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CHEMICAL AND BIOCHEMICAL PROPERTIES OF OXISOLS AFTER SEWAGE SLUDGE APPLICATION FOR 16 YEARS

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ABSTRACT

The large production of sewage sludge (SS), especially in large urban centers, has led to the suggestion of using this waste as fertilizer in agriculture. The economic viability of this action is great and contributes to improve the environment by cycling the nutrients present in this waste, including high contents of organic matter and plant nutrients. This study evaluated the chemical and biochemical properties of Dystrophic and Eutroferric *Latossolos Vermelhos* (Oxisols) under corn and after SS application at different rates for 16 years. The field experiment was carried out in Jaboticabal, São Paulo State, Brazil, using a randomized block design with four treatments and five replications. Treatments consisted of control - T1 (mineral fertilization, without SS application), 5 Mg ha^{-1} SS - T2, 10 Mg ha^{-1} SS - T3, and 20 Mg ha^{-1} SS - T4 (dry weight base). The data were submitted to variance analysis and means were compared by the Duncan test at 5 %. Sewage sludge increased P extracted by resin in both the *Latossolos Vermelhos*, Dystrophic and Eutroferric, and the organic matter content in the Dystrophic *Latossolo Vermelho*. The waste at the rate 20 Mg ha^{-1} on a dry weight basis promoted increases in acid phosphatase activity in Eutroferric *Latossolo Vermelho*, basal respiration and metabolic quotient in Dystrophic *Latossolo Vermelho*. The rate 20 Mg ha^{-1} sewage sludge on a dry weight basis did not alter the soil microbial biomass in both the *Latossolos Vermelhos*; in addition, it improved corn yields without inducing any symptoms of phytotoxicity or nutrient deficiency in the plants.

Keywords: soil fertility, enzymatic activity, soil basal respiration, microbial biomass, urban waste.

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RESUMO: *ATRIBUTOS QUÍMICOS E BIOQUÍMICOS DE LATOSSOLOS APÓS APLICAÇÃO DE LODO DE ESGOTO POR 16 ANOS*

A grande produção de lodo de esgoto (LE), principalmente em grandes centros urbanos, tem sugerido a disposição desse resíduo como fertilizante na agricultura. Trata-se de uma ação de grande viabilidade econômica, além de contribuir para melhorar o meio ambiente pela ciclagem dos nutrientes presentes nesse resíduo, que possui elevado teor de matéria orgânica e de nutrientes para as plantas. O objetivo deste estudo foi avaliar atributos químicos e bioquímicos de dois Latossolos Vermelhos, um Eutroférrico (LVef) e outro Distrófico (LVd), cultivados com milho, após aplicação do LE em diferentes doses por 16 anos. O experimento foi instalado em condições de campo em Jaboticabal, São Paulo. O delineamento experimental foi em blocos casualizados com quatro tratamentos e cinco repetições. Os tratamentos foram: T1 = testemunha (fertilização mineral, sem aplicação de LE), T2 = 5 Mg ha^{-1} de LE, T3 = 10 Mg ha^{-1} de LE e T4 = 20 Mg ha^{-1} de LE (base seca). Os resultados obtidos para os diferentes atributos avaliados foram submetidos à análise da variância e utilizou-se o teste de Duncan para comparação de médias ($p \le 0,05$). O lodo de esgoto aumentou o P extraído pelo método da resina em ambos os Latossolos, e o teor de matéria orgânica no Latossolo Vermelho Distrófico. Na dose 20 Mg ha^{-1}, base seca, o resíduo promoveu aumento na atividade da fosfatase ácida no Latossolo Vermelho Eutroférrico, respiração basal e quociente metabólico no Latossolo Vermelho Distrófico. A dose 20 Mg ha^{-1} de lodo de esgoto, base seca, não alterou a biomassa microbiana em ambos os Latossolos e aumentou a produção de grãos no Latossolo Vermelho Eutroférrico, sem causar sintomas de fitotoxicidade ou deficiência nutricional nas plantas.

Palavras-chave: fertilidade do solo, atividade enzimática, respiração basal, biomassa microbiana, resíduos urbanos.

