



Soil microbial biomass after two years of the consecutive application of composted tannery sludge

Iuna Carmo Ribeiro Gonçalves¹, Ademir Sergio Ferreira Araújo^{1*}, Luís Alfredo Pinheiro Leal Nunes¹ and Wanderley José de Melo²

¹Centro de Ciências Agrárias, Laboratório de Solos, Universidade Federal do Piauí, Campus da Socopo, 64000-000, Teresina, Piauí, Brazil.

²Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil.

*Author for correspondence. E-mail: asfaruaj@yahoo.com.br

ABSTRACT. Because the agricultural use of tannery sludge may cause increased risks to soils, composting is recognized as one of the most suitable alternative for tannery sludge recycling. Experiments were conducted under field conditions to evaluate the effects of composted tannery sludge (CTS) on the soil microbial biomass and trace elements after two years of consecutive applications. The following five treatments were used: 0 (without CTS application), 5, 10, 20 and 40 ton ha⁻¹ of CTS (dry basis). Soil samples were collected at 60 days after the CTS application at 0-20 cm depth. The CTS application promoted changes in the soil microbial biomass C (SMB-C) and N (SMB-N). In the first year, significant increases in the SMB-C and SMB-N were observed with the application of 10 ton ha⁻¹. Furthermore, CTS application increased the Cr content in the soil after two years of application.

Keywords: composting, industrial waste, soil microorganisms.

Biomassa microbiana do solo após dois anos de aplicações sucessivas de lodo de curtume compostado

RESUMO. O uso agrícola do lodo de curtume pode aumentar os riscos para os solos. Assim, a compostagem é reconhecida como um dos mais adequados métodos para reciclagem do lodo de curtume. Experimentos foram conduzidos em condições de campo para avaliar os efeitos do lodo de curtume compostado (LCC) sobre a biomassa microbiana do solo e o conteúdo de elementos traço após dois anos de aplicações sucessivas. Cinco tratamentos foram usados: 0 (sem LCC), 5, 10, 20 e 40 t ha⁻¹ de LCC (base seca). As amostras de solo foram coletadas 60 dias após a aplicação de LCC, a 0-20 cm. A aplicação de LCC promoveu mudanças na biomassa microbiana do solo (BMS). No primeiro ano, significantes aumentos no C e N da BMS foram observados com a aplicação de 10 t ha⁻¹. A aplicação de LCC aumentou o conteúdo de Cr no solo após dois anos de aplicação.

Palavras-chave: compostagem, resíduo industrial, microrganismos do solo.

Introduction

The tannery industry occupies an important place in the Brazilian economy with assets of 21 billion dollars per year (SILVA et al., 2010). However, this industry releases 1 million tons of tannery sludge annually, of which 3% is solid waste (SANTOS et al., 2011). However, there are no established methods for tannery sludge disposal in Brazil, and landfilling is commonly used. Alternatively, the application to soil has been suggested as a suitable method (SINGH; AGRAWAL, 2008). However, both methods have a series of associated economic, social and environmental problems. Therefore, it is necessary to develop new methods for tannery sludge recycling as an alternative to landfilling and soil application.

Tannery sludge is usually high in organic matter, chemical nutrients and trace elements, mainly chromium (Cr). In particular, due to the likelihood of food chain contamination and risks to human health, it is the occurrence of trace elements, such as Cr, Cd, Ni and Pb, in tannery sludge that causes serious concern (GUPTA; SINHA, 2007; SILVA et al., 2010; SINGH; AGRAWAL, 2008). Therefore, composting has been suggested as an alternative method for tannery sludge recycling before its application to the soil (SILVA et al., 2010; SANTOS et al., 2011). Indeed, this method has been used to process industrial sludge of different origin, including the textile (ARAÚJO; MONTEIRO, 2006; ARAÚJO et al. 2007) and tannery industries (SANTOS et al., 2011).

However, the knowledge of the effects of composted tannery sludge (CTS) on the soil microbial biomass and accumulation of trace elements is important to maintain the environmental quality of the soil. The soil microbial biomass (SMB), the living component of the soil organic matter (SOM) (JENKINSON; LADD, 1981), is considered an early and sensitive indicator of soil stress caused by wastes (ARAÚJO; MONTEIRO, 2006).

