Soil Biology & Biochemistry 39 (2007) 1341–1347
www.elsevier.com/locate/soilbio

Nickel in a tropical soil treated with sewage sludge and cropped with maize in a long-term field study

Wanderley José de Melo*, Priscila de Stéfani Aguiar, Gabriel Maurício Peruca de Melo, Valéria Peruca de Melo

Laboratório de Biogeoquímica, Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias, Universidade do Estado de São Paulo. Via de Acesso Prof. Paulo Donato Castellane, Km 5. CEP 14884-900. Jaboticabal, SP, Brazil

Available online 26 December 2006

Abstract

Sewage sludge produced by the SABESP wastewater treatment plant (Companhia de Saneamento Básico do Estado de São Paulo), located in Barueri, SP, Brazil, may contain high contents of nickel (Ni), increasing the risk of application to agricultural soils. An experiment was carried out under field conditions in Jaboticabal, SP, Brazil, with the objective of evaluating the effects on soil properties and on maize plants of increasing rates of a sewage sludge rich in Ni that had been applied for 6 consecutive years. The experiment was located on a Typic Haplorthox soil, using an experimental design of randomized blocks with four treatments (rates of sewage sludge) and five replications. At the end of the experiment the accumulated amounts of sewage sludge applied were 0.0, 30.0, 60.0 and 67.5 t ha^{-1}. Maize (*Zea mays* L.) was the test plant. Soil samples were collected 60 d after sowing at depths of 0–20 cm for Ni studies and from 0 to 10 cm and from 10 to 20 cm for urease studies. Sewage sludge did not cause toxicity or micronutrient deficiencies to maize plants and increased grain production. Soil Ni appeared to be associated with the most stable fractions of the soil organic matter and was protected against strong extracting solutions such as concentrated and hot HNO_3 and HCl. Ni added to the soil by sewage sludge increased the metal concentration in the shoots, but not in the grain. The Mehlich 3 extractor was not efficient to evaluate Ni phytoavailability to maize plants. Soil urease activity was increased by sewage sludge only in the layer where the residue was applied.

Keywords: Sewage sludge; Nickel; Soil pollution; Mehlich 3; Urease; Phytoavailability

1. Introduction

Sewage sludge is a residue from domestic and industrial wastewater treatment. Increasing costs of commercial fertilizers and large amounts of sewage sludge produced worldwide have made cropland application of this residue an attractive disposal option. Chemical and biological compositions of sewage sludges depend on the wastewater composition (Melo et al., 2002). Usually, it is rich in organic matter (OM) and plant nutrients such as nitrogen (N), phosphorus (P) and calcium (Ca) (Hue, 1988), and can improve soil physical, chemical and biological properties, such as porosity, aggregate stability, bulk density, soil fertility, water movement and retention (Silveira et al., 2003). In the tropics, where soils are generally poor in minerals with high cation exchange capacity (CEC) and in OM, sewage sludge could be an important soil amendment (Melo et al., 2002). A problem to be solved is the hazardous constituents, such as heavy metals, among them nickel (Ni), and persistent organic pollutants (POPs) normally present in sewage sludge. These may cause soil and water pollution and toxicity to crops, animals, and humans through the trophic chain (McBride, 1995). Ni concentrations in sewage sludge vary widely, depending on the origin of the sludge and on the processes of treatment in the sewage treatment plant (Mattigod and Page, 1983); its uptake by plants depends on the form in the soil and on the plant species. The legislation regulating sewage sludge application to soils generally considers only the total content of heavy metals in the residue. The legislation

*Corresponding author. Tel.: +55 016 3209 2675x228; fax: +55 016 3209 2675.
E-mail address: wjmelo@fcav.unesp.br (W.J. de Melo).

