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Growth of tropical tree species and absorption of copper in soil artificially contaminated

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Abstract

Reclamation of copper contaminated sites using forest species may be an efficient alternative to reduce the negative impact. The aim of this study was to quantify the growth and evaluate the quality of seedlings of native species at different doses of copper in the soil. The experimental design was completely randomized, with seven replications in a factorial arrangement (3×9), using three indigenous species of plants (*Anadenanthera macrocarpa*, *Mimosa scabrella* and *Apuleia leiocarpa*) and nine doses of copper in the soil (0, 60, 120, 180, 240, 300, 360, 420 and 480 mg kg^{-1}). The experiment was carried out in a greenhouse which the seedlings were grown for 180 days. The experimental units were plastic pots of 125 cm^3 filled with Oxisol. The results indicated that the levels of copper applied to the soil decreased the quality of seedlings and growth of *Apuleia leiocarpa*to a lesser extent compared with *Mimosa scabrella* and *Anadenanthera macrocarpa*. *Anadenanthera macrocarpa* was the forest species that resulted in the lowest copper translocation from roots to shoots. In addition, the *Apuleia leiocarpa* exhibited high resistance and tolerance for copper in the soil and also, it is highlighted an ability for copper phytoremediation.

Keywords: revegetation, native species, copper contamination, depredated areas, phytoremediation, bioremediation.

Crescimento de espécies arbóreas tropicais e absorção de cobre em solo artificialmente contaminado

Resumo

A recuperação de áreas contaminadas com cobre utilizando espécies florestais pode ser uma alternativa eficiente para reduzir o impacto negativo deste elemento nestas áreas. O objetivo deste estudo foi quantificar o crescimento e avaliar a qualidade de mudas de espécies nativas em diferentes doses de cobre no solo. O delineamento experimental foi inteiramente casualizado, com sete repetições, em um esquema fatorial (3×9), utilizando-se três espécies nativas de plantas (*Anadenanthera macrocarpa*, *Mimosa scabrella* e *Apuleia leiocarpa*) e nove doses de cobre no solo (0, 60, 120, 180, 240, 300, 360, 420 e 480 mg kg^{-1}). O experimento foi realizado em casa de vegetação, onde as plantas foram cultivadas por 180 dias. As unidades experimentais foram vasos de plástico de 125 cm^3, preenchidos com Latossolo Vermelho distrófico. Os resultados indicaram que o nível de cobre aplicado ao solo reduziu a qualidade de plântulas e crescimento de *Apuleia leiocarpa* para um menor grau comparado com bracatinga e angico. *Anadenanthera macrocarpa* foi a espécie florestal que apresentou menor translocação de cobre a parte aérea das mudas. Além disso, a *Apuleia leiocarpa* exibiu elevada tolerância para o cobre no solo e também destaca-se mostrando uma capacidade para fitoremediação de áreas contaminadas com cobre.

Palavras-chave: recuperação, espécies nativas, contaminação com cobre, áreas degradadas, fitorremediação, biorremediação.

1. Introduction

Copper is an essential micronutrient for plant development; however, in high concentrations in the soil, it can promote physiological and biochemical disorders in plants, reducing plant growth potential (Panou-Filotheou et al., 2001; Marschner, 2011). This reduction in plant growth was found in a vineyard, when using Bordeaux mixture to control fungal diseases (Chaignon and Hinsinger, 2003; Nachtigall et al., 2007) and in plantations with forest species in copper-contaminated soil, which can inhibit the growth of seedlings of *Eucalyptus grandis* and *Peltophorum dubium* as demonstrated by Antoniolli et al. (2010).

