CHEMICAL AND MICROBIOLOGICAL ATTRIBUTES OF AN OXISOL TREATED WITH SUCCESSIVE APPLICATIONS OF SEWAGE SLUDGE(1)

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SUMMARY

Studies on sewage sludge (SS) have confirmed the possibilities of using this waste as fertilizer and/or soil conditioner in crop production areas. Despite restrictions with regard to the levels of potentially toxic elements (PTE) and pathogens, it is believed that properly treated SS with low PTE levels, applied to soil at adequate rates, may improve the soil chemical and microbiological properties. This study consisted of a long-term field experiment conducted on a Typic Haplorthox (eutroferric Red Latosol) treated with SS for seven successive years for maize production, to evaluate changes in the soil chemical and microbiological properties. The treatments consisted of two SS rates (single and double dose of the crop N requirement) and a mineral fertilizer treatment. Soil was sampled in the 0–0.20 m layer and analyzed for chemical properties (organic C, pH, P, K, Ca, Mg, CEC, B, Cu, Fe, Mn, Zn, Cd, Ni, and Pb) and microbiological properties (basal respiration, microbial biomass activity, microbial biomass C, metabolic quotient, microbial quotient, and protease and dehydrogenase enzyme activities). Successive SS applications to soil increased the macro- and micronutrient availability, but the highest SS dose reduced the soil pH significantly, indicating a need for periodic corrections. The SS treatments also affected soil microbial activity and biomass negatively. There were no significant differences among treatments for maize grain yield. After seven annual applications of the recommended sludge rate, the heavy metal levels in the soil had not reached toxic levels.

Index terms: municipal waste, organic matter, soil microbes, maize.

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RESUMO: *ATRIBUTOS QUÍMICOS E MICROBIOLÓGICOS DE UM LATOSSOLO TRATADO COM APLICAÇÕES SUCESSIVAS DE LODO DE ESGOTO*

Estudos realizados com lodo de esgoto (LE) têm demonstrado o potencial desse resíduo como fertilizante e, ou, condicionador do solo em áreas agrícolas. Apesar das restrições quanto ao conteúdo de elementos potencialmente tóxicos (EPT), como os metais pesados, e de patógenos, a aplicação de LE com baixos teores de EPT e devidamente higienizado poderá melhorar os atributos químicos e microbiológicos do solo. Em um Latossolo Vermelho com caráter férrico cultivado com milho, avaliaram-se as alterações nos atributos químicos e microbiológicos do solo em dois tratamentos com LE aplicado sucessivamente por sete anos: na dose recomendada para suprir o N requerido pela cultura do milho e no dobro da dose recomendada, bem como em um tratamento com adubação mineral. Foram coletadas amostras compostas de solo, na profundidade de 0–0,20 m, para determinação de atributos químicos (C-orgânico, pH, P, K, Ca, Mg, CTC, B, Cu, Fe, Mn, Zn, Cd, Ni e Pb) e microbiológicos (respiração basal, atividade microbiana, C da biomassa microbiana, quociente metabólico, quociente microbiano e atividade das enzimas protease e desidrogenase). A adição sucessiva de LE ao solo aumentou a disponibilidade de macro e micronutrientes para as culturas. Entretanto, na maior dose do LE houve redução significativa do pH, indicando a necessidade de correções periódicas da acidez do solo. A adição do LE causou efeito negativo sobre a atividade e biomassa da microbiota do solo. Não houve diferença significativa para a produção de grãos de milho entre os tratamentos. Após sete aplicações anuais de LE baseadas na necessidade do milho em N, os níveis de metais pesados no solo não atingiram níveis tóxicos.

Termos de indexação: resíduos urbanos, matéria orgânica, microbiota do solo, milho.

INTRODUCTION

The fast development of metropolitan regions generates ever- increasing amounts of all sorts of residues. Among these, sewage sludge (SS) has been studied as a possible nutrient source for crops in agricultural areas. The proposal of using SS in crop fields as a sustainable way of SS disposal is based on the high organic C and nutrient levels.

A typical SS usually contains around 40 % organic matter, 4 % N, 2 % P and lower amounts of other nutrients (Bettiol & Camargo, 2006). Sewage sludge has been used as organic fertilizer at rates equivalent to its NPK contents, such as the commercial fertilizer formula 4: 2.5: 1 (N: P: K). Also, this organic residue has been recommended as soil conditioner, since it increases soil water retention capacity, porosity and aggregate stability, due to its high organic matter content (Jorge et al., 1991).

