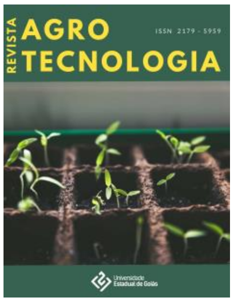
KERNEL GERMINATION AND SEEDLING GROWTH OF CORN SUBMITTED TO DIFFERENT CONCENTRATIONS OF ZINC

GERMINAÇÃO DE SEMENTES E CRESCIMENTO DE PLÂNTULAS DE MILHO SOB DIFERENTES CONCENTRACÕES DE ZINCO

Rafael Dal Bosco Ducatti[1], Fabio Pilon[1], Ivan Pedro Murari[1], Samuel Mariano-da-Silva[1]



Abstract: Zinc, which is naturally present in soils, is derived from the weathering of its parental material and is essential for the good growth of plants. However, the levels of this metal in soils have been reaching high concentrations mainly due to the input of liquid pig manure, used as fertilizer. The goal of this study was to investigate the effects of zinc concentrations on the kernel germination and the seedling growth of corn. We carried out an experiment in test plots sub-divided in randomized blocks, with cross-classification and three replicates. The kernels of the cultivar were divided into three lots with 12 replicates of 50 kernels each. Kernels were sown in pre-moistened Germitex paper. The water used to soak the three lots was added with different concentrations of zinc chloride ($ZnCl_2$), so that the final zinc concentrations were 0, 100, 200 and 400 mg L^{-1}. The rolls of paper were kept in germination chambers at constant temperature (25 °C) and the evaluations were carried out, for each treatment, on the fifth, sixth and seventh days after sowing. Zinc affected the germination percentage and the root size of corn, however, it did not affect the shoot size.

KEY-WORDS: Germination test, heavy metal, zinc contamination.

Resumo: Zinco, o qual é naturalmente encontrado nos solos, é derivado da intemperização de material de origem e essencial para o bom desenvolvimento das plantas. No entanto, os níveis deste metal nos solos tem alcançado altas concentrações, principalmente devido a aplicação de desejos suínos líquidos. O objetivo deste trabalho foi verificar os efeitos da concentração de zinco sobre a germinação de sementes e crescimento de plântulas de milho. Foi realizado um ensaio em esquema de parcelas subdivididas em blocos ao acaso, com classificação cruzada e três repetições. As sementes da cultivar foram divididas em três lotes com 12 repetições de 50 sementes cada, com cada repetição semeada em substrato de papel Germitex previamente umedecido. A água utilizada para a embebição dos lotes foi acrescida de diferentes concentrações de cloreto de zinco ($ZnCl_2$), de maneira que as concentrações finais de zinco fossem 0, 100, 200 e 400 mg L^{-1}. Os rolos de papel foram mantidos em germinadores a temperatura constante de 25 ºC, sendo as avaliações realizadas para cada tratamento no quinto, sexto e sétimo dias após a semeadura. O zinco afetou a porcentagem de germinação e o tamanho das raízes do milho, não apresentando influência no tamanho da parte aérea.

PALAVRAS-CHAVE: Contaminação por zinco, ensaio de germinação, metal pesado.

[1]Department of Agronomy. Federal University of Fronteira Sul (UFFS) – Campus Chapecó. Chapecó, Santa Catarina, Brazil. rafaelducatti1007@gmail.com

Revista Agrotecnologia, Ipameri, v.10, n.1, p.54-61, 2019
Doi: 10.12971/2179-5959/agrotecnologia.v10n1p54-61

INTRODUCTION

Currently, Brazil has the fourth largest pig production in the world, with a herd of about 40 million heads (IBGE, 2016). The state of Santa Catarina is the largest producer of pigs in Brazil, accounting for 27% of the national production (GATIBONI et al., 2015). Despite the undeniable importance of this activity for the regional development, the environmental impacts caused by this intense activity have raised concern of public and private agencies (MATTIAS et al., 2010).

Due to the high amount of animals in the region, pig manure as a fertilizer, when applied in excess, can exceed the absorptive capacity of local ecosystems, becoming a potential cause of soil contamination by metals such as zinc (Zn) (ASADA et al., 2010; MATTIAS et al., 2010). According to Basso et al. (2012), Zn is the element that is most likely to accumulate in soils that receive successive applications of liquid pig manure. Asada et al. (2010), reported that the mobility and leaching of Zn in soils amended with excessive amounts of pig manure is increased, leading to heavy metal accumulation and harming the development of certain plant species.

