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Effect of Bentonite in Zinc Contaminated Soils on Plant Development

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Abstract: The objective of this study was to evaluate the ability of bentonite to the remediation of artificially zinc (Zn) contaminated soils, having the lettuce, corn and grass as test plants. The experiment for each plant consisted of bentonite: 0. 30. 60 and 90 t ha^{-1} (4 repetitions) in a completely randomized design totaling 16 experimental units. Soil samples mixed with the bentonite plus NPK and 500 mg Zn kg^{-1} to lettuce and 250 mg Zn kg^{-1} to corn and grass were placed in plastic pots with 5 kg, 14 kg and 8 kg capacity for lettuce, corn and grass, respectively and incubated for 20 days; after this period the crops were seeded; At 70; 60 and 70 days of experimental period the plants lettuce, corn and grass, respectively, were harvested and prepared for plant analysis. The cumulative amount of Zn in dry biomass of the shoot and roots, the translocation index, translocation factor and the bioaccumulation factor in the plant and in the root, were evaluated. Bentonite promoted the retention of zinc in the soil. Translocation index and factor and bioaccumulation factors of zinc decreased as a function bentonite. The application of bentonite to the contaminated soils favored their remediation.

Key words: Heavy metals · Contamination · Accumulation · Bioaccumulation

INTRODUCTION

Numerous industrial activities have significantly increased concentrations of heavy metals in the environment contributing to the degradation. The exaggerated use of agricultural inputs and the use of sewage sludge in agriculture as a source of nutrients for the plants represent dangerous sources of pollution, especially as regards the addition of heavy metals to the soil. These metals can express their pollutant potential through availability to plants at phytotoxic levels and the possibility of transfer to the food chain through the plants themselves [1].

Contamination of the soil by heavy metals can occur immediately due to the large release of metals in the environment or over time through accumulation of the same for years in nature, often causing irreversible damage.

Zinc (Zn) is one of the most abundant trace elements and has been proved to be an essential nutrient for human, animal, plant and bacterial growth [2]. Thus, Zn deficiency can reduce grain yield and weaken the resistance of cereals to diseases, decrease the nutritional quality of grains; drastic reduction of protein synthesis (due to its relation with RNase activity); cause delay and reduction in growth; small and poorly shaped leaves; chlorophytum (due to the participation of Zn in the formation of chlorophyll), besides necrosis in the apical meristem of the root [3, 4]. At high concentrations, this metal is potentially toxic [5]. The toxicity of Zn in plants leads to a reduction in both shoot dry matter production and root biomass; necrosis of the radicle upon contact with the soil; planting and plant growth inhibition [6, 7]. The maximum tolerable value for vegetables roots and tubers and other fresh foods, according to the Brazilian legislation, for the element zinc is 50 mg kg^{-1} [8], while the heavy metals content in the dry matter causing symptoms of plant phytotoxicity is 70-400 mg kg^{-1} [9].

Several methods have been used to remove zinc from water and/or soil, for example, adsorption, precipitation, ion exchange and reverse osmosis, being that adsorption on solid surfaces is the most common one. The use of clay as an adsorbent for removing heavy metals is due to its cation exchange capacity (CEC), selectivity, regenerability and abundance compared to other natural and synthetic adsorbents, in addition to using low cost adsorbents [10, 11].

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Bentonite is a rock formed of highly colloidal and plastic clays composed mainly of montmorillonite (smectite group). The industrial application of bentonite clay depends on the composition of their clay and non-clay minerals. The clay minerals in bentonite are smectite such as montmorillonite, beidellite, saponite, hectorite, etc. On the other hand, the non-clay minerals include silica (for example, quartz and opals), feldspars, zeolites, carbonates, sulphites, sulphides, sulphates, oxides and hydroxides. According to Sen and Gomez [12], the use of bentonite has proved its effectiveness in the adsorption of heavy metals.

The objective of this study was to evaluate the ability of bentonite to the remediation of artificially zinc (Zn) contaminated soils, having the lettuce, corn and grass as test plants.

MATERIALS AND METHODS

Soil, Bentonite and Experimental Site: This study was carried out under greenhouse conditions from July 2016 to October 2016 at the Agricultural Engineering Department, Federal University of Campina Grande, Paraiba, Brazil.

Three independent experiments were carried out with lettuce (*Lactuca sativa* L.), corn (*Zea mays* L.) and grass (*Brachiaria brizantha* cv.) on loam sandy soil classified as Eutrophic Red Latosol [13], collected in Campina Grande, PB region at a 0-20 cm soil depth with chemically characterized according to Embrapa [14].

The bentonite clay used in this study was collected from a Paraiba State region. The samples were air dried and sieved with 2 mm and 0.074 mm mesh in order to proceed chemical and X-ray diffraction analyzes, respectively.

