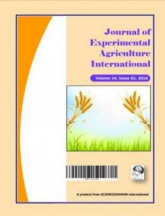

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Growth, Gas Exchange and Mineral Nutrition of Xaraés Grass (*Brachiaria brizantha* cv. Xaraés) in a Haplic Cambisol with Increasing Copper Doses

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MLC and ESD designed the study and managed the literature searches. Authors MLC, ESD and CSM wrote the protocol. Authors ESD and CSM managed the analysis of the study. Author ESD wrote the manuscript. Author DJM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To calculate the lower and upper critical doses of Cu applied to a Haplic Cambisol on growth, gas exchange and mineral nutrition of xaraés grass plants (*Brachiaria brizantha* cv. Xaraés).
Study Design: The experiment was arranged in a completely randomised design with four replications.
Locality of Study: Department of Soil Science of the Santa Catarina State University, Lages, Brazil, between February and March 2015.
Methodology: Xaraés grass plants were cultivated in a greenhouse in pots with soil containing Cu doses in the following increasing order: 0, 30, 60, 120 and 200 mg kg^{-1}. Before plant cultivation, the Cu availability in the soil was determined. During plant growth, the net CO_2 assimilation rate,

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transpiration rate, stomatal conductance, SPAD index and extended plant height were measured. After harvest, the plant leaf area, specific leaf area, plant dry mass, nutrient accumulation in the shoots and Cu concentration in the roots and shoots were evaluated.
Results: Application of Cu in increasing doses to the soil resulted in a linear increase in the Cu availability which led to parabolic functions for plant growth, gas exchange and nutrient accumulation in the shoots. There was an exponential increase in the Cu concentration in the roots. Despite the increase in the Cu concentration in the shoots, the values do not exceed 12.70 mg kg^{-1}.
Conclusion: The lower and upper critical doses of Cu for xaraés grass plants cultivated in Haplic Cambisol are 62.60 and 128.92 mg kg^{-1}, respectively.

Keywords: Micronutrients; critical doses; forage grass.

1. INTRODUCTION

Copper (Cu) is an essential element to plants. It participates in photosynthetic and respiratory electron transport chains as a constituent of plastocyanin and cytochrome c oxidase enzymes. It is also linked to carboxylation reactions of photosynthesis due to its association with ribulose 1,5 biphosphate carboxylase/oxygenase (RuBisCO) and related to oxidative stress responses, once it is a structural component of Cu/Zn superoxide dismutase [1,2,3,4]. Copper also plays a key role in cell wall metabolism and hormone signalling. At the cellular level, it develops essential functions in signalling of protein transcription, oxidative phosphorylation and Fe mobilisation [5]. Optimum Cu concentrations in the shoots may vary from 2- 5 to 15- 30 mg kg^{-1} (dry mass) depending on plant species [6,7,8].

Photosynthesis is the primary physiological process affected by Cu deficiency in plants due to decreased electron transport from the photosystem II (PSII) to photosystem I (PSI) and plastocyanin, a copper-containing mobile protein located in the thylakoid lumen. Decreased PSII activity also occurs in Cu deficient chloroplasts as a result of alterations in thylakoid membranes. In addition, Cu-deficient plants present lower chlorophyll and carotenoid content and plastoquinone synthesis [9]. Typical symptoms of Cu deficiency first appear at the tips of young leaves and then extend downward along the leaf margins. Leaves may also be twisted or malformed and show chlorosis or even necrosis [10].

At toxic concentrations in plants, Cu induces lipid peroxidation by accelerating the degradation rate of cellular components and inhibits the regeneration rate of these components, affecting photosynthesis, respiration, synthesis and activities of enzymes and proteins, among others physiological processes [5,11,12]. Photosynthesis is affected by decreased chlorophyll content and structural alterations in chloroplasts and thylakoid membranes, which impair the conformation and photochemical activity of the PSI and PSII [9]. Thickening and shortening, inhibition of secondary formations and tissue necrosis are the symptoms of Cu phytotoxicity in roots. In the shoots, Cu toxicity is evidenced by the development of chlorosis, diminished stem and leaf length and a decrease in size and number of leaves [8,13,14]. Cu toxicity to roots can result in a dramatic reduction of water and nutrient uptake, leading to the inhibition of plant growth and development [15].

Plants should maintain not only an adequate Cu content in the tissues but also a balance with other nutrients for optimum development. Many complex interactions between Cu and other elements are observed in the external root media and also within plant tissues. Ions with an affinity to proteins and other compounds similar to that of Cu^{2+} may show antagonistic interrelationships [16,17,18,19].

Brachiaria brizantha (Hochst. Ex A. Rich.) Stapf. presents high biomass production, vigorous and deep root system and high tolerance to water deficiency. It develops well in adverse environmental conditions to most plants used for grazing or soil cover. This species is mainly recommended for medium to high fertile soils, however, it is also adapted for acidic soils with low fertility [20,21]. Due to these characteristics, *Brachiaria brizantha* is one of the most common species used as forage grasses in tropical countries [22].

