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Copper translocation and tolerance in seedlings of tree species grown in contaminated soil

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A B S T R A C T

High concentrations of copper (Cu) may be toxic to plants, yet some species can be tolerant to soils contaminated by this metal. The study aimed to evaluate Cu translocation and tolerance in seedlings of 'timbaúva' (*Enterolobium contortisiliquum* (Vell.) Morong), 'pata-de-vaca' (*Bauhinia forficata* Link) and 'amendoim-bravo' (*Pterogyne nitens* Tul) subjected to Cu-contaminated soil. The study was conducted in a greenhouse for 120 days, using as substrate a clayey Oxisol. The experiment was set in a completely randomized design, using a 3 x 6 factorial scheme, corresponding to three forest species ('timbaúva', 'pata-de-vaca' and 'amendoim-bravo') and six Cu doses in the soil (0, 60, 120, 180, 240 and 300 mg kg^{-1}), with six replicates. The following variables were analysed: total dry matter, Cu content and its accumulation in roots and shoots, tolerance and translocation indices and the coefficient of impact of the contamination on the relative contents of Cu in roots and shoots. 'Timbaúva' showed lower reduction in total dry matter with the increase of Cu doses in the soil, proving to be able to decrease Cu translocation to the shoots and tolerate doses of up to 236 mg kg^{-1} of this metal in the soil. 'Timbaúva' seedlings are more tolerant to contamination of Cu in soil, compared with seedlings of 'amendoim-bravo' and 'pata-de-vaca'.

Palavras-chave:
metal pesado
fitorremediação
reflorestamento
cobertura do solo

Translocação e tolerância de cobre em mudas de espécies arbóreas cultivadas em solo contaminado

R E S U M O

Concentrações elevadas de cobre (Cu) podem ser tóxicas às plantas; contudo, algumas espécies podem ser tolerantes a solo contaminado por este metal. Objetivou-se avaliar, no trabalho, a translocação e a tolerância de Cu em mudas de timbaúva, pata-de-vaca e amendoim-bravo submetidos a solo contaminado com o referido metal. O trabalho foi conduzido em casa de vegetação por 120 dias utilizando-se, como substrato, um Latossolo Vermelho com textura argilosa. O delineamento experimental foi inteiramente casualizado em arranjo fatorial (3 x 6), sendo três espécies florestais (timbaúva, pata-de-vaca e amendoim-bravo) e seis doses de Cu no solo (0, 60, 120, 180, 240 e 300 mg kg^{-1}), com seis repetições. Foram avaliados a massa seca total, os teores e a quantidade acumulada de Cu no sistema radicular e, na parte aérea, índice de tolerância, índice de translocação e o coeficiente de impacto no teor relativo. A timbaúva evidenciou menor redução da massa seca total com a elevação das doses de cobre no solo indicando ser uma espécie capaz de diminuir a translocação do metal para a parte aérea e tolerar doses de até 236 mg kg^{-1} do metal no solo. As mudas de timbaúva são mais tolerantes à contaminação do solo com cobre que as de amendoim-bravo e pata-de-vaca.

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Introduction

Copper (Cu) is a chemical element considered as essential for plants, participating as a catalyzer of biochemical reactions in the metabolism of carbohydrates and nitrogen, in the synthesis of chlorophyll and in the constitution of protein in plants (Taiz & Zeiger, 2013). However, it is also one of the main soil-polluting heavy metals (Andreazza et al., 2010), since its excess can inhibit cell elongation and prevent important cell processes, such as the transport of electrons in the photosynthesis (Taiz & Zeiger, 2013). According to Accioly & Siqueira (2000), Cu concentrations above 100 mg kg^{-1} of soil can cause toxic effects on plants in clayey soils.

Some studies report that plants are not able to avoid the absorption of heavy metals completely, showing different mechanisms of tolerance (Alves et al., 2008; Caires et al., 2011; Silva et al., 2011). This tolerance is directly related to the physiological and biochemical response of the species, as well as to the capacity of translocation of the element in the plant (Souza et al., 2011). Thus, some plants are tolerant to heavy metals because they have developed a complex mechanism of homeostasis, controlling the absorption, accumulation and translocation of heavy metals in the tissues (Santos et al., 2006).