Although Zn is an essential element for plant growth and shows no defined structural function, it participates in numerous enzymatic reactions (TAIZ; ZEIGER, 2017), and is recognized as a precursor of tryptophan, an essential amino acid for the synthesis of auxin in the plant (SOMKUWAR et al., 2013). Alloway (2008), describes Zn as being essential for carbohydrate and protein metabolism in plants, as well as for the maintenance of biological membranes and resistance to infections.

Zinc requirements, for most cultures, does not reach 1 kg ha^{-1}, and its content in the soil ranges depending on 1) the region of the world, 2) the use of the soil and 3) the factors controlling Zn inputs in the soil (ALLOWAY, 2008). Many are the forms in which Zn inputs take place in soils, going from the decomposition of the soil's parent material, to atmospheric deposition and agricultural inputs (manures, fertilizers, industrial wastes, etc.) (ALLOWAY, 2008; MATTIAS et al., 2010; SADEGHZADEH, 2013). Alloway (2008), stated that levels ranging from 100 to 500 mg kg^{-1} can be toxic to plants and cause a reduction in yield of up to 25%.

Due to the low requirements of this micronutrient, the input of high doses in soils can cause toxicity in plants, leading to metabolic disorders, decrease of roots and shoots growth and deficiency in photosynthetic parameters, which reflect the photo-inhibition, and causes oxidative stress (TIECHER et al., 2016). In corn, the major symptom of toxicity is the appearance of yellow stripes on the leaves, however, reddish brown pigments on the leaves can be found when corn is grown in dark sandy or organic soils (ALLOWAY, 2008).

There are few studies in the literature on the effects of nutritional disorder related to Zn toxicity in corn seedlings. Considering this context and the importance of this crop, we aimed at verifying the effects of toxic and sub-toxic concentrations of Zn on kernel germination and seedling growth of corn grown in germination chambers.

MATERIAL AND METHODS

The experiment was entirely conducted at the plant physiology laboratories of the Federal University of Fronteira Sul (UFFS), Campus Chapecó, in 2018. Corn kernels of the cultivar "Crioulo Fortuna" were obtained from the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI) - Chapecó.

The experiment was planned under a scheme of sub-divided plots with an experimental design in randomized blocks, with cross-classification and 3 replicates (ARES; GRANATO 2014). The kernels were divided into 3 lots with 12 replications of 50 kernels each. The kernels were sown in pre-moistened Germitex paper with a volume of water corresponding to 2.5 times the paper weight. The water used to soak the 3 lots was added with different concentrations of zinc chloride ($ZnCl_2$), so that the final Zn concentrations were 0.0, 100.0, 200.0 and 400.0 mg L^{-1}. The rolls of paper were kept in germination chambers at constant temperature (25 °C), and the evaluations carried out for each treatment on the fifth, sixth and seventh days after sowing (BRASIL, 2009).

Kernel vigor and viability was assessed on the fifth, sixth and seventh days after sowing, and the data collected were converted to percentage of normal seedlings (BRASIL, 2009). The velocity of kernel germination was calculated according to the formula of Edmond and Drapala (1958):

$$SG = \frac{[(D1 \times P1) + (D2 \times P2) + (D3 \times P3)]}{(P1 + P2 + P3)} \quad (1)$$

where,
SG = the velocity of germination expressed in days;
D1 = number of days after sowing in the first counting;
D2 = number of days after sowing in the second counting;
D3 = number of days after sowing in the third counting;
P1 = number of normal seedlings in the first counting;
P2 = number of normal seedlings in the second counting;
P3 = number of normal seedlings in the third counting;

The growth assessment took place on the fifth, sixth and seventh days after sowing, eliminating abnormal seedlings and dead kernels. With the aid of a millimeter ruler, we measured the size of the primary root and the size of the shoot. The mean results were expressed in cm $seedling^{-1}$ (BRASIL, 2009).

