Fractionation of Zn, Cd and Pb in a Tropical Soil After Nine-Year Sewage Sludge Applications[*1]

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ABSTRACT

A long-term field experiment was carried out in the experiment farm of the Sao Paulo State University, Brazil, to evaluate the phytoavailability of Zn, Cd and Pb in a Typic Eutrorthox soil treated with sewage sludge for nine consecutive years, using the sequential extraction and organic matter fractionation methods. During 2005–2006, maize (*Zea mays* L.) was used as test plants and the experimental design was in randomized complete blocks with four treatments and five replicates. The treatments consisted of four sewage sludge rates (in a dry basis): 0.0 (control, with mineral fertilization), 45.0, 90.0 and 127.5 t ha^{-1}, annually for nine years. Before maize sowing, the sewage sludge was manually applied to the soil and incorporated at 10 cm depth. Soil samples (0–20 cm layer) for Zn, Cd and Pb analysis were collected 60 days after sowing. The successive applications of sewage sludge to the soil did not affect heavy metal (Cd and Pb) fractions in the soil, with exception of Zn fractions. The Zn, Cd and Pb distributions in the soil were strongly associated with humin and residual fractions, which are characterized by stable chemical bonds. Zinc, Cd and Pb in the soil showed low phytoavailability after nine-year successive applications of sewage sludge to the soil.

Key Words: heavy metals, humic substance, sequential extraction, *Zea mays* L.

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The large amount of sewage sludge production, especially in metropolitan regions, led researchers up to intensive investigation on the beneficial use for agriculture. Therefore, nowadays worldwide scenery for the sewage sludge recycling tends to be the soil disposal for crop production. Although the sewage sludge use in agriculture is apparently one of the most viable alternatives for this residue disposal, the presence of heavy metals can limit its application, mainly due to the risk of soil contamination. This aspect is of great concern, because environmental security is required for the correct use of such residue in agriculture (Nogueira *et al.*, 2008).

Soil heavy metal availability to plants has been evaluated through the use of sequential chemical extraction methods (Tessier *et al.*, 1979; Shuman, 1985; Mann and Ritchie, 1993; Silveira *et al.*, 2006), which allow determining the metal proportions associated to each component of the soil solid phase and, thus, to predict its phytoavailability (Mattiazzo *et al.*, 2001). The sequential chemical extraction procedure has been used especially for determining metal forms added to soil *via* sewage sludge (Sposito *et al.*, 1982; Kim and McBride, 2006). However, little information on this subject is found in the Brazilian literature. Studies have demonstrated that metals are more associated to the soil fractions with more stable bonds. This means that metals appear to be bound to oxide and residual soil fractions

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(Silveira, 2002; Borges and Coutinho, 2004; Araújo and Nascimento, 2005; Revoredo, 2005). The soil heavy metal availability must also be evaluated in the soil organic matter (OM), by means of the soil OM fractionation, in order to determine the metal distribution in the various organic fractions: fulvic acids (soluble in alkaline and diluted-acid media) that present large amount of oxygenated functional groups; humic acids (soluble in alkaline, but insoluble in strong-acid media due to the collapse of the macromolecule structure by the functional group protonation, causing precipitation); and humin fraction (insoluble residue in alkaline and acid media) that represents the organic matter closely bound to the soil mineral fraction (Canellas *et al.*, 2001).

Nevertheless, the data found in the literature are related to the N and C concentrations in the organic matter fractions (Pinheiro *et al.*, 2003; Farias *et al.*, 2005), with little information on heavy metals, particularly, after applications of sewage sludge to the soil. Melo *et al.* (2007) studied the effects of sewage sludge application to a Typic Haplorthox for six consecutive years and observed that most soil Ni was present in the humic substances in the decreasing order: humin > fulvic acid > humic acid fraction.

In this context, the objective of the present study was to evaluate the phytoavailability of Zn, Cd and Pb by means of organic matter fractionation and sequential extraction procedures of a tropical soil classified as Typic Eutrorthox after nine years of sewage sludge application.

MATERIALS AND METHODS

Field procedures

A nine-year field experiment was carried out in the experiment farm of the Sao Paulo State University, at Jaboticabal, State of São Paulo, Brazil (21° 15′ 22″ S and 48° 15′ 18″ W, altitude 618 m), beginning in 1997. The experimental design was in randomized complete blocks with four treatments and five replications.

