RESEARCH

Heavy metals in cowpea (*Vigna unguiculata* L.) after tannery sludge compost amendment

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Repeated soil amendment with industrial waste can affect the accumulation of chemical elements, mainly heavy metals, in plants. We therefore evaluated the accumulation of Cr, Cd, Ni, and Pb in cowpea (*Vigna unguiculata* [L.] Walp.) shoots and grains in soil amended for three consecutive years with tannery sludge compost (TSC). Tannery sludge compost was applied annually starting in 2009 at 0, 2.5, 5, 10, and 20 t ha^{-1} and cowpea was sown. At 40 and 60 d after cowpea sowing, the accumulation of Cr, Cd, Pb, and Ni was evaluated in the shoots and grains, respectively. The experiment used completely randomized design with four replicates and data were subjected to ANOVA and F-test (5%). Only Cr accumulation was significant ($P < 0.05$) in the cowpea shoots after 3 yr of TSC amendment; accumulation increased as TSC rates were applied. However, there was no significant Cr, Cd, Ni, and Pb accumulation in grains. After 3 yr of consecutive TSC soil amendments, Cr accumulated in the shoots, but it was not translocated to the grains.

Key words: Organic residues, trace elements, *Vigna unguiculata*.

INTRODUCTION

The tannery industry represents an important sector in the Brazilian economy and processes approximately 44 million units of leather per year (Santos et al., 2011). However, this high leather production promotes increased industrial waste, known as tannery sludge (Silva et al., 2010). Tannery sludge has a high content of inorganic elements, such as Cr, Cd, Ni, and Pb, and these elements can lead to environmental pollution and then affect human health (Gupta and Sinha, 2007).

Tannery sludge is mainly disposed of in sanitary sites, which increases soil pollution load with numerous environmental consequences (Chandra et al., 2008; Silva et al., 2010). On the other hand, agricultural use of tannery sludge has been promoted because of its high organic matter and plant nutrient content (Hargreaves et al., 2008; Silva et al., 2010). Some studies have already been conducted using tannery sludge in different crops, such as soybean and maize (Ferreira et al., 2003) and cowpea (Teixeira et al., 2006). These studies showed an increase in plant yield after tannery sludge amendment. However, Cr content increased in the soil and plant.

Nowadays, the composting process has been proposed as a suitable way to treat industrial sludge before applying it to the soil (Araújo and Monteiro, 2006; Araújo et al., 2007; Singh et al., 2010; Santos et al., 2011). This process promotes nutrient stability in sludge (Castaldi et al., 2004) and can decrease sludge toxicity (Araújo and Monteiro, 2005). However, the composting process did not decrease heavy metal content and it is necessary to monitor these elements in the soil and plant system after consecutive applications.

The behavior of heavy metals in the soil and plant system varies and depends on the type of element, in which form it occurs, its concentration in the sludge, the soil properties, and plant species (Singh and Agrawal, 2008; Nogueira et al., 2010). When soil pH is neutral or alkaline, heavy metals are usually inert in the soil under low mobility forms (Hayes and Traina, 1998). This is especially true for Cr with pH values above 5.0 where Cr is in the trivalent form (Cr^{3+}), which is more stable, and has a low solubility and mobility (Alcântara and Camargo, 2001).

Heavy metal accumulation in plants can occur without any toxicity symptoms and decrease crop yield (Rao and Shantaram, 1996). However, successive amendments with composted waste can promote heavy metal concentration in plants and cause negative or positive effects on plant growth (Singh and Agrawal, 2009; 2010a; 2010b; Santos et al., 2011). Heavy metal accumulation in plants varies between different plant parts or among different

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plant species (Singh and Agrawal, 2007; 2009; 2010a; 2010b). In maize, Zn accumulates in shoots, while Cr and Pb can accumulate in grains and shoots, respectively (Pierrisnard, 1996). Cowpea is an important legume crop in the tropical regions and heavy metal accumulation after tannery sludge compost (TSC) amendment is not clear. In addition, there are no studies evaluating TSC on heavy metal accumulation in plants after consecutive amendments.

The suitability of TSC for agricultural use should therefore be evaluated in accordance with heavy metal accumulation in plants after consecutive amendments. The aim of this study was to evaluate the accumulation of Cr, Cd, Ni, and Pb in cowpea (*Vigna unguiculata* [L.] Walp.) after 3 yr of consecutive TSC amendment.

