Critical limits for zinc to forage species

This study aimed to determine the critical limit (LC) of Zn for plants of *Brachiaria Humidicola*, *Panicum maximum* cultivars Aruana and Tanzania and *Paspalum notatum* cv. Pensacola, grown in a Cambisol fertilized with zinc sulfate. Methods: The experiment was realized in the municipality of Lages, Brazil. The experimental design was completely randomized, with four replications. The treatments consisted of applying doses of Zn (0, 25, 50, and 75 mg Kg⁻¹) to the soil and four forage species. Plant height, leaf length, leaf SPAD index, shoot dry mass (MSPA), Zn, Cu, Ca, Mg, Fe, and P contents were analyzed and the LC was determined. Results: For Pensacola, a decrease of 79.63% was found for leaf length and an increase of 147.48% for MSPA. Aruana showed a reduction of 15.43% and 67.05% for MSPA and SPAD index, respectively. An interaction was observed between the doses of Zn and the leaf contents of Fe and Zn for *Humidicola* reaching 175.08% and 564.18% respectively. For Tanzania, in the leaf contents, there was an increase of Zn by 442.6% and a reduction by Mg 80.34%. For Aruana, there was an increase in Ca and Zn by 127.07% and 422.22%, respectively, and a reduction in Fe levels by 64.6%. The LC observed for Aruana, Tanzania and *Humidicola* were 13.0; 9.1 and 29.1 mg Kg⁻¹. Conclusion: Among the forage species, the *Humidicola* has the potential to be used in places with a zinc deficiency because it has the highest LC and the highest concentration of this micronutrient.

Keywords: Cambisol; Micronutrient; Fertilizer; Critic level; Forage grasses.

Limites críticos para zinco para espécies forrageiras

Este trabalho teve como objetivo determinar o limite crítico (LC) de Zn para plantas de *Brachiaria Humidicola*, *Panicum maximum* cultivares Aruana e Tanzânia e *Paspalum notatum* cv. Pensacola, cultivada em Cambissolo fertilizado com sulfato de zinco. Métodos: O experimento foi realizado no município de Lages, Brasil. O delineamento experimental foi inteiramente casualizado, com quatro repetições. Os tratamentos consistiram na aplicação de doses de Zn (0, 25, 50 e 75 mg Kg⁻¹) ao solo e quatro espécies forrageiras. Foram analisados ??os teores de altura de planta, comprimento de folha, índice SPAD foliar, massa seca da parte aérea (MSPA), Zn, Cu, Ca, Mg, Fe e P e determinada a LC. Resultados: Para Pensacola, uma diminuição de 79,63% foi encontrada para o comprimento da folha e um aumento de 147,48% para a MSPA. Aruana apresentou redução de 15,43% e 67,05% para os índices MSPA e SPAD, respectivamente. Foi observada interação entre as doses de Zn e os teores foliares de Fe e Zn para *Humidicola* atingindo 175,08% e 564,18% respectivamente. Para a Tanzânia, no conteúdo foliar, houve aumento de Zn em 442,6% e redução de Mg em 80,34%. Para Aruana, houve um aumento no Ca e Zn em 127,07% e 422,22%, respectivamente, e uma redução nos níveis de Fe em 64,6%. O LC observado para Aruana, Tanzânia e *Humidicola* foi de 13,0; 9,1 e 29,1 mg Kg⁻¹. Conclusão: Dentre as espécies forrageiras, a *Humidicola* tem potencial para ser utilizada em locais com deficiência de zinco por apresentar o maior CL e a maior concentração desse micronutriente.

Palavras-chave: Cambisol; Micronutriente; Fertilizante; Nível crítico; Gramíneas forrageiras.

Topic: Ciências do Solo

Received: 08/06/2021

Approved: 24/06/2021

Reviewed anonymously in the process of blind peer.

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DOI: 10.6008/CBPC2179-6858.2021.006.0008

Referencing this:

SANTOS, A. P.; MATIAS, C. A.; CANTONI, F.; MIQUELLUTI, D. J.; CAMPOS, M. L.. Critical limits for zinc to forage species. *Revista Ibero Americana de Ciências Ambientais*, v.12, n.6, p.97-107, 2021. DOI: <http://doi.org/10.6008/CBPC2179-6858.2021.006.0008>

INTRODUCTION

The main source of feed for cattle in Brazil comes mainly from pastures, consisting mainly of grasses. For formation and maintenance of pastures, it is important that the supply and balance of nutrients are adequate, directly influencing the quality of forage. The practice of fertilization is indispensable to obtain good productivity of pastures, especially micronutrients such as zinc (HERNANDES et al., 2009).