0038-0717/$ - see front matter
doi:10.1016/j.soilbio.2006.12.010

adopted by CETESB (Companhia de Tecnologia e Saneamento Ambiental) (1999) in São Paulo State, which is the same as that of the Environmental Protection Agency (EPA 503) legislation for the United State of America, establishes that the upper concentration of Ni in sewage sludge for agricultural purposes is 420 mg kg^{-1} (dry weight basis). Sewage sludge produced by SABESP (Companhia de Saneamento Básico do Estado de São Paulo), which treats the sludge produced in the Metropolitan Region of São Paulo, sometimes exceeds that limit. Considering that Brazilian soils are quite different from US soils, the aim of this work was to estimate the effect of rates of application of a sewage sludge produced by SABESP on the Ni forms in the soil and on its potential availability to maize (*Zea mays* L.) plants cropped in a sandy soil in a field experiment carried out for 6 consecutive years.

2. Materials and methods

The experiment was carried out under field conditions, using a randomized block design with four treatments (rates of sewage sludge) and five replications, in Jaboticabal, SP, Brazil (21°15′20″S, 48°19′02″W), from 1997 to 2003. A sandy soil (clay, 26%; silt, 7%; sand, 67% at a depth of 0 to 20 cm) classified as a Typic Haplorthox, was used for applying sewage sludge and maize was cropped. In the first year, the rates of sewage sludge applications were 0.0 (control, with no sewage sludge and no mineral fertilization), 2.5, 5.0 and 10.0 t ha^{-1} (dry weight basis). Starting in the second year, the control plots were fertilized with mineral fertilizers according to the soil chemical analysis and the recommendations of Raij et al. (1997). Starting in the fourth year, the 2.5 rate was replaced by 20.0 t ha^{-1} in order to increase the concentration of Ni in the soil. Therefore, the accumulated amounts of sewage sludge over six years were 0.0, 30.0, 60.0 and 67.5 t ha^{-1}. The soil chemical properties of the control plots before each growing season are shown in Table 1.

Sewage sludge was obtained from the sewage treatment plant operated by SABESP, located in Barueri, SP, and its chemical composition in the 6-yr experiment is presented in Table 2.

Total N in sewage sludge was determined by the microKjeldahl method, potassium (K) by flame photometry, P by the vanado-molybdate spectrophotometric method, and the heavy metals, including Ni, by atomic absorption spectrometry (AAS) in the extract obtained by digestion with $HNO_3 + H_2O_2 + HCl$ (USEPA, 1986).

Before starting the experiment (September, 1997), the area was plowed, harrowed, and limed with 2.5 t ha^{-1} of dolomitic limestone to raise the base saturation (BS) to 70%. Thirty days after liming, the plots were marked out (54 m^2 each and 28.8 m^2 of useful area for soil and plant sampling), including six 10-m long rows, spaced 0.90 m apart; the two central rows were reserved for evaluation of grain production, and the others for plant and soil sampling.

Sewage sludge (77.6% moisture) was spread on the soil surface annually and incorporated into the top10 cm layer with a rotary hoe (first year), or a light harrowing (last five years). Plots treated with sewage sludge received supplementary P and K as mineral fertilizers, when necessary (Table 3), in order to supply them with the same amounts of NPK as the control. The mineral fertilizer was applied in the plow furrow. The mineral fertilization applied to the control plots in the 6 years of experimentation is shown in Table 3. N fertilization of the control plots was divided and the side dressing was applied 45 d after sowing. The test plant in all 6 yr was maize (*Zea mays* L.), grown at a density of 5–7 plants m^{-1} (about 55,000 plants ha^{-1}).

Plant samples (5 plants per plot) were taken 100 d after sowing. The plants were cut just above the soil surface, washed with diluted neutral detergent, water, distilled water, deionized water, dried at 70 °C in a forced air oven, ground and, after nitric-perchloric digestion, the extract was analyzed for total Ni by AAS (Bataglia et al., 1983).

For evaluation of crop production, plants were harvested 120 d after sowing from the two central rows of each plot, dried at 65 °C in a forced air oven, and weighed. The data for grain production were corrected to 13% moisture. Ni determination in the grain was processed by the same method described for the shoots.

