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Phytomass of beans and grain production as affected by zinc, copper and cadmium doses and bentonite application[1]

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ABSTRACT

The objective of this study was to determine the effect of zinc, copper and cadmium on phytomass and grain production and to evaluate indirectly the adsorbent effect of bentonite clay by determining the accumulation of these heavy metals in bean plants. The study consisted of three separate experiments (one for each cation) with the application of three doses of bentonite (0, 30 and 60 t ha^{-1}) in pots containing separately 50 mg of Zn; Cu and Cd kg^{-1} of soil, with three replicates. Thus, each experiment consisted of 9 experimental units. In each one, a bean plant was cultivated and after 65 days, samples of leaves, stems, roots and grains were collected. Afterwards, the samples were dried, weighed, grinded and the concentrations of Zn, Cu and Cd were determined. The phytomass of leaves of the plant growing on the soil with Zn, the Zn concentration in the whole plant and the Cd concentration in the leaves, stem and grains were significantly influenced by the bentonite application. Zn and Cd concentration in leaves, stem and grains decreased with the application of bentonite, indicating a positive effect of the clay application to the soil on the adsorption of these metals, decreasing the availability for plants. Zn was the most accumulated element in the plant, followed by Cd and Cu. The Zn, Cu and Cd accumulation in the plant obeyed the following sequence: stem > leaves > grains > roots; roots > stem > grains > leaves; roots > stem > leaves > grains, respectively.

Key words: heavy metal, clay, *Vigna unguiculata* L.

Fitomassa e produção do feijão afetadas pelas doses de zinco, cobre e cádmio e aplicação de bentonita

RESUMO

Objetivou-se, com este trabalho, estudar o efeito do cobre, zinco e cádmio em fitomassa e produção de grãos e avaliar indiretamente o efeito adsorvente da argila bentonita, determinando o acúmulo de metais pesados em plantas de feijão. O estudo consistiu de três experimentos separados (um para cada cátion), com a aplicação de três doses de bentonita (0, 30 e 60 t ha^{-1}) em vasos contendo, separadamente, 50 mg de Zn, 50 mg de Cu e 50 mg de Cd kg^{-1} de solo, com três repetições. Assim, cada experimento consistiu de nove unidades experimentais e em cada uma delas se cultivou uma planta; aos 65 dias após o plantio, colheram-se folhas, caules, raízes e grãos que, depois de secos, pesados e moídos, foram analisados para Zn, Cu e Cd. A fitomassa das folhas das plantas cultivadas no solo com Zn, a concentração de Zn na planta inteira e a concentração de Cd nas folhas, caule e grãos foram significativamente influenciados pela aplicação de bentonita. As concentrações de Zn e Cd nas folhas, caule e grãos, diminuíram com a aplicação de bentonita indicando efeito positivo da aplicação de argila no solo sobre a adsorção desses metais, diminuindo a disponibilidade para as plantas. Zn foi o elemento que mais se acumulou nas plantas, seguido pelo Cd e Cu. O acúmulo de Zn, Cu e Cd na planta obedeceu a seguinte sequência: caule > folha > grãos > raiz; raiz > caule > grãos > folha; raiz > caule > folha > grãos, respectivamente.

Palavras-chave: metal pesado, argila, *Vigna unguiculata* L.

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Introduction

The accelerated increase of population, city urbanization, industrial and technological development, indiscriminate use of fertilizers (mineral or organic), soil amendments (including also the use of residual water coming from water treatment stations) has contributed to the increase of heavy metals in the soil and water (Rangel et al., 2006; Cunha et al., 2008).

The use of contaminated soils and water is more worrying, when they are used for agriculture and horticultural products and harvested parts of these plants are consumed by humans, because plants are the main entry of the heavy metals in the food chain (Alcarde & Rodella, 2003). Heavy metals, such as zinc (Zn), copper (Cu) and cadmium (Cd), frequently accumulate in the surface layer of the soil (0–20 cm), also called the “agricultural layer”, making these elements available to the roots and consequently accumulated in the plant, reaching toxic levels when present in high concentrations.

In this context, several procedures have been proposed to reduce the mobility and bioavailability of heavy metals: microorganisms (bioremediation), plants (phytoremediation) or soil conditioners (lime, soluble sources of P, Fe and Mn and clay materials) (Silveira et al., 2008). The use of adsorbent surfaces, such as clay minerals (specifically bentonite) has been studied (Rodrigues et al., 2004). Bentonite is primarily a 2:1 layered silicate Na-montmorillonite; the isomorphic substitution of Al^{3+} by Si^{4+} and Mg^{2+} by Al^{3+} results in a net negative surface charge on the clay, which gives to the bentonite the ability to adsorb cations and therefore heavy metals. The low cost and high availability of these materials (Chui, 2005; Lacin et al., 2005) and their adsorption properties has caught the attention for its use as an alternative adsorbent material. Lacin et al. (2005) has shown that bentonite is highly efficient for the removal of zinc ions and adsorption of toxic heavy metal cations such as Cu, Cd and Pb.

