

## Germination and initial development of *Brachiaria brizantha* and *Brachiaria decumbens* on exposure to cadmium, lead and copper<sup>1</sup>

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**ABSTRACT** – The objective was to evaluate the germination and initial development of three cultivars of *Brachiaria brizantha* and *Brachiaria decumbens* in the presence of trace elements of Cd, Pb and Cu and quantify the Cd and Cu contents in plant tissue of these species. First, seed germination occurred in towel paper containing cadmium, lead, copper and the control. We calculated the percentage of germination, germination speed index, vigor index and seedling length. Later, the seeds were germinated in soil pots contaminated with Cd and Cu, where the Soil-Plant Analysis Development index (SPAD index) was evaluated, as well as dry matter of shoot and root, and the Cd and Cu metal content in plant tissue was quantified. *B. decumbens* showed sensitivity to the elements studied, with decreases in all parameters. Cu was the element that caused more toxic effects on germination and early seedling development of the species studied. In contaminated soil, the species studied showed no differences in dry matter production of shoots, but the exposure to Cd and Cu caused reductions in SPAD index in all species. The highest Cd and Cu contents were found in the roots.

Index terms: inhibition, phytotoxicity, heavy metals.

## Germinação e desenvolvimento inicial da *Brachiaria brizantha* e *Brachiaria decumbens* em exposição de cádmio, chumbo e cobre

**RESUMO** – O objetivo no trabalho foi avaliar a germinação e o desenvolvimento inicial de três cultivares de *Brachiaria brizantha* e a *Brachiaria decumbens* em presença dos elementos-traço Cd, Pb e Cu e quantificar os teores de Cd e Cu no tecido vegetal dessas espécies. A germinação das sementes ocorreu em papel germitest®, contendo cádmio, chumbo, cobre além da testemunha. Foram calculadas a porcentagem de germinação, índice de velocidade de germinação, índice de vigor e comprimento da plântula. Posteriormente, as sementes foram germinadas em vasos de solo contaminado com Cd e Cu, onde se avaliou o índice Soil Plant Analysis Development (SPAD), matéria seca da parte aérea e da raiz e quantificado o teor de metais Cd e Cu no tecido vegetal. A *B. decumbens* apresentou sensibilidade aos elementos estudados, apresentando decréscimos de todos os parâmetros avaliados. O Cu foi o elemento que mais causou efeitos tóxicos à germinação e desenvolvimento inicial das plântulas das espécies estudadas. Em solo contaminado, as espécies estudadas não apresentaram diferenças na produção de massa seca de parte aérea, porém a exposição de Cd e Cu ocasionou reduções do índice SPAD em todas as espécies. Os maiores teores de Cd e Cu foram encontrados nas raízes.

Termos para indexação: inibição, fitotoxicidade, metais pesados.

### Introduction

Human activity has increasingly raised trace element levels in the environment. Plants are the main entry for these elements into the food chain. Most trace elements are known as growth inhibitors and exert negative effects on plants, which

may lead to broader phytotoxicity responses and decrease the yield and quality in agricultural crops (Gratão et al., 2005; Yang et al., 2010).

Some trace elements, such as Cd (cadmium) and Pb (lead), are considered toxic even in minute concentrations, causing deleterious effects on plants. Such deleterious

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effects can be observed on seedling growth, changes in the structure of chloroplasts, inhibition of photosynthesis, chlorosis, induced lipidic peroxidation, suppression of germination, reduction of the root system, disturbances in plant metabolic activities and reduction of plant biomass (Guimarães et al., 2008; Gill et al., 2013).

In addition, plants may exhibit indirect effects caused by these trace elements such as inhibition of water absorption and nutrient deficiency. Mineral nutrition disorders arise from deleterious effects caused by trace elements on the metabolism of essential elements, including calcium, magnesium, potassium, iron, zinc, manganese and copper (Kabata-Pendias, 2011).

Cu (copper), on the other hand, plays a significant role in physiological processes such as photosynthesis and respiration, among others (Yruela, 2009; Karimi et al., 2012). However, in environmental conditions where copper is found in excess in the soil, plants may exhibit symptoms of toxicity which culminates in physiological disturbances inhibiting plant growth (Karimi, et al., 2012; Kabata-Pendias, 2011). Toxicity caused by Cu causes damage and disturbance in the integrity of the thylakoid membranes and photosynthesis impairment, which result in chlorosis or necrosis and inhibition of root and shoot growth (Yruela, 2009; Fidalgo et al., 2013).

