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A Comparative Study of the Effect of a Single Sewage Sludge Addition on Soil Microbial Activity in Cultivated Land and Disturbed Saprolite

Eva VIDAL VÁZQUEZ[1], Adriana P. D. SILVEIRA[2], Marlene C. ALVES[3], Cleide A. ABREU[2], Jorge PAZ FERREIRO[1],
[1]Facultad de Ciencias. Universidade da Coruña. Campus de A Zapateira s/n 15071 A Coruña, Spain.
[2]Instituto Agronômico de Campinas. Av. Barão de Itapura 1481. Campinas-SP, Brazil.
[3]Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista. Ilha Solteira-SP, Brazil.
Corresponding author: evidal@udc.es

Abstract: The addition of sewage sludge to agricultural land has been recognized as an effective method of waste disposal, a useful source of organic material, and a means of improving soil physical properties. The impact of biossolids from municipal residues in field plots of a Ferralsol devoted to agriculture and in a disturbed saprolite resulting from depth excavation of the soil profile was assessed after a single application. The biossolids were rich in organic matter, macronutrients (N, P and K) and micronutrients (Fe, Mn, B, Cu and Zn), and showed low contents of heavy metals such as Pb, Cd and Cr. The study areas are located in Selviria-MS, Brazil, in the wet/dry tropical savannah zone, known as “cerrado”. Different treatments each with four replications were investigated in the agricultural area and the disturbed saprolite, and in addition, natural forest was also analized as a reference. Soil samples were taken at 0-0.20 m depth. Soil microbial biomass, basal respiration, and dehydrogenase and cellulase activity were determined in order to evaluate the potential use of these parameters as indicators of soil disturbances. Microbial biomass C contents discriminate between the agricultural and the degraded saprolite area. Mean values of this parameter in the cerrado were slowly lower than agricultural plots with biossolids addition. Dehidrogenase activity was significantly higher in the cultivated area after sewage sludge application than in the other treatments. Regression analysis among the studied biological properties showed the highest correlation coefficient (r = 0.82) between dehidrogenase and the cellulase activity.

Key words: biosolid, sewage sludge, microbial activity

INTRODUCTION

In recent years, composted urban wastes have been added to agricultural land for both waste disposal and to improve soil fertility. Compost is rich in organic matter and means an important source of nutrients for plants (Gallardo-Lara and Nogales, 1987). For these reasons soil scientists are meeting the challenge of sewage sludge management by preventing soil contamination and increasing soil fertility in different land uses.

Organic waste compost addition promotes the soil microbiological activity. Microbial activity and soil fertility are usually related because the mineralization of the important elements (C, N, P and S) occurs through the biomass (Frankenberger and Dick, 1983; García Gil et al., 2000). Microbial biomass carbon and enzyme activities may have a potential as sensitive indicators of soil restoration (Dick and Tabatabai, 1992; García Gil et al., 2000).

Microbial and enzyme activities provide information on the biochemical processes occurring in the soil and these parameters may have a potential as early and sensitive indicators of soil ecological stress and restoration (Dick and Tabatabai, 1992).

The determination of the microbial and enzymatic activities together with the use of general soil parameters seems to be the best approach for evaluating the state of soil microbial activity and for understanding its response to compost amendments, cultivation practices, and environmental factors (Nannipieri et al., 1990; Perucci, 1992).

Soils of decapitated areas have a very low microbial activity, low levels of microbial biomass and a low organic matter content. Microbial biomass, being the living part of soil organic matter, can be a good index for comparing natural and degraded ecosystems.

A single biosolids application was made in field plots of a Ferralsol devoted to agriculture and in a disturbed saprolite resulting from depth excavation of the soil profile.

The aim of the present research was to compare the microbial activity of an agricultural soil, a disturbed saprolite, and a soil under natural “cerrado” vegetation after sewage sludge application. The soil microbial biomass, basal respiration, metabolic quotient and two enzymatic activities (dehydrogenase and cellulase) were measured.

MATERIALS and METHODS

Experimental site

The experiment was performed in Selviria (MS, Brazil), 51º22’ W and 20º22’ S. The study field is about 327 m above sea level and the environmental conditions define the area as humid tropical with an average annual rainfall of 1370 mm and a mean annual temperature of 23.5ºC.