With respect to the native tree species, the use of plants from the Fabaceae family is a practice recommended for the recovery of soil cover due to their capacity to incorporate nitrogen and the consequent improvement of soils through the increase in the deposition of organic material and improvement of soil physical properties (Manhães et al., 2007). The species 'timbaúva' (*Enterolobium contortisiliquum* (Vell.) Morong), 'pata-de-vaca' (*Bauhinia forficata* Link) and 'amendoim-bravo' (*Pterogyne nitens* Tul) represent this family of fast-growing plants, which arises the interest in their use to recover degraded areas (Lorenzi, 2009; Pellizzaro et al., 2011; Behling et al., 2013).

Studies have described the forest species of the Fabaceae family as promising for the revegetation of degraded soils and for the acceleration of the ecological succession process (Freire et al., 2010; Chaer et al., 2011). In this context, besides improving soil characteristics, the species of this family may be tolerant to high Cu contents and show the capacity to immobilize metals in their tissues. 'Timbaúva' has shown tolerance to Cu-contaminated soil (Silva et al., 2011), but there are no studies on the behavior of 'pata-de-vaca' and 'amendoim-bravo'. Therefore, this study aimed to evaluate Cu translocation and tolerance in seedlings of 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo' subjected to a Cu-contaminated soil.

Material and Methods

The experiment was carried out in a greenhouse of the Department of Agronomic and Environmental Sciences of the Federal University of Santa Maria, campus of Frederico Westphalen-RS, Brazil. The soil used in the experiment was classified as Red Latosol (Santos et al., 2013) and its chemical and physical analyses (Table 1) were performed according to EMBRAPA (1997).

Table 1. Chemical analysis of the soil used for the development of seedlings of 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo' in greenhouse

pH water	Ca + Mg	Al	H + Al	P	K	Cu	OM	Clay
(1:1)	$cmol_c\ dm^{-3}$			mg dm^{-3}			g dm^{-3}	
5.4	5.4	0.4	4.3	6.6	110	15.1	24	810

The seeds of the forest species 'timbaúva' (*Enterolobium contortisiliquum* (Vell.) Morong), 'pata-de-vaca' (*Bauhinia forficata* Link) and 'amendoim-bravo' (*Pterogyne nitens* Tul) were subjected to the overcoming of tegument dormancy through mechanical scarification on the opposite side of the hilum, using a Norton 80 sandpaper. Seeding was performed at a depth of 0.5 cm in 125-cm^3 polypropylene recipients, filled with sieved soil (0.5-cm grid), by planting three seeds per tube. Thinning was performed when the seedlings showed one pair of definitive leaves, leaving only one plant per recipient and considering phytosanitary aspects and vigor, which constituted the experimental unit.

The experiment was set in a completely randomized design with six replicates, using a 3 x 6 factorial scheme, which represented three native forest species and six Cu doses added to the soil (0, 60, 120, 180, 240, 300 mg kg^{-1}). The Cu doses were applied in the form of a solution of copper sulfate ($CuSO_4.5H_2O$) and diluted in 50 mL of distilled water for later mixture with the soil.

The study was conducted for 120 days after seeding, with three daily irrigations, maintaining the soil at approximately 80% of field capacity by weighing the experimental units. After this period, the following variables were analyzed: total dry matter, Cu contents and its accumulation in roots, shoots and in the entire plant, translocation and tolerance indices and the coefficient of impact of the contamination on the relative contents of Cu in roots and shoots. Shoots and roots were separated in the base of the plant, kept in a forced-air oven at 65 ± 1 °C until constant weight and then weighed using an analytical scale. Total dry matter was obtained by the sum of shoot and root dry matters. The Cu contents in shoot and root dry matter of the seedlings were obtained through wet digestion with HNO_3 + $HClO_4$ (3:1) and determined through atomic absorption spectrophotometry (Miyazawa et al., 2009).

