COPPER, NICKEL AND ZINC PHYTOAVAILABILITY IN AN OXISOL AMENDED WITH SEWAGE SLUDGE AND LIMING

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ABSTRACT: Contents of heavy metal on agricultural soils have been raised by land applications of sewage sludge and may constitute a hazard to plants, animals and humans. A field experiment was carried out from 1983 to 1987, to evaluate the long-term effect of sewage sludge application, with and without liming, on heavy metal accumulation and availability in a Rhodic Hapludox soil grown with maize (HMD 7974 hybrid). Trials were set up in a completely randomized blocks design with four replications. Each block was split in two bands, one with and another without liming. The sludge was applied in each band at rates: 0, 20, 40, 60 and 80 Mg ha^{-1} (dry basis) in a single application; and 40, 60 and 80 Mg ha^{-1} split in two, three and four equal yearly applications, respectively. The soil was sampled for chemical analysis each year after harvest. Soil samples were analysed for Cu, Ni and Zn in extracts obtained with DTPA and Mehlich-3 solutions, and in extracts obtained by digestion with nitric-perchloric acid (total metal contents), using an inductively coupled plasma (ICP) spectrometer. In general, Zn, Cu and Ni concentrations in DTPA and Mehlich-3 extracts increased linearly with sludge application. Total Cu and Zn concentrations increased when sludge was applied, whereas total Ni concentrations were not affected. Both extractants were suitable to evaluate Cu and Zn availability to corn in the soil treated with sewage sludge. Liming reduced the DTPA extractability of Zn. DTPA-extractable Cu concentrations were not significantly affected by liming. Mehlich-3-extractable Cu and Zn concentrations increased with liming. Only DTPA extractant indicated reduction of Ni concentrations in the soil after liming.
Key words: DTPA, Mehlich-3, heavy metals, extractability, corn

FITODISPONIBILIDADE DE COBRE, NÍQUEL E ZINCO EM UM LATOSSOLO VERMELHO TRATADO COM LODO DE ESGOTO E CALAGEM

RESUMO: A reciclagem agrícola do lodo de esgoto tem provocado o acúmulo de metais pesados no solo e na água, podendo atingir níveis tóxicos e causar danos às plantas cultivadas, aos animais e ao homem, por meio da cadeia trófica. Foi desenvolvido um experimento, em condições de campo entre 1983 e 1987, onde foram avaliados os efeitos da aplicação de lodo de esgoto por quatro anos, com e sem calagem, sobre a acumulação e disponibilidade de metais pesados em um Latossolo Vermelho distrófico típico cultivado com milho (HMD 7974). O delineamento utilizado foi o de blocos completos ao acaso, com quatro repetições. Cada bloco foi dividido em duas faixas nas quais foram aplicados os tratamentos com e sem calcário, e em cada faixa foi aplicado o lodo nas seguintes doses: 0, 20, 40, 60 e 80 Mg ha^{-1} (material seco) de lodo de esgoto, em aplicação única; e as doses de 40, 60 e 80 Mg ha^{-1}, parceladas em dois, três e quatro anos, respectivamente. A fitodisponibilidade dos metais foi avaliada pelos extratores DTPA e Mehlich-3. Os teores extraíveis de Cu, Ni e Zn no solo aumentaram linearmente com as doses de lodo de esgoto, para os dois extratores usados. A adição de lodo de esgoto elevou os teores totais de Cu e Zn, mas não afetou os teores de Ni no solo. Os extratores DTPA e Mehlich-3 foram igualmente eficientes na predição da fitodisponibilidade de Cu e Zn. A adição de calcário reduziu a extratabilidade de Zn-DTPA, não alterou a de Cu-DTPA e aumentou os teores de Cu e Zn extraíveis do solo por Mehlich-3. Somente o extrator DTPA mostrou a redução do teor de Ni extraível do solo com a adição de calcário.
Palavras-chave: DTPA, Mehlich-3, metais pesados, extratibilidade, milho