Borges et al. [23], evaluating the effects of two doses of Cu (0 and 200 mg kg^{-1}) applied to a Haplic Cambisol on *Brachiaria* grasses, verified that plants of *Brachiaria brizantha* cvs. Marandu, Piatã and Xaraés (MG-5) grown at 200 mg kg^{-1}

Cu did not present differences in the dry mass of roots and shoots compared to the dose of 0 mg kg^{-1}. Among these cultivars, xaraés grass presented the best production of the dry mass of shoots at the highest dose. Nevertheless, more information is needed regarding the effects of Cu application on the soil on xaraés grass plants within the range evaluated in that work.

In this way, the aim of this work was to evaluate the effects of increasing Cu doses applied to a Haplic Cambisol on xaraés grass plants, considering Cu availability in the soil and growth, gas exchange, nutrient accumulation in the shoots and Cu concentration in the plants, in order to calculate the lower (deficiency) and upper (toxicity) critical doses.

2. MATERIALS AND METHODS

A pot experiment was carried out under greenhouse conditions in Lages, Santa Catarina State, Brazil (27°48'58 "S and 50°19'34" W, 941 m a.s.l.), from February to March 2015. The soil used was a composite sample collected in high-altitude grassland within the same municipality, according to a grid of 32 equally spaced points on a 12 hectares area and at a depth of 0-20 cm of a Haplic Cambisol. This soil was selected because of its typical characteristics for livestock in Southern Brazil. The soil was dried in a forced-air circulation oven for 72 h at 65°C and sieved through a 2 mm stainless steel sieve.

A sub-sample (500 g) was taken from the composite soil sample to determine physical and chemical properties (Table 1). The pH was measured potentiometrically (soil: water 1:1, v/v). P and K were extracted by Mehlich-1 and determined by colourimetry (P) (SPEKOL 1300, Analytik Jena, Jena, Germany) and atomic emission spectroscopy (K) (DM-62, Digimed, Campo Grande); Ca, Mg and Al were extracted by 1 mol L^{-1} KCl and determined by flame atomic absorption spectrophotometry (Ca and Mg) (ContrAA 700, Analytic Jena, Jena, Germany) and titration with 0.0125 mol L^{-1} NaOH (Al). The acidity (H + Al) was extracted with 0.5 mol L^{-1} calcium acetate buffered at pH 7.0 and quantified by titration with 0.0125 mol L^{-1} NaOH. The organic carbon was determined by the Walkley-Black method [24]. The cation exchange capacity (CEC) was calculated by the following Equation (1).

$$CEC = \sum K, Ca, Mg, H + Al \quad (1)$$

The available Cu in the soil was extracted by HCl 0.1 mol L^{-1}, while the semi total Cu was extracted by aqua-regia according to ISO11047/1998 (E) [25]. Both were determined by flame atomic absorption spectrophotometry. The textural characterisation was performed using the densimeter method.

Table 1. Chemical and textural characterisation of the soil used in the experiment

Property	Unit	Value
pH in water	-	4.5
P	mg dm^{-3}	0.6
K	$cmol_c$ dm^{-3}	0.13
Ca	$cmol_c$ dm^{-3}	3.31
Mg	$cmol_c$ dm^{-3}	1.04
Al	$cmol_c$ dm^{-3}	7.72
CEC	$cmol_c$ dm^{-3}	15.18
Organic carbon	g kg^{-1}	14.28
Available Cu	mg dm^{-3}	2.53
Semi-total Cu	mg dm^{-3}	12.37
Sand	g kg^{-1}	707.59
Silt	g kg^{-1}	87.41
Clay	g kg^{-1}	205.00

The soil was fertilised according to N, P and K levels suitable for warm-season grasses: 200 kg ha^{-1} of N, 120 kg ha^{-1} of P_2O_5 and 100 kg ha^{-1} of K_2O [26].

The experimental units consisted of plastic pots containing 0.5 kg of soil and five plants of xaraés grass. The plants developed from seeds sown directly in the treated soil. The treatments were established by the addition of aliquots of $Cu(NO_3)$ stock solution to the soil, resulting in Cu doses of 0, 30, 60, 120 and 200 mg kg^{-1}. These doses were defined based on the Brazilian soil quality guideline [27]. The values 60 and 200 mg kg^{-1} represent the prevention limit in soils and the maximum permissible limit in agricultural soils for Cu in Brazil respectively. Before sowing, the soil remained incubated with moisture near the field capacity (FC) until pH stabilisation, which occurred at 4.3. The experiment was arranged in a completely randomised design and replicated four times.

The greenhouse was equipped with a control structure that maintained the temperature between 15 and 25°C and the air humidity between 70 and 95%. The luminosity and

photoperiod were provided by natural sunlight. The soil moisture was kept in FC by periodic irrigation with distilled water. The randomisation of pots was performed every five days. From sowing to harvesting, the plants were grown for 42 days. The available Cu in the soil was evaluated before plant cultivation by extraction with HCl 0.1 mol L^{-1} and determination by flame atomic absorption spectrophotometry.