This work had as its main objective the determination of the effects of zinc, copper and cadmium on the phytomass and grain production of bean plants (*Vigna unguiculata* (L) Walp.) and indirectly to infer the adsorbent capacity of the bentonite when applied to the soil by determining the metals concentration in the plant.

Material and methods

The study was carried out from August to October 2008 in a semi-controlled greenhouse conditions of the Agricultural Engineering Department of the Federal University of Campina Grande, Campina Grande, Paraiba State, Brazil. Temperatures ranged from approximately 32 °C during the day to 27 °C during the night.

The study consisted of three separated experiments. The first with the application of three doses of bentonite (0, 30 and 60 t ha^{-1}) in recepients containing 50 mg kg^{-1} of soil of Zn; the second the same three doses of bentonite in recepients containing 50 mg kg^{-1} of soil of Cu, and the third one the same three doses of bentonite in recepients containing 50 mg kg^{-1} of soil of Cd, with three replicates. The experimental design was completely randomized.

Each experimental unit consisted of a plastic recepients filled with 15 kg of a gray Distrophic Argisol with the following chemical characteristics (determined using, EMBRAPA (1997) procedures): pH (H_2O) = 5.8; Ca = 2.61 $cmol_c$ kg^{-1}; Mg = 0.83 $cmol_c$ kg^{-1}; Na = 0.03 $cmol_c$ kg^{-1}; K = 0.05 $cmol_c$ kg^{-1}; H + Al = 1.48 $cmol_c$ kg^{-1}; OM = 16.2 g kg^{-1}; P = 18.3 mg kg^{-1}; Zn = 0.264 mg kg^{-1}; Cu = 0.074 mg kg^{-1}; Cd = 0.05 mg kg^{-1}. The soil was dried, passed through a 5 mm mesh and mixed with the bentonite. According to the references, a soil pH of 5.5 to 6.2 is preferred for beans. Before the planting the soil was fertilized according to the standard recommendations: 100 mg kg^{-1} of N; 300 mg kg^{-1} of P_2O_5 and 150 mg kg^{-1} of K_2O. After the soil was set in the recepients, the metals Zn, Cu and Cd were applied and the soil water content brought to about 80% of the field capacity. The soil was left during ten days to allow the bentonite to interact with the soil and metals applied; afterwards seeds of bean (*Vigna unguiculata* (L) Walp.) were planted leaving finally on plant per recepients. When the experiment was finalized, the plants were collected and separated into leaves, stems, roots and grains, washed with deionized water, oven-dried at 65 °C to a constant weight, grinded and weighed. The plant samples suffered nitro-perchloric acid digestion and the Zn, Cu and Cd concentrations were measured using an atomic absorption spectrophotometer. The plants were irrigated daily with tap water and the water volumes calculated using the gravimetric method. The water was applied manually using a graduated beaker.

Results and discussion

The Technological Company for Environmental Sanitation of São Paulo (CETESB) established the following values as maximum limits of heavy metals in agricultural soils: Cu, 200 ; Zn, 450 and Cd 3 mg kg^{-1} (Fernandes et al., 2007). In general, the data show that the soil utilized in the study was not contaminated by these heavy metals.

Bean plants cultivated in Zn and Cu presence had similar leaves, stem, roots and grain phytomass production (Table 1). Carvalho et al. (2008) working with beans on a soil containing 54 mg dm^{-3} of Zn found an aerial phytomass of 10.06 g $recepients^{-1}$, lower than the present study, for similar Zn application and without bentonite application. This fact seems to show that bentonite applied to the soil adsorbed in some way the Zn, and prevented the adverse effects of excess Zn in the soil.

Table 1. Phytomass of leaves, steam, roots and grains of the bean plant growing in soils containing Zn, Cu and Cd (Mean of the three bentonite treatments)

Cation per Kg of soil	Leaves	Steam	Roots	Grains
	g $recepients^{-1}$			
50 mg Zn	9.43	10.48	2.88	11.55
50 mg Cu	10.38	10.64	3.30	9.72
50 mg Cd	6.60	5.96	2.16	8.91

Regarding the presence of Cd, the phytomass production was lower than when the plants were cultivated in soils with Zn an Cu applications (Table 1). Carvalho et al. (2008), evaluating the effect of the Cd on beans, found an aerial phytomass and grain production of 5.87 and 4.3 g, respectively, with an application to the soil of 20 mg dm^{-3} of Cd. It is interesting to observe that the phytomass and grain production of beans in this work practically doubled that obtained by Carvalho et al. (2008), even when the Cd application in the former research was more than twice. This fact again allows thinking about the beneficial effects of the bentonite, adsorbing the Cd and therefore decreasing the availability for plants and the pollution effects of the ecosystem.

