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RESEARCH ARTICLE

BENTONITE APPLICATION IN THE REMEDIATION OF CADMIUN IN CONTAMINATION SOIL

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ABSTRACT

The objective of this work was to evaluate the effect of bentonite application on the melioration of cadmium contaminated soils, by evaluating the production of beets, radish and grass, as test plants. The experiment was conducted in a greenhouse in a completely randomized design, with four replicates. Red Eutrophic Latossol was contaminated with 3 mg kg^{-1} of Cd and mixed to treatments which consisted of four doses of bentonite 0; 30, 60 and 90 t ha^{-1}. After that was planted beet, radish and grass. The cadmium content in the plants, as well as the translocation index in the plants was evaluated. The present study led to demonstrate that addition of bentonite in soil contaminated with Cd had a significant positive effect on development of beet and radish. The increasing doses of bentonite applied to soil contaminated with Cd decreased the concentration of this metal in the edible parts of the sugar beet plants and grass. The amount of bentonite applied to contaminated soils, in general, was not enough on its improvement despite having decreased the concentration of cadmium in plants. The translocation index of cadmiun in the grass was reduced with the bentonite application, allowing to find a great quantity of cadmium in the grass roots.

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INTRODUCTION

The concentrations of heavy metals in agricultural soils, most of the time, are from the rock, but there are some activities that contribute to increasing the concentration of heavy metals in these soils as excessive and improper use of fertilizers and the addition of contaminated sludge. The contamination of such soils may occur immediately due to the large release of metals into the environment or over time through the accumulation of metals for years or decades in nature causing often irreparable damage because the main properties of metals heavy are the high levels of reactivity and bioaccumulation (Calabuig and Cañadas, 2004). Soil contamination with heavy metals is increasingly common and worrying because of the negative impact of these elements in the ecosystem. The main concerns about the effects of heavy metals are their participation in the food chain (Kumar *et al*., 2012; Marin *et al*., 2010), reduced agricultural productivity due to phytotoxic effects, accumulation in the soil, changes in microbial activity and contamination of water resources (Wowk and Melo, 2005). According to Guimarães *et al*. (2008), the plants are the main entry point of heavy metals in the food chain, as they are readily absorbed by the roots being a risk to human health and the environment. Therefore, it is important to control the metal concentrations in plants, especially in the edible parts to ensure food safety. Recovery of heavy metals contaminated soils requires the adoption of techniques to mitigate the bioavailability thereof. These technologies are very variable, according to the contaminated matrix, the nature of the contaminant, the level of contamination and the availability of resources (Tavares *et al*., 2013). In this sense, studies by Bhattachryya and Gupta (2007), Ghorbel-Abid *et al*. (2010) and Jiang *et al*. (2010) have pointed out the potential of clays for the removal of heavy metals from soils. Because of large specific surface area, high cation exchange capacity, low cost and wide spread availability, bentonite is probably the most promising material interacting with many heavy metal ions in contaminated soil (Hamidpour *et al*., 2010). Bentonite is composed predominantly of smectite clay group and quartz impurities and some varieties present caulinite and illite (Sdiri *et al*., 2011). Bentonite is found in great amounts in Boa Vista Municipal region, State of Paraiba. Cadmium is a heavy metal that is regarded as an element of high toxicity. It is toxic plants even at low concentrations, and when added to soil, is rapidly and readily absorbed by plants, dramatically increasing the level of the accumulated element (Paganini *et al*., 2004). Likewise, as the cadmium ions are not biodegradable, they can be easily accumulated in living tissue, thus can be readily absorbed into the human body throughout the food chain.

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According to Malavolta (1994), soils with Cd levels above 3 mg kg^{-1} are considered toxic, unfit for the cultivation of plants intended for food. According to ABIA (1985), the maximum tolerable level for vegetables, roots and tubers and other fresh foods, according to Brazilian law for cadmium element is 0.5 mg kg^{-1}. While the heavy metal content of dry matter that cause symptoms of phyto toxicities in the plant is 2-8 mg kg^{-1} (Marques *et al*., 2002). The objective of the present work was to evaluate the effect of bentonite application on the melioration of cadmium contaminated soils, by evaluating the production of beets, radish and grass, as test plants.

