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Bentonite application in the remediation of copper contaminated soil

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The concern on heavy metals from commercial fertilizers for fertilization of crops when they are intended for human consumption has increased specifically, horticultures and grains. Several procedures have been proposed to reduce the concentration of heavy metals in the soil; among these are, the application of materials that are able to adsorb these elements, making them less available to plants. This study aimed to evaluate the bentonite for remediation of artificially copper contaminated soils, grown with beets, radish and corn. The experiment was conducted in a greenhouse in a completely randomized design, with four replicates. A loamy sand soil planted with radish and corn was contaminated with 100 mg kg^{-1}of copper, while for beets, the soil was contaminated with 250 mg kg^{-1} of copper as copper sulphate (Cu_2SO_4). Bentonite treatments consisted of four doses of bentonite: 0, 30, 60 and 90 t ha^{-1}. The copper content in the soil and in plants, as well as the translocation index in the plants was evaluated. The results were analyzed by the F test and polynomial regression was used for adjustment of significant data. Bentonite decreased the copper content in the dry phytomass of the plants, affected significantly the copper accumulated in the roots of beets and radish, and in the aerial part of radish. The copper translocation index in beets reduced with bentonite doses, and consequently the quantity of copper on beets was higher than those levels permitted for human consumption. Application of bentonite in contaminated soils grown with radish and corn improved their amelioration; on the other hand, the soil grown with beets did not present any amelioration.

Key words: Heavy metal, accumulation, vegetables.

INTRODUCTION

The concern on heavy metals from commercial fertilizers is still more preoccupant when they are intended for human consumption, specifically horticultures and grains. Plants are the main entrance of heavy metals in the

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feeding chain due the relative absorption ability of their roots (Guimarães et al., 2009).

Soil contamination by heavy metals can occur immediately by the great release of heavy metals to the environment. In addition, the contamination can be observed with the accumulation of metal in nature throughout decades, producing irreversible damages in most cases. Thus, several researches have been conducted to study, prevent or minimize health problems due to heavy metals contamination and to remediate areas already contaminated.

Remediation of soils contaminated with heavy metals needs the adoption of technics that provide a decrease in the availability of these metals for the plants. These technologies vary according to the characteristics of the soil, the nature of the contaminant, the degree of contamination and the financial conditions (Tavares et al., 2013). Several adsorbent materials, such as clay minerals have been evaluated to reduce the contaminant effect of heavy metals (Ghorbel-Abid et al., 2010; Jiang et al., 2010; Bhattacharyya and Gupta, 2007), indicating some advantages of these materials such as low cost, availability and efficiency when compared with other adsorbent materials. Bentonite can also be used as a chemical and physical conditioner due to the high cationic exchange capacity, as it has been already reported by Tito et al. (1997) and Tito et al. (2001).

Mainly, minerals from the smectite clay group and quartz impurities constitute bentonite clays, and some bentonites present caulinite and illite (Sdiri et al., 2011). In the State of Paraiba, Brazil, mainly in Boa Vista Municipal region, there are great amounts of a bentonite mineral known as “bofe”, whose characteristics are not adequate to the industry requirements, and therefore less commercialized. In this sense, this “bofe” bentonite could be used to meliorate contaminated soils with heavy metals.

Copper is an essential element for plant growth; however, elevated concentrations can produce drastic alterations in plant cells, affecting plant development (Girotto, 2010). According to Kabata-Pendias and Pendias (1992), copper content greater than 100 mg kg^{-1} is considered excessive in the soil and may cause phytotoxicity. The maximum content of Cu from which there is risk to human health and to the environment varies greatly and depends on the laws of each country. In Brazil, the permissible maximum Cu value for agricultural soils is 200 mg kg^{-1} (CONAMA, 2009). Copper moves slowly in the soil, generally as an organic complex and remains at the soil surface (Paganini et al., 2004). Tito et al. (2012) evaluating the effect of the bentonite on the zinc and copper mobility in an Argissol verify that the copper was strongly adsorbed as a soil/bentonite complex.

The objective of the present work was to evaluate the contamination of soils and plants with copper and the effect of “bofe” bentonite application on the melioration of copper contaminated soils, by evaluating the production of beets, radish and corn.

MATERIALS AND METHODS

This study was carried out under semi controlled greenhouse conditions, from March 2014 to June 2014 at the Agricultural Engineering Department, Federal University of Campina Grande, Paraiba, Brazil. The experiments were conducted with beets (*Beta vulgaris*), radish (*Raphanus sativus*) and corn (*Zea mays* L.) on a loamy sand soil classified as a Red Eutrophic Latossol (Embrapa, 2006), collected in Campina Grande region at a 0-20 cm soil depth. After collecting the soil, samples were air-dried, crushed, sieved through a 2 mm 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 Cu = 0.355 mg kg^{-1}.