In areas contaminated by trace elements, grasses are more promising in their establishment because they are relatively easy to develop, promote rapid growth in soil cover, improve soil physical and chemical structures, help in the cycling of nutrients and increase the soil organic matter content (Amaral et al., 2012).

Among grasses, genus *Brachiaria* spp. stands out due to high dry matter, easy cultivation and adaptation to different soils, allowing cultivation throughout the year, and low maintenance cost of the cultivated area (Lucena et al., 2010). However, few studies are carried out with this genus on trace element contamination (Gomes et al., 2011).

The present work aims to evaluate the germination and initial development of three cultivars of the species *Brachiaria brizantha* (cv. Piatã, Marandu and MG 5) and the species *Brachiaria decumbens* in the presence of trace elements of Cd, Pb and Cu, and quantify the content of Cd and Cu in its plant tissue.

## Materials and Methods

The experiment was conducted in two distinct stages. In the first step, the experiment was conducted in a Seed Testing Laboratory. The second part of the experiment was carried out in a greenhouse and analyses were performed in a Laboratory of Environmental Survey.

Four different *Brachiaria* varieties were used, being three cultivars of *Brachiaria brizantha* (cv. Piatã, Marandu, MG 5)

and one of *Brachiaria decumbens*. The seeds used came from a commercial agriculture and livestock farm in the Brazilian municipality Lauro Müller, SC. Seeds purity was determined following the protocol suggested by Rules for Seed Testing (Brasil, 2009) (Table 1). Before all the tests, seeds were submitted to a sanitary treatment in a 3% solution of sodium hypochlorite for five minutes and afterwards they were washed in distilled water.

Table 1. Characterization of the physical purity of seeds of *B. brizantha* (cv. Piatã, Marandu, MG 5) and *B. decumbens*.

| Species                           | Percentage of purity % |
|-----------------------------------|------------------------|
| <i>B. brizantha</i> (cv. Piatã)   | 71.5                   |
| <i>B. brizantha</i> (cv. Marandu) | 55.6                   |
| <i>B. brizantha</i> (cv. MG 5)    | 73.5                   |
| <i>B. decumbens</i>               | 66.6                   |

### 1<sup>st</sup> step – Germination test in the presence of Cd, Cu and Pb.

Seeds were placed on towel paper rolls for germination. In order to moisture the paper, 2.5 times the paper dry weight was used with solutions containing Cd(NO<sub>3</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub> or Cu(NO<sub>3</sub>)<sub>2</sub>. The concentrations used followed the agricultural research values proposed by CONAMA (National Council for the Environment) Resolution no. 420 (2009) (3, 180 and 200 mg.L<sup>-1</sup> respectively), besides the control (0 mg.L<sup>-1</sup>). Then the rolls were kept in a Mangelsdorf-type germinator at a temperature of 25 °C with a natural photoperiod. For the experimental unit, 50 seeds were considered, with four replicates for each treatment. The seeds were evaluated by the following parameters:

*Germination Percentage (GP)* – It was calculated by the number of normal seedlings (shoot and root) identified on the last day of the experiment, following the recommendations by the Rules for Seed Testing (Brasil, 2009).

*Germination speed index (GSI)* – Evaluations were performed every 24 hours until germination stabilization, which occurred on the tenth day. Seedlings were considered germinated when they reached root length greater than 5 mm and there was plumule rupture. To obtain the index, the formula proposed by Maguire (1962) was applied.

*Length of shoot and main root* – At the end of the germination test, the length of shoot and root of each seedling was measured in centimeters.

*Vigor Index (VI)* – Measurement of root length multiplied by the germination percentage (Dezfuli et al., 2008).

The germination percentage data were transformed to  $\arcsen\sqrt{x}/100$  and trace element content data were analyzed

after logarithmic transformation,  $Y = \log(X + 1)$ . All data were submitted to analysis of variance ( $p \leq 0.05$ ) considering a factorial arrangement of the treatments (varieties and trace elements). When statistical significance was found, the Tukey's test ( $p \leq 0.05$ ) was used to verify the magnitude of the difference among treatments.

