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Growth of *Brachiaria decumbens* in Latosol contaminated with copper

Crescimento de *Brachiaria decumbens* em Latossolo contaminado com cobre

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ABSTRACT

Brachiaria decumbens presents high rusticity, rapid growth, as well as easy implantation and management. The aim of the study is to evaluate the growth of brachiaria in soil contaminated with copper. An experiment following a 3 x 4 factorial design was carried. It comprised three plant ages (30, 45 and 60 days after transplanting) and four copper concentrations (0, 20, 40 and 80 mg of Cu per kg of soil). Plant age and copper concentrations have influenced the main pseudostem length, the number of leaves, as well as the leaf area, chlorophyll (SPAD), fresh and dry shoot matter, root system volume, and fresh and dry root system matter of plants. The lowest dry shoot matter accumulation was found at the concentration 51.24 mg Kg^{-1}. The herein investigated copper concentrations have influenced the growth of *Brachiaria decumbens*. The lowest growth variable values were between 45.52 and 57.63 mg.Kg-1. Thus, brachiaria has shown potential to be used in Copper phytoremediation at concentrations below 45.52 mg Kg^{-1}.

Index terms: Adsorption; Cerrado; phytoremediation; heavy metal.

RESUMO

A *Brachiaria decumbens* apresenta alta rusticidade, crescimento rápido, facilidade na implantação e manejo. Avaliou-se o crescimento de braquiária em solo contaminado com cobre. Implantou-se um experimento em arranjo fatorial 3 x 4, sendo três idades das plantas (30; 45 e 60 dias após o transplante) e quatro dosagens de cobre (0; 20; 40 e 80 mg de Cu por kg de solo). A idade das plantas e as doses de cobre influenciaram no comprimento do pseudocolmo principal, número de folhas, área foliar, clorofila (SPAD), massa fresca e seca da parte aérea, volume do sistema radicular, e massa fresca e seca do sistema de radicular de plantas de Brachiária. O menor acúmulo de massa seca da parte aérea de plantas foi obtido da concentração de 51,24 mg Kg^{-1}. A *Brachiaria decumbens* demonstrou tolerância e potencial de cultivo em solos contaminados com cobre.

Termos para indexação: Adsorção; Cerrado; fitorremediação; metal pesado.

INTRODUCTION

Copper is one of the oldest known metals and the twenty-fifth most abundant element in the earth's crust. It is also a good electricity conductor. Copper was defined as essential to vegetables in 1930; however, high copper concentrations cause toxicity, mainly in plant roots (Kopsell; Kopsell, 2007).

Agricultural and industrial activities may increase the amount of copper in the soil, fact that may change the microorganism population, the biological activity (Li et al., 2016), and consequently, the soil productive capacity. When it comes to plants, Cu is associated with the transport of electrons between membranes during the photosynthetic process; it is found complexed with proteins and, when it is available in its free form, it produces free radicals with high oxidation capacity, damages cell membranes (Williams, 2015) and inhibits electron transport and primary metabolism. Overall, the excess of copper reduces plant growth, the number and thickness of roots, and biomass accumulation. However, many plants have defense mechanisms comprising the expression and activation of antioxidative enzymes, as well as the formation of chelating agents due to heavy metals. Plants also range from highly sensitive to tolerant to heavy metal accumulation (Ovečka; Takáč, 2014).

Currently, there are two prevailing segments able to minimize the impact heavy metals have on the soil. The first segment concerns the ability of each species to absorb the heavy metals available in the soil through phytoextraction and phytoremediation. The second one refers to plants' resistance to toxicity, which allows them to grow and produce even in soils containing high contaminant concentrations (Carmo et al., 2008).

According to studies about heavy metal remediation mechanisms, the use of *Brachiaria decumbens* may be an

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interesting alternative to identify and select copper-tolerant plants, because the species presents good biomass yield, tolerance to acidic soils (Arroyave et al., 2013), easy implantation, rapid growth, high photosynthetic efficiency, and efficient use of nutrients. The species may be even intercropped with other annual or perennial crops.