MATERIALS AND METHODS

The study was carried out under semi controlled greenhouse conditions at the Agricultural Engineering Department of the Federal University of Campina Grande, Paraiba, Brazil. The experiments were conducted with beets (*Beta vulgaris*), radish (*Raphanus sativus*) and *Brachiaria* grass (*Brachiaria brizantha* cv.) on a loamy sand soil classified as a Red Eutrophic Latossol (Embrapa, 2006), collected in the Campina Grande Municiple to a 0-20 cm soil depth. After the collect, soil samples were air dried, crushed, sieved through a 2mm sieve and analyzed using the procedures recommended by Embrapa (1997). The following attributes were found: 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 = 45.0 mg $kg^{-1}$and Cd = 0.015 mg kg^{-1}. Bentonite samples used in these experiments are of low quality clays and rejected for industrial, purposes, called vulgarly as “bofe”. They were collected in the orebody Primavera, located in the municipality of Boa Vista, Paraiba State, Brazil. These samples were air dried, crushed, and sieved through a 2 mm sieve to be used in experiments and with 0.074 mm sieve for semi-quantitative chemical analysis, performed via x-ray fluorescence (EDX) using EDX equipment 720 (Table 1).

Table 1. Chemical constituents of bentonite determined by x-ray fluorescence

Compounds	Results (%)
SiO_2	76.784
Al_2O_3	13.339
Fe_2O_3	6.347
MgO	2.225
CaO	0.759
Other oxides	0,545

The experiments were conducted in 5 kg plastic containers for beets and radish and in 8 kg containers for grass. Soil seeded with the plants received 3 mg kg^{-1} of cadmium ($CdSO_4$ 8/3 H_2O). Nitrogen, phosphorus and potassium fertilization for beets and radish was 1.11 g de urea, 1.25 g of potassium chloride (KCl) and 8.3g of super phosphate (P_2O_5). Nitrogen, phosphorus and potassium fertilization for grass was 1.78 g of urea, 2.0 g of potassium chloride (KCl) and 13.33 g of super phosphate (P_2O_5). After the NPK fertilization, cadmium and bentonite application the soil was conditioned in plastic containers, irrigated to field capacity and incubated during 20 days. Finished the incubation process the crops were seeded and 8 days after the emergency a thinning was conducted leaving two plants per vase. Each experimental unit received 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. The irrigation was conducted using tap water maintaining the soil to field capacity. At 30; 90 and 70 days of experiment, the radish, beets and grass plants, respectively, were collected separating the aerial and root part, washed with distillated water, conditioned in paper sacks and dried in forced air stove at 65°C during 48 hours. After drying, the plants were triturated and weighed for foliar analyses. Cadmium determination was conducted after nitroperchloric digestion, according to Embrapa procedures (Embrapa, 1997), using a spectrometer of opticalemission with plasma - ICP OES, as described by Oliva *et al*. (2003). The cumulative amount of Cd in dry biomass of the aerial part (CADBAP) and roots (CADBR) plant (mg / pot) was calculated by the expression

CADBAP or CADBR = {DAP, or DR (g) x element concentration (mg kg^{-1})} / 1000.

The translocation index (TI) was determined by using the follow expression (Abichequer and Bohnen, 1998):

$$TI= \frac{\text{Amount of cadmium accumulated in the aerial part of the plant}}{\text{Amount of cadmium accumulated in the whole plant}} \times 100$$

The experimental design was a completely randomized with four replicates, totalizing thus 16 experimental units (plastic containers). The results were analyzed statistically by the F test and regressions using the SISVAR program (Ferreira, 2011).

