risk for both the environmental and human health. In this context, phytoremediation has become an efficient, accessible technological solution to extract or remove inactive metals and metal pollutants from contaminated soils. Our objective is to assess the behavior of different native plants of the Cerrado of Maranhão, ipê roxo (*Tabebuia impetiginosa*) and aroeira (*Myracrodrun urundeuva*), in the phytoremediation of soils contaminated with Zinc (Zn) (II) under controlled greenhouse conditions. Between the species investigated, *Tabebuia impetiginosa* presented a higher biomass production (aerial part/root) than *Myracrodrun urundeuva* for the period of 25 days. In turn, in the 45-day period, root biomass was higher for *Tabebuia impetiginosa* in doped soil, while lower biomass production was found in the aroeira control group. The highest Zn concentrations in the root appeared in the period of 25 days for doped soil for both species, with an absorption of 142.10 mg kg-1 for Tabebuia impetiginosa and 150.57 mg kg-1 for *Myracrodrun urundeuva*. In the aerial part, the aroeira species had higher Zn concentration in doped soil in the 45-day period, 398.97 mg kg-1. Regarding the TF and BF, the species presented higher efficiency in zinc phytoextraction. Both species were able to absorb and tolerate the metals in the soil induced to stress. Finally, we conclude that *Tabebuia impetiginosa* and *Myracrodrun urundeuva* can be applied in phytostabilization and phytoextraction processes, with hyperaccumulating potential for the ions of Zn.

Keywords: Phytoextraction; Potentially toxic metals; *Myracrodrun urundeuva*; *Tabebuia impetiginosa*.

RESUMO

O acúmulo de metais pesados nos solos e nas águas representa um risco tanto para o meio ambiente quanto para a saúde humana. Neste contexto, a fitorremediação tornou-se uma solução tecnológica eficiente e acessível para extrair ou remover metais inativos e poluentes metálicos de solos contaminados. Objetivou-se avaliar o comportamento de diferentes plantas nativas do Cerrado maranhense, ipê roxo (*Tabebuia impetiginosa*) e aroeira (*Myracrodrun urundeuva*), na fitorremediação de solos contaminados com Zinco (Zn) (II) sob condições controladas de casa de vegetação. Entre as espécies investigadas, *Tabebuia impetiginosa* apresentou maior produção de biomassa (parte aérea/raiz) que *Myracrodrun urundeuva* no período de 25 dias. Por sua vez, no período de 45 dias, a biomassa radicular foi maior para Tabebuia impetiginosa em solo dopado, enquanto menor produção de biomassa foi encontrada no grupo controle aroeira. As maiores concentrações de Zn na raiz apareceram no período de 25 dias para solo dopado para ambas as espécies, com absorção de 142,10 mg kg-1 para *Tabebuia impetiginosa* e 150,57 mg kg-1 para *Myracrodrun urundeuva*. Na parte aérea, a espécie aroeira apresentou maior concentração de Zn em solo dopado no período de 45 dias, 398,97 mg kg-1. Em relação ao TF e BF, as espécies apresentaram maior eficiência na fitoextração de zinco. Ambas as espécies foram capazes de absorver e tolerar os metais do solo induzidos ao estresse. Por fim, concluiu-se que *Tabebuia impetiginosa* e *Myracrodrun urundeuva* podem ser aplicadas em processos de fitoestabilização e fitoextração, com potencial hiperacumulador para os íons de Zn.

Palavras-chave: Fitoextração; Metais potencialmente tóxicos; *Myracrodrun urundeuva*; *Tabebuia impetiginosa*.

INTRODUÇÃO

After iron, Zinc is the second most abundant element in biological systems among metals of transitions (Marschner, 2011). It has an important role in the development, reproduction, and plant signaling processes due to its catalytic activation structure (Lehmann *et al*., 2014). Zinc also functions as a cofactor for many enzymes in carbonic anhydrase, carboxypeptidase, and Zn superoxide dismutase (Zhang *et al*., 2014; Castillo-González *et al*., 2018), therefore crucial for the activity, regulation, and stabilization of protein structure.

Nonetheless, at concentrations higher than 300 mg kg-1 in the soil (Viçosa, 2017), Zn becomes a contaminant regarded as very reactive and potentially absorbed by plants. Its high concentrations in the soil, especially resulting from human activities, have toxic potential to cause morphological, biochemical, and physiological disturbances, in addition to decreasing the production of dry matter in both the aerial and root parts, root necrosis biomass when in touch with the soil, seedling death, and inhibition of plant growth (Balafrej *et al*., 2020).