Nevertheless, the fact that potentially toxic elements may be present in SS residues requires constant monitoring of the SS composition, prior to application to agricultural soils, to ensure the preservation of the environmental quality. This is particularly relevant because some toxic elements such as heavy metals may persist indefinitely in the soil and alter the environment quality. This means that plants can absorb toxic elements in quantities high enough to damage not only crop plants but also the health of animals and/or humans and to contaminate the entire food chain (Berton, 2000).

In Brazil, the few studies in the literature that describe the effects of long-term successive SS field applications on soil chemical, physical and biological properties, are generally positive reports. Fernandes et al. (2005) evaluated the effects of two SS types on soil microbiological properties after four years of successive applications and found a significant increase in microbial activity related to the applied SS rates. Nogueira et al. (2008) studied soil treated with SS for nine years and found similar maize dry matter and grain yield as on chemically fertilized plots. Also, these authors found that among the investigated heavy metals, only the Zn contents increased in the soil, as a consequence of SS application. De Maria et al. (2010) observed lower soil density, greater porosity and improved soil physical quality after six successive SS applications.

Nevertheless, the results may vary not only according to the SS quantity applied to the soil but also to the SS type, which depends on the residue origin and treatment method.

The purpose of this study was to quantify the accumulated effects of seven successive annual SS (unlimed) applications on chemical and microbiological soil properties, and also to determine whether the SS rate calculated according to the maize N demand improves soil fertility and stimulates microbial activity and if the maize yield remains stable even without additional mineral fertilizer (N-P) and excessive accumulation of potentially toxic elements.

MATERIALS AND METHODS

The experiment was carried out at the experimental station of the Instituto Agronômico Campinas, State of São Paulo, Brazil (22 º 52 ' 44 " S, 47 º 04 ' 56 " W; 650 m asl). The soil was classified as a clayey eutroferric Red Latosol, according to the Brazilian System of Soil Classification (Embrapa, 2006), somewhat similar to Typic Haplorthox (USDA) and Rhodic Ferrasol (FAO). The climate was defined as a humid tropical with a clearly defined rainy summer and dry winter, with an average rainfall of 1,400 mm year^{-1} and average annual temperature of 20.5 °C.

The experiment was arranged in a randomized complete block design with three treatments and four replications. The slope of the 4 x 25 m plots had 10 % declivity and the treatments consisted of two sewage sludge (SS) rates (L1 and L2) and a single annual mineral fertilizer rate (as control treatment), applied from 2001 to 2007.

The SS used in the experiment came from the municipal Wastewater Treatment Plant of the city of Jundiaí, State of São Paulo. This domestic waste sludge is treated biologically and aerobically in complete-mix aerated lagoons and then a settling pond for solids removal. The process is continued by centrifugation and dewatering for 120 days, using a mechanical rotator. The pathogen concentration in this residue was classified as B and there are no restrictions to agricultural use with regard to the heavy metal concentrations, according to the Brazilian law (directive P 4230, CETESB, 1999). The chemical characteristics of the sewage sludge used in this long-term experiment are presented in table 1.

The SS rates (L1 and L2) were calculated in the first year of the experiment, based on maize N demand. Thus, according to the directive P 4230 (CETESB, 1999) the recommended SS rate was calculated by equation 1:

$$\text{SS application rate (t ha}^{-1}\text{)} = \text{N recommended rate (kg ha}^{-1}\text{)/SS available N (kg t}^{-1}\text{)} \qquad (1)$$

where, the recommended N quantity for maize is 80 kg ha^{-1}, according to Raij et al. (1996); and the SS available N was calculated by equation 2, considering a surface application followed by incorporation:

Table 1. Chemical composition of the sewage sludge applied in a Red Latosol cultivated for maize cultivation during the period of 2001-2007