Previous studies regarding the effects of composted wastes, such as textile (ARAÚJO; MONTEIRO, 2006; ARAÚJO et al. 2007) and tannery (SANTOS et al., 2011) sludge, on the SMB have been conducted under controlled laboratory conditions. Santos et al. (2011) evaluated the effects of CTS on the SMB in a short-term study using a pot experiment with soil and reported no negative effects on the microbial biomass and activity. However, the same authors suggested that field studies are necessary to demonstrate the effects of CTS on the SMB after years of applications.

Therefore, the aim of this study was to evaluate the effects of two years of composted tannery sludge amendment on the soil microbial biomass. Our hypothesis is that, after two consecutive years, the applications of composted tannery sludge may promote strong effects on the soil microbial biomass as a result of the accumulation of this waste.

Material and methods

The tannery sludge was collected from the wastewater treatment plant of a tannery located at Teresina city, Piauí State, Brazil. The compost was produced with tannery sludge and structuring materials (sugarcane straw and cattle manure) mixed at a ratio 1:1:3 (sludge: sugarcane: cattle manure). The composting process was performed using the aerated-pile method for 85 days. The size of the pile was 2 m long by 1 m wide by 1.5 m high. The pile was turned twice a week during the first 30 days and was turned twice a month during the remainder of the composting process. The temperature of the compost was approximately 70°C during the first month and decreased and remained at approximately 30°C until the end of the process. At the end of the composting process, twenty subsamples were randomly collected from the CTS to produce a composite sample. The N, P and K contents were evaluated using 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 presented in Table 1.

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

Properties	CTS (2009)	CTS (2010)	Limits of heavy metal permitted ¹
pH	7.8	7.2	-
C _{org} (g kg ⁻¹)	187.5	195.3	-
N (g kg ⁻¹)	1.28	1.39	-
P (g kg ⁻¹)	4.02	3.83	-
K (g kg ⁻¹)	3.25	3.51	-
Ca (g kg ⁻¹)	95.33	84.28	-
Mg (g kg ⁻¹)	6.80	5.71	-
Cu (mg kg ⁻¹)	17.80	19.51	1500
Fe (mg kg ⁻¹)	5171	4932	-
Mn (mg kg ⁻¹)	1848	1958	-
Zn (mg kg ⁻¹)	141.67	128.31	2800
Mo (mg kg ⁻¹)	9.28	14.87	-
Ni (mg kg ⁻¹)	21.92	28.61	420
Cd (mg kg ⁻¹)	2.87	3.93	39
Cr (mg kg ⁻¹)	2255	2581	1000
Pb (mg kg ⁻¹)	42.67	38.54	300

¹Brasil (2006).

The experiment was performed under field conditions at the “Long-Term Experimental Field” of the 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, a rainy summer and dry winter, with an annual average temperatures of 30°C and a rainfall level of 1,200 mm. The rainy season extends from January to April during which 90% of the total annual rainfall occurs. The soil is classified as fluvic Neossol of the following composition at a 0-20 cm depth: clay, 10%; silt, 28% and sand, 62%. The average values of the soil chemical properties (0-20 cm depth) before the installation of experiment were as follows: pH – 6.7; available P – 8.04 mg kg⁻¹; exchangeable K – 0.06 cmol_c kg⁻¹; Ca – 1.76 cmol_c kg⁻¹ and Mg – 0.37 cmol_c kg⁻¹.

The experiments were conducted in 2009 and 2010 using five treatments: 0 (without CTS), 5, 10, 20 and 40 ton ha⁻¹ of CTS (dry basis). The experiment was arranged in a completely randomized design with four replications. The plots were marked out (20 m² each with 12 m² of useful area for the soil and plant sampling) and included rows spaced 1.0 m apart.

In each year, the CTS was applied ten days before the sowing of cowpea (*Vigna unguiculata*). The CTS was spread on the soil surface with incorporation into the 20 cm layer using a harrow. The cowpea plants were grown at a density of 5 plants m⁻¹ (approx. 62,000 plants ha⁻¹). Two months after the CTS amendment, the cowpea was harvested and five soil samples at the 0-20 cm depth were randomly collected from each plot using a soil auger and then pooled.