However, some plant species have the capacity to bioaccumulate high quantities of zinc, including *Myracroduon urundeuva* (Freitas *et al.*, 2019), *Jatropha curcas* L (Garófalo Chaves; Sousa, 2015), and *Brassica juncea* (Islan *et al*., 2020), which characterizes them as zinc phytoremediator. Phytoremediation is defined as the use of green plants to remove, immobilize, contain or render harmless organic and inorganic contaminants present in soil or water (Laghlimi *et al*., 2015; Silva *et al*., 2019). Its advantages include the absence of damages to the plant specimens, low operational costs and investment, stabilization of the area for decontamination, and capacity to remedy different contaminants simultaneously (Amado; Chaves Filho, 2015).

According to Herzig *et al.* (2014), the main characteristics of an optimum plant phytoextractor must include tolerance to high concentrations of metals in the soil, fast growth, low demand of water, efficiency at removing pollutants, non-invasive nature, resistance to plagues and diseases, low use of inputs, and easy crop.

Tabebuia impetiginosa is a deciduous arboreal species popularly known as ipê-roxo and belongs to the family Bignoniaceae. It is native to the biomes of Atlantic Forest, Cerrado, and Caatinga in Brazil (Lorenzi, 1992) and commonly found in vegetation areas in the Brazilian Northeast and Southeast. Its wood has high economic value and good durability, especially for furniture production, and has been indicated as an alternative for the recovery of degraded areas and landscape (Silva *et al*., 2015).

Myracrodruon urundeuva Allemão, known as aroeira, aroeira-do-sertão or urundeúva, is a deciduous, halophytic, selective xerophytic species belonging to the family Anacardiaceae (Lorenzi, 1992). Its study demonstrated zinc accumulation in the roots to the detriment of the aerial part, indicating a great potential for use in processes of environmental recovery of areas contaminated with high Zn concentrations (Freitas *el al.*, 2019).

In this sense, our objective is to assess the behavior of *Tabebuia impetiginosa* and *Myracrodruon urundeuva Allemão* to investigate their phytoremediation potential in soils contaminated with Zn (II) under greenhouse conditions. The applicability of this study is to suggest a low-cost alternative with the use of a green technology that does not imply environmental impacts.

METHODS

We carried out the experiment in a greenhouse belonging to the State University of Região Tocantina do Maranhão between December 2018 and May 2019. We used plastic bags of three-liter capacity filled with 2kg of substrate collected from the soil surface layer (0 - 0.20 m in depth) at two sites in conserved Cerrado areas in the city of Imperatriz/MA (5º 25’ 34.2” S 47º 32’ 26.4” W).

All soil samples were air-dried and sieved (3nm) before determining their organic matter chemical attributes (EMBRAPA, 2010), pH_{H2O} and pH_{KCl} (Raij, 2011), and cation exchange capacity (CEC) (Cotta *et al*., 2006). The procedure of flame atomic absorption spectrophotometry (FAAS) VARIAN-model Espectra AA/55 (Table 1) was applied to determine the bioavailable concentration of Zn.

Table 1 - Average values of the soil chemical attributes in the preserved area of Imperatriz/MA point 1 (P1) and point 2 (P2)

	pHH2O	**pHKCl**	**ΔpH**	**CTC (cmolc dm^{-3})**	**M.O (%)**	**Zn (mg kg^{-1})**
P1	5.2	4.3	-1	0.21	6.08	7.69
P2	5.3	4.4	-0.8	1.02	8.45	7.76

Source: authors (2024).

The soil used presented characteristics of pH_{H2O} with average acidity (Cordeiro *et al*., 2018), the values of ΔpH suggest predominance of negative charge in the soil, that is, the soil retains more cations than anions. CEC values point to a soil with low cation exchange capacity (Prezotti; Guarçoni, 2013), predominance of inorganic matters and incorporated minerals, according to Alcântara *et al.* (2011). The contents of zinc available were above the standard for extensive production in the Cerrado (1.1 to 1.6 mg dm^{-3}) (Resende *et al.*, 2016). For the experiment, we contaminated the soil with 60 mg L^{-1} of Zn. Adsorption tests in the studied soil and respective pH conditions indicated an adsorption efficiency of 98%.

The experiment was installed based on a completely randomized design, including 12 treatments (6 controls, 3 for ipê-roxo, and 3 for aroeira) and three repetitions per treatment, 2x2 factorial, with two treatments (T1= non-doped soil; T2= doped soil) for the periods of 25 and 45 days, encompassing a total of 36 experimental units. We produced seedlings of the studied species from the seeds obtained with the Consórcio Estreito Energia (CESTE). The aroeira seedlings were subjected to break of dormancy according to methodology by Oliveira *et al.* (2013), which consists of a process of surface disinfection in the seeds using a sodium hypochlorite solution at 1% for three minutes. The seeds of ipê-roxo were immersed in water for 18 hours, washed in distilled water, and sterilized. Finally, we removed excess moisture in the seeds on filter paper.