Zinc is essential for plant growth, being responsible for nutritional regulation, chlorophyll synthesis, reproductive growth, carbohydrate production and seed and fruit development (TRIPATHI et al., 2015). However, the presence of Zn in tropical soils, in a natural way, is scarce, with a deficiency of this micronutrient (FAQUIN, 2005), which has limited the production of agricultural crops in Brazil (PRADO et al., 2008), caused crop yield losses that can exceed 40% (ALLOWAY, 2008; SADEGHZADEH, 2013).

In plants, Zn acts on protein synthesis and plant growth, participating in the formation of the amino acid tryptophan, which forms indole acetic acid, which is essential for apical growth and cell expansion. When a plant is deficient in zinc, it has reduced height, internodes, leaves and productivity (SANTOS et al., 2009a; MUNER et al., 2011).

Although there is no zinc deficiency in pastures, it is observed that the content of zinc in plant tissue in many forages tends to be lower than the minimum requirement for cattle (ALLOWAY, 2008), which is 30 mg kg⁻¹ Zn of dry matter for beef cattle (NRC, 1996). Thus, it is recommended that when the Zn levels are below 20 mg kg⁻¹, an inorganic (or organic) fertilization is carried out through foliar applications or in the Zn soil (CHATZISTATHIS, 2014).

The concept of toxic level or critical limit (LC) is related to the content of a certain nutrient in which there is a reduction in production based on the maximum level of productivity (FAGERIA et al., 1997). In general, the LC of an element can be correlated with parameters related to crop production, such as: photosynthesis, growth rate and dry mass production (MALAVOLTA et al., 1997). The toxicity level is reached when these parameters are reduced between 80 to 95% of the optimum production (BATAGLIA et al., 1992). Upon reaching toxicity, the concentration of the nutrient is higher than sufficiency, reaching the critical physiological level, exceeding the entire range of luxury consumption and causing negative effects on crop yields (KURIHARA et al., 2005). This study determined the LC of Zn for plants of *Brachiaria humidicola*, *Panicum maximum* cultivars Aruana and Tanzania and *Paspalum notatum* cv. Pensacola, grown in a Humic Cambisol fertilized with zinc sulphate.

METHODOLOGY

An experiment was conducted from February to May 2017, in a greenhouse, on the premises of the Department of Soils and Natural Resources of CAV/UDESC - Lages/SC. The soil used was a leptic Aluminium Humic Cambisol, collected at a depth of 0 to 20 cm. The region has well-defined seasons, with average annual temperatures of 15 to 25 °C, average annual rainfall distribution of 1500 mm, characterized by the climate type Cfb - Temperate (humid mesothermal and mild summer) by the Koeppen classification (EPAGRI). Two

soil samples were sent to the Soil Chemical Analysis Laboratory - LASQ of CAV-UDESC, the average values being reported: pH in water 5.0; SMP index 4.8, CTC at pH 7.0 of 23.3 $\text{cmol}_c \text{dm}^{-3}$; base saturation of 25.8%, contents of exchangeable Al, Ca and Mg 2.75; 3.16 and 2.55 $\text{cmol}_c \text{dm}^{-3}$ respectively; K, P, Fe and Mn contents of 122, 3, 55.4 and 3.8 mg dm^{-3} , respectively; and content of organic matter and clay of 25 and 400 g dm^{-3} , respectively. The Zn content was 0.3 mg dm^{-3} , being below the critical level of 0.5 mg dm^{-3} (CQFS-RS/SC).

The pH correction was carried out with the addition of dolomitic lime, and during the incubation period the field capacity was maintained at 60%. The pH stabilization at 5.9 occurred on the 30th day. After this period, 0.48 g kg^{-1} of urea were applied; 0.18 g kg^{-1} of potassium chloride and 0.5 g kg^{-1} of triple superphosphate, as recommended in the fertilization and liming manual (CQFS-RS/SC). Concomitantly, increasing doses of Zn were applied to the soil (0, 25, 50 and 75 mg Kg^{-1}), using zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$).

The experimental design was completely randomized, with four replications. The treatments consisted of applying increasing doses of Zn (0, 25, 50 and 75 mg Kg^{-1}) to the soil and four forage species, namely: *Panicum maximum* Jacq. cultivars Aruana and Tanzania, *Paspalum notatum* Flüggé cv. Pensacola and *Brachiaria humidicola*.