Based on total dry matter (TDM), Cu contents (mg kg^{-1}) in shoots (CuS), roots (CuR) and total (CuT), on Cu accumulation (μg $plant^{-1}$) in shoots (CuAS), roots (CuAR) and total (CuAT), on the zero dose of Cu (d_0) and on the doses from 60 to 300 mg kg^{-1} (d_n), the indices of tolerance (ITOL) and translocation (ITRA) and the coefficient of impact on the relative content (CIRC) were calculated. ITOL (Eq. 1) measures the ability of the seedlings to grow in environments with high Cu concentrations (Wilkins, 1978), ITRA (Eq. 2) corresponds to the total percentage of absorbed Cu transported to the shoots (Abichequer & Bohnen, 1998) and CIRC (Eq. 3.) represents the impact of the contamination on the relative contents of Cu in roots and shoots (Marques et al., 2000).

$$ITOL = \frac{TDM_{dn}}{TDM_{d0}} \times 100 \quad (1)$$

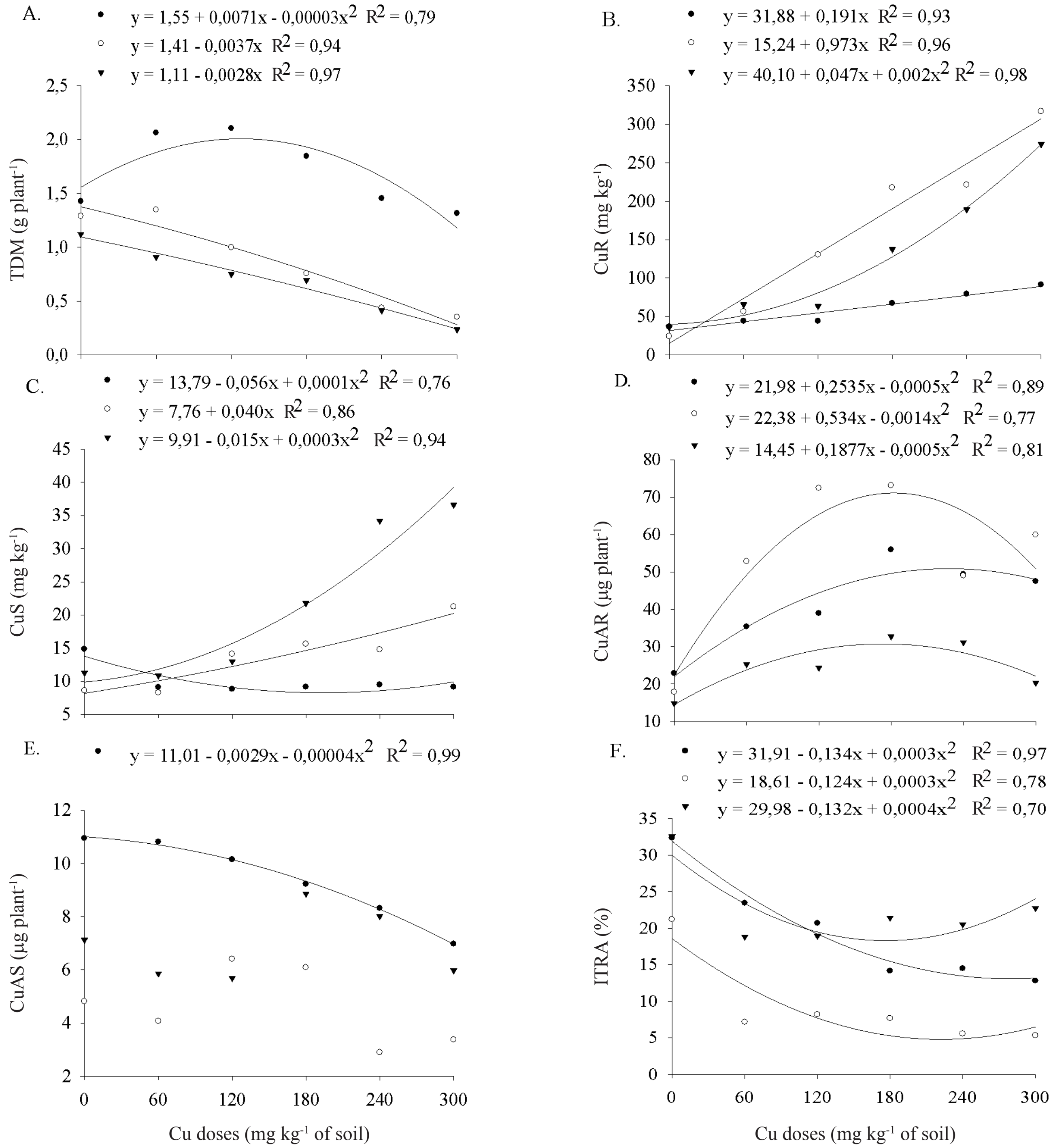
$$ITRA = \frac{CuAS_{dn}}{CuAT_{dn}} \times 100 \quad (2)$$

