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Silicon application and mycorrhiza inoculation promoted *Leucaena leucocephala* growth in a soil highly contaminated by manganese

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ABSTRACT: Arbuscular mycorrhizal fungi (AMF) can increase the acquisition of silicon (Si) and, therefore, alleviate the problems caused by metallic toxicity in plants, but this effect remains poorly understood. The objective was to evaluate the influence of Si application on the growth of *Leucaena leucocephala* inoculated with AMF (*Claroideoglomus etunicatum*) in a soil contaminated by manganese (Mn). We exposed plants to increasing levels of Si (0, 100, 200 and 400 mg kg^{-1}) in the soil for 90 days. Intermediate levels of Si and AMF inoculation significantly increased shoot and root dry mass, the number of sheets, root system length and mycorrhizal colonization. The abundance of AMF spores decreased linearly with increasing levels of Si applied to the soil, suggesting a low correlation with mycorrhizal colonization. In addition to the higher Mn contents in the shoots and, mainly, in the roots, the combined application of Si and inoculation with AMF significantly reduced foliar toxicity by more than 40%, when compared to the absence of Si and AMF inoculation. Our results demonstrated a synergistic effect of AMF and Si in improving the growth and tolerance of *L. leucocephala* plants in soil contaminated by Mn.
Keywords: heavy metals; stress; remediation; AMF; Mn toxicity.

Aplicação de silício e inoculação de micorriza promove crescimento de *Leucaena leucocephala* em solo altamente contaminado por manganês

RESUMO: Os fungos micorrízicos arbusculares (FMA) podem aumentar a aquisição de silício (Si) e, portanto, amenizar os problemas causados pela toxidez metálica nas plantas, mas esse efeito ainda é pouco conhecido. O objetivo foi avaliar a influência da aplicação de Si no crescimento de *Leucaena leucocephala* inoculada com FMA (*Claroideoglomus etunicatum*) em solo contaminado por manganês (Mn). Expusemos as plantas a níveis crescentes de Si (0, 100, 200 e 400 mg kg^{-1}) no solo por 90 dias. Níveis intermediários de Si e inoculação de FMA aumentaram significativamente a massa seca da parte aérea e radicular, o número de folhas, o comprimento do sistema radicular e a colonização micorrízica. A abundância de esporos de FMA diminuiu linearmente com o aumento dos níveis de Si aplicados ao solo, sugerindo uma baixa correlação com a colonização micorrízica. Além dos maiores teores de Mn na parte aérea e, principalmente, nas raízes, a aplicação combinada de Si e inoculação com FMA reduziu significativamente a toxidez foliar em mais de 40%, quando comparada à ausência de Si e inoculação de FMA. Nossos resultados demonstraram um efeito sinérgico de FMA e Si na melhoria do crescimento e tolerância de plantas de *L. leucocephala* em solo contaminado por Mn.
Palavras-chave: metais pesados; estresse; remediação; FMA; toxidez por Mn.

1. INTRODUCTION

Mining is one of the oldest productive activities of humanity. Brazil is one of the largest producers and exporters of mining products, with an enormous mineral heritage. According to the Brazilian Mining Institute/IBRAM (2020), mining activity represents 4% of the country's Gross Domestic Product, being one of the pillars of Brazil's economic support. However, despite its economic importance, income generation and employment, factors that drive the country's growth, mining can cause environmental damage and constitute an imminent risk to human life (HADDAWAY et al., 2019).

Manganese (Mn), despite being an essential micronutrient for all living organisms (TANG et al., 2021), presents a highly toxic reaction when present in high concentrations (LI et al., 2019). In plants, Mn toxicity can change metabolic processes and, consequently, limit their growth and development (LI et al., 2019). The most visible effects of plant exposure to high Mn concentrations in the soil are, in general, described by brown spots, chlorosis and leaf necrosis, which can limit the photosynthetic efficiency (FARIA et al., 2020; GARCIA et al., 2020). In humans, excessive Mn exposure can damage to the nervous system and intellectual deficit in children (BUDINGER et al., 2021).

In mining areas, Mn can reach high concentrations through the deposition of tailings residues, which can release large amounts of Mn into the soil (HUANG et al., 2014). These conditions, in addition to the low soil fertility commonly found in these areas, make the establishment of plant species an important challenge. In this sense, strategies that mitigate this impact and improve soil revegetation are extremely necessary.

A viable alternative for the revegetation contaminated soil is to stimulate the plant association with beneficial soil microorganisms, such as arbuscular mycorrhizal fungi (AMF) (GARCIA et al., 2020). The use of leguminous plants has been recommended in the literature, mainly due to the association with nitrogen-fixing bacteria and mycorrhiza, attributes that increases soil quality and the potential of plant survival (BALIEIRO et al., 2017).

AMF are able to associate symbiotically with more than 80% of terrestrial plants (YOU et al., 2021). This association can increase nutrient absorption, water, attenuate the negative effects of heavy metals (HM) and promote plant growth in environments under contamination (SPAGNOLETTI et al., 2017). It is known that AMF can protect plants against HM through several mechanisms, including the retention and immobilization of these elements in roots of colonized plants (SPAGNOLETTI et al., 2017), chelation through glomalin synthesis (VODNIK et al., 2008) and accumulation of metals on the surface of AMF-spores (GARCIA et al., 2020). In addition, they can increase the absorption of other elements present in the soil solution, such as silicon (Si), a beneficial micronutrient, which can also mitigate the stress caused by HM in plants (ZEHRA et al., 2020).