Parameter[(1,2)]	Year						
	2001	2002	2003	2004	2005	2006	2007
pH	6.6	7.2	-	7	-	6.8	5.4
Moisture (%)	68.2	65.7	-	73.4	-	65.6	67.2
Volatile solids (%)	54.8	57.3	51.2	62.3	57.9	58.9	56.3
Organic – C (g kg^{-1})	325	298	225	288	292	214	313
Nitrogen – total N (Kjeldahl, g kg^{-1})	28.3	27	-	28.9	31	32.1	28.6
Ammonium – N (mg kg^{-1})	577	438	-	266	-	287	289
Nitrate – nitrite N (mg kg^{-1})	37.2	138.7	-	2.3	-	33.4	30.6
Aluminum – Al (g kg^{-1})	16.4	18.8	19.5	23.3	18.14	21.3	18.6
Arsenium – As (mg kg^{-1})	<0.01	<0.01	<0.01	<0.01	<0.5	<0.01	<0.5
Boron – B (mg kg)	12.3	11.7	4.3	12.7	19.8	77.8	31.3
Cadmium – Cd (mg kg^{-1})	5.8	6.6	6.4	13.5	12.5	9.4	3.9
Calcium – Ca (g kg^{-1})	12.3	9.8	8.09	7.32	38.2	18.3	9.2
Lead – Pb (mg kg^{-1})	283	207	134	100	157	129	183
Copper – Cu (mg kg^{-1})	284	865	576	872	540	237	304
Chromium – Cr (mg kg^{-1})	149	188	153	<0.01	150	166	200
Sulfur – S (g kg^{-1})	26.1	26.8	25.6	12.1	14.5	16.3	18
Iron – Fe (g kg^{-1})	26	24.1	21.3	17.2	23.3	22.3	21.9
Phosphorus – P (g kg^{-1})	6.6	7.2	5.6	13	6.3	10.7	7.1
Magnesium – Mg (g kg^{-1})	2.1	1.7	1.4	1.6	1.7	1.6	1.5
Manganese – Mn (mg kg^{-1})	677	693	476	441	560	648	749
Mercury – Hg (mg kg^{-1})	<0.01	<0.01	<0.01	<0.01	<1.0	<0.01	<0.5
Molybdenum – Mo (mg kg^{-1})	<0.01	<0.01	<0.01	<0.01	7.5	11.3	6.1
Nickel – Ni (mg kg^{-1})	41.8	35.5	37.4	35.4	29.5	42.7	189
Selenium – Se (mg kg^{-1})	<0.01	<0.01	<0.01	-	<1.0	<0.01	<0.5
Zinc – Zn (mg kg^{-1})	1365	1738	1295	941	1039	1551	1450
Potassium – K (mg kg^{-1})	1500	1000	826	11470	967	932	1074
Sodium – Na (mg kg^{-1})	900	1700	943	8102	1138	1282	1385
Barium – Ba (mg kg^{-1})	-	-	-	-	-	235	460

[(1)] Ammonium and nitrate N were analyzed on wet samples by digestion with dichromate and determined by colorimetry. Metals were analyzed according to recommendations of US-EPA, SW-846 and method 3051. Sodium (Na) and potassium (K) were determined by flame photometry and the other elements by ICP-AES. SS moisture and volatile solids were determined by mass loss at 60 and 500 °C, respectively. SS pH was determined in water extract (1:5). [(2)] The concentration values were calculated on a dry matter basis.

$$\text{SS available N} = (\text{MF}/100) \times (N_{Kj} - N_{NH_3}) + N_{NH_3} + (N_{NO_3^-} + N_{NO_2^-}) \quad (2)$$

where N_{Kj} = total nitrogen (Kjeldahl) in mg kg^{-1}; N_{NH_3} = ammonium-nitrogen in mg kg^{-1}; $N_{NO_3^-}$= nitrate-nitrogen in mg kg^{-1}; $N_{NO_2^-}$ = nitrite-nitrogen in mg kg^{-1}; MF = mineralization fraction, determined in a laboratory incubation test.

Based on this equation, the available N was around 1/3 of the total N (8 kg t^{-1}) in the SS; consequently, the recommended SS rate (L1) was 10 t ha^{-1} $year^{-1}$ and L2, twice as much (20 t ha^{-1} $year^{-1}$), on a dry matter basis. The control treatment consisted of SS-free mineral fertilizer (MF) at the recommended rate (equivalent to L1) for comparison (80 kg ha^{-1} N).

The mineral fertilizer (MF) treatment consisted of N (6 kg ha^{-1}), P_2O_5 (21 kg ha^{-1}) and K_2O (12 kg ha^{-1}) at sowing and N side dressing (75 kg ha^{-1}). Treatments (L1 and L2) with sewage sludge were complemented with mineral K fertilizer at the same rate as in the MF treatment (12 kg ha^{-1} K_2O), at each sowing.

The plant residues on all experimental plots were incorporated into the soil. Sewage sludge was also manually applied and hoed into the soil to a depth of 10 cm. Maize was grown on the plots in the summer (0.90 m between rows and five plants per meter). In fall and winter, the soil was left fallow with plant residues on the surface.

