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## *Senna multijuga* and peat in phytostabilization of copper in contaminated soil

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### Key words:

tree species  
heavy metal  
pau-cigarra  
soil decontamination

### ABSTRACT

Phytoremediation is a technique that uses plants, whether associated or not to ameliorating agents, for phytostabilization of contaminated soil. The aim of this study was to evaluate the use of *Senna multijuga* associated with peat in the phytostabilization of copper contaminated soil. The experiment was carried out in a greenhouse for 120 days, using an entirely randomized design in a factorial arrangement (2 x 6), with and without peat (200 mL L<sup>-1</sup> soil) and six doses of copper (0, 60, 120, 180, 240, 300 mg kg<sup>-1</sup>), with six repetitions of each treatment. At 120 days after seedling, the plants were evaluated for height, stem diameter, root and aerial dry matter, contents and accumulated amount of copper in the root system and aerial part. Also, the Dickson quality indexes, as well as tolerance and translocation indexes were analyzed. The results showed that the use of 200 mL of peat L<sup>-1</sup> of soil was inefficient as an ameliorating agent for copper-contaminated soil, but it acted as a soil conditioner, increasing the morphological parameters of *S. multijuga*. In its initial development period, the *S. multijuga* species presented low copper phytostabilization potential in contaminated soil.

### Palavras-chave:

espécies de árvores  
metal pesado  
pau-cigarra  
descontaminação de solo

## *Senna multijuga* e turfa em fitoestabilização do cobre em solo contaminado

### RESUMO

A fitorremediação é uma técnica que usa plantas associadas ou não a agentes de melhoria, para fitoestabilização em solo contaminado. O objetivo deste estudo foi avaliar o uso de *Senna multijuga* associada à turfa em fitoestabilização de cobre em solo contaminado. O experimento foi conduzido em casa de vegetação durante 120 dias, usando-se o delineamento inteiramente casualizado em esquema fatorial (2 x 6), com e sem turfa (200 mL L<sup>-1</sup> de solo) e seis doses de cobre (0, 60, 120, 180, 240, 300 mg kg<sup>-1</sup>), com seis repetições por tratamento. Avaliaram-se, aos 120 dias após a semeadura, as plantas quanto à altura, ao diâmetro, massa seca radicular e aérea, conteúdo e quantidade acumulada de cobre no sistema radicular e na parte aérea; índice de qualidade de Dickson, índice de tolerância e de translocação também foram analisados. Os resultados mostraram que a utilização de 200 mL de turfa L<sup>-1</sup> de solo não foi eficaz como agente melhorante para solo contaminado por cobre, mas atuou como condicionador do solo aumentando os parâmetros morfológicos de *Senna multijuga*. A espécie *Senna multijuga* apresentou baixo potencial de fitoestabilização de cobre em solo contaminado, em seu período inicial de desenvolvimento.



## INTRODUCTION

Anthropogenic activities, such as mining, industrial and agricultural, have negative impacts on the ecosystem (Gomes et al., 2011). Among the inorganic elements, copper (Cu) is considered a pollutant and its toxic effect reduces plant growth (Taiz & Zeiger, 2013). The application of inadequate copper-based pesticides is among the main causes of soil contamination by this metal (Mackie et al., 2012).

The use of woody species is an important strategy for recovering metal contaminated areas. Trees can immobilize large amounts of metals absorbed in the tissues, as a result of the long life cycle and high biomass production (Domínguez et al., 2009; Jensen et al., 2009). A representative of the Atlantic Forest, pau-cigarra (*Senna multijuga* (Rich.) Irwin and Barn.) is a pioneer species that represents the Fabaceae family, and presents a fast initial growth.

When there are high concentrations of metals in the soil, phytoremediation becomes limited as a result of the toxic effects on plants. In this case, it is primarily recommended that the contaminant be stabilized, thereby promoting the adsorption of metals, and reducing its available content to a level that is tolerated by plants (Gabos et al., 2011). Organic matter is regarded as the main binder for heavy metals (Jorge et al., 2010), thereby reducing their availability to plants (Ribeiro Filho et al., 2001). This is as a result of the generation of complexes and formation of chemical bonds with the polar oxygen functional groups.

Among the organic matter sources, peat is a good option for use as a conditioner agent for Cu-contaminated soil, because it is widely available, has low cost and high metal adsorption capacity (Franchi et al., 2003). Thus, the aim of this study was to determine the use of *S. multijuga* associated with peat in the phytostabilization of Cu-contaminated soil.