Mycorrhizal plants often show greater tolerance to excess Mn in the soil (GARCIA et al., 2020). In this sense, evidence suggests that one of the reasons for the attenuation of Mn toxicity in the presence of AMF is due to the increase in the absorption of Si, which in turn may be involved in mitigating the adverse effects of metals in plants metabolism, especially Mn (HORST; MARSCHNER, 1978). Although some studies report the effects of Si on the attenuation of toxicity by HM in plants, to date, there are no publications on the role of Si, as well as a possible synergistic effect between Si and AMF in mitigating Mn toxicity in *L. leucocephala*.

In our study, we tested the effect of Si doses and AMF inoculation on the initial development of *L. leucocephala* cultivated in a soil degraded by Mn mining. Our hypothesis was that the interaction between Si and mycorrhiza inoculation attenuates phytotoxicity by Mn and promotes better plant growth conditions, thereby increasing its efficiency in the revegetation process.

2. MATERIAL AND METHODS

2.1. Localization area and experimental soil

The experiment was conducted in a greenhouse, located at the Fortaleza, Ceará, Brazil. For plants growth, we collected soil at a depth of 0-20cm in a manganese mining area located in Ocara, Ceará, Brazil. Subsequently, the soil was sieved with the aid of a 2mm sieve and then autoclaved at 121ºC and 1 atm of pressure for 2h, to eliminate fungal propagules and other existing microorganisms. Soil chemical characterization analysis (Table 1) was made according to the methods described by Teixeira et al. (2017).

Briefly, the pH was measured in water (1:2.5) by potentiometry; exchangeable aluminum (Al^{3+}), was extracted with 1M KCl solution and determined by titration; Ca^{2+} and Mg^{2+} were extracted with 1M KCl solution and determined by atomic absorption spectrometry; Potential acidity (H+Al), was extracted with calcium acetate buffered (pH 7.0) and determined by titration; Phosphorus (P), sodium (Na^+) and potassium (K^+) were extracted with Mehlich 1 solution, with P determined by colorimetry and K^+ and Na^+ by flame photometry; N was determined by the semimicro Kjeldahl method; Mn, Fe, Cu, Zn, were extracted with Mehlich's solution 1 and determined by atomic absorption spectrometry. The Si was extracted with 0.2 mol L^{-1} ammonium acid oxalate solution (pH 3.0) and determined by ICP-OES.

Table 1. Soil chemical characterization collected at the manganese mining area, Ocara, CE.
Tabela 1. Caracterização química do solo coletado na área de mineração de manganês, Ocara, CE.

pH	(Water)	6.7
Al^{3+}	$(cmol_c\ kg^{-1})$	0.1
Ca^{2+}		5.7
Mg^{2+}		2.08
Na^+		0.04
K^+		0.35
H+Al		0.9
P	$(mg\ kg^{-1})$	6.8
N	$(g\ kg^{-1})$	1.25
Si		0.00
Mn	$(mg\ kg^{-1})$	332
Fe		5.15
Cu		1.82
Zn		5.23

2.2. Cultivated plant and AMF inoculum

The plant species chosen in our study was *Leucaena leucocephala*. We chose this species because it is to be of multiple use and because it has potential for use in revegetation practices in soils contaminated by HM (JUSON et al., 2016). In addition, this species is able to grow well in soils with low fertility, probably due to its ability to fix atmospheric nitrogen and to associate with AMF, thus making it a promising plant species. The AMF used was the species *Claroideoglomus etunicatum*. To obtain the inoculum soil, we used corn plants (*Zea mays* L.) as trap crop, cultivated in sterile sand. We chose this AMF species for its good performance in increasing plant growth in soil contaminated by Mn (GARCIA et al., 2020). A volume of 40g inoculum, containing spores, fragments of colonized roots, mycelium and sand, were used as AMF inoculum soil. We performed a preliminary count of spores in the inoculum soil, which showed ~300 viable spores in 40g soil.

2.3. Experimental setup and plant growth conditions

The experiment consisted of a completely randomized design, in a 4x2 factorial scheme, with four replications. Thus, our study consisted of four doses of Si (0; 100; 200; 400 mg kg^{-1}) and two mycorrhizal treatments (inoculated with *Claroideoglomus etunicatum* and control–non-inoculated). The parameters mycorrhizal colonization and abundance of AMF spores in the soil were analyzed without the presence of the uninoculated treatment (control), since such plants did not show evidence of colonization by AMF, as well as no spores were observed in their rhizospheres, confirming the effectiveness of the adopted sterilization process.

The soil was distributed in plastic pots with a capacity of 1.5 liters, using 1 kg of soil per pot. Soil fertility correction was performed by applying 10 mg kg^{-1} of N, 15 mg kg^{-1} of P, 100 mg kg^{-1} of K and 3 mg kg^{-1}, mixed with the substrate. The sources of nutrients used were $CO(NH_2)_2$, P_2O_5, KCl and $CaSO_4$, respectively for nitrogen, phosphorus, potassium and calcium. One day after correction, Si doses (0, 100, 200, and 400 mg kg^{-1}) were applied in the pots containing the soil, in the form of sodium silicate (Na_2SiO_3). The doses applied were chosen following the methodology proposed by Garg and Singh (2018).