The net CO_2 assimilation rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration in the leaves were measured at 41st day of plant growth by a portable photosynthesis system (LI-6400XT, LI-Cor Inc., Lincoln, USA). The measurements were performed from 9:00 to 10:00 at ambient CO_2 concentrations (ranging from 375 to 385 µmol CO_2 mol^{-1}), chamber temperature of 25°C, and light intensity of 1,000 µmol photons m^{-2} s^{-1} provided by a light-emitting diode (6400-02B Red-Blue; Li-Cor Inc., Lincoln, USA). These evaluations considered the youngest fully expanded leaf of each experimental unit.

At 42nd day of growth, the SPAD index of leaves was measured using a portable chlorophyll meter (SPAD-502, Konica Minolta, Osaka, Japan). This variable is highly correlated with the chlorophyll content in the leaves. Values close to 0 (zero) or 100 indicate low and high content, respectively. The SPAD index was measured on the lower, middle and upper thirds of nine fully expanded leaves of each experimental unit between 9:00 and 10:00 am. On this occasion, measurements of the extended height of plants (from the base of the stem to the apex of the longest leaf) were also taken.

The plants were harvested, washed with distilled water and divided into leaves, stems (stems + pseudostems) and roots. The plant leaf area (PLA) was determined using a leaf area meter (LI-3050A, LICOR, Lincoln, USA). The roots and shoots were dried in a forced-air circulation at 65ºC until constant weight. The dry mass of roots (DMR), stems (DMS) and leaves (DML) were determined by using an analytical balance (0.0001 g). The data of the dry mass of leaves and plant leaf area were used to obtain the specific leaf area (SLA), according to equation (2).

$$SLA = PLA / DML \quad (2)$$

The dried plant tissues were ground into a fine powder using a Wiley type mill. Samples were submitted to acid digestion according to USEPA 3052 method [28] aiming to quantify the Cu concentration in the roots and shoots. 0.25 g sample was transferred to PTFE (polytetrafluoroethylene) tubes with 9 mL of nitric acid (65%). The tubes were kept in a closed system consisting of a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) at a 10 minutes temperature ramp, the time required to reach 180 ºC, which was sustained for another 20 minutes. After cooling, the samples were transferred to 25 mL volumetric flasks, which were topped up with distilled water, and the extracts immediately filtered through quantitative filter paper (Whatman no. 42).

The Cu concentration was determined in triplicate by flame atomic absorption spectrophotometry. The detection and quantification limits were calculated as the blank signal plus three or ten times its standard deviation, corresponding to 0.02 and 0.07 mg L^{-1}, respectively. The quality control was ensured using the certified reference material NIST SRM 1573a (Table 2).

The accumulation of Ca, Mg, K, N and P was evaluated in the shoots. Samples (0.2 g) were added into glass vessels and digested by a mixture of concentrated sulfuric acid (98%) and hydrogen peroxide (30%) (2:1, v/v). The vessels were kept at 350°C on aluminium heating blocks for 3 h or longer until the solution became clear. The digested samples were adjusted to a volume of 50 mL with distilled water. The determination of nutrient concentration was performed by flame atomic absorption spectrophotometry for Ca and Mg, atomic emission spectroscopy for K, micro-Kjeldahl method [29] for N and colourimetry for P. The concentration of Fe, Mn and Zn were also evaluated in the shoots using the same methodology as described for Cu. The accumulation of each nutrient in the shoots was calculated according to equation (3).

$$\text{Nutrient accumulation in the shoots} = \text{Nutrient concentration} \times (DMS + DML) \quad (3)$$

The data were tested for normality by the Shapiro-Wilk test and for homogeneity of variances by the Bartlett's test, and then subjected to Analysis of Variance (ANOVA). Regression equations were adjusted between the Cu doses applied to the soil and the analysed variables by SigmaPlot software [30], considering $p = .05$.

Table 2. Cu concentration recovered from the reference material by the method used to determine the Cu concentration in the samples of xaraés grass plant tissues

NIST SRM	Certified Cu concentration (mg kg^{-1})	Triplicate	Determined Cu concentration (mg kg^{-1})	Recovery (%)
1573a	4.70	1	4,89	104.04
		2	4,84	102.98
		3	4,75	101.06

3. RESULTS

The available Cu in the soil was significantly affected by the doses applied, showing a linear increase from 0 to 200 mg kg^{-1} (Fig. 1). The observed values ranged from 5.60 to 43.82 mg kg^{-1}, which corresponded to 32 and 54%, respectively, of the total Cu in the soil (original content + applied dose).