Soil samples were placed in 5 kg plastic pots for beets and radish; and for corn, 14 kg pots were used. The doses of copper used in this study were; for beets, 250 mg kg^{-1}; for radish and corn, 100 mg kg^{-1}. Nitrogen, phosphorus and potassium fertilization for beets and radish was 1.11 g of urea, 1.25 g of potassium chloride (KCl) and 8.3 g of super phosphate (P_2O_5). Nitrogen (N), phosphorus (P) and potassium (K) fertilization for corn was 3.11 g of urea, 3.5 g of potassium chloride (KCl) and 23.33 g of super phosphate (P_2O_5). The 100 mg kg^{-1} copper dose applied to the soil was based on not published results of the effect of copper doses on the emergency of radish and corn.

After the NPK fertilization, copper and bentonite were applied to the soil, which was conditioned in the plastic containers. Water was applied to the soil until the field capacity of soil and it remained incubated during 20 days under this soil moisture. After the incubation period, the seeds of each crop were sown and, 8 days after the emergency of seedlings, a thinning was conducted leaving two plants per pot. Four does of bentonite were used: 0.0; 10.7; 21.4 and 32.1 g kg^{-1}, corresponding to 0, 30, 60 and 90 t ha^{-1}, respectively. The bentonite used in this study was “bofe” bentonite clay from a Paraiba State region. The X-rays diaphactogram of this bentonite is presented in Figure 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.

The irrigation was carried out using tap water to maintain the soil moisture to field capacity. At 30 and 60 days of experimental period, the plants 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 h. After drying, the plants were triturated and samples were weighed for foliar analyses. Plant samples were submitted to cooper determination conducted after nitric-perchloric digestion, according to Embrapa procedures (Embrapa, 1997), using a Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES), as described by Oliva et al. (2003). The translocation index (TI) was determined by using the follow expression (Abicheque and Bohnen, 1998):

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

Soil samples were collected from each experimental unit and the copper content was determined using the Mehlich-1 extractor (Embrapa, 1997).

The experimental design was a completely randomized design

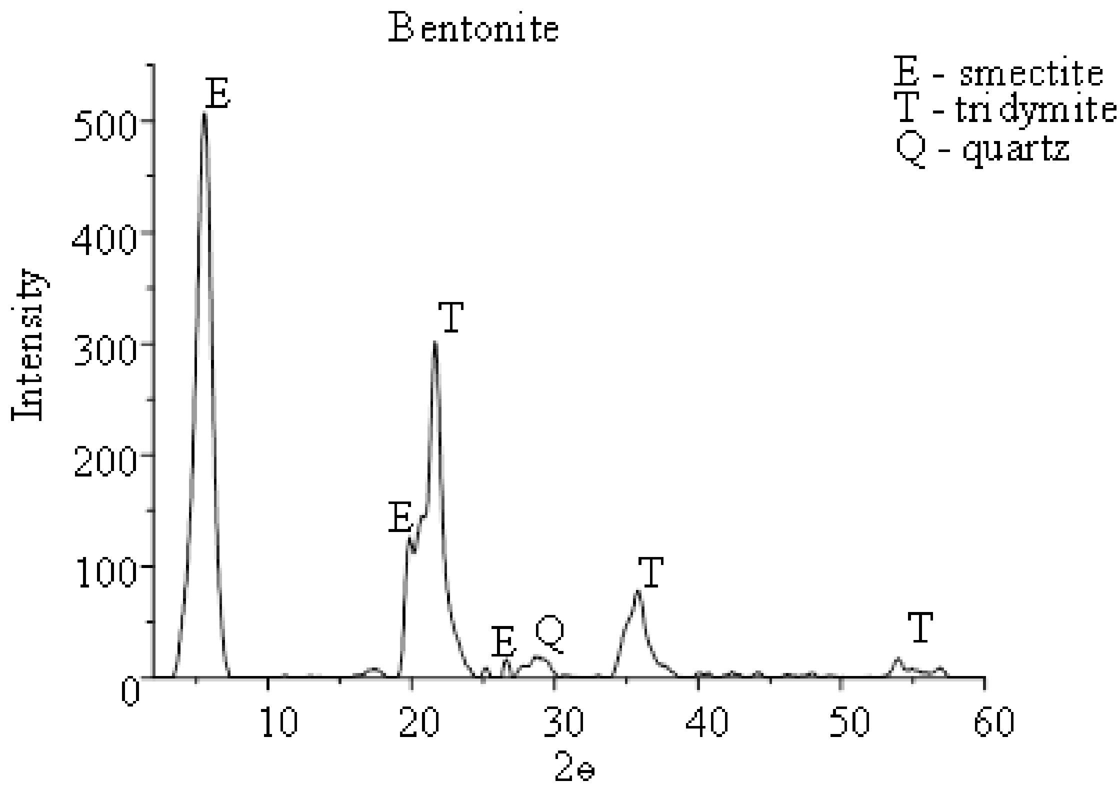

Figure 1. X-rays diaphactogram of the "bofe" bentonite clay.