Table 2. Chemical characteristics and clay in the soil sample (Haplic Cambisol) used in the experiment.

| pH H <sub>2</sub> O | CTC <sub>pH 7.0</sub> | Al <sup>3+</sup><br>Cmolc.dm <sup>-3</sup> | Ca  | Mg  | K                   | P   | Clay | CO <sup>1</sup> |
|---------------------|-----------------------|--|-----|-----|---------------------|-----|------|-----------------|
|                     |                       |  |     |     | mg.dm <sup>-3</sup> |     | %    |                 |
| 5.0                 | 35.2                  | 56.1                                       | 3.3 | 1.0 | 52                  | 0.6 | 34   | 1.4             |

Note: <sup>1</sup>Organic carbon.

For soil contamination, the concentrations of the elements also followed the values of agricultural research proposed by CONAMA (2009), 3 mg.kg<sup>-1</sup> for Cd and 200 mg.kg<sup>-1</sup> for Cu. Soil contamination occurred after soil drying and sieving in a 2-mm sieve, where each trace element represented a treatment. The soil, after being contaminated, was incubated for 30 days at humidity above field capacity to stabilize the chemical conditions.

A completely randomized design with four replications was used, with each pot receiving 10 seeds. For plants full development, soil moisture was maintained in field capacity and luminosity in natural conditions of intensity and photoperiod. The temperature remained between 15 and 25 °C and air moisture controlled between 70 and 95%.

Ten days after the plants emergence, thinning was carried out, keeping five plants in each pot, making up the experimental unit. On the 30<sup>th</sup> day, the leaves green intensity values were measured [Soil-Plant Analysis Development index (SPAD)], obtained by means of the SPAD 502 (Konica Minolta®, Tokyo, Japan) chlorophyll content portable meter in the period between 8-10 h am. The green intensity value considered was the average of readings carried out on five leaves having no physical damages or symptoms of pest and disease attack, randomly sampled in the five plants of each experimental unit.

After the plants development, these were collected and separated in shoot and root. The vegetative samples were washed with distilled water and dried in a forced-air circulation oven at a temperature of approximately 65 °C until reaching constant weight. After drying, the material was weighed to evaluate the shoot dry matter (SDM) and root dry matter (RDM) yields and processed in a plant tissue grinder. Quantification of the Cd and Cu elements in the plant was carried out in the shoot and root. Samples were submitted to acid digestion in Teflon® [polytetrafluoroethylene (PTFE)] tubes, according to the USEPA (The United States Environmental Protection Agency) 3051

## 2<sup>nd</sup> step – Development in a greenhouse.

Plastic pots contained 0.5 kg of Haplic Cambisol (profile from 0 to 10 cm), collected in a natural environment in the Brazilian municipality of Lages, SC. The chemical characteristics of this soil are presented in Table 2.

method in a Multiwave 3000 (USEPA, 1994) microwave oven. In each battery a NIST 1573A Tomato Leaves reference sample was inserted, where 0.77 mg.kg<sup>-1</sup> of Cd were obtained while what was expected was 1.52 ± 0.04 mg.kg<sup>-1</sup> and 4.01 mg.kg<sup>-1</sup> of Cu while what was expected was 4.70 mg.kg<sup>-1</sup> ± 0.14 mg.kg<sup>-1</sup>. The contents of the trace elements of Cd and Cu were quantified in a high resolution atomic absorption spectrometer and electrothermal atomization (CONTRAA 700 – Analytik Jena). Reading conditions were those indicated by the manufacturer. For the available soil contents, the Tedesco (1995) methodology was used. The contents were quantified in atomic absorption spectrophotometry (AA Perkin Elemer – A Analyst 100).

Data were submitted to the Shapiro-Wilk normality tests and the homogeneity of variance Levene's test, applying the required scale transformations when appropriate. Then the data were submitted to analysis of variance according to the completely randomized design in a factorial arrangement. The Tukey's averages comparison test was used at 5% probability to discriminate the effect of factor levels when this was the case.

## Results and Discussion

### *Germination test in the presence of Cd, Cu and Pb.*

For species *B. decumbens* there reductions in GP in exposure to all trace elements studied (Figure 1 A). The cv. Piatã, Marandu and MG 5 of *B. brizantha* have not presented difference in the germination percentage (GP) in exposure to Cd. In exposure to Pb, cultivars Piatã and Marandu have not presented GP differences either. However, cv. MG 5 has had 34% reduction in GP and *B. decumbens* 12% when comparing with the control.