INTRODUCTION

The large production of sewage sludge (SS), mainly in urban areas, has led to the alternative suggestion of using it as fertilizer in farming activities. This option is economically highly viable, for protecting the environment by cycling the elements contained in the waste, which has high amounts of organic matter and nutrients for crop plants.

Organic material and nutrients of SS play an important role in sustaining crop yields and soil fertility (Ceolato, 2007). Therefore, it can be considered a highly beneficial residue for use in agriculture as soil physical, chemical and biological conditioner due to the high organic matter content and the presence of plant nutrients (Melo et al., 1994).

As chemical and physical properties underlie soil fertility and crop yield, mechanisms that regulate biological activities are becoming increasingly important for the maintenance of the soil quality (Stuczynski et al., 2007).

Soil microbial activity is primarily responsible for organic material decomposition, nutrient cycling and energy flow, so that soil biochemical properties are more sensitive to soil quality changes than physical and chemical properties. Such alterations can be a result of changes in soil use and management practices, as those promoted by organic residue use (Debosz et al., 2002). Thus, microbial biomass, basal respiration, metabolic quotient ($q CO_2$) and activities of enzymes involved in C, N, P and S cycles have been used to assess SS application in soils (Garcia-Gil et al., 2000).

Analyzing different SS under varied conditions and for different crops, Melo et al. (2001) observed a positive effect on soil fertility and plant nutrition. The use increased basal respiration, as reported by Cardoso and Fortes Neto (2000), who evaluated the effect of rates of 0, 10, 20, 40, 80, and 160 Mg ha^{-1} SS on soil microbial flora. Moreover, this residue changed the environment by alterations in microbial community and microorganism activities.

Microbiological indices, such as microbial biomass carbon (MBC), readily mineralized C and enzyme activity in areas treated with SS has been used to monitor environmental impacts of this waste. Increases in MBC, mineralizable C and enzyme activity were observed using different sludge rates from the Wastewater Treatment Plant of the municipality of Barueri - SP, Brazil (Fernandes et al., 2005).

The hypothesis of this study is that application of sewage sludge to soil leads to improved soil fertility and improved soil biological and biochemical properties and can thus at least partially replace mineral fertilizers.

This study evaluated chemical and biochemical properties of two *Latossolos Vermelhos*, Dystrophic and Eutroferric, under corn cultivation and after 16 years of annually sewage sludge application in different rates.

MATERIAL AND METHODS

Treatments were installed in 2012 in two areas. Area 1 consisted of a Eutroferric *Latossolo Vermelho*

(LVef) and Area 2 consisted of a Dystrophic *Latossolo Vermelho* (LVd). For both the areas, the same plots of the experiment initiated in the 1997/98 growing season in Jaboticabal, São Paulo, Brazil (21° 15' 22" S and 48° 15' 18" W, altitude 618 m asl) were used. The local climate is classified as Aw type, according to Köppen, i.e., tropical climate with dry winter (Volpe and Cunha, 2008). Mean monthly rainfall and temperature in the 2012/2013 growing season are shown in figure 1. The chemical analysis prior to the experiment was shown in table 1.

A randomized block design was used with four treatments (SS rates) and five replications, in 60 m² (6 × 10 m) plots.

In the 1st year, SS rates were zero (control without SS application and no mineral fertilization); 2.5; 5.0 and 10.0 Mg ha^{-1} SS on a dry weight basis. The rate of 5 Mg ha^{-1} SS was set to supply the corn N demand, assuming that ⅓ of N contained in SS would become available to the crop. From the 2nd year, it was decided to fertilize the control according to soil analysis and recommendations of Raij et al. (1997). From the 4th year onwards, plots of 2.5 Mg ha^{-1} started to receive 20 Mg ha^{-1}, which were used until the 16th year. Thus, in the 16th year, treatment rates consisted of a control without SS but with mineral fertilization; 5; 10 and 20 Mg ha^{-1} SS (dry weight basis).

Mineral fertilizers were applied to the SS treatments, if necessary, to ensure the supply of equal amounts of N, P, K.