Composition of Soil and Bentonite: The following attributes of soil samples were as follows: pH (H_2O) = 6.0; electrical conductivity = 0.16 mmhos cm^{-1}; Ca = 2.10 cmol$_c$ kg^{-1}; Mg = 2.57 cmol$_c$kg^{-1}; Na = 0.06 cmol$_c$kg^{-1}; K = 0.14 cmol$_c$kg^{-1}; H+ Al = 1.78 cmol$_c$kg^{-1}; organic carbon = 5.5 g kg^{-1}; P = 45 mg kg^{-1} and Zn = 14.16 mg kg^{-1}.

The X-rays diaphactogram of this bentonite is presented in Fig. 1. The diaphactogram picks observed are typical of the smectite (E) clays and picks of tridymite (T), a silicate mineral and polymorph of high temperature of quartz. Picks of quartz (Q) are observed although in a low quantity.

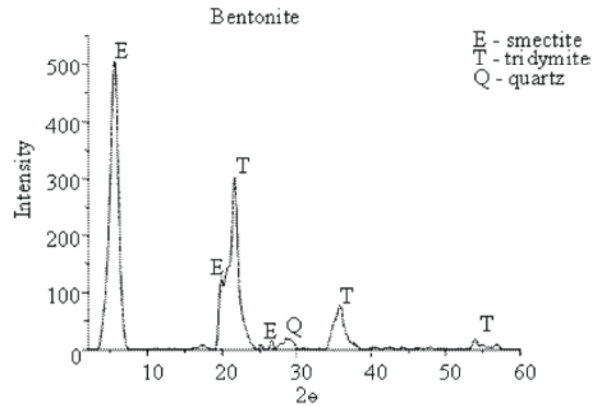

Fig. 1: The diaphactogram picks observed are typical of the smectite (S) clays and picks of tridymite (T), a silicate mineral and polymorph of high temperature of quartz. Picks of quartz are observed although in a low quantity.

Treatments and Doses: The experiment for each plant consisted of four doses of bentonite: 0.0; 10.7; 21.4 and 32.1 g kg^{-1}, corresponding to 0. 30. 60 and 90 t ha^{-1}, respectively, with 4 repetitions in a completely randomized design totaling 16 experimental units (plastic pots).

Incubation Condition and Time after Treatments: Soil samples mixed with the bentonite dose corresponding to treatment were placed in plastic pots with 5 kg, 14 kg and 8 kg capacity for lettuce, corn and grass, respectively. These mixtures were incubated for 20 days with moisture corresponding to field capacity.

Conduct of the Study: Soil seeded with lettuce received 500 mg kg^{-1} of zinc plus 1.11 g of urea, 1.25 g of potassium chloride (KCl) and 8.3 g of super phosphate (P_2O_5) per pot; that seeded with corn and grass received 250 mg kg^{-1} of zinc plus 3.11 and 1.78 g of urea, 3.5 and 2.0 g of potassium chloride (KCl) and 23.3 and 13.33 g of super phosphate (P_2O_5) per pot, respectively to these plants [15].

After the incubation period and NPK fertilization, the seeds of each crop were sown and 8 days after the emergency a thinning was conducted leaving two plants per pot. The irrigation was conducted using tap water maintaining the soil to field capacity.

Collection of Vegetal Material: At 70; 60 and 70 days of experimental period the plants lettuce, corn and grass, respectively, were harvested and separated into aerial part and roots, washed with distilled water and placed in paper bags in order to be dried in forced air stove at 65°C during 48 hours. After drying, the plants were triturated and samples were weighed for foliar analyses.

Determination of Zinc in Shoots and Roots of the Plants and Calculated the Factors Used: Zinc determination was conducted after nitroperchloric digestion, using a spectrometer of optical emission with plasma - ICP OES, as described by Oliva *et al.* [16].

The cumulative amount of Zn in dry biomass of the aerial part (shoot) and roots (mg / pot) was calculated by the expression Zn accumulated shoot or Zn accumulated root = {Dry Biomass of shoot or Dry Biomass of root (g) x element concentration (mg kg^{-1})} / 1000.

The translocation index (TI) was determined by using the follow expression [17]: (Zn accumulated shoot/ Zn accumulated in the complete plant) x 100.

The translocation factor (TF) gives the leaf/root zinc concentration and depicts the ability of the plant to translocate the metal species from roots to leaves (shoot) at different concentrations. This index was calculated by the relationship: TF = (zinc concentration in the aerial part of the plant (mg kg^{-1}) (shoot)/ zinc concentration in the root (mg kg^{-1})) [18].