Table 1. Summary of the analyses of variance for the dry phytomass of the aerial part and roots of the beets, radish and corn according to the different bentonite treatments.

Source of variation	DF	Mean Square					
		Beets		Radish		Corn	
		ADPB	RDPB	ADPR	RDPR	ADPC	RDPC
Bentonite	3	0.514^{ns}	43.02**	0.80**	1.01**	84.50**	4.85ns
Linear	1	1.169^{ns}	120.95**	1.53**	2.48**	236.25**	14.12*
Quadratic	1	0.154^{ns}	4.67^{ns}	0.68	0.005^{ns}	6.91^{ns}	0.02^{ns}
Error	12	0.579	1.69	0.60	0.14	5.09	2.86
VC (%)		10.17	19.58	9.35	19.71	2.53	10.49
Mean (g)		7.48	6.64	2.70	1.90	89.29	16.14

DF = Degree of freedom, ^{ns}, * and ** not significant, significant at 5 and 1% levels, respectively. VC = variation coefficient. ADPB, RDPB, ADPR, RDPR, ADPC and RDPC: Aerial dry phytomass of beets, root phytomass of beets, aerial dry phytomass of radish, root phytomass of radish, aerial dry phytomass of corn and root phytomass of corn, respectively.

with four replicates, totaling 16 experimental units (plastic pots). SISVAR statistical program (Ferreira, 2011) 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 DISCUSSION

With the exception of beets, the dry phytomass of the aerial part of the radish (ADPR) and corn (ADPC) was significantly affected by the bentonite application at 1% significance level. With the exception of the corn, the bentonite treatments affected significantly the dry phytomass of aerial part of the beets (ADPB) and radish (ADPR) at 1 and 5% significance levels, respectively (Table 1).

