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SHORT COMMUNICATION

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Growth and nodulation of cowpea after 5 years of consecutive composted tannery sludge amendment

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Abstract

Tannery industry releases high amounts of tannery sludge which are currently composted and used in agricultural soils. The consecutive amendment of such composted tannery sludge (CTS) may affect soil microrganisms, such as rhizobia. In this study, we evaluated the effects of 5-year repeated CTS amendment on growth, nodulation, and yield of cowpea (*Vigna unguiculata* L.) CTS was applied in different amounts (0, 2.5, 5, 10 and 20 Mg ha^{-1}) to a sandy soil. Amendment of CTS increased soil pH, electrical conductivity (EC), sodium and chromium content. Plant growth, nodulation, N accumulation, and cowpea yield increased up to 10 Mg ha^{-1}; however, above this rate, these variables decreased. After 5 years of CTS amendment, the increase in soil chemical properties, particularly EC and Na content, exerted negative effects on the growth, nodulation, and yield of cowpea.

Additional key words: wastes; sandy soil; rhizobia; composting; *Vigna unguiculata* L.

The tannery industry is important to the Brazilian economy, with annual assets of 21 billion dollars (Silva *et al.,* 2013). However, it releases 1 million tons of tannery sludge, 3% of which is solid waste (Santos *et al.,* 2011). Landfilling is the main established method for tannery sludge disposal in Brazil, although application to soil has been suggested as an alternative method (Singh & Agrawal, 2008). However, tannery sludge contains salts and trace elements such as chromium, which can affect soil biochemical processes. Recently, composting has been used for tannery sludge recycling (Santos *et al.,* 2011; Silva *et al.*, 2013; Gonçalves *et al.,* 2014). Composting is an alternative method to recycle organic wastes and has been used to process industrial sludge such as tannery and textile sludge (Santos *et al.,* 2011; Araujo *et al.,* 2007).

The application of composted waste to agricultural soils should be carefully performed to minimize negative effects on soil microorganisms, particularly rhizobia (Singh & Agrawal, 2007). Rhizobia play an important role in biological nitrogen fixation (BNF), a process by which atmospheric nitrogen (N_2) is converted to ammonia (NH_3) and subsequently becomes available to plants. This process occurs in nodules where the bacteria fix atmospheric nitrogen that is supplied to the plant. Nodulation is thus an indicator of BNF and may simultaneously be used as an important parameter to evaluate the toxic effects of pollutants from compost application (Wetzel & Werner, 1995). Till date, a small number of studies have been conducted on the effects of composted industrial sludge on nodulation of legumes (Araujo *et al.,* 2007; Santos *et al.,* 2011). However, studies employing repeated application of composted wastes such as composted tannery sludge (CTS) under field conditions are scarce, and the limited information from earlier studies does not provide a clear picture.

We accordingly began a long-term study of CTS application, aiming to evaluate its effects on soil micro-

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Abbreviations used: BNF (biological nitrogen fixation); CTS (composted tannery sludge); EC (electrical conductivity); SOM (soil organic matter).

bial properties after consecutive CTS amendment. In particular, we hypothesized that nodulation of native rhizobia is affected by consecutive and cumulative addition of CTS as a consequence of increased the soil salinity and Cr content. In this study, we evaluated the effects of 5-year repeated CTS amendment on growth, nodulation from native rhizobia and yield of cowpea (*Vigna unguiculata* L.) in a tropical soil.

The experimental site is located at the Long-Term Experimental Field of Agricultural Science Center, Teresina, Piauí state (05°05 S; 42°48' W, 75 m). The regional climate is dry tropical (Köppen), and it is 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 the total annual rainfall occurs. The soil is classified as Fluvisol with the following composition at 0-20 cm depth: 10% clay, 28% silt, and 62% sand.

Composted tannery sludge was annually produced with tannery sludge mixed with sugarcane (*Saccharum officinarum* L.) straw and cattle manure (ratio 1:3:1; v:v:v). The composting process was performed using the aerated-pile method for 85 days. 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 days and twice a month during the remaining 55 days. At the end of the composting process, 20 subsamples were randomly collected from CTS to produce a composite sample. The water content was determined after oven drying the samples at 105°C for 24 h, the pH and electrical conductivity (EC) were directly read, and total solids were measured by drying the samples at 65°C (APHA, 2005). The total organic C content was evaluated by dichromate oxidation of the samples under external heating (Nelson & Sommers, 1996). Nitrogen, P, and K were evaluated in this sample using Kjeldahl, colorimetry, and photometry methods, respectively. The other elements and trace elements (Cr, Cd, Ni, and Pb) were evaluated by atomic absorption spectrophotometry according to the method 3050B (USEPA, 1996), which consists in the solubilizing of the sample with HNO_3, HCl and H_2O_2 concentrated under heating and the absorbance lecture using air-acetylene-nitrous oxide or air-acetylene flame (Cr, Cd, Ni, and Pb).