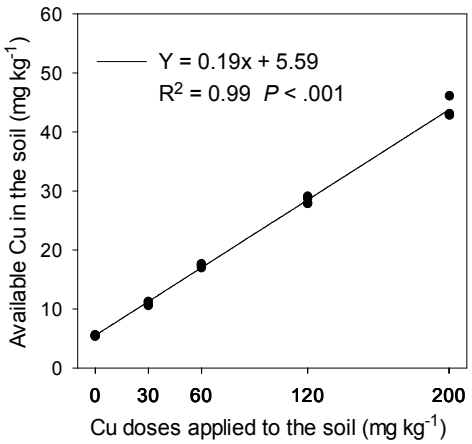

Fig. 1. Availability of Cu (mg kg^{-1}) as a function of application of Cu doses to the soil

The application of increasing Cu doses in the soil resulted in significant effects and quadratic response on leaf area, specific leaf area, SPAD index and extended height for xaraés grass plants (Fig. 2). There was an increase of 5% between 0 -65.07 mg kg^{-1}, and a decrease of 22% between this dose and the dose of 200 mg kg^{-1} in the plant leaf area (Fig. 2). The specific leaf area was reduced by 17% between 0 - 130.51 mg kg^{-1} and enhanced by 6% between this dose and the dose of 200 mg kg^{-1} (Fig. 2). The SPAD index was increased by 1% between 0 - 77.71 mg kg^{-1}, and reduced by 3% between this dose and the dose of 200 mg kg^{-1} (Fig. 2). There was an increase of 27% between 0- 93.59 mg kg^{-1} in the extended plant height, and a decrease of 27% between this dose and the dose of 200 mg kg^{-1} (Fig. 2).

A significant quadratic relationship was observed between Cu doses applied to the soil and the dry mass of leaves, stems (stems + pseudostems) and roots for xaraés grass plants within the tested range (Fig. 3). There was an increase of 23% between 0 - 94.89 mg kg^{-1}, and a decrease of 23% between this dose and the dose of 200 mg kg^{-1} in the dry mass of leaves (Fig. 3). The dry mass of stems enhanced by 38% between 0 - 106.92 mg kg^{-1}, and decreased by 21% between this dose and the dose of 200 mg kg^{-1} (Fig. 3). The dry mass of roots increased by 19% between 0 - 80.08 mg kg^{-1}, and decreased by 36% between this dose and the dose of 200 mg kg^{-1} (Fig. 3). There was an increase of 24% between 0 - 91.22 mg kg^{-1} in the total dry mass, and a decrease of 27% between this dose and the dose of 200 mg kg^{-1} (Fig. 3).

The application of increasing Cu doses in the soil resulted in significant effects and quadratic response on net CO_2 assimilation rate, transpiration rate and stomatal conductance (Fig. 4). There was an increase of 29% between 0 - 84.25 mg kg^{-1} and a decrease of 42% between this dose and the dose of 200 mg kg^{-1} in the net CO_2 assimilation rate (Fig. 4). The transpiration rate increased by 29% between 0 - 82.30 mg kg^{-1} and decreased by 33% between this dose and the dose of 200 mg kg^{-1} (Fig. 4). The stomatal conductance increased by 24% between 0 - 75.12 mg kg^{-1} and decreased by 54% between this dose and the dose of 200 mg kg^{-1} (Fig. 4). The intercellular CO_2 concentration in the leaves was not affected by the Cu doses applied to the soil (Fig. 4).

A significant quadratic relation was observed between the Cu doses applied to the soil and the accumulation of nutrients in the shoots of xaraés grass (Fig. 5).

The accumulation of Ca, Mg, Zn and Mn increased 94, 80, 107 and 76% between 0 and the doses of 106.19, 111.79, 111.81 and 117.65 mg kg^{-1}, respectively, in which maximum values were observed. From these doses to the dose of 200 mg kg^{-1}, the accumulation of Ca, Mg, Zn and

Mn decreased 38, 28, 32 and 21%, respectively (Fig. 5). The accumulation of P, K, Fe and N increased 78, 17, 19 and 19% between 0 and the doses of 107.80, 75.24, 95.19 and 76.50 mg kg^{-1}, respectively, in which maximum values were observed. From these doses to the dose of 200 mg kg^{-1}, the accumulation of P, K, Fe and N decreased 32, 41, 19 and 42%, respectively (Fig. 5).

The application of increasing Cu doses in the soil resulted in significant effects on Cu concentration in the plant tissues. The Cu concentration increased exponentially in the roots from 17.56 to 113.24 mg kg^{-1} (6.4 times) (Fig. 6) and increased according to the Michaelis-Menten model in the shoots from 8.24 to 12.70 mg kg^{-1} (1.5 times) (Fig. 6), respectively from 0 to 200 mg kg^{-1} of Cu applied to the soil.

Based on the 90% cut-off value of the maximum values estimated for the variables of growth, gas exchange and nutrient accumulation, the lower and upper critical doses (LCD and UCD) of Cu applied to the soil were calculated (Table 3). These values represent the limits of deficiency and toxicity for xaraés grass plants.

4. DISCUSSION

In acidic vineyard soils with sandy surface horizons and a history of Cu-bearing fungicides application, Brunetto et al. [31] found Cu availability around 72%, corroborating the results of the present work that there is a high proportion of Cu in the available soil fraction of acidic and sandy soils (Fig. 1).

The increase in the SPAD index up to the dose of 77.71 mg kg^{-1} concomitant to the decrease in the specific leaf area is due to the increase of chlorophyll content in the leaves, once Cu is essential to plastocyanin synthesis, protein involved in the electron transport from chlorophylls of the PSII to PSI [12] (Fig. 2). From that dose to the dose of 130.51 mg kg^{-1}, the decrease in the SPAD index concomitant to the decrease in the specific leaf area suggests an increase in the proportion of non-chlorophyll parenchyma or an increase of mesophyll cell size (Fig. 2).

