



RESEARCH ARTICLE

BENTONITE EFFECT ON COPPER ADSORPTION ON PLANTS IRRIGATED WITH LOW QUALITY WATER

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ABSTRACT

Bentonite is a clay mineral with high cation exchange capacity and it has been evaluated as an alternative sorbent in removing metals in diverse environments. This study aimed to evaluate the effect of bentonite clay in adsorbing copper present in lower quality water in radish, corn and pasture grass. The experiments were carried out in a greenhouse, with a completely randomized design with four replications. The experimental units were plastic pots with a capacity of 5, 14 and 8 kg of soil for radish corn and grass, respectively. The soil was mixed with increasing doses of bentonite equivalent to 0; 30; 60 and 90 t ha⁻¹. The sowing was done directly on the pot, leaving two plants per pot after thinning. They have been irrigated with poor quality water with a concentration of 1 mg L⁻¹ Cu. Plants were harvested at 30, 60 and 70 days, separated in shoot and roots and placed in air circulation oven. The data were submitted to analysis of variance. Bentonite improved the development of radish and pasture grass crops; promoted the retention of copper in the soil, and favored the reduction of copper translocation to the shoot of corn, thereby reducing the concentration of this element in the aerial part.

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INTRODUCTION

The effluents from industrial activities, from treatment of municipal sewage and agricultural sector, discharged in water bodies have contributed to the aggravation of environmental problems, especially regarding the preservation of surface and groundwater. The use of wastewater in agriculture is gaining importance due to reduced supply of water of good quality (Dantas *et al.*, 2014). However, these effluents, most often contain toxic metals in their compositions, and although low concentrations occur in successive applications can cause their accumulation in the soil representing, over time, a threat to ecosystems. Depending on the physical and chemical form such as heavy metals are in the effluent, they may be bioavailable to living beings and the environment in which they are providing certain toxicity in wastewater. Therefore, the release of these metals to the soil solution, making them available for plant uptake (Bertoncini and Mattiazzo, 1999), reduces agricultural productivity and/or affects the entire food chain due to the accumulation of metals (Cunha Filho *et al.*, 2014). According to Guimarães *et al.* (2008), plants constitute the main entry point of heavy metals in the food chain, as the roots readily absorb them.

Consequently, the need for new techniques and solutions that can reduce the concentration of heavy metals in soil and waters is paramount. Conventional treatments for decontamination of large volumes of effluent, which contains potentially toxic metal ions, are related to the chemical precipitation, sedimentation and filtration. However, these treatments often are inadequate and of high cost (Nerbitt and Davis, 1994), so alternatives for remediation of wastewater contaminated are being used, for example, the use of clay (Feng *et al.*, 2004). The application of clays has been evaluated for efficiency in removing heavy metals and toxic substances in wastewater (Eloussaief and Benzina, 2010). Among these clays, there is bentonite, which is a clay mineral from smectite group with adsorbent properties, able to remove the metal ions from soil / wastewater systems, making them less available to plants. According to Sdiri *et al.* (2011), this clay can be effectively used for the treatment of contaminated wastewater. Copper, despite being one of the metals occurring in lower quality water may harm the ecosystems, it is an essential element for plant growth. This element moves slowly in the soil generally in the form of organic complex (Paganini *et al.*, 2004). Tito *et al.* (2012) evaluated the effect of the bentonite on the mobility of zinc and copper in crop and found that copper was strongly adsorbed by the soil complex / bentonite. It is generally known that different clay minerals have greater or lesser adsorption capacity for the various metal cations. In this

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sense, it is important to evaluate the effectiveness of bentonite in copper adsorption. For these reasons, this study aimed to evaluate the effect of different doses of the clay bentonite applied to the soil irrigated with low quality of water in the copper uptake by plants.