With the exception of the dry phytomass of the aerial

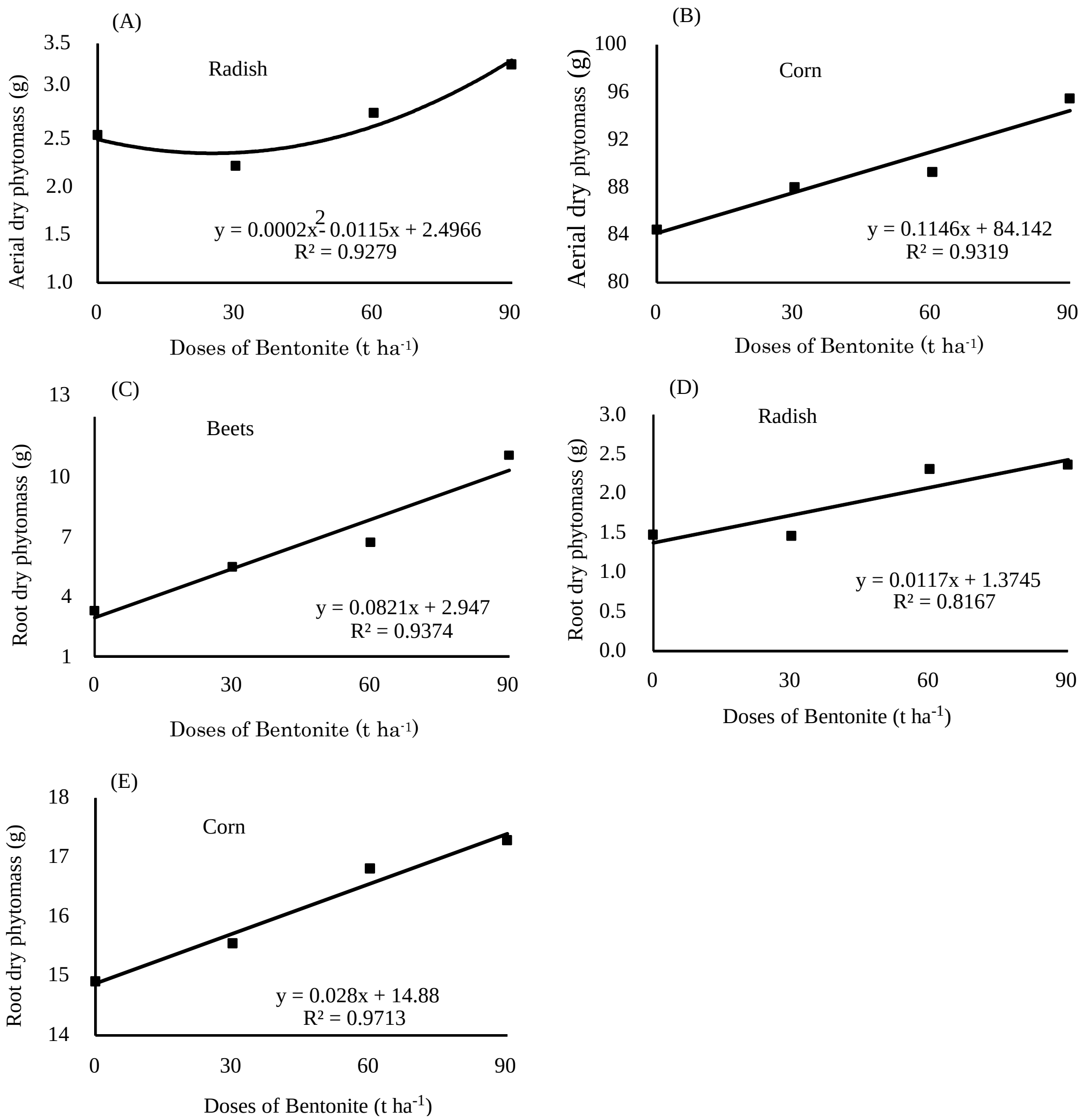

Figure 2. Dry phytomass of the aerial part for the radish (2A) and corn (2B) and dry phytomass of the roots for beets (2C), radish (2D) and corn (2E) according to bentonite doses.

part of the corn, which was adjusted to a quadratic model, the significant bentonite effects were adjusted to linear regression models (Figure 2). The dry phytomass of the aerial part of the radish (ADPR) presented the maximum value with the highest bentonite treatment, which was 23.43% greater than the control (dose 0) (Figure 2A). The dry phytomass of the aerial part of corn (ADPC) presented the maximum value (17.40 g) with the highest bentonite treatment; and the lowest value (14.88 g) with the control, an increase of 14.48% (Figure 2B). The dry phytomass of beet beets, radish and corn roots increased with the bentonite application, reaching increases of 250.00, 76.61 and 16.93% when comparing the zero bentonite dose (the lowest phytomass) to the higher bentonite dose (Figures 2C, D and E, respectively). The increase of the dry phytomass of the aerial part and roots for beets, radish and corn with the bentonite application can be explained because the clay increased the adsorption of the copper in soil reducing its availability to the plant roots. The results corroborate with Kabata-Pendias and Sadurski (2004), who found that the mobility of Cu from soil to plant decreased due the presence of bentonite in the soil.

The increase of the soil adsorption capacity for copper due to bentonite application reduced the availability of copper in the soil solution, and therefore favored the growth of the crops and increased the dry phytomass of the aerial part and roots, corroborating Llorens et al. (2000) and Qian et al. (2005). According to these authors, the presence of high copper concentrations in

Table 2. Summary of the analyses of variance for the copper concentration in the aerial and root part of the beets, radish and corn for the different bentonite treatments.

Source of variation	DF	Mean Square					
		Beets		Radish		Corn	
		BACC[1]	BRCC	RACC	RRCC	CACC	CRCC
Bentonite	3	0.31^ns	178.37*	2.20**	1615.77**	2.86^ns	2046.37*
Linear	1	0.33^ns	365.51**	5.55**	3158.59**	2.58^ns	5474.74**
Quadratic	1	0.0003^ns	169.26^ns	0.24^ns	364.49**	4.12^ns	664.09**
Error	12	0.43	37.49	0.10	24.47	1.46	518.52
VC (%)		8.43	9.97	18.49	14.05	19.13	19.93
Mean (mg kg^{-1})		7.80	61.44	1.70	35.21	6.32	114.23

^ns, * and ** not significant, significant to the 5 and 1% level, respectively, VC = Variation coefficient. [1] Data transformed in √x. BACC, RACC, CACC = Copper concentration in the aerial part of beets, radish and corn, respectively. BRCC, RRCC, CRCC = Copper concentration in the roots of beets, radish and corn, respectively.

soil can influence the plant metabolism and the proper absorption of others nutrients, affecting negatively, the growth of plants.