Exposure to Cu has reduced GP in all species studied. However, it was for *B. decumbens* that Cu promoted the highest germination inhibition, because GP in exposure to Cu was only 1%, i.e., a reduction of 99% compared to the control.

The absorption of trace elements in brachiaria promotes oxidative stress, compromising the epidermis walls, which become thinner in relation to exodermis, resulting in the

roots cellular degradation. In addition, the presence of trace elements accelerates maturation of the cell wall in mesoderm and endoderm (Gomes et al., 2011).

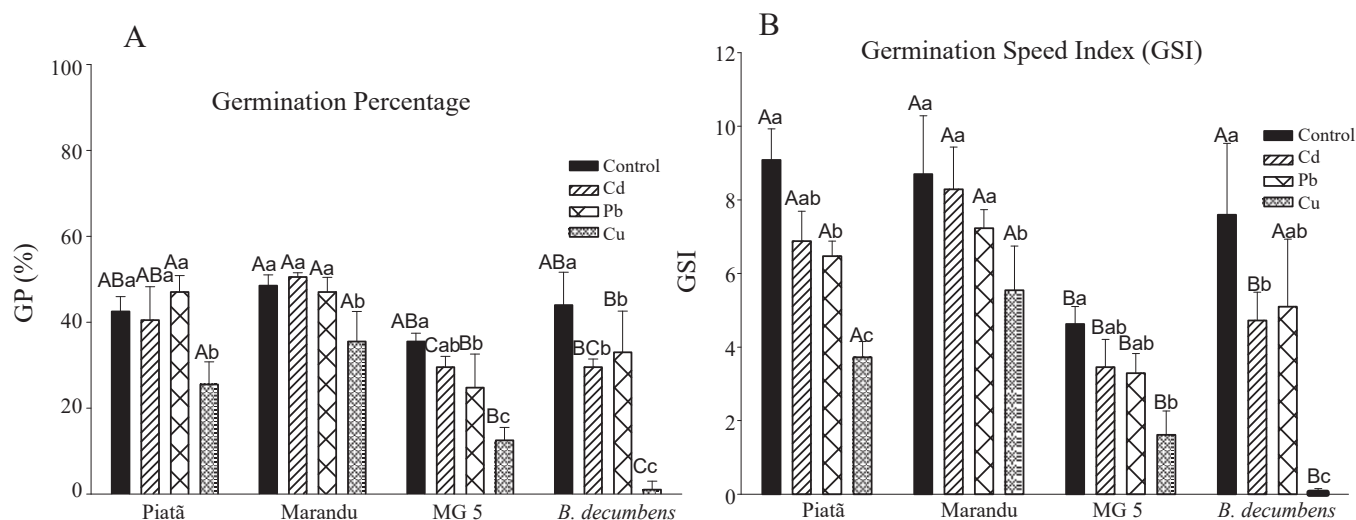


Figure 1. Germination percentage (GP) (A) and Germination speed index (GSI) (B) of species *Brachiaria brizantha* (cv. Piatã, Marandu and MG 5) and *Brachiaria decumbens* in the presence of Cd, Pb and Cu.

Note: Uppercase letters statistically compare species within the same treatment and lowercase letters compare treatments in the same variety.

The presence of trace elements may block the transport of water to the seeds due to the increase in the osmotic potential of the solution, resulting in a secondary effect caused by the low water absorption and not the element toxic effects on the embryo (Kranner and Colville, 2011). Nonetheless, Kalai et al., (2014) state that germination inhibition may occur due to a failure in mobilization of the endosperm caused by the decline of  $\alpha$ -amylase, acid phosphatase activity and alkaline phosphatase, as well as a small modification of  $\beta$ -amylase activity resulting in a failure in the mobilization of Cd and Cu in the endosperm.

In exposure to Cd, cv. Piatã, Marandu and MG 5 of *B. brizantha* have not presented reductions in the germination speed index (GSI). However, in exposure to Pb, for cv. Piatã has caused reduction of GSI from 9 (control) to 6.5 (Pb). This reduction can be explained by germination delayed beginning on exposure to this element, which occurred two days after germination start in the control (Figure 1 B). As it occurred in GP, *B. decumbens* presented GSI reductions in exposure to Cd and Cu. In the exposure to Cu, all species studied had a germination delay of five days compared to that of the germination beginning without the presence of trace elements. Germination delay can occur by the protective role which integument can exert on seeds, being able to block and retain trace elements on its surface. However, when the trace element is absorbed, it is deposited in the endosperm, the

organ responsible for providing nutrients to the germination process, thus being able to be translocated to the embryo (Sun and Luo, 2014). On the other hand, the GSI delay when exposed to Cu may be the result of a reduction in nitrogen availability in the embryonic axis. This is due to the inhibition of protein synthesis resulting in decreased availability of amino acids present in the endosperm (Karmous et al., 2012).