MATERIALS AND METHODS

The study was carried out under semi controlled greenhouse conditions, from March 2014 to June 2014 at the Agricultural Engineering Department of the Federal University of Campina Grande, Paraiba, Brazil. The experiments were carried out with radish (*Raphanus sativus*), pasture grass (*Brachiaria brizantha* cv.) and corn (*Zea mays* L.), on a loamy sand soil classified as a Red Eutrophic Latosol (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 2mm mesh and analyzed using the procedures recommended by Embrapa (1997). The following attributes were found: pH (H₂O) = 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. 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.

Figure 1 shows the bentonite X-ray diffractogram showing characteristic peaks of smectite clay mineral or montmorillonite, main components of bentonite, tridymite peaks (silicate mineral and a high temperature quartz polymorph), as well as the presence of minor amount of quartz.

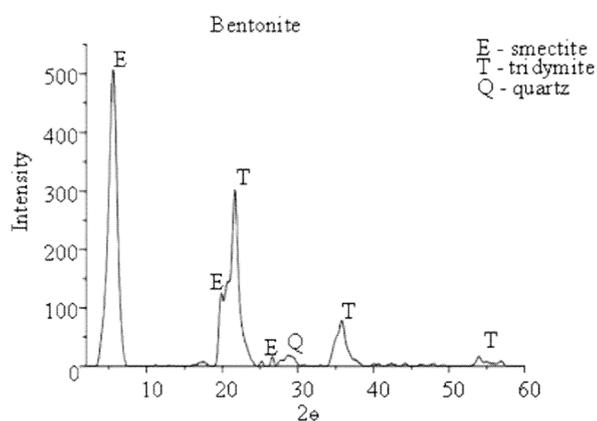


Figure 1. Diffractogram of Bentonite obtained by X-ray Diffraction

The bentonite doses (treatments) used in these studies were 0; 30; 60 and 90 t ha⁻¹, corresponding to 0; 10.7; 21.4 and 32.1 g kg⁻¹ of soil, respectively, with 4 repetitions in a completely randomized design totaling 16 experimental units (plastic pots). Each experimental unit found a plastic pot with 5, 14 and 8 kg of soil for radish (*Raphanus sativus*), maize (*Zea mays* L.) and pasture grass (*Brachiaria brizantha* cv.), respectively. The soil was previously dried, sieved and mixed with the bentonite dose corresponding to each treatment. The soil mixtures with bentonite were placed in plastic pots, moistened with water supply to reach field capacity, and incubated for 20 days. According to Novais *et al.* (1991), the radish was fertilized with 1.11 g of urea, 1.25 g of potassium chloride (KCl) and 8.3g of super phosphate (P₂O₅); corn was

fertilized with 3.11 g of urea, 3.5 g of KCl and 23.33 g of P₂O₅; and the pasture grass was fertilized with 1.78 g of urea, 2.0 g of KCl and 13.33 g of P₂O₅. After fertilization, the seeds of each crop were sown and 8 days after the emergency of seedlings, a thinning was conducted leaving two plants per pot. The irrigation was carried out using lower quality water, with concentration of copper (Cu) of 1.0 mg L⁻¹, to maintain the soil moisture to field capacity. According to the total volume of water used in irrigation of radish, corn and grass, the amount of copper applied to the soil was 1.2; 2.32 e 2.63 mg kg⁻¹ respectively. At 30; 60 and 70 days of experimental period, corresponding to radish, corn and pasture grass, respectively, plants were harvested and separated into aerial part and roots, washed with distilled water, 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 of Cu content in plant tissue after nitric-perchloric tissue digest (3:1) (Embrapa, 1997). Then, the readings were made by using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES), as described by Oliva *et al.* (2003). Soil samples were collected from each experimental unit and the copper content was determined using the Mehlich-1 extractor (Embrapa, 1997). The amount of Cu accumulated in the aerial part (AAP) and roots (AR) of the plant (mg/pot) was calculated by the expression AAP or AR = {dry biomass of the aerial part (g) or dry biomass of roots (g) x concentration of element (mg kg⁻¹)} / 1000. The translocation index (TI) was determined by using the follow expression (Abichequer and Bohnen, 1998): (AAP/ amount of Cu accumulated in the complete plant) x 100. 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