The soil microbial biomass C (SMB-C) and N (SMB-N) were determined according to Vance et al. (1987) with the 0.5 M K_2SO_4 extraction of the organic C and total N from the fumigated and unfumigated soils. The qCO_2 was calculated as the ratio of the basal respiration to the SMB-C. Fluorescein diacetate (FDA) hydrolysis was measured according to Schnurer and Rosswall (1982). The dehydrogenase (DHA) activity was determined using the method described by Casida et al. (1964), as based on the spectrophotometric determination of the triphenyltetrazolium formazan released by 5 g of soil after 24h incubation at 35°C.

The soil pH (1:2.5 soil:water), P, K and Mg were evaluated in accordance with Tedesco et al. (1995). The soil organic C (SOC) was determined using the Walkey-Black method. The Cr, Cd, Ni and Pb contents were analyzed according to USEPA (1996) after the digestion of the soil with HNO_3 , HCl and H_2O_2 . The soil extracts were analyzed for Cr, Cd, Ni and Pb using atomic absorption spectrophotometry. As the digestion method does not promote the complete dissolution of the soil minerals and the Cr, Cd, Ni and Pb contents do not show the total values, these values were considered the pseudo-total content (ANDRADE et al., 2009).

The data were subjected to an analysis of variance (ANOVA) and the means were compared using the Student's test (5% level) and regression analyses.

Results and discussion

The chemical properties of the CTS used during the two years showed a high amount of organic matter and Cr, Cd, Ni and Pb contents. Because the CTS was found to be rich in organic matter, its application increased the SOC content in both years (Figure 1).

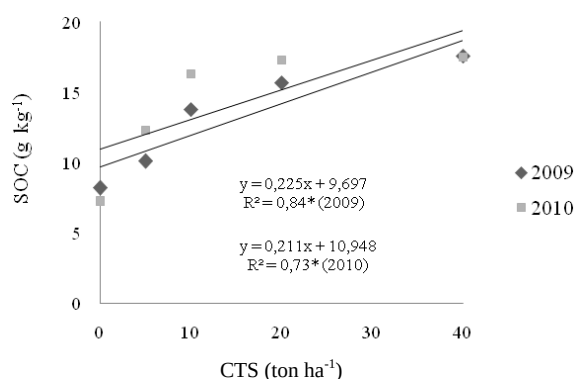


Figure 1. Soil organic C (SOC) in soil after two years of CTS amendment. ns - no significant; * - significant $p < 0.05$.

The increase in the SOC content after the application of CTS is a direct response to the high organic matter content in the waste, as reported by Gupta and Sinha (2007) who found an increase in the SOC after the application of tannery sludge to soils in India. This increase in the SOC is important for tropical soils that are naturally poor in organic matter (MELO et al., 2007). In addition, an increase in the SOC stimulates the soil microbial biomass and may regulate the availability of the soil trace elements (KARACA, 2004; PARTELLI et al., 2012).

Similarly, the CTS was found to be rich in trace elements, particularly Cr; thus, after two years, the application of CTS increased the Cr pseudo-total content (Figure 2). In contrast, the application of the CTS did not increase the Cd, Ni and Pb pseudo-total contents in the soil. The soil Cr contents were found to be higher at the highest rates of application, showing a linear response. In addition, the increase was more prominent in the second year of application.

The increase of the Cr content in the soil after the CTS amendment is due to the high concentration of Cr in the composted waste. Cavallet and Selbach (2008) also found an increase in the Cr content of the soil from Rio Grande do Sul after the application of tannery sludge rich in Cr. Although the Cr content in the CTS was higher than the values permitted by CONAMA (BRASIL, 2006), this element was probably in its trivalent form, and therefore more stable and with a low solubility and mobility (ALCÂNTARA; CAMARGO, 2001). Furthermore, Cr is in the insoluble form of $Cr(OH)_3$ at pH values above 5.0, as found in the CTS and soil (AQUINO NETO; CAMARGO, 2000), thus reducing its toxic potential.

Conversely, the values of Cr, Cd, Ni and Pb found in the soil after two years were below those permitted by USEPA (1996) for trace elements in soil, which are 1530, 20, 230 and 180 $mg\ kg^{-1}$ for Cr, Cd, Ni and Pb, respectively.