The bentonite doses had significant effect on beets, radish and corn roots at 5, 1 and 5%, respectively, but there was no significance for beet and corn aerial part (Table 2). According to Marques et al. (2002), copper concentration in roots that is toxic for plants ranges from 60 to 125 mg kg^{-1}, thus the high concentrations found on the beet roots (mean of 61.44 mg kg^{-1}) and corn roots (mean of 114.23 mg kg^{-1}) can be considered as non-toxic for the plants. However, the root concentrations of copper found in the beets and corn are toxic for human consumption, according to the Brazilian Association of Feeding Industries (ABIA) (ABIA, 1985). According to the ABIA (1985), the tolerant limit of copper for roots, horticultures, tubercles and other fresh foods is 30 mg kg^{-1}.

The copper concentration of the beet roots decreased linearly from 69.95 to 54.43 mg kg^{-1} when bentonite application varied from 0 to 90 t ha^{-1} (Figure 3A). For the aerial part, copper concentration also decreased linearly with the bentonite application decreasing from 2.5 to 0.91 mg kg^{-1} when the bentonite doses varied from 0 to 90 t ha^{-1} (Figure 3B). The copper concentration in the roots of the radish varied exponentially with the bentonite doses, by increasing from 44.96 to 50.60 mg kg^{-1} for doses of 0 to 30 ton ha^{-1}; and decreased to 7.34 mg kg^{-1} when the bentonite dose was 90 ton ha^{-1} (Figure 3C). In the corn roots, copper concentration varied linearly with the bentonite doses, by decreasing from 139.05 to 89.41 mg kg^{-1} when the bentonite dose was 90 ton ha^{-1} (Figure 3D). It is shown in Figures 3A, C and D that copper concentration in the roots of beets, radish and corn were higher than the permitted levels for human consumption recommended by ABIA (1985). According to ABIA (1985), the tolerant limit of copper for roots, horticultures, tubercles and other fresh foods is 30 mg kg^{-1}. It is also observed that the copper concentration of the roots of radish decreased to below 30 mg kg^{-1} for bentonite doses greater than 60 t ha^{-1}, pointing out that this application increase the soil adsorption capacity of copper, decreasing the availability of copper in the soil solution and, consequently, decreasing the absorption of copper by plants.

The results found for corn corroborates Marques et al. (2002) and Mantovani (2009). Mantovani (2009) evaluated corn grown in a soil contaminated with 202 mg kg^{-1} Cu and a great copper concentration was observed in the roots (502 mg kg^{-1}); however, the aerial part presented low copper concentrations, below 30 mg kg^{-1}, which is the toxic limit for human consumption according to the ABIA (1985).

The copper concentration in the roots was much higher than in that the aerial part of the plants as evaluated in this study (Table 2). This fact can be attributed to physiological mechanisms presented by plants in order to prevent the translocation of the copper from the roots to the aerial part (Cornu et al., 2007). According to Marsola et al. (2005), this phenomenon would be a tool that plants present as a protection for copper intoxication. Loneragan (1981) and Tiffin (1972) observed that root tissues present a higher capability to hold copper and prevent the copper translocation to shoots, both for copper deficiency and excess. These authors concluded that the copper excretion from root cells to xylem and phloem is a key process for plant nutrition. Bentonite affected significantly at 1% probability level the accumulation of copper in roots of beets and on the aerial and roots of the radish (Table 3). The regression curve presented in Figure 4A shows a linear increase for copper accumulation in the beet roots with bentonite doses, ranging from 0.219 mg with the 0 t ha^{-1} to 0.562 mg for 90 t ha^{-1}, corresponding to an increase of 156.16%. It is important to highlight that although the copper concentration in the root decreased with the bentonite application, the accumulated copper found in the roots increased, this is because the accumulative