Composted tannery sludge was annually applied from 2009 using five rates: 0 (without CTS application), 2.5, 5, 10, and 20 Mg ha^{-1} of CTS (dry basis). The experimental site was arranged in a completely randomized design with four replicates. Plots were 20 m^2 each, with 12 m^2 of usable area for soil and plant sampling, and rows were spaced 1.0 m apart. In the fifth year (2013), CTS was applied 10 days before cowpea sowing. It was spread on the soil surface and incorporated into the 20 cm layer with a harrow. Cowpea was grown at a density of 5 plants m^{-1} (approximately 62,000 plants ha^{-1}). Plant growth and nodulation were recorded 40 days after plant emergence (flowering period), and plants were harvested 60 days after plant emergence. Growth, nodule number, shoot dry weight, and N accumulation (Keeney & Nelson, 1982) were measured. Nodule, shoots and roots were dried at 60°C for 72 h before dry weight determination. N was extracted with 40 mL 2 M KCl followed by filtration through Whatman's No. 42 filter paper. The N contents of the extract were determined using the steam distillation and titration method of Keeney & Nelson (1982). Aliquots (40 mL) of the extracts were added via pipette into a distillation flask, and steam distillation. The distillate was then collected in 5 mL of boric acid containing bromocresol green/ methyl red mixed indicator and titrated against 0.05 M hydrochloric acid (HCl).

Three soil samples per treatment were collected, air-dried, crushed, sieved (2 mm), and stored separately for further chemical analyses. Soil pH (1:2.5 soil:water) and available P were evaluated according to the method of Tedesco *et al.* (1995). The soil organic matter (SOM) content was determined according to the method of Yeomans & Bremner (1998). Chromium concentration in soil was analyzed using acid digestion with HNO_3, HCl and H_2Cl_2 concentrated under heating and the absorbance lecture in AAS using air-acetylene-nitrous oxide flame (USEPA, 1996). The validation of the methodology was carried out with certificate reference material (soil contaminated with sewage sludge - RTC CRM 005-050). It is important to remember that the method USEPA (1996) does not estimate all the trace elements present in the soil.

The data were subjected to analysis of variance (ANOVA), and the means were compared by Student's *t* test (5% level). All statistical analyses were performed with the SPSS (vers. 15.0) software package.

The chemical characteristics of CTS are shown in Table 1. CTS showed high values of pH, Ca, Na, and Cr content. Therefore, soil chemical properties such as soil pH, EC, and Cr content markedly increased after CTS amendment (Table 2). At the highest CTS rate, soil pH increased 1.3 units, while soil EC and Na

Table 1. Chemical properties of composted tannery sludge (CTS)

	CTS	Maximum limit permitted[a]
Water content (g kg^{-1})	680	
pH	7.5	
EC (dS m^{-1})	19.2	
Organic C (g kg^{-1})	201.2	
Total N (g kg^{-1})	15.1	
C/N ratio	13.3	
Total P (g kg^{-1})	4.91	
Total K (g kg^{-1})	2.90	
Total Ca (g kg^{-1})	121.18	
Total Mg (g kg^{-1})	7.21	
Total S (g kg^{-1})	10.20	
Total Na (g kg^{-1})	49.1	
Total Cu (mg kg^{-1})	16.38	1500
Total Ni (mg kg^{-1})	23.26	420
Total Cd (mg kg^{-1})	1.93	39
Total Cr (mg kg^{-1})	1943	1000
Total Pb (mg kg^{-1})	40.31	300

[a] Following CONAMA (2009).

content increased six and thirteen times, respectively, after 5 years of CTS amendment. Similarly, compared with the control, Cr content increased 1.8 times after amendment with 20 Mg ha^{-1} CTS.

Composted tannery sludge showed high pH, Ca and Na content owing to the use of hydroxides, carbonates, and salts during the tanning process (Santos *et al.,* 2011). Also, the residue has high Cr content because this metal is used during the tanning process. The Cr content in CTS was twice the upper limit for Cr prescribed by Brazilian regulations (CONAMA, 2009) and it is characteristic of raw tannery sludge, which is not pretreated in the industry. However, as shown in Table 2, soil pH increased to above 7.0 as CTS rates increased and when soil pH is alkaline, Cr remains inert in soil in forms with low mobility (Gonçalves *et al.*, 2014).

Plant growth, nodulation, N accumulation, and cowpea yield increased even at 10 Mg ha^{-1}; however, above this rate, these variables decreased (Table 3). Compared with unamended soil, the shoot and root dry weight increased by approximately twofold, whereas the nodule number and dry weight increased by 90% and 70%, respectively, after amendment with 10 Mg ha^{-1} CTS. Similarly, nitrogen accumulation increased by 120% and the cowpea yield correspondingly increased by more than 100% after amendment with 10 Mg ha^{-1} CTS.