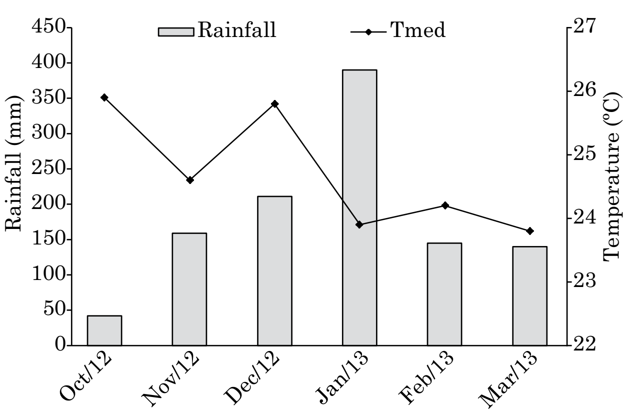

Figure 1. Mean monthly rainfall and temperature in the 2012/2013 growing season.

The test plant was corn; however, in the 2004/2005 and 2008/2009 growing seasons, crop rotations with sunflower and in 2005/2006, with crotalaria were applied, which are both appropriate techniques for sustainable agriculture (Table 2).

Sewage sludge was supplied by various sewage treatment plants of the Basic Sanitation Company of São Paulo State (SABESP) (Table 3).

The chemical characterization of sewage sludge was done in samples formed by six sub-samples collected from different points of the waste mass (ABNT, 2004). Nitrogen concentration was determined by Kjeldahl method (Melo, 1974); P by spectrophotometry (Malavolta et al., 1997); K by flame photometry (Sarruge and Haag 1974); S by turbidimetry (Vitti, 1989); and the other elements by atomic absorption spectrophotometry in digestion extract with $HNO_3 + H_2O_2 + HCl$, according to method 3050B (USEPA, 1996).

The results were N 46.9 g kg^{-1}; P_2O_5 20.84 g kg^{-1}; K_2O 2.8 g kg^{-1}; Ca 20.1 g kg^{-1}; Mg 1.33 g kg^{-1}; Cl 1.14 g kg^{-1}; S 2.38 g kg^{-1}; Cu 372.55 mg kg^{-1}; Fe 4,100 mg kg^{-1}; Mn 529 mg kg^{-1}; Zn 748 mg kg^{-1}; B 51.65 mg kg^{-1}; Mo 1.77 mg kg^{-1}; Cd 1.27 mg kg^{-1}; Cr 15.45 mg kg^{-1}; Pb 57.28 mg kg^{-1}; Ba 2.36 mg kg^{-1}; Ni 34.53 mg kg^{-1}; Co 15.04 mg kg^{-1}; As 4.50 mg kg^{-1}; Hg 3.20 mg kg^{-1}; Se <0.05 mg kg^{-1}, on a dry weight basis.

Sewage sludge was applied by broadcasting at 5 % moisture level, and evenly distributed over the whole area, and incorporated by light harrowing (to a depth of 0.10 m).

After sewage sludge application, planting furrows were drawn in the plots, spaced 0.90 m apart, and mineral fertilizer (NPK) was applied along them (Table 4).

The corn hybrid (2B710PW DOW) was sown after mineral fertilization, using seven to eight seeds per linear meter.

Sidedressing was performed 40 days after sowing (Table 4).

At 68 days after emergence (DAE), soil samples were collected from the 0.00-0.20 m layer, with a Dutch auger. Ten single samples were collected from each plot, in and between rows, and then mixed to form one composite sample per plot. Thereafter, the material was maintained in plastic bags in

Table 1. Chemical analysis of Eutroferric *Latossolo Vermelho* (LVef) and Dystrophic *Latossolo Vermelho* (LVd) prior to the experiment in the growing season 2012/2013

Soil	P-resin	OM	pH($CaCl_2$)	K^+	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	V
	mg dm^{-3}	g dm^{-3}		mmol$_c$ dm^{-3}						%
LVd	44	20	5.7	2.2	25.7	10.6	16	38.5	54.5	70.7
LVef	67	34	5.7	4.9	41.8	18.6	22	65.3	87.3	74.8

P-resin: extracted by anionic resin; OM: organic matter, Walkley-Blake method; Ca, Mg: extracted by 1 mol L^{-1} KCl; H+Al: by titration after extraction with 0.5 mol L^{-1} calcium acetate at pH 7; SB: sum of bases; CEC: cation exchange capacity; V: base saturation.