The application of increasing doses of bentonite significantly influenced at 1% probability the dry biomass of roots (DBR) of the radish and pasture grass, and in dry biomass of aerial part (DBAP) of pasture grass irrigated with lower quality water (Table 1). The exception was of DBAP of grass, which was fitted to quadratic model. All significant effects of bentonite were adjusted to linear regression model (Figure 2). The dry biomass of roots of radish ranges from 1.32 g (0 t ha⁻¹ bentonite) to 2.80 g (90 t ha⁻¹ bentonite), corresponding to an increase of 112.12% for the higher dose of bentonite compared to control (Figure 2A). For pasture grass, the application of bentonite in soil also had a beneficial effect, i.e., it promoted an increase of greater treatment (90 t ha⁻¹ of bentonite) compared to control (0 t ha⁻¹ of bentonite) approximately 12.0 and 21.6% for dry biomass of shoots (Figure 2 C) and roots (Figure 2 B), respectively. In this study, the incorporation of bentonite clay in the soil did not present significant effect on biomass of corn; however, Hassan and Mahmoud (2013) found that bentonite and zeolite mixed with soil provided an increase in biomass and development of corn and bean. Likewise, Youssef (2013) working with these clays found a significant increase in growth, yield and chemical composition of potato (*Solanum tuberosum* L.). According to these authors, the significant effect of adding bentonite to the soil in the growth of various plants may be due to the increase in water retention capacity in soil (Iskander *et al.*, 2011), by reducing leaching of nutrients through their colloidal content (Sittaphanit *et al.*, 2010), especially in sandy soils, that is the soil used in this study.

Table 1. Summary of the analyses of variance for the dry biomass of the aerial (DBAP) and root part (DBR) of the radish, corn and pasture grass irrigated with lower quality water with increasing doses of bentonite

Source of Variation	DF	Mean squares					
		Radish		Corn		Grass	
		DBAP	DBR	DBAP	DBR	DBAP	DBR
Bentonite	3	0.01ns	1.648**	47.85ns	10.96ns	26.36**	13.20**
Linear	1	0.011ns	4.871**	108.9ns	21.57ns	54.21**	37.98**
Quadratic	1	0.017ns	0.003ns	31.8ns	1.35ns	22.70*	1.50ns
Error	12	0.10	0.119	28.099	15.468	4.35	2.15
VC (%)		12.27	16.80	5.79	18.09	5.08	6.92
Mean (g)		2.632	2.057	91.561	21.75	41.066	21.209

DF= Degree of Freedom, ^{ns}, * and ** no significant, significant at 5 and 1% level, respectively. VC = Variation Coefficient. DBAP, DBR= Dry biomass of aerial part, dry biomass of root, respectively.

Table 2. Summary of the analysis of variance of copper concentration in the shoot (CAP) and copper concentration in the root (CR) of radish, corn and pasture grass, irrigated with lower quality water with increasing doses of bentonite

Source of Variation	DF	Mean squares					
		Radish		Corn		Grass	
		CAP ²	CR	CAP ¹	CR	CAP	CR
Bentonite	3	0.006*	57.01**	2.83**	41.13**	0.99**	10.62**
Linear	1	0.014**	127.31**	5.77**	74.27**	2.44*	16.09**
Quadratic	1	0.001ns	29.92**	2.31**	43.96**	0.073ns	9.06**
Error	12	0.001	0.93	0.10	1.88	0.119	0.43
VC (%)		10.47	12.11	16.87	24.68	12.63	13.07
Mean (mg kg ⁻¹)		0.33	7.95	1.88	5.56	2.73	5.02

DF= Degree of Freedom, ^{ns}, * and ** no significant, significant to the 5 and 1% level, respectively. ¹e² transformed data in \sqrt{x} and $1/\sqrt{x}$, respectively. VC = Variation Coefficient. CAP, CR= Concentration in shoot, concentration in roots, respectively.