Initially, the tropical soil (classified as a Typic Eutrorthox, clayey texture) was plowed, harrowed and limed with 2.5 t ha^{-1} of dolomitic limestone to raise the base saturation to 70%, according to recommendations of Van Raij and Cantarella (1997). Afterwards, sewage sludge (SS) was applied to the soil at the following rates: 0.0 (control), 2.5, 5.0 and 10.0 t ha^{-1} on a dry basis. The rate of 5.0 t ha^{-1} was calculated to supply the nitrogen (N) required for the crop, assuming that 1/3 of the residue-N would be available to the plants. In the following years (second and then on), the control plot was treated with mineral fertilizer (N, P, K) based on soil analysis and recommendations of Van Raij and Cantarella (1997). Starting in the fourth year, the SS rates were increased from 2.5 to 20.0 t ha^{-1}, and after nine years, the accumulated residue doses applied to the soil were: 0.0 (control = no residue, with mineral N-P-K), 45.0, 90.0 and 127.5 t ha^{-1} of sewage sludge, on a dry basis.

Maize (*Zea mays* L.) was used as test plant up to the sixth year, sunflower (*Helianthus annuus* L.) in the seventh year, crotalaria (*Crotalaria juncea* L.) in the eightieth, and maize again in the ninth year. The present work reports the results of the nine years.

In 2004, before the cropping year, soil samples for all the experimental area (a clayey texture Typic Eutrorthox) were taken at 0–20 cm depth for chemical analysis according to procedures described by Van Raij *et al.* (2001) (Table I).

Based on the soil analysis results, the base saturation (BS) was raised to 70% in the control plot and in the 20 t ha^{-1} SS-treated plot by adding 1.8 and 2.5 t ha^{-1} of dolomitic limestone, respectively (Van Raij and Cantarella, 1997).

The SS-residue used during the long-term experiment proceeded from the Wastewater Treatment Plant of SABESP (Company of Basic Sanitation of the State of São Paulo) located at Barueri, State of São Paulo, Brazil.

Chemical characteristics of the SS-residue (Table II) were determined as follows: total N by micro-kjeldahl method (Bremner *et al.*, 1996); total P and K by vanado-molybdate spectrophotometry and

TABLE I

Chemical attributes[a] of the Typic Eutrorthox soil after eight-year applications of sewage sludge

Sewage sludge rate[b]	pH (CaCl$_2$)	OM	P (resin)	K	Ca	Mg	H+Al	SB	CEC	BS
t ha^{-1}		g dm^{-3}	mg dm^{-3}			mmol$_c$ dm^{-3}				%
0.0[c]	5.1	25	58	4.8	29	12	42	46	88	52
40.0	5.3	27	65	3.9	41	13	38	58	96	60
80.0	5.6	28	132	4.1	58	13	31	75	106	71
107.5	5.0	28	88	4.1	32	10	52	46	98	47

[a]OM = organic matter; SB = sum of bases; CEC = cation exchange capacity; BS = base saturation; [b]Accumulated doses after eight-year annual applications; [c]Control = no residue, with mineral N-P-K.

TABLE II

Chemical composition of sewage sludge[a], on a dry basis, used for the nine-year field experiment

Cropping year	N	P	K	Cu	Mn	Zn	Cr	Cd	Ni	Pb
		g kg^{-1}					mg kg^{-1}			
1997/1998	32	17	4.8	664	228	1 800	290	8	268	152
1998/1999	37	11	1.7	551	294	3 810	1 190	12	595	371
1999/2000	29	17	1.5	660	257	2 328	764	8	360	180
2000/2001	29	15	1.8	719	263	1 745	699	10	354	171
2001/2002	37	15	2.7	627	287	2 354	778	9	350	155
2002/2003	34	22	1.9	722	222	2 159	808	11	231	186
2003/2004	41	19	0.1	690	194	2 930	736	10	297	173
2004/2005	34	19	1.3	998	206	2 474	798	8	299	169
2005/2006	34	19	1.3	998	206	2 474	798	8	299	169

[a]Samples obtained from the Wastewater Treatment Plant of SABESP (Company of Basic Sanitation of the State of São Paulo), at Barueri, State of São Paulo, Brazil.

flame-photometry, respectively, in nitric-perchloric extracts (Malavolta *et al.*, 1997); and the heavy metals by atomic absorption spectrophotometry (AAS) in an acetylene-air flame (Model: AVANTA GBC, Australia) in extracts obtained by digestion with $HNO_3 + H_2O_2 + HCl$ (USEPA, 1986).

In 2005, the sewage sludge was manually spread out on the soil surface and incorporated by harrowing into the top-10 cm-layer. The control plots received mineral fertilizers manually applied in the plow furrows before sowing, as follows (kg ha^{-1}): N = 150, as ammonium sulfate (200 g N kg^{-1}); P = 277, as simple-superphosphate (180 g P_2O_5 kg^{-1}); and K = 86, as potassium chloride (580 g K_2O kg^{-1}). In the SS-treated plots, K was added when necessary based on soil analysis, in order to supply all plots with the same N-P-K levels. The N and P quantities in the sludge-treated plots were within the range required by the crop (Van Raij and Cantarella, 1997).