INTRODUCTION

Recicling sewage sludge in agriculture has resulted in accumulation of heavy metals in soils, specially Cd, Cr, Cu, Ni and Zn, and mainly when the residue is originated from industrial areas (Williams et al., 1980; Chang et al., 1984; Krebs et al., 1998). This practice polutes the soil and water resources and causes toxicity to crops, animals and humans through the trophic chain (Logan & Chaney, 1983; McBride, 1995). The extension

of the damage resulting from the presence of heavy metals will depend, among other factors, on the quantities of sewage sludge added to the soil and to the chemical forms in which they will occur (Chaney & Giordano, 1977; Latterel et al., 1978; Anjo & Mattiazzo, 2001).

Several factors control the availability of soil metals to the plants. Even though high quantities of heavy metals are added to soils by application of sewage sludge, the absorption of such metals by plants represent less than 1.0% of the total introduced by the residue input (Chang et al., 1984).

Heavy metals may adsorb strongly to the soil organic and mineral coloids like clay, iron hydroxides and manganese oxides, form insoluble inorganic compounds with hydroxides, carbonates and sulfides, and form complex molecules with soil organic matter (Chaney & Giordano, 1977). Metals applied to the soil in the form of salts are more available to plants and more extractable by the DTPA extractant than metals applied in equivalent quantities by sewage sludge (Dowdy & Larson, 1975; Korcak & Fanning, 1985). Liming-induced higher pH may reduce the heavy metal availability because of the formation of less soluble oxides and carbonates (Heckman et al., 1987; Berton et al., 1997).

The control of the soil availability of heavy metals to safe levels for humans, animals, plants and environment, mainly after a long period of sludge application in a specific soil, is still a matter of concern among scientists. During the initial period of application, the sewage sludge organic matter not only strongly protects the plant from metal acquisition, but also improves soil properties and provides a greater control on metal solubility (McBride, 1995). These benefits are usually reduced along time with the mineralization of the sludge organic fraction. Neverthless, McGrath & Cegarra (1992) observed that 20 years after the application of sludge in a soil, when 70% of the organic matter was already decomposed, a relatively constant percentage of metals (Cd, Cr, Ni, Pb and Zn) extracted from soil fractions was found. Other authors have reported a decrease on metal availability with time (Bidwell & Dowdy, 1987).

Several extractant solutions have been tested to evaluate the phytoavailability of heavy metals in soil samples from field experiments using sewage sludge (Simonete & Kiehl, 2002). Korcak & Fanning (1978) found correlations between the amounts of Cd, Cu, Ni and Zn extracted by DTPA and Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) and the amounts absorbed by maize plants. Berton et al. (1997), using maize as test plant, observed that DTPA predict with efficacy the availability of Cu and Zn, but did not to predict Ni. None of the extractants used by Haq et al. (1980) - aqua regia, DTPA, NTDA, EDTA, acetic acid, ammonium acetate, hydrochloric acid and aluminum chloride – was adequate to evaluate Cu availability for plants of swiss chard. The extractant solutions Mehlich-1, Mehlich-3 and DTPA were not efficacious to evaluate the availability of Pb, Cd, Cr and Ni for wheat plants and of Pb and Cd for common bean plants. Correlations between soil and plant contents were obtained only for Ni and Cr when using the common bean as test plant (Abreu et al., 1995). The efficacy of the extractant solutions depends mainly on the type of soil, the element under concern and on the species used as plant test. The extractant DTPA has shown to be superior in discriminating the liming effect on the availability of soil metals (Korcak & Fanning, 1978; Singh & Narwal, 1984; Bataglia & Raij, 1994).

Long term field studies under tropical or subtropical conditions to evaluate the effects of sewage sludge on the accumulation and availability of heavy metals in soils are scarce in the literature. The present research aimed at studying the effects of sewage sludge application, in a long-term field experiment, on the accumulation of heavy metals (Cu, Zn and Ni) in the soil and their availability to maize plants, with and without liming.