In general, plant species adapt differently to the concentrations of heavy metals in soil (Rascio and Navari-izzo, 2011). Brunner et al. (2008) highlight that forest tree species may be an alternative for use in metal-contaminated soil because of their high capacity to immobilize metals in their tissue, mainly in the roots; delaying the return of such metals to the soil (Soares et al., 2000) and minimizing the contamination of surface and subsurface waters (Wuana and Okieimen, 2011). This is called phytostabilization which use plants to stabilize the metal in the soil (Andreazza et al., 2013b), and some studies have been demonstrated efficiency for copper phytostabilization on vineyards soils contaminated with high copper concentrations and copper mine waste (Andreazza et al., 2011, 2013a).

One of the required characteristics for phytoremediation is the fast-growing plant, once native species as *Anadenanthera macrocarpa* already has the adaptation and rusticity capacity, this species has high potential use for phytoremediation; and also this specie is widely used for restoration of riparian forests and reclamation of degraded areas (Carvalho, 1994). Also, Pereira et al. (2012) demonstrated that the concentration of heavy metals in tree species used for revegetation of contaminated areas, indicating that *Anadenanthera macrocarpa* efficiently accumulates copper in its tissue, reducing the availability of this metal in the soil.

Mimosa scabrella belongs to the Fabaceae family, and it is considered a suitable species for reclamation of degraded areas because it fixes significant amounts of nitrogen-rich organic material in the soil (Carpanezzi et al., 1988). Regensburger et al. (2008) studied the interactions among techniques of soil, plants and animals to recover degraded areas and found that *Mimosa scabrella* showed a survival rate, higher than 92% when used in degraded areas. It demonstrates a high potential for phytoremediation, once the initial growth is the most important phase for this technique success.

Apuleia leiocarpa is a native tree species, belonging to the Fabaceae family, with wide geographical distribution in the Brazil (Mattos, 2002) and has a potential to be used in reforestation of areas contaminated with heavy metals (Carvalho, 1994). In general, the native tree species have some interesting characteristics for growth, adaptation and stabilization on copper contaminated areas and recuperate these environments; however, this issue lacks in studies of different conditions. So, this study aimed to evaluate the growth and quality of seedlings of *Anadenanthera macrocarpa*, *Mimosa scabrella* and *Apuleia leiocarpa* at different doses of copper applied in the soil and use for further studies on phytoremediation.

2. Material and Methods

The study was conducted in the greenhouse of the Agronomic and Environmental Sciences Department of the Federal University of Santa Maria, Frederick Westphalen campus, RS, Brazil. The experiment was conducted in a completely randomized design in a factorial arrangement (3×9) with seven replicates. Three native species were used: *Anadenanthera macrocarpa* (Benth.), *Mimosa scabrella* Benth. (Brenan) and *Apuleia leiocarpa* (Vogel) J.F. Macbr.; and nine doses of copper in the soil (0, 60, 120, 180, 240, 300, 360, 420 and 480 mg kg^{-1}) as cupric sulfate ($CuSO_4.5H_2O$).

The experimental units were root trainers with a volume of 125 cm^3, filled with soil classified as Oxisol (Soil Survey Staff, 1999) with the following chemical and physical properties: pH_{water}: 4.9; Ca + Mg = 5.4 $cmol_c$ kg^{-1}; Al = 4.3 $cmol_c$ kg^{-1}; H+Al = 6.6 $cmol_c$ kg^{-1}; P = 6.6 mg dm^{-3}; K = 111.0 mg dm^{-3}; Zn = 1.6 mg dm^{-3}; Cu = 15.1 mg dm^{-3}; organic matter = 24 g dm^{-3} and clay = 810 g dm^{-3}.

Three seeds were sown in the pots and thinning was performed 15 days after seedling, leaving only one plant per pot. The seeds were provided by Forest Research Station of FEPAGRO from Santa Maria, RS, Brazil. The chemical fertilizations followed the recommendations proposed by Gonçalves and Benedetti (2005). Irrigations were performed three times a day, keeping the soil at approximately 80% of field capacity.