Soil samples (0–20 cm layer) for Ni analysis were collected 60 d after sowing in the sixth year of experimentation. In the

Table 1
Chemical properties in the 0–20 cm layer of the control plots of a Typic Haplorthox soil before sewage sludge application in each of the 6 yr of experimentation[a]

Year	pH $CaCl_2$	O.M. (g dm^{-3})	P_{resin} (mg dm^{-3})	K (mmol$_c$ dm^{-3})	Ca (mmol$_c$ dm^{-3})	Mg (mmol$_c$ dm^{-3})	H + Al (mmol$_c$ dm^{-3})	SB[b] (mmol$_c$ dm^{-3})	CEC (mmol$_c$ dm^{-3})	BS[c] (%)
1997/98	5.7	20	44	2.2	26	11	16	39	55	71
1998/99	6.1	18	47	1.7	35	10	15	47	62	75
1999/00	5.4	16	43	1.6	20	10	19	32	51	71
2000/01	5.0	16	71	2.3	25	9	29	36	66	55
2001/02	5.1	24	54	4.8	23	10	38	38	76	50
2002/03	4.8	20	31	1.7	18	10	34	30	64	47

[a]Values on an air dried basis.
[b]SB, sum of bases.
[c]BS, base saturation.

Table 2
Chemical composition of sewage sludge in each of the six years of experimentation

Element	Unit[a]	1997/98	1998/99	1999/00	2000/01	2001/02	2002/03
Nitrogen	$g\,kg^{-1}$	6.40	37.31	28.72	28.94	36.75	34.08
Phosphorus	$g\,kg^{-1}$	3.32	11.30	17.41	15.58	15.54	21.62
Potassium	$g\,kg^{-1}$	0.97	1.70	1.47	1.85	2.74	1.90
Manganese	$mg\,kg^{-1}$	228	294	257	263	287	222
Zinc	$mg\,kg^{-1}$	1800	3810	2328	1745	2354	2159
Copper	$mg\,kg^{-1}$	664	551	660	719	627	722
Nickel	$mg\,kg^{-1}$	268	595	360	354	350	231
Lead	$mg\,kg^{-1}$	152	371	180	171	155	186
Cadmium	$mg\,kg^{-1}$	8	12	8	10	9	11
Chromium	$mg\,kg^{-1}$	290	1190	764	699	778	808

[a]Values on an air dried basis.

Table 3
Chemical fertilization applied to a Typic Haplorthox soil cropped with maize during the six years of experimentation

Growing season	Rate ($t\,ha^{-1}$)	Nutrient ($kg\,ha^{-1}$)				
		N		P_2O_5	K_2O	
		S[a]	D[b]	S	S	D
1997/98	0.0	—	—	—	—	—
	2.5 (20)	—	—	50	29	—
	5.0	—	—	44	26	—
	10.0	—	—	33	20	—
1998/99	0.0	16.7	60	30	30	—
	2.5 (20)	—	—	—	25	—
	5.0	—	—	—	20	—
	10.0	—	—	—	10	—
1999/00	0.0	30	110	50	50	40
	2.5 (20)	—	—	—	46	40
	5.0	—	—	—	41	40
	10.0	—	—	—	32	40
2000/01	0.0	30	120	50	50	40
	5.0	—	—	32	39	40
	10.0	—	—	2	28	40
	20.0	—	—	—	5	40
2001/02	0.0	30	140	70	50	40
	5.0	—	—	—	34	40
	10.0	—	—	—	17	40
	20.0	—	—	—	—	40
2002/03	0.0	30	140	70	50	40
	5.0	—	—	—	38	40
	10.0	—	—	—	25	40
	20.0	—	—	—	—	40

[a]Sowing stage.
[b]Side-dressing stage.

area reserved for soil sampling of each plot, 12 single samples were collected 10 cm from the sowing furrow, at the side of a maize plant, and composited. Soil samples were air dried and sieved (2 mm) before chemical analysis. For urease activity evaluation, soil samples were collected from the layers 0–10 and 10–20 cm.