RESULTS AND DISCUSSION

The dry biomass of the aerial part of all plants was not influenced by increasing doses of bentonite. In contrast, the dry biomass of the roots of beet and grass were significantly influenced to the 1% probability and radish 5% probability by applying bentonite soil (Table2). With the exception of dry biomass of grass root, which was adjusted to a quadratic model, all significant effects of bentonite were adjusted to linear regression models. Dry biomass of root beet ranged from 5,87 g (0 t ha^{-1} bentonite) to 9,21 g (90 t ha^{-1} bentonite), corresponding to an increase of 56.85% in the highest dose compared to control (Figure 1A). Likewise, the dry biomass of radish root increase of 28.54% of bentonite higher dose compared to the control (Figure 1B). The positive effect of bentonite addition on plant growth may be due to its positive effect on the water retention capacity (Iskander *et al*., 2011) and the growth of surface area, increasing the metal cation adsorption capacity present in the soil (Tito *et al*., 2011). The increasing linear behavior of the dry biomass of the grass root was influenced by increasing doses of bentonite, ranging from 4.70 to 7.64 g, promoting an increase of around 62.55%. On the other hand, the shoot dry biomass had a small growth, but was not significant. With the exception of the radish, the cadmium concentration in the aerial part and in the roots of the beet and grass was significantly affected by the bentonite application (Table 3). The quadratic behavior of the cadmium concentration in the aerial part of beet, shows the decrease of the same with increasing doses of bentonite (Figure 2A), ranging from 103.78 a 55.40 mg kg^{-1}.

Table 2. Summary of the analyses of variance for the dry biomass of the aerial (DAP) part and root (DR) of the beets, radish and grass cultivated on soil contaminated with cadmium for the different bentonite treatments

Source of Variation	DF	Mean Square					
		Beets		Radish		Grass	
		DAP	DR	DAP	DR	DAP	DR
Bentonite	3	1.64ns	9.87**	0.30ns	0.28*	14.41ns	6.35**
Linear	1	-	24.77**	-	0.53*	-	14.09**
Quadratic	1	-	0.46ns	-	0.03ns	-	4.86*
Error	12	2.33	0.49	0.19	0.06	8.48	0.81
VC (%)		14.70	9.29	14.48	12.36	11.27	14.90
Mean (g)		10.39	7.54	3.03	1.95	25.84	6.06

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

Table 3. Summary of the analyses of variance for the cadmium concentration in the aerial part (CAP) and root (CR) of the beets, radish and grass for the different bentonite treatments

Source of Variation	DF	Mean Square					
		Beets		Radish		Grass	
		CAP	CR[1]	CAP	CR	CAP	CR
Bentonite	3	2172.03**	2.20**	20.72ns	2.19ns	22.86**	70.49*
Linear	1	212.57**	5.54**	-	-	51.52**	131.58*
Quadratic	1	767.29*	0.47ns	-	-	17.06ns	33.98ns
Error	12	93.73	0.30	18.24	3.98	3.73	17.67
VC (%)		13.33	14.69	9.30	8.86	12.16	14.87
Mean (mg kg^{-1})		72.64	3.65	45.91	22.51	15.89	28.27

DF= Degre of Fredom, ns, * and ** no significant, significant to the 5 and 1% level, respectively.VC = Variation Coefficient. [1] Data transformed in √x.

Table 4. Summary of the analyses of variance for the accumulated cadmium in the aerial part (CADBAP) and root (CADBR) of the beets, radish and grass for the different bentonite treatments

Source of Variation	DF	Mean Square					
		Beets		Radish		Grass	
		(CADBAP)	(CADBR) [1]	(CADBAP)	(CADBR)	(CADBAP)	(CADBR)
Bentonite	3	0.250**	0.008**	0.00013ns	0.00009ns	0.017*	0.0021ns
Linear	1	0.588**	0.021**	-	-	0.016ns	-
Quadratic	1	0.152*	0.002ns	-	-	0.0226*	-
Error	12	0.020	0.0013	0.0006	0.00003	0.005	0.0007
VC (%)		19.09	11.64	17.82	12.69	16.15	16.88
Mean (mg)		0,75	0,31	0,14	0,04	0,42	0,17

DF= Degre of Fredom, ns, * and ** no significant, significant to the 5 and 1% level, respectively.VC = Variation Coefficient. [1] Data transformed in √x.