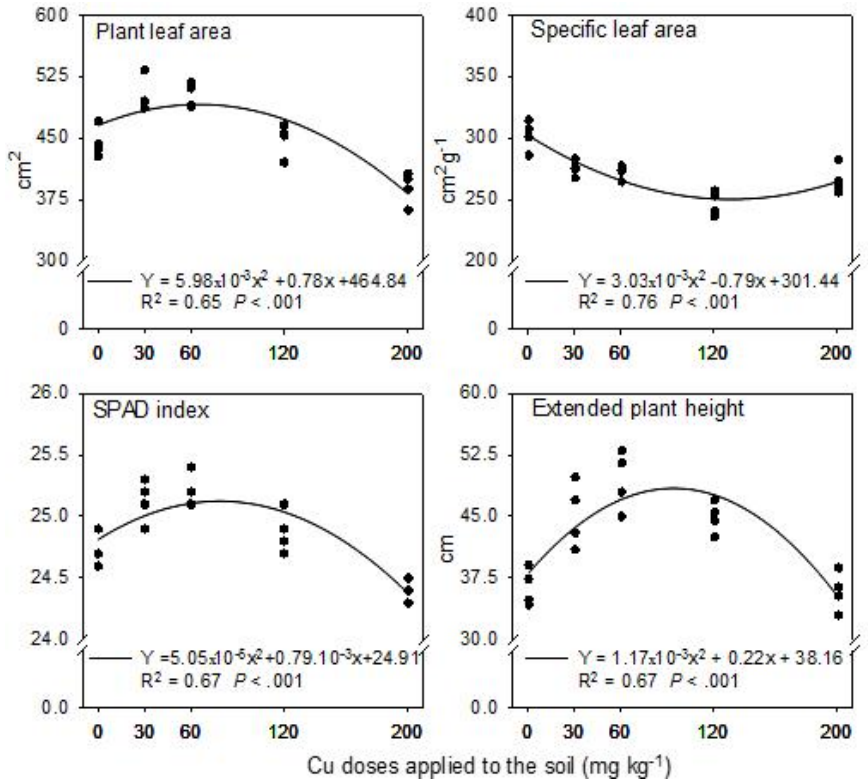

Fig. 2. Plant leaf area (cm^2), specific leaf area (cm^2 g^{-1}), SPAD index and extended plant height (cm) of xaraés grass plants grown in soil with increasing Cu doses

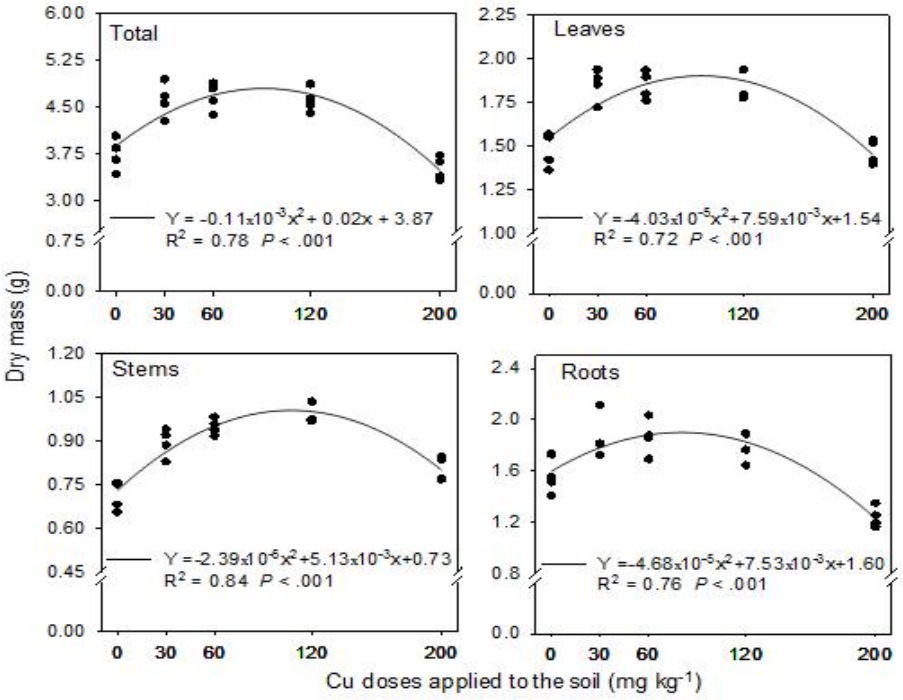

Fig. 3. Total dry mass, dry mass of leaves, stems and roots of xaraés grass plants grown in soil with increasing Cu doses