In November 2008, the 0–20 cm soil layer was sampled using a hand auger. Each composite soil sample was represented by 15 subsamples. The composite soil samples were homogenized, air dried, passed through a 2 mm sieve and submitted to chemical analyses, as described by Raij et al. (2001): pH in 0.01 mol L^{-1} $CaCl_2$ solution; organic C by humid digestion and colorimetric determination; P, K, Ca, and Mg by ion exchange resin extraction and determination by colorimetry (P) and flame photometry (K, Ca and Mg); potential acidity by the SMP buffer solution method; S by extraction with 0.01 mol L^{-1} $Ca(H_2PO_4)$ solution and determination by spectrophotometry; B by hot water extraction and colorimetric determination; Cu, Fe, Mn, Zn Cd, Pb, Ni, and Cr by extraction with DTPA (pH 7.3) solution and determination by atomic absorption spectrometry.

An aliquot sample of the original soil sample (before air-drying) was cooled (5 °C) for the microbiological analyses. Basal respiration (BR) was measured as follows: 100 g soil was moistened to 60 % of the maximal water retention capacity and incubated at 25–30 °C for 5 days in the dark. The released CO_2 was captured in 10 mL of 0.01 mol L^{-1} NaOH solution, to which 1 mL of $BaCl_2$ (50 % m/v) was added, plus 2 drops of phenolphthalein and titrated with 0.1 mol L^{-1} HCl solution.

Microbial activity (MBA) was measured according to the method suggested by Werf & Vestrate (1987), modified as follows: before the beginning of the second sample incubation period, the sample was incubated for 10 h with 120 mg glucose, 30 mg yeast extract, 45 mg NH_4Cl, 12 mg $MgSO_4.7H_2O$ and 10 mg KH_2PO_4 and titrated with 0.01 mol L^{-1} HCl solution, as reported by Islam (2000). The data were multiplied by a coefficient (0.283) convert the "respired C" into microbial activity. Microbial biomass C (MBC) was determined by the method of fumigation-extraction (Vance et al., 1987). The metabolic quotient (qCO_2) was obtained by the MBC/BR ratio and indicates the efficiency of the microbial community to incorporate C into its own biomass (Anderson & Domsh, 1989). The microbial quotient (Qmic) was obtained by the MBA/org-C ratio (Tótola & Chaer, 2002). The protease enzyme activity was determined using the method described by Ladd & Butler (1972) and the dehydrogenase activity (DHA) according to Casida et al. (1964).

The data were subjected to analysis of variance by the F test and treatment means were compared by the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

Samples of the sewage sludge (SS) used during the long-term experiment were analyzed and the data used to estimate the total amounts of chemical elements applied to the soil, considering both SS rates 10 and 20 t ha^{-1} (Table 2). The main SS contribution to soil fertility was related to C addition, calculated to be around 20 t ha^{-1} after seven years in the L1 treatment. Soil macronutrient accumulation was ranked in the following decreasing order: Ca > P > K > Mg.

The P/K ratios (5:1) of sewage sludge were well above the recommended ratio (1.5:1) for maize in Oxisols, according to Raij et al. (1996). These high P/K ratios evidenced the need for K complementation in fields fertilized with SS, to supply enough K to maize and N:P:K in adequate proportions, as proposed by Pratt et al. (1977) and confirmed by Simonete et al. (2003) under greenhouse conditions. In this experiment, the plots with sewage sludge were complemented with mineral K, as previously described.

Calcium and Mg quantities added by SS rates reached ratios of up to 10:1 and indicated the need for monitoring soil Mg concentrations. According to Souza et al. (2007), adequate Ca/Mg ratios can vary according to crop and soil, but should usually be around 3:1 or 4:1 (molar ratio). Muñoz Hernandez & Silveira (1998) observed that Ca:Mg ratios > 3:1 caused a decrease in maize growth and grain yield, due to the antagonistic effect of Ca on Mg uptake by plants. Obviously, these nutrient ratios must be evaluated, based on the available rather than the total element concentrations in the soil, once the ratios based on the latter are not relevant. Over the course of time, the amounts of S

Table 2. Total amount of chemical elements disposed in the soil by the sewage sludge rates of 10 and 20 t ha^{-1} year^{-1}, respectively L1 and L2, during the period of 2001-2007