The CTS promoted significant changes in the soil microbial biomass only in the first year, whereby the waste promoted an increase in the SMB-C and SMB-N contents with the application of 10 $ton\ ha^{-1}$ (Figure 3). Above this rate, the CTS amendment decreased the SMB-C and SMB-N. In the second year, we did not find differences in the SMB-C and SMB-N with regard to the rate of amendment.

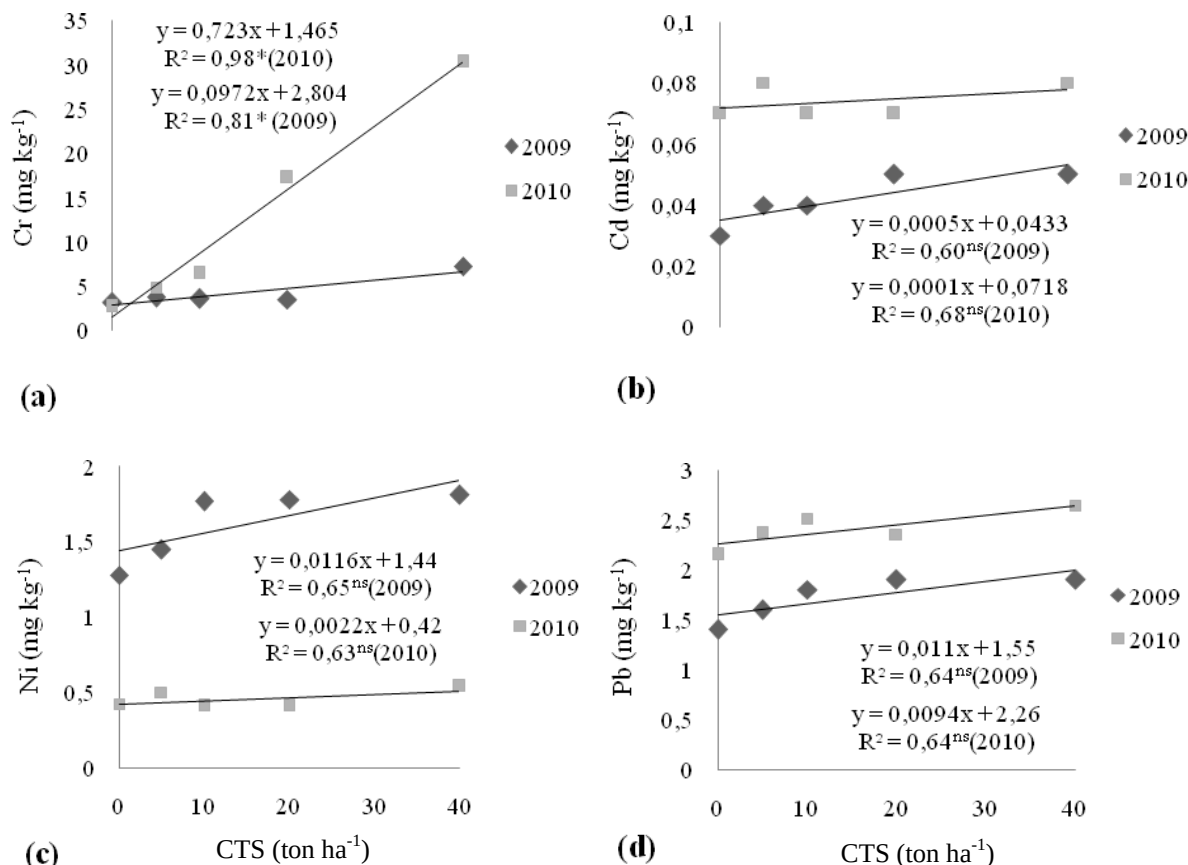


Figure 2. Pseudo total content of Cr (a), Cd (b), Ni (c) and Pb (d) in soil after two years of TSC amendment. ns – no significant; * – significant $p < 0.05$.