Table 3. Summary of the analysis of variance of amount copper accumulated in the shoot (AAP) and root (AR) of radish, corn and pasture grass, irrigated with lower quality water with increasing doses of bentonite

Source of Variation	DF	Mean squares					
		Radish		Corn		Grass	
		AAP ¹	AR ¹	AAP ¹	AR ¹	AAP	AR
Bentonite	3	0.002ns	0.0003ns	0.244**	0.026**	0.0012*	0.0025**
Linear	1	0.004*	0.0001ns	0.476**	0.043**	0.0020**	0.0025*
Quadratic	1	0.0005ns	0.0004ns	0.220**	0.029**	0.0005**	0.0026*
Error	12	0.0006	0.0003	0.0108	0.0021	0.0002	0.0003
VC (%)		15.54	13.62	18.29	13.77	13.04	16.58
Mean (mg/pot)		0.16	0.12	0.57	0.33	0.111	0.104

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

These increases in the specific surface area improve the adsorption capacity of the nutrients released by the fertilizer, maintaining balance with the crop needs. In addition, it has function as fertilizer, increasing soil micronutrients and macronutrients in addition to other various biochemical processes associated with plant growth (Marschner, 1995). Additionally to these effects, the treatments increased dry biomass of aerial parts and/or roots of radish and pasture grass crops, indicating that bentonite, probably by adsorption mechanism, reduced copper levels available in soil solution, thus promoting the growth of these crops, particularly the roots by reducing the toxic effect of copper. As Yruela (2005) stated, Cu toxicity in plants inhibits their growth and prevents important cellular processes such as, for example, electron transfer in photosynthesis. According to Llorens *et al.* (2000), the presence of high concentrations of Cu in the root environment can influence the absorption and the metabolism of other nutrients affecting plant growth. The increasing doses of bentonite had a significant effect on the concentration of Cu in the shoot (CAP) and roots (CR) of all three crops (Table 2). Regarding the radish, the Cu concentrations in shoots and roots ranged from 14.18 to 7.41 mg kg⁻¹ (data not transformed) and from 13.52 to 5.12 mg kg⁻¹ presenting better fit in linear and quadratic model, respectively (Figure 3A and 3D). According to ABIA (1985), the maximum tolerable level for human consumption of vegetables, root crops and other fresh foods is 30 mg kg⁻¹ of copper.

However, it can be seen that the general average of Cu concentration in both roots (9.82 mg kg⁻¹, data not transformed) and aerial parts (7.95 mg kg⁻¹) of radish were well below the maximum permissible value, making them acceptable for human consumption (Table 2), corroborating Khan *et al.* (2008). These authors studied the soil contamination by heavy metals and absorption in food crops through irrigation water containing heavy metals. These authors found that the contamination in the soil and in the edible parts of plants, for copper and zinc were substantially lower the permissible limits set by the State Environmental Protection Administration (SEPA) in China for all food crops grown. The same behavior was observed by Silva *et al.* (2012), who studied the irrigated maize with wastewater containing heavy metals (Cu, Cd, Mn, and Zn), and they found that concentrations in the leaf biomass was not able to cause toxicity to the plant or human health, according to Governing law (Conama, 1996). The general average for Cu concentration in the shoot (4.13 mg kg⁻¹, data not transformed) and roots (5.56 mg kg⁻¹) of corn (Figure 3B and 3E), had a reduction in the range of 78.26 and 60.56%, respectively, due to increasing doses of bentonite, with the best fit for quadratic model. For pasture grass, the general average of Cu concentration in the shoot (2.73 mg kg⁻¹) and roots (5.02 mg kg⁻¹) were also reduced in the order of 32.06 and 41.96% (Figure 3C and 3F), indicating a possible decrease in the availability of this element in the soil due to adsorption by bentonite.