MATERIAL AND METHODS

Field experiments were carried out in Cordeirópolis, SP, Brazil (22º32'S, 47º27'W, 639 m altitude). Sewage sludge was applied to a Rhodic Hapludox soil cultivated with maize (HMD 7974 hybrid), from 1983 to 1987. The chemical analyses (Raij & Quaggio, 1983) of soil samples taken from the 0-20 cm layer, indicates the following results: pH ($CaCl_2$) - 3,9; organic matter - 48 g kg^{-1}; P-resin: 5 mg dm^{-3}; K^+, Ca^{2+}, Mg^{2+} and $H^+ + Al^{3+}$: 1.6; 3.0; 1.0; and 97 $mmol_c\ dm^{-3}$ of soil, respectively. The physical analyses of the soil samples (Camargo et al., 1986) indicated: coarse sand – 75; fine sand – 200; silt – 145; and clay - 580 g kg^{-1} soil, respectively.

Sewage sludge anaerobically digested and not treated with calcium monoxide and ferric chloride, was sampled in São Paulo, during 1983 to 1986. The sludge was chemically characterized yearly (Table 1) and the analysis followed the procedure: 1.000 g samples were digested with 50 mL of 4 mol L^{-1} HNO_3 solution, at 180ºC, for one hour; the digested samples stood at room temperature and after that, the volume was adjusted to 50 mL with deionized water and filtered through filter paper. The filtered digested solutions were analysed for K by flame photometry; P by the vanado-molybdate spectrophotometric method; Ca, Mg, Fe, Cu, Ni, Mn, and Zn, by atomic absorption spectrophotometry.

The total N concentration in sludges was determined by the micro-Kjeldhal method and the total C by the oxidation of organic matter using 0.17 mol L^{-1} potassium dichromate solution and titration of the excess bichromate with a 0.5 mol L^{-1} ammonium ferrous sulfate solution, using diphenylamine as indicator (Raij & Quaggio, 1983). Cd and Pb were also determined by

atomic absorption spectrofotometry, but their total contents were below the detection limit (< 0.01 mg kg^{-1}).

Trials were set up in a totally randomized, complete blocks design (n = 4). Each block was split in two bands, one with and another without liming. The sludge was applied in each band at rates: 0, 20, 40, 60 and 80 Mg ha^{-1} (dry basis) in a single application; and 40, 60 and 80 Mg ha^{-1} split in two, three and four equal yearly applications, respectively. Each experimental unit consisted of 42-m^2 plots, with six rows (7 m length, 1 m spacing) cultivated with maize, the central rows used at harvest (10m^2) for plant yield evaluations.

The sewage sludge was applied by hand and incorporated into the soil in the 15 cm depth layer, during October, 1983. The plots corresponding to the split doses of sludge, received new applications in October 1984, 1985 and 1986. The band of each plot treated with lime, received two applications of 6.0 and 3.5 Mg ha^{-1} calcinated lime (43.8% CaO e 27.2% MgO) to rise base saturation to 70%, during October 1983 and September 1984. All plots received a P-K fertilization consisting of 100 kg P_2O_5 ha^{-1} as simple superphosphate and 60 kg K_2O ha^{-1} as potassium chloride, applied to rows before sowing.

Soil samples (ten subsamples) were taken during the four planting years from each treated plot after harvest. Soil samples were analysed for pH ($CaCl_2$ 0.01 mol L^{-1}); Cu, Ni, Zn concentrations in extracts obtained with DTPA (Lindsay & Norwell, 1978) and Mehlich-3 (Mehlich, 1984), and for the total metal concentrations in extracts obtained by digestion with concentrated nitric-perchloric acids (Abreu et al., 1996). Concentrations of metals were determined in all extracts using an inductively coupled plasma spectrometer (ICP-OES).

Plant samples were collected in two stages: (1) at 60-65 days of age, leaves from position +4 were taken from 20 plants per plot, and the one-third mid-leaf of the limbs used for chemical analyses; (2) at harvest, four maize plants per plot (central rows) were separated into shoots (leaves + stalks) and grain. Plant samples were dried in a forced air oven at 65ºC until constant mass for dry matter determination and ground in a Willey type grinder; 1.000 g samples were then incinerated at 500ºC for two hours and extracts were obtained by suspending the ashes in 2 mol L^{-1} HCl and filtering in filter paper (Bataglia et al., 1978). Leaf Cu, Ni and Zn concentrations were determined by atomic absorption spectrometry.