## MATERIAL AND METHODS

The experiment was conducted in a heated greenhouse (with 12 h of light and 12 h of darkness), with 80% relative humidity, temperature of 20 °C at night and 28 °C ( $\pm 3$  °C) during the day. The soil used in this experiment was characterized as Rhodic Eutrudox; and peat Green<sup>®</sup> was used as organic material. Its chemical and physical analyses (Table 1) were performed according to EMBRAPA (1997).

The experimental units were polyethylene plastic bags with volume capacity of 600 cm<sup>3</sup>. The seeds of pau-cigarra (*S. multijuga* (Rich.) Irwin and Barn.) were provided by the Forestry Research Center from the State Foundation of Agriculture and Cattle Raising Researches (FEPAGRO), Santa Maria, RS, Brazil. The seeding was carried out in seedbeds and, when presenting a definitive pair of leaves, the seedlings were

transplanted to the experimental units, with one seedling in each bag.

The experimental outline was entirely randomized in a factorial arrangement (2 x 6): with and without peat (200 mL L<sup>-1</sup> soil (v:v); and six doses of Cu(II) amended to the soil (0, 60, 120, 180, 240, 300 mg kg<sup>-1</sup>), with six repetitions for each treatment. The Cu(II) doses were applied 30 days in the soil before the transplantation of seedlings. Copper (Cu) was added as a solution containing doses of Cu(II) (CuSO<sub>4</sub>·5H<sub>2</sub>O), diluted in 50 mL of water and homogenized with the soil by agitation in a plastic bag. The Cu(II) doses were mixed in pure soil (without peat) and incubated for 15 days; so as to stabilize before the addition of peat (200 mL L<sup>-1</sup> of soil). After the addition of peat, the soil remained in incubation for another 15 days at 80% of moisture. Before transplanting the seedlings, a soil sample of each treatment was obtained to determine the contents of the pseudo-total Cu(II) (USEPA, 1996).

After transplanting the seedlings, the experiment was conducted for 120 days. At the end of the experiment, plant shoot height (H) was measured with a graduated scale from the base of the seedlings to the top of the shoots; stem diameter (SD) was measured using a digital caliper with a precision of 0.01 mm. To determine the dry mass of the roots (DMR) and shoots (DMS), both fractions were separated in the cervical region of the plant. The roots were washed with water and then with distilled water. Subsequently, the material was dried in an oven with forced-air at 60  $\pm$  1 °C. The total dry matter (TDM) was determined by summarizing the DMR and DMS. According to the methodology described by Tennant (1975), the specific surface area (SSA) of the roots was estimated; and through Eq. 1, the Dickson quality index (DQI) was determined.

$$DQI = \frac{TDM}{\frac{H}{SD} + \frac{DMS}{DMR}} \quad (1)$$

After weighing the roots and aerial part of the dry matter, the material was ground using a Wiley grinder (10 mesh sieve) to determine the Cu(II) contents through nitric-perchloric digestion (3:1) and atomic absorption spectrophotometry (Miyazawa et al., 2009).

Based on the TDM, in the Cu(II) contents (mg kg<sup>-1</sup>) of the roots (CuR) and shoots (CuS), in the accumulated amounts of Cu(II) ( $\mu$ g plant<sup>-1</sup>) in the roots (CuAR), in the shoots (CuAS) and in the total of seedlings (CuAT), in the zero dose of Cu(II) (d0) and in the doses of 60 to 300 mg kg<sup>-1</sup> (dn), the tolerance index (Toi) was calculated according to Eq. 2 and the translocation index (Tri) with Eq. 3.

$$Toi = \frac{TDM_{dn}}{TDM_{d0}} \cdot 100 \quad (2)$$

Table 1. Physicochemical analysis of the soil used

Substrate	pH <sub>water</sub> 1:1	Ca + Mg <sup>(1)</sup>	Al <sup>(1)</sup>	H + AL	P <sup>(2)</sup>	K <sup>(2)</sup>	Cu <sub>soluble</sub>	OM	Clay
		cmol <sub>c</sub> kg <sup>-1</sup>							
Soil	5.2	4.23	0.33	5.34	2.16	61.52	0.21	1.15	65.00
Soil + peat*	5.3	15.21	0.21	6.11	43.28	368.53	0.12	4.38	57.00