Following the break of dormancy, the seeds were planted in greenhouse using commercial substrate for forest species inside 500ml disposable glasses. The seedlings were transferred to definitive vases when reaching approximately 20cm in height. During cultivation, the vases were irrigated with distilled water to not exceed the maximum limit of 70% of the soil field capacity. The vases were irrigated with 50 ml of nutrient solution composed of Na, Ca, k, and Mg at weekly intervals. After the periods of 25 and 45 days of cultivation, the species were harvest, washed, stored in paper bags and dried out in forced circulation air oven at a temperature between 65 and 70°C until reaching constant weight. Subsequently, we separated their aerial parts and root systems to determine biomass, the species were then ground in a mill and followed for analyses to establish their Zn contents (II) phytoextracted at the Laboratory of Environmental Chemistry of UEMASUL.

The sample was broken according to adaptations of the method described by Santos (1999) and Silva *et al*. (2000). We transferred the grinded material to a Becker with capacity of 80 mL and then added nitric acid and perchloric acid at a 3:1 proportion (v/v). The mixture was left to rest during the night and subsequently mineralized (160° ±5º C).

Following a brief cooling, 2 mL of H_2O_2 were added. Digestion was made until the content of the flask gained a light shade. The acid extracts were then cooled at room temperature and filtered in quantitative filter paper (Whatman 44). The filtered solution was collected using volumetric flasks of 50 mL, whose volume was completed with deionized water. During the extraction processes, we performed a blank control of the extracting solutions for each set of samples analyzed on the same day. The analyses were performed in triplicate.

We applied the procedure of Flame Atomic Absorption Spectrometry (FAAS) with background corrector with a deuterium lamp to determine the Mn (II) and Zn (II) metals in the samples of soil and arboreal species. Standard solutions used to calibrate the instrument were prepared based on the rates of a stock solution of 1000 mg L^{-1}.

Calculating the Bioaccumulation Factor (BF) and Translocation Factor (TF) is required to estimate the phytoextraction potential of a plant, which, in this study, was based on Novo *et al.* (2015) and Sun, Zhou, and Diao (2008) given the equations:

$$Bioacumulation\ \text{Factor}\ (BF) = \frac{Metal\ concentration\ in\ plant\ tissues}{Metal\ concentration\ in\ soil}$$

$$Translocation\ Factor\ (TF) = \frac{Metal\ concentration\ in\ aerial\ part}{Metal\ concentration\ in\ root}$$

We calculated the residues and applied the procedures of Levene's variance homogeneity tests and Kolmogorov-Smirnov normality test. Subsequently, we generated the variance analyses for independent samples (ANOVA) and compare the averages through t test at a probability of 5% for the occurrence of significant differences between the treatments. The statistical analyses were performed on IBM SPSS Statistic (ver. 22.0, IBM Corp., Armonk, New York, USA).

