GROWTH, NODULATION AND NITROGEN FIXATION OF COWPEA IN SOILS AMENDED WITH COMPOSTED TANNERY SLUDGE[1]

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SUMMARY

Tannery wastes generation is increasing every year and a suitable method for tannery sludge management is necessary in order to decrease this environmental problem. The composting is recognized as a suitable method for sludge recycling.. The effect of tannery sludge compost (TSC) rates on growth, nodulation and N fixation of cowpea was investigated. Sandy and clayey soils were amended with TSC at rates of 0, 7.5, 15, 30, and 60 t ha^{-1}. The shoot dry weight of cowpea plants 45 days after emergence (DAE) was greater in the TSC-amended than in the unamended soil. In the sandy soil, nodule dry weight increased with TSC application 45 DAE. In the clayey soil, 45 DAE, nodule dry weight decreased with TSC amendment levels greater than 7.5 t ha^{-1} compared to the unamended control. The application of TSC increased N accumulation in the cowpea plants. The results suggest that cowpea responds differently to TSC depending on the amendment rate and initial soil type.

Index terms: waste, soil texture, composting, heavy metal.

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RESUMO: *CRESCIMENTO, NODULAÇÃO E FIXAÇÃO DE NITROGÊNIO PELO FEIJÃO-CAUPI EM SOLO COM COMPOSTO DE LODO DE CURTUME*

A geração de resíduos de curtume está aumentando a cada ano e um método adequado para o manejo do resíduo de curtume é necessário para diminuir o problema ambiental. A compostagem é reconhecido como um método adequado para a reciclagem do lodo. O objetivo do trabalho foi investigar o efeito da aplicação de composto de lodo de curtume (CLC) sobre o crescimento, nodulação e acumulação de nitrogênio do feijão-caupi. Solos arenoso e argiloso foram adubados com CLC em doses de 0, 7,5, 15, 30 e 60 t ha^{-1}. O comprimento da parte aérea do feijão-caupi foi maior, aos 45 dias após a emergência das plantas (DAE), no solo com aplicação de CLC do que no solo sem aplicação. No solo arenoso, a massa dos nódulos, aos 45 DAE, aumentou com a aplicação do CLC. No solo argiloso, aos 45 DAE, em doses superiores a 7,5 t ha^{-1}, houve um decréscimo na massa nodular quando comparado ao solo sem aplicação. A aplicação do CLC aumentou a acumulação de N nas plantas de feijão-caupi. Os resultados sugerem que o feijão-caupi responde diferentemente ao CLC sendo dependente da dose de aplicação e do tipo de solo.

Palavras-chave: resíduo; textura do solo, compostagem, metal pesado.

INTRODUCTION

The leather tanning industry plays an important role in the Brazilian economy, with assets of about 21 billion dollars. However, this industry is also responsible for the release of more than 1,000,000 tons of tannery sludge each year (Pacheco, 2009), of which 3 % are solid residues (Silveira et al., 2002). The high annual production of tannery sludge has created a series of economic, social and environmental problems. In Brazil, there is currently no appropriate disposal method for tannery sludge, so most often the residue is simply sent to a landfill. Tannery sludge usually contains high levels of organic matter, chemical nutrients and heavy metals, mainly Cr^{3+} (Castilhos et al., 2002). The occurrence of metals in complex form, especially Cr, Fe, Mn, Zn, Cu, Pb, Ni, and Cd in tannery sludge is a cause of serious concern because of the risks they pose to human health, particularly the danger of food chain contamination (Gupta & Sinha, 2007).

Landfilling and land application of the sludge are commonly the most recommended disposal techniques (Singh & Agrawal, 2008, 2009). However, landfills are often not a suitable disposal option in view of the large volume of soil required to cover the waste to prevent leaching of potentially toxic compounds. In addition, landfills may be economically unfeasible because of the large land area required (Chandra et al., 2008; Singh & Agrawal, 2008). Thus, new methods for recycling and recovery of this organic waste are required (Ahlberg et al., 2006). Composting has long been recognized as one of the most cost-effective and environmentally sound alternatives for organic waste recycling (Araújo & Monteiro, 2006; Araújo et al., 2007; Singh et al., 2010). During composting, organic matter decomposes into carbon dioxide, water vapor, inorganic nutrients and stable organic material, containing humic substances (Senesi, 1989; Singh et al., 2010). Additionally, composting can reduce the concentration of pathogens and toxic organic compounds (Araújo & Monteiro, 2005). This method has also been used to process sludge of different origins, including sewage and textile sludge (Bernal et al., 1998; Araújo & Monteiro, 2006; Araújo et al., 2007; Singh et al., 2010).