The soil, developed over clay sediment, was classified as Oxisol according to the Soil Survey Staff (1998), Ferralsol (FAO, 1998) or Latossolo Vermelho Distrofico in the Brazilian Soil Classification System (EMBRAPA, 1999).

The original native area is known as “cerrado” and covers approximately 204 million hectares in tropical Brazil. The vegetation of this ecosystem, of which about 35 percent has been cleared, was fire-climax savannah. The vegetation ranges from open grassland to grassland with shrubs and small twisted trees, to light, low tree cover. Cerrado soils, which can be up to 25 meters or more deep, are among the oldest and least fertile in the world. Clearing costs are low and crop production easily mechanized, but high fertilizer and soil improvement costs are incurred.

In the ’60 decade, an 8.6 m deep layer of soil of the experimental area was removed due the construction of a power station on the Parana River. Soil excavation resulted in an abandoned area with saprolite materials. A revegetation experience took place since 2003.

Soil samples were taken in both studied areas from control and sludge amendment plots and also from soil under natural “cerrado” vegetation. An agricultural field under soybean culture was also analyze.

Experimental design

The experiment was arranged in completely randomized design, in a 6 x 4 factorial scheme with four replicates as follows:

T1: Natural area of cerrado next to the experimental plots of the saprolite and the agricultural field. This treatment reflects the ancient natural conditions of the zone.

T2: Disturbed saprolite, without any revegetation techniques.

T3: Saprolite planted with Eucaliptus (*Eucalyptus citriodora* Hook) and cropped with Brachiaria (*Brachiaria decumbens*) with sewage sludge. Previous the sludge application, it was made two subsoiled with harrowing at 0.40 m depth. Later, the soil acidity pH was corrected with 800 kg of $CaMg(CO_3)_2$ to raise the base saturation ratio to 70% of the soil cation exchange capacity. The dolomite was incorporated into the soil with a harrow.

T4: Native specie (*Astronium fraxinifolium* Schott) with sludge application. Before the compost addition it was made one subsolated and a harrowing level operation.

T5: Soybean (*Glycine Max* (L.) Merrill) culture without sewage sludge.

T6: Soybean (*Glycine Max* (L.) Merrill) culture with sludge. After sewage sludge addition the agricultural area under soybean was ploughed with a disc plough. Then, 70 kg ha^{-1} of P_2O_5 and 80 $kgha^{-1}$ of K_2O were applied as fertilizers. Finally, this area was harrowed; therefore conventional tillage was carried out.

Urban waste products composted under aerobic conditions was supplied by the Municipal Waste Treatment Plant in Araçatuba (São Paulo, Brazil) and it contained an 88% of humidity.

The sewage sludge showed high concentration of organic matter, macronutrients (N, P and K) and micronutrients (Fe, Mn, B, Cu and Zn) and low heavy metals content (Table 1).

Table 1. Concentration of organic matter (O.M.), macronutrients, and micronutrients in the sewage sludge.

O.M.	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Humidity
g dm^{-3}	mmol$_c$ dm^{-3}						mg dm^{-3}					kg kg^{-1}
200	71.26	18.79	15.14	11.06	3.44	7.78	16.37	160.04	960.6	115.74	583.48	0.88

The sludge was manually distributed on the area; it was incorporated to the soil with a disc harrow at 0.10 m depth and exposed to soil radiation at 35-40 °C during 7 days to reduce the organic N content.

The applied dose of sludge was 60 Mg ha^{-1} except in the soybean culture area that was 30 Mg ha^{-1}.

Soil sampling was performed at 0-0.20 m depth in each plot with four replicates, resulting in 24 individual samples. Soil samples were dried and sieved through a 2 mm sieve.

Microbiological analysis

Soil microbial biomass, basal respiration and dehydrogenase and cellulase activity were determined in order to evaluate the potential use of these parameters as indicators of soil disturbances.

Soil microbial biomass C was performed using the fumigation-extraction method according to Vance et al. (1987). Soil samples were incubated for five days at 28 ± 2 ^oC. Carbon was extracted using 0.5 mol L^{-1} K_2SO_4 in a ratio of 1:4 (w:v) of soil extractor. Organic C in the filtered soil extracts was determined by acid oxidation with 0.0667 mol L^{-1} $K_2Cr_2O_7$ and subsequently titration with 0.0333 mol L^{-1} $(NH_4)_2Fe(SO_4)_2$. Microbial biomass C was calculated as 2.64 Ec where Ec is the difference between organic C extracted from the fumigated and non-fumigated samples, both expressed as μg C g^{-1} oven dry soil.