At 180 days of growth in greenhouse, the indigenous species were harvested and quantified: a) height, obtained by the distance of the stem up to the last leaf axils; b) stem diameter (SD), measured with a digital caliper; c) shoot dry weight (SDW) and root dry weight (RDW), quantified after shoots and roots separated by/at the stem of the seedlings and dried at 60 °C until constant weight; d) main root length (MRL); e) specific surface area of roots (SSA), obtained by the software SAFIRA (Jorge and Silva, 2010); f) copper content in the shoots, determined according to the methodology of Silva (2009); g) ratio between the copper contents of the root and the shoot at zero dose of copper (R/S_0) and the maximum dose of copper (R/S_{480}); h) impact coefficient of relative copper content (ICRC), calculated by the ratio $(R/S_{480})/(R/S_0)$; i) seedling quality index (H/D), calculated by the ratio between plant height and stem diameter; j) Dickson quality index (Dickson et al., 1960).

Data were subjected to analysis of variance at 5% probability of error by the software SISVAR (Ferreira, 2011) and multivariate analysis of main components with the software InfoStat, version 2013 (Di Rienzo et al., 2013). The mean of the qualitative factor (species) were compared by Tukey test at 5% probability of error and the means of the quantitative factor (doses) were subjected to regression analysis by the software SISVAR (Ferreira, 2011), with a level of 5% probability of error. Estimation of the dose of copper required to cause 50% of injury to seedlings (DR_{50}) was based on linear regression equations.

3. Results and Discussion

The results showed a significant interaction between species and doses of copper (Figure 1). *Apuleia leiocarpa* was the species that promoted substantial growth, with the highest development in the different copper concentrations. This species showed high resistance for copper concentrations with more biomass production compared to the other two species. When there was an increase on copper concentrations, the *Apuleia leiocarpa* slight reduced the growth parameters, and the other two species substantial