The application of composted waste to agricultural soils requires caution due to the possibility of food chain contamination and negative effects on soil microorganisms, particularly rhizobia (Singh & Agrawal, 2009, 2010). Little information is available about the toxic effects of composted industrial waste on soil microbiology and biochemical processes (Araújo & Monteiro, 2007). Soil bacteria are important because of their role in biological N_2 fixation, a process that has been identified as an important indicator of soil disturbances (Viser & Parkinson, 1992). Brookes (1995) recommended the measurement of biological N_2 fixation as an indicator of soil stress resulting from pollutants. Wetzel & Werner (1995) reported that nodulation is an important property when examining the toxic effects of pollutants from compost application. There is currently a small number of studies on the effects of tannery sludge on soil microbial biomass and activity (Andre & Mattiazzo, 1997; Konrad & Castilhos, 2001); however, none of these focused specifically nodulation and biological N_2 fixation in tropical legumes. Therefore, the purpose of this study was to evaluate the effect of tannery sludge compost (TSC) on growth, nodulation and N_2 fixation of the cowpea plant. This study was an attempt to understand the response of the tested leguminous plant (cowpea) to the conflicting stimulation at on the one hand the beneficial effects of increased nutrient availability, on the other the harmful effects of heavy metal accumulation.

MATERIAL AND METHODS

Tannery sludge was collected from the wastewater treatment plant of the leather company "Curtume

Europa" in Teresina, PI, Brazil. The sludge was composted for 85 days using the Beltsville aerated-pile method (USDA, 1980), stacked in two piles (sides 2 x 1 m, height 1.5 m). The compost piles were turned over twice a week during the first and second week and once a week during the remaining bio-oxidative phase. The bio-oxidative phase of composting was considered finished when the temperature in the piles stabilized at around 30 °C, the temperature of the surrounding environment. This stage was reached after 55 days of composting. At this point, the compost was allowed to mature, without turning, for an additional 30 days. On the 85th day, 20 subsamples were collected from several locations in each pile, to produce two composite samples. The chemical properties of both composite samples were determined by the EPA 3051 method (USEPA, 1986) (Table 1).

Two different soil types were collected with low and high-clay soils (sandy soil and clayey soil, respectively). Both soil types were sampled from the surface layer of the soil down to a depth of 10 cm. The soil samples were air-dried, crushed and sieved (2 mm) to remove large fragments and the main physico and chemical properties of the soils assessed (Table 2). The soils were then amended with tannery-sludge compost at rates of 0 (control), 7.5, 15, 30 and 60 t ha^{-1}.

The compost was also air-dried and ground before mixing with the soils. Before filling the pots with the respective soil treatments, the separate heaps were mixed again to ensure that the compost was evenly distributed through the soil. The water-holding capacity of the final mixture was 40 %, using distilled water. A randomized complete block design with five treatments (tannery sludge rates of 0, 7.5, 15, 30, and 60 t ha^{-1}) and four replicates per treatment was used for each soil type. For each treatment, soil types and replicates, 5 kg pots were filled to the respective level and left to stand for 10 days.

The cowpea seeds were inoculated with the commercial strain BR3262 of *Bradyrhizobium elkanii* (1 kg per 50 kg of seeds). Three seeds were sown per pot, at a depth of 0.5 cm. After emergence, the seedlings were thinned to one plant per pot. The plants were harvested 30 and 45 days after emergence (DAE). The growth, nodule number and shoot dry weight and N accumulation (Keeney & Nelson, 1982) were then measured. Shoots and roots were dried at 60 °C for 72 h before dry weight determination.

Table 2. Soil pH, soil organic matter, total P, total N and exchangeable K contents in the sandy and clayey soils after amendment with tannery sludge compost

Rate	pH	SOM	Total P	K	Total N
t ha^{-1}		g kg^{-1}	mg kg^{-1}		
		Sandy soil			
0	6.4 b	4.8 c	1.2 b	7.1 c	0.11 b
7.5	7.6 a	5.7 b	1.5 b	7.9 c	0.34 b
15	7.7 a	5.8 b	1.8 b	7.7 c	0.41 b
30	7.7 a	6.1 a	2.3 a	10.8 b	0.80 a
60	7.8 a	6.3 a	2.5 a	14.1 a	0.95 a
		Clayey soil			
0	6.5 c	4.6 c	1.1 b	7.5 b	0.09 c
7.5	7.0 b	5.5 b	1.0 b	7.6 b	0.26 b
15	7.6 a	5.8 b	1.3 a	7.6 b	0.37 b
30	7.7 a	5.7 b	1.4 a	13.2 a	0.75 a
60	7.7 a	6.2 a	1.4 a	13.9 a	0.90 a

In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$ according to SNK's multiple range test.