The bioaccumulation factor (BF), an index of the plant ability to accumulate a particular metal with respect to its concentration in the soil substrate [19] was calculated as follows: BFP = zinc concentration in the complete plant (mg kg^{-1}) / zinc concentration in the soil (mg kg^{-1}), or to calculate metal bioaccumulation only in the root was: BFR = zinc concentration in the plant root (mg kg^{-1}) / zinc concentration in the soil (mg kg^{-1}).

Statistical Analysis: The Assistat Software version 7.7 [20] was employed to analyze the obtained results, by using the F test and regression polynomials, which were used to adjust the data when significant.

RESULTS AND DISCUSSIONS

Shoot and Root Dry of the Plants: According to the analysis of variance the application of increasing doses of bentonite significantly influenced at 1% probability the dry biomass of shoots of lettuce and corn and the dry biomass of roots of lettuce, while these doses influenced at 5% probability the dry biomass of shoot of the grass and the dry biomass of roots of corn and grass (Table 1).

Dry weight of shoots lettuce increased by around 48.3% as a function of treatments ranging from 0.578 g/pot (0 t ha^{-1} bentonite) to 0.857 g/pot (90 t ha^{-1} bentonite) (Fig. 2A); on the other hand, the dry biomass of roots varied from 0.084 to 0.129 g/pot according to these treatments corresponding an increase of 53.6% (Fig. 2B). The application of bentonite in soil resulted in an increase of dry biomass of shoot and root of corn from 69.98 to 94.48 g/pot and from 10.92 to 14.64 g/pot, around 35.0 and 34.0%, respectively (Fig. 2C and 2D). The dry biomass of grass shoot (Fig. 2E) and roots (Fig. 2F) were influenced by increasing doses of bentonite ranging from 36.36 to 41.62 g and from 17.40 to 21.34 g / pot, promoting an increase of around 14.5 and 22.7% respectively. These increases are smaller than those observed in the other crops studied. In general, increases in dry biomass of shoots and/or roots of these plants, indicate that bentonite, probably by adsorption mechanism, reduced the zinc content available in the soil decreasing the deleterious effect of this metal on plants, thus promoting the development of these cultures corroborating [21]. The authors observed positive effect of bentonite in soil contaminated with cadmium on development on beet and radish.

Table 1: Summary of the analyses of variance for the dry biomass of the shoot and root of the lettuce, corn and grass cultivated on soil contaminated with zinc for the different bentonite treatments.

		Mean Square					
		Lettuce		Corn		Grass	
Source of Variation	DF	shoot[1]	root	shoot	root	shoot	root
Bentonite	3	3.99**	0.002**	429.19**	15.80 *	23.1.5*	12.21*
Linear	1	11.95**	0.005**	1175.20**	30.63*	61.32**	34.66**
Quadratic	1	0.008ns	0.0003ns	73.36ns	5.31ns	5.88ns	1.80ns
Error	12	0.49	0.0002	18.84	4.86	5.49	2.54
VC (%)		12.18	14	5.28	17.25	6.01	8.23
Mean (g)		5.83	0.11	82.23	12.78	38.99	19.37

DF= Degree of Freedom. ^{ns}, * and **, no significant, significant to the 5 and 1% level, respectively. VC = Variation Coefficient; [1] Data transformed in vx.