$$CITR = \frac{\left(\frac{CuR_{dn}}{CuS_{dn}}\right)}{\left(\frac{CuR_{d0}}{CuS_{0}}\right)} \qquad (3)$$

The results were subjected to analysis of variance and, when significant, means of quantitative treatments were analyzed through regression and means of qualitative treatments through Tukey test at 0.05 probability level, using the procedures available in the statistical program SISVAR.

Results and Discussion

The analysis of variance showed significant interaction ($p \leq 0.05$) between the forest species and the Cu doses for all the analyzed variables. 'Pata-de-vaca' and 'amendoim-bravo' showed linear reduction in TDM with the Cu doses, while 'timbaúva' showed maximum TDM at the Cu dose of 118 mg kg^{-1} (Figure 1A). TDM values for 'timbaúva' were significantly higher compared with 'pata-de-vaca', for all the doses, and with 'amendoim-bravo', from the Cu dose of 60 mg kg^{-1} on (Table 2). Chaves et al. (2010) also reported reduction of dry



*Only regression equations with $R^2 > 0.60$ are presented

Figure 1. Regression equations for total dry matter – TDM (A), copper contents in roots – CuR (B) and shoots – CuS (C), copper accumulation in roots – CuAR (D) and shoots – CuAS (E) and index of translocation of copper – ITRA (F) in seedlings of 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo' subjected to doses of copper (Cu) in the soil

Table 2. Total dry matter (TDM), copper contents in roots (CuR) and aerial parts (CuS), copper accumulation in roots (CuAR) and aerial parts (CuAS) and index of translocation (ITRA) of copper in seedlings of 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo' subjected to doses of copper (Cu) in the soil