Leucena seeds were superficially disinfected in 95% alcohol. Subsequently, they were heated to a temperature of 80ºC for 5 minutes, in order to break dormancy. The production of seedlings to be used in our study took place in plastic trays, by placing one seed per cell at a depth of 2 cm. The substrate used to produce the seedlings was sterilized sand.

At 14 days after sowing (DAS), the plants were transplanted into the pots. Inoculation with AMF occurred during the transplant process. Soil moisture in the experimental trial was maintained at approximately 60% of the soil's water holding capacity and plants were allowed to grow for 90 days after transplanting (DAT).

2.4. Measurements and analytical determinations

At the end of the experimental conduction, growth data were measured evaluating shoot and root dry mass (g), number of sheets (plant^{-1} unit) and root length (cm). To obtain the shoot dry mass, the plants freshly harvested were immediately dried in an oven with forced air circulation at a temperature of around 65ºC. The number of sheets was obtained through direct counting and the root length was measured from the plant neck to the tip of the main root, using a millimeter ruler. After obtaining the dry mass of shoots and roots, the material was ground to determine Mn levels in shoots and roots. Mn contents were determined by atomic absorption spectrophotometry after digestion of HNO_3-$HClO_4$ (TEIXEIRA et al., 2017).

Arbuscular mycorrhizal colonization was determined following the methodological procedures described by Phillips and Hayman (1970). The root fragments were washed in running water and, later, the root cortex was clarified by heating (60 ºC) in a water bath in a 10% KOH solution. Then the, roots were stained with 5% acidified Parker® pen ink (VIERHEILIG et al., 1998). AMF spore abundance in soil was determined by extracting 100 g of soil through wet sieving (GERDEMANN; NICOLSON, 1963).

Leaf toxicity by Mn was performed by counting the number of sheets with symptoms and calculated according to the formula: (number of sheets with symptoms / total number of sheets) x 100 (GARCIA et al., 2020).

2.5. Statistical analysis

Data set was submitted to analysis of variance (ANOVA), using the F test. When significant differences were observed, the quantitative data were adjusted with fitted with regression models and the qualitative data were compared using the Scott-Knott test, using the software Sisvar 5.6 (FERREIRA, 2011).

3. RESULTS

3.1. Plant growth

All plants that received silicon doses above 200 mg kg^{-1} significantly reduced shoot dry mass production (Figure 1a). On the other hand, when they were inoculated with AMF, they showed an increase in the production of shoot dry mass, when compared to plants without inoculation, mainly in the presence of Si, regardless of the applied dose. A similar pattern was found for root dry mass. However, plants without AMF inoculation showed a significant reduction, even with the application of increasing doses of Si (Figure 1b).

The number of sheets of the inoculated treatments with AMF was significantly (~362%) higher, especially when compared to plants without AMF inoculation (Figure 1c). The root length increased as a function of increasing doses of Si applied in the soil in both treatments (with and without AMF) (Figure 1d). However, the difference between inoculated and non-inoculated treatments was only evidenced at the dose of 200 mg kg^{-1} of Si, where plants inoculated with AMF showed a significant increase of 15.7% in root length when compared to plants without AMF.

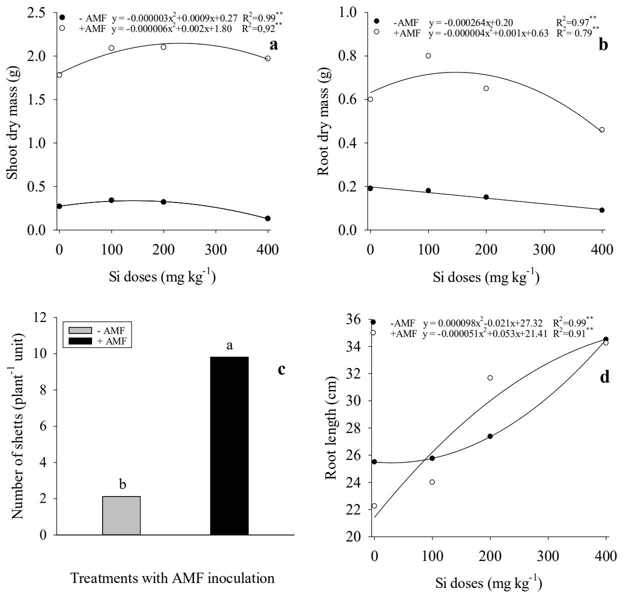

Figure 1. Dry mass of shoot (a) and root (b) as a function of Si doses and treatments with AMF inoculation. Number of sheets as a function of treatment with AMF inoculation (c). Root length as a function of Si doses and treatments with AMF inoculation (d). Means followed by the same letters do not differ by Scott-Knott test ($p \leq 0.05$).

Figura 1. Massa seca da parte aérea (a) e raíz (b) em função das doses de Si e tratamentos com inoculação de FMA. Número de folhas em função do tratamento com inoculação de FMA (c). Comprimento da raiz em função das doses de Si e tratamentos com inoculação de FMA (d). As médias seguidas pelas mesmas letras não diferem pelo teste de Scott-Knott ($p \leq 0,05$).