Total contents of heavy metals in maize plants were estimated from concentration and dry matter yield data of shoots and grain. The total dry matter and grain yield data was obtained at harvest, using all plants from the central rows of each plot.

The effects of sewage sludge on the extractable (DTPA, Mehlich-3) metal concentrations and on the total-metal concentrations in the soil samples were interpreted based on the polynomial regression analysis for the data obtained in the treatments correspondent to the doses 0 to 80 Mg ha^{-1} sludge in a single application at the beginning of the field experiments. The liming effects were interpreted from the analysis of variance and the efficacy of the extractants in predicting the soil availability of heavy metals for plants were evaluated by means of simple linear correlation analysis between leaf metal concentrations, plant total metal contents and extractable (DTPA, Mehlich 3) soil metal concentrations.

RESULTS AND DISCUSSION

Extractability of heavy metals from soil

Concentrations of Zn in DTPA and Mehlich-3 extracts increased linearly with increasing sludge doses, with or without liming (Figure 1). These results are similar to those obtained by Korcak & Fanning (1985) and Rappaport et al. (1988) for the DTPA extractant, and by Cavallaro et al. (1993) and Oliveira & Mattiazzo, (2001) for the Mehlich-3 extractant. The increase in the extractable soil Zn concentrations was proportional to the increase in the sewage sludge rates applied to the soil (Table 2). Rappaport et al. (1988) also observed this relationship between the extractable soil Zn concentrations and the sewage sludge rates applied to the soil.

Liming reduced the extractability of Zn-DTPA and the inverse effect was observed for Zn-Mehlich-3. Similar results were obtained by Korcak & Fanning (1978) and Bataglia & Raij (1994) when using DTPA. The small range of values observed for the extractable Zn soil concentrations due to liming, may in part be explained by the effect of the sludge on soil pH (Table 3). Although the addition of lime resulted in steady increase in soil pH along the years, pH values were only significantly higher in 1984 and 1985, but still remained below

Table 1 - Characterization of sewage sludge samples utilized in the experiment.

Year	C	N	P	Ca	Mg	K	Fe	Cu	Ni	Mn	Zn	Moisture
	g kg^{-1}							mg kg^{-1}				g kg^{-1}
1983	217	14	8	24	9	4	38	791	322	315	1,888	605
1984	265	16	2	15	6	2	44	975	401	423	2,904	570
1985	261	16	2	20	7	3	41	1,148	459	479	3,326	610
1986	286	9	2	21	12	2	84	989	1.240	223	4,035	600

6.0. The unexpected increase in the Zn-Mehlich-3 extract concentrations in the limed treatments can be related to the acid characteristic of this extractant solution (acetic, nitric and fluoridric acids + EDTA), which might be strong enough to overpass the liming effect.

Table 2 - Quantities of Cu, Ni and Zn added to the soil by the sewage sludge, in single or split applications.

Sludge	Cu	Ni	Zn
Mg ha^{-1}	----------	kg ha^{-1}	----------
20	15.8	6.4	38
40	31.6	12.8	76
60	47.4	19.2	114
80	63.2	25.6	152
40[p]	35.3	14.4	96
60[p]	58.3	23.6	163
80[p]	78.1	48.4	244

[p]Total doses of 40, 60 e 80 Mg ha^{-1} split in 2, 3 and 4 years, respectively, adding 20 Mg ha^{-1} year^{-1}

Similarly, extractable Cu concentrations with both extractants also increased linearly with the increasing sewage sludge doses (Figure 1), corroborating the results of Korcak & Fanning (1985) and Rappaport et al. (1988). The addition of lime did not alter the concentrations of Cu-DTPA but induced an increase in the Cu-Mehlich-3 concentrations. Krebs et al. (1998) observed that Cu concentrations in soil extracts obtained with 0.1 mol L^{-1} $NaNO_3$ solution were not affected by liming, possibly because Cu forms stable complexes with soluble organic molecules. In such complexes, Cu would be protected from adsorption by soil mineral particles and, consequently, more easily extracted.