RESULTS AND DISCUSSION

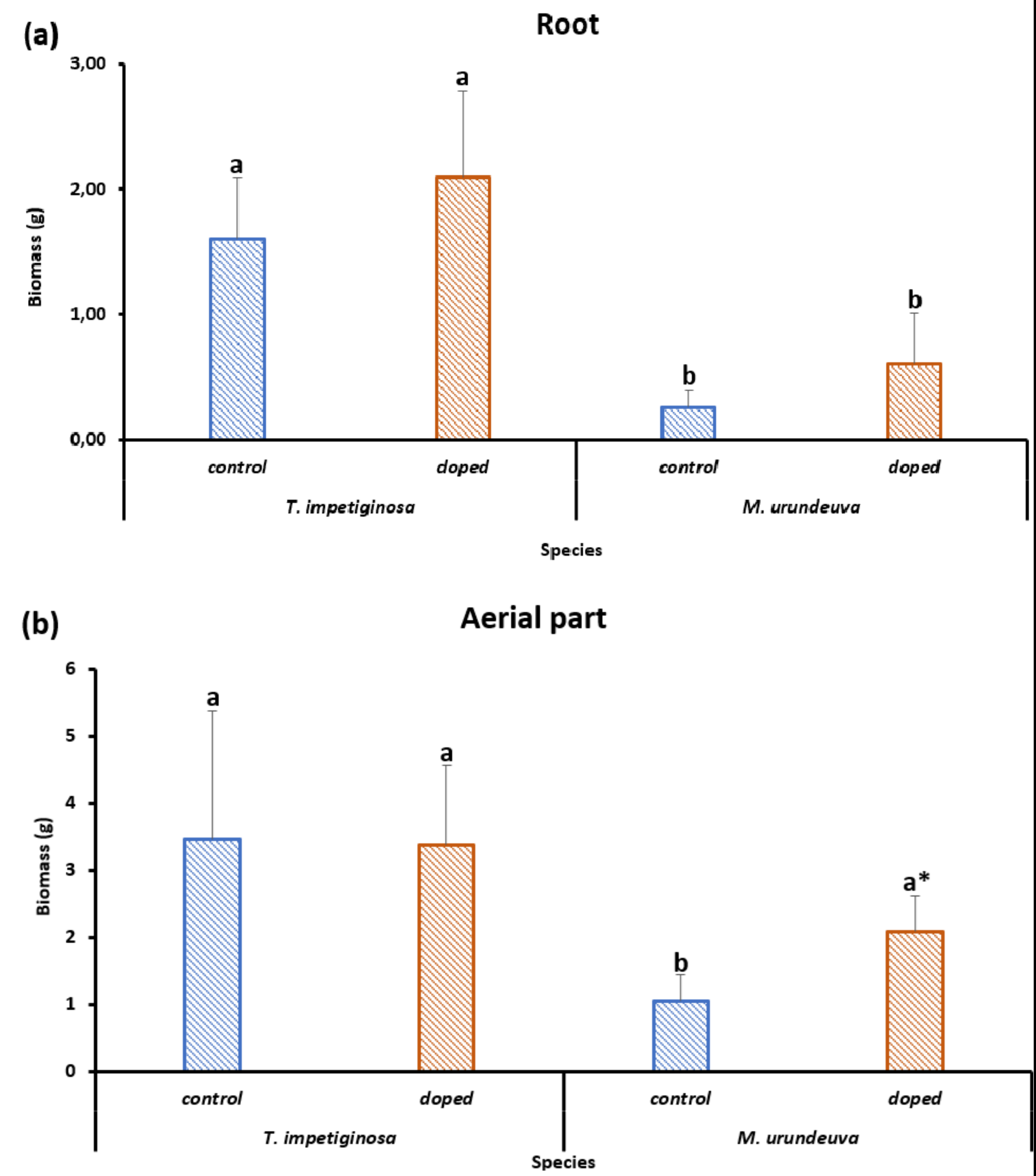
PRODUCTION OF BIOMASS IN THE ROOT AND AERIAL PART

For better comparing the development of the plant species in the soils, we analyzed the produced dry biomass (in grams) per sample. Biomass production is associated with the tolerance of a species to a contaminant and is obtained through different biochemical adaptations that allow the plant to tolerate high concentrations of these elements (Taiz; Zeiger, 2013).

Regarding the production of dry mass, *Tabebuia impetiginosa* had a higher value in the root and aerial part in the 25-day period in relation to *Myracrodroun urundeuva*. However, no statistical difference in the biomass production of root and aerial part of *T. impetiginosa* was indicated in the applied treatment, with a dry mass production in the root in control soil of 1.60g and 2.09g in doped soil, while the production in the aerial part reached 3.45g and 3.37 g in in control and doped soils, respectively (Figure 1.B).

Regarding the dry mass production in the aerial part, the treatments of *M. urundeuva* presented significant statistical difference ($p < 0.05$) with higher biomass in doped soil. Dry mass production in the aerial part is a favorable characteristic for the use of these species in zinc phytoremediation. Freitas *et al.* (2019) studied the behavior of *M. urundeuva* in soils contaminated with Zinc and found the same tendency of biomass increase in the aerial parts in soils with higher Zn concentrations. Nonetheless, control soil did not show significant difference in biomass production between the aerial part and root, 1.05g and 0.25g, respectively, which was the opposite in doped soils, indicating significant difference ($p<0.05$) in biomass production in the root, 0.61g, and aerial part, 2.07 g.

Figure 1 - Values of biomass weights in different parts of *Tabebuia impetiginosa* and *Myracrodroun urundeuva* in the period of 25 days. **1.a)** Root biomass weight in the control group and doped group (g). **1.b)** Aerial part biomass weight in the control group and doped group (g).



The values are the averages ± of the standard deviation. Different letters indicate significant differences among all treatments for both species. Asterisk indicates significant differences between the control and doped groups for the same species, $p<0.05$.