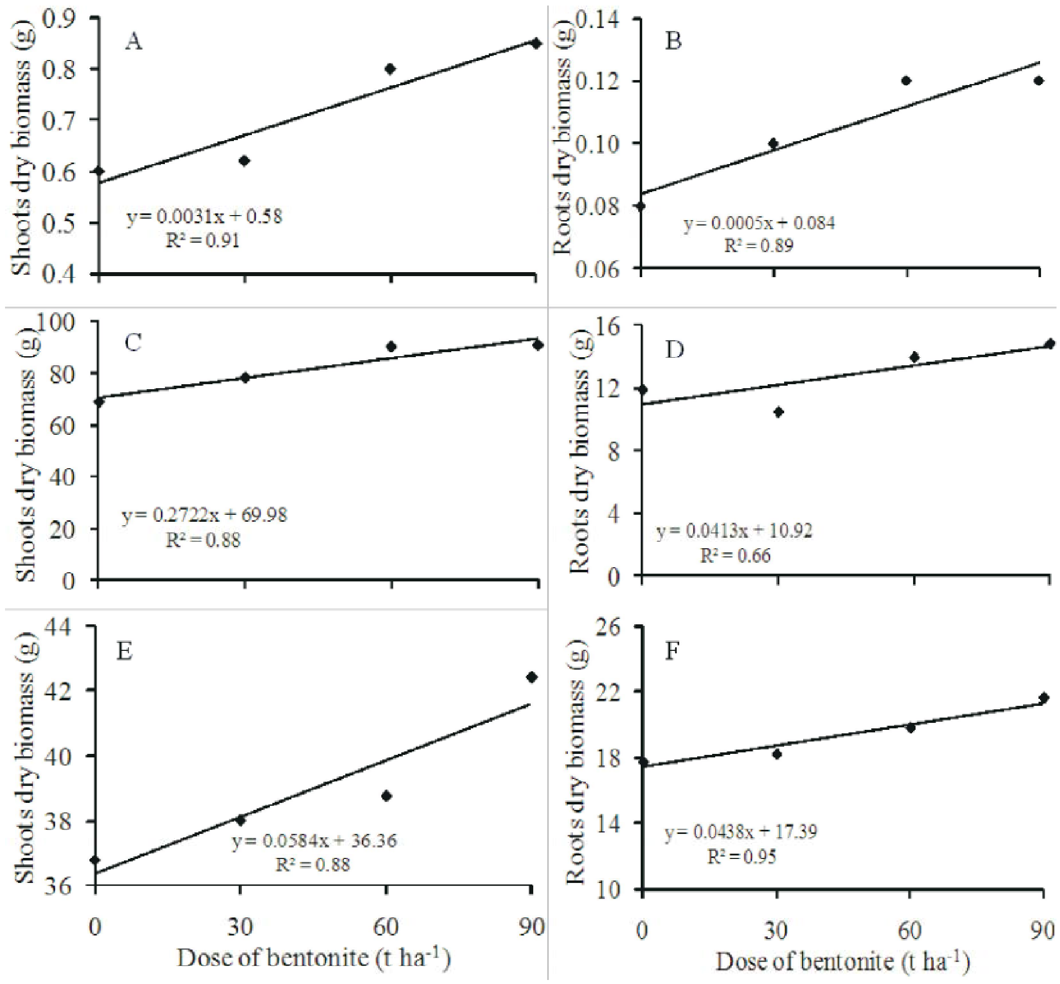

Fig. 2: Dry biomass of the lettuce (A), corn (C) and grass (E) shoots and dry biomass of lettuce (B), corn (D) and grass (F) roots cultivated on soil contaminated with zinc for the different bentonite treatments.

Zinc concentration in shoot and root: Increasing bentonite doses had a significant effect on the aerial part and root concentration of all three crops studied (Table 2).

Table 2: Summary of the analyses of variance for the zinc concentration in the shoot and root of the lettuce, corn and grass cultivated on soil contaminated with zinc for the different bentonite treatments.

		Mean Square					
		Lettuce		Corn		Grass	
Source of Variation	DF	shoot	root	shoot	root	shoot	root
Bentonite	3	213491.2**	1019510.3**	135621.6*	456827**	1672.44*	2114.52**
Linear	1	629557.1**	2317851.7**	384864.8**	1157574.7**	4936.08**	5752.83**
Quadratic	1	10363.2ns	580796.4**	18279.04ns	55696.00ns	62.41ns	163.84ns
Error	12	32015.29	7408.44	24155.39	23478.6	401.52	149.98
VC (%)		7.37	3.30	13.97	9.95	15.45	11.10
Mean (mg kg^{-1})		2427.15	2612.07	1112.70	1539.30	129.67	110.35

DF= Degree of Freedom. ns, * and ** no significant, significant to the 5 and 1% level, respectively.VC = Variation Coefficient.