3.2. Mycorrhizal Colonization and AMF Spore Abundance in Soil

Mycorrhizal colonization showed a maximum increase (~32%) up to the estimated dose of 181.54 mg kg^{-1} of Si, which started to decrease from this value onwards (Figure 2a). On the other hand, the abundance of AMF spores in the rhizosphere decreased as a function of the increasing doses of Si (Figure 2b).

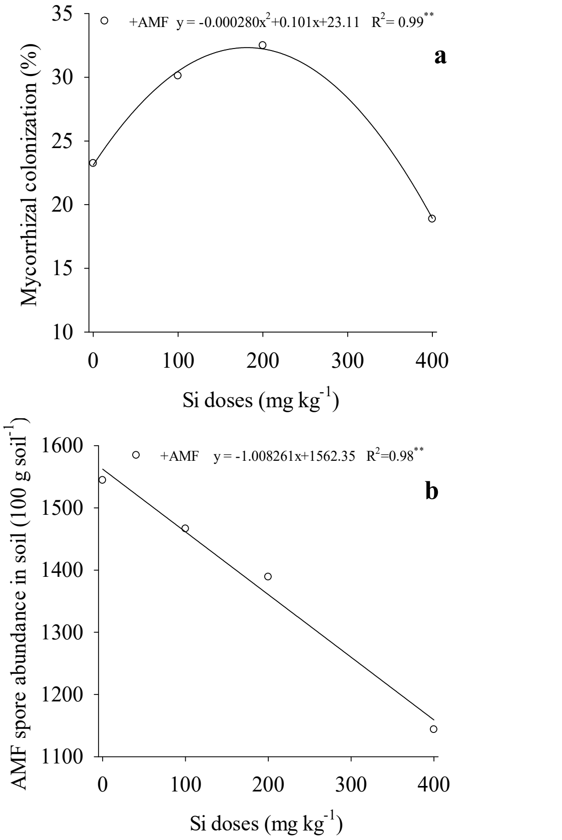

Figure 2. Mycorrhizal Colonization (a) and AMF Spore Abundance in Soil (b) in *L. leucocephala* as a function of the doses of Si applied to the soil.
Figure 2. Colonização micorrízica (a) e abundância de esporos de FMA no solo (b) em *L. leucocephala* em função das doses de Si aplicadas no solo.

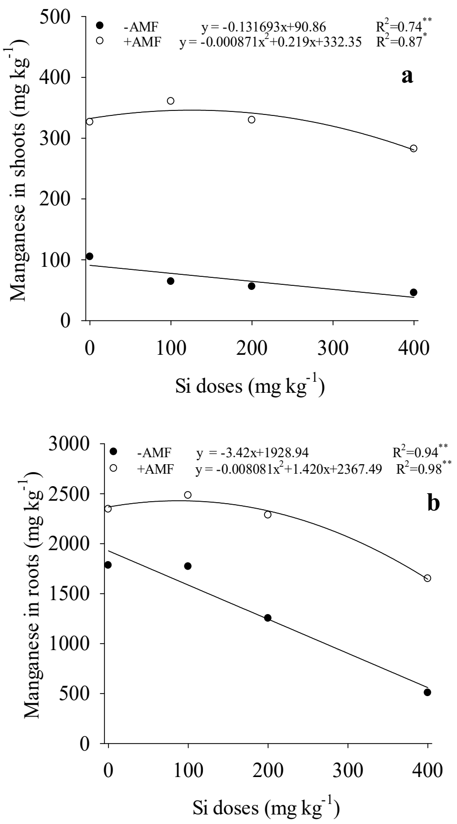

Figure 3. Manganese content in shoots (a) and roots (b) of *L. leucocephala* as a function of Si doses and treatments with AMF inoculation.
Figura 3. Teor de manganês na parte aérea (a) e nas raízes (b) de *L. leucocephala* em função de doses de Si e tratamentos com inoculação de FMA.

3.3. Manganese in shoots and roots

The levels of manganese in shoots of plants inoculated with AMF showed a maximum increase (346.13 mg kg^{-1}) up to the estimated dose of 125.78 mg kg^{-1} of Si, which started to decrease from this value onwards (Figure 3a). On the treatment without AMF inoculation decreased linearly as a function of increasing doses of Si (Figure 3a). All plants inoculated with AMF showed higher manganese content in shoots than plants without AMF.

In the roots, the Mn contents were higher than in the shoot, mainly in plants inoculated with AMF (Figure 3b). Inoculation with AMF provided a maximum increase (2367.49 mg kg^{-1}) in the manganese content in roots up to the estimated dose of 88 mg kg^{-1} of Si, which started to decrease from this value onwards, while the treatment without AMF decreased linearly as a function of increasing doses of Si (Figure 3b).

3.4. Leaf toxicity

Leaf toxicity showed a linear decrease as a function of increasing doses of Si applied to the soil in both treatments (with and without AMF) (Figura 4). However, the inoculation of plants with AMF was able to significantly decrease the leaf toxicity by Mn, when compared to plants without AMF, up to the dose of 200 mg kg^{-1} of Si, while at the maximum dose of this element (400 mg kg^{-1} of Si) there was no difference in leaf toxicity when comparing plants with and without AMF (Figure 4).