Two side-dressing applications were conducted, each at 156 kg ha^{-1} N (as urea, 450 g N kg^{-1}) only in the control plots; and 69 kg ha^{-1} of K (as potassium chloride, 580 g K_2O kg^{-1}) in all plots, at 27 and 40 days after sowing. The N, P and K quantities applied to the soil during the nine-year experiment are presented in Table III. Seeds of the maize were sowed in November 26, 2005, and spaced 0.9 m between rows, using 8 plants per meter, in 60 m^2 plots.

Laboratory analysis

Sixty days after seedling emergence, soil samples from 0–20 cm depth layer were taken to the laboratory, sieved and air-dried for heavy metal (Zn, Cd and Pb) analysis, by sequential extraction fractionation according to procedures developed by Shuman (1985), excluding the manganese oxide fraction (considered negligible in the studied soil).

The fractionation consisted of five sequential extractions, discriminating metals from the following fractions: exchangeable (Exch), organic matter (OM), amorphous Fe oxides (AFeO$_x$), crystalline Fe oxides (CFeO$_x$) and residual (Res).

TABLE III

Mineral fertilization applied to the Typic Eutrorthox soil during the nine-year experiment

Cropping year	Sewage sludge rate[a]	N		P	K	
		Sowing	Side-dressing	Sowing	Sowing	Side-dressing
	t ha^{-1}			kg ha^{-1}		
1997/1998	0.0	-	-	-	-	-
	2.5	-	-	278	50	-
	5.0	-	-	244	45	-
	10.0	-	-	183	34	-
1998/1999	0.0	75	136	167	52	-
	5.0	-	-	-	43	-
	10.0	-	-	-	34	-
	20.0	-	-	-	17	-
1999/2000	0.0	150	245	278	86	69
	7.5	-	-	-	79	69
	15.0	-	-	-	71	69
	30.0	-	-	-	55	69
2000/2001	0.0	150	267	278	86	69
	20.0	-	-	89	67	69
	27.5[b]	-	-	-	48	69
	40.0	-	-	-	9	69
2001/2002	0.0	150	311	278	86	69
	25.0	-	-	-	59	69
	47.5	-	-	-	29	69
	50.0	-	-	-	-	69
2002/2003	0.0	150	311	278	86	69
	30.0	-	-	-	66	69
	60.0	-	-	-	47	69
	67.5	-	-	-	7	69
2003/2004	0.0	50	89	111	34	-
	35.0	-	-	-	-	14
	70.0	-	-	-	-	-
	87.5	-	-	-	-	-
2004/2005	0.0	-	-	100	31	-
	40.0	-	-	-	-	-
	80.0	-	-	-	-	-
	107.5	-	-	-	-	-
2005/2006	0.0	150	311	278	86	69
	45.0	-	-	-	41	69
	90.0	-	-	-	-	69
	127.5	-	-	-	-	69

[a] Annual accumulated doses. [b] Since 2000/2001, it was decided to substitute 2.5 by 20 t ha^{-1} of sewage sludge, on a dry basis.

Exchangeable fraction (Exch).   Five grams of air-dried soil and 20 mL of 1 mol L^{-1} Mg(NO$_3$)$_2$ solution were shaken for 2 hours in a 50 mL centrifuge tube. The supernatant was filtered through Whatman grade No. 42 quantitative filter paper and 10 mL of deionized water was added to the tube, which was shaken again for 3 minutes, centrifuged and filtered. This procedure was repeated once and the three resultant supernatants were combined and sent to metal analysis.

Organic matter fraction (OM).   Ten milliliters of 0.7 mol L^{-1} NaOCl solution, pH 8.5 (unstable pH adjusted at the moment of use) were added to the same tube and the sample was heated in water-bath to 100 °C for 30 min, and occasionally shaken. The sample was centrifuged and filtered, and this procedure was repeated twice and the three supernatants were combined. 10 mL of deionized water was added to the tube. The sample shaken, filtered and the filtrate was added to the previous NaOCl extracts.

Amorphous Fe oxide fraction (AFeO$_x$).   Thirty milliliters of a solution containing 0.25 mol L^{-1} hydroxylamine hydrochloride (NH$_2$OH·HCl) + 0.25 mol L^{-1} hydrochloric acid (HCl), pH 3.0, were added to the centrifuge tube; and this sample was heated in water bath at 50 °C during 30 min, and occasionally shaken. The sample was centrifuged, filtered and rinsed thrice, combining the three supernatants as previously described.

Crystalline Fe oxide fraction (CFeO$_x$).   Thirty milliliters of a solution containing 0.2 mol L^{-1} (NH$_4$)$_2$C$_2$O$_4$, 0.2 mol L^{-1} H$_2$C$_2$O$_4$, and 0.01 mol L^{-1} ascorbic acid, pH 3.0, were added to the sample in the centrifuge tube and heated in water bath for 30 min at 100 °C, and occasionally shaken. Then the sample was centrifuged, filtered and rinsed thrice, combining the three supernatants as described above.