Element	L1	L2
	kg ha^{-1} (1)	
Organic - C	19550	39100
Nitrogen - N (Kjeldahl)	1759	3518
Iron	1561	3122
Sulfur	1394	2788
Aluminum	1360	2721
Calcium	1032	2064
Phosphorus	565	1130
Potassium	178	355
Sodium	155	309
Magnesium	116	232
Zinc	94	188
Manganese	42	85
Copper	37	74
Ammonium - N	19	37
Lead	12	24
Chromium	10	20
Barium	7	14
Nickel	4	8
Nitrate – nitrite - N	2	5
Boron	2	3
Cadmium	1	1
Molybdenum	0.25	0.50
Arsenium	ND(2)	ND
Mercury	ND	ND
Selenium	ND	ND

(1) On a dry matter basis. (2) ND: not determined, due to the low concentration in the original residue which was below the detection limit by the analytical method used.

applied to the soil also became significant, which is an important aspect, since usually no specific S source is included in commercial fertilizers for commercial crops.

The soil organic C concentration was significantly higher in the SS-treated than in the chemically fertilized (MF) plots (Table 3). This result was expected, since the SS is a high org-C content residue. Soil org-C increased 23.8 % in SS-treated plots (10 t ha^{-1}) compared to MF-plot, corroborating results reported by Melo et al. (1994), Oliveira (2000), Melo et al. (2004) and Chiba et al. (2008). These authors observed increasing soil org-C contents with the increasing sewage sludge rates applied.

Similarly, the L1 and L2 SS rates induced increases in soil P concentrations (resin extract, available P) of 120 and 240 %, respectively, compared to the MF-treatment. Similar increases in soil available P were reported by Berton et al. (1989), in SS-treated soils growing maize in a greenhouse and by Simonete (2003), in a field experiment. Chiba et al. (2009) pointed out that SS contributes significantly to the P crop supply. These authors reported that 30 % of the sugarcane P demand was supplied by SS applied to the soil.

It is important to emphasize the high P application to soil during this long-term experiment to explain the increase in P availability in the SS-treated plots, calculated as around 565 and 1,130 kg ha^{-1} of total P in the SS applications, corresponding to 80.71 and 161.43 kg ha^{-1} year^{-1} against 21 kg ha^{-1} P in the MF-treatment. The higher soil available P contents found in the SS plots may be attributed to: (a) the presence of water-soluble P in the SS (Berton & Pratt, 1997); (b) mineralization of org-P present in the soil after SS application (Sattel & Morris, 1992); (c) organic acid release from SS decomposition and subsequent reaction with P in soil solution, resulting in P-organic acid complex molecules; this reaction would prevent precipitation of soluble-P as well as block the adsorption site for P in the solid phase, thus decreasing the soil adsorption capacity for P (Nagajarah et al., 1970); and (d) the higher soil CEC values observed in SS-treated plots might also have contributed to increase the repulsion charges between phosphate anion and the adsorbent surface, thus decreasing the electrostatic potential of the anionic adsorption surface (Barrow, 1985). It is highlighted that the increase in soil-available P concentrations occurred after seven annual SS applications, with simultaneous pH decrease, which may also have induced Fe-P and Al-P solubilization. During the first two years of this field experiment, the available P concentrations in SS-treated soils were similar to those found in MF-treated plots (Galdos et al., 2004).

The significant pH reduction observed in SS-treated plots (Table 3) was followed by a significant reduction in the soil-available Ca and Mg concentrations and increased potential acidity (H + Al). According to Raij et al. (1996), the soil acidity values in the MF plots can be classified as medium (pH 5.1 to 5.5) for field crops, but the values in the SS-treated plots were classified as high (pH 4.4–5.0, L1 treatment) and very high (pH < 4.4, L2 treatment).

SS application to agricultural areas can increase or decrease soil acidity, depending on the type of SS treatment in the Wastewater Treatment Plants (WTP). SS treated with hydrated calcium oxide during the disinfection or coagulation process is an alkaline residue that increases soil pH, as observed by Berton et al. (1989).

The soil pH decrease observed in the SS-treated plots might also be due to the H$^+$ release during org-N mineralization from organic residues and to the increment of soil org-C added by SS applications, also causing pH decreases as well. Thus, the decreased pH values observed in this study might be attributed to the type and composition of sewage sludge used, which specifically was only centrifuged with polyelectrolytes and not treated with hydrated CaO.