Species	Cu doses (mg kg^{-1})						LSD	CV (%)
	0	60	120	180	240	300		
				TDM (g plant^{-1})				
Timbaúva	1.43 a*	2.06 a	2.10 a	1.85 a	1.45 a	1.32 a	0.19	8.86
Pata-de-vaca	1.29 ab	1.35 b	1.00 b	0.76 b	0.44 b	0.35 b		
Amendoim-bravo	1.12 b	0.91 c	0.75 c	0.69 b	0.41 b	0.24 b		
				CuR (mg kg^{-1})				
Timbaúva	36.88 a	44.18 b	44.15 c	67.38 c	79.26 c	91.42 c	19.13	8.24
Pata-de-vaca	24.39 a	56.57 ab	130.40 a	217.72 a	221.28 a	316.73 a		
Amendoim-bravo	35.98 a	65.64 a	63.46 b	137.50 b	189.23 b	274.09 b		
				CuS (mg kg^{-1})				
Timbaúva	14.86 a	9.12 ab	8.83 b	9.16 c	9.48 c	9.15 c	2.19	7.31
Pata-de-vaca	8.61 c	8.28 b	14.13 a	15.62 b	14.81 b	21.26 b		
Amendoim-bravo	11.28 b	10.86 a	13.00 a	21.77 a	34.21 a	36.60 a		
				CuAR (μg plant^{-1})				
Timbaúva	22.90 a	35.35 b	38.91 b	55.94 b	49.36 a	47.50 b	6.72	8.39
Pata-de-vaca	17.87 ab	52.83 a	72.40 a	73.11 a	48.99 a	59.91 a		
Amendoim-bravo	14.81 b	25.27 c	24.36 c	32.70 c	31.14 b	20.31 c		
				CuAS (μg plant^{-1})				
Timbaúva	10.95 a	10.82 a	10.15 a	9.23 a	8.32 a	6.98 a	0.83	5.95
Pata-de-vaca	4.81 c	4.08 c	6.41 b	6.09 b	2.90 b	3.37 c		
Amendoim-bravo	7.13 b	5.86 b	5.69 b	8.87 a	8.02 a	5.98 b		
				ITRA (%)				
Timbaúva	32.37 a	23.45 a	20.69 a	14.17 b	14.51 b	12.82 b	2.48	7.28
Pata-de-vaca	21.21 b	7.17 c	8.22 b	7.69 c	5.58 c	5.35 c		
Amendoim-bravo	32.56 a	18.85 b	18.95 a	21.45 a	20.53 a	22.75 a		

*Means followed by the same letter in the column do not differ by Tukey test at 0.05 probability level; LSD – least significant difference; CV (%) – coefficient of variation

matter production of jatropha (*Jatropha curcas* L.) as Cu doses increased. Similar results were obtained by Silva et al. (2011), in seedlings of 'timbaúva' grown in sandy soil contaminated with Cu. The results of this study evidence that the TDM of 'timbaúva' is negatively affected only by high Cu doses in the soil.