The response of all the variables was analyzed using the statistical program "R" version 3.2.3. Two-way and One-way ANOVA were used to analyze the average of the variables, and when significantly different, the averages were compared with the use of Tukey's HSD multiple comparison method. Regressions for the days of assessment as a function of Zn concentrations were also established as to characterize the effect of Zn on the different variables analyzed on this study (ARES; GRANATO, 2014).

RESULTS AND DISCUSSION

Analyzing the germination (%) of corn kernels contaminated with different amounts of Zn concentrations we found no significant interaction between the Zn concentration used to start the experiment and the day of assessment (Table 1), therefore we dropped this interaction and rerun the analysis. We found that the day of assessment didn't affect the germination of kernels (Table 1), however, the Zn concentrations used to start the experiment had a large impact on the germination (%) of corn (Table 2).

Table 1. F-statistics and *P* values for analyses of germination as a function of the day of assessment, concentration of zinc and the interaction between these variables

Germination percentage	Degrees of freedom	F-value	*P*-value
Day of assessment	02	1.496	0.265
Zinc concentration	03	10.676	$5.13E^{-5}$
Day of assessment x Zinc concentration	06	1.641	0.164

Revista Agrotecnologia, Ipameri, v.10, n.1, p.54-61, 2019
Doi: 10.12971/2179-5959/agrotecnologia.v10n1p54-61

Table 2. Germination (%) of normal seedlings as a function of the zinc concentration and the day of assessment

Days	mg L^{-1} of Zn			
	0	100	200	400
Fifth	59.0	68.5	71.0	34.0
Sixth	58.5	61.5	60.0	33.0
Seventh	63.5	71.5	54.0	54.0
Mean	60.3 A	67.1 A	61.6 A	40.3 B
	Standard deviation (%) = 16.3259			

Averages followed by same letters are not significantly different according to Tukey's HSD multi comparison tests (alpha = 5%)

Zinc concentrations affected the percentage germination of normal seedlings. The germinations obtained for the highest Zn concentration used were 43, 50.4 and 54% lower than the percentage obtained at the 0, 100 and 200 concentrations, respectively. Our findings go in accordance to those reported by Baran (2013), who reported that Zn can influence the germination of corn by up to 50%, due to its toxic effects.

According to Appenroth (2010), it is difficult to define the dose in which heavy metals become toxic to plants, as their toxicity vary depending upon their density, soil properties and the element properties. However, we can say that heavy metals are only toxic when they exceed a certain threshold of concentration in the plant.

The effect of the different concentrations of Zn on the percentage of germination can be represented by the estimates of second-degree polynomial equations shown in Figure 1.