There was no significant difference in shoot length for cultivars *B. brizantha* (cv. Piatã, Marandu and MG 5) in the presence of Cd, Pb and Cu and for *B. decumbens* in the presence of trace elements Cd and Pb (Figure 2 A). However, in the presence of Cu, *B. decumbens* presented inhibition in shoot growth, which reduced from 5.5 to 1.0 cm. The low translocation of trace elements from the root to the shoot causes the shoot to be little influenced by the seedlings exposure to the trace elements (Fidalgo et al., 2013).

In the presence of Cd there was a reduction of root length of 2.5 and 1.0 cm for cv. Piatã of *B. brizantha* and in *B. decumbens* respectively (Figure 2 B). However, for cv. Marandu and MG 5 of *B. brizantha* there was no reduction in root length in the presence of Cd.

In exposure to Pb, cultivars Piatã, Marandu of *B. brizantha* and *B. decumbens* presented reductions in root length. As for exposure to Cu, the reductions in root length could be observed in cultivars Piatã, MG 5 of *B. brizantha* and *B. decumbens*. In cultivars Piatã and Marandu it was observed



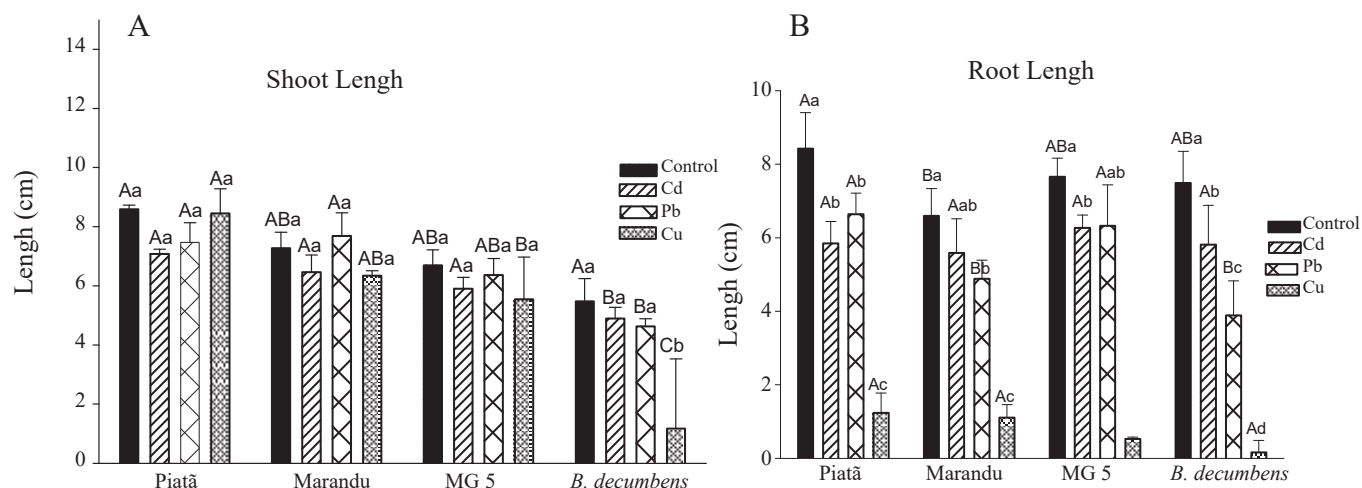


Figure 2. Shoot length (A) and root length (B) of seedlings of *Brachiaria brizantha* (cv. Piatã, Marandu and MG 5) and *Brachiaria decumbens* in the presence of Cd, Pb and Cu.

Note: Uppercase letters statistically compare varieties within the same treatment and lowercase letters compare treatments in the same variety.

that even when not presenting differences in GP, exposure to Pb caused reductions in root length. The greatest reduction of root length was observed in exposure to Cu, with growth not exceeding 1.5 cm in the species studied.

Blockade of enzyme activation and reduction or direct blockage of cell division and interference in mitosis formation may explain the reduction in root growth. Repression of protein synthesis, a DNA replication, can also block cell division (Moosavi et al., 2012).