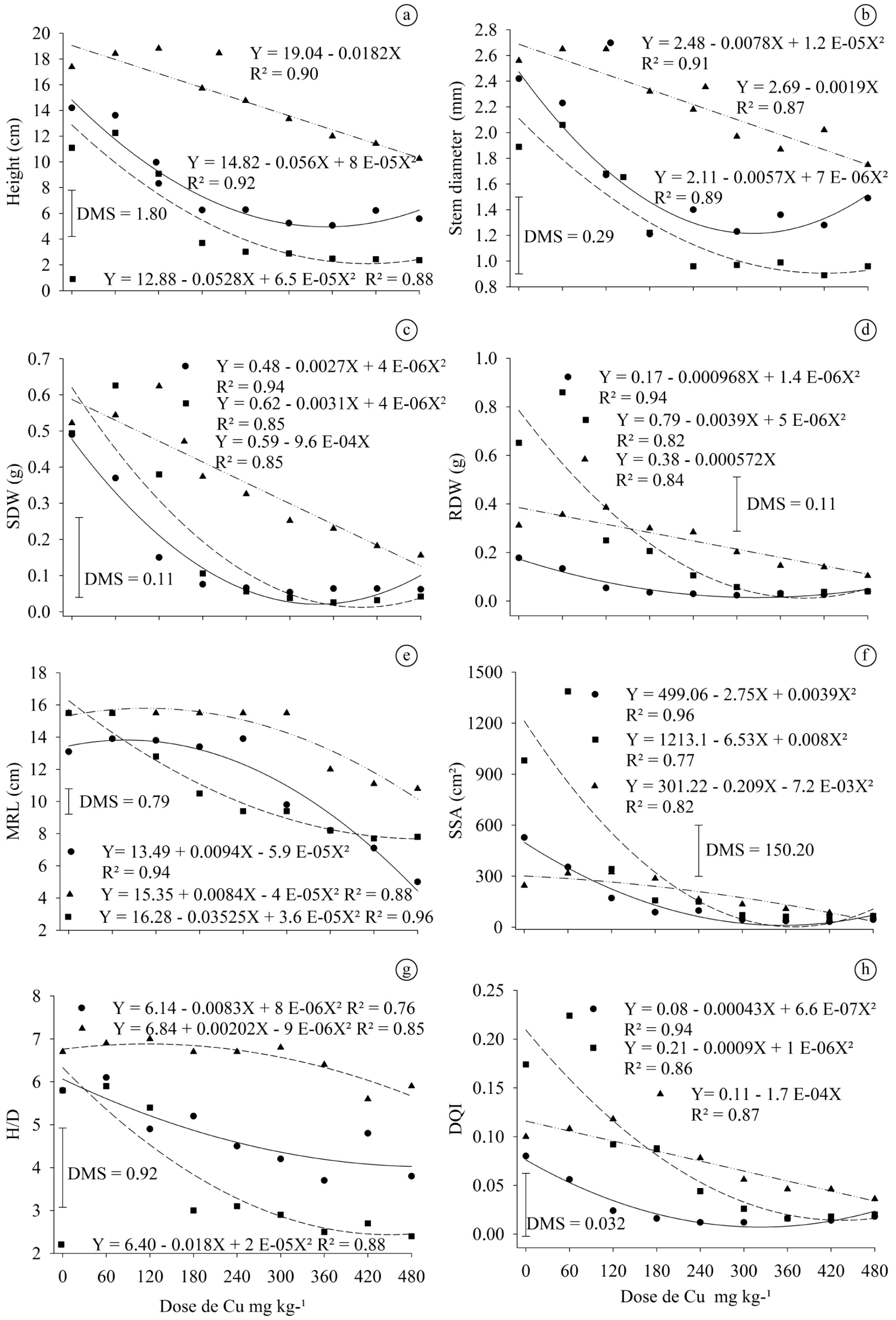

Figure 1. Regression equations for height (a), stem diameter (b), shoot dry weight – SDW (c), root dry weight – RDW (d), main root length – MRL (e), specific surface area – SSA (f), height/diameter ratio – H/D (g) and Dickson quality index – DQI (h) of the *Anadenanthera macrocarpa* (●), *Mimosa scabrella* (■) and *Apuleia leiocarpa* (▲) seedlings with diferent copper doses. Level of 5% probability of error ($P \leq 0.05$).

reduced the same parameters, once the *Anadenanthera macrocarpa* plant exhibited high sensibility for high copper concentrations. The *Mimosa scabrella* maintained the average of growth between the *Apuleia leiocarpa* and *Anadenanthera macrocarpa*. Shoot dry weight, root dry weight and main root length were also significantly higher in *Apuleia leiocarpa* after the addition of 120 mg kg^{-1} of copper in the soil. Results of previous studies showed that there is a susceptibility for copper toxicity among plant species (Guo et al., 2010), and it has been attributed to the fact that plants develop mechanisms to adapt to toxic metals (Lequeux et al., 2010).

Apuleia leiocarpa showed a significantly higher height/stem diameter ratio than the other species studied with a dose of 60 mg kg^{-1} of copper, with values ranging from 5.6 to 7.0, whereas doses higher than 120 and 180 mg kg^{-1} of copper for *Anadenanthera macrocarpa* and *Mimosa scabrella*, respectively, allowed values of height/stem diameter ratio below 5.4 (Figure 1a and b). This ratio refers to seedlings etiolating, and it is an important criterion used by researchers to evaluate the quality of seedlings in the arboretum (Souza et al., 2006; Jacobs et al., 2012). The values of this ratio for seedlings of *Apuleia leiocarpa* are in proper range proposed by Carneiro (1995), which showed values from 5.4 to 8.1. Thus, small doses of copper in the soil contribute to the growth of *Apuleia leiocarpa* without etiolating. High doses of copper indicate that *Apuleia leiocarpa* has some mechanisms that allow the reduction of the toxic effects of copper when compared to *Anadenanthera macrocarpa* and *Mimosa scabrella*, indicating tolerance to this heavy metal.