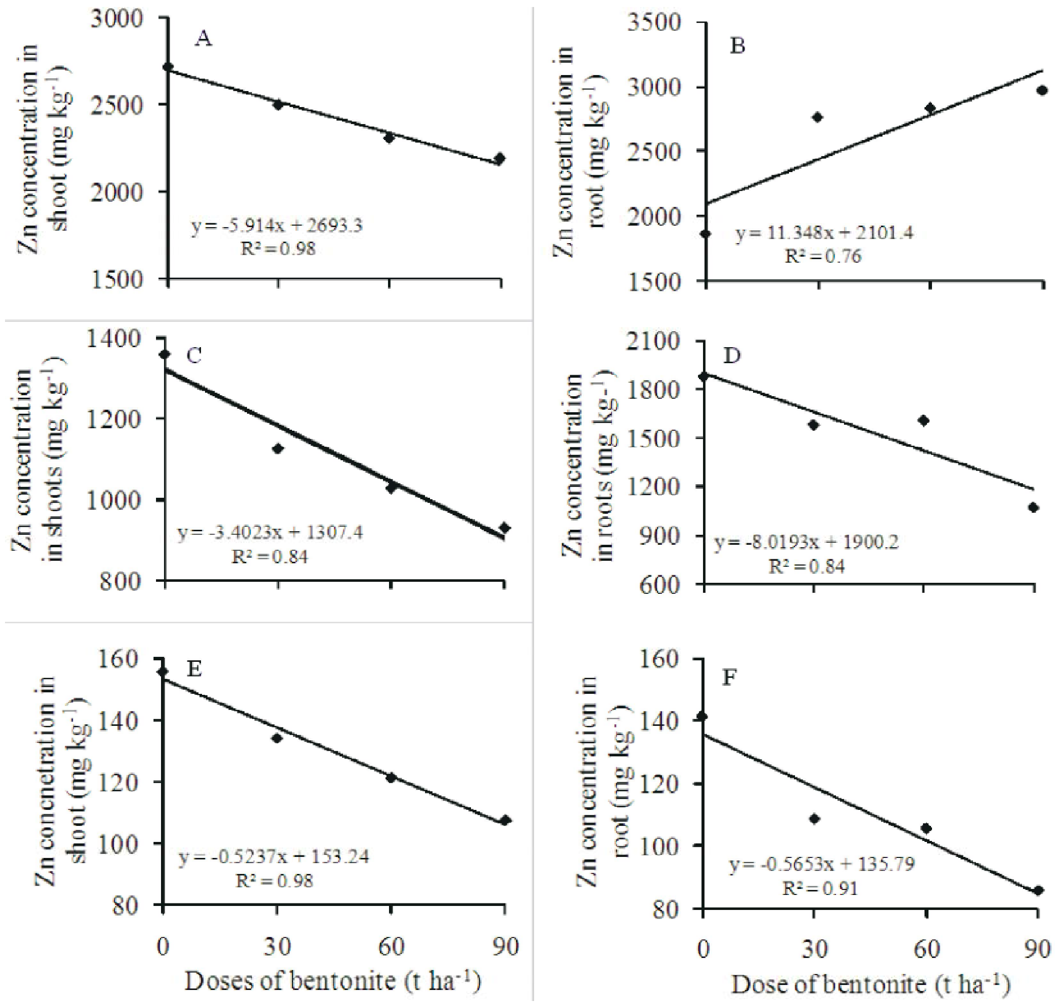

Fig. 3: Zinc concentration in the shoots of lettuce (A), corn (C) and grass (E) and Zn concentration in the root of (B), corn (D) and grass (F) cultivated on soil contaminated with zinc for the different bentonite treatments.

The Zn concentrations in shoots and roots lettuce ranged from 2693.3 to 2161.04 mg kg^{-1} and from 2101.4 to 3122.72 mg kg^{-1} presenting better fit in the linear model (Fig. 3A, 3B). In the case of the zinc concentration in the aerial part (comestible part) there is a decrease of 19.8% of the highest dose in relation to the control, however, this decrease, promoted by the addition of bentonite, was not sufficient for lettuce leaves to be suitable for human consumption. According to ABIA [8], the maximum tolerable value for human consumption of vegetables, roots and tubers and other fresh foods is 50 mg kg^{-1} of zinc. The zinc concentration in the roots had an increase with the bentonite doses around 48.6%. It is worth noting that the concentration of the soil where the lettuce was grown was slightly above the maximum value allowed for agricultural soils, where the limiting concentration is 450 mg kg^{-1} [22].

The incorporation of bentonite in the soil influenced the Zn concentration in the corn, reducing by 23.4% and 38.0% in shoots and roots, respectively, in relation to the increasing doses of bentonite (Fig. 3C, 3D).

It is verified that the Zn concentration in the root was higher than in the aerial part corroborating [23, 24] that observed similar behavior in bean and corn cultures, respectively. According to Cornu *et al*. [25] some plant species have in their root system mechanisms that can prevent or reduce the translocation of the absorbed metal to the aerial part of the same. For Marsola *et al.* [23] the great difference between shoot and root concentrations are a way of protecting plant from intoxication.

In the case of grass, even if it is not on the human food list, the concentration of zinc in its plant tissues is worrying as it is consumed by the animal and consequently can contaminate the entire food chain.

Table 3: Summary of the analyses of variance for the accumulated zinc in the shoot and root of the lettuce, corn and grass cultivated on soil contaminated with zinc for the different bentonite treatments.