Total Ni was determined by AAS on the extract obtained by the two methods. In the USEPA (1986) method, since named the USEPA method, the extract was obtained as follows: the soil sample (2.00 g) was placed in a 100 ml beaker and 10 ml of 1 + 1 (v/v) HNO_3 were added, followed by heating on a hot plate at 90–95 °C for 15 min. After heating, concentrated HNO_3 (6 ml) was added and heated for a further 30 min. This operation was repeated until no more brown fumes were formed and the volume of the sample reached about 5 ml. Deionized water (2 ml) and 3 ml of 30% (w/w) H_2O_2 were added and the sample was heated with repeated additions of 1 ml of H_2O_2 (maximum of 10 ml) until the reaction stopped and the volume of the extract reached about 5 ml. Then concentrated nitric acid (10 ml of 35%, w/w) was added and the extract heated at 90–95 °C for 15 min. The extract was transferred to a 50 ml volumetric flask and made up to volume with deionized water. In the method described in Jackson (1958), since named the Jackson method, the soil extract was obtained as follows: Soil (100 mg) was placed in a 50 ml Teflon crucible. The sample was wetted with a few drops of deionized water. Then, 0.5 ml of concentrated $HClO_4$ and 5 ml of 48% HF were added. The crucible, with the lid covering nine-tenths of the top, was placed on a sand bath at a temperature of 200–225 °C, and the acids were evaporated to dryness. The crucible was then removed from the sand bath, cooled, 6 M HCl (5 ml) was added and the suspension was diluted with 15 ml of deionized water. The crucible was then put on a hot plate and the suspension boiled gently for 5 min. When the soil was totally dissolved, the solution was cooled, transferred to a 100 ml volumetric flask and made up to volume with deionized water.

Soil organic matter (SOM) was extracted and fractionated by a modified method of Dabin (1971), which consisted of extracting the humic substances (HS) from the soil samples with 0.1 M NaOH, followed by acidifying the alkaline extract to pH 1 with concentrated H_2SO_4. The soluble fraction was the fulvic acids (FAs) and the precipitated fraction was the humic acids (HAs). Ni was determined by AAS in the extracts obtained from the different fractions by the USEPA method.

Ni potentially available to maize plants was estimated by the Mehlich 3 extraction method (Mehlich, 1984). Soil

(5.00 g) was placed in a 125 ml Erlenmeyer flask and 25 ml of Mehlich 3 extractor were added. The flask was shaken for 5 min and the suspension filtered through a Whatman number 1 filter paper. Ni was determined in the extract by AAS. A control was carried out in the absence of soil.

Soil urease activity was evaluated by the method of May and Douglas (1976) with the modifications of Longo and Melo (2005a). Soil (30.00 g) was placed in a 125 ml Erlenmeyer flask and held at 30 °C for 10 min with 0.5 ml of toluol and 12 ml of deionized water. Then urea solution (3 ml of 3.5 g l^{-1}) was added and a new incubation of 60 min at 30 °C and agitated. After incubation, 2 M KCl (15 ml) containing phenylmercury acetate (5 Mm) was added and the filtrate (10 ml) was used for NH_4-N determination by the steam distillation method (Bremner and Keeney, 1965). A control was carried out in the same way, but urea solution was added after the 60-min incubation.

The data were analyzed for variance and when the *F*-test was significant at $P<0.01$, the Tukey's test ($P<0.05$) was used for comparison of means. The correlation coefficients (R) between Ni extracted from the soil and the amounts taken up by maize plants (grain + shoots) were calculated (Pimentel Gomes, 1966).

3. Results

3.1. Soil properties and sewage sludge composition

The Typic Haplorthox soil was selected for this study because it occurs in large areas in the State of São Paulo. The chemical properties of samples obtained from the control plots for the 6 yr of experimentation are presented in Table 1. These data were used to calculate the mineral fertilization to be applied to those plots.

The sewage sludge generated in the sewage treatment plant operated by SABESP in Barueri, SP, is rich in OM, plant nutrients (except K) and also in heavy metals such as Ni, since the plant treats the sludge collected from a highly industrialized region (Table 2). The content of Ni ranged from 231 (2002/2003) to 595 mg kg^{-1} (1998/1999), values sometimes higher or very close to the limit established by CETESB (1999), where sewage sludge is applied to agricultural areas.