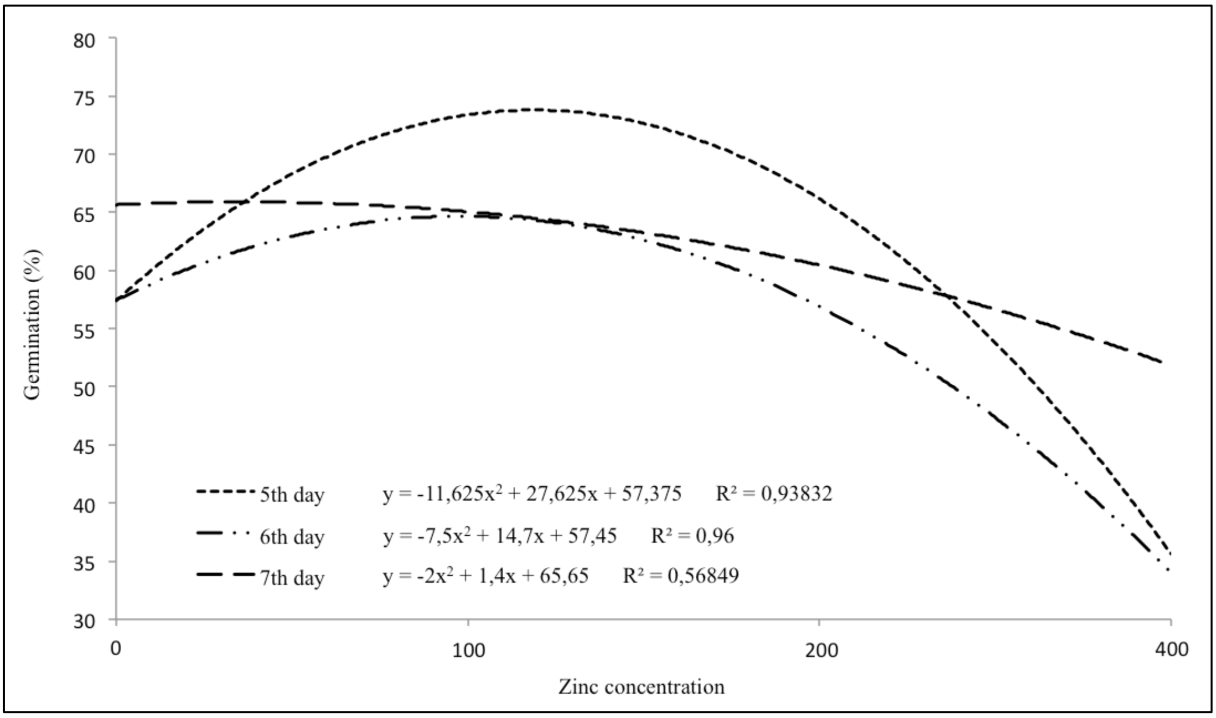


Figure 1. Second-degree polynomial equation related to the effect of Zn doses for the percentage of germination.

Revista Agrotecnologia, Ipameri, v.10, n.1, p.54-61, 2019
Doi: 10.12971/2179-5959/agrotecnologia.v10n1p54-61

The quadratic behavior of the percentage of germination demonstrates the role of Zn in the physiology of corn as a promoter of germination (Figure 1), perhaps showing its participation as an enzyme activator (TAIZ; ZEIGER, 2017).

The coefficient of velocity of germination can be seen in Table 3. We used the Edmond and Drapala (1958) equation to come up with the velocity of germination. This equation is based on the average germination time of kernels attempting to reduce the tendencies of encountering biased results (RANAL; SANTANA, 2006).

Table 3. Coefficient of velocity for germination (SD).

mg L^{-1} of Zn	SD[1]
0	6,0304
100	6,0142
200	5.9105
400	6.1666

[1]SD expresses the average of days need for germination to take place

It is possible to verify that there was no variation throughout the treatments, i.e., Zn does not affect the velocity of germination of kernels, once the germination does not depend on the content of nutrients present in the bedding media, but, on water, oxygen and temperature (SHABAN, 2013).

When analyzing the root size, we found a significant interaction between the day of assessment and the concentration of Zn used to start the experiment (P = 0.0031). It shows that not only did the concentrations influenced the size of the root, but also the amount of time given to the plant to grow (Table 4).

Table 4. Root size (cm $root^{-1}$) measured during the germination tests.

Days	mg L^{-1} of Zn			
	0,0	100	200	400
Fifth	10.20 Aab	10.44 Aa	10.73 Aa	8.57 Ab
Sixth	12.00 Ba	12.41 Ba	11.76 Aa	11.27 Ba
Seventh	13.75 Ca	12.72 Ba	13.70 Ba	10.81 Bb
	Standard deviation (%) = 3.3449			

Averages followed by same letters (lowercase on the same line or uppercase on the same column) are not significantly different according to F and Tukey's HSD multi comparison tests (alpha = 5%)

By looking at table 4 we can clearly see that the 400.0 mg L^{-1} treatment caused toxic effects on the development of roots, at least for the fifth and seventh days of assessment. Baran (2013), shows that increases from 0.0 to 750.0 mg kg^{-1} of Zn can reduce the root size up to 29.2%. A similar, but not so abrupt, tendency was observed in our study.

As reported by Sadeghzadeh (2013), corn kernels have the ability of storing large amounts of Zn, which can be beneficial when plants are grown in soils with Zn deficiency. In this case, Zn reserves may favor the establishment of the crop and improve vegetative growth. As stated by the same author, increasing the density of micronutrients in kernels can confer greater resistance to root diseases.

Table 4 also shows that, for most of the Zn concentrations used, when the time allowed for the development of the plant is broadened, longer root systems were obtained, going in accordance to the findings of Chilian et al., (2015). The effect of the different concentrations of Zn on the root size of corn can be represented by the estimates of second-degree polynomial equations shown in Figure 2.