		Mean Square					
		Lettuce		Corn		Grass	
Source of Variation	DF	Shoot[1]	root	shoot	Root[1]	shoot	root
Bentonite	3	0.08*	0.04**	72.30ns	0.63ns	1.13ns	0.33ns
Linear	1	0.21**	0.11**	-	-	-	0.71*
Quadratic	1	0.003	0.01*	-	-	-	0.08ns
Error	12	0.02	0.001	231.51	0.30	0.94	0.10
VC (%)		12.71	13.83	16.86	12.56	19.29	14.96
Mean (mg/pot)		1.11	0.28	90.27	4.37	5.04	2.11

DF= Degree of Freedom, [ns], * and ** no significant, significant to the 5 and 1% level, respectively.VC = Variation Coefficient. [1]Data transformed in vx

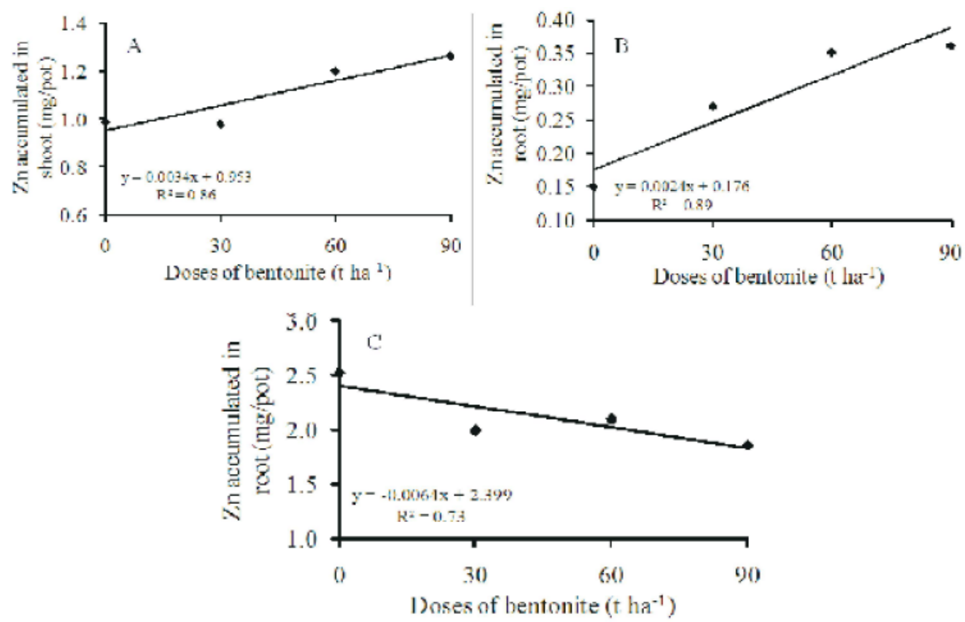

Fig. 4: Amount of Zn accumulated in shoots of lettuce (A) and amount Zn accumulated in the root of lettuce (B) and grass (C) cultivated on soil contaminated with zinc for the different bentonite treatments.

According to Kumar *et al.* [26] and Marin *et al*. [27] the main concerns regarding the effects of heavy metals are their participation in the food chain.

Concerning zinc contents in dry biomass causing plant phytotoxicity symptoms, it is in the range of 70-400 mg kg^{-1} Zn [9]. Table 2 shows that the overall mean of all plants is above this range, that is, all concentrations were considered toxic to plants.

Cadmium accumulated in shoot and root: Increasing doses of bentonite significantly influenced the cumulative amount of Zn in shoot and root of lettuce (Table 3). On the other hand, the amount accumulated in the grass root presented significance in the regression.

The Zn accumulated in the aerial part and the lettuce root (Fig. 4A, 4B) had an increase ranging from 0.953 to 1.259 mg / pot and 0.176 to 0.392 mg / pot, respectively, when compared the control with the higher dose of bentonite. This increase is due to the higher development of the aerial part and root of the lettuce with the increase of the doses of bentonite, giving a high value of the biomass and consequently the accumulated amount of the metal, since the calculation is proportional (biomass x concentration) even though the lettuce concentration of the lettuce has decreased with the doses of bentonite.

The Zn accumulated in root grass (Fig. 4C) had a reduction of about 23.9%, ranging from 2.396 (0 t ha^{-1} de bentonite) to 1.823 mg / pot (90 t ha^{-1} de bentonite). This reduction, through the incorporation of bentonite, has been beneficial, since excessive accumulation of heavy metals in agricultural soils, such as zinc, through wastewater irrigation can result in contamination of the soil and increased absorption of metals heavy by cultures, affecting food quality and safety [28].

Zinc translocation index, translocation factor and bioaccumulation factor of cadmium in plant and in root: Although the bentonite did not influence the TI and TF of radish (Table 5), the values ??of this last factor > 1 indicated a very efficient ability to transport cadmium from roots to shoots, most likely due to efficient metal transport systems. It worth noting that radish is a suitable plant for phytoextraction for this metal. This can be confirmed by the concentration data and the amounts of cadmium (Table 1) which were higher in the aerial part than in the roots. This is important because the comestible part of plant is the root.