3.2. Forms of Ni in soil

Total soil Ni, estimated according to the USEPA method (Table 4), ranged from 6.40 (control) to 16.56 mg kg^{-1} (in the 60.0 t ha^{-1} accumulated sewage sludge treatment) while the total soil Ni evaluated according to the Jackson (1958) method ranged from 17.28 (control) to 27.43 mg kg^{-1} (in the 60.0 t ha^{-1} accumulated sewage sludge treatment). When total Ni was determined by the Jackson method, all the data obtained from treatments that received sewage sludge differed from the control, but no difference was detected among the treatments that received the sludge.

Soil Ni extracted by the Mehlich 3 extractor method ranged from 0.35 (control) to 3.65 mg kg^{-1} (60.0 t ha^{-1} sewage sludge plots). This is a low fraction of the Ni evaluated by both the methods used for estimating total Ni (23.4% and 9.0% related to the methods of USEPA and Jackson, respectively).

The major part of the soil Ni associated with OM was found in the humin fraction (Table 5), where concentration increased from 4.10 (control) to 13.82 mg kg^{-1} (60.0 t ha^{-1} sewage sludge plots). This value is 3.4 times the content in the control. Ni in the FA fraction increased from 2.07 (control) to 2.89 mg kg^{-1} (67.5 t ha^{-1} sewage sludge plots), and Ni in the HA fraction decreased from 0.23 (control) to 0.18 mg kg^{-1} (60 t ha^{-1} sewage sludge plots). For the most part, the Ni added to the soil by sewage sludge appeared as humin, the most stable fraction of the SOM. The distribution of Ni in the SOM is humin > FA > HA. This fact is important to consider because of the solubility of these soil constituents. It is also important to observe that the concentrations of Ni extracted by the Mehlich 3 extractor and the Ni present as FA were very close.

3.3. Plant growth and Ni uptake

Maize plants showed normal growth in the field. They did not present any symptoms of Ni toxicity or micronutrient deficiency induced by an excess of Ni in the soil.

Table 4
Forms of Ni in a Typic Haplorthox soil treated with sewage sludge and cropped with maize for 6 consecutive years

Sewage sludge (t ha^{-1})	Total Ni		Mehlich 3 (mg kg^{-1} soil[a])
	USEPA (mg kg^{-1} soil[a])	Jackson (mg kg^{-1} soil[a])	
0	6.40d[b]	17.28b	0.35c
30.0	10.58c	26.14a	2.00b
60.0	16.56a	27.43a	3.65a
67.5	13.36b	28.13a	3.40a

[a]Values on an air dried basis.
[b]Means followed by the same letters in the same column are not different by the Tukey's test at $P<0.05$.

Table 5
Ni in the soil organic matter fractions of a Typic Haplorthox soil treated with sewage sludge and cropped with maize for 6 consecutive years

Sewage sludge (t ha^{-1})	Humin Ni (mg kg^{-1} soil[a])	Fulvic acid Ni (mg kg^{-1} soil[a])	Humic acid Ni (mg kg^{-1} soil[a])
0	4.10d[b]	2.07c	0.23ab
30.0	8.21c	2.32bc	0.05b
60.0	13.82a	2.47bc	0.18ab
67.5	10.33b	2.89a	0.14a

[a]Values on an air-dried basis.
[b]Means followed by the same letters in the same column are not different by the Tukey's test at $P<0.05$.

Dry matter production by the aerial part of the maize plants sampled 100 d after sowing was not affected by the treatments (Table 6), but the grain production increased from the control (5.57 t ha^{-1} at 13% moisture) to the highest rate of sewage sludge (8.60 t ha^{-1} at 13% moisture), an increase of 3.03 t ha^{-1}.

Sewage sludge increased the Ni concentration in maize shoots (Table 6) from 6.28 (control) to 11.48 mg kg^{-1} (67.5 t ha^{-1} sewage sludge), but it did not affect Ni concentration in the maize grain.