In each experimental unit, 15 seeds were sown. Thinning was carried out ten days after emergence, leaving two plants per pot. At 70 days after sowing, plant height, leaf length, SPAD index of leaves were determined using a portable chlorophyll meter (SPAD 502; Konica Minolta®, Tokyo, Japan). The readings of the SPAD index were made in the middle third (SPADme) of three leaves.

The plants were harvested at 72 days, when growth stabilization occurred. The aerial part of the plant was dried in a forced air circulation oven, at 65 °C, for 72 hours.

For the extraction of Zn, Ca, Cu, Fe, Mg and P from the aerial part the acid digestion of the samples was carried out using the method 3051A in a Multiwave 3000 microwave oven (Anton Paar, Graz, Austria). After cooling, the samples were transferred to falcon tubes, with a volume of 25 mL with ultrapure water. The extraction and quantification were performed in duplicates. The levels of Zn, Cu, Ca, Mg, Fe and P were quantified by an inductively coupled plasma emission spectrometer (ICP-OES) (Optima® 8300, Perkin Elmer, USA). The reliability of the analytical method used to determine the levels of Zn in plants was guaranteed by the use of standard reference material from the National Institute of Standards and Technology (NIST - SRM Pine Needles 1575a). The recovered levels of Zn in the reference sample (NIST) were 84% of Zn.

To determine the LC of Zn, the MSPA production data of the aerial part were used. The LC was calculated based on a 10% reduction in dry matter production after it reached its maximum level (Fageria et al. 1997). To find the toxic level, therefore, the calculation was performed based on the adjusted regression equation and with $y = 90\%$. Data were subjected to analysis of variance using the F test, at 5% probability of error. The models were fitted into regression equations using the Sigma Plot 12.5 software.

RESULTS

There was an interaction between Pensacola and Zn levels in the soil for leaf length and MSPA, as shown in Figure 1. There was a linear decrease of 79.63% for leaf length and polynomial increase of 147.48%

for MSPA, with a minimum of 47.48% found in the dose of 25 mg Kg⁻¹. There was no significant difference for the SPAD index (Table 1).

Table 1: Average values of parameters that did not show a significant difference.

ARUANA						
Doses of Zn	Content in the leaf			Leaf Length		
	Mg	P	Cu			
mg kg ⁻¹	mg kg ⁻¹			Cm		
0	10380.8	1746.4	13.9	16.2		
25	10001.4	1587.2	14.4	14.9		
50	8795.0	1739.5	14.1	16.3		
75	10704.4	1912.7	11.1	18.7		
TANZANIA						
Doses of Zn	Content in the leaf				Leaf Length	
	Ca	P	Cu	Fe		
mg kg ⁻¹	mg kg ⁻¹				Cm	
0	4692.0	1959.4	1.3	302.4	18.3	
25	5130.2	1810.1	11.1	301.6	22.2	
50	5008.8	1748.5	11.1	216.7	19.5	
75	5714.2	1881.7	11.2	237.6	18.9	
PENSACOLA						
Doses of Zn	Content in the leaf					SPAD Index
	Ca	Mg	P	Cu	Fe	
mg kg ⁻¹			mg kg ⁻¹			
0	5532.5	7641.8	2258.0	14.9	1193.3	31.7
25	6554.6	7945.5	2452.8	14.7	1332.1	31.1
50	5783.1	6845.9	2431.4	16.5	1161.6	31.4
75	5571.5	7020.3	2516.4	14.4	495.4	28.1
HUMIDICOLA						
Doses of Zn	Content in the leaf				Leaf Length	SPAD Index
	Ca	Mg	P	Cu		
mg kg ⁻¹	mg kg ⁻¹				cm	
0	4128.4	6817.9	2360.6	12.9	11.2	27.0
25	2610.4	4911.6	1856.6	11.0	13.7	28.7
50	3557.2	6929.9	2355.9	12.6	9.8	25.9
75	4600.6	8190.9	2236.4	11.2	11.6	25.5

Fig. 1: Leaf length and dry mass of aerial part (MSPA) of *Paspalum notatum* Flüggé cv. Pensacola subjected to different doses of Zn applied to the soil.

There was an interaction between the doses of Zn and MSPA for Aruana (Figure 2) and, SPAD index for Aruana and Tanzania (Figure 3). For MSPA there was a linear reduction of 15.43%. While for the SPAD index there was a linear decrease of 67.05% and 50.09% for Aruana and Tanzania respectively. There was no significant interaction for soil Zn and MSPA doses, leaf length and SPAD index for *Brachiaria humidicola* (Table 1).