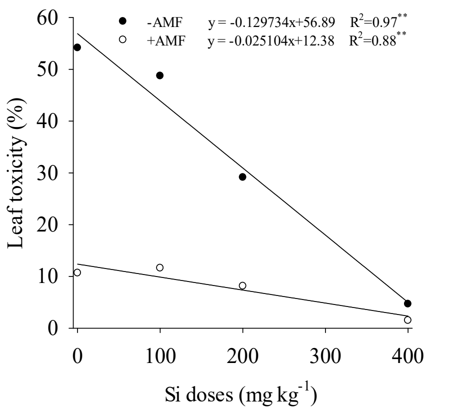

Figure 4. Leaf toxicity of *L. leucocephala* as a function of Si doses and treatments with AMF inoculation.
Figura 4. Toxidez foliar de *L. leucocephala* em função de doses de Si e tratamentos com inoculação de FMA

4. DISCUSSION

The results obtained point to a benefit generated by the association of AMF with *L. leucocephala,* since, there was an increase in the production of shoot dry mass, root dry mass and number of sheets, suggestive of the participation of the mycorrhizal fungus in the increase of Si absorption. It is known that high concentrations of Mn in the soil can impair plant growth (FARIA et al., 2020). It can be affirmed that AMF increased the tolerance of *L. leucocephala* to the Mn excess present in the soil, since, AMF in association with plants improve their nutritional status, including in relation to Si, contributing to its growth and decreasing the availability of some metals for plants, for example the Mn (GARG; SINGH, 2018; GARCIA et al. 2020).

According to Emamverdian et al. (2018), Si can act on the complexation of metal ions in the cell wall, decreasing, in turn, the translocation of ions from the root to the shoot of the plant. This fact may have contributed to the improvement in the growth of *L. leucocephala* plants under Mn stress in the present study. In the same way, Wang et al. (2020) also observed that the application of Si in the soil increased the growth of *Brassica chinensis* L. grown in soil contaminated with several HM.

Regarding root length, there is little differentiation between treatments (with and without AMF) and both obtained positive results in relation to root system growth, evidencing possible action of Si in increasing the tolerance of plants in the presence of Mn. However, the mechanisms of Si responsible for this possible protection are still not well understood (VACULÍK et al., 2021). Ur Rahman et al. (2021) state that Si supplementation in wheat plants grown under cadmium stress increased the length and volume of their roots.

It is worth mentioning that the relevant results obtained with the addition of Si, in terms of benefits for plants, are more evident under stress conditions (BHAT et al., 2019). It is also important to note that, in addition to plants, microorganisms are also affected when exposed to high levels of HM in the soil. Such a fact can explain why, in this study, inoculation with AMF did not have much influence on root length, being possible that the high concentrations of Mn have reduced the ability of the fungus to influence the greater growth of these roots. Except for the root length, the results found suggest that AMF inoculation and Si application in the soil, act synergistically and alleviate Mn stress, increasing the growth of *L. leucocephala*.

With regard to mycorrhizal colonization, it was observed that, despite its decrease in plants under higher Si doses in the soil (Figure 5), the symbiosis with *C. etunicatum* was effective and did not limit the development of these plants.

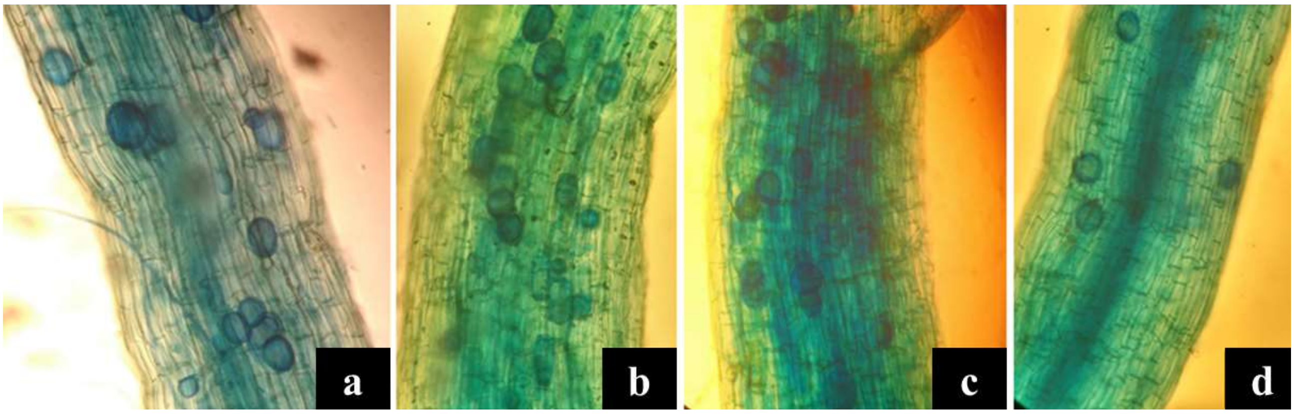

Figure 5. Mycorrhizal colonization of *L. leucocephala* at different silicon doses applied to the soil (a – 0 mg kg^{-1}; b – 100 mg kg^{-1}; c – 200 mg kg^{-1}; d – 400 mg kg^{-1}).
Figura 5. Colonização micorrízica de *L. leucocephala* em diferentes doses de silício aplicadas ao solo (a – 0 mg kg^{-1}; b – 100 mg kg^{-1}; c – 200 mg kg^{-1}; d – 400 mg kg^{-1}).