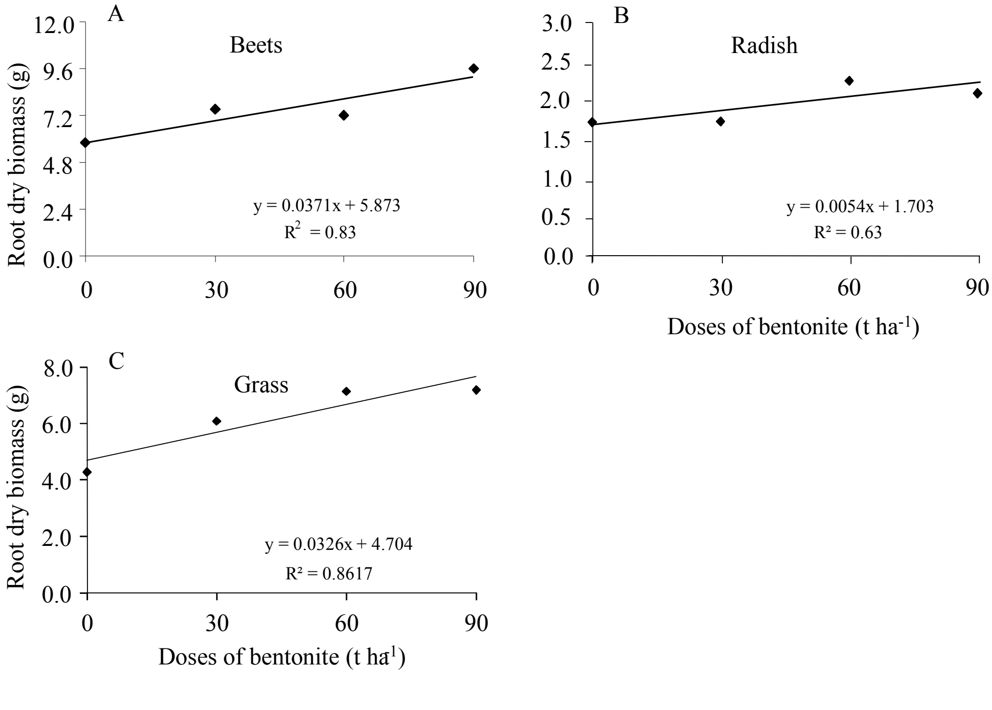

Figure 1. Dry biomass of the roots for beets (A), radish (B) and grass (C) cultivated in soil contaminated with cadmium, for the different bentonite treatments