When plants are exposed to excess Cu, this element can affect the metabolism of N by the reduction of nitrate reductase enzyme responsible for the root length, decrease of the amount of leaves, decrease of the plant biomass caused by the increase of Cu concentration in plant tissues (Xiong et al., 2006).

The presence of trace elements of Cd, Pb and Cu has caused reductions in the Vigor Index (VI) in all species studied (Figure 3). The decrease in VI, which is a result of the low germination percentage and/or decrease in root length, can be caused by the inhibition of mitosis and the synthesis reduction of the cell wall components and in the metabolism of polysaccharides (Heidari and Sarani, 2011). As with the data obtained here, Saderi and Zarinkamar (2012) have obtained germination reductions and low root growth of *Matricaria chamomilla* exposed to Cd and Pb. In seeds of *Populus alba* contaminated by Cd, Madejón et al. (2015) have not found differences in vigor of these seeds comparing with non-contaminated seeds. It is worth noting that the contamination tested by these authors was in seeds and not in the environment.

Germination is the most sensitive phase in a plant life cycle and there is no consistent test or measurable parameter valid for all possible conditions at the time of sowing, i.e., in

vitro germination does not refer to the plants field development conditions (Madejón et al., 2015). Germination tests serve as a preliminary parameter to seedlings development in exposure to trace elements (Di Salvatore et al., 2008). Thus, the experiment was conducted in a second step to evaluate these conditions.

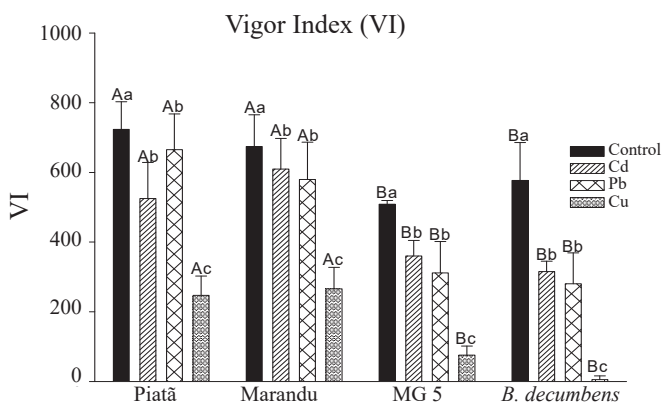


Figure 3. Vigor Index (VI) of seedlings of *B. brizantha* (cv. Piatã, Marandu and MG 5) and *B. decumbens* in the presence of Cd, Pb and Cu.

Note: Uppercase letters statistically compare varieties within the same treatment and lowercase letters compare treatments in the same variety.

#### Greenhouse

The root dry matter (RDM) results showed no interaction among the species studied and the presence or absence of Cd and Cu. By means of the test of means it was observed that there were no differences among the species studied nor for the treatments studied (Table 3).

There was an interaction between the shoot dry matter (SDM) and cultivars of *B. brizantha* and *B. decumbens*. Exposure to Cd has caused an increase of SDM from 1028.2 to 1457.8 mg for cv. MG 5 of *B. brizantha* (Table 3). The presence

of Cu has increased SDM from 483.4 to 836.2 mg in cv. Marandu of *B. brizantha*, an increase of 57.8% when compared to the control. However, for cv. Piatã of *B. brizantha* and for *B. decumbens* there was no difference among treatments.

Table 3. Dry matter (mg.kg<sup>-1</sup>) of the plant tissue of the species *B. brizantha* (cv. Piatã, Marandu and MG 5) and *B. decumbens* (Dec) in the absence and presence of trace elements of Cd and Cu.

| Cultivars | Shoot (SDM) |           |           |        | Root (RDM) |         |         |         |
|-----------|-------------|-----------|-----------|--------|------------|---------|---------|---------|
|           | Control     | Cd        | Cu        | Mean   | Control    | Cd      | Cu      | Mean    |
| Piatã     | 893.8 Ba    | 902.5 Aa  | 902.3 Ba  | 899.6  | 767.5      | 729.5   | 736.7   | 744.6 A |
| Marandu   | 483.4 Cb    | 836.2 Aa  | 891.3 Ba  | 737.0  | 751.3      | 681.8   | 922.4   | 785.2 A |
| MG5       | 1457.9 Aa   | 1028.2 Ab | 1596.7 Aa | 1361.0 | 776.4      | 805.8   | 850.2   | 810.8 A |
| Dec       | 1135.8 ABa  | 1180.8 Aa | 1129.5 Ba | 1148.7 | 569.5      | 697.3   | 773.8   | 680.2 A |
| Mean      | 992.7       | 987.0     | 1130.00   |        | 716.2 a    | 728.6 a | 820.8 a |         |

Note: The representation of the uppercase letters statistically compare species within the same treatment and lowercase letters compare treatments within the same variety.