Revista Agrotecnologia, Ipameri, v.10, n.1, p.54-61, 2019
Doi: 10.12971/2179-5959/agrotecnologia.v10n1p54-61

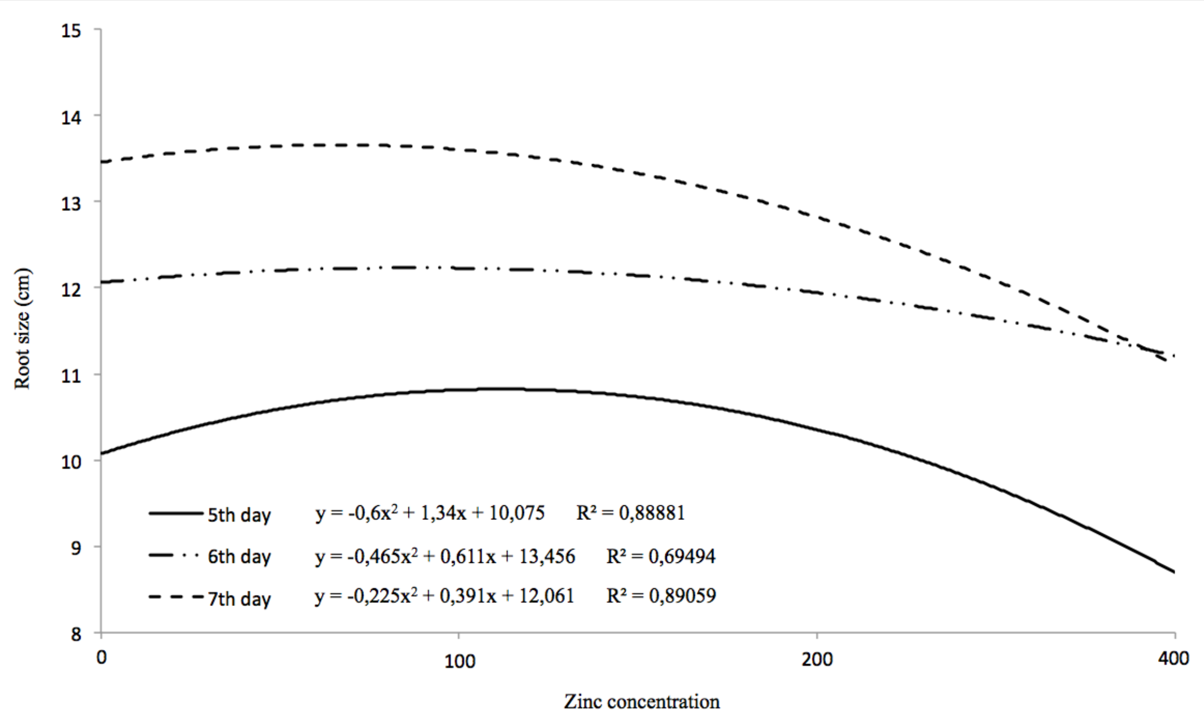


Figure 2. Second-degree polynomial equations related to Zn doses for the root size.

The quadratic behavior of the root size demonstrates the importance of Zn in the physiology of corn as a growth promoter (Figure 2), showing its participation as a precursor of auxins (SOMKUWAR et al., 2013).

The shoot size was also analyzed taking into consideration the interaction between the concentrations of Zn used to start the experiment and the day of assessment (Table 5). We found a significant interaction within the averages of these variables ($P = 5.46E^{-11}$), which shows that both variables influenced the shoot size of corn seedlings.

Table 5. Shoot size (cm seedling^{-1}) measured during the germination tests.

Days	mg L^{-1} of Zn			
	0,0	100	200	400
Fifth	3.11 Aa	3.82 Ab	3.06 Aa	3.25 Aab
Sixth	3.26 Aa	4.13 Ab	3.42 ABa	3.75 ABab
Seventh	3.69 Aabc	3.09 Ba	3.86 Bbc	4.19 Bc
Standard Deviation(%) = 1.5877				

Averages followed by same letters (lowercase on the same line or uppercase on the same column) are not significantly different according to F and Tukey's HSD multi comparison tests (alpha = 5%)

Similar results were found by Baran (2013) and Chilian et al., (2015). These authors reported that higher contents of Zn affected the growth of corn, however, even in high Zn concentrations the shoot size of corn plants was higher when plants were allowed more time to grow. The effect of the different concentrations of Zn on the shoot size of corn can be represented by the estimates of second-degree polynomial equations shown in Figure 3, showing the importance of Zn as a promoter of growth (TAIZ; ZEIGER, 2017; SOMKUWAR et al., 2013).