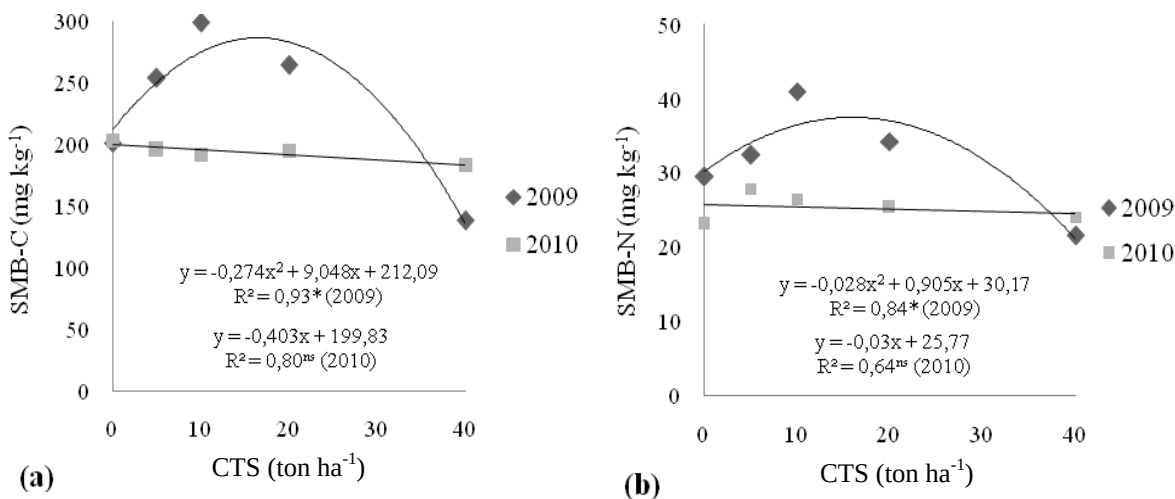


Figure 3. Soil microbial biomass C (SMB-C) (a) and N (SMB-N) (b) in soil after two years of CTS amendment. ns – no significant; * – significant $p < 0.05$.

In the first year, the application of 10 ton ha⁻¹ CTS increased the SMB-C and SMB-N due to growth of the soil microorganisms as a direct response to the available C and nutrients in the waste and the raw materials (straw and cattle

manure) used in the composting process. These results agree with previous studies using sewage (SELIVANOVSKAYA et al., 2001), textile (ARAÚJO et al., 2007) and tannery (SANTOS et al., 2011) sludge composts, with the authors finding an

increase in the soil microbial biomass with the application of these wastes and in soils managed using organic agriculture (PARTELLI et al., 2012).

However, the high rates of CTS application (20 and 40 ton ha⁻¹) decreased the SMB-C and SMB-N due, probably due to the higher concentration of Cr in the soil, suggesting possible toxic effects on the soil microbial biomass of CTS applied at high rates.

In the second year, similar values were found for the SMB-C and SMB-N at all of the rates, indicating a possible adaptation of the soil microbial biomass to the elements present in the CTS. In addition, except for the Cr content, there were no increases in the Pb, Cd and Ni contents in the soil after two years of CTS application. This result suggests that the increases in the Cr content were insufficient to cause negative effects on the soil microbial biomass, as reported by Sullivan et al. (2006) and Souza et al. (2009) after applications of sewage sludge for two years. These authors suggested an adaptation of the soil microbial by the permanent application of these wastes.

The FDA hydrolysis and DHA activity results showed similar responses to the soil microbial biomass, with changes only in the first year. However, the FDA hydrolysis and DHA activity decreased linearly after the CTS application in the first year, showing significant differences from the unamended soil only at 20 and 40 ton ha⁻¹ CTS (Figure 4). In the second year, the enzymes activities were not affected by the CTS application.

The soil enzymatic activities are indicators of the natural and anthropogenic disturbances occurring in the soil ecosystem (SANTOS et al., 2011). FDA hydrolysis and DHA activity decreased after the application of 20 and 40 ton ha⁻¹ CTS, probably due to the increased Cr content in the soil. According to Barajas-Aceves et al. (2007), the increase in trace elements, mainly Cr, in the soil may reduce the DHA activity.

The results of the enzyme analyses demonstrated a similar behavior those observed for the soil microbial biomass. However, the soil microbial biomass showed a positive quadratic response, whereas the enzyme activities had a negative linear response as the CTS rates were increased. These findings indicate that the CTS did not negatively affect the size of the microbial biomass but its activity, as measured by the enzyme activity. Studying the effect of Cr on the soil microbial biomass and activity, Shi et al. (2002) found that the

soil microbial activity decreased after Cr application, though the soil microbial biomass was not affected.