3.4. Urease activity

Soil urease activity ranged from 6.52 (control) to 12.49 mg NH_4-N kg^{-1} h^{-1} (67.5 t ha^{-1} sewage sludge) in the samples obtained from 0 to10 cm layer. For the samples obtained from 10 to 20 cm layer, urease activity initially decreased from 11.77 (control) to 8.91 (60.0 t ha^{-1} sewage sludge) and then increased to 12.01 NH_4-N kg^{-1} h^{-1} (67.5 t ha^{-1} sewage sludge) as can be seen in Fig. 1.

The enzyme activity was affected differently by the treatments depending on the sampling depth. For samples taken from the upper layer, the layer where sewage sludge was incorporated, urease activity increased with the rate of sludge application. On the other hand, for the samples obtained from 10 to 20 cm layer, the enzyme activity was not affected by the treatments.

Table 6
Dry matter, grain production and grain Ni content in maize plants cropped in a Typic Haplorthox soil treated with increasing rates of sewage sludge for 6 consecutive years

Sewage sludge (t ha^{-1})	Shoots[a]		Grain[b]	
	Dry matter (t ha^{-1})	Ni (mg kg^{-1})	Mass (t ha^{-1})	Ni (mg kg^{-1})
0	14.67a[c]	6.28b	5.57b	3.23a
30.0	15.39a	6,87b	6.19ab	3.14a
60.0	17.21a	8,11b	7.20ab	2.91a
67.5	15.88a	11,48a	8.60a	2.65a

[a]Values on an oven dried basis.
[b]Data adjusted to 13% moisture.
[c]Means followed by the same letters in the same column are not different by the Tukey's test at $P<0.05$.

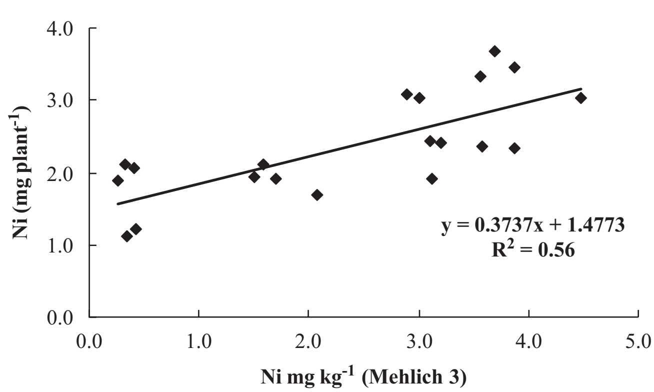

Fig. 1. Determination coefficients between Ni extracted by the Mehlich 3 extractor and the metal absorbed by maize plants cropped in a Typic Haplorthox soil treated with increasing rates of sewage sludge for 6 consecutive years.

4. Discussion

4.1. Soil properties and sewage sludge composition

The chemical properties of the soil used in this experiment (Table 1) classify it as a soil with medium fertility according to the concentrations established in Raij et al. (1997).

The Ni content in sewage sludge (Table 2), in the second year of experimentation (1998/1999), it was higher than the 420 mg kg^{-1} (dry weight basis) established by CETESB (1999) in its legislation regulating sludge application to agricultural soils. However, in most of the years, the concentration of Ni in the sewage sludges was very close to that limit, and so Ni is a heavy metal that can limit the application to agricultural soils. of the sewage sludges generated by the SABESP source. As the limiting value of 420 mg kg^{-1} was not established for tropical soils, it is very important to know the Ni behavior in soil when sewage sludge is added, the forms in which it occurs, the availability to plants, and the translocation to the grain in order to prepare guidelines for applications that will have low risk for the environment.