Andrade et al. (2014) have observed that exposure of *B. decumbens* to Ba and Pb has not resulted in differences in dry matter weight either. Exposure of brachiaria to trace elements may result in the production of phytochelatins and antioxidant compounds such as ascorbate and tocopherol, which minimizes the toxic effects of trace elements (Santos et al., 2011).

An indication of tolerance to plant exposure to trace elements is the dry matter weight proportionality between the absence and presence of these contaminants (Andrade et al., 2014; Tolentino et al., 2014). This has been observed in this study for *B. decumbens* and for cultivar MG 5.

There was a reduction in the SPAD index in all species studied in exposure to Cd and Cu (Figure 4). The negative effect caused by plant exposure to Cd and Cu is reflected in the indirect measure of the chlorophyll content by the SPAD index (Marchiol et al., 2004). The cv. Piatã of *B. brizantha* was the one which presented the highest reductions in the SPAD index from 27 in the absence of trace elements to 19 in the presence of Cd and 18 in the presence of Cu. Santos et al. (2013) have found reductions in the SPAD index in *Phaseolus vulgaris* L. in exposure to increasing doses of Cd. The uptake of Cd in plants can cause disorganization of chloroplast structures and reduction of chlorophyll biosynthesis, interfering with photosynthesis, respiration and water relations (Gill et al., 2013). The levels of chlorophyll, of the enzymes activity of the Calvin cycle and photosystem II (or water-plastoquinone oxidoreductase) are susceptible to trace elements. In combination, both directly and indirectly, these factors contribute to the reduction of carbon assimilation (Gomes et al., 2011).

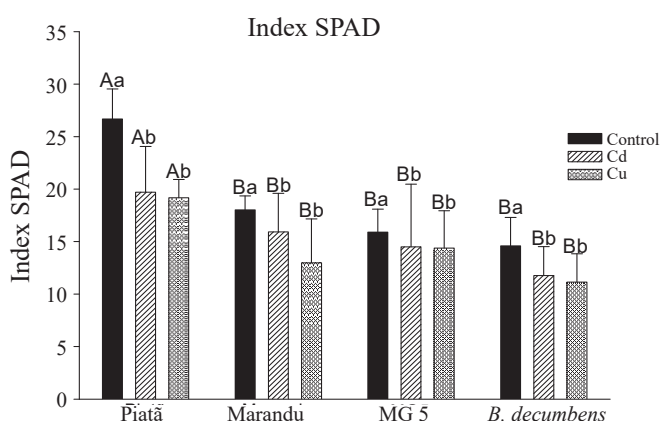


Figure 4. SPAD (Soil Plant Analysis Development) index of species *Brachiaria brizantha* (cv. Piatã, Marandu and MG 5) and *Brachiaria decumbens* in the presence of Cd and Cu.

Uppercase letters statistically compare varieties within the same treatment and lowercase letters compare treatments in the same variety.

The root tissue showed higher levels of Cd and Cu compared to the shoot for all species studied, as can be seen in Table 4. There was no statistical difference between the levels of Cd found in the roots of all cultivars of *B. brizantha* and *B. decumbens*. Cd contents in shoot have not differed among cv. Piatã, Marandu of *B. brizantha* and *B. decumbens*. As for cv. MG 5 of *B. brizantha* it presented the lowest content of Cd. Resistance to trace elements may be based on an exclusion mechanism in which the element accumulates in the roots and its translocation to the shoot is prevented (Rui et al., 2015).

The presence of trace elements may cause changes in size, shape and arrangement of cortical parenchyma in *B. decumbens* cells. These changes indicate an interference of

these trace elements in the root maturation rate, possibly caused by the ability of these contaminants to disturb the plant hormonal balance. The cell walls thickening in the

root may indicate a greater area supply for retention of the trace elements, reducing their translocation to the shoot (Gomes et al., 2011).