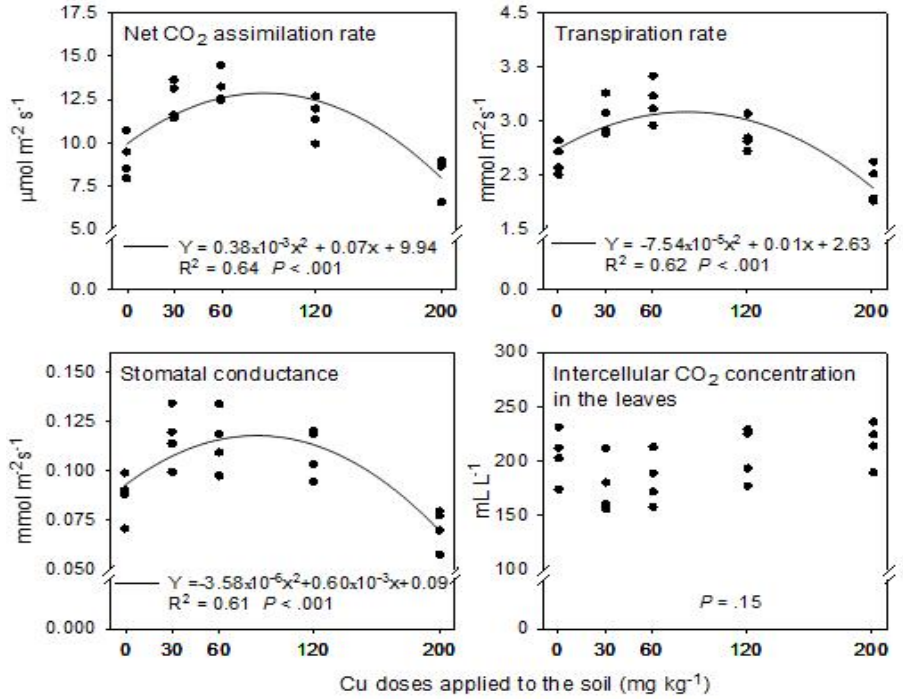

Fig. 4. Net CO_2 assimilation rate (µmol m^2 s^{-1}), transpiration rate (mmol m^2 s^{-1}), stomatal conductance (mmol m^2 s^{-1}) and intercellular CO_2 concentration in the leaves (mL L^{-1}) of xaraés grass plants grown in soil with increasing Cu doses

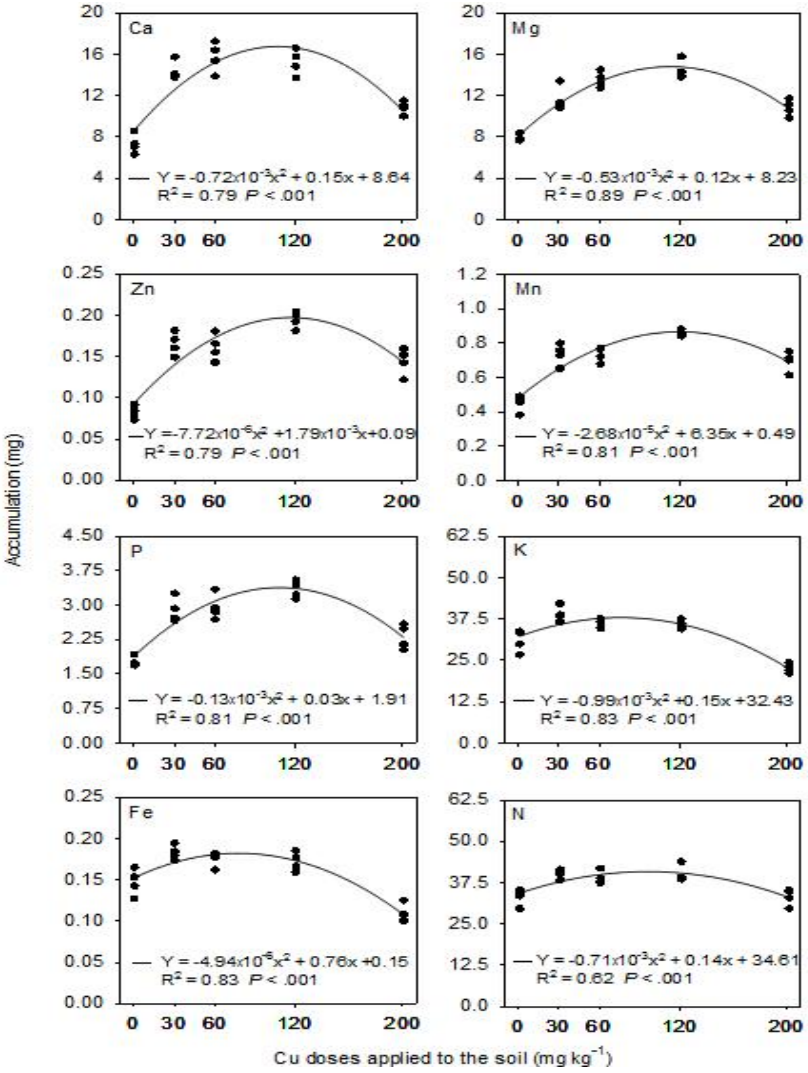

Fig. 5. Accumulation of Ca, Mg, Zn, Mn, P, K, Fe and N (mg) in the shoots of xaraés grass plants grown in soil with increasing Cu doses