4.2. Forms of Ni in soil

Sewage sludge application to the soil significantly increased total Ni content determined by the USEPA (1986) method up to the rate of 60.0 t ha^{-1} and then decreased the rate of 67.5 t ha^{-1} (Table 4). It is important to consider that the accumulated rate of 60.0 t ha^{-1} was obtained after 6 yr of applications of 10 t ha^{-1}, while the accumulated rate 67.5 t ha^{-1} was obtained in a shorter time, since 60.0 t ha^{-1} was applied in the last 3 years. This finding shows that the interaction of the components of sewage sludge with soil fractions is time dependent and that the USEPA method is not able to measure some forms of Ni in soil. Other authors, estimating total Ni by the USEPA method have also found an increase in total soil Ni by increasing rates of sewage sludge (F.C. Oliveira, unpublished Ph.D. Thesis, ESALQ, São Paulo University, Piracicaba, SP, Brazil, 2000; M. Marchiori Jr, unpublished Ph.D. Thesis, FCAV, University of the State of São Paulo, Jaboticabal, SP, Brazil, 2002). When total Ni was measured by the method of Jackson (1958), all the rates of sewage sludge showed an increase in the Ni concentration as compared to the control, but the different rates of sewage sludge did not show different totals of soil Ni concentrations. The USEPA method extracted about 47% of the Ni extracted by the Jackson method. Thus the Ni extracted by the USEPA method really is not the total Ni.

One possible explanation for this finding is that heavy metals in soils may be occluded in iron hydroxides and manganese oxides, in which tropical soils are rich (Malavolta, 1994). Whereas the USEPA method does not dissolve the entire soil sample, the Jackson method does.

Mehlich (1984) proposed a variation of the Mehlich 1 extractor, which is a mixture of two diluted acids ($H_2SO_4 + HCl$) to estimate the availability of heavy metals present in soils. This extractor also includes complexing agents and is known as the Mehlich 3 extractor. In our study, the application of sewage sludge to a Typic Haplorthox soil increased extractable Ni up to an accumulated rate of 60.0 t ha^{-1} and then decreased at the highest accumulated rate. This behavior is similar to that obtained for total Ni evaluated by USEPA (1986), confirming that at that rate, accumulated in a shorter time, the content of easily extractable Ni is lower. Other authors, like Martins et al. (2003), also have observed that increasing sewage sludge rates induced a linear increase in the Ni concentration of soil extracts obtained with the Mehlich 3 extractor.

Ni distribution in the fractions of the SOM was in the following order: humin > FA > HA. Considering that the FA fraction is soluble both in acid and in alkaline media, it may be an important fraction for the availability of Ni to plants or for increasing the movement of the metal down in the soil profile. On the other hand, Ni associated with humin, a fraction that is not extracted by an alkaline solution, may be greatly unavailable to plants and a way to maintain Ni in the upper soil layers.

The findings that soil Ni is not totally extracted by the USEPA method, and that soil Ni is concentrated in the humin fraction of the SOM, can explain why most authors have found that Ni accumulates in the top layers of the soil. For example, Sidla et al. (2003) found that more than 93% of all heavy metals applied over a 2-yr period were accumulated in the surface soil. Earlier, Anderson and Nilson (1972) observed that practically all the Ni remained in the 0 to 20 cm layer of the soil, after 12 yr of adding 84 t ha^{-1} of sludge. The accumulation of Ni in the top layer of the soil can be attributed to the high affinity of the metal to OM (McGrath and Lane, 1989). Heavy metals like Ni may be adsorbed strongly to the soil organic and mineral colloids like clay, iron hydroxides and manganese oxides and to SOM (Chaney and Giordano, 1977).

4.3. Plant growth and Ni uptake

Metals and other toxic products did not affect maize plant growth after an application of 67.5 t ha^{-1} of sewage sludge in a period of 6 consecutive years. The plants did not present any symptoms of Ni toxicity or of deficiency in other nutrients caused by the presence of an excess of Ni in the soil. Dry matter content of the shoots was not affected (Table 6), but the grain production increased in the presence of sewage sludge. This increase in grain production may be explained by the improvement in soil properties due to the OM and plant nutrients present in sewage sludge (Melo et al., 2002).