MATERIALS AND METHODS

The experimental site is located at the Long-Term Experimental Field of the Agricultural Science Center, Teresina, Piauí State (05º05' S; 42º48' W, 75 m a.s.l.) The regional climate is dry tropical (Köppen) characterized by two distinct seasons: rainy summer and dry winter with annual average temperatures of 30 °C and rainfall of 1200 mm. The rainy season extends from January to April when 90% of total annual rainfall occurs. Soil pH (1:2.5 soil:water), P, K, and Mg were evaluated according to Tedesco et al. (1995). Soil organic matter (OM) was determined by the method described by Yeomans and Bremner (1998). The Cr, Cd, Ni, and Pb contents were analyzed in accordance with USEPA (1996) after soil digestion with HNO_3, HCl, and H_2O_2. Soil extracts were analyzed for Cr, Cd, Ni, and Pb with atomic absorption spectrophotometry. The physical properties are soil density, -1.81 kg dm^{-3}; particle density, -2.62 kg dm^{-3}; and soil porosity, -0.29 m^3 m^{-3}.

Tannery sludge compost was produced annually with tannery sludge mixed with sugarcane straw and cattle manure (ratio 1:3:1; v:v:v). The composting process was carried out by the aerated-pile method for 85 d. The pile was 2 m long, 1 m wide, and 1.5 m high. The pile was turned twice a week during the first 30 d. Afterwards, it was turned twice a month for an additional 55 d. At the end of the composting process, 20 subsamples were randomly collected from TSC to produce a composite sample. The N, P, and K contents were evaluated by the Kjeldahl, colorimetry, and photometry methods, respectively. The other elements and trace elements (Cr, Cd, Ni, and Pb) were evaluated by spectrophotometry with atomic absorption (USEPA, 1996). The results are shown in Table 1.

The experiments were conducted annually starting in 2009 with five treatments: 0 (TSC not applied), 2.5, 5, 10, and 20 t ha^{-1} TSC (dry basis). The experimental site was arranged in a completely randomized design with four replicates. The plots are marked out (20 m^2 each and 12 m^2 of useful area for soil and plant sampling) with row spacing of 1.0 m.

Table 1. Chemical properties of tannery sludge compost (TSC).

Properties	TSC 2009[(1)]	TSC 2010[(1)]	TSC 2011	Permitted heavy metal limits[a]	Maximum heavy metal limits permitted in sludge-based compost[b]
pH	7.8	7.2	7.5		-
C, g kg^{-1}	187.5	195.3	201.2		-
N, g kg^{-1}	1.28	1.39	1.51		-
P, g kg^{-1}	4.02	3.83	4.91		-
K, g kg^{-1}	3.25	3.51	2.90		-
Ca, g kg^{-1}	95.33	84.28	121.18		-
Mg, g kg^{-1}	6.80	5.71	7.21		-
S, g kg^{-1}	9.39	8.43	10.20		A - B
Cu, mg kg^{-1}	17.80	19.51	16.38	1500	100 - 1000
Zn, mg kg^{-1}	141.67	128.31	127.81	2800	200 - 2000
Ni, mg kg^{-1}	21.92	28.61	23.26	420	20 - 80
Cd, mg kg^{-1}	2.87	3.93	1.93	39	2 - 8
Cr, mg kg^{-1}	2.255	2.581	1.943	1000	120 - 600
Pb, mg kg^{-1}	42.67	38.54	40.31	300	100 - 300

[a]Conama, 2006.
[b]Conama, 2003.

In the third year, TSC was applied 10 d before cowpea (*Vigna unguiculata* [L.] Walp.) sowing. It was spread on the soil surface and incorporated into the 20 cm layer with a harrow. Cowpea is grown at a density of 5 plants m^{-1} (approximately 62 000 plants ha^{-1}). In both years data were collected at 40 d (flowering period) and 60 d (harvest) after plant emergence. The contents of Cr, Cd, Ni, and Pb were evaluated in the shoots in the first sampling (40 d). Shoots were collected in 10 plants from the plots. The contents of Cr, Cd, Ni, and Pb were evaluated in the grains and plant yield was measured in the second sampling (60 d). Plant yield was evaluated by sampling 10 plants from the plots and grains were dried to 13% moisture. To extract heavy metals in plant samples, a 1 g oven-dried sample was digested in 10 mL tri acid mixture (HNO_3:H_2SO_4:$HClO_4$ at 5:1:1) until a transparent color occurred (Allen et al., 1986). In all cases after complete digestion, the solution was filtered and the filtrate was analyzed separately for heavy metals with an atomic absorption spectrophotometer.