Basal respiration was determined as the CO_2 evolved from 100 g of soil trapped by NaOH 0.1 mol L^{-1}, precipitation with $BaCl_2$ and titration with standard HCl, five days after soil incubation in the dark at 28 ± 2 ^oC, as described by Alef (1995).

The metabolic quotient (qCO_2), also called the specific respiratory rate, is defined as the microbial respiration rate per unit microbial biomass and was calculated according to Anderson and Domsch (1993) based in the relation μg C-CO_2 h^{-1}/ μg C-biomass g^{-1} of dry soil.

Dehydrogenase (DH) activity analysis was performed according to Casida et al. (1964) method with modifications: 5 g of soil was incubated in tubes with 5 mL of 10 g L^{-1} 2,3,5-tripheniyltetrazolium chloride (TTC) at 37 °C for 24 hours in the darkness. During the incubation the tubes were occasionally mixed on a Vortex mixer. After incubation the triphenyl-formazan (TTF) formed by the reduction of TTC was extracted twice with 10 mL of methanol by centrifugation for 10 minutes (7320 g). TTF in the supernatant was determined spectrophotometrically at 485 nm against a calibration curve. The results were expressed as μL H g^{-1} soil day^{-1} considering that 1 mg of TTF is equivalent to 150.35 μL of H.

Cellulase activity was determined by the method of Hope and Burns (1987) based on the determination of liberated reducing sugars after soil incubation in acetate buffer with Avicel for 24 hours at 40°C in a water bath. Results were expressed as μg glucose g^{-1} soil day^{-1}.

Obtained data were submitted to analysis of variance, Tuckey test for comparison of means and regression and correlation analyses for doses treatments and among variables, respectively.

All data was subjected to an analysis of variance using the least significant difference test and comparing the differences between specific treatments.

RESEARCH RESULTS

The highest contents of microbial biomass C were observed in the treatments with soybean culture, particularly in the T6 with sewage sludge application (479 μg g^{-1} soil). It was a 5% higher than T5 without sludge amendment and a 6% higher than the natural area of “cerrado” (T1) (Figure 1). The lowest values of soil microbial biomass were found in the area with eucalyptus (T3) (176 μg g^{-1} soil). The other two treatments had intermediate values (239 μg g^{-1} soil and 244 μg g^{-1} soil for the native specie treatment and the exposed area, respectively).

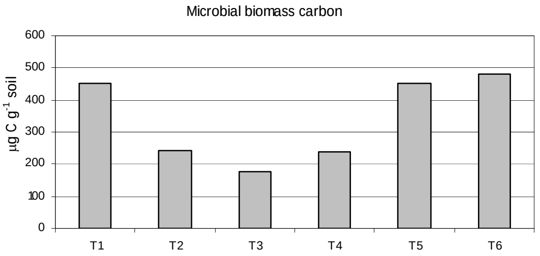

Figure 1. Soil microbial biomass carbon.

Higher soil basal respiration values were found for the T1, T3, T4 and T6 treatments (Figure 2). The lowest value of this parameter (4.9 µgC g^{-1} soil day^{-1}) occurs for the area with disturbed saprolite (T2). The culture area of soybean without sewage sludge (T5) showed a value of 11.7 µgC g^{-1} soil day^{-1}). These two treatments, without amendment were significatively differents to rest of the treatments and between them.

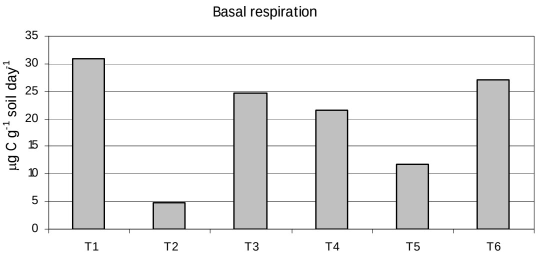

Figure 2. Soil basal respiration.

From microbial biomass carbon and soil basal respiration values, metabolic quotient was calculated. The metabolic quotient (qCO_2), the equivalent of respiration per unit of biomass, was higher in the area with eucalyptus and brachiaria (T3). The lowest value occurs in the saprolite area (T2).