Table 2 shows a summary of the variance analysis for the Zn, Cu and Cd concentrations in bean leaves, stem, roots and grains when submitted to the bentonite treatments. It is observed that Zn concentration of leaves, stem, roots and grains were significantly affected ($p < 0.01$) by the increasing doses of bentonite. No significant effect, however, the application of bentonite had on Cu content of the plant. With the exception of the roots, bentonite application affected significantly ($p < 0.01$) the concentration of Cd on plant tissues.

Table 2. Summary of the variance analysis for the Zn, Cu and Cd concentrations in bean leaves, stem, roots and grains when submitted to bentonite applications

Effect of bentonite	Mean square			
	Leaves	Stem	Roots	Grain
Zinc	35.39 **	51.47**	112.74 **	12.89**
Copper	0.32 ns	0.42 ns	0.34 ns	0.23 ns
Cadmium	25.31**	25.06**	4.29 ns	33.46**
	Means ($mg\ kg^{-1}$)			
Bentonite	Zn			
0 t ha^{-1}	132.75 a	143.75 a	154.50 a	48.50 a
30 t ha^{-1}	130.00 a	114.25 b	133.50 b	45.50 b
60 t ha^{-1}	113.50 b	110.17 b	111.83 c	44.78 b
	Cu			
0 t ha^{-1}	2.60 a	3.17 a	47.47 a	3.20 a
30 t ha^{-1}	2.47 a	2.92 a	44.27 a	3.10 a
60 t ha^{-1}	2.60 a	3.03 a	43.35 a	3.15 a
	Cd			
0 t ha^{-1}	24.80 a	41.55 a	148.75 a	1.85 a
30 t ha^{-1}	20.80 c	34.45 b	133.47 a	1.17 b
60 t ha^{-1}	22.80 b	36.67 b	110.83 a	1.35 b

** significant at 1% and ^{ns} non significant at 1% (F test); means followed by the same case letters in columns for each element are not different ($p > 0.01$)

Mean heavy metal concentration (Table 2) show that, in general, Zn concentration in leaves, stem, roots and grains decreased with the application of bentonite. The same occurred with Cd concentration in leaves, stem and grain. The decrease of Zn and Cd in the plant indicates a positive effect of the clay application to the soil on the adsorption of these metals. The decrease of the metals in the plant tissues probably would be due to the decrease of the cations in the soil, due to the adsorption by the bentonite. Although not significant, there was also an apparent decrease of Cu with the bentonite application. It is also observed that Cu and Cd were higher in the roots when compared with the rest of the plant. The high concentration of Cu encountered in the roots are in agreement with the results obtained by Xiaohai et al. (2008) and Camargo & Muraoka (2007) working with castor bean and cashew nuts of Brazil, respectively. In general the Zn, Cu and Cd concentrations in the grain were always the smallest, results also found by Oliveira et al. (2005) and Carvalho et al. (2008).

It is also observed in Table 2 that, although the doses of Zn, Cu and Cd applied to the soil were the same (50 mg kg^{-1}), the concentrations of Zn found on the different parts of the plant were higher than those of Cu and Cd. One of the reasons for this can be the fact that bean plants have more facility to absorb Zn and a low capacity to absorb Cu (Marsola et al., 2005). Another reason is the lowest adsorption of Zn by the bentonite (Tito, 2009) resulting in a higher quantity of Zn in the soil solution available for the plants. Working with the same treatments she observed that the bentonite adsorbed more Cu than Zn and Cd. The steepest decline of the adsorption isotherm for Cu shows that, for the same concentration of metal, there is a greater amount of Cu adsorbed, showing a high affinity of the bentonite for this metal. Prado & Juliatti (2003), studying the leaching of Cd in two different soil columns, did not observe the presence of this metal in the leached solution, indicating that Cd was adsorbed in both soils.

Figure 1 shows the accumulation of Zn, Cu and Cd in the different parts of the plant for the bentonite treatments. The application of bentonite to the soil which received Zn had a significant negative effect ($p < 0.01$) on the accumulation of Zn in the leaves and roots of the bean, decreasing it in about 12%. No effect of the bentonite application was observed on the Cu and Cd accumulated in the different parts of the plant. However, a decreasing trend was observed for the Cd when the bentonite was applied. It can be seen on Figure 1 also, that Zn was the element which most accumulated in the plant, followed by Cd and Cu. The low content of Cu in the plant was also observed in beans by Marsola et al. (2005). The phenomenon was attributed, by the authors, to the high affinity of the Cu with the organic matter of the root tissues, resulting in higher Cu content in these, preventing the translocation to the rest of the plant.