Santos et al. (2006) have highlighted that *Brachiaria decumbens* meets the requirements to be used in cadmium (Cd), zinc (Zn) and lead (Pb) phytoextraction due to its rapid growth, biomass accumulation and tolerance to aluminum. Thus, it is worth assessing the tolerance of plants adapted to Cerrado conditions to Cu levels in the soil, since there are no studies about species with aggressive growth behavior and adapted to Cerrado conditions, such as brachiaria plants. Santos et al. (2015) found that the cultivation of eucalyptus with *Brachiaria* between lines showed better results for recovery of soil contaminated with heavy metals.

The aim of the current study is to assess the growth of *Brachiaria decumbens* in latosol contaminated with copper.

MATERIAL AND METHODS

The current study was carried out from September to December 2015, in a greenhouse belonging to Instituto Federal Goiano (Goiano Federal Institute) - Campus Ceres, Ceres County - Goiás State, located at the geographic coordinates 15°21'18.65 "S and 49°36'26.26" W, 600 m altitude. The soil used in the experiment was medium texture red latosol. The region has tropical semi-humid climate and mean temperature 28 ºC.

The soil used in the current study was collected at Goiano Federal Institute - Campus Ceres, and it showed the following chemical and physical properties: 69.8% sand; 8.4% silt and 21.8% clay; pH (in water) = 7.0; O.M. = 14.2 g m^{-3} (colorimetric); P = 160.0 mg dm^{-3}, K = 1.0 cmolc dm^{-3}, Cu = 1.7 mg kg^{-1} (Mehlich-1); Ca = 4.1 cmolc dm^{-3}, Mg = 1.8 cmolc dm^{-3} (KCl mol.1^{-1}); H^+ Al = 0.0 cmolc dm^{-3} (SMP buffer at pH 7.5); CTC = 7.9 and V = 87.3%. The methodology used in all soil analyses has followed the recommendations by EMBRAPA (2011). The analyses were carried out at Goiano Federal Institute Soil Laboratory - Ceres Campus. The preparation of the collected soil was based on drying the samples in open air for physical and chemical analysis purposes. The clods were crushed and the samples were sieved in a 2mm mesh to obtain the air-dried fine earth (ADFE).

The pH values were obtained by electronically measuring the Hydrogen potential using a combined electrode immersed in soil: water suspension (1:2.5). The solution was based on 10 cm^3 ADFE, using a 25 ml water dosing scoop in a 50ml beaker. It was stirred in a TECNAL Te-145 horizontal stirrer (orbital stirring table) at 180 rpm, for 5 minutes. A glass stick was used to stir the sample after 30 minute rest and the reading was carried out in a LOGEN LS-300 HH potentiometer with combined electrode.

The (H^+ Al) estimate and the soil acidity extraction used buffered calcium acetate determined through combined electrode immersed in 0.01 moL^{-1} buffered $CaCl_2$ solution. The reading was performed in a potentiometer with combined electrode and, at the end, a table was used to estimate the H^+ Al values.

The total fraction of phosphorus content in the soil corresponded to the content used by the plants. The extraction was based on 5 cm^3 ADFE and 50 mL Mehlich-1 extracting solution (0.05 moL^{-1} HCl + 0.0125 moL^{-1} H_2SO_4). After the sample was left to rest for at least 16 h, 5 mL of working reagent was added to it and the phosphorus content was estimated in the BEL Photonice UV/Vis spectrophotometer, Model LGS 53, with wavelength 730 nm and frequency 50.

The potassium estimate was based on 5 cm^3 ADFE and 50 ml Mehlich-1 extracting solution. The reading was carried out in the Analiser flame photometer, Model 910 MS.

The organic matter (O.M.) estimate was based on 1 cm^3 ADFE and 10 mL digesting solution ($Na_2Cr_2O_7.2H_2O$ + H_2SO_4). The reading was performed in the BEL Photonice UV/Vis Spectrophotometer, Model LGS 53.

The Ca and Mg estimates were based on 5 cm^3 ADFE and 50 ml of 1 mol^{-1} KCl extracting solution. The solution was stirred and left to rest for at least 16 hours; after this period, a 1 ml aliquot of the supernatant was added with 17 ml of La_2O_3 solution at 0.1%. The Ca and Mg were estimated in a GBC atomic absorption spectrophotometer, model SAVANT AA.

The Al estimate was based on 5 cm^3 ADFE and 50 ml of 1 moL^{-1} KCl extracting solution. After the solution was left to rest for at least 16 hours, it was titrated with 0.025 moL^{-1} NaOH using bromothymol blue.