Residual fraction (Res).   The left over precipitate of the CFeO$_x$ fraction in the centrifuge tube was re-suspended and transferred to 100 mL Becker flasks, oven-dried at 105 °C, weighed, triturated in mortar and analyzed for heavy metals, using USEPA (United States Environmental Protection Agency) method 3050B (USEPA,1986).

The extract volumes of each Exch, OM, AFeO$_x$ or CFeO$_x$ fraction were made to 50 mL with deionized water and the Res fraction, to 25 mL, and then, analyzed for Zn, Cd and Pb by AAS.

The procedure used to determine Zn, Cd and Pb distribution in the soil organic matter fractions was based on the modified method of Dabin (1971), which consisted of the use of deionized water for the soluble fraction and 0.1 mol L^{-1} NaOH solution for the humic substances (fulvic and humic acids and humin). The extract was fractionated in fulvic and humic acids by acidifying the alkaline extract with concentrated H$_2$SO$_4$ until pH 1.0. Heavy metals (Zn, Cd and Pb) were determined using USEPA method 3050B (USEPA, 1986) and AAS.

The data were submitted to analysis of variance and means and compared by Tukey's test ($P < 0.05$), using the SAS system of analysis (SAS Institute Inc., 2002). In the case of significant F tests, correlation coefficients were determined between the soil metal data and plant metal concentrations and maize grain yields.

RESULTS AND DISCUSSION

Sequential extraction

Zinc (Zn) was associated to all soil fractions studied, but higher Zn concentrations were found in the plots treated with 90 t ha^{-1} of sewage sludge (SS), indicating that the applications of residue contributed to increased Zn in the soil (Table IV).

Although Zn bound to the exchangeable and organic matter fractions was small as compared to the total concentration, it is indicative of Zn-phytoavailability. This is not only a benefit from the point of view that Zn is a micronutrient, but also a potential risk for the biological chain. Similar results were observed by other authors (Ma and Rao, 1997; André, 2003). In similar long-term experiments there are reports of increased Zn bound to the exchangeable (Exch) and organic matter (OM) fractions after ten years of sewage sludge applications to the soil (McGrath and Cegarra, 1992). Increased Zn associated to the OM fraction with consequent decrease in the Zn-Res fraction has been also observed in seven-year-SS-treated soils (Chang *et al.*, 1984). Soil Zn distribution in percentage decreased in the various fractions in the following order: Res > AFeO$_x$ > CFeO$_x$ > Exch > OM (Fig. 1a).

Soil organic matter constituents show high affinity for heavy metals, thus, it is estimated that 1.5% to 2.3% of the total soil Zn is associated to the organic matter fraction; and, the sewage sludge is known to contain considerable percentage of organic compounds (Kabata-Pendias and Pendias, 2001). In a Typic Haplorthox soil treated with urban waste vermicompost (0, 25, 50, 75 and 100 t ha^{-1}) combined with lime to raise the soil base saturation to 40% and 80%, André (2003) observed the following soil Zn distribution in the various fractions: Zn-Res (82.6%) > Zn-FeO$_x$ (11.6%) > Zn-MnO$_x$ (3.0%) > Zn-OM (2.2%) > Zn-Exch (0.6%).

TABLE IV

Zinc, cadmium and lead concentrations[a] of the Typic Eutrorthox soil applied with sewage sludge (SS) annually for nine consecutive years (1997–2005) obtained by means of sequential extraction

Sewage sludge rate[b]	Exch	OM	$AFeO_x$	$CFeO_x$	Res	SF	Total
t ha^{-1}				mg kg^{-1}			
				Zinc			
0.0[c]	0.75 c[d]	0.30 b	7.79 c	2.43 b	54.00 b	65.27	52.99
45.0	1.21 bc	0.32 ab	9.82 bc	5.78 a	53.15 b	70.28	58.47
90.0	3.32 a	0.56 a	16.76 a	7.46 a	68.08 a	91.17	75.01
127.5	1.67 b	0.39 ab	13.33 ab	5.86 a	63.78 ab	85.03	72.81
CV (%)	21.1	34.7	22.6	30.71	11.31	-	9.7
				Cadmium			
0.0	< 0.01	< 0.01	< 0.01	< 0.01	1.08 a	1.08	1.87
45.0	< 0.01	< 0.01	< 0.01	< 0.01	1.05 a	1.05	1.90
90.0	< 0.01	< 0.01	< 0.01	< 0.01	1.11 a	1.11	1.98
127.5	< 0.01	< 0.01	< 0.01	< 0.01	1.09 a	1.09	1.97
CV (%)	-	-	-	-	6.5	-	6.4
				Lead			
0.0	< 0.09	< 0.05	1.83 a	1.46 a	8.98 a	12.27	14.49
45.0	< 0.09	< 0.05	1.63 a	1.90 a	8.86 a	12.39	15.19
90.0	< 0.09	< 0.05	2.68 a	2.20 a	9.65 a	14.53	16.53
127.5	< 0.09	< 0.05	2.66 a	1.76 a	9.60 a	14.02	16.65
CV (%)	-	-	30.5	26.3	10.8	-	9.6