Plant height, shoot dry weight and stem diameter of all species were reduced with the addition of increasing doses of copper in the soil (Figure 1a, b and c). The *Anadenanthera macrocarpa* and *Apuleia leiocarpa* showed greater reductions with lower height at the doses of 350 and 406 mg kg^{-1} of copper, respectively, compared to *Apuleia leiocarpa*, which had a linear reduction, achieving 50% of injury at dose 569 mg kg^{-1} of copper (Figure 1a). According to Kabata-Pendias (2011), the excess of copper reduces the photosynthetic rate by interfering with the electron transport chain of photosystem I, decreasing the production of photoassimilates by plants, resulting in reduced plant growth. Similar results were found by Caires et al. (2011), where shoot dry weight of *Cedrela fissilis* was significantly reduced by doses of copper in seedlings grown in Oxisol with medium texture.

Root dry weight of *Apuleia leiocarpa* seedlings also showed lower reduction with increasing doses of copper compared to *Anadenanthera macrocarpa* and *Mimosa scabrella* (Figure 1d). According to Kahle (1993), copper toxicity of the root system induces the reduction of secondary roots, reducing the ability of plants to absorb nutrients, resulting in reduction in plant growth. According to Taiz and Zeiger (2006), plants may have different biochemical adaptations that enable tolerance to high concentrations of copper in the soil. Thus, *Apuleia leiocarpa* seedlings seem to possess biochemical adaptations because they have higher root dry weight values than *Anadenanthera macrocarpa* and *Mimosa scabrella* on high doses of copper.

Main root length was reduced at the doses tested in the three species studied (Figure 1e). Similar results were found in other studies which increasing concentrations of copper in the nutrient solution reduced the root length of seedlings of *Phytolacca americana* (Zhao et al., 2012) and two species of *Eucalyptus* (Soares et al., 2000). Thus, the presence of copper in the soil may have promoted a negative effect on the main root length of our native species studied.

Mimosa scabrella had higher SSA than *Anadenanthera macrocarpa* and *Apuleia leiocarpa* in the lowest doses of copper, and did not differ from *Apuleia leiocarpa* after the dose of 120 mg kg^{-1} (Figure 1f). Plants whose root system consists of fine roots have a higher SEA (Tennant, 1975) and, thus, they have greater ability to absorb nutrients from the soil (Silva et al., 2011). So, the greater SSA of *Mimosa scabrella* and *Apuleia leiocarpa* seedlings, observed in larger doses of copper can contribute to their growth in contaminated soil compared with of *Mimosa scabrella* seedlings.

Mimosa scabrella exhibited higher values for DQI than *Anadenanthera macrocarpa* and *Apuleia leiocarpa* seedlings, at the control and at 60 mg kg^{-1} treatments (Figure 1h). Different responses in this index in indigenous species were also observed by Ferraz and Engel (2011) in Multiplant® substrate, in which seedlings of *Tabebuia serratifolia* seedlings had higher DQI than *Peltophorum dubium* and *Hymenaea courbaril* seedlings grown in tubes with volume of 300 cm^3. DQI considers the production of total dry weight (root and shoot), stem diameter and plant height (Dickson et al., 1960), determining the strength and balance of the biomass distribution of seedlings (Fonseca et al., 2003). Thus, the increase in the shoot dry weight of the *Mimosa scabrella* seedlings produced at doses up to 60 mg kg^{-1} seems to have contributed considerably to the increase in DQI. It shows that in this dose is properly great to produce this specie.

The specific surface area of the roots of *Anadenanthera macrocarpa* and *Mimosa scabrella* were reduced in the doses of 355 and 408 mg kg^{-1} of copper, respectively, while *Apuleia leiocarpa* showed an increase in surface area up to 145 mg kg^{-1} of copper (Figure 1f). Results of *Apuleia leiocarpa* were similar to Silva et al. (2011), which copper doses up to 128 mg kg^{-1} in the soil stimulated the specific surface area of *Enterolobium contorstisiliquum* seedlings. The fact that *Apuleia leiocarpa* seedlings had stimulated the surface area in the smaller doses of copper can be attributed to excess of copper in the rhizosphere, causing the reduction in longitudinal growth of the roots and the formation of secondary roots (Taiz and Zeiger, 2006).