The textural analysis used the pipet method and it was based on 10 cm^3 ADFE and 50 ml NaOH. After the solution was stirred and left to rest for at least 16 hours, it was transferred to a 1000 mL beaker and topped with distilled water. Subsequently, it was stirred and left to rest for 4 minutes. The temperature of 25 mL suspension was measured and the sedimentation time of the fractions was calculated.

The contamination was carried out through soil spraying with copper sulfate pentahydrate solution ($CuSO_4.5H_2O$) at the concentrations 0, 20, 40 and 80 mg Kg^{-1}; a cement mixer was used to homogenize the Cu in the soil. The contaminated soil was distributed in 5.0 L plastic containers.

Brachiaria seedlings were produced in a 128 cell polystyrene tray filled with commercial substrate using *Brachiaria decumbens* seeds produced by the company "Sementes Moeda" - Quirinópolis - GO and sold by the local stores. After the seedlings presented four leaves, approximately fifteen days after sowing, they were transferred to the pots containing the contaminated soil and kept in a greenhouse under micro sprinkler irrigation at two-hour intervals, during the experimental period.

The experiment has followed a randomized complete block design comprising a 3 x 4 factorial arrangement, three plant ages (30, 45 and 60 days after transplantation), four copper concentrations (0, 20, 40 and 80 mg kg^{-1}) and four repetitions.

The number of tillers and leaves, the leaf area, the pseudostem length, the longest root length, the root system volume, the chlorophyll in the leaves, the fresh and dry shoot matter, and the fresh and dry root system matter were assessed in each collection.

An analytical scale (Shimadzu AY Precision Series) with 220g capacity and 0.0001g accuracy - Inmetro certification - was used to measure the fresh shoot and root matters. The roots were separated from the soil (through washing), and weighed and measured after the water was drained.

A CID INC laser meter, model CI-203 (Bio-Science®, Camas, WA, USA) was used to measure the leaf area, whereas the chlorophyll content (SPAD) was measured in a Minolta portable chlorophyll meter, Mod. SPAD 502 (Soil and Plant Analysis Development).

The data were subjected to ANOVA; the means of the assessment period were compared through Tukey test at 5% error probability. A linear regression analysis was carried out to find the plants' response tendencies according to the copper concentrations, which should be explained through the quadratic polynomial linear equation.

RESULTS AND DISCUSSION

The copper concentrations and the plant age have influenced the number of leaves, leaf area, pseudostem length, root system volume, chlorophyll (SPAD), fresh and dry shoot matter, and fresh and dry root system matter. On the other hand, the number of tillers was influenced by the interaction between copper concentrations and sampling times. The pseudostem length, root system volume, fresh and dry shoot matter, and dry root system matter have increased as the plants grew older (Table 1). However, the chlorophyll has shown reversed response (Table 2).

Santos et al. (2011) have estimated the life span of *Brachiaria decumbens* leaves in 37.8 days. According to Bianco et al. (2000), the biomass accumulation has increased for 160 days after the plants emerged. It has reached 38.27 g of total dry matter and 9.67 g of leaves per plant. *Brachiaria* plants have continuous and indeterminate growth; new leaves emerge simultaneously to the senescence of the older leaves. The dry matter of the plants was higher than that found by Bianco et al. (2010). By considering the growth

Table 1: Fresh shoot matter (FSM), dry shoot matter (DSM), fresh root system matter (FRSM) and dry root system matter (DRSM) of *Brachiaria decumbens* plants at different ages, cultivated in soil contaminated with copper.

Plant's age (days)	FSM (g)	DSM (g)	FRSM (g)	DRSM (g)
30	31.33 c	6.44 c	38.02 b	4.50 c
45	55.49 b	24.56 b	81.15 a	14.63 b
60	77.28 a	36.86 a	81.27 a	30.23 a

*Mean treatment values followed by the same letter in the column do not statistically differ from each other, according to the Tukey test, at 5% probability.

Table 2: Number of leaves, leaf area, chlorophyll (SPAD), main pseudostem length (MPSL), and root system volume (RSV) of *Brachiaria decumbens* plants at different ages, cultivated in soil contaminated with copper.