[a] Exch = exchangeable fraction; OM = organic matter fraction; $AFeO_x$ = amorphous Fe oxide fraction; $CFeO_x$ = crystalline Fe oxide fraction; Res = residual fraction; SF = sum of fractions; Total = total element concentration (USEPA, 1986); [b] Accumulated SS rates after nine-year annual applications; [c] Control = no residue, with mineral N-P-K; [d] Means followed by the same letter(s) are not significantly different at $P < 0.05$ by Tukey's test.
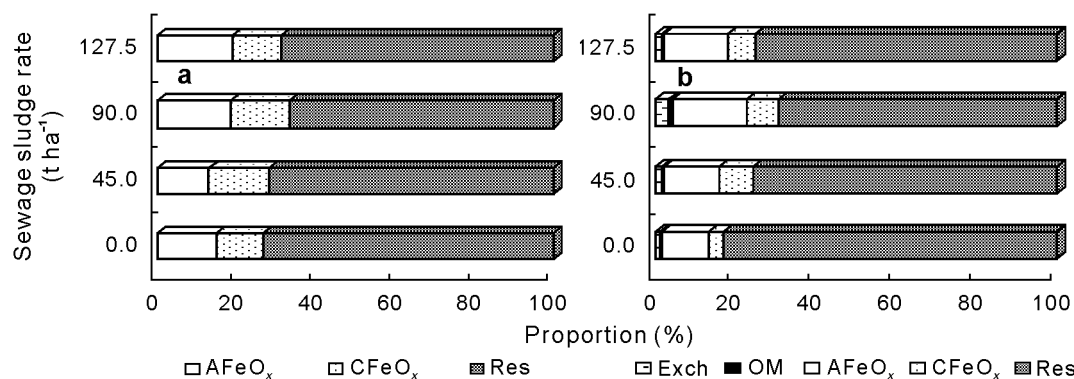

Fig. 1 Proportions of exchangeable (Exch), organic matter (OM), amorphous Fe oxide ($AFeO_x$), crystalline Fe oxide ($CFeO_x$) and residual (Res) Pb (a) and Zn (b) fractions in the soil applied with sewage sludge annually for nine consecutive years.

In the present work, the major part of soil Zn was found associated with the residual fraction, which represents the Zn present in primary and secondary mineral structures, and therefore, unavailable to the plants. Araújo and Nascimento (2005), after 180 days of SS-incubation with soil, observed that the highest Zn concentrations were found in the residual fraction. In another study with nineteen soils with different chemical and physical attributes, the residual fraction was also evidenced as the larger Zn reservoir (Iyengar *et al.*, 1981). Similar results were also reported by Davis-Carter and Shuman (1993), André (2003) and Consolini (2003).

Besides the residual fraction, Zn was found mainly associated with the amorphous ($AFeO_x$) and crystalline Fe oxide ($CFeO_x$) fractions, in decreasing order, respectively. Such results disagreed with the ones obtained by Borges and Coutinho (2004). However, some authors reported that 14% to 38% of the

total soil Zn is associated mainly with Fe and Al oxides (Kabata-Pendias and Pendias, 2001). This value range is close to the one obtained in the present study (15% to 26%), by calculating the ratio between the percent Zn in $AFeO_x + CFeO_x$ fractions and the total Zn (Table IV and Fig. 1a), implying the importance of those two fractions in soil Zn adsorption. Research data on Zn adsorption and retention in soils revealed that the soil clay minerals and organic matter are able to strongly bind and retain Zn (Kabata-Pendias and Mukherjee, 2007).

Cadmium was mainly bound to the residual fraction (Table IV). In a soil treated with siderurgical residue, Amaral Sobrinho *et al.* (1997) observed that, after seven weeks of incubation with alkaline and acid residue, 50% and 21%–27% of the total Cd were associated with the Exch fraction, respectively. However, after 18 months of incubation, for both alkaline and acid residues, 100% of the total Cd was bound to the Res fraction. Such high Cd insolubility observed in long-term experiments (Brams and Anthony, 1988) have been mainly attributed to the Cd co-precipitation with Fe and Mn oxides (Bell *et al.*, 1991).

Also, no effect of SS annual applications on the Res Cd concentration was observed (Table IV). In studies of heavy metal fractionation in a clayey soil treated with sewage sludge for nine years, researchers observed that less than 1% of the added metals were found in available fractions (soluble and exchange-able), and that the residue splitting application resulted in higher metal concentrations in the studied fractions (Taylor *et al.*, 1995).