Increasing doses of bentonite significantly reduced Zn translocation index (TI) to the lettuce shoot of 5.2% (Table 4) when compared to control (85.86%) up to 90t ha^{-1} (81.41%). This reduction was beneficial because it reduced the concentration of Zn in the aerial part of the plant, that is, in the edible part of the lettuce (Fig. 5). This behavior was similar to that observed by Tito *et al*. [29] researching on the application of bentonite in the reduction of the available levels of copper of the soil.

Table 4: Summary of the analyses of variance for the zinc translocation index (TI), translocation factor (TF), bioaccumulation factor of zinc in plant (BFP) and bioaccumulation factor of zinc in root (BFR) of the lettuce, corn and grass cultivated on soil contaminated with zinc for the different bentonite treatments.

		Mean Square		
Source of Variation	DF	Lettuce	Corn	Grass
			translocation index	
Bentonite	3	52.08 *	14.32ns	8.63ns
Linear	1	40.40ns	-	-
Quadratic	1	84.51*	22,03ns	32.93ns
VC (%)		3.94	5.71	8.19
Mean		81.49	82.21	70.08
			translocation factor	
Bentonite	3	0.43**	0,047ns	0,016ns
Linear	1	1.014**	0.042ns	0,022ns
Quadratic	1	0.228**	0.069ns	0,001ns
VC (%)		6.47	21.17	20.76
Mean		0.98	0.74	1,19
			bioaccumulation factor of zinc in plant	
Bentonite	3	0.24ns	2.31**	2.44**
Linear	1	0.652*	6.57**	7.07**
Quadratic	1	0.022ns	0.15ns	0.12ns
VC (%)		6.61	11.42	10.87
Mean		4.77	4.43	4.52
			bioaccumulation factor of zinc in root	
Bentonite	3	3.85**	6.55**	2.84**
Linear	1	8.75**	16.59**	7.73**
Quadratic	1	2.20**	0.80ns	0.22ns
VC (%)		3.29	9.95	11.10
Mean		5.08	5.83	4.04

DF= Degree of Freedom, ^{ns}, * and ** no significant, significant to the 5 and 1% level, respectively. VC = Variation Coefficient.

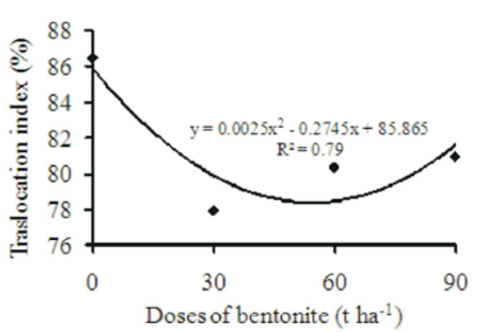

Fig. 5: Zinc translocation index in lettuce plants according to the increasing doses of bentonite.

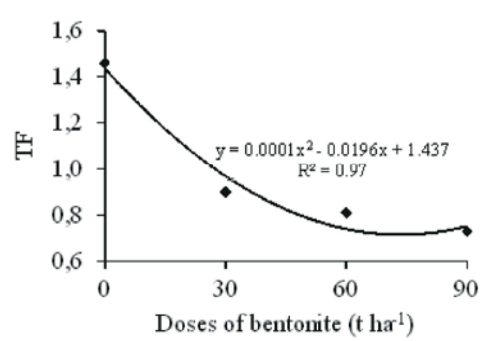

Fig. 6: Zinc translocation factor in lettuce plants according to the increasing doses of bentonite.