The highest Zn accumulation occurred in the stem and leaves, results agreeing with those found by Soares et al. (2001) which indicate that the accumulation of Zn in the stem can contribute to its immobilization in the plant. The highest Cu and Cd accumulation occurred on the roots (Figure 1).

According to Arduini et al. (1996) the absorption of heavy metals by the rizosphere, the accumulation of these in the roots and the low translocation to the aerial part of the plant are considered the mechanisms by which the root system of the plant contributes to the regulation of the presence of heavy metals in the plant and the tolerance to them.

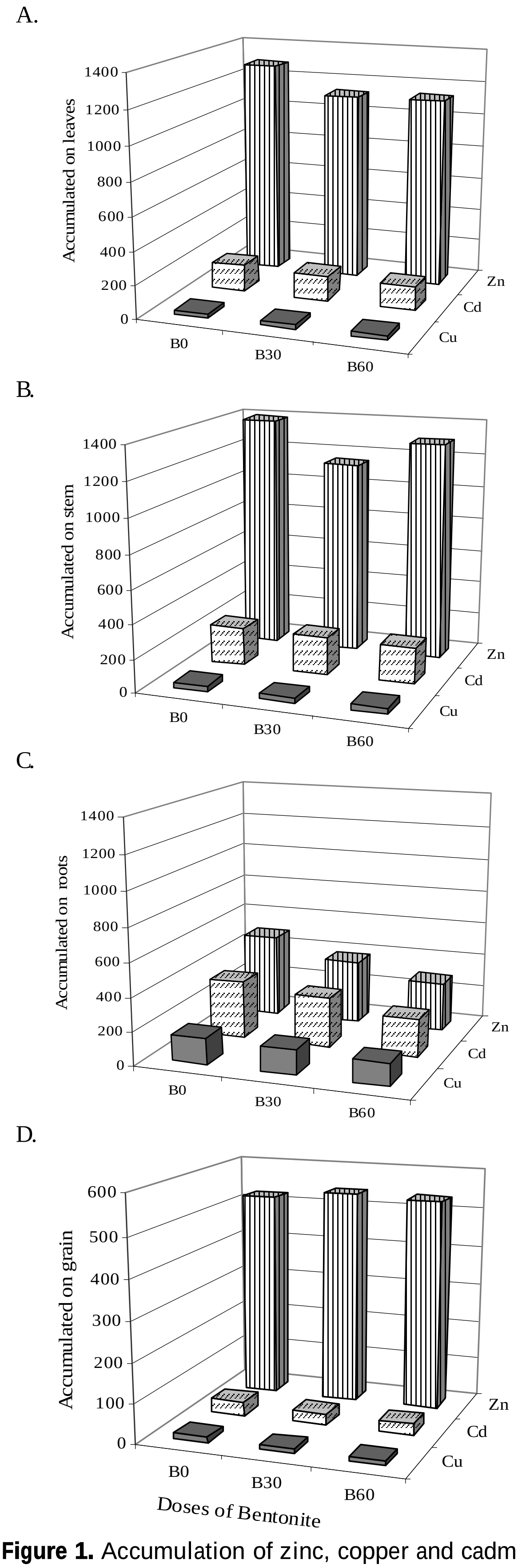

Figure 1. Accumulation of zinc, copper and cadmium (in μg) on the different parts (A-leaves; B – stem; C – roots; D – grain) of the plant for the bentonite treatments (B0; B30; B60 t ha^{-1})

Conclusions

1. The phytomass of leaves of the plant growing on the soil with Zn, the Zn concentration in the whole plant and the Cd concentration in the leaves, stem and grains were influenced by bentonite application.

2. Zn and Cd concentration in leaves, stem and grains decreased with the application of bentonite, indicating a positive effect of the clay application to the soil on the adsorption of these metals, decreasing the availability for the bean plants.

3. Zn was the most accumulated element in the plant, followed by Cd and Cu.

4. The accumulation of zinc, copper and cadmium on the whole plant obeyed the following decreasing sequences: stem > leaves > grains > roots for the zinc; roots > stem > grains > leaves for the copper and roots > stem > leaves > grains for the cadmium.

5. The low cost and high availability of bentonite in the region and its apparently and efficient adsorption properties leads to recommend its use for the removal of toxic heavy metals such as the studied zinc, copper and cadmium.

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