Table 4. Cd and Cu content in the plant tissue of species *B. brizantha* cv. Piatã, Marandu, MG 5 and *B. decumbens* (Dec) in the absence and presence of these elements.

|          | Cultivars | Content of Cd mg.kg <sup>-1</sup> |         | Content of Cu mg.kg <sup>-1</sup> |          |
|----------|-----------|-----------------------------------|---------|-----------------------------------|----------|
|          |           | Shoot                             | Root    | Shoot                             | Root     |
| Absence  | Piatã     | 0.40 Ab                           | 1.19 Ab | 0.94 Cb                           | 8.22 Bb  |
|          | Marandu   | 1.30 Ab                           | 1.47 Ab | 5.88 Ab                           | 12.26 Bb |
|          | MG5       | 0.56 Ab                           | 0.87 Bb | 2.30 Bb                           | 12.77 Ba |
|          | Dec       | 0.86 Ab                           | 1.44 Ab | 5.40 Ab                           | 14.20 Ab |
| Presence | Piatã     | 1.95 Aa                           | 5.22 Aa | 8.25 Aa                           | 13.15 Ba |
|          | Marandu   | 2.99 Aa                           | 3.22 Aa | 9.03 Aa                           | 15.77 Ba |
|          | MG5       | 1.43 Ba                           | 3.35 Aa | 5.32 Aa                           | 12.96 Ba |
|          | Dec       | 2.41 Aa                           | 2.77 Aa | 9.06 Aa                           | 30.80 Aa |

Note: The representation of the uppercase letters statistically compare species within the same treatment and lowercase letters compare treatments in the same variety.

Differences of accumulation in different cultivars of the same species were also verified by Rui and contributors (2015). These authors have observed that cultivars of species *Vicia sativa* L. have presented sensitivity differences to Cd. In another study with wheat, concentration differences of Cd, Pb, Zn, Cu, Ni and Cr were found in different cultivars cultivated in soils contaminated with domestic sewage sludge (Jamali et al., 2009).

Santos et al. (2006) have observed an increase of up to 2.7 times in the absorption of Cd in *Brachiaria decumbens* after the application of phytochelins, showing that this species can also increase its remediation power in contaminated soils.

Contrary to germination observed in laboratory, the presence of a high concentration of Cu has not caused a phytotoxic effect for the plants evaluated in a greenhouse. This is possibly the result of Cu ability to be complex with organic matter, binding to the functional carboxylic and phenolic groups (Kim et al., 1999). Cu content available, observed in Table 5, is four times lower than the dose applied to the soil (200 mg.kg<sup>-1</sup>). The same was not observed for Cd, where the content available obtained was of 2.8 mg.kg<sup>-1</sup>, similar to the dose applied to the soil (3 mg.kg<sup>-1</sup>).

Table 5. Available cadmium and copper (mg.kg<sup>-1</sup>) content found in Haplic Cambisol in the absence and presence of contamination.

|          | Cd   | Cu    |
|----------|------|-------|
| Absence  | 0.83 | 1.64  |
| Presence | 2.88 | 52.97 |

In exposure to Cu, *B. decumbens* was the species with the highest Cu content in its root tissue and no phytotoxic effect was observed in plants of *B. decumbens* when in exposure to Cu. This result may be related to lower Cu availability in the soil and low Cu translocation for the shoot.

## Conclusions

*B. decumbens* is more sensitive to the exposure of Cd, Pb and Cu.

*B. decumbens* presents germination inhibition, decrease in germination speed index and vigor index. These effects were less evident for cultivars Piatã, Marandu and MG 5 of *B. brizantha*.

The Vigor Index reduction measured by root length is more sensitive to the presence of Cd, Pb and Cu for the species studied. Shoot length is not affected in the presence of the same elements.

In the germination test, Cu is the trace element that caused the most damage to the germination and initial development of *B. brizantha* (cv. Piatã, Marandu and MG 5) and *B. decumbens* seedlings.

The exposure to Cu causes toxicity to all species studied.

There is no difference in root dry matter production among plants cultivated in the absence and presence of Cd and Cu in the soil.

The presence of Cd and Cu in the soil results in a reduction of the SPAD (Soil Plant Analysis Development) index for *B. brizantha* (cv. Piatã, Marandu and MG 5) and *B. decumbens*.

The highest contents of Cd and Cu are found in roots of all species studied.

*B. decumbens* presents the highest contents of Cu in its root.

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