The pH of the soil was determined in water (1:1); <sup>(1)</sup> Extractor KCl 1 mol L<sup>-1</sup>; H+Al determined by SMP index; <sup>(2)</sup> Extractor Mehlich-1. OM: Sulphochromic solution with exterior hit; Clay was determined with hydrometer after soil dispersion in sodium hydroxyl solution. \*200 mL of peat L<sup>-1</sup> of soil

$$\text{Tri} = \frac{\text{CuAS}_{\text{dn}}}{\text{CuAT}_{\text{dn}}} \cdot 100 \quad (3)$$

The results were submitted to variance analysis and when a significant interaction was found, analysis was conducted regarding the regression of the quantitative factor within each qualitative factor level. In the case of parameters without a significant interaction, simple effects were deployed, the means of the qualitative factor were compared by the Tukey test at 0.05 error of probability, while the quantitative factor was subjected to polynomial regression analysis by the Sisvar program (Ferreira, 2011).

## RESULTS AND DISCUSSION

After the addition of metal doses, the pseudo-total contents of Cu(II) in the soil increased (Figure 1). The contents of Cu(II) in the soil with values above 200 mg kg<sup>-1</sup> are regarded as a minimum limit for the investigation of agricultural soils, according to resolution no. 420 (CONAMA, 2009). The reason is that they cause toxic effects in the root system, such as the deformation of roots (Yruela, 2009) and reduce plant growth in the aerial part (Taiz & Zeiger, 2013). Data showed that the process of soil contamination with Cu(II) was efficient, enabling the development of this experiment.

The interaction between peat and doses of Cu(II) was significant ( $p \leq 0.05$ ) for the height, dry matter of the aerial part, Cu(II) content and Cu(II) accumulated in the root system and aerial part, and for the Cu(II) translocation index in the seedlings of *S. multijuga* (Figures 2 and 3).

The height of the plants and dry matter of the aerial part were statistically superior with the use of 200 mL of peat L<sup>-1</sup> of soil (Figures 2A, B). By increasing the doses of Cu(II) in the soil in the absence of peat, there was a quadratic reduction of the height and dry matter of the aerial part, with their respective minimum point at 228 and 256 mg of Cu kg<sup>-1</sup> of

soil, respectively (Figures 2A, B). Even when using 200 mL of peat L<sup>-1</sup> of soil, the Cu(II) doses caused a linear reduction in the heights of *S. multijuga* seedlings and quadratic reduction for the dry matter of the aerial part. Copper (Cu) interferes with the electron transport chain of photosystem I (Taiz & Zeiger, 2013), reduces the production of assimilates and significantly decreases apical growth. Results have shown that the addition of peat may have reduced the toxic effect of Cu(II) on the height and dry matter of the aerial part of the *S. multijuga* seedlings.

The contents of Cu(II) in the root system and aerial parts of the *S. multijuga* seedlings were higher with the use of 200 mL peat L<sup>-1</sup> soil (Figures 2C, D). This result is unexpected because peat is considered a Cu(II) toxicity ameliorating agent (Santos & Rodella, 2007). Hence, it reduces the amount of the metal available for plant uptake, since this material contains a natural high amount of humic substances, which are responsible for the adsorption of metals (Franchi et al., 2003). However, Brown et al. (2000) reported that the elementary composition of peat, as well as their properties, depend on factors such as the nature of the vegetation, the climate in the region and the degree of decomposition. Also, there is a difference between peats and peatlands.

The results showed a higher accumulation of Cu(II) in the root system and in the aerial parts of *S. multijuga* seedlings, after using 200 mL of peat L<sup>-1</sup> of soil (Figures 2E, F). This result can be attributed to the capacity of peat in increasing the soil chemical properties (Table 1). In addition, macroporosity, hydraulic conductivity and the decreasing apparent density of the soil area improved. These factors are relevant for the circulation of fluids and for the development of roots, favoring a higher volume of exploited soil and, therefore, greater absorption of water and chemical elements such as Cu(II) (Franchi et al., 2003). The use of peat did not achieve the goal of reducing the Cu(II) content to an acceptable value for the plants, but it could be used to help in metal extraction by plants due to the significant increase in the accumulation of Cu(II) in their tissues. Especially in the root system which has greater accumulation (Figure 2E) than the aerial parts of the *S. multijuga* seedlings (Figure 2F).

In phytoremediation studies, the greater accumulation of a contaminant in the roots is interesting (Caires et al., 2011). This is because the accumulation of a contaminant in the root system, favors non-translocation to the aerial part. Therefore, it avoids greater risks to the plants due to the low activity of the contaminant in the plants (Marques et al., 2000). However, according to the results obtained for the accumulation of Cu(II), the species of *S. multijuga* presented low phytostabilization potential when compared to other studies with different plant species such as *Brassica juncea* and *Cedrela fissilis* (Santos & Rodella, 2007; Caires et al., 2011).