Lead (Pb) was the element found associated to more stable fractions ($AFeO_x$, $CFeO_x$ and Res fractions) and not to the Exch and OM fractions (Table IV). Besides, no effect of SS-rates on the soil Pb fractions was observed. Lead usually show high affinity to the oxide and residual fractions (Silva and Vitti, 2008), where it is strongly bound (Amaral Sobrinho *et al.*, 1997; Nogueira *et al.*, 2008), which in fact decreases its mobility (Sheppard and Thibault, 1992) and soil availability to plants (Alloway, 1995). The soil Zn percent distribution decreased in the various fractions in the following order: Res > $AFeO_x$ > $CFeO_x$ > Exch > OM (Fig. 1b).

Except for cadmium (Cd), of which average recovery rate was close to 56%, satisfactory results were obtained from the metal sequential extraction in the soil samples, since most Pb and Zn were recovered, that is, the sum of Pb and Zn percentages found in the various fractions was close to the total concentrations of these elements (Table IV). Thus, the average recovery value was 85% for Pb and 120% for Zn. Tessier *et al.* (1979), Sposito *et al.* (1982) and André (2003) studied Zn fractionation and found average Zn recovery values of 99%, 105%, and 111%, respectively. Nevertheless, Silveira (2002) has observed that Zn, Cu and Cd recovery in soil samples in general show a variation range close to ±15%.

In the case of Cd and Pb, even low recovery values are acceptable, because the total recovery is rarely achieved in studies of soil metal fractionation (Bertoncini, 2002). This is the main criticism to the sequential extraction methods, that is, the extraction errors of each fraction are accumulated to the errors of the subsequent fractions, resulting in a relatively large error variation range (Pierrisnard, 1996). Besides, the fractionation methods are not yet adequately standardized and researchers use procedures of their own or modifications of others (Kim and McBride, 2006), impairing comparison between results obtained from different laboratories.

This study evidenced that the major parts of soil Zn, Cd and Pb were linked to the Res fraction. Although Ma and Rao (1997) obtained similar results, part of the studied metals were found associated with more phytoavailable fractions, indicating that studies on metal fractionation are highly relevant when the target is to evaluate the soil contamination by heavy metals.

The soil organic matter fractionation showed that the major Zn, Cd and Pb contents were connected to the humin fraction, the most stable fraction of the soil humic substances (Table V).

However, Zn concentrations in the organic fractions were affected by the SS treatments, except for the humin fraction. In the soluble fraction, the lowest Zn concentration was found for the 45.0 t ha^{-1} SS-rate treatment. In the fulvic acid fraction, Zn concentrations increased as SS-rates increased, varying

TABLE V

Zinc, cadmium and lead concentrations of the Typic Eutrorthox soil applied with sewage sludge (SS) annually for nine consecutive years (1997–2005) obtained by means of organic matter fractionation

Sewage sludge rate[a]	Soluble	Fulvic acid	Humic acid	Humin
t ha^{-1}		mg kg^{-1}		
		Zinc		
0.0[b]	0.277 a[c]	0.776 b	0.492 c	51.447 a
45.0	0.166 b	1.503 b	0.883 b	55.926 a
90.0	0.234 ab	3.947 a	1.525 a	69.310 a
127.5	0.229 ab	4.386 a	1.150 b	67.083 a
CV (%)	21.4	21.4	16.9	17.7
		Cadmium		
0.0	< 0.004	0.593 a	0.224 a	0.877 a
45.0	< 0.004	0.499 a	0.203 a	1.202 a
90.0	< 0.004	0.553 a	0.203 a	1.220 a
127.5	< 0.004	0.584 a	0.230 a	1.155 a
CV (%)	-	18.3	13.5	25.4
		Lead		
0.0	< 0.047	< 0.190	1.725 a	12.766 a
45.0	< 0.047	< 0.190	1.619 a	13.575 a
90.0	< 0.047	< 0.190	1.566 a	14.968 a
127.5	< 0.047	< 0.190	1.763 a	14.888 a
CV (%)	-	-	12.0	13.4

[a] Accumulated SS doses after nine-year annual applications; [b] Control = no residue, with mineral N-P-K; [c] Means followed by the same letter are not significantly different at $P < 0.05$ by Tukey's test.