Fig. 2: Dry mass of the aerial part (MSPA) observed in *Panicum maximum* Jacq. cv. Aruana, subjected to increasing concentrations of Zn.

Fig. 3: SPAD index observed for *Panicum maximum* Jacq. cv. Aruana (a) and cv. Tanzania (b), subjected to increasing doses of Zn applied to the soil.

Nutrient content in species

The forage species showed a differential response to the nutrient content in the leaf when exposed to the Zn doses. For Aruana, there was a change in Ca, Fe and Zn behaviour as shown in Figure 4. The increase in the concentration of the element linearly increased the levels of Ca in the leaf by 127.07%. In addition, the regression of Fe and Zn levels showed a polynomial response with an increase of Zn by 422.22% and a reduction of 84.66% for Fe, reaching a maximum reduction of 69.29% in the dose of 25 mg kg⁻¹. There was no significant difference for P, Mg and Cu, as shown in Table 1.

There was an interaction between the doses of Zn and the leaf contents of Fe and Zn for humid crops, reaching 175.08% and 564.18% respectively, as shown in Figure 5. For the other nutrients there was no significant interaction (Table 1).

The increase in the concentration of Zn did not significantly differ the levels of Ca, P, Cu and Fe, for Tanzania, as shown in Table 1. However, it provided a linear increase for the leaf content of Zn in 442.60% and reduction for the leaf content of Mg by 80.34%, as shown in Figure 6.

Fig. 4: Calcium, Iron and Zinc contents in the aerial part of the species *Panicum maximum* Jacq. cv. Aruana grown in soil with increasing doses of Zn.

Fig. 5: Leaf contents of Iron and Zinc observed in *Brachiaria humidicola* submitted to increasing doses of Zn.

Fig. 6: Magnesium and Zinc contents found in the aerial part of *Panicum maximum* Jacq. cv. Tanzania, submitted to different doses of Zn in the soil.

Critical limit for Aruana, Tanzania and Humidicola

The LC expresses the concentration of Zn applied to the soil, which reduces 10% of production, being important to assess productive losses and optimize fertilization (NARDIS et al., 2017). The critical limits observed for Aruana, Tanzania and Humidicola were 13.0; 9.1 and 29.1 mg Kg⁻¹, respectively, as shown in Figure 7. Confirming the different levels of Zn sensitivity by forage species, which can be explained by the intrinsic characteristics between them.

Fig. 7: Critical limit observed for *Panicum maximum* Jacq. cv. Aruana (a), *Panicum maximum* Jacq. cv. Tanzania (b) and *Brachiaria humidicola* (c) exposed to increasing doses of Zn in the soil.

DISCUSSION

Zn acts as a cofactor, activating different hormones (auxin), necessary for plant growth and development (BEGUM et al., 2016), thus, the increase in MSPA yield with the increase in Zn doses for Pensacola may be associated with the function of enzymatic activation of metabolic processes (HERNANDES et al., 2009). While the reduction of MSPA observed in Aruana may indicate low tolerance of the species to the increase in the Zn content in the soil, since the reduction of the leaf area and consequent reduction of photosynthesis and decrease of production due to the triggered toxicity (CHERIF et al., 2010; ALEXANDRE et al., 2012). The decrease in MSPA with excess Zn consists of the difficulty of rising sap as a consequence of

the accumulation of callose present in the xylem (PEDROSA et al., 2013). Nardis et al. (2017) evaluated the effects of increasing doses of Zn (0, 100, 300 and 900 on the soil in Aruana, Tanzania, Marandu and Xaraes, verifying a reduction in MSPA for all grasses.

The inhibition of the absorption and translocation of Mg to the chloroplast triggers the reduction of the SPAD index, as a consequence of the decrease in the biosynthesis of photosynthetic pigments such as chlorophyll (a, b) as seen for Tanzania (TSONEV et al., 2012). Also, the reduction in the SPAD index for Aruana and Tanzania may be associated with the formation of buffers in the xylem, as a result of excess Zn, making it difficult for the crude sap to rise, reducing the concentration of chlorophyll (a, b) (SILVA et al., 2010). For both situations there is a decline in the activity of photosystem II (PS II). The potential of PS II is compromised by reducing the transfer of electrons from the primary plastoquinone receptor (Qa) to the secondary plastoquinone receptor (Qb) on the PSII acceptor side (SHU et al., 2016). Thus, the use of electrons is reduced in primary processes, such as the production of NADPH in chloroplasts during biochemical CO₂ fixation. With the reduction of NADPH availability, there is a decrease in chlorophyll synthesis by the enzyme divinyl chlorophyllide α 8-vinylreductase, which uses NADPH as a substrate (Silva et al. 2012). Still, the excess of Zn in the aerial part favours the production of reactive O₂ species causing oxidative stress, since these substances are toxic to chloroplasts (SANTOS et al., 2009b).