Plants' age (days)	Number of leaves	Leaf area (cm^{-2})	Chlorophyll (SPAD)	MPSL (cm)	RSV (cm^{-3})
30	50.00 b	571.33 b	39.49 c	65.85 c	39.93 c
45	60.43 b	1064.01 ab	31.14 b	86.19 b	71.25 b
60	118.08 a	1548.01 a	22.64 a	100.90 a	110.00 a

*Mean treatment values followed by the same letter in the column do not statistically differ from each other, according to the Tukey test, at 5% probability.

dynamics of *Brachiaria decumbens*, Velasco (2011) has found that plants cut at 56 days presented better nutritive value than plants cut at 84 and 112 days.

According to Table 1, there was increased FSM, DSM and DRSM in all plant ages investigated in the current study. The increase rates in ages between 30 and 45 days were higher than those in ages between 45 and 60 days; they presented differences higher than 24%.

There was increase in the number of leaves, leaf area, pseudostem length and root system volume according to the plants' age increase (Table 2). However, the chlorophyll content did not follow the same trend; it has shown approximately 21% decline in the age interval between 30 and 45 days and subsequent 27.3% decline in the age interval between 45 and 60 days.

Figures 1 to 8 show responses to variables such as the number of tillers, number of leaves, leaf area, shoot length, chlorophyll, fresh and dry shoot matter, root system volume, fresh and dry root system matter. The response trends for these variables are explained through the quadratic polynomial linear equation.

Figure 1 shows that older plants have shown greater sensitivity to copper concentrations. This fact may be associated with the Cu absorption during the time the plants were cultivated in contaminated soil. The lowest number of tillers was found in 30, 45 and 60-day-old plants at the concentrations 58.03, 49.12 and 40.30 mg kg^{-1}, respectively.

The smallest number of leaves was found at the concentration 51.61 mg kg^{-1}. There was positive correlation (0.82) between the number of leaves and the leaf area. Thus, concentrations above 51.61 mg kg^{-1} have reduced the number of leaves, compromised the leaf area and, consequently, compromised the production of photoassimilates and biomass accumulation in the plant.

Zanine and Vieira (2006) have reported that the number of leaves the grasses keep per tiller is relatively constant under adequate growth conditions, since the emergence of new leaves coincides with the death of the oldest ones. Thus, soils contaminated with Cu concentrations above 51.61 mg kg^{-1} (Figure 2) compromise the metabolism of brachiaria plants.

The lowest main pseudostem length was found at the concentration 45.52 mg kg^{-1} (Figure 3). The increased pseudostem length has favored the increase in the number of leaves and in leaf area, since there was 0.74 and 0.71 positive correlation between pseudostem length and number of leaves, as well as between pseudostem length and leaf area, respectively.

The lowest leaf area (756.26 cm^{-2}) was found at the concentration 53.54 mg kg^{-1} (Figure 4). The leaf area accounts for the production of photoassimilates necessary to meet the metabolic demand and for the formation of new plant structures. Thus, the leaf area reduction led to reduction in fresh and dry shoot matter, with 0.83 and 0.75 positive correlation, respectively.

The lowest chlorophyll content (SPAD) was found at the concentration 38.23 mg kg^{-1} (Figure 5). According to Maranhão et al. (2009), nitrogen application has increased the chlorophyll content in SPAD values in *Brachiaria decumbens*. The authors have associated crude protein increase with dry matter production in plants showing increased chlorophyll content. They have also reported that the chlorophyll content (SPAD index) can be used as plant protein level indicator.