Table 1. Chemical properties of composted dry tannery sludge (TSC)

Property	TSC	Heavy metal limit
pH	7.8	-
Moisture content a 65 °C (%)	42.7	-
C_{org} (g kg^{-1})	187.58	-
Total N (g kg^{-1})	1.28	-
Total P (g kg^{-1})	4.02	-
Exchangeable K (g kg^{-1})	3.25	-
Ex. Ca (g kg^{-1})	95.33	-
Ex. Mg (g kg^{-1})	6.80	-
Total S (g kg^{-1})	9.39	-
Cu (mg kg^{-1})	17.83	4,300
Fe (mg kg^{-1})	5,171.67	-
Mn (mg kg^{-1})	1,848.73	-
Zn (mg kg^{-1})	141.67	7,500
Mo (mg kg^{-1})	9.28	-
Ni (mg kg^{-1})	21.92	420
Cd (mg kg^{-1})	2.87	85
Cr (mg kg^{-1})	2,255.0	3,000
Pb (mg kg^{-1})	42.67	75

(1) CETESB (1999).

Three soil samples per treatment were collected, air-dried, crushed, sieved (2 mm) and then stored separately for further chemical analyses. The pH of the soil samples and composted tannery sludge was measured in a 1:5 (w/v) suspension of soil to water. The organic C content of the soil was measured using the loss-on-ignition method at 550 °C. Total N was measured by the Kjeldahl and total P by the Olsen method. Exchangeable K, Ca and Mg were measured by extracting the soil sample with 1 mol L^{-1} CH_3COONH_4. Extractable Zn, Cu, Fe, Mo, Mn, Ni, Cd, Cr, and Pb were measured by extraction using DTPA, with quantitative determination by atomic absorption spectrometry (USEPA, 1986). Data were analyzed with analysis of variance (ANOVA) at 5 % probability, using the Student-Newman-Keuls (SNK).

RESULTS

The tannery sludge compost (TSC) had a high pH (7.8) and high levels of organic C (187.58 g kg^{-1}), total N (1.28 g kg^{-1}) and total P (4.02 g kg^{-1}), compared to both unamended soils (Tables 1 and 2). Additionally, high levels of all heavy metals studied were detected in the TSC, including Ni, Cd, Cu, Cr, and Pb. The soil properties of the sandy and clayey soils were altered by TSC application (Tables 2 and 3).

The TSC also raised the heavy metal contents compared to unamended soil (Table 3). The Cr, Cu, Cd, Ni and Pb content of the soils increased due to TSC application and increased with increasing amendment rates.

The shoot and root dry weight of cowpea plants in both soils was determined 30 and 45 DAE (Table 4). The shoot dry weight of cowpea did not vary significantly between soil types 30 DAE. However, 45 DAE in clayey soil, dry weight was higher than in unamended soil. In sandy soil, 45 DAE, cowpea shoot dry weight increased at TSC rates of 30 t ha^{-1}, but decreased at 60 t ha^{-1}. The cowpea root dry weight did not vary significantly between soil types 30 or 45 DAE (Table 4).

The number of cowpea root nodules did not vary significantly in either evaluation (Table 5). However, the nodule dry weight in the sandy soil did respond to TSC. Nodule dry weight was greater than in unamended soil 45 DAE, but did not vary significantly between treatment levels. In the clayey soil, 30 DAE, nodule dry weight did not vary significantly with increasing TSC amendment rate. However, nodule dry weight decreased 45 DAE for TSC amendment rates greater than 7.5 t ha^{-1} compared to the unamended soil (Table 5).

Nitrogen accumulation increased for the cowpea in both soil types with increasing TSC amendment rates in both evaluations, compared with unamended soil (Table 6). However, the variation between the treatments was insignificant, with the exception of the 60 t ha^{-1} treatment 45 DAE in/ clayey soil.