At low pH values, SS-treated soils tend to have a higher availability of most heavy metals, i.e., SS applications must be monitored to prevent food chain

Table 3. Soil chemical attributes at 0–0.20 m depth layer after addition of sewage sludge (SS) (L1 = 10 t ha^{-1} and L2 = 20 t ha^{-1}) and mineral fertilizer treatments

Treatment	Org-C	pH	P-res	K^+	Ca^{2+}	Mg^{2+}	H + Al	CEC	V
	g kg^{-1}		mg dm^{-3}	mmol$_c$ dm^{-3}					%
L2	17.74A	3.9C	52.75A	1.48B	8.50B	2.25C	109.75A	122.20A	10.25C
L1	15.75A	4.6B	39.00B	2.05AB	19.25A	7.25B	52.25B	80.90B	35.25B
MF	12.72B	5.3A	25.50C	2.68A	25.50A	14.50A	28.00C	70.53B	60.25A
CV (%)	12.15	3.39	12.88	14.03	17.59	23.84	14.43	9.41	9.88
F test	0.0246	0.0001	0.0008	0.0030	0.0007	0.0003	0.0001	0.0003	0.0001

Means followed by the same letter in the same column do not differ by Tukey test ($p < 0.05$).

contamination. Berton et al. (1997) does not recommend residue applications containing high amounts of Cu, Cd, Ni and Zn to the soil, at soil pH < 5.3.

In relation to micronutrients, higher soil concentrations were observed in SS-treated plots (Mn > Fe > Zn > Cu >> B, Table 4). SS application increased the availability of these elements in comparison to MF treatment. Despite the 200 % increase in soil Cu-DTPA concentrations observed in SS-treated plots, and the fact that these values were well above the level considered high for agricultural areas (> 0.8 mg dm^{-3}, according to Raij et al., 1996), the available Cu concentrations were below the toxicity limit for plants (20 mg dm^{-3}) established by Malavolta (2006). Similar results were found for the available concentrations of Mn, Zn and Fe (Table 4).

Increased soil basal respiration (BR) was observed with the SS rates, reaching 36 and 78 % higher values in SS than in MF plots, with L1 and L2 rates, respectively (Table 5). This confirmed results reported by Cardoso & Fortes Neto (2000) and Fernandes et al. (2005). Positive correlations were observed between BR values and the soil concentration increases of P ($r = 0.65$; $p \le 0.02$), B ($r = 0.59$; $p \le 0.04$); Fe ($r = 0.55$; $p \le 0.05$) and CEC ($r = 0.74$; $p \le 0.006$), as a consequence of the SS chemical composition and the org-C quantity supplied by SS addition ($r = 0.84$; $p \le 0.001$). Nevertheless, according to Islam & Weil (2000) and Fernandes et al. (2005), high soil respiration indices might indicate an ecological disequilibrium or a high level of ecosystem productivity. Therefore, a more reliable interpretation by means of the metabolic quotient ($q\mathrm{CO_2}$) is suggested, that is, the soil respiration rate per unit of microbial biomass.

A significant decrease in microbial activity (MBA) was observed in SS-treated than in MF plots, whereas no significant differences were observed among microbial biomass carbon values (Table 5). MBA and MBC were positively correlated ($r = 0.53$; $p \le 0.05$), that is, the biomass activity was proportional to its mass quantity, but not related with basal respiration. These results suggested a possible stress caused by increases in soil heavy metal contents in SS-treated plots, or by higher soil acidity as observed by Melo (2006). Nevertheless, Cardoso & Fortes Neto (2000) and Fernandes et al. (2005) observed MBC increase with increasing SS rates even at high heavy metal levels. Therefore, it was inferred that the MBA and MBC decrease observed in the this study might be attributed to the lower soil pH values found in SS-treated plots (Table 3), which were below the values reported by those authors.

Besides, a significant increase in $q\mathrm{CO_2}$ was observed in the SS over the MF-treatment (Table 5). This metabolic quotient may be considered a potential environmental stress index, since high $q\mathrm{CO_2}$ values indicate a great energy demand of the microbial community for its maintenance, with consequent decrease of the microbial quotient (Qmic), which represents the efficiency of org-C conversion into biomass. According to Islam & Weil (2000) and Fernandes et al. (2005), $q\mathrm{CO_2}$ is negatively correlated with soil quality, i.e., high $q\mathrm{CO_2}$ values indicate microbial community stress, disturbance or functional

Table 4. Soil micronutrient availability at the 0–0.20 m depth layer, in plots treated with sewage sludge at rates of 20 t ha^{-1} (L2), 10 t ha^{-1} (L1) and mineral fertilizer (MF)

Treatment	B	Cu	Fe	Mn	Zn
	mg dm^{-3}				
L2	0.57A	15.73A	83.75A	72.18A	23.50A
L1	0.35B	12.38B	45.50B	80.75A	20.08A
MF	0.21C	5.78C	11.00C	27.68B	1.40B
CV (%	11.26	9.54	20.53	29.35	14.95
F test	0.0001	0.0001	0.0001	0.0112	0.0001

Means followed by the same letters in the same column do not differ by Tukey test ($p < 0.05$).