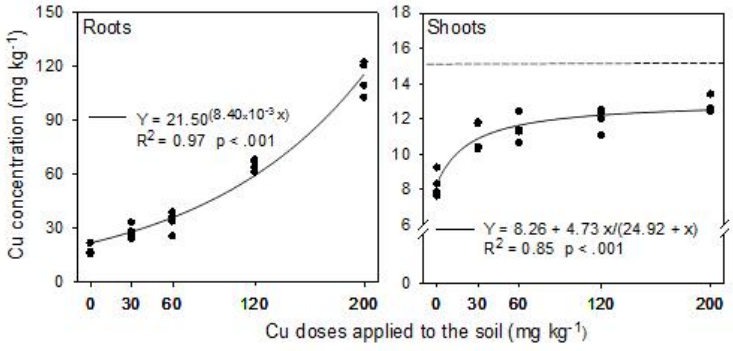

Fig. 6. Cu concentration in the roots and shoots (mg kg^{-1}) of xaraés grass plants grown in soil with increasing Cu doses

Table 3. Lower and upper critical doses (LCD and UCD) of Cu applied to the soil for variables of growth, gas exchange and nutrient accumulation for xaraés grass plants

Variable	LCD (mg kg^{-1})	UCD (mg kg^{-1})
Plant leaf area	-	155.62
Specif leaf area	39.70	-
SPAD index	-	-
Extended plant height	29.38	157.81
Total dry mass	25.52	156.92
Dry mass of leaves	25.93	163.85
Dry mass of stems	42.27	171.56
Dry mass of roots	16.54	143.64
Net CO_2 assimilation rate	27.71	140.79
Transpiration rate	17.62	146.98
Stomatal conductance	21.32	128.92
Ca accumulation	57.92	154.45
Mg accumulation	58.72	164.85
Mn accumulation	61.10	174.20
Zn accumulation	62.60	161.02
P accumulation	56.31	159.30
K accumulation	13.45	137.04
Fe accumulation	16.04	136.96
N accumulation	19.43	170.95

The increase in the plant leaf area up to the dose of 65.07 mg kg^{-1} concomitantly to the decrease in the specific leaf area suggests a beneficial effect of Cu to leaves, which may occur due to the increase of photosynthesis and/or amino acid and proline synthesis [6,7] (Fig. 2). On the other hand, the decrease in the plant leaf area concomitantly to the increase in the specific leaf area from the dose of 130.51 mg kg^{-1} confers Cu toxicity status to leaves (Fig. 2). The exposure of plants to high doses of trace elements leads to a reduction in the size of mesophyll cells and a collapse of the palisade and spongy parenchyma cells [32,33].

Once the intercellular CO_2 concentration in the leaves became stable, the variation in the stomatal conductance had no influence on the net CO_2 assimilation rate. The application of Cu doses to the soil resulted in similar responses on net CO_2 assimilation rate and SPAD index, indicating that the net CO_2 assimilation rate is closely related to the chlorophyll content in the leaves. Cu is a plastocyanin component, the protein responsible for the electron transport from chlorophylls of the PSII to PSI [12]. The increase in the net CO_2 assimilation rate up to the dose of 84.25 mg kg^{-1} (Fig. 4) could also be explained due to the association of Cu with RuBisCO [1]. The decrease in the net CO_2 assimilation rate from that dose (Fig. 4) may occur due to the adverse effects of Cu on chlorophyll content, RuBisCO activity, plastocyanin synthesis and PSII activity [9,6,34,35,36,37].

The increase in the transpiration rate up to the dose of 82.30 mg kg^{-1} and the decrease between this dose and the dose of 200 mg kg^{-1} (Fig. 4) occurred as a function of the stomatal conductance. Variations in stomatal conductance are related to the supply of K to stomatal guard cells [38].

Plenderleith and Bell [39], studied the effects of increasing Cu doses in the soil (ranging from 2 to 600 mg kg^{-1}) in pearl millet (*Pennisetum americanum*), and verified a decrease in the dry mass of shoots between the doses of 95 and 175 mg kg^{-1}. This decrease occurred between the doses of 50 and 95 mg kg^{-1} for Sabi grass (*Urochloa mosambicensis*), Rhodes grass (*Chloris gayana*), buffel grass (*Cenchrus ciliaris*), setaria (*Setaria sphacelata*), African lovegrass (*Eragrostis curvula*), signal grass (*Brachiaria decumbens*), Makarikari grass (*Panicum coloratum* var. *makarikariense*) and green panic (*Panicum maximum* var. *trichoglume*). For green couch (*Cynodon dactylon*) and black speargrass (*Heteropogun contortus*), the decrease occurred between 2-50 mg kg^{-1} doses.

Vassilev et al. [40], verifying the effects of increasing Cu doses in the sand (ranging from 0 to 15 mg kg^{-1}) to barley plants, observed a significant decrease in the dry mass of roots

(42%) and shoots (31%) at the dose of 15 mg kg^{-1}. Borges et al. [23] did not identify significant differences in the dry mass of roots and shoots for xaraés grass plants grown at 0 and 200 mg kg^{-1} of Cu applied in the soil.