DISCUSSION

The application of TSC affected the soil physical and chemical properties in this experiment. Because of the high pH of the compost, its addition to the soil raised the soil pH according to the different TSC amendment rates. The high levels of organic matter,

Table 3. Heavy metals contents in the sandy and clayey soils after amendment with tannery sludge compost

Rate	Cr	Cd	Cu	Ni	Pb
t ha^{-1}	mg kg^{-1}				
			Sandy soil		
0	2.1 e	0.052 c	1.46 b	0.782 c	17.5 b
7.5	32.4 d	0.068 c	1.62 b	0.912 b	15.7 b
15	89.8 c	0.130 b	1.53 b	1.028 b	20.4 a
30	117.8 b	0.138 b	1.68 b	1.150 a	18.3 a
60	162.0 a	0.162 a	2.31 a	1.188 a	18.8 a
			Clayey soil		
0	2.3 e	0.062 d	1.44 b	0.612 a	16.5 a
7.5	24.1 d	0.074 c	1.43 b	0.754 a	15.2 a
15	54.2 c	0.094 b	1.22 b	0.586 a	13.6 a
30	85.4 b	0.096 b	1.20 b	0.368 a	11.7 a
60	148.4 a	0.102 a	1.79 a	0.476 a	15.6 a

In the columns, means followed by the same letter do not differ statistically from each other at $p < 0.05$, according to the SNK test.

Table 4. Changes in shoot and root dry weight of cowpea grown in soils amended with composted tannery sludge

Rate	Time (Day after emergence)			
	30	45	30	45
	Sandy soil		Clayey soil	
t ha^{-1}	g/pot			
	Shoot			
0	1.14 a	1.70 b	1.40 a	1.53 b
7.5	1.30 a	4.71 a	1.23 a	1.92 b
15	1.42 a	3.93 a	1.18 a	2.08 b
30	1.06 a	3.60 a	1.31 a	2.28 ab
60	1.28 a	2.46 b	1.64 a	2.78 a
	Root			
0	0.30 a	0.47 a	0.22 a	0.37 a
7.5	0.29 a	0.55 a	0.25 a	0.34 a
15	0.33 a	0.41 a	0.31 a	0.33 a
30	0.24 a	0.65 a	0.30 a	0.24 a
60	0.32 a	0.68 a	0.27 a	0.31 a

In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$, according to the SNK test.

Table 5. Changes in nodule number (nodules per pot) and nodule dry weight (mg per pot) of cowpea growing in soils amended with composted tannery sludge

Rate	Time (Day after emergence)			
	30	45	30	45
	Sandy soil		Clayey soil	
$t\ ha^{-1}$	Nodule number (nodules per pot)			
0	14 a	53 a	15 a	68 a
7.5	24 a	25 a	25 a	62 a
15	17 a	33 a	10 a	37 a
30	28 a	40 a	18 a	54 a
60	14 a	40 a	12 a	52 a
	Nodule dry weight (mg per pot)			
0	26.9 a	42.1 a	30.3 b	65.2 a
7.5	30.4 a	137.2 a	39.1 a	69.5 a
15	30.1 a	119.4 a	5.1 c	12.9 b
30	27.3 a	139.6 a	3.4 c	3.4 c
60	19.9 a	141.5 a	6.0 c	11.3 b

In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$ according to the SNK test.

Table 6. Changes in N accumulation (mg/pot N) of cowpea growing in soils amended with composted tannery sludge

Rate	Time (Day after emergence)			
	30	45	30	45
	Sandy soil		Clayey soil	
$t\ ha^{-1}$	N content (mg per pot)			
0	26.5 b	32.0 a	73.0 b	66.0 c
7.5	31.5 a	28.2 b	84.0 a	72.8 b
15	31.6 a	28.3 b	92.6 a	76.0 b
30	31.9 a	29.2 b	94.5 a	76.8 b
60	31.2 a	29.7 b	95.8 a	83.9 a

In each column, means followed by the same letter do not differ statistically from each other at $p < 0.05$ according to the SNK test.

nutrients and heavy metals in TCS increased the concentrations of these components at the different compost rates.