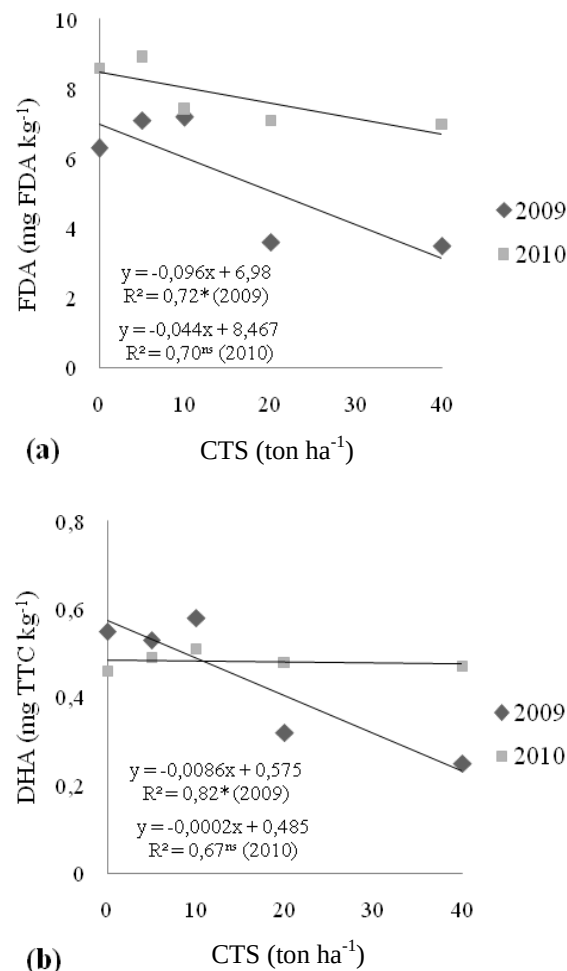


Figure 4. Fluorescein diacetate hydrolysis (FDA) (a) and dehydrogenase activity (DHA) (b) in soil after two years of CTS amendment. ns – no significant, * – significant $p < 0.05$.

In the second year of our study, the similar values for these enzymes suggest neither negative nor positive effects of the CTS on the soil enzymes, probably due to a possible adaptation of the soil microorganism to the CTS or Cr content. Jezierska-Tys and Frac (2006) did not observe negative or positive effects on the DHA activity after applications of sewage sludge with low levels of heavy metals.

In the first year, the metabolic quotient (qCO_2) increased when the CTS rates increased (Figure 5), and the highest qCO_2 values were found in the soils that had been amended with 20 and 40 ton ha⁻¹. In contrast, the lowest values were observed with the applications of 0, 5 and 10 ton ha⁻¹. In the second year, the qCO_2 values did not vary between the treatments.

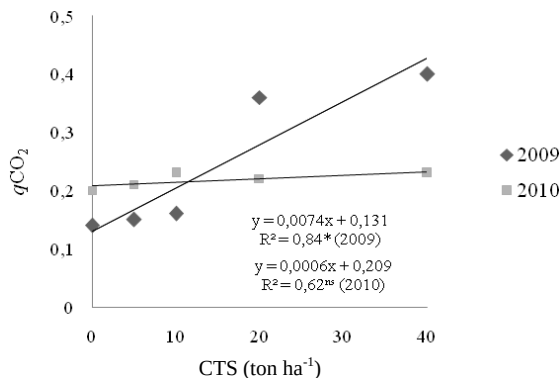


Figure 5. Soil respiratory quotient ($q\text{CO}_2$) in soil after two years of CTS amendment. ns – no significant, * – significant $p < 0.05$.

The results of the $q\text{CO}_2$ showed that the $q\text{CO}_2$ decreased when the soil microbial biomass increased; this occurred after the application of 10 ton ha^{-1} CTS, thus the soil microbial biomass was efficient in its C at this rate. In contrast, the $q\text{CO}_2$ increased when soil microbial biomass decreased, as observed after the applications of 20 and 40 ton ha^{-1} CTS. In this case, the soil microbial biomass directed its C pool to support cell integrity and maintenance (MOSCATELLI et al., 2007), suggesting a stress on the soil microbial community. The respiration rate per unit of soil microbial biomass, or respiratory quotient ($q\text{CO}_2$), is a variable that is used to indicate stress in the soil environment (FERNANDES et al., 2005). Thus, the CTS rates above 10 ton ha^{-1} caused stress to the system. These results are accordance with Fernandes et al. (2005) and Araújo and Monteiro (2007) who found higher $q\text{CO}_2$ values for soils with high sewage and textile sludge rates.