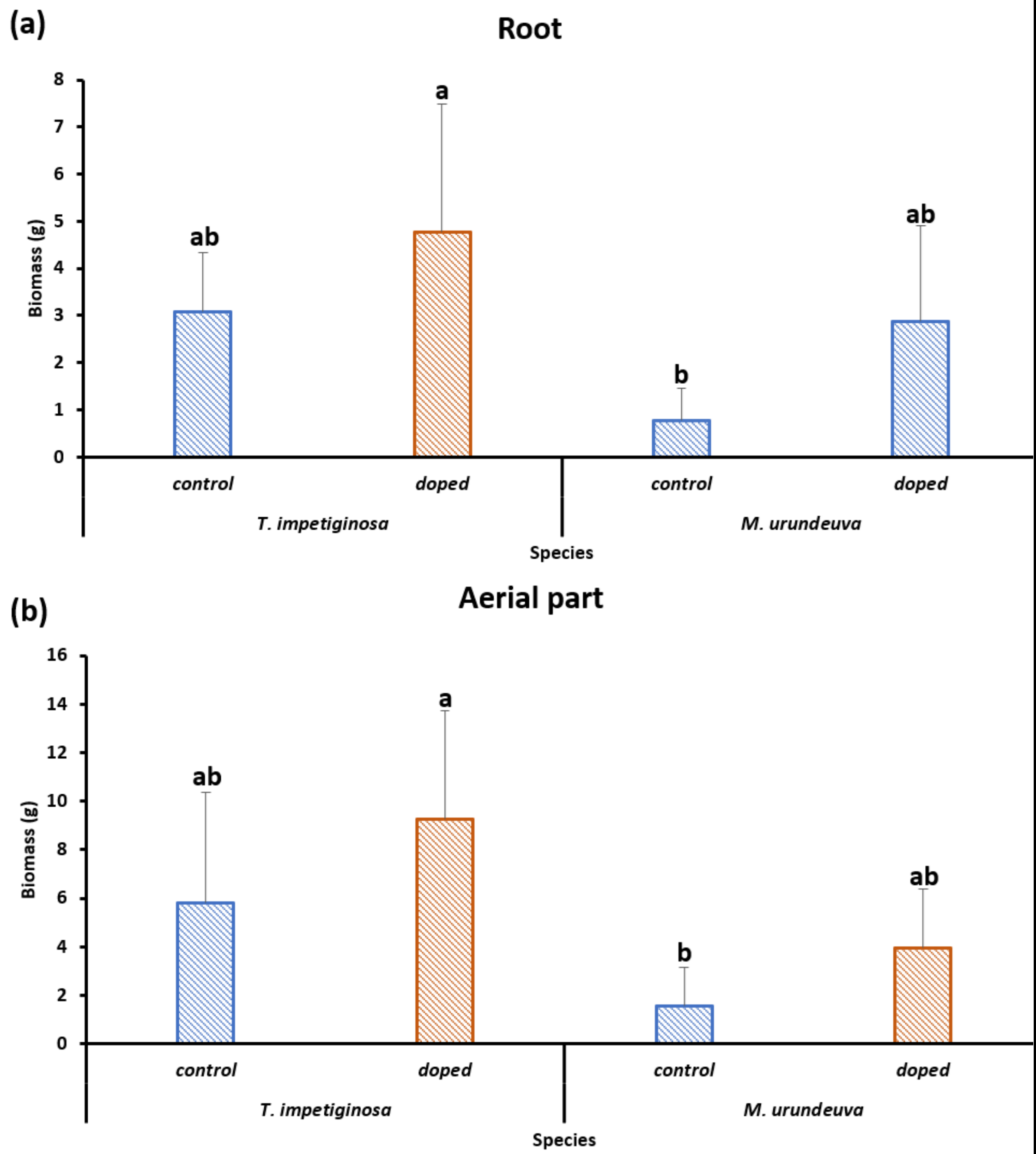
Source: authors (2024).

The assessments referent to the 45-day crop period revealed a higher root biomass for *T. impetiginosa* (4.75 g) in doped soil, and lower root biomass production for the control group (1.55 g) (Figure 2).

T. impetiginosa stands out with the highest total biomass production, while *M. urundeuva* showed a sharp reduction in biomass production in both the roots and aerial part. It is worth mentioning that the latter has slow to moderate growth pattern and poorly developed secondary roots at the seedling phase (Carvalho, 2003).

In the aerial part (Figure 2.b), doped soils presented values of biomass productions of 9.27g and 3.93g for *T. impetiginosa* and *M. urundeuva*, respectively. *T. impetiginosa* had the highest biomass weights in doped soil. In the 45-day period, biomass production showed no significant differences ($p < 0.05$) between root and aerial part for either of two soils.