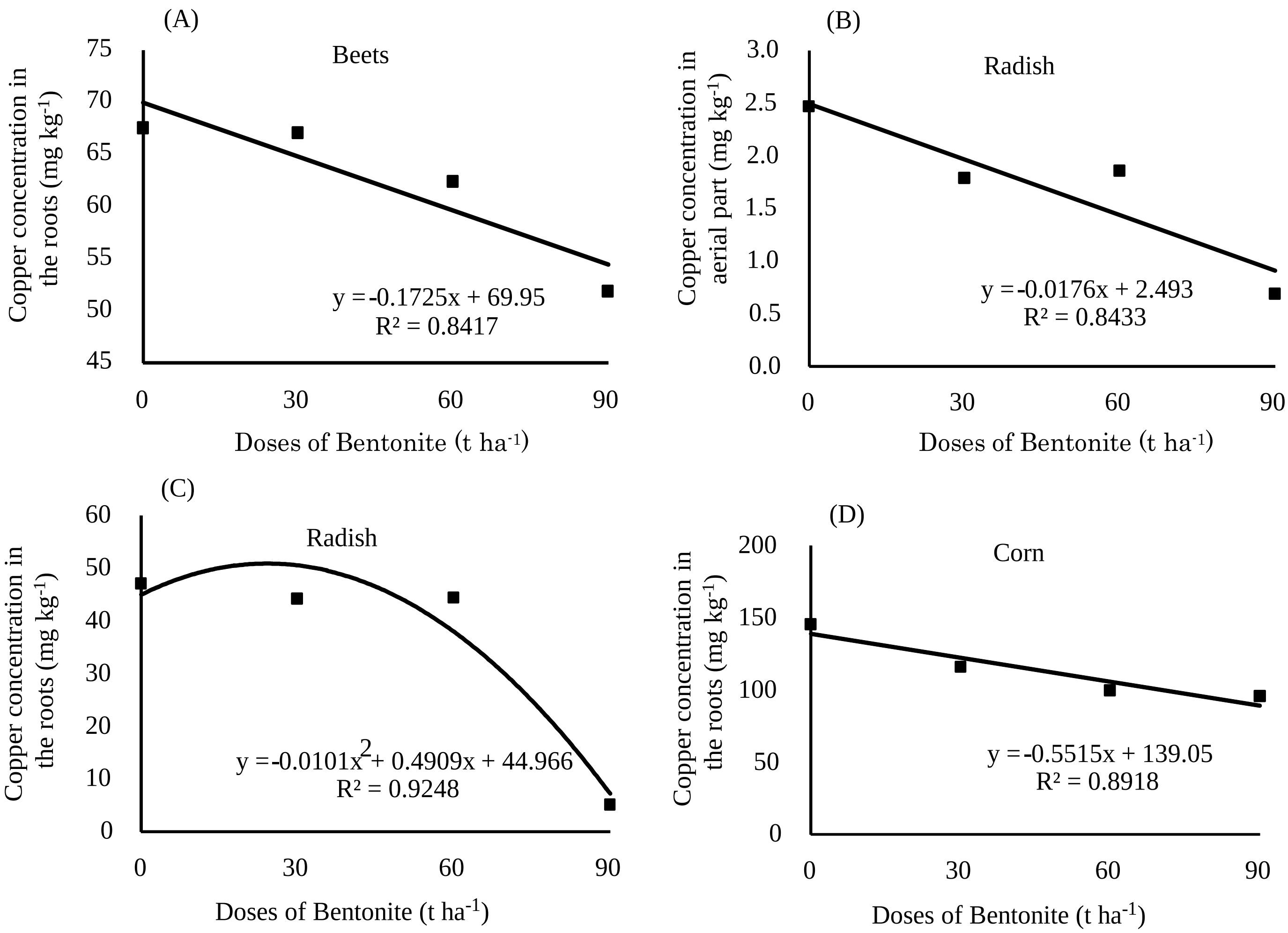

Figure 3. Copper concentration in the roots of beets (3A), in the aerial part of the radish (3B), in the roots of radish (3C) and in the roots of corn (3D) at the end of the experiment, according to bentonite doses.

copper was calculated based on the plant dry phytomass, which increased with bentonite application. The regression curve in Figure 4B shows an increase of the copper accumulated in beet roots until 40 ton ha^{-1} approximately, and a decrease of 68.35% for the 90 ton ha^{-1} when compared with the results. The regression curve in Figure 4C shows a linear decrease of copper accumulated in the aerial part of the radish with the bentonite doses, varying from 0.0873 mg with 0 t ha^{-1} to 0.051 for the 90 t ha^{-1} of bentonite, corresponding to a decrease of 41.58%. As a result of the accumulated copper calculated based on the dry phytomass of the plant, similar amount of copper accumulation was observed in the roots and shoots because the dry phytomass in the aerial part of the plant (2.70 g) was much higher than that in the roots (1.90 g).