Apuleia leiocarpa seedlings had an increase in the H/D ratio at 112.3 mg kg^{-1} of copper, while for *Mimosa scabrella*, this ratio is reduced to 440.5 mg kg^{-1} and for *Anadenanthera macrocarpa* to 516 mg kg^{-1} (Figure 1g). Similar results were also observed by Silva et al. (2012) in which *Lafoensia pacari* seedlings had reduced H/D ratio when subjected to doses of copper in the soil. Therefore, doses of copper caused a greater reduction in the H/D ratio in *Mimosa scabrella* seedlings than in *Apuleia leiocarpa* seedlings.

Dickson quality index (DQI) was reduced at doses of copper and only the *Mimosa scabrella* seedlings produced at small doses had a DQI greater than 0.2 (Figure 1h). This index is indicative of seedling quality for transplantation, and the minimum value recommended is 0.2 (Dickson et al., 1960; Cruz et al., 2004; Melo et al., 2008). According to Dechen and Nachtigall (2006), copper participates in the metabolism of nitrogen; however, when in excess, it can cause disturbance in plant metabolism. So, there is a negative effect of copper on the DQI of *Mimosa scabrella* and *Apuleia leiocarpa* seedlings, even at low doses.

Multivariate analysis of the data indicated that applications of copper in the soil caused both negative and positive effect on seedlings of native forest species (Figure 2). In *Anadenanthera macrocarpa* and bracatinga, doses above 120 mg kg^{-1} reduced seedling height, root dry mass, stem diameter, specific surface area of the roots, main root length, H/D ratio and DQI (Figure 2a and Figure 2b). In *Apuleia leiocarpa* seedlings, doses above 240 mg kg^{-1} influenced seedling height, stem diameter, shoot dry weight and root dry weight, specific surface area of the roots and DQI (Figure 2c).

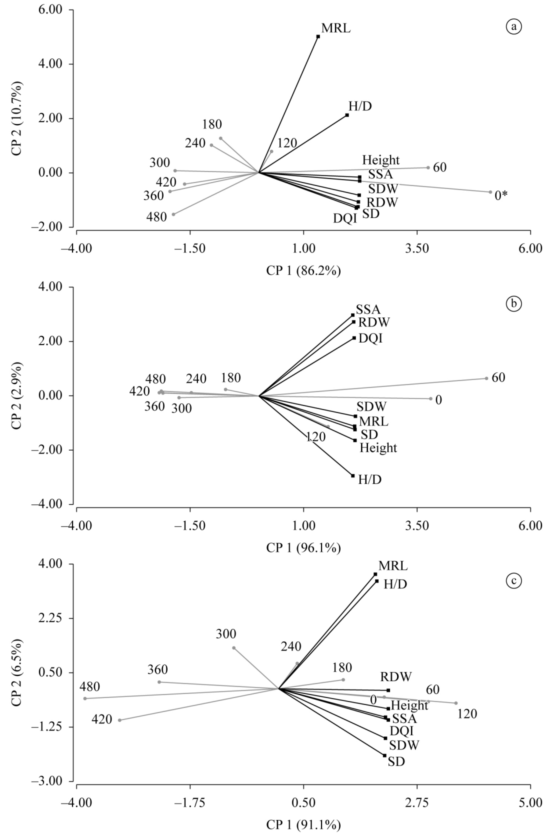

Figure 2. Multivariate analysis of height, stem diameter (SD), shoot dry weight (SDW), root dry weight (RDW), main root length (MRL), specific surface area (SSA), height/diameter ratio (H/D) and Dickson quality index (DQI) of *Anadenanthera macrocarpa* (a), *Mimosa scabrella* (b) and *Apuleia leiocarpa* (c) seedlings with different doses of copper (* mg kg^{-1}of soil).