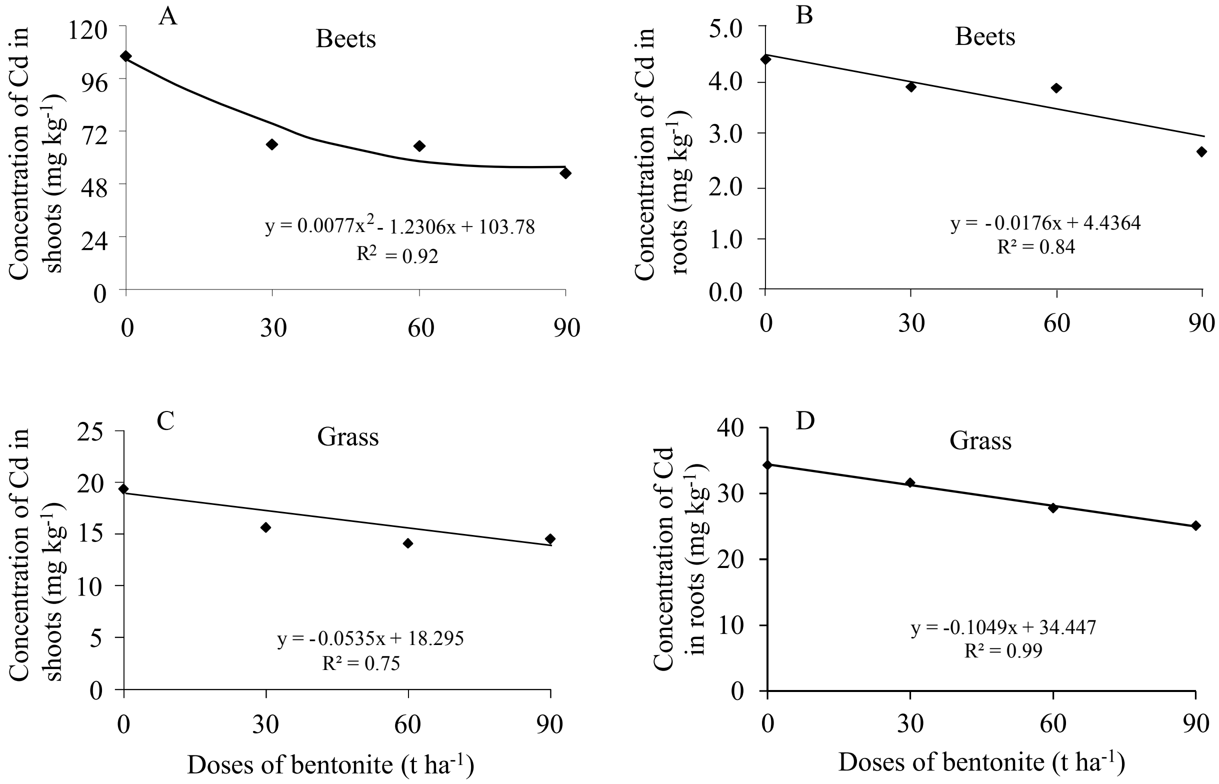

Figure 2. Cadmium concentration in the aerial part of the beets (A) and grass (C) and in the roots of beets (B) and grass (D), cultivated in soil contaminated with cadmium, for the different bentonite treatments