Moreover, the foliar symptoms of Mn toxicity did not increase with the decrease in colonization, on the contrary, there was a decrease in the percentage of foliar toxicity as the Si doses were increased, indicating that there was no isolated effect of AMF. Garg and Sing (2018) also point out that this interaction does not decrease the ability of AMF to colonize plant roots. It is worth mentioning, that another factor that may be related to the decrease in mycorrhizal colonization is the density of the roots, since decreases in the dry mass of plants' roots were also observed under higher Si doses.

Increasing Si doses significantly influenced AMF spore abundance in soil, which decreased with increasing amounts of applied Si in the soil. However, this factor was not reflected in greater damage to the plants, since they all showed similar development. Was noticed that mycorrhizal colonization had a low relationship with spore abundance, this is consistent with the fact that the number of spores in the soil minimally impacts the potential for mycorrhizal colonization in the roots (BAREA et al., 1991). The few studies that evaluated the interaction between Si and mycorrhiza in plants under stress conditions due to the presence of large amounts of Mn did not correlate mycorrhizal colonization with AMF spore abundance in soil and with the influence of Si (YOST; FOX, 1982). In general, what can be inferred from the results obtained is that Si had a negative influence on AMF spore abundance in soil, since increasing the applied doses resulted in a decrease in the number of spores in the rhizosphere of *L. leucocephala*.

All plants inoculated with AMF showed higher levels of Mn in the shoot than uninoculated plants, a result that differs from some studies found with other HM (ULTRA et al., 2021). Garcia et al. (2020) explain that often the uptake of Mn and its concentrations in plants are lower in mycorrhizal plants. Although not well understood, this behavior has been attributed to different mechanisms of AMF protection to plants against excess HM in the soil, including the retention and immobilization of these elements on the surface of AMF spores (GARCIA et al., 2020). The result for this study, however, is not an indicator of inefficiency for the treatment with mycorrhiza, since this association obtained the best results for all the variables analyzed in this study. Even though high levels of Mn were obtained in the shoots, the concentration of this element did not exceed the level considered toxic to plants, which is 400 mg kg^{-1} of Mn in the dry mass of the shoot (KABATA-PENDIAS, 2010).

Moreover, it is also worth mentioning that Si can help against the effects of HM contamination in plants. Among the mechanisms that act in this process are the decrease of metal ions in the soil and their absorption in the plant, chelation of metals, gene regulation related to the transport of metals, stimulation of antioxidants and structural changes in plants (BHAT et al., 2019).

In general, in both treatments (with and without AMF) the highest levels of Mn were found in the roots compared to the shoot of the plants, with emphasis on the mycorrhizal plants. This greater accumulation of Mn in the roots may explain the lower levels found in the shoot, which may characterize this plant species as a potential indication for use in phytostabilization programs. Motaharpoor et al. (2019) also observed high concentrations of Cd in the roots of plants inoculated with AMF, having the authors attributed these results to the sequestration of metals in the cell wall and in compartments in the structures of the AMF. With respect to Si, it is notable that this element, as well as AMF, can also help in the retention of metals in the roots. According to Khan et al. (2021), Si can help to increase the cell wall thickness of roots, forming a physical barrier that binds and restricts the transport of HM.

The treatments with AMF inoculation showed the lowest leaf toxicity percentage in relation to the treatments without inoculation. These results may be partially related to the decrease in Mn content observed in the shoots of the plants (Figure 3a) as a result of the interaction with Si. However, the decrease in Mn content in the shoots of the plants is apparently not related to the greater absorption by the roots observed, since Mn concentrations in roots also tended to decrease with increasing Si supplementation (Figure 3b).

The decrease in leaf toxicity percentage in these plants may probably be associated with a decrease in the availability of this metal in the soil and, consequently, its lower absorption. Some authors recognize the action of Si in the immobilization of HM in the soil (VACULÍK et al., 2021). Bhat et al. (2019) explain that Si can stimulate plant roots to release a greater amount of flavonoids and organic acids that can act in the chelation of metals in the soil and, therefore, reduce their phytotoxicity. Another effect of Si in the soil would be to favor an increase in the pH of the soil solution, resulting consequently in a decrease in the availability of metallic elements (KHAN et al., 2021). Analyzing especially the action of AMF, results similar to those found in this study were observed by Garcia et al. (2020) while studying the attenuation of foliar toxicity in mycorrhizal *L. leucocephala* plants under increasing levels of Mn in the soil.

The interaction of Si doses with AMF for the leaf toxicity variable was of great value, from the lowest to the highest applied Si dose. It can be affirm that the association of AMF *C. etunicatum* with *L. leucocephala*, in conjunction with Si, significantly attenuates the foliar symptoms of toxicity caused by Mn excess.

5. CONCLUSIONS

The inoculation of AMF and the application of Si in the soil, together, alleviate the stress caused by Mn and increase the growth of *L. leucocephala.* Furthermore, Si proved to be effective, up to a dose of 200 mg kg^{-1}, in maximizing the growth of mycorrhizal plants under Mn stress. It is likely that this dose of Si may differ depending on the type of soil, metal, plant species, and AMF. In a future perspective, our study can serve as a basis to assist in revegetation practices in mining areas with Mn excess.