One of the most important benefits of sludge application to agricultural soil is the increased availability of beneficial nutrients (Singh & Agrawal, 2007, 2009, 2010). Gupta & Sinha (2007) reported an increase in different chemical properties, such as organic matter and available nutrient and heavy metal contents, at different tannery sludge soil amendment rates. Other authors reported an increase in soil chemical properties due to sewage sludge amendment (Logan & Harrison, 1995; Moreno et al., 1997; Singh & Agrawal, 2007, 2009, 2010). The exchangeable concentration of phytoavailable heavy metals, such as DTPA, increased significantly with each TSC amendment rate. Phytoavailability of heavy metals is determined by several factors, such as the nature of the metal species, its interaction with soil colloids, soil properties and the duration of contact with the surface of the metals (Naidu et al., 2003). Concentrations of phytoavailable heavy metals in both soils increased along with higher amendment rates. Ailincai et al. (2007) found a similar trend for Cu, Ni and Cr at sewage sludge amendment rates of 40 and 60 $t\ ha^{-1}$. The heavy metal concentration of the tannery sludge used in this study was found to be lower than in sludge produced by a tannery studied by Gupta & Sinha (2007) in India. In addition, after amendment the heavy metal concentrations were below the thresholds established by the Brazilian legislation (CETESB, 1999). The Cr content of the sludge did however not meet the more restrictive standards set by Brazilian law for use in agricultural soil. The values of Cr, Cd, Ni and Pb were found to be far below the range proposed by Lake (1987) for agricultural soils. Especially for Cr, the content observed after the TSC application of 7.5 $t\ ha^{-1}$ was below the limit of 50 $mg\ kg^{-1}$ proposed by Barceló & Poschenrieder (1992). On the other hand, the pH values of the soils in this study ranged from 6.4 to 7.8 (Table 2), indicating that almost all Cr was insoluble (Bartlett & James, 1988).

The plant response to environmental stress is determined by the response of its individual cells. Cell integrity as well as structure are affected (Ciamporova & Mistrik, 1993; Ouzounidou et al., 1995). The plant response to heavy metal stress may also depend on how the metal ions are distributed between the plant tissues and within the cells that constitute these tissues (Ouzounidou et al., 1995). The decrease in shoot dry weight 45 DAE in the sandy soil for the samples with highest TSC rate indicated a toxic effect of heavy metals on cowpea in sandy soil. These results are in agreement with the findings of Eivazi (1990) and of Araújo & Monteiro (2006), who observed a decrease in dry weight after applying high rates of sewage sludge or textile sludge compost. Singh & Agrawal (2007) also reported significant reductions in the root and shoot dry weight of palak plants growing at 20 and 40 % sewage sludge amendment rates, compared to the unamended control. Alvarez et al. (1995) reported that plant growth significantly increased compared to control plants when manure compost was added to soil. Wong et al. (1999) reported that the addition of manure compost increased total organic matter, macronutrients and micronutrients according to the compost application rate. Increased plant growth has been attributed to increased levels of organic matter and nutrients in amended soils.

There have been conflicting reports on the effects of sludge on nodulation and N fixation of legumes (Angle et al., 1992). Some papers reported that heavy metals inhibited nodulation significantly (Abd-Alla et al., 1999). Singh & Agrawal (2010) reported that the number of nodules was significantly higher in *Vigna radiata* at sewage sludge rates of 6 and 9 kg m^{-2} than in unamended control plants. At higher levels however, nodulation decreased. In this study, it was shown that nodule numbers were not negatively affected by the heavy metals present in TSC. Nodule mass, however, was negatively affected by the application of TSC at high rates in the clayey soil. In the clayey soil, heavy metals are bounded to clay particles which decreased the availability of some important metals that promote increase in nodule mass. The higher accumulation of N in plant shoots grown in amended soil, compared to the control, is most likely a result of the high N levels of the composted tannery sludge.

In the light of this study appear several opportunities for further study. It is important to verify whether the inoculation of cowpea with strain 3262 resulted in increases plant growth. It is fundamental to further study the effects of tannery compost on yields. It is therefore essential to evaluate the heavy metal contents transferred to the plants, to avoid any negative impact (Hue, 1995). Additionally, applications at lower rates would be important for both economic and environmental reasons.

In legume production, some environmental and economic advantages are associated with N_2-fixing microorganisms. Due to these microorganisms, inoculated legumes have an increased capacity to survive, compared to non-inoculated plants, because they have an additional nutritional alternative. Sewage sludge applied to an inoculated legume crop increase the availability of plant nutrients; however, the toxic heavy metals present in the sludge can inhibit biological N fixation (Broos et al., 2005).

CONCLUSIONS

1. Application of tannery sludge compost increased shoot N accumulation and organic C, N, P and heavy metal contents.

2. The shoot biomass was highest at TCS rates of 30 and 60 t ha^{-1} in sandy and clayey soil, respectively.

3. Nodule weight was affected positively by TSC amendment of the sandy soil, but was negatively affected in the clayey soil at TSC rates higher than 7.5 t ha^{-1}.

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