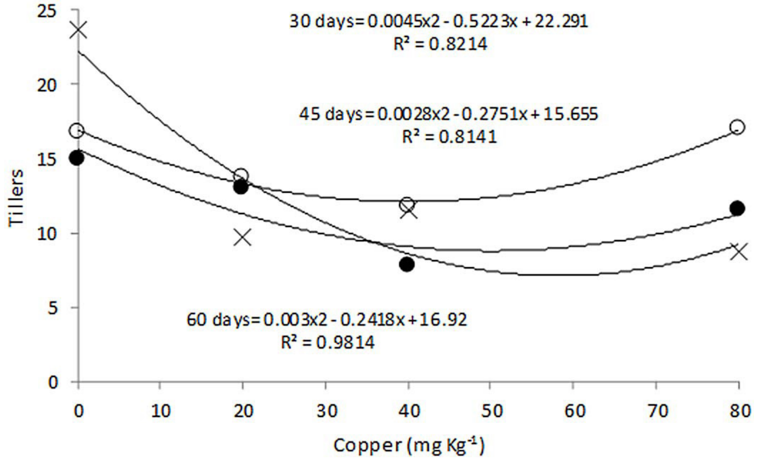

Figure 1: Number of tillers in *Brachiaria decumbens* plants cultivated in soil contaminated with copper ("X" 30 day-old plants; "●"45-day-old plants; and "O" 60-day-old plants).

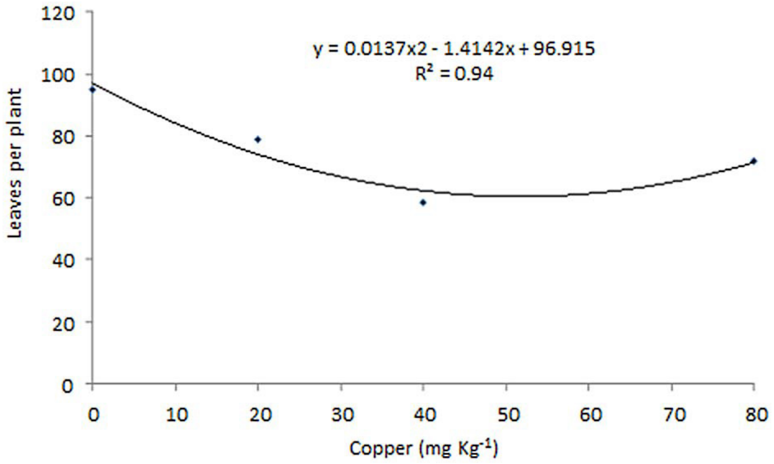

Figure 2: Number of leaves in brachiaria plants cultivated in soil contaminated with copper.

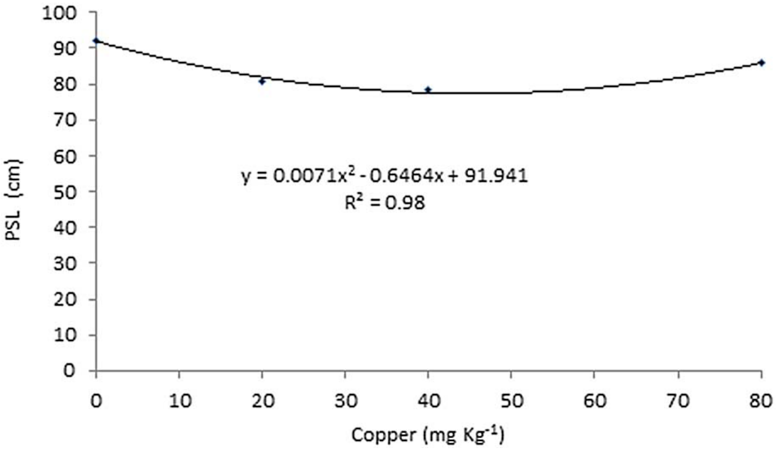

Figure 3: Pseudostem length (PSL) of brachiaria plants cultivated in soil contaminated with copper.

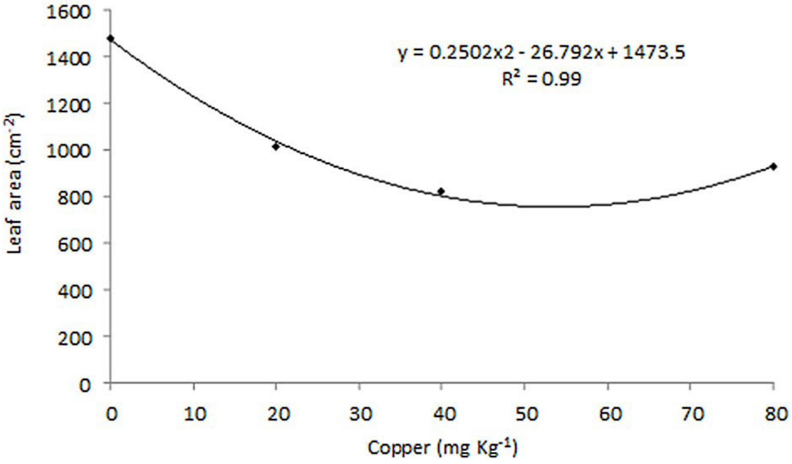

Figure 4: Leaf area of brachiaria plants cultivated in soil contaminated with copper.