Conclusion

The application of 10 ton ha^{-1} of composted tannery sludge increased the microbial biomass, organic C and Cr contents in the soil, whereas there was a decrease in the soil microbial biomass at rates above 10 ton ha^{-1} . However, the application of the composted tannery sludge, at all rates, decreased the soil microbial activity, as measured by the enzyme activity.

Acknowledgements

This research was Funded by “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq-Brazil) and “Fundação de Amparo a Pesquisa do Estado do Piauí” (FAPEPI). A.S.F Araújo and W.J. Melo are supported by a personal grant from CNPq-Brazil.

References

- ALCÂNTARA, M. A. K.; CAMARGO, O. A. Movement of trivalent chromium as influenced by pH, soil horizon and sources of chromium. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 5, n. 2, p. 497-501, 2001.
- AQUINO NETO, V.; CAMARGO, A. Growth and accumulation of Chromium in lettuce sowed in two oxisols treated with CrCl_3 and tannery waste. **Brazilian Journal of Soil Science**, v. 24, n. 1, p. 225-235, 2000.
- ANDRADE, M. G.; MELO, V. F.; SOUZA, L. C. P.; GABARDO, J.; REISSMANN, C. R. Metais pesados em solos de área de mineração e metalurgia. II- formas e disponibilidade para plantas. **Revista Brasileira de Ciencia do Solo**, v. 33, n. 10, p. 1889-1897, 2009.
- ARAÚJO, A. S. F.; MONTEIRO, R. T. R. Microbial biomass and activity in a Brazilian soil plus untreated and composted textile sludge. **Chemosphere**, v. 64, n. 6, p. 1043-1046, 2006.
- ARAÚJO, A. S. F.; MONTEIRO, R. T. R.; CARVALHO, E. M. S. Effect of textile sludge composted on growth, nodulation and nitrogen fixation of soybean and cowpea. **Bioresource Technology**, v. 98, n. 6, p. 1028-1032, 2007.
- BARAJAS-ACEVES, M.; OCAMPO, V. R.; RODRIGUEZ, V. R. Effects of Cr^{3+} , Cr^{6+} and tannery sludge on C and N mineralization and microbial activity in semi-arid soils. **Journal of Hazardous Materials**, v. 143, n. 3, p. 522-531, 2007.
- BRASIL. Conselho Nacional do Meio Ambiente.. Resolução n.º 375/2006, de 29 de agosto de 2006. **Diário Oficial da República Federativa do Brasil**. Available from: <<http://www.mma.gov.br/port/conama/legiano>>. Access on: Apr. 30, 2012.
- CASIDA, L. E.; KLEIN, D. A.; SANTORO, T. Soil dehydrogenase activity. **Soil Science**, v. 98, n. 3, p. 371-376, 1964.
- CAVALLET, L. E.; SELBACH, P. A. Populações microbianas em solo agrícola sob aplicação de lodos de curtume. **Revista Brasileira de Ciência do Solo**, v. 32, n. 12, p. 2863-2869, 2008.
- FERNANDES, A. P.; BETTIOL, W.; CERRI, C. C. Effect of sewage sludge on microbial biomass, basal respiration, metabolic quotient and soil enzymatic activity. **Applied Soil Ecology**, v. 30, n. 1, p. 65-77, 2005.
- GUPTA, A. K.; SINHA, S. Phytoextraction capacity of the plants growing on tannery sludge dumping sites. **Bioresource Technology**, v. 98, n. 12, p. 1788-1794, 2007.
- JENKINSON, D. S.; LADD, J. N. Microbial biomass in soil: measurement and turnover. In: PAUL, E. A.; LADD, J. N. (Ed.). **Soil Biochemistry**. New York: Marcel Dekker, 1981. p. 415-471.
- JEZIERSKA-TYS, S.; FRAC, M. Enzymatic activity in gray-brown podzolic soil enriched with sewage sludge from a dairy plant. **Polish Journal of Soil Science**, v. 39, n. 1, p. 33-42, 2006.
- KARACA, A. Effect of organic wastes on the extractability of cadmium, copper, nickel and zinc in soil. **Geoderma**, v. 122, n. 2, p. 297-303, 2004.