Figure 2 - Values of biomass weights in different parts of *Tabebuia impetiginosa* and *Miracrodrun urundeuva* in the period of 45 days. **2.a)** Root biomass weight in the control group and doped group. **2.b)** Aerial part biomass weight in the control group and doped group.



The values are the averages ± of standard deviation. Different letters indicate significant differences among all treatments for both species. Asterisk indicates significant differences between the control and doped groups for the same species, $p<0.05$.

Source: authors (2024).

The literature features some studies demonstrating different behaviors of plant species in contaminated soils regarding their biomass production. Garófalo Chaves and Sousa (2015) found that higher doses of heavy metals applied to the soil decrease the production of dry matter in sunflower, castor bean plant, and pepper. Considering the effects of soil contamination with Zinc, Andrade *et al*. (2009) described that the growth of the plants was severely reduced with high Zn concentrations in the soils. The presence of Zn at high concentrations interferes with several metabolic processes and induces oxidative stress, possibly damaging the structure and function of proteins due to its strong affinity to bind with sulfhydryl groups, processes that influence general cell metabolism (White; Pongrac, 2012).

CONCENTRATION OF ZINC IN THE ROOT AND AERIAL PART

For being a structure component that activates several proteins, Zinc is essential for plant development (Li *et al.*, 2011), however, at high levels it can become harmful to plants by interfering in different metabolic processes and inducing oxidative stress that can damage the structure and function of proteins due to its strong affinity to bind with sulfhydryl groups, a process that affect cell metabolism (White; Pongrac, 2012).

Table 2 - Concentration of Zn in different parts of *Tabebuia impetiginosa* and M*yracrodruon. urundeuva* in the periods of 25 and 45 days in the control and doped groups (mg kg^{-1}).

Root	*Tabebuia impetiginosa*		*Myracrodroun urundeuva*	
	25 days	**45 days**	**25 days**	**45 days**
Control	40.97	30.04	82.99	23.79
Doped	233.01	8.54	150.57	49.01
Aerial part	*Tabebuia impetiginosa*		*Myracrodroun urundeuva*	
	25 days	**45 days**	**25 days**	**45 days**
Control	64.6817	62.05	193.4583	37.23
Doped	229.59	145.13	27.4583	398.8717

Source: authors (2024).

The quantification of Zn content absorbed by both species suggest significant accumulations of Zn ($p < 0.05$) in their roots in doped soil during the period of 25 days (Figure 3a). Zn concentration in the root of *T. impetiginosa* was significantly higher than in *M. urundeuva* in doped soil. In control soil, M. *urundeuva* accumulated 82.99 mg kg^{-1} and *T. impetiginosa*, 40.97 mg kg^{-1}, while in doped soil, we found 150.57 mg kg^{-1} and 233.01 mg kg^{-1}, respectively (Table 2). For the 45-day period, we observed that Zn concentration in the root of both species also presented significant variation ($p<0.05$) between the treatments; however, *Tabebuia impertignosa* had a lower concentration in doped soil.