The positive effect of doses of copper was observed with addition of 60 mg kg^{-1}, which provided increase on specific surface area, root dry weight, shoots dry weight, stem diameter and DQI of *Mimosa scabrella* seedlings (Figure 2b). These results indicate that small amounts of copper added to the soil can be an essential micronutrient for plant development, it has a positive effect on plant growth and plant quality; however, it depends on the plant species. High doses in the soil reduced growth and affected the quality, regardless of plant species. In the most of crop species, the sufficient range of copper in the shoots is 5-20 mg kg^{-1} of dry weight, and the critical toxicity level of copper in the leaves is above 20-30 mg kg^{-1} of dry weight (Marschner, 2011). In this case, it is possible that the dose of 60 mg of copper kg^{-1} of soil had inhibited the seedlings of *Mimosa scabrella* achieve the sufficiency level of copper content in dry mass.

The coefficient of impact on relative copper content (IRCC) was 1.63 for *Apuleia leiocarpa*, 11.96 for *Mimosa scabrella* and 25.2 for *Anadenanthera macrocarpa* (Table 1). According to Marques et al. (2000), this coefficient indicates the ability of species to limit the translocation of elements from root to shoot and, when IRCC is greater than the values of one unit, it is indicative of a greater proportion of copper content in the roots than in the shoots. Similar results were also found in *Anadenanthera macrocarpa* (Silva et al., 2011), and in *Sesbania virgata* (Branzini et al., 2012) which showed high ability to accumulate copper in the root system, reducing copper translocation to the shoots. Thus, *Anadenanthera macrocarpa* has greater capacity to limit copper translocation to the shoots compared with other species.

Apuleia leiocarpa showed lower value for IRCC compared to *Anadenanthera macrocarpa* and *Mimosa scabrella*, indicating that *Apuleia leiocarpa* has higher copper translocation to shoots compared to other species (Table 1). Tolerance to copper toxicity has been proposed in the literature and includes mechanisms such as enzyme activity in the detoxification of free radicals, copper chelation by organic compounds and/or compartmentalization in the vacuole (Qian et al., 2005; Colzi et al., 2011). Thus, the results of this study indicate that *Apuleia leiocarpa*

Table 1. Copper ratio of the roots and shoots at the control (without copper) (R/S_0), at the maximum copper dose (R/S_{480}) and the impact coefficient of relative copper content (ICRC) for *Anadenanthera macrocarpa*, *Mimosa scabrella* and *Apuleia leiocarpa* seedlings.

Species	(R/S_0)	(R/S_{480})	ICRC[1]
A. macrocarpa	0.34	8.64	25.20
M. scabrella	2.20	26.33	11.96
A. leiocarpa	2.68	4.39	1.63

[1]ICRC = (R/S_{480})/(R/S_0).

may have copper detoxification mechanisms, allowing for greater growth than *Anadenanthera macrocarpa* and *Mimosa scabrella* in contaminated soil.

4. Conclusions

Applications of increasing doses of copper in the soil affect the quality and growth all species; however, the *Apuleia leiocarpa* seedlings was lower affected than the *Anadenanthera macrocarpa* and *Mimosa scabrella*.

The *Anadenanthera macrocarpa* enables higher copper accumulation in the roots than in the shoots, compared to *Mimosa scabrella* and *Apuleia leiocarpa*.

The *Apuleia leiocarpa* exhibited high resistance and tolerance for copper in the soil and strongly characteristics for growth and phytomass produce in copper contaminated soil. Also, it is highlighted this plant tolerance and ability for copper phytoaccumulation, and it is a potential culture to grow in copper contaminated areas for reforestation and or use of inappropriate areas as these sites.

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