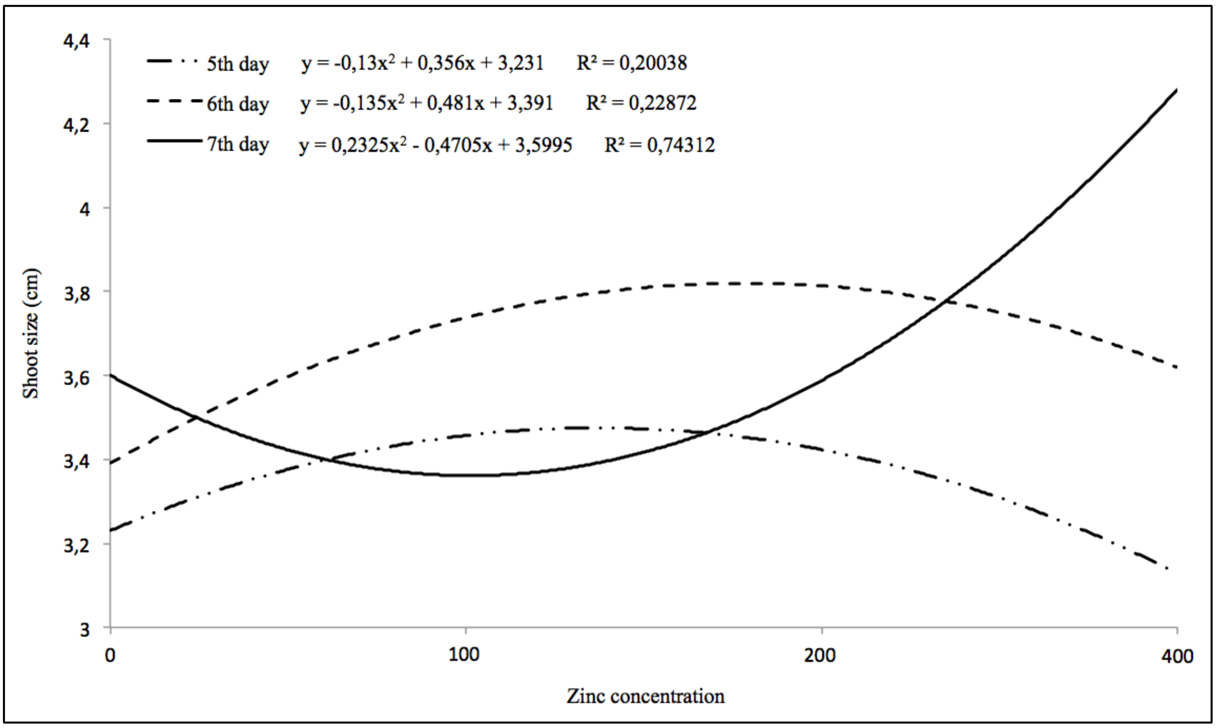


Figure 3. Second-degree polynomial equations related to Zn doses for the shoot size.

Looking at Figure 3 we can infer that, for the seventh day, there is a positive correlation between the dose of Zn used and the shoot growth, however, this outcome may have been favored by the brief period of assessment used in the study.

CONCLUSION

Zinc doses affected the percentage of germination, shoot size and root size of corn seedlings, however it didn´t affect the velocity of kernel germination. More studies are necessary to assess what the Zn threshold is for corn and its cultivars, and to confirm if Zn, even is high doses can negatively affect the development of shoot after the seventh day of observation.

REFERENCES BIBLIOGRAPHICAL

ALLOWAY, B. J. **Zinc in Soils and Crop Nutrition**. France: IZA/IFA, 2008. 137p.

APPENROTH, K-J. What are "heavy metals" in plant sciences? **Acta Physiologiae Plantarum**, v.32, n.4, p.615-619, 2010.