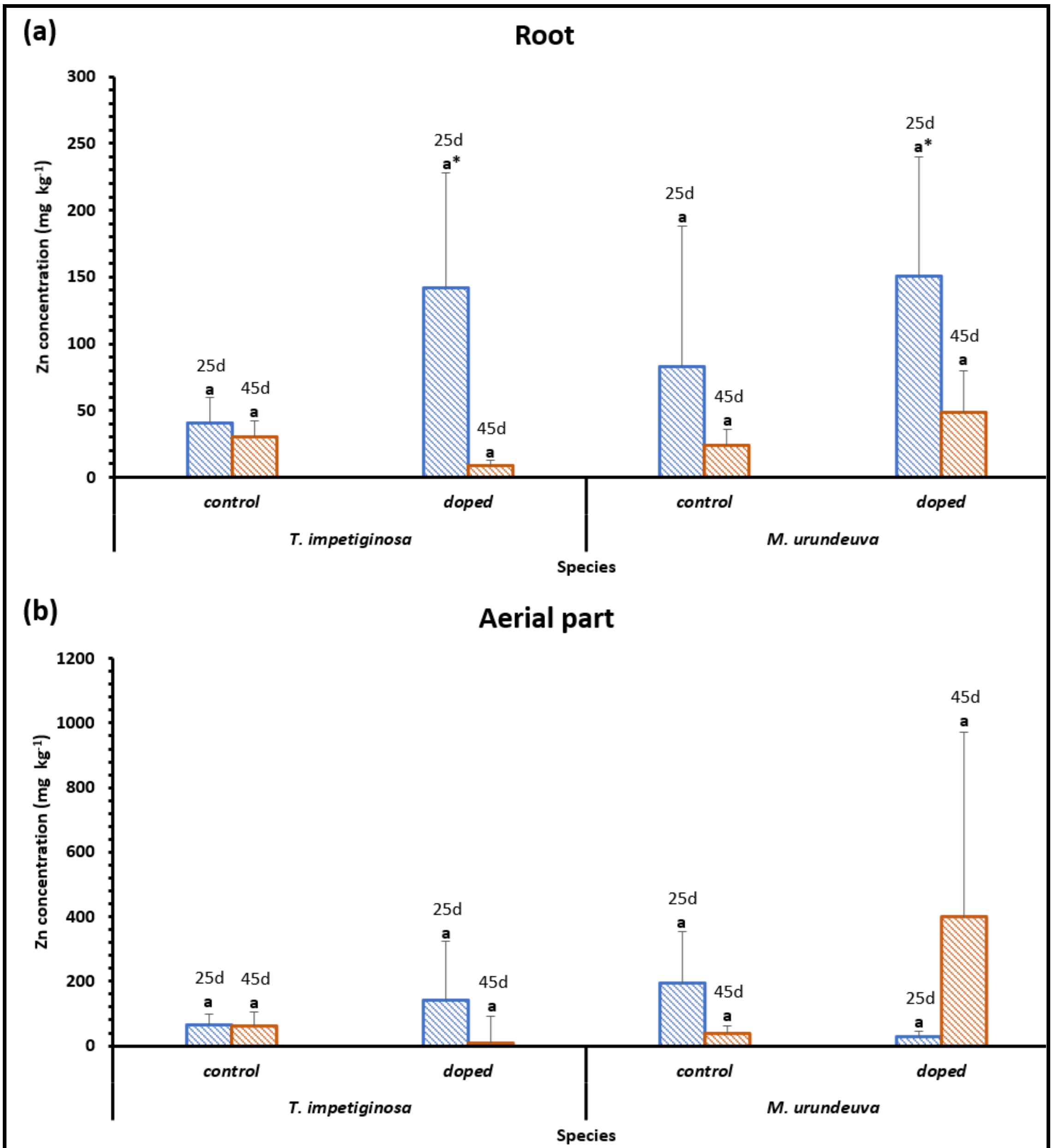
Regarding the aerial part (Figure 3b), the highest Zn concentrations in control soil and doped soil occurred for the 25-day period, with 64.68 mg kg^{-1} and 229.59 mg kg^{-1} for

T. impertignosa. *M. urundeuva*, in turn, did not show such behavior in the same period, with control soil presenting higher concentration (193.45 mg kg^{-1}) than doped soil, while in the 45-day period, doped soil had the highest concentration (398.87 mg kg^{-1}). Zinc concentrations in the aerial part were not significant ($p<0.05$) for any of the plant species when comparing control and doped soils.

The assessment of the 25-day period indicated that *T. impetiginosa* was able to accumulate the highest zinc contents both in the root and aerial part in relation to the 45-day period, while the control showed a weaker reduction.

M. urundeuva in control soil had higher accumulation in the aerial part in the 25-day and 45-day collections in relation to the root. In doped soil, the 25-day sampling presented a higher accumulation in the root, while the 45-day period showed higher accumulation in the aerial part. Regarding the doping of the soil, we assume that the different parts of the plants had different responses according to the cultivation period.

Figure 3 - Zn concentration in different parts of *Tabebuia impetiginosa* and M*yracrodruon urundeuva* in the periods of 25 and 45 days in the control and doped groups.
2.a) Zn concentration in the root (mg kg^{-1}). **2.b)** Zn concentration in the aerial part (mg.kg^{-1}).



Error bars indicate the standard deviation, all values are averages ± standard deviation. Different letters indicate significant differences among the treatments for the same crop at p<0.05. Asterisk indicates significant differences among the crops for the same treatment in p<0.05. All values are calculated from six repetitions.

Source: authors (2024).

Kabata-Pendias and Pendias (1992) state that plants generally contain much more zinc in the roots than in the aerial part, which, in large quantities, is transferred from the roots and accumulated in the aerial part. We calculated the quantity of zinc accumulated in *T. impetiginosa* and *M. urundeuva* by the sum of the Zn quantities accumulated in each part of the plant. By comparing the two species, we found higher Zn accumulation in *M. urundeuva* in control soil, while doped soil had higher accumulation for *T. impetiginosa*. For the 45-day collection, the highest accumulation values appeared for ipê roxo in control soil and aroeira in doped soil.