In order to understand the soil to plant behavior of heavy metals due to TSC amendment after 3 yr, we calculated both the bioaccumulation and translocations factors.

Heavy metal bioaccumulation factors (BF) were estimated by the following equation:

$$BF = C_2W_2 - C_1W_1/T_2 - T_1$$

where C_1 and C_2 are heavy metal concentration in plants (shoots or grains) and W_1 and W_2 are leaves or grain weight at time T_1 and T_2 (year).

Heavy metal translocation factors (TF) from shoots to grains were estimated by

$$TF\ (\%) = C_{grain}/C_{shoot}$$

where, C_{grain} and C_{shoot} are heavy metal concentrations in grains and shoots, respectively.

Data were subjected to ANOVA and F-test (5%). When F was significant, data were adjusted to regression analyses as functions of TSC amendment rates (SAS Institute, 1996).

RESULTS AND DISCUSSION

Tannery sludge compost had lower Cu, Pb, and Zn concentrations than the limits set by Conama (2003) for type A compost (Table 1). The Ni and Cd concentrations were slightly higher than the maximum limits for type A compost (20 and 200 μg g^{-1} soil, respectively). However, Cr concentration was 3 to 4 times higher than the maximum limits for type B compost (600 μg g^{-1} soil) (Conama, 2003) and from 1.9 to 2.2 higher than the upper limits for Cr set by Conama (2006) (Table 1).

Data in Table 2 show that Cd, Ni, and Pb concentrations after 3 yr of TSC amendment were lower than the upper limits for soils (European Union, 1986). The European Union (1986) does not have an upper limit for Cr. The lower upper limit concentration allowed in agricultural soils treated with sewage sludge is 250 mg kg^{-1} recommended by EPA (Harrison et al., 1999). The Cr concentration is also lower than the upper limits for agricultural soils amended with sewage sludge (Table 2).

Tannery sludge compost amendment promoted a quadratic response of Cr concentration in cowpea shoots (Figure 1a) where the values of shoot Cr concentration ranged from 0.11 to 8.78 mg kg^{-1} for 0 and 10 t ha^{-1} TSC, respectively. For grains, Cr concentration did not vary between amended and unamended soils and mean value of Cr in grains was 0.44 mg kg^{-1}.

Increased Cr concentrations in shoots are related to the high Cr concentration in TSC (Table 1). Aquino Neto and Camargo (2000) also observed an increase in Cr concentration in lettuce shoots after applying tannery sludge as a direct response to the increase in tannery sludge rates. Thus, for some crops, Cr is translocated from the roots to the top parts of the plant and is influenced by plant species, although Cr is usually retained in the plant root system (Dudka et al., 1991; Piotrowska et al., 1991). For example, Castilhos et al. (2001a; 2001b) observed that the increase in Cr concentration in the soybean plant root promoted increased Cr in the shoots.

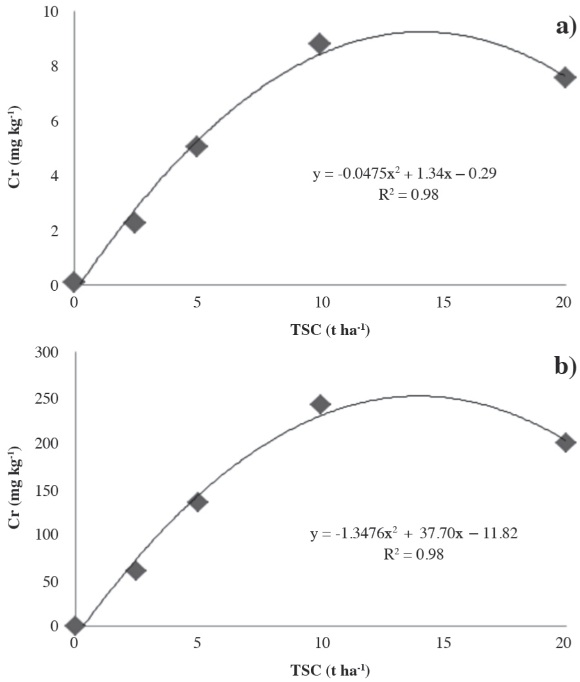

Figure 1. Cr concentration (a) and accumulation factor (b) in cowpea plant shoots grown in tannery sludge compost (TSC) in both unamended and amended soil after 3 years.