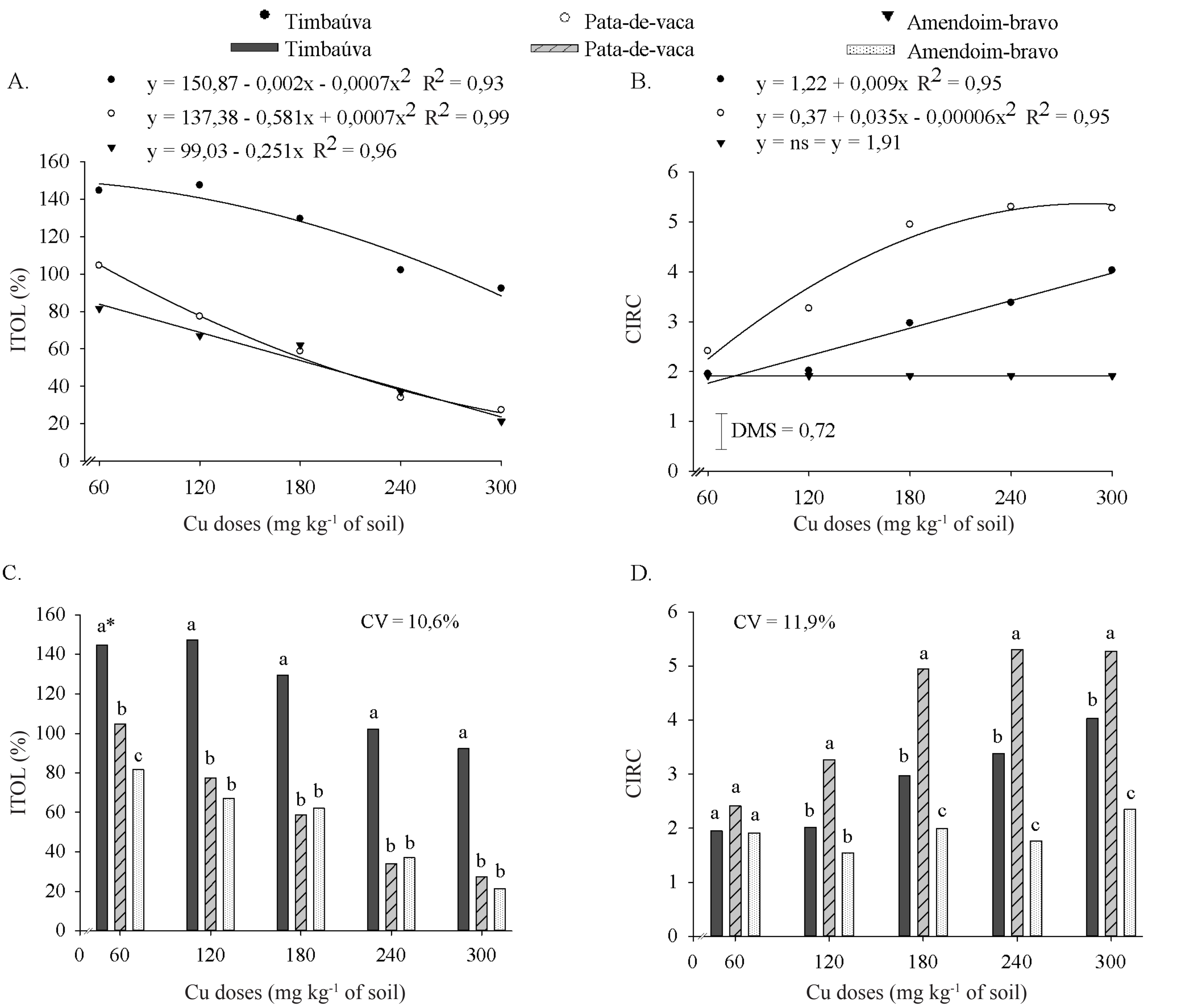
'Pata-de-vaca' and 'amendoim-bravo' showed a significant increment in the CuR and CuS, compared with 'timbaúva' at the highest Cu doses in the soil (Figure 1B, C and Table 2). Silva et al. (2011) also observed increase in Cu contents in the roots of 'timbaúva', 'angico-vermelho' (*Parapiptadenia rigida* (Benth.) Brenan) and 'canafístula' (*Peltophorum dubium* (Spreng.) Taub.), and a reduction only in the shoots in the 'angico-vermelho', as a function of the applied doses. The results of this study may indicate the existence of a biochemical mechanism in 'timbaúva' that allows lower Cu contents in roots and shoots, compared with the other tested species. For Lequeux et al. (2010), this has been attributed to the fact that plants develop adaptive mechanisms in response to the toxicity of metals. In this sense, Yruela (2009) reported some cell mechanisms that may be involved in the tolerance, such as the reduction of the absorption through the release of extracellular exudates by the roots and the flow of the metal due to higher pumping of the plasmatic membrane and to the chelation by acids.

The results evidence the increase in CuAR until Cu doses of 250, 190 and 180 mg kg^{-1} for 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo', respectively (Figure 1D and Table 2). For 'timbaúva', the accumulation of Cu may be associated with the increase in dry matter or Cu content in root tissues (Figures 1A, 1B), which indicates that it is a promising species to be used in soil with higher Cu contents. Greger (2003) claims that plants with low biomass production and high accumulation of metals are considered as hyperaccumulators, while the accumulators produce more biomass, but accumulate less metal compared to the former ones.

The values CuAS were lower than those of CuAR, with quadratic reduction for the species as a function of the Cu doses added to the soil. However, for 'pata-de-vaca' ($y = 4.44 + 0.18x - 0.00008x^2$) and 'amendoim-bravo' ($y = 6.25 + 0.011x - 0.00003x^2$), the index of determination was very low (0.47 and 0.08, respectively) and precise considerations were not possible (Figure 1E and Table 2). Plants with higher accumulation of metals in the root system, compared with the shoots, are classified as tolerant to the absorption of metals (Garbisu & Alkorta, 2001), since the capacity to limit the translocation to the shoots is one of the mechanisms through which the root system can contribute to the tolerance of some plant species to heavy metals (Chaves et al., 2010). In this case, 'timbaúva' may have higher ability to accumulate Cu in the root system than the other tested species.