from a range of 0.776 (control plot) to 4.386 mg kg^{-1} of Zn (127.5 t ha^{-1} SS rate plot). In the humic acid fraction, the Zn concentrations varied from 0.492 (control plot) to 1.525 mg kg^{-1} (90.0 t ha^{-1} SS rate plot). Therefore, Zn concentrations in the organic matter fractions decreased in the following order: humin > fulvic acid > humic acid > soluble (Fig. 2a).
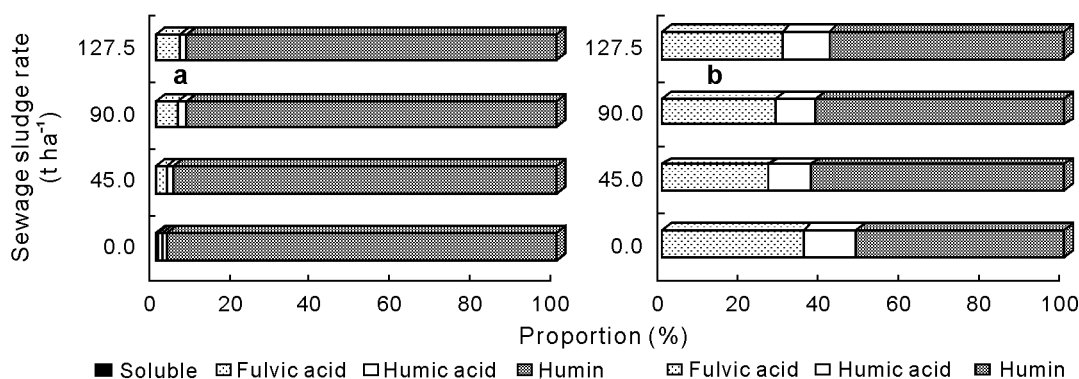

Fig. 2   Proportions of Zn (a) and Cd (b) in the soil organic matter fractions in relation to the total sum.

Cadmium concentrations in soluble and other fractions were not affected by the increased SS rates (Table V). The highest Cd concentrations were found in the fractions of decreased order: humin > fulvic acid > humic acid (Fig. 2b).

Lead (Pb) was generally below the limit of detection in the soluble and fulvic acid fractions by the referred methods used, and Pb concentrations in the other fractions were not affected by SS-rates (Table V). Lead is highly reactive with humic substances forming insoluble complex molecules, meanwhile Cd and Zn react with low molecular weight mobile organic substances (Angehrn-Bettinazzi *et al.*, 1989). In this aspect, some authors observed that Pb-humic acid complex molecules showed higher stability than

the Cd and Zn-organic molecules (Waller and Pickering, 1993; Pinheiro *et al.*, 1994).

In the present study, the highest Zn, Cd and Pb concentrations were found in the humin fraction, evidencing these elements being low phytoavailability. In contrast, Boruvka and Drábek (2004), in a study on highly polluted soils, found that Zn, Cd and Pb are predominantly distributed in the fulvic acid fraction.

As already discussed, the data in the literature have reported only N and C concentrations in the humic substance fractions (Canellas *et al.*, 2001; Pinheiro *et al.*, 2003; Farias *et al.*, 2005), and little information is available on heavy metal distribution in soil, especially after long-term application of sewage sludge.

Nevertheless, the results obtained in the present work are corroborated by some authors (Revoredo, 2005; Melo *et al.*, 2007), clearly evidencing the importance of such studies in front of a scenery of demands for sewage sludge recycling mainly through agriculture soil disposal.

Correlations

Correlation coefficients (r) were calculated for the Zn and Pb in soil fractions obtained by sequential extraction, and the Zn and Pb accumulated in maize plant parts (Table VI). There was a positive correlation of Zn bound to Exch fraction with the Zn accumulated in all maize plant parts, except for stalk and grains. The Zn bound to OM fraction correlated only with the Zn in husk, grains and shoot. Iyengar *et al.* (1981) observed that despite most Zn was present in the residual and Fe-oxide fractions, positive correlations of maize-Zn were only obtained with the Zn bound to Exch and OM fractions.

TABLE VI

Correlation coefficients between the soil Zn and Pb fractions (obtained by sequential extraction) and the element quantities ($\mu g \ plant^{-1}$) accumulated in plants and grain yield of maize

Sequential extraction[a]	Stalk	Leaves	Husk	Cob	Grains	Shoot[b]	Grain yield
				Zinc			
Exch	0.35	0.45*	0.51*	0.57**	0.38	0.49*	0.28
OM	0.39	0.26	0.56*	0.08	0.45*	0.55*	0.15
AFeO$_x$	0.47*	0.44	0.61**	0.66**	0.54*	0.62**	0.21
CFeO$_x$	0.48*	0.44	0.51*	0.41	0.21	0.41	0.03
Res	0.50*	0.55*	0.65**	0.07	0.43	0.58**	−0.15
				Lead			
Exch	-	-	-	-	-	-	-
OM	-	-	-	-	-	-	-
AFeO$_x$	0.41	0.45	0.64**	0.69**	-	0.66**	0.31
CFeO$_x$	0.26	0.36	0.11	0.30	-	0.27	0.31
Res	0.33	0.32	0.47*	0.43	-	0.49*	0.09

*,**Significant at $P < 0.05$ and $P < 0.01$, respectively.
[a]Exch = exchangeable fraction; OM = organic matter fraction; AFeO$_x$ = amorphous Fe oxide fraction; CFeO$_x$ = crystalline Fe oxide fraction; Res = residual fraction; [b]Shoot = sum of all aerial plant parts.