Table 2. Crop plants tested in Eutroferric and Dystrophic *Latossolos Vermelhos* (Oxisol), which have been fertilized with sewage sludge for 16 years

Period	Crop
1997/1998 to 2003/2004	Corn
2004/2005	Sunflower
2005/2006	Crotalaria
2006/2007 to 2007/2008	Corn
2008/2009	Sunflower
2009/2010 to 2013/2014	Corn

Table 3. Sewage treatment plants that provided sewage sludge for the experiment throughout 16 years

Period	Municipality
1997 to 2006	Barueri
2007 and 2008	Franca
2009 and 2010	Barueri
2011 to 2013	Monte Alto

Table 4. Contents of N, P_2O_5 and K_2O applied to the treatments as mineral fertilizers in the 16th year

Nutrient	Sewage sludge rate (Mg ha^{-1})			
	0	5	10	20
	At sowing (kg ha^{-1})			
N	30	-	-	-
P_2O_5	50	-	-	-
K_2O	50	36	22	-
	40 days after sowing (kg ha^{-1})			
N	140	-	-	-
P_2O_5	-	-	-	-
K_2O	40	40	40	40

N: ammonium sulfate (18 % N), P: single superphosphate (18 % P_2O_5) and K: potassium chloride (60 % K_2O).

polystyrene boxes filled with ice, which were sent to the laboratory, where they were sieved (<2 mm), and stored in a refrigerator until biological and biochemical analysis. The samples were air-dried in the shade, and sieved (<2 mm) for chemical analysis.

Soil chemical properties were determined by methods described by Raij et al. (2001). We assessed pH ($CaCl_2$), contents of Ca, Mg, K, P, organic matter, H+Al, sum of bases (SB), cation exchange capacity (CEC) and base saturation (V).

Microbial biomass carbon (MBC) was evaluated by a method proposed by Mendonça and Matos (2005), in which electromagnetic energy (microwaves) was used to cell disruption releasing the intracellular compounds. For each sample, we used two subsamples, a fumigated and a non-fumigated. The fumigated subsamples were irradiated in a microwave oven at 700 W for 12 min. Organic C content was determined in irradiated and non-irradiated subsamples by the Walkley-Black method (Embrapa, 1997).

Soil samples were incubated with and without triphenyl tetrazolium chloride (TTC) at 30 °C for 24 h, and followed by quantification of triphenyl formazan (TPF) by spectrophotometry to determine dehydrogenase activity (Melo et al., 2010).

To assess acid phosphatase activity, soil samples were incubated with and without sodium *p*-nitrophenyl phosphate, at 37 °C and pH 6.5 for 30 min. After incubation, the amount of *p*-nitrophenol produced was determined (Eivazi and Tabatabai, 1977).

For arylsulfatase activity assessment, we applied a method similar to that for acid phosphatase, using potassium *p*-nitrophenyl sulfate as substrate in acetate buffer medium, at 37 °C and pH 5.8 for 1 h, and then quantifying *p*-nitrophenol content.

The potential of the fluorescein diacetate hydrolysis (FDA) was assessed by soil sample incubation with and without FDA, evaluating the content of fluorescein produced (Melo et al., 2010).

Soil basal respiration was determined by incubating fresh soil samples in airtight jars for 48 h, and respired CO_2 was trapped in NaOH solution, which was titrated after the incubation period for the quantification of the respired CO_2 (Araújo et al., 2010). The metabolic quotient was calculated from the data of C of microbial biomass and basal respiration (Anderson and Domsch, 1993).

The data were subjected to variance analysis using SAS software (SAS, 2009); and means were compared by the Duncan test at 5 %.