6. REFERENCES

BALIEIRO, F. de C.; COSTA, C. A.; de OLIVEIRA, R. B.; de OLIVEIRA, R.; DONAGEMMA, G. K.; de ANDRADE, A. G.; CAPECHE, C. L. Carbon stocks in mined area reclaimed by leguminous trees and sludge. **Revista Arvore**, v. 32, p. 1-10, 2017. https://doi.org/10.1590/1806-90882017000600010

BAREA, J. M.; AZCÓN-AGUILAR, C.; OCAMPO, J. A.; AZCÓN, R. Morfologia, anatomia y citologia de las micorrizas vesiculo-arbusculares. In: BAREA, J. M. & LIVARES, J. (Eds). **Fijación y movilización biológica de nutrientes**, Madrid, p. 149-173, 1991.

BHAT, J. A.; SHIVARAJ, S. M.; SINGH, P.; NAVADAGI, D. B.; TRIPATHI, D. K.; DASH, P. K.; SOLANKE, A. U.; SONAH, H.; DESHMUKH, R. Role of silicon in mitigation of heavy metal stresses in crop plants. **Plants**, v. 8, p. 1-20, 2019. https://doi.org/10.3390/plants8030071

BUDINGER, D.; BARRAL, S.; SOO, A. K. S.; KURIAN, M. A. The role of manganese dysregulation in neurological disease: emerging evidence. **The Lancet Neurology**, v. 20, p. 956-968, 2021. https://doi.org/10.1016/S1474-4422(21)00238-6

EMAMVERDIAN, A.; DING, Y.; XIE, Y.; SANGARI, S. Silicon mechanisms to ameliorate heavy metal stress in plants. **BioMed Research International**, v. 2018, p. 1-10, 2018. DOI: https://doi.org/10.1155/2018/8492898

FARIA, J. M. S.; MARTINS, D.; PAULA, A.; BRITO, I.; BARRULAS, P.; ALHO, L.; CARVALHO, M. Toxic levels of manganese in an acidic Cambisol alters antioxidant enzymes activity, element uptake and subcellular distribution in *Triticum aestivum*. **Ecotoxicology and Environmental Safety**, v. 193, p. 1-9, 2020. https://doi.org/10.1016/j.ecoenv.2020.110355

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia***,* v. 35, p. 1039-1042, 2011. https://doi.org/10.1590/S1413-70542011000600001

GARCIA, K. G. V., MENDES FILHO, P. F.; PINHEIRO, J. I.; CARMO, J. F. do; PEREIRA, A. P. de A.; MARTINS, C. M.; ABREU, M. G. P. de; OLIVEIRA FILHO, J. de S. Attenuation of Manganese-Induced Toxicity in *Leucaena leucocephala* Colonized by Arbuscular Mycorrhizae. **Water, Air and Soil Pollution**, v. 231, p. 1-15, 2020. https://doi.org/10.1007/s11270-019-4381-9

GARG, N.; SINGH, S. Arbuscular Mycorrhiza Rhizophagus irregularis and Silicon Modulate Growth, Proline Biosynthesis and Yield in Cajanus cajan L. Millsp. (pigeonpea) Genotypes Under Cadmium and Zinc Stress. **Journal of Plant Growth Regulation**, v. 37, p. 46-63, 2018. https://doi.org/10.1007/s00344-017-9708-4

GERDEMANN, J. W.; NICOLSON, T. H. Spores of mycorrhizae endogone species extracted from soil by wet sieving and decanting. **Transactive British Mycology Society***,* v. 46, p. 235-244, 1963. https://doi.org/10.1016/S0007-1536(63)80079-0

HUANG, J.; NARA, K.; ZONG, K.; WANG, J.; XUE, S.; PENG, K.; SHEN, Z.; LIAN, C. Ectomycorrhizal fungal communities associated with Masson pine (Pinus massoniana) and white oak (Quercus fabri) in a manganese mining region in Hunan Province, China. **Fungal Ecology**, v. 9, p. 1-10, 2014. https://doi.org/10.1016/j.funeco.2014.01.001

IBRAM_Instituto Brasileiro de Mineração. **Setor mineral 1° trimestre 2020**, 43. 2020. Acesso em: 16 de março de 2022. Disponível em: https://ibram.org.br/wp-content/uploads/2021/02/PDF_DADOS_1oTRIM20_16ABR20_FINAL-1.pdf

KABATA-PENDIAS, A. **Trace elements in soils and plants** (4th ed.). Boca Raton: CRC Press, 2010. 548p.

KANG, X.; YU, X.; ZHANG, Y.; CUI, Y.; TU, W.; WANG, Q.; LI, Y.; HU, L.; GU, Y.; ZHAO, K.; XIANG, Q.; CHEN, Q.; MA, M.; ZOU, L.; ZHANG, X.; KANG, J. Inoculation of Sinorhizobium saheli YH1 leads to reduced metal uptake for Leucaena leucocephala grown in mine tailings and metal-polluted soils. **Frontiers in Microbiology**, v. 9, p. 1-13, 2018. https://doi.10.3389/fmicb.2018.01853

KHAN, I.; AWAN, S. A.; RIZWAN, M.; ALI, S.; HASSAN, M. J.; BRESTIC, M.; ZHANG, X.; HUANG, L. Effects of silicon on heavy metal uptake at the soil-plant interphase: A review. **Ecotoxicology and Environmental Safety**, v. 222, p. 1-15, 2021. https://doi.org/10.1016/j.ecoenv.2021.112510