Working with 24 different types of soil (Camargo et al., 1982), observed that the DTPA extractant discriminated the variation in Cu concentrations re-

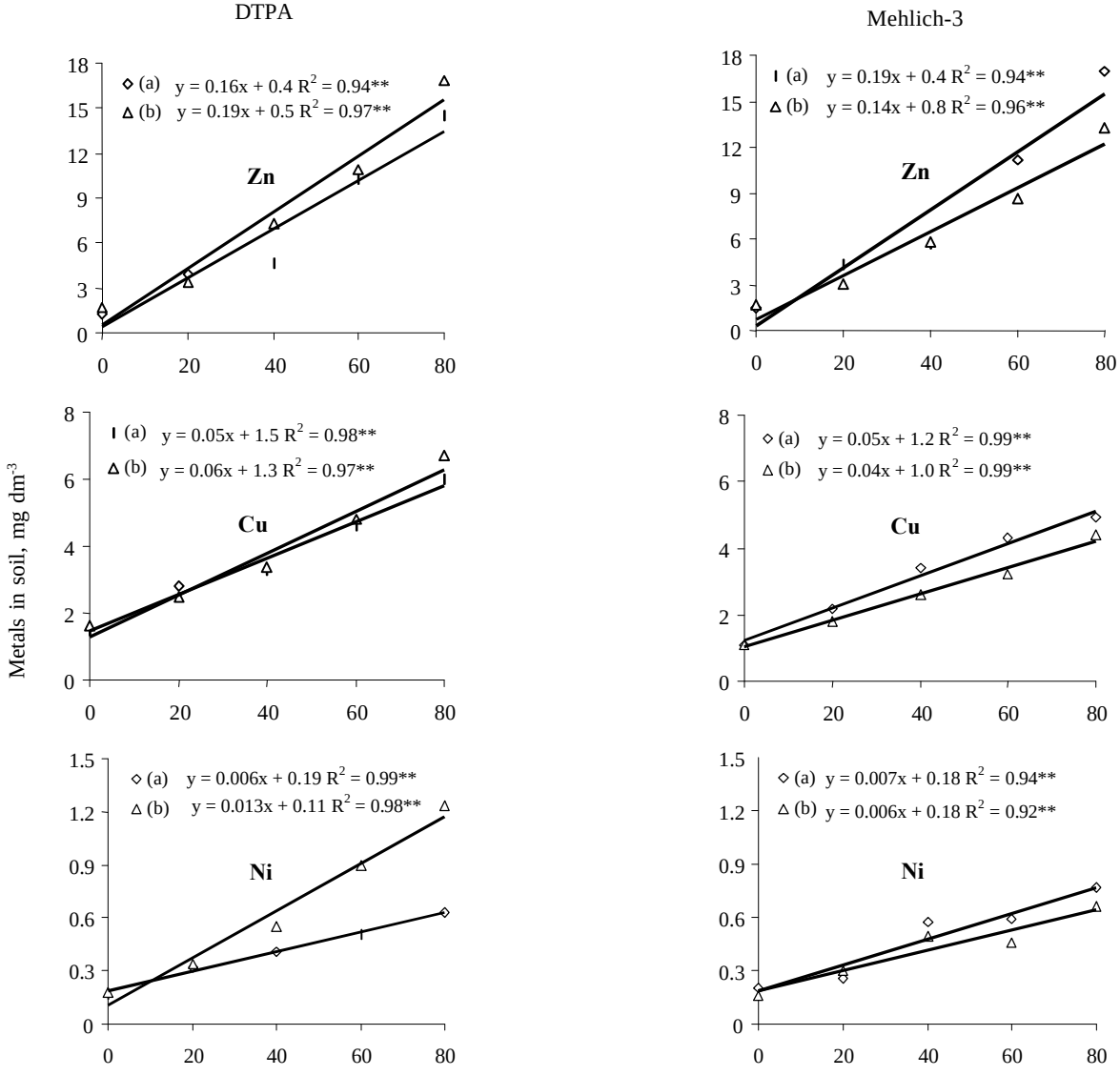

Figure 1 - Concentrations of heavy metals extracted with DTPA and Mehlich-3 solutions, from a soil treated with increasing sewage sludge doses, with (a) and without (b) liming.