RESULTS AND DISCUSSION

The pH($CaCl_2$) ranged from 4.4 to 4.8 for LVef and, from 4.3 to 4.5 for LVd. The treatment effect was only detected in LVef, and the highest pH was observed at rate 10 Mg ha^{-1} of SS, which differed from 5 Mg ha^{-1} (Table 5). Despite the below ideal pH for corn, the yield was above the national average, which may be related to waste application to the soil. Some authors reported a pH increase and some a decrease in response to organic matter addition to the soil. The pH decrease may be due to nitrification process (Yan et al., 1996), in which ammonium is oxidized to nitrite and nitrate, releasing H^+ ions, resulting in pH reduction. A reduction of 0.4 in pH in soils supplied with SS compared to control was observed by Stamatiadis et al. (1999). Other authors reported increased pH when applying organic material (Hoyt and Tuner, 1975; Hue and

Amien, 1989; Franchini et al., 1999). In this case, H^+ ions from soil solution are exchanged with cations from carboxyl groups of organic matter (Miyazawa et al., 2000).

Soil pH is important in mobility and availability of potentially toxic elements. At increasing pH levels, H^+ ions are dissociated from OH groups of organic matter and Fe and Al oxides. It increases negative charges, which enables a greater adsorption of cation trace elements (Alleoni et al., 2005).

Soil organic matter (OM) content varied from 25.8 to 26.4 g dm^{-3} in LVef, and from 18.4 to 21.8 g dm^{-3} in LVd. Treatment differences were only observed for LVd, in which OM content was only greater at the rate of 20 Mg ha^{-1} SS compared with control; however, there were no differences between rates (Table 5).

Significant, but temporary, effects on OM content were observed at rates of up to 32 Mg ha^{-1} SS (Melo et al., 1994). Only at high applications, above 75 Mg ha^{-1}, the effect of SS on OM contents stayed longer (Santos et al., 2010). This information is important because it reinforces SS as an alternative to enhance OM levels, especially in OM poor soils. On the other hand, it is not advisable to apply SS to sandy soils, since it can cause contamination of groundwater by trace elements and nitrate leaching.

Phosphorus content varied from 45.2 to 67.8 mg dm^{-3} in LVef, and between 41.8 to 79.8 mg dm^{-3} in LVd (Table 5). Significant differences were observed among LVd treatments; the rates of 10 and 20 Mg ha^{-1} SS induced higher P contents than the other rate. In the LVef, SS addition resulted in higher P contents than in the control. Phosphorus increment has been observed in soils treated with SS, indicating this waste as a good source of P for soils (Nascimento et al., 2004). Organic waste can improve P availability, as it is source of the element, be it in mineral form or by organic P mineralization, and by providing ions that compete with phosphate for adsorption sites (Abreu Jr et al., 2002).

Despite a balanced K content during fertilization of the treatments, significant differences were detected between the two soils. In LVef, the highest content was observed after application of 10 Mg ha^{-1} SS, compared to the control. Potassium leaching in the control treatment, in which this element had been added in a completely soluble form, can explain this fact. Even though SS also enriched the soil with K in combination with organic material, no significant increase in CEC was detected. Rainfall records of the crop cycle showed a rainy period in January 2013, which was the time of soil sampling. On the other hand, in LVd, the highest K content was observed in the control treatment, which differed only from 20 Mg ha^{-1} SS. This was rather unexpected, since in this sandy soil, a larger amount of K leaching was expected than in LVef. This behavior may be related to the high biological activity observed in control, characterized by dehydrogenase activity (Table 6), where the K absorbed by microbial flora would not have been measured by the used method. These results confirm other authors, who stated that SS application must be performed together with K supplementation (Melo et al., 2007).