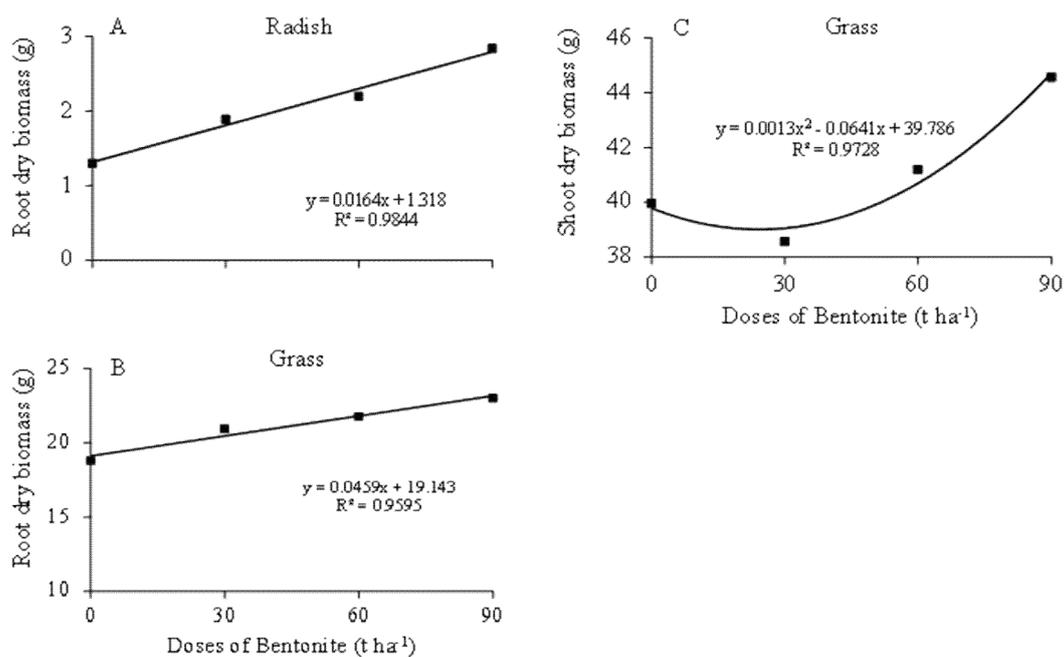


Figure 2. Dry biomass of the radish (A) and pasture grass (B) roots, and dry biomass of the aerial part of pasture grass (C) irrigated with lower quality water, due to increasing doses of bentonite