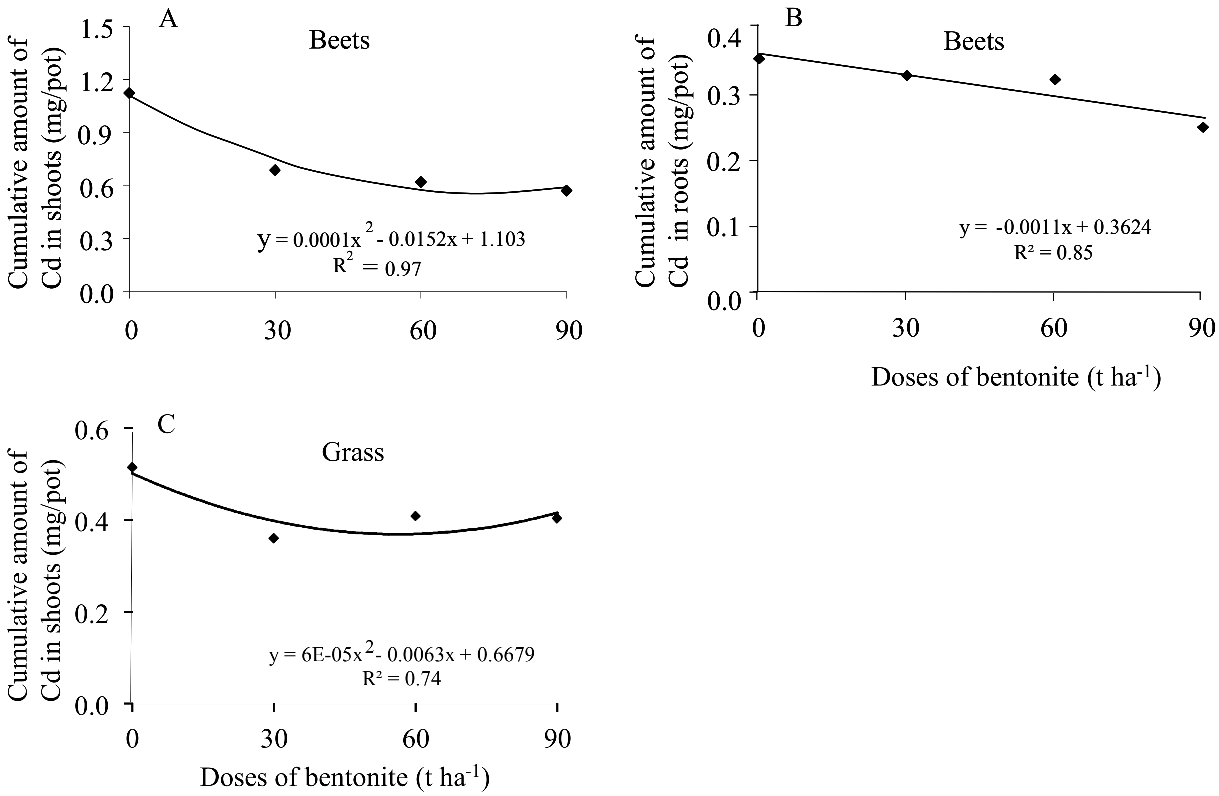

Figure 3. Accumulated cadmium in the aerial part of beets (A) and grass (C) and in the root of beets (B), cultivated in soil contaminated with cadmium, for the different bentonite treatments

While this decrease had the linear behavior in the roots (Figure 2B) ranging from 4.44 to 2.91 mg kg^{-1} occurring a decrease of 46.62% and 34.49%, respectively, the control regarding the higher dose. Cadimum concentrations in the aerial part (CAP) and root (CR) of the grass were reduced by around 26.32% and 27.41%, respectively, compared with the witness the highest dose of bentonite (Figure 2C and 2D). The concentration of Cd in the roots was higher than in the shoot corroborating Hamidpour *et al*. (2010), who observed a higher concentration of this element in the roots than in the shoots of corn grown in contaminated soil mixed with bentonite indicating a relatively limited translocation of metal ions from the roots to the part aerial. According to Cornu *et al*. (2007), some plant species have in their root system mechanisms that can prevent or reduce the translocation of absorbed metal to shoot them. For Marsola *et al*. (2005), the big difference between the shoot and root concentrations is a plant form to protect from poisoning. The reduction of concentration Cd in these plants, beet and grass (Figure 2), indicates a possible decrease in the availability of the element in the soil, due probably to its adsorption by bentonite. However, despite promoting the reduction in concentration, bentonite was not effective enough to reduce the concentration of Cd in the plant below the maximum allowed value for food, i.e. below of 0.5 mg kg^{-1} of cadmium (ABIA, 1985). The concentration of Cd in grass is also worrying, since it is consumed by the animal and therefore can contaminate the food chain. According to Kumar *et al.* (2012) and Marin *et al*. (2010), the main concern for the effects of heavy metals is their participation in the food chain. It is noteworthy that these plants were grown in soil with the maximum concentration of Cd allowed for agricultural land. Even then it is very worrying consumption of food grown in soils with the presence of this metal. As Marques *et al*. (2002), cadmium content of dry matter that cause symptoms of phytotoxicities in plants is in the range of 2-8 mg kg^{-1} of Cd in dry weight. Appears that the overall average of all test plants (including radish which was not significant) are above this range, ie, all concentrations were considered toxic to plants (Table 3). Increasing doses of bentonite significantly influenced the level of 1% the cumulative amount of Cd in aerial part (CADBAP) and root (CADBR) beet and at 5% in the accumulated amount in the shoot (CAAP) of grass (Table 4). The cumulative amount of cadmium in the aerial part of the beet had the best fit in the quadratic form (Figure 3A). It is observed that there was a reduction of Cd accumulation in shoots of beet ranging from 1.103 mg/pot (0 t ha^{-1} bentonite) to 0,545 mg/pot (90 t ha^{-1} bentonite), corresponding to a decrease 50.6%. On the other hand, the accumulated amount in the root (Figure 3B) ranged from 0.362 to 0.263 mg/pot, decreasing linearly 27.3% from the control in relation to higher dose of bentonite applied to the soil. The cumulative amount of cadmium in shoots of grass had the best fit in the quadratic form (Figure 3C). It is found that there was a reduction of Cd accumulation in grass leaves ranging from 0.50 mg/pot (0 t ha^{-1} bentonite) to 0.42 mg/pot (90 t ha^{-1} bentonite), corresponding to a decrease of 14.5%, occurring the lowest value to the CADBAP, with the dose of 30 t ha^{-1}.