The percentage of Cu(II) translocated to the aerial part was significantly higher with the use of 200 mL of peat L<sup>-1</sup> of soil, and was further reduced with increasing doses of Cu(II) (Figure 3). According to Kabata-Pendias (2011), Cu(II) is strongly bound to the cellular walls of the roots, not being immediately mobile in the plant, thereby enabling greater quantities of Cu(II) to remain in the root system. The results indicate that the transfer of Cu(II) ions from the root to the

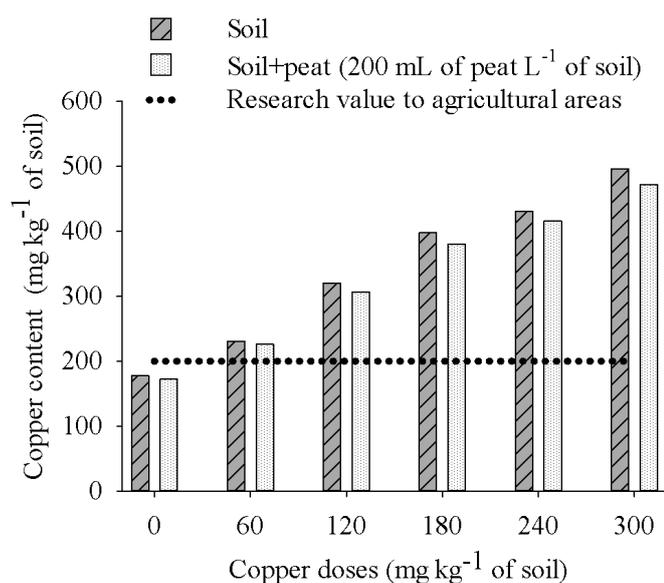
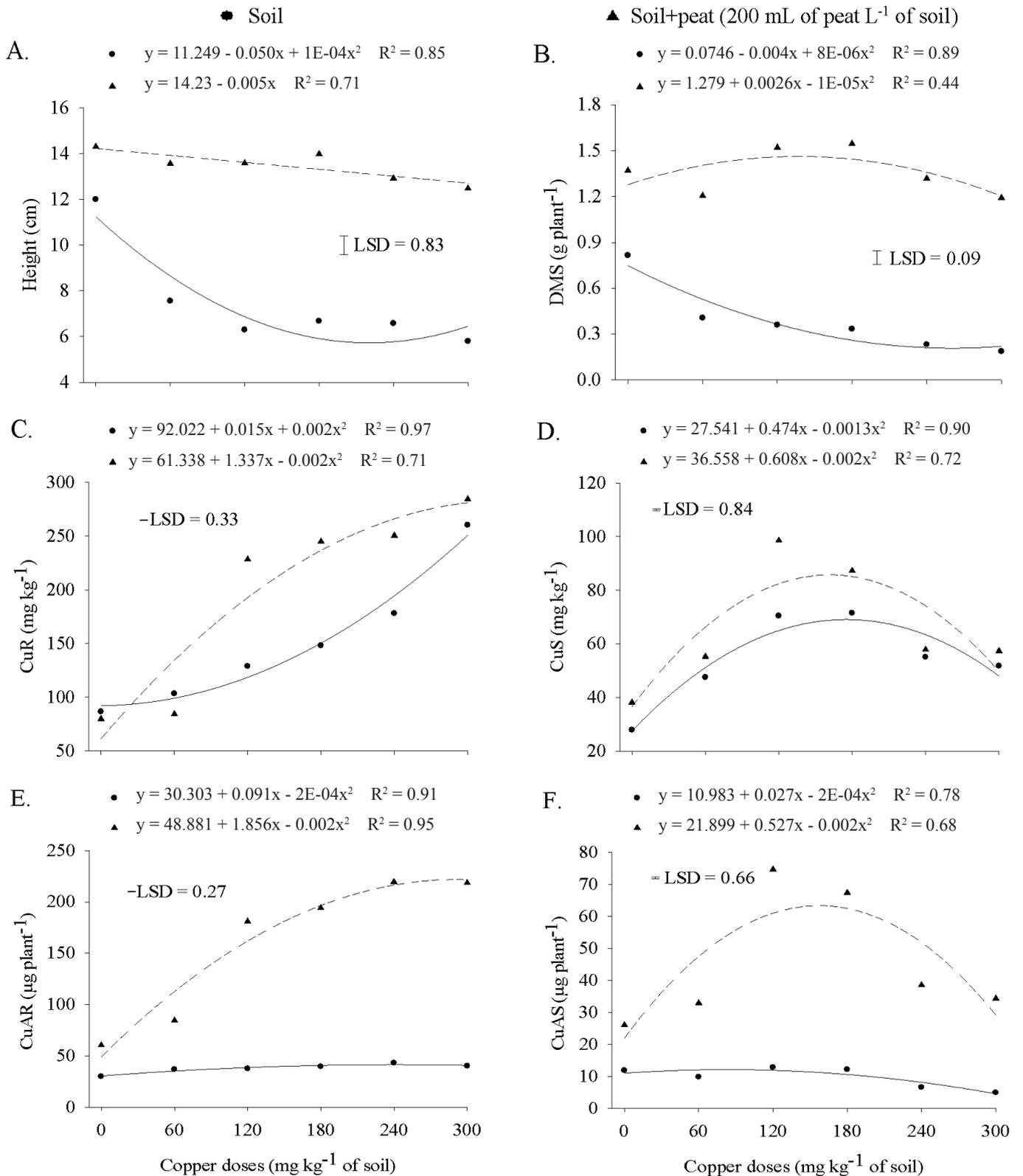