LI, J.; JIA, Y.; DONG, R.; HUANG, R.; LIU, P.; LI, X.; WANG, Z.; LIU, G.; CHEN, Z. Advances in the mechanisms of plant tolerance to manganese toxicity. **International Journal of Molecular Sciences**, v. 20, p. 1-15, 2019. https://doi.org/10.3390/ijms20205096

MOTAHARPOOR, Z.; TAHERI, H.; NADIAN, H. *Rhizophagus irregularis* modulates cadmium uptake, metal transporter, and chelator gene expression in *Medicago sativa*. **Mycorrhiza**, v. 29, p. 389-395, 2019. https://doi.org/10.1007/s00572-019-00900-7

PHILLIPS, J. M.; HAYMAN, D. S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. **Transactions of the British Mycological Society**, v. 55, p. 158-161, 1970. https://doi.org/10.1016/s0007-1536(70)80110-3

SPAGNOLETTI, F.; CARMONA, M.; TOBAR, N. E.; CHIOCCHIO, V.; LAVADO, R. S. Arbuscular mycorrhiza reduces the negative effects of *M. phaseolina* on soybean plants in arsenic-contaminated soils. **Applied Soil Ecology**, v. 121, p. 41-47, 2017. https://doi.org/10.1016/j.apsoil.2017.09.019

TANG, T.; TAO, F.; LI, W. Characterization of manganese toxicity tolerance in Arabis paniculata. **Plant Diversity**, v. 43, p. 163-172, 2020. https://doi.org/10.1016/j.pld.2020.07.002

TEIXEIRA, P. C. T.; DONAGEMMA, G. K.; FONTANA, A.; TEIXEIRA, W. G. **Manual de Métodos de Análise de Solo** (3rd ed.). Brasília: Embrapa, 2017. 576p.

ULTRA, V. U.; MANYIWA, T. Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three Acacia species grown in Cu–Ni mine soil. **Environmental Geochemistry and Health**, v. 43, p. 1337-1353, 2021. https://doi.org/10.1007/s10653-020-00627-x

UR RAHMAN, S.; XUEBIN, Q.; KAMRAN, M.; YASIN, G.; CHENG, H.; REHIM, A.; RIAZ, L.; RIZWAN, M.; ALI, S.; ALSAHLI, A. A.; ALYEMENI, M. N. Silicon elevated cadmium tolerance in wheat (*Triticum aestivum* L.) by endorsing nutrients uptake and antioxidative defense mechanisms in the leaves. **Plant Physiology and Biochemistry**, v. 166, p. 148-159, 2021. https://doi.org/10.1016/j.plaphy.2021.05.038

VACULÍK, M.; KOVÁČ, J.; FIALOVÁ, I.; FIALA, R.; JAŠKOVÁ, K.; LUXOVÁ, M. Multiple effects of silicon on alleviation of nickel toxicity in young maize roots. **Journal of Hazardous Materials**, v. 415, p. 1-13, 2021. https://doi.org/10.1016/j.jhazmat.2021.125570

VIERHEILIG, H.; COUGHLAN, A. P.; WYSS, U.; PICHÉ, Y. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. **Applied and Environmental Microbiology**, v. 64, p. 5004–5007, 1998. https://doi.org/10.1128/aem.64.12.5004-5007.1998

VODNIK, D.; GRČMAN, H.; MAČEK, I.; VAN ELTEREN, J. T.; KOVAČEVIČ, M. The contribution of glomalin-related soil protein to Pb and Zn sequestration in polluted soil. **Science of the Total Environment**, v. 392, p. 130-136, 2008. https://doi.org/10.1016/j.scitotenv.2007.11.016

WANG, B.; CHU, C.; WEI, H.; ZHANG, L.; AHMAD, Z.; WU, S.; XIE, B. Ameliorative effects of silicon fertilizer on soil bacterial community and pakchoi (*Brassica chinensis* L.) grown on soil contaminated with multiple heavy metals. **Environmental Pollution**, v. 267, p. 1-10, 2020. https://doi.org/10.1016/j.envpol.2020.115411

YOST R. S.; FOX R. L. Influence of mycorrhizae on the mineral contents of cowpea and soybean grown in an oxisol. **Agronomy Journal**. v. 74, p. 475-481, 1982. https://doi.org/10.2134/agronj1982.00021962007400030018x

YOU, Y.; WANG, L.; JU, C.; WANG, G.; MA, F.; WANG, Y.; YANG, D. Effects of arbuscular mycorrhizal fungi on the growth and toxic element uptake of *Phragmites australis* (Cav.) Trin. ex Steud under zinc/cadmium stress. **Ecotoxicology and Environmental Safety**, v. 213, p. 1-10, 2021. https://doi.org/10.1016/j.ecoenv.2021.112023

ZEHRA, A.; CHOUDHARY, S.; WANI, K. I.; NAEEM, M.; KHAN, M. M. A.; AFTAB, T. Silicon-mediated cellular resilience mechanisms against copper toxicity and glandular trichomes protection for augmented artemisinin biosynthesis in Artemisia annua. **Industrial Crops and Products**, v. 155, p. 1-9, 2020. https://doi.org/10.1016/j.indcrop.2020.112843