sulting from treatments with and without liming, except for three soils, two of them having high organic matter content, and the other, low Cu concentration (< 0.4 mg dm^{-3}). The soil and sewage sludge organic matter might play important role in the control of Cu availability, possibly through complexation reactions (McBride, 1989).

Several authors have demonstrated that the lower Cu response to pH changes as compared to Zn (Jeffery & Uren, 1983; Sims & Kline, 1991), might be related to the different chemical performance of these elements in the soil and in the sludge. Luo & Christie (1998) obtained different chemical fractions (exchangeable fraction, carbonates, Fe and Mn oxides, organic and residual fractions) in two soils (sandy and claiy) before and after treatment with rural or urban sewage sludge. In the sludges, the major part of the Cu was associated to the organic fraction (30.4 e 54.7 %), followed by the residual fraction (12.8 e 30.7 %), carbonates (7.2 e 25.5 %), Fe and Mn oxides (9.3 e 1.7 %) and the exchangeable fraction (16.0 e 11.6 %). The major part of the Zn was associated to the residual fraction (72.2 e 55.9), followed by the Fe and Mn oxides fraction (23.4 and 15.6 %), organic (9.5 e 7.1 %), carbonates (1.6 e 13.6 %) and exchangeable fraction (1.1 and 0.1 %). After application of sludge, approximately 74% and 80% of the added Cu and Zn were found in the residual fraction, for both soils (Luo & Christie, 1998). The major part of the extractable Cu and Zn was associated to the organic fraction and to the Fe and Mn oxides fraction, respectively.

Increasing sewage sludge doses induced a linear increase in the Ni concentrations of soil extracts obtained with both extractants (Figure 1). The results obtained for Ni-EDTA were similar to those obtained by Valadares et al. (1983) and were correlated to the total quantities of Ni added to the soil by the sludge. On the other hand, Williams et al. (1980) observed that Ni concentration in the soil was not affected by increasing rates of sewage sludge (0, 45, 90, 135, 180 e 225 Mg ha^{-1}). In this research, the analyses run in the sludge samples taken in 1985 and 1986 (Table 1) indicated that total Ni concentrations exceeded the critical limits permitted for use in agriculture, according to Norm CETESB 4230 (CETESB, 1999). Consequently, large quantities of this element were applied to the field experiment (Table 2). Although Ni concentrations increased with increasing sludge doses, values obtained for both extractants remained within the regular range for soils of the State of São Paulo (0.5 a 1.4 mg dm^{-3}), according to Rovers et al. (1983).

Only the DTPA extractant showed reduction in the extractable Ni concentration of samples from limed treatments (Figure 1). In studies of Ni adsorption-desorption in the soil, Pombo et al. (1989) observed that liming-induced higher soil pH increased soil adsorption capacity. Both soils (Hapludox and Red Yellow Podzol) presented higher Mg concentrations in the extracts of Ni adsorption, indicating that Ni competes with Mg by the same exchange soil sites. On the other hand, the high quantities of desorbed Ni (with 0.01 mol L^{-1} HCl solution) found for the Red Yellow Podzol under pH 5.5, rose doubts about the soil pH management concept to imobilize heavy metals added to soils. Camargo et al. (1989) observed also an increase in the soil capacity for Ni adsorption after liming.