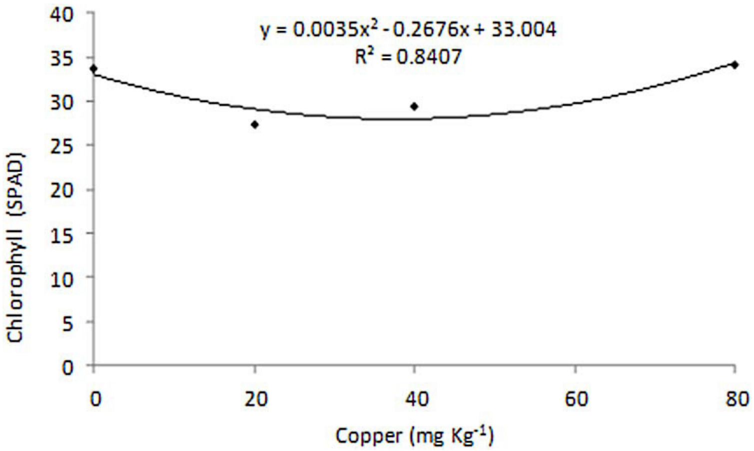

Figure 5: Relative chlorophyll content (SPAD) in leaves of brachiaria plants cultivated in soil contaminated with copper.

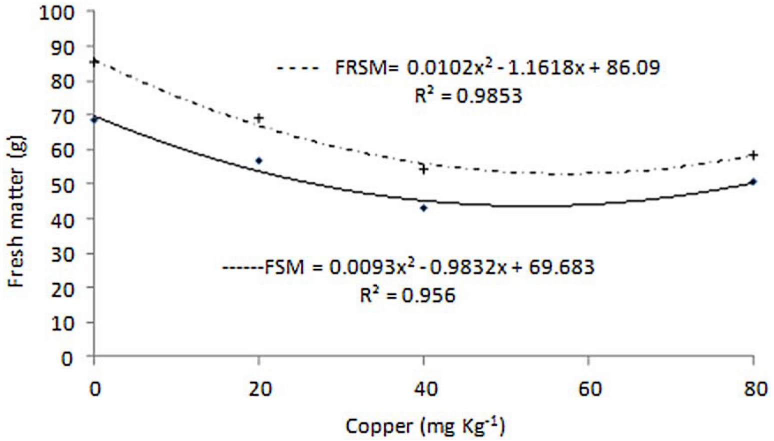

Figure 6: Biomass accumulation in the shoot and root system of brachiaria plants cultivated in soil contaminated with copper.

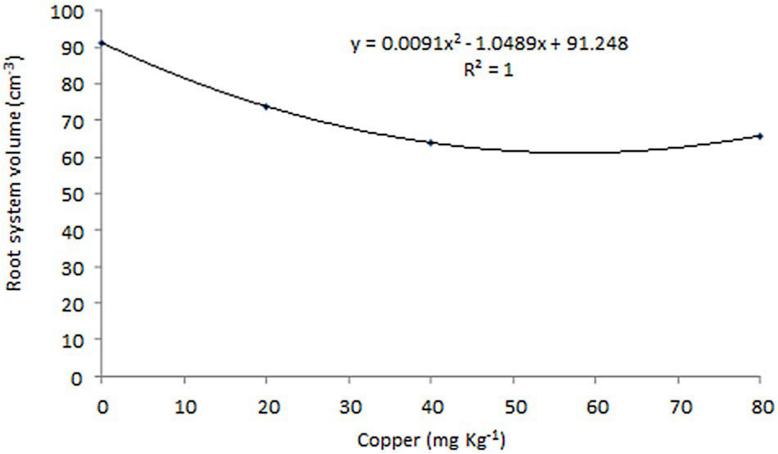

Figure 7: Root system volume (cm^{-3}) in brachiaria plants cultivated in soil contaminated with copper.