- MELO, W. J.; AGUIAR, P. S.; MELO, G. M. P.; MELO, V. P. Nickel in a tropical soil treated with sewage sludge and cropped with maize in a long-term field study. **Soil Biology and Biochemistry**, v. 39, n. 9, p. 1341-1347, 2007.
- MOSCATELLI, A.; CIAMPOLINI, F.; RODIGHIERO, S.; ONELLI, E.; CRESTI, M.; SANTO, N.; IDILLI, A. Distinct endocytic pathways identified in tobacco pollen tubes using charged nanogold. **Journal of Cell Science**, v. 120, p. 3804-3819, 2007.
- PARTELLI, F. L.; VIEIRA, H. D.; FERREIRA, E. P. B.; VIANA, A. P.; MARTINS, M. A.; URQUIAGA, S. Chemical and microbiological soil characteristics under conventional and organic coffee production systems. **Communications in Soil Science and Plant Analysis**, v. 43, n. 5, p. 847-864, 2012.
- SANTOS, J. A.; NUNES, L. A. P. L.; MELO, W. J.; ARAÚJO, A. S. F. Tannery sludge compost amendment rates on soil microbial biomass in two different soils. **European Journal of Soil Biology**, v. 47, n. 1, p. 146-151, 2011.
- SCHNURER, J.; ROSSWALL, T. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. **Applied Environmental Microbiology**, v. 43, n. 8, p. 1256-1261, 1982.
- SELIVANOVSKAYA, S. Y.; LATYPOVA, V. Z.; KIYAMOVA, S. N.; ALIMOVA, F. K. Use of microbial parameters to assess treatments methods of municipal sewage sludge applied to grey forest soils of Tatarstan. **Agriculture, Ecosystem and Environment**, v. 86, n. 1, p. 145-153, 2001.
- SHI, W.; BISCHOFF, M.; TURCO, R.; KONOPKA, A. Long-term effects of chromium and lead upon the activity of soil microbial communities. **Applied Soil Ecology**, v. 21, n. 1, p. 169-177, 2002.
- SILVA, J. D. C.; LEAL, T. T. B.; ARAÚJO, A. S. F.; ARAUJO, R. M.; GOMES, R. L. F.; MELO, W. J.; SINGH, R. P. Effect of different tannery sludge compost amendment rates on growth, biomass accumulation and yield responses of *Capsicum* plants. **Waste Management**, v. 30, n. 12, p. 1976-1980, 2010.
- SINGH, R. P.; AGRAWAL, M. Potential benefits and risks of land application of sewage sludge. **Waste Management**, v. 28, n. 2, p. 347-358, 2008.
- SOUZA, C. A.; REIS JUNIOR, F. B.; MENDES, I. C.; LEMAINSKI, J.; SILVA, J. E. Lodo de esgoto em atributos biológicos do solo e na nodulação e produção de soja. **Pesquisa Agropecuária Brasileira**, v. 44, n. 9, p. 1319-1327, 2009.
- SULLIVAN, T. S.; STROMBERGER, M. E.; PASCHKE, M. W.; IPPOLITO, J. A. Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. **Biology and Fertility of Soils**, v. 42, n. 2, p. 258-266, 2006.
- TEDESCO, M. J.; GIANELLO, C.; BISSANI, C. A. **Análises de solos, plantas e outros materiais**. Porto Alegre: UFRGS, 1995.
- USEPA-United States Environmental Protection Agency. **Acid digestion of sediments, sludges and soils**. Metod 3050b. Washington, D.C.: EPA, 1996. Available from: <<http://www.epa.gov/sw-846/pdfs/3050b.pdf>>. Access on: Apr. 18, 2010.
- VANCE, E. D.; BROOKES, P. C.; JENKINSON, D. S. An extraction method for measuring soil microbial biomass C. **Soil Biology and Biochemistry**, v. 19, n. 3, p. 703-707, 1987.

Received on May 8, 2012.

Accepted on August 21, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.