According to data presented in Table 5, the bentonite dose influenced significantly at 5% probability in Cd translocation index only in grass showing a linear behavior (Figure 4), ranging from 76.74% (control) to 70.15% (B90).

Table 5. Summary of the analyses of variance for the cadmium translocation index of the beets, radish and grass for the different bentonite treatments

Source of Variation	DF	Mean Square		
		Beets	Radish	Grass
Bentonite	3	15.02ns	11.91ns	65.84*
Linear	1	-	-	90.50*
Quadratic	1	-	-	103.47*
Error	12	49.02	29.58	15.67
VC (%)		8.28	7.25	5.58
Mean		84.59	74.99	71.01

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

The translocation index represents the percentage of the total amount of absorbed element that was transferred to the shoot (Abichequer and Bohnen, 1998). Based on this definition, increasing doses of bentonite promoted reduction in translocation of Cd to aerial part of grass of 12.3 and 8.6% when compared to the witness regarding the doses of 60 and 90 t ha^{-1} of bentonite, respectively, thus promoting the reduction of Cd in the edible part of the grass (Figure 4). According Wang *et al*. (2006) this decrease in translocation could be due the very high affinity of grass roots for Cd, whereby most of the Cd in solution could be trapped in the root tissue mainly bounded to the root cell walls.

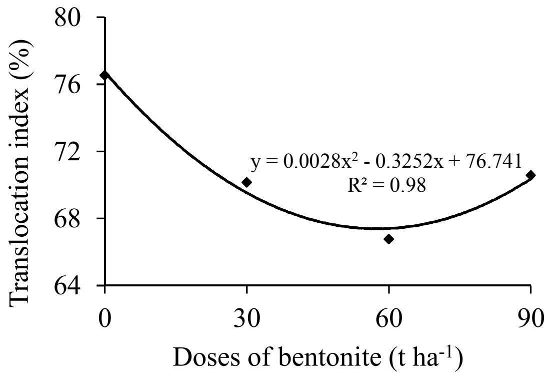

Figure 4. Translocation index of Cd for the grass for the applied bentonite

Conclusion

- The present study led to demonstrate that addition of bentonite in soil contaminated with Cd had a significant positive effect on development of beet and radish;
- The increasing doses of bentonite applied to soil contaminated with Cd decreased the concentration of this metal in the edible parts of the sugar beet plants and gras; with it can infer that the bentonite adsorbed cadmium;
- The amount of bentonite applied to contaminated soils, in general, was not enough on its improvement despite having decreased the concentration of cadmium in plants;

- The translocation index of cadmiun in the grass was reduced with the bentonite application, allowing to find a great quantity of cadmium in the grass roots.

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