The values observed in this experiment indicate that the two species tended to suffer with Zn toxicity. White and Pongrac (2012) demonstrated that critical Zn concentrations in leaves can cause toxicity between 100 and 700 $mg.kg^{-1}$, depending on the species and ionic composition of soil solution. It is worth highlighting that we did not find any visual symptoms of phytotoxicity during the entire experimental period for either species. Mattiazzo-Prezotto (1994) reported that concentrations of 400 $mg.kg^{-1}$ of Zn influence the development of *M. urundeuva* and cause phytotoxic effects, reflecting in foliar chlorosis and lower development.

BIOCONCETRATION FACTOR AND TRANSLOCATION FACTOR

Bioconcetration Factor (BF) indicates the relation between the metal concentration in plants tissues and in the soil, while the capacity of metal transference from the roots to the aerial part is given by Translocation Factor (TF). BF values above 1 and FT values below 1 indicate that the species is more susceptible to phytostabilization, while BFs and TFs higher than 1 point to a good efficiency at the translocation from the roots to the aerial part, suggesting that the species is a promising candidate to phytoextraction.

Table 3 - Translocation Factor and Bioconcetration Factor of Zinc (Zn) for *Tabebuia impetiginosa* and *Myracrodruon urundeuva* in the control group and doped group in the periods of 25 and 45 days.

Plants	**Zn (mg.kg-1)**	**Translocation factor**		**Bioconcentration factor**	
		25 days	45 days	25 days	45 days
Tabebuia impetiginosa	Control group	2.16±1.66[a]	2.29±1.96[b]	7.23±2.52[b]	5.64±2.81[a]
	Doped group	0.89±0.78[a]	20.76±7.58[a*]	3.39±1.66[b]	1.94±1.04[a]
Myracrodruon urundeuva	Control group	14.69±18.60[a]	1.15±0.92[b]	22.67±17.15[a]	4.19±1.59[a]
	Doped group	0.20±0.10[a]	2.56±1.78[b]	0.84±0.36[b]	5.80±9.44[a]

Values indicate the averages ± standard deviation. Different letters indicate significant differences among all treatments for the same crop in both species at p<0.05. Asterisk indicates significant differences among the crops for the same treatment at p<0.05. All values are calculated from six repetitions.

Source: authors (2024).

In general, both species presented FT >1 in the two periods for both control and doped soils (Table 3). However, doped soil in the crop period of 25 days reached a Translocation Factor value below 1. Translocation Factor in the doped group of ipê-roxo showed significant difference ($p < 0.05$) for the 45-day period (Table 3), which is confirmed by the high Zn concentrations in the aerial parts of the species in the period.

T. impetiginosa reached a Bioconcetration Factor for Zn of BF>1 in all soils and crop periods. The analyses the two factors allow to conclude that the ipê-roxo species behaved differently as phytostabilizator and phytoextractor, presenting a TF above and below 1 and a BF above 1, depending on the crop period.

M. urundeuva had higher BF values than *Tabebuia*. Generally, plants with high bioconcentration values are the best choices for phytoextraction (Ghosh; Singh, 2005). Nevertheless, the doped group of aroeira in the 25-day period presented FBC<1. A lower BF value shows that the plant aerial part excludes metallic ions and enhances the phytostabilization potential in soils contaminated with metals (Zhao *et al.*, 2016).

Considering the two factors, in control soil in the two crop periods, *M. urundeuva* demonstrated good efficiency for phytoextraction, while in doped soil for the 25-day crop period, its factors reached values below 1, thus showing a phytostabilizator behavior. In the period of 45 days, the species behaved as phytoextractor.

CONCLUSION

Both species were able to absorb and tolerate the metals in the soil induced to stress over a period of up to 45 days. The two studied species demonstrated good tolerance to zinc in the soils (control/doped) used in the experiment. We found high concentrations in the aerial part of seedlings, while the analyses of Zn suggest that the processes of absorption and translocation of metals from the roots to the aerial part is sufficient to state that the studied plants absorb high metal concentrations, indicating their classification as hyperaccumulator with phytoremediation potential for areas contaminated with Zn.

In general, the results of TF and BF indicate that *Tabebuia impetiginosa* and *Myracrodruon urundeuva* proved efficient at Zn phytoextraction. *Myracrodruon urundeuva* had the most satisfactory phytoextractor behavior. Finally, we conclude that ipê-roxo and aroeira can be applied in phytostabilization and phytoextraction processes for showing hyperaccumulator potential for the ions of Zn.

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