Table 5. Chemical properties of Eutroferric and Dystrophic *Latossolos Vermelhos* in the 0.00-0.20 m layer, treated for 16 years with sewage sludge at different rates

Treatment	P	OM	pH($CaCl_2$)	K^+	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	V
Mg ha^{-1} SS	mg dm^{-3}	g dm^{-3}		mmol$_c$ dm^{-3}						%
				Eutroferric *Latossolo Vermelho*						
0	45.2 b	25.8 a	4.5 ab	3.9 b	16.2 a	5.2 a	43.6 a	25.3 a	68.9 a	37.1 a
5	61.2 a	26.2 a	4.4 b	5.1 ab	16.4 a	5.2 a	52.8 a	26.7 a	79.5 a	34.0 a
10	67.0 a	26.4 a	4.8 a	5.7 a	19.0 a	6.2 a	43.2 a	30.9 a	74.1 a	41.6 a
20	67.8 a	26.0 a	4.6 ab	4.4 ab	19.0 a	5.8 a	45.2 a	29.2 a	74.4 a	40.1 a
Mean	60.4	26.1	4.6	4.8	17.7	5.6	46.2	28.0	74.2	38.2
CV (%)	14.7	6.9	4.9	17.9	17.4	20.8	20.6	15.3	10.6	19.5
				Dystrophic *Latossolo Vermelho*						
0	41.8 c	18.4 b	4.5 a	5.8 a	9.2 a	2.2 a	31.4 a	17.2 a	48.6 a	36.0 a
5	61.2 b	19.6 ab	4.5 a	3.7 ab	11.6 a	3.2 a	37.0 a	18.5 a	55.5 a	34.9 a
10	79.6 a	21.6 ab	4.3 a	3.6 ab	12.6 a	3.0 a	38.4 a	19.2 a	57.6 a	33.6 a
20	79.8 a	21.8 a	4.3 a	3.0 b	11.0 a	2.4 a	40.6 a	16.4 a	57.0 a	28.6 a
Mean	66.1	20.3	4.4	4.0	11.1	2.7	36.8	17.8	54.7	33.3
CV (%)	12.5	11.2	6.3	45.9	24.2	31.3	26.4	22.6	15.4	28.2

SS: sewage sludge on a dry weight basis; OM: organic matter, Walkley-Blake method; Ca, Mg: extracted by 1 mol L^{-1} KCl; H+Al: by titration after extraction with 0.5 mol L^{-1} calcium acetate at pH 7; SB: sum of bases; CEC: cation exchange capacity; V: base saturation; CV: coefficient of variation. Means followed by the same letter in the column do not differ from each other by the Duncan test at 5 %.

The other chemical properties related to soil fertility, such as Ca^{2+}, Mg^{2+}, H+Al, SB, CEC, and V were not affected by treatments (Table 5).

The Ca^{2+} content varied from 16.2 to 19.0 $mmol_c$ dm^{-3} in LVef, and from 9.2 to 12.6 $mmol_c$ dm^{-3} in LVd, which are considered very high values by Raij et al. (1997) but without affecting corn yield. A significant increase in Ca availability was found using SS rates up to 60 Mg ha^{-1} in two soil types, although the waste had been treated with lime (Nascimento et al., 2004).

The Mg^{2+} contents varied from 5.2 to 6.2 $mmol_c$ dm^{-3} in LVef, and from 2.2 to 3.2 $mmol_c$ dm^{-3} in LVd, which are considered mean values for LVef and low for LVd (Raij et al., 1997), which may have influenced corn yield negatively, especially in LVd, which was the most productive. Studies showed that sludge addition increases Mg concentrations in sugarcane, corn and sorghum leaves (Melo et al., 2000).

Base saturation varied from 34.0 to 41.6 % in LVef, and from 28.6 to 36.0 % in LVd, considered unfit for corn cultivation (Raij et al., 1997). This may be related to the low content of Mg^{2+} and high H+Al, which can be corrected for the following season by liming.

For potential acidity (H+Al), values ranged from 43.2 to 52.8 $mmol_c$ dm^{-3} for LVef, and from 31.4 to 49.6 $mmol_c$ dm^{-3} for LVd, but no differences were found among treatments. An increase in H+Al concentration at higher SS rates was observed by Trannin et al. (2007).

The sum of bases (SB) and cation exchange capacity (CEC) did not differ among treatments for both soils, but the improved cation retention by organic load from sludge becomes extremely important for soils with low CEC and poor OM content. It is the case in tropical climate and for soils in Northeastern Brazil, which are particularly OM poor (Oliveira et al., 2002). Organic matter increase contributes to CEC values, by generating negative charges because of the high concentration of OM in SS (Silva et al., 1995).