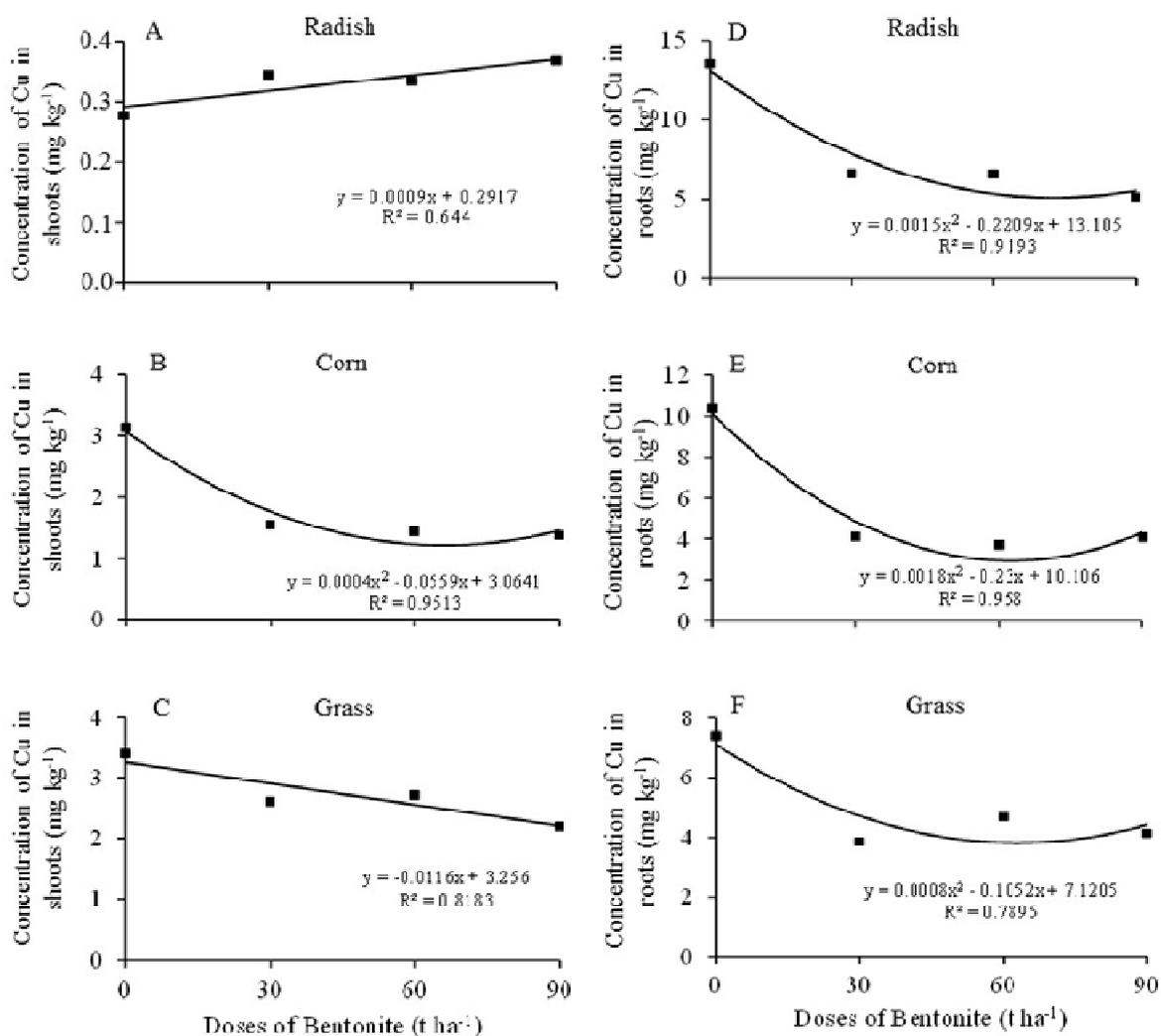


Figure 3. Cu concentration in the shoots of radishes (A), corn (B) and pasture grass (C) and Cu concentration in the root of radish (D), corn (E) and pasture grass (F) in function of increasing doses of bentonite

The general average of Cu concentrations (Table 2) for corn and pasture grass were higher in roots, corroborating Marsola *et al.* (2005), who stated that there is a mechanism to reduce the spread of the cation inside the tissue, protecting it from intoxication. This was observed by Mantovani (2009) studying corn in contaminated environment with Cu; and by Hamidpour *et al.* (2010), evaluating the corn crop in pots containing bentonite and sand contaminated with cadmium. Likewise Seidel *et al.* (2009), studying the phytoavailability of copper in corn, found that the roots have absorbed part of applied Cu in excess in treatments, but there was no proportional translocation to the shoot, showing that corn roots have mechanisms that prevent translocation part of the Cu surplus absorbed. Increasing doses of bentonite significantly influenced the amount accumulated in the shoot (AAP) and root (AR) of corn and pasture grass (Table 3). With the exception of the accumulated amount in the aerial part of the radish (linear adjustment), the best fit of the data corn and pasture grass was provided by the quadratic regression model (Figure 4).

Bentonite applied to the soil significantly influenced at 5% of probability in the accumulated amount of Cu in the shoot (AAP) of radish (Table 3), whose behavior followed the linear model (Figure 4A), ranging from 0.18 to 0.14 mg/pot, that is, from 0.0369 to 0.0196 mg/pot, averaging around 0.026 mg/pot (data not transformed). The general average of Cu accumulation in roots was approximately 0.015 mg/pot, i.e., much less than it was accumulated in the shoots. The amounts of Cu accumulated in the shoots (AAP) and the root (AR) of corn decreased according to increasing doses of bentonite applied to the soil (Figures 4B and 4D), ranging from 0.92 to 0.21 mg/pot (0.88 to 0.20 mg/pot, data not transformed) and 0.45 to 0.32 mg/pot (0.21 to 0.09 mg/pot, data not transformed), respectively. However, it can be observed in this figure that there was practically no difference between the doses 30, 60 and 90 t ha⁻¹ despite having been reduced around 77 and 28% of the control (without bentonite) and the dose of 90 t ha⁻¹ of bentonite, respectively.