The well known effect of soil reaction on micronutrient and heavy metal availabilities has lead some scientists to establish minimum critical values of pH for soils treated with residues suspected to contain such elements. Mattiazzo-Prezotto (1994) suggested to maintain the pH (H_2O) at the minimum of 5.3 for acid tropical soils, before application of residues containing heavy metals. Higgins (1984) reported that minimum pH (H_2O) values around 6.2 or above, are required to control the phytoavailability of heavy metals and recommended the utilization of low-heavy-metal sewage sludges. In the State of São Paulo, Brazil, the Norm CETESB P4230, which controls the sewage sludge application to soils of the State, establishes that the pH ($CaCl_2$) values ought to be in the range 5.5 to 7.0, in areas submitted to such sludge treatment (CETESB, 1999). In the present experiments, limed subplots presented values of pH as high as 6.3 during the year of 1985; however, during most of the period, values of pH ranged from 5.0 to 6.0 (Table 3). In unlimed subplots, values of pH stood below 4.5.

Total metal concentrations in the soil

Total Cu and Zn concentrations increased linearly with the increasing doses of sewage sludge, however, the total Ni concentration was not affected (Table 4). Chang et al. (1984) observed that sewage sludge applications, at the rate of 90 Mg ha^{-1} $year^{-1}$ resulted in heavy metal accumulation in the soil profile, increasing the concentrations of Cd, Cr, Cu, Ni and Zn, extracted by 4 mol L^{-1} HNO_3 solution, from 0.4; 19; 16; 7 and 110 mg kg^{-1}, determined at the beginning of the experiment, to 8.3; 168; 146; 59 e 764 mg kg-1, respectively, in six years. Similar results were reported by Baxter et al. (1983) using the same extractant solution, and by Oliveira (2000) for the Cu, Cr and Zn extracted with aqua regia in microwave oven.

In the present research, the increases observed in total Cu and Zn concentrations in the soil samples were correlated to the quantities of these elements added by the sewage sludge (Table 2). However, total Ni concentrations, despite of the high quantities of Ni added by the sewage sludge applications, remained within the regular range found by Rovers et al. (1983) for soils of the State of São Paulo (< 10 a 127 mg dm^{-3}) (Table 2).

Table 3 - Values of pH in 0.01 mol L^{-1} $CaCl_2$ in a Rhodic Hapludox soil treated with sewage sludge, in single or split application, with or without liming, during four consecutive years (average of four replications).

Sludge doses	1984		1985		1986		1987	
	a	b	a	b	a	b	a	b
Mg ha^{-1}								
0	4.8	3.9	5.1	4.1	5.9	4.1	5.6	4.1
20	4.6	4.0	5.4	4.1	5.3	4.1	5.6	4.0
40	4.8	4.0	5.4	4.4	6.0	4.4	5.5	4.1
60	5.2	4.0	6.3	4.3	5.6	4.4	5.2	4.2
80	5.2	4.2	5.0	4.4	5.8	4.4	5.2	4.3
Regression	L**	ns	Q*	ns	ns	ns	ns	ns
Liming	**		**		**		**	
C.V. (%)	5.6		6.0		5.0		5.5	
40[p]	4.5	3.9	5.9	4.5	5.5	4.5	5.3	4.2
60[p]	4.7	4.0	5.3	4.3	5.5	4.3	5.5	4.1
80[p]	4.7	3.9	6.0	4.2	5.4	4.3	5.1	4.2

a: with liming; b: without liming; L: linear effect; Q: quadratic effect; *,**: significant at 0.05 and 0.01 level, respectively; ns: not significant; [p]: total doses of 40, 60 and 80 Mg ha^{-1} of sludge, split in 2, 3 and 4 years, respectively, adding 20 Mg ha^{-1} $year^{-1}$.

Table 4 - Total Cu, Ni and Zn concentrations in a Rhodic Hapludox soil , treated with sewage sludge, in single or split application (average of eight replications).