Generally, the qCO_2 is found to be higher when adverse conditions for the microbial population occur because in this state more oxidable C is used for the microbiota maintenance instead of cellular C incorporation. This usually happens in acid soils and in soils with a recent addition of organic substrates or heavy metals (Valsecchi et al., 1995).

Dehydrogenase (DH) is an intracellular enzyme that is involved in microbial oxidoreductase metabolism. The activity of this enzyme depends on the metabolic state of the soil biota.

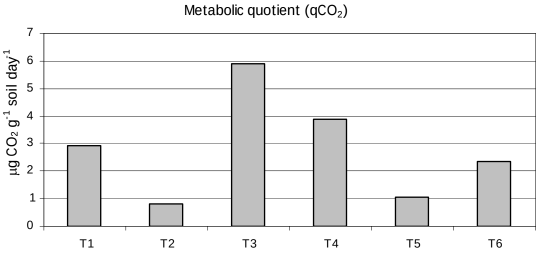

Figure 3. Metabolic quotient.

The additions of organic residues in the cultivated area increased DH and cellulase activity. This activity was significantly higher for the T6 treatment, the cultivated area with sludge addition. The lower values of enzymatic activities occur in the exposed area of saprolite without sludge application (T2). The “cerrado” area (T1) showed higher enzymatic activities than T3 and T4 treatments, of saprolite planted with Eucaliptus and the native specie, respectively.

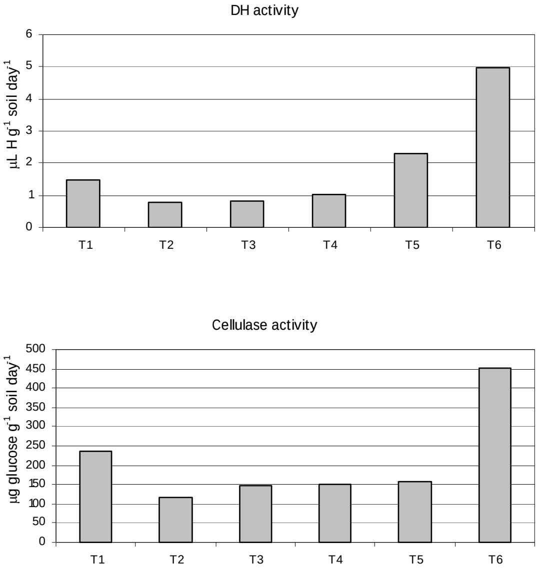

Figure 4. Dehidrogenase and cellulase activity.

A correlation matrix (Table 2) shows the relationships among the studied variables. There was a strong positive correlation between the DH activity and the cellulase activity (r = 0.82). Soil basal respiration was correlated with all the other parameters except the soil microbial biomass carbon.

Table 2. Correlation coefficients

	DH	qCO_2	Resp	C bio
Cellu	0.82**	0.04	0.60**	0.58**
DH		-0.08	0.41*	0.55**
qCO_2			0.65**	-0.52**
Resp				0.23

(*, ** Indicate significance at the 5 and 1% level, respectively. Cellu=cellulase activity; DH=dehydrogenase activity; qCO_2= metabolic quotient; Resp=soil basal respiration; C bio=soil microbial biomass C).

DISCUSSION AND CONCLUSIONS

Sewage sludge of municipal waste treatment plants was rich in organic matter and nutrients, and can be used in agriculture as fertilizer.

Dehydrogenase (DH) activity was significantly correlated with soil microbial biomass C ($P<0.01$). This means that DH activity could be a good indicator of soil microbial activity in this area.

Both dehydrogenase activity and cellulase activity measurements were valid indicators of an ecosystem disturbance. Dehydrogenase and cellulase activities were significantly correlated with soil basal respiration and soil microbial biomass carbon.

The addition of organic residues to agricultural, natural and saprolitic soils has a variety of effects on microbial biomass C, basal respiration and enzyme activities. Soils amendment with municipal solid waste compost can improve soil quality, increasing the organic matter content of degraded soils and improving soil biological and biochemical properties. However, the wide variety of substances such as heavy metals and other potential pollutants in municipal solid wastes limits the use of these residues in compost. Consequently, there must be a quality control of these organic amendments in order to minimize the risk of inhibiting essential biogeochemical processes as well as contributing to environmental pollution.

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