The increase in the plant leaf area and the decrease in the specific leaf area up to the doses of 65.07 and 130.51 mg kg^{-1}, respectively, increased the dry mass of leaves up to the dose of 94.89 mg kg^{-1}. The decrease in the plant leaf area and the increase in the specific leaf area decreased the dry mass of the leaves at the subsequent dose range (Figs. 2 and 3).

The increases in the dry mass of stems and roots as well as in the extended plant height from the dose 0 mg kg^{-1} to the doses of 106.92, 80.08 and 93.59 mg kg^{-1}, respectively, are due to beneficial effects of Cu to plants [6,7]. The decreases in the values of these variables at the subsequent dose range can be attributed to disturbances caused by Cu on the physiological process and/or direct negative effects on plant growth [15,3,18,5] (Figs. 2 and 3).

The Cu^{2+} ion binds strongly to the cell wall. When it is reduced to Cu^{+}, it can promote the Fenton reaction and increase the concentration of the hydroxyl radical, an excessively reactive molecule that breaks down structural polysaccharides, causing cell wall loosening and stimulating cell expansion [41]. This may have contributed to the increase of the plant leaf area, extended plant height and dry mass of the plant parts up to the maximum values. However, Murphy et al. [42] found that toxic levels of Cu lead to lipid peroxidation and damage to plasma membranes resulting in decreased K uptake by the roots, an essential element for cell expansion. This could explain the decrease in the plant leaf area, extended plant height and dry mass of the plant parts from the maximum values.

The increase in the K accumulation in the shoots up to the dose of 75.24 mg kg^{-1} (Fig. 5) induced by the increase of Cu in the soil may have increased the stomatal conductance and transpiration rate, once stomatal conductance is directly related to the supply of K to stomatal guard cells [38]. The uptake of N, Ca, Mg, Zn, Fe and Mn occur, to a greater or lesser extent, through mass flow [43]. Thus, the increase in the transpiration rate up to the dose of 82.30 mg kg^{-1} (Fig. 5) may have increased the accumulation of these nutrients in the shoots. At toxic levels, Cu induces K^{+} loss from root cells by efflux through K^{+} channels in the plasma membrane and by lipid peroxidation-induced membrane disruption, decreasing the K uptake [42]. This may have reduced the K accumulation in the shoots, inducing the decrease in the transpiration rate and consequently in the accumulation of N, Ca, Mg, Zn, and Fe from that dose.

The decrease in the accumulation of Ca, Mg, Mn, Zn, Fe and K in the shoots may also be due to the displacement of cationic ions from plant tissues by Cu at toxic levels [16,17]. The decrease in the P accumulation in the shoots from the dose 107.80 mg kg^{-1} (Fig. 5) could be linked with an inhibition of phosphatase activity by the excess Cu, decreasing the P availability to plants [44].

Corroborating these findings, Lidon and Henriques [45], studying the growth of rice plants in nutrient solution with increasing Cu concentrations (from 0 to 6.25 mg L^{-1}), observed an increase in the accumulation of N, P, K, Ca, Mg and Zn from 0 to 0.25 mg L^{-1} and a decrease at the subsequent dose range. Similar results were obtained by Vassilev et al. [40] for the concentration of Ca, Fe and Mn in barley plants grown in sand with increasing Cu doses (from 0 to 15 mg kg^{-1}). Manivasagaperumal et al. [46] studied the growth of cowpea (*Vigna unguiculata*) plants in soil with increasing Cu doses (from 0 to 250 mg kg^{-1}) and observed an increase in the concentration of N, P, K, Ca, Mg, Fe, Mn and Zn in the shoots from 0 to the dose of 50 mg kg^{-1}, and a decrease at the subsequent dose range. However, Kopittke et al. [18] reported a decrease in the concentration of K, Mg, Fe, Zn and Mn of Sabi grass shoots between 0.12 and 2.3 µmol L^{-1} of Cu in the nutrient solution.

The Cu concentration was higher in the roots compared to shoots over the whole dose range (Fig. 6). In plants, Cu is retained mainly in the root epidermis or in vacuoles of cortical cells adjacent to the root endoderm [18,47]. The Cu concentration in the shoots did not exceed the maximum tolerable level of 15 mg kg^{-1} for animal feeds [48] over the whole dose range tested (Fig. 6).

The highest LCD was obtained for Zn accumulation at 62.60 mg kg^{-1}, and the lowest UCD was obtained for stomatal conductance at 128.92 mg kg^{-1} (Table 3). At these doses, the Cu availability in the soil reached 17.56 and 30.26

mg kg^{-1}, and the Cu concentration in the plants reached 36.38 and 63.59 mg kg^{-1} in the roots and 11.64 and 12.22 mg kg^{-1} in the shoots, respectively.

5. CONCLUSION

Xaraés grass plants growing in Haplic Cambisol with increasing Cu doses present lower critical dose to overcome the deficiency at 62.60 mg kg^{-1}, and upper critical dose to cause toxicity at 128.92 mg kg^{-1}, which correspond to 17.56 and 30.26 mg kg^{-1} of Cu available in the soil, respectively.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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