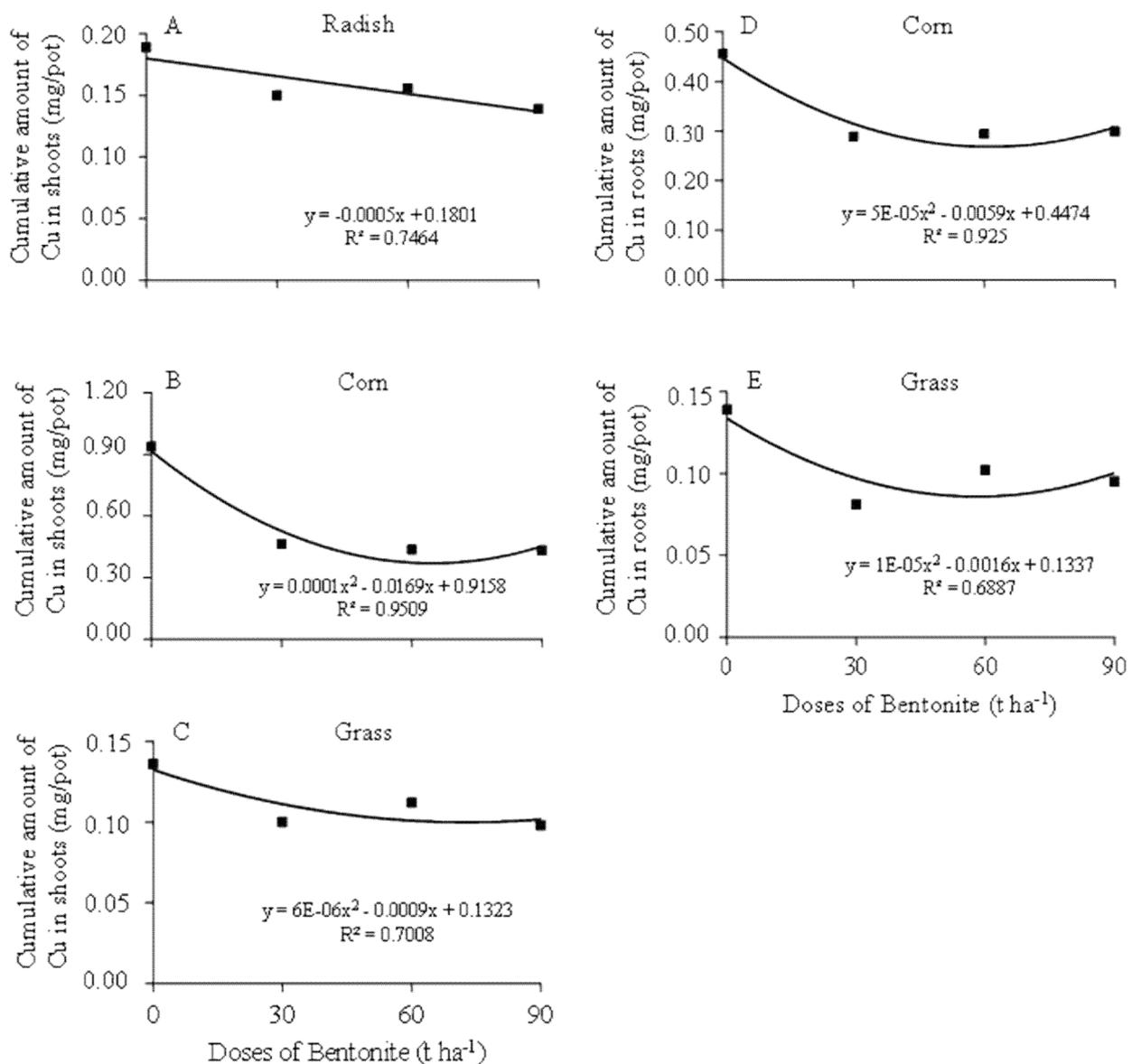


Figure 4. Amount of Cu accumulated in shoots of radishes (A), corn (B) and grasses (C) and the amount Cu accumulated in the root of corn (D) and pasture grass (E), depending on dose increasing bentonite

Table 4. Summary analysis of variance of translocation index (TI) of copper in radish, corn and pasture grass, irrigated with lower quality water with increasing doses of bentonite

Source of variation	DF	Mean squares		
		TI		
		Radish	Corn	Grass
Bentonite	3	47.97ns	179.92ns	12.45ns
Linear	1	138.25ns	487.95 *	0.49ns
Quadratic	1	1.25ns	40.23ns	14.03ns
Error	1	4.39ns	11.59ns	23.73ns
Residue	12	117.14	74.53	15.96
VC (%)		17.65	12.12	7.81
Mean (%)		61.321	71.258	51.159

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

For pasture grass (Figures 3C and 3E), a reduction in the accumulated amount of Cu in both the aerial part and the root was also observed, ranging from 0.132 to 0.10 mg/pot and 0.134 to 0.071 mg/pot, respectively, corresponding a decrease in the order of 47.12 and 25.2% for AAP and AR pasture grass, respectively. The irrigation of crops with lower quality water can accumulate heavy metals in soil and/or increase absorption of these metals by plants causing major health risks to consumers (Khan *et al.