Table 5. Basal respiration (BR), microbial activity (MBA), microbial biomass carbon (MBC), metabolic quotient ($q\mathrm{CO}_2$), microbial quotient (Qmic), and soil enzyme activities of protease and dehydrogenase, at the 0–0.20 m depth layer, in plots treated with sewage sludge at rates of 20 t ha^{-1} (L2), 10 t ha^{-1} (L1) and mineral fertilizer (MF)

Treatment	BR	MBA	MBC	$q\mathrm{CO}_2$	Qmic	Protease	Dehydrogenase
	—— μg CO_2 g^{-1} day^{-1} ——		μg g^{-1} C	μg CO_2 μg^{-1} C day^{-1}	μg CO_2 μg^{-1} C	μg H g^{-1} solo h^{-1}	mg of H
	50.15 A	1.114 B	56.79	0.89 A	3.24 B	0.85	1.63
L1	37.91 B	1.288 B	63.85	0.63 AB	4.01 B	1.06	3.36
MF	27.96 B	1.623 A	88.55	0.35 B	7.10 A	1.08	3.26
CV (%)	13.86	10.97	27.85	21.34	28.66	26.44	30.46
Teste F	0.0033	0.0075	0.1283	0.0039	0.0161	0.4331	0.0462

Means followed by the same letter in the same column do not differ by the Tukey test ($p < 0.05$).

disequilibrium. However, the $q\mathrm{CO}_2$ measured in the SS-L1 treatment (recommended SS dose based on maize N demand) did not significantly differ from the value found in the MF-treatment. In general, these values are similar to the results of Fernandes et al. (2005) in an experiment with increasing rates of SS from Barueri and Franca (cities in the State of São Paulo); and of Cardoso & Fortes Neto (2000), who evaluated the effects of increasing SS soil applications (up to 160 t ha^{-1}) on the soil microbiota, and reported increases in the metabolic quotient.

Therefore, a decreasing microbial quotient (Qmic) was observed as SS rates increased (L1, L2) compared to the MF-treatment (Table 5), corroborating the results of Melo (2006) in a eutroferric Red Oxisol. According to Tótola & Chaer (2002), Qmic provides a measure of the soil organic matter quality; thus, the higher the Qmic value the higher the efficiency of soil org-C conversion into MBC (Melo, 2006). For the latter author, this relationship might be understood as an organic matter mineralization potential, and thus, the lower the relation between MBA and org-C, the lower the mineralization rate.

The $q\mathrm{CO}_2$ and Qmic values are coherent and highly negatively correlated ($r = -0.89$; $p \leq 0.0001$), indicating a stress scenario with increasing energy demand for carbon metabolism concomitantly with decreasing soil organic matter quality. This means that SS applications produced unfavorable conditions for soil organic matter mineralization. In the plots treated with six annual SS applications (a total of 298 t ha^{-1} on a dry basis), soil org-C in the form of oils and greases was 11.9 %, compared to the 1.7 % found in the MF plots, indicating that SS application induced accumulation of these substances considered recalcitrant (Hohla et al., 1978).

The protease activity has the function to catalyze the hydrolysis of protein-N, giving rise to a mixture of amino acids which in turn decompose to ammonium-N (ammonification reaction), available to plants and microorganisms (Karaca et al., 2002), or are oxidized to nitrate (nitrification reaction); no significant treatment effects were observed on the enzyme activity. These results disagree with those of Melo (2006), who reported increased protease activity in two Oxisols treated with increasing SS rates up to 60 t ha^{-1} (SS from Barueri, State of São Paulo).