Figure 1. Initial contents of copper in the soil: Pseudo-total due to the application of copper doses to the soil with and without the amendment of peat (200 mL peat L<sup>-1</sup> soil) (USEPA, 1996)



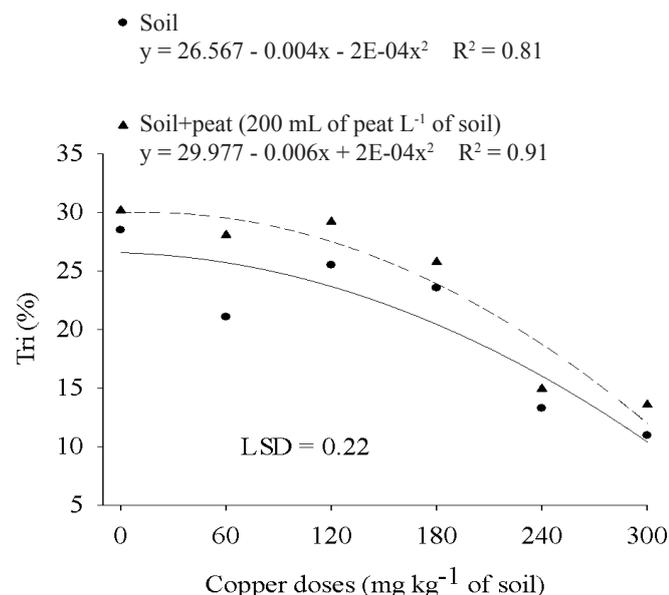
LSD = Least significant difference

Figure 2. Regression equations for the height – (A); dry matter of the aerial part (DMS) – (B); content of copper in the root (CuR) – (C); and aerial part (CuS) – (D); accumulated copper in the root (CuAR) – (E); and in the aerial part (CuAS) – (F) in the *S. multijuga* seedlings subjected to copper doses in the absence and presence of peat (200 mL peat L<sup>-1</sup> soil)

aerial parts in the *S. multijuga* seedlings was facilitated with the use of 200 mL of peat L<sup>-1</sup> of soil, and possibly stimulated the increase in height and dry mass of the shoots.

There was no significant relationship ( $p > 0.05$ ) between the addition or non-addition of peat and the Cu(II) doses applied to the soil for the stem diameter, root dry matter,

specific superficial area, Dickson quality index and tolerance index in the *S. multijuga* seedlings (Table 2). After analyzing the isolated effect of the variation factor of peat addition, it was found that with the use of 200 mL of peat L<sup>-1</sup> of soil, there was a significant increase in the stem diameter, root dry matter, specific superficial area, Dickson quality index and tolerance



LSD = Least significant difference

Figure 3. Regression equations for the translocation index (Tri) in *S. multijuga* seedlings subjected to copper doses in the absence and presence of peat (200 mL peat L<sup>-1</sup> of soil)

index in the *S. multijuga* seedlings (Table 2). This can be justified because peat improved the physical (Franchi et al., 2003) and chemical properties of the soil (Table 1), thereby allowing expansion of the root system.