The increases in shoot and root dry weight of cowpea after amendment with 10 Mg ha^{-1} suggest a direct response of plants to the high organic matter and nu-

Table 2. Soil pH, electrical conductivity (EC), sodium (Na), and Cr content after 5 years of composted tannery sludge (CTS) amendment

CTS (Mg ha^{-1})	Soil pH	EC (dS m^{-1})	Na (cmol$_c$ kg^{-1})	Cr (mg kg^{-1})
0	6.64 c	6.73 e	2.7 e	17.88 c
2.5	7.04 c	1.7 d	7.4 d	19.12 c
5	7.32 bc	2.8 c	19.4 c	23.41 b
10	7.73 ab	3.4 b	25.7 b	25.34 b
20	7.94 a	3.69 a	33.2 a	28.18 a

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

Table 3. Growth, nodulation, accumulation of N, and cowpea yield after 5 years of composted tannery sludge (CTS) amendment

CTS (Mg ha^{-1})	Shoot dry weight (g pl^{-1})	Root dry weight (g pl^{-1})	Nodule number (No. pl^{-1})	Nodule dry weight (mg pl^{-1})	N accumulated (mg pl^{-1})	Yield (Mg ha^{-1})
0	2.3 c	0.29 b	19 c	17.3 c	71.8 d	0.78 d
2.5	2.9 b	0.31 b	33 b	32.0 b	79.3 d	1.18 c
5	3.5 b	0.32 b	29 b	28.3 b	97.9 c	1.75 b
10	5.0 a	0.49 a	36 a	35.6 a	158.9 a	2.04 a
20	3.8 b	0.37 b	30 b	29.7 b	140.2 b	1.64 b

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

trients in CTS. Wong *et al.* (1998) reported that the addition of organic compost increased the total organic matter, macronutrients, and micronutrients according to the rate of compost application and attributed the increased plant growth to the organic matter and nutrients added to soil. The decrease in the shoot and root dry weight with the highest application of CTS suggests that soil salinity influences cowpea growth. According to Murillo-Amador *et al.* (2006), the salinity-induced stress promotes reduction in plant height and dry weight of cowpea. Patel *et al.* (2010) found a reduction of 50% in cowpea growth with the increase in salinity level (2 to 10 dS m^{-1}).

A similar effect of soil salinity was found for nodulation and N accumulation in cowpea where. According to Angle *et al.* (1992) when adverse effects of sludge are observed on symbiotic parameters or plant growth, the effects are probably due to the salts added to soil in the sludge. Also, root growth is highly affected by salinity levels (Patel *et al.,* 2010) and it influences plant nodulation through reduction in the root development and consequently rhizobial infection (Ferreira & Castro, 1995). According to Singleton & Bohlool (1984), nodulation and N accumulation are reduced at EC values above 1.0 dS m^{-1} (Singleton & Bohlool, 1984). However, in our study, nodulation and N accumulation decreased at EC values above 3.4 dS m^{-1}, and it suggest that cowpea has some tolerance to soil salinity. This result indicates that cowpea may be used as a tolerant crop to amendment of tannery sludge in long-term once this waste increases the soil salinity.

Although Cr increased in soil after CTS amendment, the nodulation and N accumulation of cowpea were not affected by the Cr toxicity. Wani *et al.* (2007) and Wani & Khan (2010) reported that Cr toxicity affected the nodulation due to the decreasing of the water and nutrients uptake, and cellular injuries to the roots. They evaluated the effect of soil Cr concentration (34, 68, and 136 mg kg^{-1}) on the growth and nodulation of *Vigna radiata* and *Cicer arietinum*, respectively, and the plant growth and nodulation increased in soil with Cr as compared with the control. However, in our study the highest Cr content (28.18 mg kg^{-1}) found in soil was lower to the lowest concentrations used by Wani *et al.* (2007) and Wani & Khan (2010) and it was not sufficient to cause damage on plant nodulation. On the other hand, consecutive applications of composted tannery sludge will increase Cr content in soil and, consequently, may affect plant nodulation and N accumulation. Therefore, the monitoring of these applications should be done to observe the behavior of this waste on cowpea growth and nodulation.

The cowpea yield obtained after amendment with 10 Mg ha^{-1} CTS was twice the yield obtained in control soil (0 Mg ha^{-1} CTS), and this yield was higher than those reported in Brazil by Ferreira *et al.* (2008) and Gualter *et al.* (2008) using chemical fertilization. This result indicates that CTS has the potential to increase cowpea yield and may be an alternative to chemical fertilization.

In conclusion, the amendment of 10 Mg ha^{-1} CTS increased the growth, nodulation, and yield of cowpea. Soil amendment with CTS over 5 years promoted an increase in soil pH, electrical conductivity, Na, and Cr content. Particularly, the increase in soil salinity, as indicated by electrical conductivity and Na content, exerted negative effects on the growth, nodulation, and yield of cowpea at the highest CTS rate.

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