There is no information for cowpea about Cr uptake after tannery sludge amendment, although some studies found a decrease in plant dry weight as a direct response to tannery sludge with high Cr content (Teixeira et al., 2006; Santos et al., 2011). However, Cr concentrations in cowpea shoots remained within the normal range with levels ranging from 0.03 to 14 mg kg^{-1} (Kabata-Pendias and Pendias, 2001).

Concentrations of Cd, Ni, and Pb in cowpea shoots and grains were similar when grown in soil amended with TSC as compared with those grown in unamended soils. In these cases, the mean values of Cd, Ni, and Pb in shoots were 0.65, 1.82, and 3.59 mg kg^{-1}, respectively. For grains, mean values of Cd, Ni, and Pb were 0.08, 0.52, and 0.96 mg kg^{-1}, respectively. These values are within the normal range for plant tissue proposed by Adriano (1986) for Ni and Kabata-Pendias and Pendias (2001) for Cd and Pb. The results of Cd, Ni, and Pb accumulation

Table 2. Soil chemical properties after 3 years of tannery sludge compost (TSC) amendment.

	Properties										
TSC	EC	pH	OM	P	K	Ca	Mg	Cr	Cd	Ni	Pb
t ha^{-1}	mS m^{-1}	H_2O	g kg^{-1}	mg dm^{-3}		cmol$_c$ dm^{-3}		mg kg^{-1}			
	2011										
0.0	0.28b	6.6b	17.88c	2.97	46.8	1.19	0.36	6.7d	0.06a	0.66a	1.94a
2.5	0.40b	6.6b	18.12c	3.64	35.2	1.36	0.46	11.7c	0.05a	0.66a	1.81a
5.0	0.61b	7.1a	21.4b	4.17	31.3	1.45	0.32	16.7b	0.07a	0.73a	1.94a
10.0	1.17a	7.1a	22.34b	4.10	31.3	1.50	0.23	20.6b	0.06a	0.65a	1.91a
20.0	1.32a	7.5a	28.18a	3.04	19.5	1.56	0.17	35.7a	0.06a	0.62a	2.15a
Upper limits[a]								154	4	74	41

EC: electrical conductivity; OM: organic matter.
[a]Conama (2006)

in the shoots are a consequence of low Cd, Ni, and Pb content found in TSC (Table 1) and therefore in the soil (Table 2). These elements do not usually accumulate in untreated tannery sludge; therefore, these metals are not found in high quantities in TSC. Furthermore, previous studies with untreated tannery sludge did not find Ni, Cd, and Pb accumulation in maize (Souza et al., 2005) and soybean (Ferreira et al., 2003).

Heavy metal analyses in cowpea shoots after 3 yr of TSC amendment showed increases in Cr accumulation (Table 3). The accumulation of Cr was approximately 100% as compared with the first year of TSC amendment. It can be suggested that Cr is strongly accumulated in plant shoots as a linear and significant response to TSC amendment and is associated with the increasing Cr content in the soil after TSC amendment (Table 2). Our findings agree with Gonçalves et al. (2013), who observed an increase in Cr content in cowpea shoots after applying TSC as a direct response to increase TSC rates.

On the other hand, Cr, Cd, Ni, and Pb did not significantly accumulate in cowpea grains after 3 yr of TSC amendment (Table 4). These data are confirmed by the translocation factors which show values of less than 1 (Table 5). The translocation factor is the plant's ability to translocate heavy metals from the roots to the shoots or from the shoots to the grains (Singh and Agrawal, 2007); when the values are greater than 1, this suggests that heavy metals were transported from the shoots to the grains or from the roots to the shoots, whereas values less than 1 indicate heavy metal accumulation in the shoots or roots (Singh and Agrawal, 2007). Our results therefore confirm that Cr, Cd, Ni, and Pb were not translocated for cowpea grains. This suggests that the plants can significantly accumulate Cr, Ni, Cd, and Pb, but these heavy metals cannot be translocated to the grains (Mortvedt, 2001).