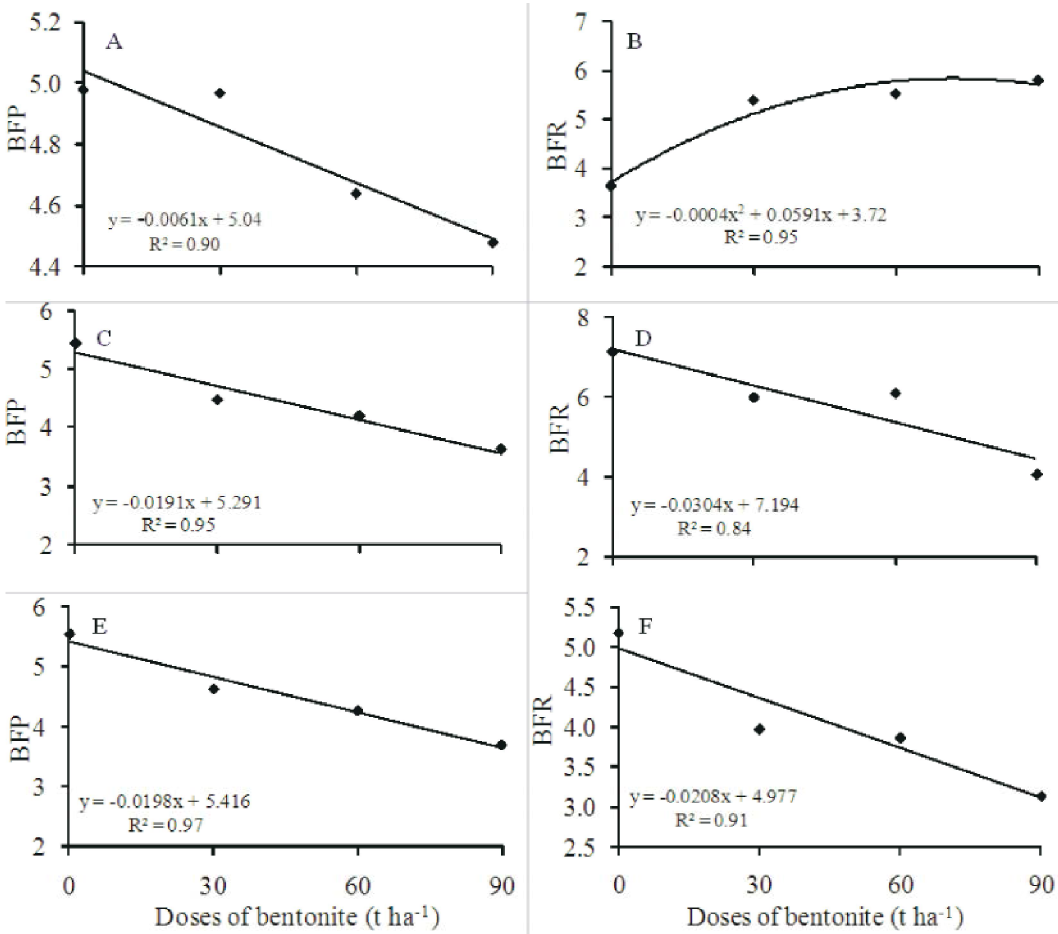

Fig. 7: Bioaccumulation factor of zinc in shoots and roots of lettuce (A, B), corn (C, D) and grass (E, F) depending on dose increasing bentonite.

Bentonite doses positively favored only the translocation factor (TF) of the lettuce (Table 4), that is, they favored zinc retention in lettuce roots since it decreased the TF by 66.4% (Fig. 6). The average value of this factor (0.98) is less than 1 indicating a low capacity for transporting zinc from roots to shoots which means that the lettuce is a suitable plant for phytoextraction for this metal. This can be observed by the concentration data and the amounts of zinc which were higher in the root than in the shoot.

Although there was no significant effect of the increasing doses of bentonite on TF of grass (Table 4) the values ??of this factor > 1 indicated a very efficient ability to transport zinc from roots to shoots, most likely due to efficient metal transport systems. This is worrying because the zinc contents were higher in the aerial part than in the roots, part commensurate to the animals.

Bentonite doses significantly favored the zinc bioaccumulation factor in the plant at a 1% probability level for corn and grass, while for lettuce, only in the regression showed significance (Table 4). Zinc bioaccumulation factor of lettuce (BFP) reduced 10.9% with incorporation of increasing doses of bentonite to the soil varying from 5.04 to 4.49 (Fig. 7A).

In the case of maize and grass the value of the reduction was practically the same 32.5 and 32.9%, respectively (Fig. 7C and 6E). This reduction was beneficial since bentonite have a great potential to adsorb pollutants due to their large specific surface area, the layered structure and high cation exchange capacity [30] reducing the transport of zinc in the soil to the edible part of the plant

The bioaccumulation factor of zinc in the root (BFR), with the exception of the lettuce, decreased significantly as a function of the increasing doses of bentonite (Table 4) (Fig 7D, 7F). Probably because the presence of bentonite in the soil retains the zinc reducing its absorption by the roots of the plants. In the case of lettuce, the reverse occurred (Fig. 7B), perhaps because it was cultivated in soil with a very high concentration (500mg kg^{-1}) or due to characteristics of the plant itself.

According to Figure 7, the BFP and BFR values generally decreased as a function of increasing doses of bentonite presenting better fit in linear model. However, these values were higher than unity, indicating high bioaccumulation potential of lettuce, corn and grass for this metal.

CONCLUSIONS

Bentonite in soil contaminated with zinc had a significant positive effect on development of shoot and roots of lettuce, corn and grass.

In general the bentonite promoted the retention of zinc in the soil, evidenced by the reduction of the zinc concentration in the shoots and roots of analyzed cultures.

Bentonite favored the reduction of translocation index and factor of zinc thereby increasing the concentration of this element in plant and roots, respectively.

Bioaccumulation factors of zinc decreased as a function bentonite increasing the concentration of this element in soil in relation to the plants.

The application of bentonite to the contaminated soils favored their remediation.

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