Sludge	Cu	Ni	Zn
Mg ha^{-1}		mg dm^{-3}	
	1984		
0	50	16	61
20	58	20	73
40	57	21	76
60	61	25	83
80	70	22	92
Regression	L**	ns	L**
C.V. (%)	7.1	9.8	10.5
40[p]	59	23	70
60[p]	55	22	70
80[p]	56	18	70
	1987		
0	56	19	79
20	60	23	79
40	31	20	84
60	61	21	88
80	79	25	123
Regression	L*	ns	L*
C.V. (%)	13.2	12.3	22.3
40[p]	64	21	103
60[p]	73	24	123
80[p]	76	29	139

[p]:total doses of 40, 60 e 80 Mg ha^{-1} of sludge, split in 2, 3 and 4 years, respectively, adding 20 Mg ha^{-1} $year^{-1}$; L: linear effect; *,**: significant at 0.05 and 0.01 level, respectively; ns: not significant.

Phytoavailability of metals

DTPA and Mehlich-3 extractant solutions were effective to evaluate availability of Zn for maize plants, using as reference the Zn leaf concentration and total Zn content of shoots (Table 5). For treatments with liming, leaf Zn concentrations were better correlated to the soil extractable concentrations than to total Zn content in the shoots, corroborating the results reported by Bataglia & Raij (1994). On the other hand for, treatments without liming, except for the year of 1986, the total Zn contents in the shoots presented higher correlation coefficients with the soil concentrations, as compared to leaf Zn concentrations, for both extractant solutions (Table 5).

Different results were observed for Cu, since high correlation coefficients were only obtained between soil extractable Cu concentrations and total Cu contents in the shoots of maize plants, during the four years for the unlimed treatments and during the first year for the limed treatment. When using the variable leaf Cu concentration to calculate the correlation coefficients, none of the extractants, were effective in evaluating Cu availability to the maize plants. This might be explained by the fact that there was no effect of increasing sewage sludge doses on the leaf Cu concentrations, whereas the soil extractable Cu concentrations, with both extractant solutions, increased linearly in response to the sludge treatments (Figure 1).

Contrarily to the results obtained in this experiment, several authors have reported the superiority of DTPA extractant solution, as compared to acid extractants, in predicting the availability of Cu (Korcak & Fanning, 1978; Camargo et al., 1982) and Zn (King & Hajjar, 1990; Bataglia & Raij, 1994), and in the ability of DTPA discriminating the effect of pH increase on the reduction of these metal availabilities. Although the high

Table 5 - Simple correlation coefficients (r) between Cu and Zn leaf concentrations or total shoot contents in maize plants and the soil extractable element concentrations, using DTPA and Mehlich-3 extractant solutions, in soil treated with sewage sludge, in single and split application, with and without liming, during four successive years.

Extractant solutions	Correlation coefficients							
	Leaf metal concentration				Absorbed quantitie			
	1984	1985	1986	1987	1984	1985	1986	1987
With liming								
Cu								
DTPA	0.192	-0.022	0.217	0.238	0.595**	0.351*	0.011	0.012
Mehlich-3	0.198	-0.063	0.21	0.305	0.669**	0.164	0.201	0.001
Zn								
DTPA	0.779**	0.547**	0.602**	0.572**	0.868**	0.246	0.236	0.620**
Mehlich-3	0.729**	0.502**	0.560**	0.534**	0.860**	0.21	0.255	0.493**
Without liming								
Cu								
DTPA	0.089	0.056	0.168	0.225	0.741**	0.570**	0.458**	0.442*
Mehlich-3	0.015	0.229	0.278	0.151	0.682**	0.479**	0.571**	0.509**
Zn								
DTPA	0.707**	0.622**	0.763**	0.797**	0.750**	0.653**	0.536**	0.882**
Mehlich-3	0.689**	0.565**	0.808**	0.758**	0.723**	0.620**	0.638**	0.894**

*, **: significant at 0.05 and 0.01 level, respectively.

Ni concentrations found in sewage sludge samples (Table1) and the increasing soil extractable Ni concentrations found in the soil samples from plots treated with increasing sludge doses (Figura 1), Ni was not detected in the analyzed plant parts (leaves, shoots and grains).

ACKNOWLEDGEMENTS

To FAPESP and CNPq for financial support and fellowships.

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Received August 12, 2002
Accepted August 12, 2003