The index of translocation (ITRA) decreased with the increase in Cu doses applied to the soil, with minimum point at 223, 207 and 165 mg kg^{-1} of soil for the seedlings of 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo', respectively (Figure 1F), and was statistically higher in 'amendoim-bravo' at high Cu doses (Table 2). Chaves et al. (2010) also observed a reduction in ITRA with the increase of Cu in the soil in seedlings of castor bean (*Ricinus communis* L.). This occurs because Cu is strongly bound to root cell walls and is not readily available in the plant (Kabata-Pendias, 2011). The results indicate that the transfer of these ions from roots to shoots in 'timbaúva' seedlings was less affected until the Cu dose of 223 mg kg^{-1}, in comparison to the other species.

Cu doses reduced the index of tolerance (ITOL) in the three forest species (Figure 2A), while 'timbaúva' was



*Means followed by the same letter do not differ by Tukey test at 0.05 probability level; CV (%) - coefficient of variation; LSD – least significant difference

Figure 2. Regression equations and means comparison test, respectively, for the index of tolerance – ITOL (A and C) and coefficient of impact of the contamination on the relative content – CIRC (B and D) of seedlings of 'timbaúva', 'pata-de-vaca' and 'amendoim-bravo' subjected to doses of copper (Cu) in the soil

significantly more tolerant compared with 'pata-de-vaca' and 'amendoim-bravo' (Figure 2C). Tolerant plants have biochemical adaptations that control absorption, accumulation and translocation of heavy metals in the tissues (Whiting et al., 2004; Santos et al., 2006), allowing these species to develop in soils in which the contents are toxic for other plants (Macnair et al., 2000). Thus, there would be a reduction in total dry matter of 'timbaúva' seedlings (ITOL < 100%) only above the estimated Cu dose of 268 mg kg^{-1} (Figure 2A), compared with the treatment without Cu application (ITOL = 100%), while for 'pata-de-vaca' and 'amendoim-bravo', the reduction in dry matter production occurs from the Cu dose of 60 mg kg^{-1} on (Figure 2C).

The coefficient of impact of the contamination on the relative content (CIRC) in the three forest species showed higher proportional retention of Cu in the roots in the contaminated soil (CIRC > 1), with linear increase for 'timbaúva' and quadratic increase for 'pata-de-vaca' (Figure 2B), being significantly higher in 'pata-de-vaca' from 120 mg kg^{-1} on (Figure 2D). Meda et al. (2007) reported that one of the tolerance mechanisms to toxic elements in plants is their higher retention in the root system, compared with the shoots. Thus, the linear increase of CIRC in 'timbaúva' suggests that its mechanism of tolerance to the excess of Cu is the capacity to absorb and retain this metal in the roots. In this case, it proved to be the species with the highest capacity to grow in soils with higher Cu contents.

Although this study has been developed using a clayey soil, which usually shows low Cu availability, since most of it is associated with more stable forms, bound to Fe and Al oxides and to the residual fraction (Borges & Coutinho, 2004), the results showed significant variation between the tested plants with respect to growth, translocation and tolerance to Cu, with the doses applied to the soil. In spite of that, the results only suggest a potential of 'timbaúva' to be used in phytoremediation of Cu-contaminated soils and further experiments, considering a longer cultivation period, are essential for more-conclusive results.

Conclusions

1. The reduction in the translocation of Cu from roots to shoots in seedlings of 'timbaúva' contributes to maintaining shoot Cu contents below the toxic level, not affecting its dry matter until the Cu dose of 236 mg kg^{-1} applied in the soil.

2. The seedlings of 'timbaúva' are more tolerant to high Cu contents in the soil, compared with seedlings of 'amendoim-bravo' and 'pata-de-vaca'.

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