The soil dehydrogenase activity reflects the total oxidative capacity of the microbiota, acting as an indicator of soil microbial activity (Andrade & Silveira, 2004). No significant effect of SS-L1 on this enzyme activity was observed, but a 50 % reduction was found in SS-L2 plots. This result contradicted Araújo et al. (2009), who observed increased soil enzyme activity with increasing SS rates (SS from Franca, State of São Paulo), probably due to the presence of toxic elements in the residue and also to a decrease in soil pH, favoring the availability of heavy metals.

Positive correlations were found between soil dehydrogenase activity and MBA ($r = 0.53$; $p \leq 0.05$) and Qmic ($r = 0.60$; $p \leq 0.04$), but negative correlations were observed between this enzyme activity and $q\mathrm{CO}_2$ ($r = -0.70$; $p \leq 0.01$), indicating again that SS application caused a stress condition to soil microbiota, mainly at the higher rate (L2).

The analytical results of the potentially toxic elements (PTE) indicated that none of these elements reached the intervention value for agricultural use established by CETESB (2005). The conclusion was drawn that the SS applied for seven years in this study resulted in no restrictions for agriculture with regard to heavy metal contents, even at the highest SS rate applied to maize (Table 6).

The maize grain yield in the 2008/2009 growing season was highest in the SS-L1 plots, followed by MF and L2-SS treatments (Figure 1). Maize shoot dry matter yield was higher in the SS-treatments. After seven years of successive SS applications, based on the maize N demand, the residue efficiently supplied maize N and P demands.

Table 6. Soil total concentrations of potentially toxic elements, at 0–0.20 m depth layer, from SS-treated plots (L1 and L2 sewage sludge rates) and MF-treated plots

Element	Guide value(1)		
	Reference		Intervention
Arsenium (mg kg^{-1})	15		35
Barium (mg kg^{-1})	150		300
Cadmium (mg kg^{-1})	1.3		3
Lead (mg kg^{-1})	72		180
Copper (mg kg^{-1})	60		200
Mercury (mg kg^{-1})	0.5		12
Nickel (mg kg^{-1})	13		30
Zinc (mg kg^{-1})	300		450
	Treatment		
	MF	L1	L2
Arsenium (mg kg^{-1})	4.78	5.48	5.23
Barium (mg kg^{-1})	14.88	30.33	38.63
Cadmium (mg kg^{-1})	1.30	1.53	1.75
Lead (mg kg^{-1})	14.13	18.65	23.23
Copper (mg kg^{-1})	64.85	75.03	91.90
Mercury (mg kg^{-1})	0.28	< 0.005	0.10
Nickel (mg kg^{-1})	9.68	10.20	11.03
Zinc (mg kg^{-1})	20.48	61.73	69.65

(1) Guide values of quality reference and intervention for agriculture established by CETESB (2005). MF: mineral fertilizer; L1: 10 t ha^{-1} of sewage sludge (recommended dose to supply maize crop demand for N); L2: 20 t ha^{-1}of sewage sludge (twice the recommended dose).

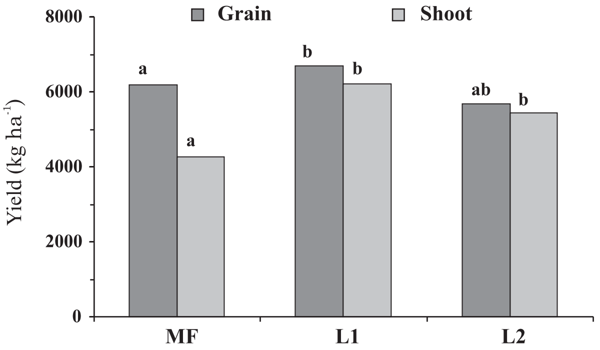

Figure 1. Maize shoot dry matter and grain yields, cultivated during summer 2008/2009, fertilized with mineral fertilizers (MF) and sewage sludge (SS) after seven successive applications of SS at the rates of 10 t ha^{-1} (L1) and 20 t ha^{-1} (L2). Means followed by the same letters within each variable do not differ by Tukey test ($p < 0.05$).

CONCLUSIONS

1. Sewage sludge applied to the soil at the recommended rates improved the availability of soil macro- and micronutrients, but some caution about Mg deficiency is advisable.

2. Sewage sludge applied at the recommended rates caused a negative effect on the soil microbial activity and biomass.

3. Sewage sludge at the recommended rates was enough to maintain maize yields without the need for complementary fertilization with mineral N, P, and micronutrients fertilizers.

4. Seven successive annual sewage sludge applications based on the maize N demand raised the concentrations of potentially toxic elements in the soil to levels permitted by environmental regulatory laws.

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