Bentonite application affected significantly at 1% level of probability, the translocation index of the copper in beets and radish (Table 4). The translocation index is the percentage of the metal absorbed by the plant and transferred to the aerial part (Abichequer and Bohnen, 1998). The bigger the index, the greater the translocation. The translocation index of the copper in the beets decreased linearly with bentonite application, varying from 67.17% for the 0 ton ha^{-1} to 43.62% for the 90 ton ha^{-1} of bentonite application, a decrease of 35.06% (Figure 5A). Based on the definition of the translocation index and the results of the significant regression (Figure 5A), it is observed that the translocation of copper on beets decreased with bentonite application, being accumulated in the roots and not transferred to the aerial part of the plant. The results corroborate Kabata-Pendias and Pendias (1992) who said that the copper is an unmoved element because it is strongly fixed by the root cellular walls. This is probably the reason why a great quantity of copper was found in the beet roots making it inappropriate for human consumption, higher than the permitted levels for human consumption as recommended by the ABIA (1985).

The application of bentonite in the soil grown with

Table 3. Summary of the analyses of variance for the accumulated copper in the aerial and root part of the beets, radish and corn for the different bentonite treatments.

Source of variation	DF	Mean square					
		Beets		Radish		Corn	
		BACAP	BACR	RACAP[1]	RACR	CACAP	CACR
Bentonite	3	0.005^{ns}	0.089**	0.0007**	0.006**	0.020^{ns}	0.220^{ns}
Linear	1	0.00002^{ns}	0.259**	0.002**	0.004**	0.001^{ns}	0.523^{ns}
Quadratic	1	0.0002^{ns}	0.00001^{ns}	0.00006^{ns}	0.007**	0.038^{ns}	0.133^{ns}
Error	12	0.009	0.006	0.0001	0.0001	0.010	0.139
VC (%)		20.86	20.01	14.06	19.23	18.43	20.45
Mean (mg)		0.460	0.390	0.065	0.060	0.560	1.821

^{ns} not significant, ** significant at 1% probability. VC = Variation coefficient. [1]Data transformed in √x. BACAP, RACAP, CACAP = Copper concentration in the aerial part of beets, radish and corn, respectively. BACR, RACR, CACR = Copper concentration in the roots of beets, radish and corn, respectively.

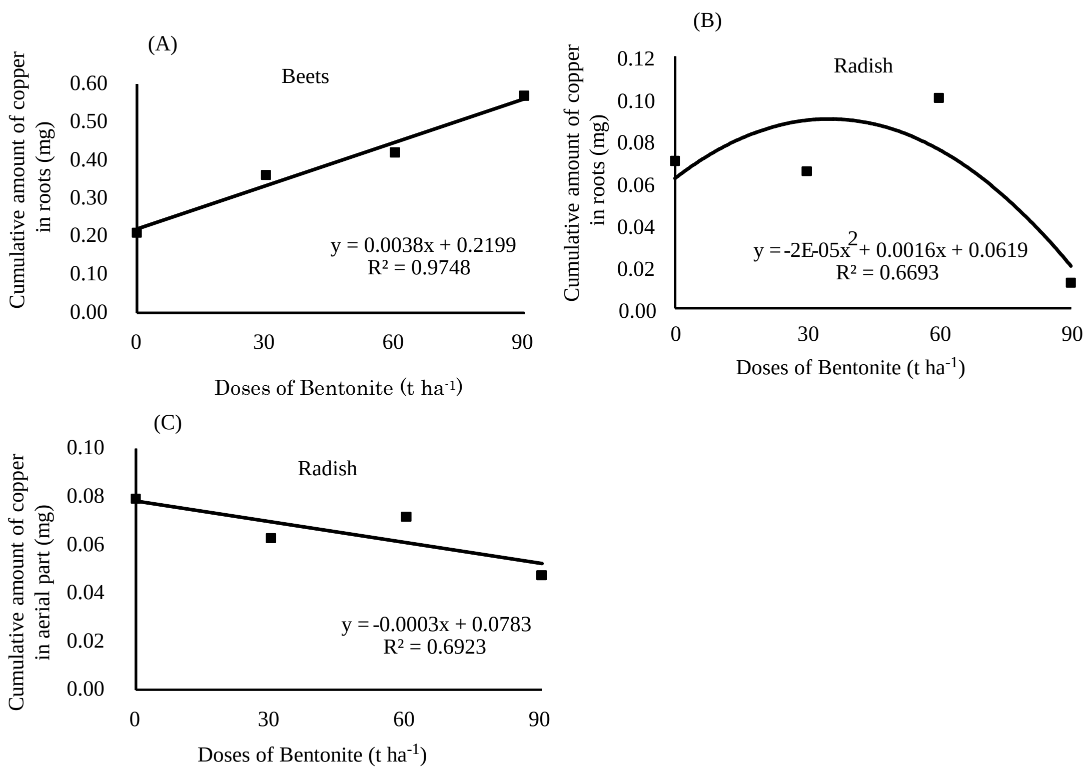

Figure 4. Accumulated copper in the root of beets and radish and in the aerial part of radish according to bentonite doses.