Table 3. Cr, Cd, Ni, and Pb (mg kg^{-1}) in cowpea shoots in the first year and after 3 years of tannery sludge compost (TSC) amendment.

	TSC		
TSC (Mg ha^{-1})	First year	After 3 years	Difference %
		Cr	
2.5	1.12	2.26	+ 101.7
5.0	2.40	5.05	+ 110.4
10.0	3.20	8.78	+ 174.3
20.0	3.83	7.55	+ 97.1
		Cd	
2.5	0.53	0.64	+ 20.7
5.0	0.53	0.65	+ 22.6
10.0	0.54	0.69	+ 27.7
20.0	0.51	0.63	+ 23.5
		Ni	
2.5	1.83	1.66	- 9.2
5.0	1.96	1.82	- 7.1
10.0	2.06	1.88	- 8.7
20.0	2.14	1.92	- 10.2
		Pb	
2.5	3.36	3.49	+ 3.8
5.0	3.51	3.67	+ 4.5
10.0	3.55	3.67	+ 3.4
20.0	3.43	3.55	+ 3.5

Table 4. Cr, Cd, Ni, and Pb (mg kg^{-1}) in cowpea grains in the first and after 3 years of tannery sludge compost (TSC) amendment.

	TSC		
TSC (Mg ha^{-1})	First year	After 3 years	Difference %
		Cr	
2.5	0.41	0.42	+ 2.4
5	0.43	0.45	+ 4.6
10	0.46	0.48	+ 4.3
20	0.46	0.42	- 8.6
		Cd	
2.5	0.08	0.07	- 12.5
5	0.08	0.07	- 12.5
10	0.09	0.08	- 11.1
20	0.10	0.11	- 10.0
		Ni	
2.5	0.43	0.47	+ 9.3
5	0.58	0.58	+ 0.1
10	0.57	0.58	+ 1.7
20	0.47	0.53	+ 12.7
		Pb	
2.5	0.67	0.87	+ 29.8
5	0.71	1.01	+ 42.2
10	0.80	1.06	+ 32.5
20	0.84	1.06	+ 26.1

Table 5. Translocation factor of heavy metals from cowpea shoots to grains at different tannery sludge compost (TSC) rates after 3 years of amendment.

TSC rates	Cr	Cd	Ni	Pb
2.5	0.09	0.11	0.28	0.25
5.0	0.16	0.11	0.32	0.28
10.0	0.19	0.12	0.31	0.29
20.0	0.14	0.16	0.28	0.30

According to Oliveira et al. (2005), shoots usually exhibit higher heavy metal accumulation than seeds and fruits. In an experiment with maize after 5 yr of sewage sludge amendment, Oliveira et al. (2005) also found increases in Cd, Zn, Cu, and Ni in shoots, while grains did not accumulate these elements. Our findings suggest that plants can exhibit biological protection to heavy metal accumulation in the reproductive organs. This is important for cowpea production with TSC amendment when these grains are used as food.

Cowpea yield after TSC amendment was similar to yields obtained with chemical fertilization (Gonçalves et al., 2013). In the grains, mean values of Cr, Cd, Ni, and Pb were 0.44, 0.08, 0.54, and 1.0 mg kg^{-1}, respectively, and these values are below the limits for grain proposed by Kabata-Pendias and Pendias (2001). Values of Cr and Pb are within the limits found by Anjos and Mattiazzo (2000) and Rangel et al. (2006) for Cr and Pb in maize grains sown in tropical soils amended with sewage sludge for 3 yr.

There was a quadratic response for the factor of Cr accumulation in the shoots where values ranged from 60.3 to 242.1 (Figure1b), while Cr was not accumulated in the grains and showed a mean value of 178 units. There was no significant accumulation of Cd, Ni, and Pb in both shoots and grains. These results confirm that Cr was translocated from the soil to the plant and increased Cr accumulation in the shoots. Similar behavior was found by Castilhos et al. (2001a; 2001b), who observed increases in soybean shoot Cr concentration after applying tannery sludge to the soil.

CONCLUSIONS

The present study indicates that consecutive tannery sludge compost (TSC) amendments in the soil increase Cr concentrations in cowpea shoots, while Cd, Ni, and Pb do not accumulate in the plants. In addition, Cr accumulation in the shoots increased as a direct consequence of high Cr uptake from the soil. However, Cr, Cd, Ni, and Pb were not translocated from the shoots to the grains.

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