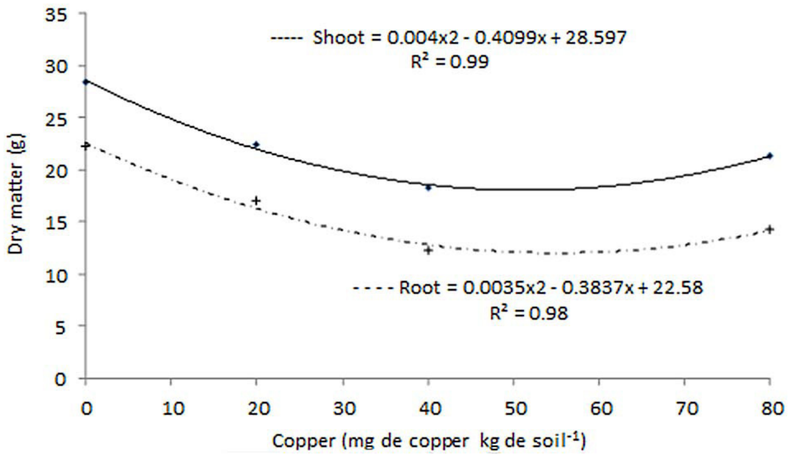

Figure 8: Biomass accumulation in the shoot and root system of brachiaria plants cultivated in soil contaminated with copper.

The lowest fresh shoot biomass of each plant (43.697 g) was found at the concentration 52.86 mg kg^{-1} and that of the root system (53.007 g) was found at the concentration 56.95 mg kg^{-1} (Figure 6). The reduced fresh shoot and root system biomass may be associated with the reduced production of photoassimilates, as well as with reduced leaf area in the plants and with the reduced chlorophyll content in the leaves, as shown in Figures 4 and 5.

The lowest root system volume was found at the concentration 57.63 mg kg^{-1} (Figure 7). Plants show relation between the shoot and the root system. Such relation ensures balance in the production of photoassimilates due to water and nutrient absorption. There was 0.79 positive correlation between the fresh shoot matter and the root system volume. The reduced root system volume in soils containing high Cu concentrations can be a mechanism used by plants to reduce the area of contact with the Cu ions in the soil in order to limit absorption. Consalter et al. (2013) have found that *Brachiaria decumbens* is an Al accumulating plant, whose shoot is not affected by these elements' presence; however, there was root-thickening tendency in presence of Al.

The dry shoot matter was higher than the dry root system matter at all copper concentrations used in the current study. The concentrations 51.24 and 54.81 mg kg^{-1} have provided the lowest shoot and root system dry matter, respectively (Figure 8). The lowest biomass accumulations in the shoot and root system were found at similar concentrations, fact that indicates that brachiaria plants have limited the shoot and root system growth when they were cultivated in soil contaminated with Cu concentrations between 51.24 and 54.81 mg kg^{-1}. There was 0.85 positive correlation between dry shoot matter and dry root system matter.

Copper concentrations above 75 mg dm^{-3} have reduced the growth of jatropha plants (Chaves et al., 2010), as well as the quality of timbó seedlings (*Ateleia glazioviana* ail.). However, Silva et al. (2014) have found that copper doses did not influence the quality of Cassia seedlings, and that the genus Stryphnodendron was able to kept seedling quality when up to 300 mg kg^{-1} copper was added to the soil. Fageria (2001) has found copper toxicity in soil treated with doses above 51 mg kg^{-1} for rice; 37 mg kg^{-1}, for beans; 48 mg kg^{-1}, for maize; 15 mg kg^{-1}, for soybeans; and 51 mg kg^{-1}, for wheat. The better growth and accumulation of biomass in seedlings of *Ricinus communis* L. was found in 40 mg L^{-1} of CuSO4, and the largest copper content in seedlings was found at a concentration of 60 mg L^{-1} CuSO4 (Kang et al., 2015).

According to Fageria (2001), brachiaria plants' tolerance to Cu was close to that of rice, maize and wheat; however, this species has shown lower tolerance when it was compared to Jatropha, Cassia and Stryphnodendron plants. Thus, the phytoremediation of soils contaminated with high Cu levels requires prioritizing species such as *Brachiaria decumbens*, which present higher tolerance to copper.