Nutrient Content in Species

The quadratic and linear increase in Fe content in Aruana and Humidicola leaves respectively as a function of the increase in the Zn dose applied to the soil is due to the strategy mechanism II commanded by phytosiderophores, which are chelating agents and have Fe affinity (PUSCHENREITER et al., 2017). Plants exude phytosiderophores to the rhizosphere, which bind to Fe³⁺ and form a chelate complex (Fe³⁺-FS) by the action of the enzyme nicotianamine synthase (ZHAO et al., 2019). The absorption of this complex occurs through the plasma membrane with the aid of transporters from the Yellow Stripe family (YS) (ALEXANDRE et al., 2012; DAI et al., 2019), following the transpiration flow to the aerial part (NOZOYE et al., 2011; ARAUJO et al., 2020).

Solubilization of Zn by grasses is similar to strategy II for Fe, since phytosiderophores have affinity for other cations (WALTER et al., 2016). Still, the accumulation of Zn concentration in the roots favours the excess to be translocated to the aerial part (NARDIS et al., 2017). This behaviour was observed for Aruana, Humidicola and Tanzania. Leaf content varied among forage species, confirming that there are different levels of absorption between them. The maximum Zn content observed, at the dose of 75 mg Kg⁻¹, was for Humidicola (138.79 mg Kg⁻¹) and the lowest content found in Aruana (33.82 mg Kg⁻¹). Zn has moderate phytotoxicity, and when it is translocated to the aerial part, it occurs, through the biochemical mechanism of plants, storage of the element in vacuoles, chloroplasts and cell membranes (NARDIS et al., 2017).

It is important to note that the absorption mechanism shared by Zn and Fe results in competitiveness for complexation, affecting the bioavailability of the elements, as observed for Aruana and Humidicola (SCHENKEVELD et al., 2014).

The differences in leaf content for Zn between species is explained by the distinct nutritional requirement, as well as the absorption efficiency depends on the amount of exudated phytosiderophores, being different between SPECIES (FLORA et al., 2010; OBURGER et al., 2014). And the lower Zn content in the leaf observed for Aruana can be attributed to the reduction in MSPA, since the extraction of Zn for the aerial part is mainly influenced by the MSPA variable (SHEORAN et al., 2010).

The reduction in Mg levels observed for Tanzania is attributed to inhibition triggered by Zn. Both are bivalent cations, so they compete for the same bioactive sites. Mg is replaced by Zn in chlorophyll molecules, causing metabolic imbalance, such as altering the photosynthetic capacity of plants (ALBUQUERQUE et al., 2020).

Critical limit for Aruana, Tanzania and Humidicola

Aerial part Zn and dry matter production data were used to find the LC for the species Aruana, Tanzania and Humidicola, which was based on a 10% reduction in the production of these species (FAGERIA, 2000). Finding the critical Zn limit of these species is essential, as zinc can affect plant growth both when its content is lower and higher than the critical level, this is due to deficiency and/or toxicity problems, respectively (NOULAS et al., 2018).

The critical levels of nutrients in the leaf provide necessary information for the assessment of the nutritional status of a given crop, assisting with fertilization recommendations and/or soil nutritional corrections if necessary (LEITE et al., 2003).

Although there is no zinc deficiency in pastures, it is observed that the Zn content in plant tissue in many forages, tends to be lower than the minimum requirement for cattle (ALLOWAY, 2008), which is 30 mg kg⁻¹ of dry matter Zn for beef cattle (NRC, 1996). Thus, it is recommended that when the Zn levels are below 20 mg kg⁻¹, an inorganic (or organic) fertilization is carried out through foliar applications or in the Zn soil (CHATZISTATHIS, 2014).

CONCLUSIONS

Pensacola showed responses to soil zinc doses, decreasing for leaf length and increasing for MSPA. The doses of zinc in the soil caused a decrease in MSPA for Aruana and the SPAD index for Aruana and Tanzania. Aruana showed an increase in Ca and Zn in the leaf when exposed to zinc doses in the soil. The species had different critical limits, Tanzania had the lowest LC and Humidicola the highest LC for zinc doses. Humidicola has potential to be cultivated in places where there is a lack of zinc in forages, since it presented a higher LC to this micronutrient and a higher concentration of zinc in the leaves.

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