radish affected significantly the translocation index of the copper; however, the indices were very small: 1.97% for the 30 ton ha^{-1} and 5.67% for the 90 ton ha^{-1} (Figure 5B). There was no defined pattern of variation for the bentonite application. The available copper content in soil at the end of experimental period, whose means for radish and corn were 28.33 and 23.93 mg kg^{-1}, respectively, when submitted to bentonite applications, they were lower than 35 mg kg^{-1}, which is the reference value corresponding to the quality level for a clean soil, with absolute no copper contamination (CETESB, 2005). It also was lower than the copper intervention level (60 mg kg^{-1}) also reported by the CETESB (2005), corresponding to the copper content in which there are risks for human health when this soil is used for human food production. Thus, the application of bentonite to these contaminated soils favored their amelioration. The mean available copper found in the soil after harvest of

Table 4. Summary of the analyses of variance for the copper translocation index of the beets, radish and corn for the different bentonite treatments.

Source of Variation	DF	Square root		
		Beets	**Radish**[1]	**Corn**
Bentonite	3	470.60**	3.25**	0.23^{ns}
Linear	1	1231.54**	3.66**	0.41^{ns}
Quadratic	1	17.07^{ns}	6.64**	0.08^{ns}
Error	12	33.83	0.24	0.28
Variation Coefficient (%)		10.50	17.55	10.91
Mean (%)		55.40	2.78	4.88

^{ns} not significant; ** significant at 1% probability. [1] Data transformed in logx.

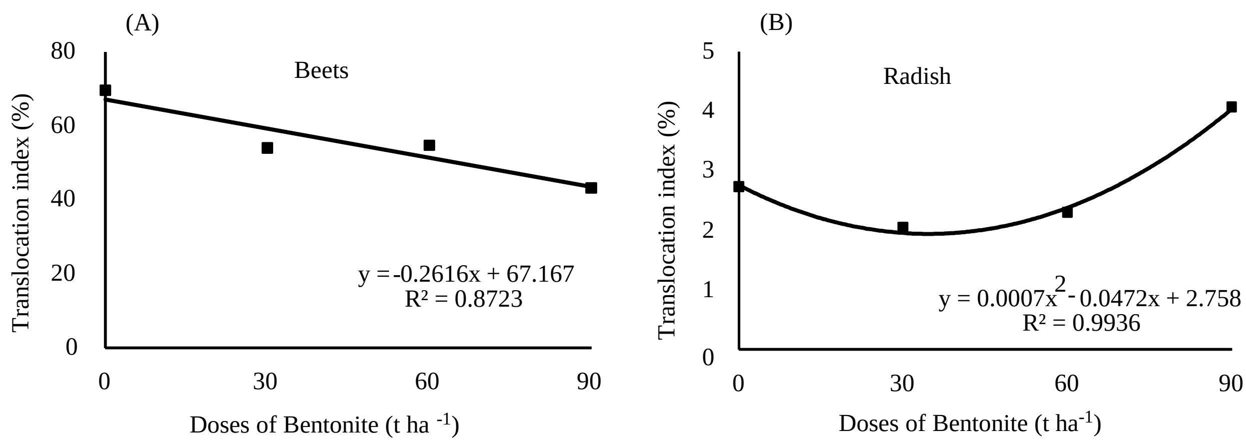

Figure 5. Translocation index for the beets and radish according to bentonite doses.

the beets was 116.49 mg kg^{-1}, corresponding to a contaminated soil, inappropriate for agricultural use because the risks for human health when used for human food production. Thus, the application of bentonite to this contaminated soil did not ameliorate it, probably due to the great quantity of copper added to the soil at the beginning of the experiment.

Conclusions

The increase of dry phytomass of the aerial part and roots of beets, radish and corn with bentonite application showed that the bentonite reduced the copper content of the plants, probably because the adsorption capacity of the soil increased with the application of bentonite. Thus, for the conditions under which the study was conducted, bentonite application favored crop development. With the exception of aerial part of the beets and corn, the copper concentration of the plant decreased significantly with the bentonite application. Bentonite affected significantly at 1% probability, the copper accumulated in the roots of beets and radish and in the aerial part of the radish.

The translocation index of copper in the beets was reduced with the bentonite application, to find a great quantity of copper in the beet roots, higher than the permitted levels for human consumption and making it inappropriate for human consumption.

The application of bentonite to the contaminated soils planted with radish and corn favored their amelioration; the application of bentonite to the contaminated soil planted with beets did not ameliorate it.

Conflict of Interests

The authors have not declared any conflict of interests.

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