Davis-Carter and Shuman (1993) obtained positive peanut plant-Zn correlation with the soil-Zn bound mainly to the Exch fraction. Borges (2000) tested maize plants in two different soils and observed that, in a sandy soil, the Exch fraction was enough to supply Zn to the plants, but in a clayey soil, both Exch and OM fractions were responsible for the Zn supply to the plants. In another experiment, André *et al.* (2003) observed that the Zn accumulated by the graminea *Cynodon* spp., cultivated in an Alfisol, was supplied by three soil fractions: Exch, OM and Mn-oxides.

In this work, there was a positive correlation of Zn bound to AFeO$_x$ fraction with maize plant-Zn accumulated in all parts, except for leaves. And, a positive correlation of Zn bound to CFeO$_x$ fraction with maize plant-Zn accumulated in stalk and husk. And finally, there was a positive correlation of Zn bound to Res fraction with plant-Zn accumulated in all parts, except for the cob and grains. However, these results did not agree with the ones found by Consolini (2003), who observed positive plant-Zn

correlations only with the Zn bound to Exch and OM fractions, for three studied soils.

The correlation coefficients for the relationships between soil-Pb and maize Pb (Table VI) were not determined for the Pb bound to Exch and OM fractions. There were positive correlations of Pb bound to $AFeO_x$ fraction with the Pb accumulated in plants, only for the corn-husks, corn-cobs and plant-tops. No correlation was observed between Pb bound to $CFeO_x$ fraction and the Pb accumulated in maize plant parts. And, positive correlations were observed between Pb bound to Res fraction and the Pb accumulated in husk and shoot.

No correlation of soil Zn and Pb concentrations with maize grain yield was obtained (Table VI). Higher correlation coefficients were obtained between Zn concentrations in soil OM fractions and Zn accumulated in plant parts when compared with the coefficient values found for Pb (Table VII). However, there was only a positive correlation of Zn in the soluble-OM fraction with the grain Zn concentration. Furthermore, Zn concentrations in the fulvic acid, humic acid and humin fractions were highly correlated with the Zn accumulated in maize plant parts, except for the grain Zn content and grain yield.

TABLE VII

Correlation coefficients between the soil organic matter Zn and Pb (organic matter fractionation) and the element quantities (μg plant^{-1}) accumulated in plant parts and grain yield of maize

OM fraction[a]	Stalk	Leaves	Husk	Cob	Grains	Shoot[b]	Grain yield
				Zinc			
Soluble	0.16	0.34	0.05	0.12	0.49*	0.41	0.12
Fulvic acids	0.69**	0.50*	0.62**	0.80**	0.37	0.61**	0.24
Humic acids	0.52*	0.50*	0.62**	0.67**	0.47*	0.60**	0.31
Humin	0.46*	0.45*	0.55*	0.57*	0.54*	0.60**	0.16
				Lead			
Soluble	-	-	-	-	-	-	-
Fulvic acids	-	-	-	-	-	-	-
Humic acids	−0.27	−0.15	−0.07	0.06	-	−0.12	−0.04
Humin	0.10	0.40	0.46*	0.47*	-	−0.21	0.16

*,**Significant at $P < 0.05$ and $P < 0.01$, respectively.
[a]Organic matter fractions; [b]Sum of all aerial plant parts.

The correlations referred to the OM-Pb bound to soluble and acid fulvic fractions were not calculated. And, in general, there were no correlations between Pb concentrations in the OM fractions and the Pb accumulated in maize plant parts. There was only a positive correlation between the Pb concentration in the humin fraction and the Pb accumulated in the husk and cob (Table VII).

Revoredo (2005) obtained positive correlation of soil Ni concentrations in organic matter fractions (except for the soluble fraction) with the Ni accumulated in sorghum plants cultivated in a SS-treated soil. Similar to the results obtained by sequential extraction, the studies of organic matter fractionation also evidenced that there was no relationship between Zn and Pb concentrations in the soil OM fractions and maize productivity (Table VII).

CONCLUSIONS

The sewage sludge rates applied during nine consecutive years to the soil did not affect the Cd and Pb concentrations in the soil fractions obtained by organic matter fractionation and sequential extraction. But they increased the Zn concentration in the soil fractions. The highest Zn, Cd and Pb concentrations were found in soil fractions with more stable chemical bonds such as: humin (organic matter) and residual fractions (sequential extraction). The metals Zn, Cd and Pb showed low phytoavailability in the soil even after nine-year annual applications of sewage sludge.

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