ARES, G.; GRANATO, D. **Mathematical and statistical methods in food science and technology**. New Jersey: John Wiley & Sons, Inc. 2014. 536p.

ASADA, K.; TOYOTA, K.; NISHIMURA, T.; IKEDA, J.; HORI, K. Accumulation and mobility of zinc in soil amended with different levels of pig-manure compost. **Journal of Environmental Science and Health Part B**, v.45, n.4, p.285-292, 2010.

BARAN, A. Assessment of *Zea mays* sensitivity to toxic content of Zinc in soil. **Polish Journal of Environmental Studies**, v.22, n.1, p.77-83, 2013.

BASSO, C. J.; CERETTA, C. A.; FLORES, E. M. M.; GIROTTO, E. Teores totais de metais pesados no solo após aplicação de dejeto líquido de suínos. **Ciência Rural**, Santa Maria, v. 42, n. 4, p.653-659, 2012.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para Análise de Sementes**. Brasília: MAPA/ACS, 2009. 399p.

CHILIAN, A.; BANCUTA, R. O.; BANCUTA, I.; SETNESCU, R.; ION, R-M.;

RADULESCO, C.; SETNESCU, T.; STIHI, C.; GHEBOIANU, A. I.; CHELARESCU, E. D. Study of the influence of Zn concentration on the absorption and transport of Fe in maize by AAS and EDXRF analysis techniques. **Romanian Reports in Physics**, v.67, n.3, p.1138-1151, 2015.

EDMOND J. B.; DRAPALA W. J. The effects of temperature, sand, soil and acetone on germination of okra seed. **Proceedings of the American Society for horticultural Science**, v.71, n.5, p.428-434, 1958.

GATIBONI, L. C.; SMYTH, T. J.; SCHMITT, D. E.; CASSOL, P. C.; OLIVEIRA, C. M. B. Soil phosphorus thresholds in evaluating risk of environmental transfer to surface waters in Santa Catarina, Brazil. **Revista Brasileira de Ciência do Solo**, v.39, n.4, p.1225-1234, 2015.

Instituto Brasileiro de Geografia e Estatística (IBGE). **Sistema IBGE de Recuperação Automática – SIDRA. Pesquisa da Pecuária Municipal**. 2016. Online. Disponível em: <https://sidra.ibge.gov.br/tabela/3939>. Acesso em: 17/03/2018.

MATTIAS, J. L.; CERETTA, C. A.; NESI, C. N.; GIROTTO, E.; TRENTIN, E. E.; LOURENZI, C. R.; VIEIRA, R. C. B. Cooper, zinc and manganese in soils of two watersheds in Santa Catarina with intensive use of pig slurry. **Revista Brasileira de Ciência do Solo**, v.34, n.4, p.1455-1454, 2010.

RANAL, M. A.; SANTANA, D. G. How and why to measure the germination process? **Brazilian Journal of Botany**, v.29, n.1, p.1-11, 2006.

SADEGHZADEH, B. A review of zinc nutrition and plant breeding. **Journal of Soil Science and Plant Nutrition**, v.13, n.4, p.905-927, 2013.

SHABAN, M. Effect of water and temperature on seed germination and emergence as a seed hydrothermal time model. **International Journal of Advanced Biological and Biomedical Research**, v.1, n.12, p.1686-1691, 2013.

SOMKUWAR, R. G.; SHARMA, J.; SATISHA, J.; KHAN, I.; ITROUTWAR, P. Effect of zinc application to mothervines of dog ridge rootstock on rooting success and establishment under nursery conditions. **International Journal of Scientific & Technology Research**, v.2, n.9, p.198-201, 2013.

TAIZ, L; ZEIGER, E. **Fisiologia e desenvolvimento vegetal**. Porto Alegre: Artmed. 2017. 858p.

TIECHER, T. L.; CERETTA, C. A.; TIECHER, T.; FERREIRA, P. A. A.; NICOLOSO, F. T.; SORIANI, H. H.; ROSSATO, L. V.; MIMMO, T.; CESCO, S.; LOURENZI, C. R.; GIACHINI, A. J.; BRUNETTO, G. Effects of zinc to a copper-contaminated vineyard soil on sorption of Zn by soil and plant physiological responses. **Ecotoxicology and Environmental Safety**, v.129, p.109-119, 2016.