The two soils differed in response to the different SS rates in the biological and biochemical properties. In LVef, only acid phosphatase activity was affected by treatments, while in LVd, basal respiration, arylsulfatase activity and metabolic quotient were affected (Table 6).

Acid phosphatase activity, basal respiration and metabolic quotient had similar behavior when SS rate increased, and the rate of 20 Mg ha^{-1} differed from control. In general, there were no differences among the sewage sludge rates. The increase in acid phosphatase in response to some SS rates was expected, since phosphate esters are part of the residue composition, and at very high rates, the presence of trace elements could cause inhibition of the enzyme. Studying lentils and barley in a greenhouse experiment, Moreno et al. (2003) also found that phosphatase activity was higher in the treatments with SS, and attributed this to the substrates containing organic P.

Basal respiration and metabolic quotient increased with increasing SS rates, which was also as expected, due to the toxic effect of trace elements and other toxic substances (Martinez et al., 2006). The arylsulfatase activity in LVd had a contrary behavior, being higher in control, with no differences among rates. This result suggests that this enzyme class is more sensitive to the toxic components of SS. Arylsulfatase was positively affected by SS application and susceptible to toxic effects of trace elements at higher rates in studies of Kunito et al. (2001) and Marschner et al. (2003). These different results show that arylsulfatase performance depends on the soil type and fertility, be it natural or improved by human action (Melo et al., 2007).

Increasing values of microbial biomass carbon was found after industrial waste application at growing rates due to the availability of organic substrates and nutrients (Santos et al., 2011). After two years in a row application of SS to corn, Trannin et al. (2007) observed alterations in soil organic matter and microbial biomass carbon. Furthermore, Lopes (2002) also noted an increase in basal respiration with increases in OM and nutrients. The application of OM enables CO_2 release, which is mainly attributed to metabolically active populations, which are the most affected by heavy metal input in soil (Insam et al., 1996).

Thus, basal respiration increase in soil under application of 20 Mg ha^{-1} SS may have occurred due to elevated concentrations of trace elements in the SS. According to Brookes (1995), qCO_2 can be understood as microbial efficiency, since it is the relation of the energy required to maintain metabolic activities to the energy used for biomass synthesis; in many cases, energy consumption is high under stressful conditions.

Therefore, the applied SS rates for 16 years were not enough to affect microbial biomass in the soil. As stated by Lo et al. (1992), organic material of SS acts in metal complexation, reducing their short-term availability and consequently its toxicity. At the same time, it provides C and energy for soil microorganisms. However, an increase in basal respiration without a rise in microbial biomass indicates a toxic effect on the soil microbial flora, mainly at the highest rates.

The values of FDA hydrolysis indicated that overall soil microbial activity was not affected by SS application, which was corroborated by the activity of dehydrogenases. In other studies, FDA was not affected by applications of increasing rates of tannery sludge, cellulose and urban waste compound (Santos et al., 2011). Studing industrial sludge applied to corn, Trannin et al. (2007) observed that after two consecutive years with up to 24 Mg ha^{-1},

Table 6. Biochemical and biological properties in Eutroferric and Dystrophic *Latossolos Vermelhos* in the 0-0.20 m layer, treated for 16 years with sewage sludge at different rates