*, 2008). In this sense, the incorporation of bentonite clay in soil was beneficial since it reduced the accumulation of copper in plants due to loss of soil copper transfer to plants. This is probably because the adsorption effect of bentonite. In relation to translocation index (TI) of copper in radish, corn and pasture grass these crops were not significantly affected by increasing the doses of bentonite (Table 4). However, regression analysis showed that bentonite applied in soil had a significant effect on the 5% level of probability in Cu TI in corn, following a linear trend (Figure 5) ranging from 78.67% to 63.86%, i.e., a reduction approximately 18.83%. This reduction, depending on the bentonite doses, was beneficial because in spite of this crop have high efficiency to translocate copper to the shoot (Tavares *et al.*, 2013), the presence of this clay reduced the shoot copper concentration where is located the edible part of corn, thus avoiding entering the food chain.

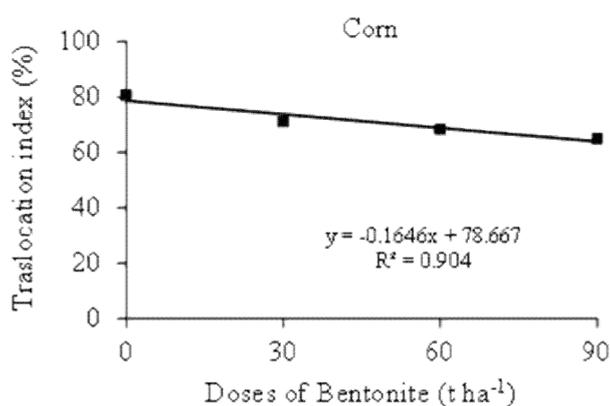


Figure 5. Translocating index of copper in corn as a function of increasing doses of bentonite

Conclusion

For the conditions shown in this research, bentonite incorporation in soil irrigated with poor quality water:

- improved the development of radish and pasture grass crops;

- promoted the retention of copper in the soil, evidenced by the reduction of the concentration and accumulation of this metal in the shoot and roots of radish, corn and pasture grass;
- favored the reduction of copper translocation to the shoot of corn, thereby reducing the concentration of this element in the aerial part.

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