CONCLUSIONS

The growth of *Brachiaria decumbens* plants varied according to the plants' age, as well as to the copper concentrations in the soil. Despite the influence copper concentrations have on plant growth, *Brachiaria decumbens* showed potential to be used in copper phytoremediation in soils contaminated with concentrations up to 45.52 mg Kg^{-1}.

REFERENCES

ARROYAVE, C. et al. Differential aluminum resistance in Brachiaria species. **Environmental and Experimental Botany**, 89:11-18, 2013.

BEZERRA NETO, E.; BARRETO, L. P. **Métodos de Análises Químicas em Plantas**. Recife: Imprensa Universitária da UFRPE. 2004, 263p.

CARMO, M. L. et al. Seleção de plantas para fitorremediação de solos contaminados com Picloram. **Planta Daninha**, 26(2):301-313, 2008.

CHAVES, L. H. G. et al. Crescimento, distribuição e acúmulo de cobre e zinco em plantas de pinhão manso. **Revista Ciência Agronômica**, 41(2):167-176, 2010.

CONSALTER, R. et al. Efeito de doses de alumínio no crescimento e nutrição de *Brachiaria decumbens* cultivada em solução nutritiva. **Cultivano o Saber**, 6(3):98-112, 2013. Available in: <http://www.fag.edu.br/upload/revista/cultivando_o_saber/526e669e783bb.pdf> Access in: February, 14, 2017.

FAGERIA, N. K. Adequate and toxic levels of copper and manganese in upland rice, common bean, corn, soybean and wheat grown on an oxisol. **Communications in Soil Science and Plant Analysis**, 32(9-10):1659-1676, 2001.

KANG, W. et al. Distribution and chemical forms of copper in the root cells of castor seedlings and their tolerance to copper phytotoxicity in hydroponic culture. **Environmental Science and Pollution Research**, 22(10):7726-7734, 2015.

LI, J. et al. Copper pollution decreases the resistance of soil microbial community to subsequent dry-rewetting disturbance. **Journal of Environmental Sciences**, 39:155-164, 2016.

MARANHÃO, C. M. A. et al. Produção e composição químico-bromatológica de duas cultivares de braquiária adubadas com nitrogênio e sua relação com o índice SPAD. **Acta Scientiarum. Animal Science**, 31(2):117-122, 2009

SANTOS, J. V. et al. Biological attributes of rehabilitated soils contaminated with heavy metals. **Environmental Science and Pollution Research**, 23(7):6735-6748, 2016.

SANTOS, F. S. et al. Chelate-induced phytoextraction of metal polluted soils with *Brachiaria decumbens*. **Chemosphere**, 65:43-50, 2006.

SANTOS, M. E. R. et al. Características morfogênicas e estruturais de perfilhos de capim-braquiária em locais do pasto com alturas variáveis. **Revista Brasileira de Zootecnia**, 40(3):535-542, 2011.

SILVA, R. F. et al. Efeito do cobre sobre o crescimento e qualidade de mudas de *Stryphnodendron polyphyllum* Mart. e *Cassia multijuga* Rich. **Ciência Florestal**, 24(3):717-725, 2014.

SILVA, R. F. et al. Crescimento e qualidade de mudas de Timbó e dedaleiro cultivadas em solo contaminado por cobre. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 16(8):8811-886, 2012.

SONMEZ, S. et al. High level of copper application to soil and leaves reduce the growth and yield of tomato plants. **Scientia Agricola**, 63(3):213-218, 2006.

VELASCO, F. O. **Valor nutricional da *Brachiaria decumbens* em três idades**. Universidade Federal de Minas Gerais, Escola de Veterinária. 2011. 141p.

Williams, M. E. Plant Nutrition 3: Micronutrients and metals. **The Plant Cell**. 27(5):1-20, 2015.

YRUELA, I. Copper. In: BARKER, A. V.; PILBEAM, D. J. **Handbook of plant nutrition**. New York, USA. 2nd Edition. 2007, p.367-398.

ZANINE, A. M.; VIEIRA, B. R. Fluxo de tecidos em gramíneas. **Revista Científica Eletrônica de Agronomia**. N. 6, 2006. Available in: <http://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/F5NRC0YPxwKc4Yn_2013-4-29-17-52-12.pdf>. Access in: February, 13, 2017.