Treatment	MBC	Basal respiration	Dehydrogenase	Arylsulfatase	Acid phosphatase	FDA hydrolysis	qCO$_2$
Mg ha^{-1} SS	mg kg^{-1} C	mg kg^{-1} h^{-1} CO$_2$	mg kg^{-1} h^{-1} TPF	mg kg^{-1} h^{-1} PNP		mg kg^{-1} h^{-1} fluorescein	mg mg^{-1}
				Eutroferric *Latossolo Vermelho*			
0	412.23 a	3.10 a	0.92 a	12.29 a	126.88 b	41.51 a	0.0074 a
5	432.39 a	3.66 a	0.90 a	19.21 a	139.28 ab	41.69 a	0.0092 a
10	451.41 a	3.60 a	1.16 a	11.18 a	169.93 ab	39.84 a	0.0082 a
20	443.97 a	3.48 a	0.98 a	20.1 a	185.95 a	44.42 a	0.0080 a
Mean	435.00	3.46	0.99	15.7	155.51	41.87	0.0082
CV (%)	16.7	15.4	41.8	40.6	24.7	18.8	22.0
				Dystrophic *Latossolo Vermelho*			
0	241.58 a	1.80 b	0.49 a	14.45 a	79.72 a	34.07 a	0.0064 b
5	275.41 a	1.74 b	0.59 a	7.03 b	125.21 a	32.23 a	0.0076 ab
10	267.41 a	2.03 ab	0.87 a	8.60 b	131.84 a	40.89 a	0.0078 ab
20	294.88 a	2.72 a	0.80 a	7.42 b	117.4 a	39.53 a	0.0098 a
Mean	269.82	2.07	0.69	9.38	113.54	36.68	0.0079
CV (%)	24.9	28.0	51.4	43.0	33.8	17.3	20.7

SS: sewage sludge on a dry weight basis; TPF: triphenilformazan; PNP: p-nitrophenol; FDA: fluorescein diacetate hydrolysis; CV: coefficient of variation. Means followed by the same letter in the column do not differ from each other by the Duncan test at 5 %.

there were increases in C and N of microbial biomass, basal respiration and FDA hydrolysis.

Both dehydrogenase activity and FDA hydrolysis are soil properties used to quantify soil microbial activity. Under stressful conditions, microbial activity can increase without a rise in microbial biomass, which increases the metabolic quotient. This was observed in LVef, but no negative effects on dehydrogenase activity and FDA hydrolysis were detected. The high variation coefficient of the dehydrogenase activity (51.4 %) can explain this fact, since in terms of activity, there was an increase with growing rates of SS. In contrast, FDA had a very low variation coefficient (17.3 %), as well as variability among treatments. It is noteworthy that FDA hydrolysis does not express a specific enzyme activity, but the activity of a whole group of enzymes including lipases, esterases and proteases (Melo et al., 2010). Fluorescein diacetate can be hydrolysed by algae, protozoa and animal tissues, so that SS was not favorable to them.

In both soils and in all treatments, corn plants showed no phytotoxicity or nutritional deficiency symptoms. However, crop yield varied from 7.51 to 10.01 Mg ha^{-1} in LVef, and from 8.80 to 11.09 Mg ha^{-1} in LVd, data expressed with 13 % grain moisture (Table 7). Sewage sludge affected crop yield in LVef, reaching the highest yield in the treatment with 20 Mg ha^{-1} SS, which differed from the control but not among other treatments. In LVd, no significant effect of treatments was detected on corn yield; however, if CV was smaller, a difference probably will be detected since the difference between the rate 20 Mg ha^{-1} SS and the control was very similar in the two soils.

Table 7. Corn yield cropped in Dystrophic (LVd) and Eutroferric (LVef) *Latossolo Vermelho* treated for 16 years with sewage sludge at different rates

Treatment	Corn yield	
	LVef	LVd
Mg ha^{-1} SS	Mg ha^{-1}	
0	7.81 b	8.80 a
5	8.62 ab	9.49 a
10	9.28 ab	10.80 a
20	10.01 a	11.09 a
CV (%)	15.6	17.7

SS: sewage sludge on a dry weight basis; CV: coefficient of variation. Means followed by the same letter in the column do not differ from each other by the Duncan test at 5 %.

CONCLUSIONS

Sewage sludge increased phosphorus extracted by resin in both the *Latossolos Vermelhos*, Dystrophic and Eutroferric, and the organic matter content in the Dystrophic *Latossolo Vermelho*.

The waste at the rate 20 Mg ha^{-1} on a dry weight basis promoted increases in acid phosphatase activity in Eutroferric *Latossolo Vermelho*, basal respiration and metabolic quotient in Dystrophic *Latossolo Vermelho*.

The rate 20 Mg ha^{-1} sewage sludge on a dry weight basis did not alter the soil microbial biomass in both the *Latossolos Vermelhos*; in addition, it improved corn yields without inducing any symptoms of phytotoxicity or nutrient deficiency in the plants.

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