The stem diameter, root dry matter and specific superficial area were linearly reduced with increase in the soil Cu(II) doses (Table 2). According to the estimated values, there was a reduction between the control treatment (natural soil) and the 300 mg dose of Cu kg<sup>-1</sup> of soil after plant growth, with 17% for the stem diameter, 39.7% for the dry matter of the root part and 43.2% for the specific superficial area. Studies with tree species have shown a reduction in the stem diameter and root system due to elevated contents of Cu(II) in the soil (Dellai et al., 2014; Silva et al., 2014). This high soil Cu content inhibits root growth, being one of the most evident symptoms of Cu(II) toxicity, just like what was observed in this study.

The Dickson quality index (DQI) was linearly reduced with the addition of Cu(II) doses to the soil, causing an estimated reduction of 33.9% between the dose zero and the dose 300 mg of Cu kg<sup>-1</sup> of soil (Table 2). Cu(II) doses have also reduced the DQI in timbó seedlings (*Ateleia glazioviana* Bail.) (Silva et al., 2012). However, Silva et al. (2014) concluded that the *S. multijuga* seedlings did not present alteration in DQI until reaching a dose of 450 mg of Cu kg<sup>-1</sup> of soil (the authors used

the botany synonymy - *Cassia multijuga* Rich). This difference between the studies of Silva et al. (2014) and this study, may be related to different soil chemical properties such as pH and the duration of the experiments.

The Cu(II) doses caused a linear reduction in the tolerance index of the seedlings to Cu(II) (Table 2). The tolerance to Cu(II) toxicity has been proposed in the literature and includes mechanisms such as the enzyme activities in the detoxification of free radicals, Cu(II) chelating by organic compounds as well as compartmentalization in the plant vacuole (Qian et al., 2005). The results of this study indicates that the *S. multijuga* species is not tolerant to Cu(II) because there was a reduction in the production of total dry matter, starting from 60 mg kg<sup>-1</sup> Cu(II) related to the non-contaminated soil (Toi < 100%).

The use of 200 mL peat L<sup>-1</sup> of soil provided a significant increase in the content and accumulated Cu(II) in the tissues and morphological parameter of the *S. multijuga* seedlings. It is important to infer that the dose of peat used did not act as an ameliorating agent against Cu(II) contamination, and it was incapable of reducing the metal contents available for plant uptake. However, it acted as a soil conditioner, enabling an increase in the morphological parameters of *S. multijuga* seedlings.

## CONCLUSIONS

1. The use of 200 mL of peat L<sup>-1</sup> of soil was not efficient as an ameliorating agent for copper-contaminated soil, but it acted as a soil conditioner, increasing the morphological parameters of *S. multijuga* seedlings.
2. The *S. multijuga* species presented low copper phytostabilization potential in the contaminated soil during its initial development period.

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Table 2. Regression analysis for copper variation factor and Tukey's test to peat variation factor for parameters without significant interaction such as stem diameter (SD), root dry matter - (DMR), specific superficial area - (SSA), Dickson quality index - (DQI) and tolerance index - (Toi) in the *S. multijuga* seedlings

Parameters	Regression analysis for copper			Tukey analysis for peat		
	Regression equation	R <sup>2</sup>	PR (%) <sup>(1)</sup>	Without	With <sup>(2)</sup>	CV (%)
SD (mm)	$\hat{y} = 3.531 - 0.0020x$	0.90	- 17.0	2.17B <sup>(3)</sup>	4.18A	12.89
DMR (g plant <sup>-1</sup> )	$\hat{y} = 0.681 - 0.0009x$	0.94	- 39.7	0.27B	0.83A	21.42
SSA (cm <sup>2</sup> )	$\hat{y} = 50.708 - 0.0730x$	0.91	- 43.2	28.83B	50.51A	18.09
DQI	$\hat{y} = 0.354 - 0.0004x$	0.94	- 33.9	0.14B	0.45A	23.58
Toi (%)	$\hat{y} = 88.349 - 0.1030x$	0.98	- 35.0	50.12B	89.56A	23.69

<sup>(1)</sup>Percentage reduction at the highest dose of applied copper (300 mg kg<sup>-1</sup>). <sup>(2)</sup>Addition of 200 mL L<sup>-1</sup> of soil. <sup>(3)</sup>Means followed by the same letter in the column do not differ from each other according to the Tukey test at 0.05 error of probability. CV - Coefficient of variation

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