From analysis of maize uptake of Ni, and the distribution of the metal in the plant parts, we can see that sewage sludge increased the metal content in the shoots (Table 6). This was not observed in the case of the grain, which shows that the translocation of Ni from leaves and stem to grain was not significant. In an experiment with *Cedrela fissilis* Vell, submitted to increasing rates of Ni in the nutrient solution, Paiva et al. (2002) found that Ni concentrations increased in roots, stems and leaves and that the metal concentration was higher in shoots than in the grain. Miller et al. (1995), in an experiment with barley, also observed that the contents of Ni were higher in the straw than in the grain. Anjos and Mattiazzo (2000) observed similar results for different parts of maize plants cropped in Oxisols treated with sewage sludge.

The increase in Ni concentration in stem + leaves is not important for human nutrition, but it is important for animal nutrition, if the material is used for silage or hay production.

The Mehlich 3 extractor was not sufficiently good for estimating the availability of Ni to maize plants ($R^2 = 0.56$), as can be seen in Fig. 2. Other authors, such as Haq et al. (1980), Abreu et al. (1995), and Anjos and Mattiazzo (2000) have also found that the Mehlich 3 extractor is not efficient for measuring soil Ni availability to plants. According to Pires and Mattiazzo (2003), the efficiency of this extractor depends on the metal to be measured, the type of soil, and the plant species.

4.4. Urease activity

Longo and Melo (2005b), studying the effect of sampling time, sampling depth and the soil coverage, obtained urease activities varying from 2 to 15 mg NH_4-N dm^{-3} of soil h^{-1}. They observed that the enzyme activity was affected by the time of sampling, the highest activity being found in hot and rainy months, by the type of soil coverage, and by the soil depth.

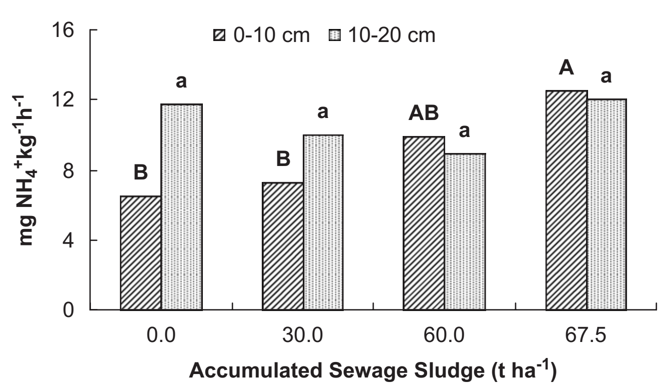

Fig. 2. Urease activity in a Typic Haplorthox soil treated with sewage sludge and cropped with maize for 6 consecutive years. Means followed by the same capital letter (0–10 cm) and lower case letter (10–20 cm) are not different by the Tukey's test at $P < 0.05$.

Urease is an enzyme that depends on Ni for its activity. Thus it is possible to suppose that the addition of sewage sludge contaminated with the metal might cause an increase in the enzyme activity, if other pollutants would not be able to inactivate the enzyme. In this experiment, it was observed that sewage sludge increased urease activity, when the samples were obtained from 0 to 10 cm layer (Fig. 1), where the residue was incorporated.

This increase in urease activity caused by sewage sludge application may be explained by the content of OM, the types of plants, and the microorganisms in sewage sludge, and also by the urea, the substrate of the enzyme, and by some urease already present in sewage sludge. Nickel may also have contributed to that increase, since the enzyme is metal-dependent. The fact that the enzyme activity was not affected by the treatments in the samples obtained from 10 to 20 cm layer suggests that OM and other factors that affect urease activity, such as Ni, did not reach that depth.

4.5. Conclusions

Sewage sludge did not cause toxicity, did not induce micronutrient deficiencies to maize plants, and did not affect shoot dry matter production, but it did increase grain production. Ni added to the soil by sewage sludge appeared to be associated with the most stable fractions of the SOM and protected against strong extracting agents such as concentrated and hot HNO_3 and HCl, justifying its concentration in the upper soil layers. Soil Ni after 6 yr of experimentation was available to maize plants, with increased concentration in the shoots, but not in the grain. The Mehlich 3 extractor was not efficient to evaluate Ni phytoavailability to maize plants. Soil urease activity was increased by sewage sludge only in the layer where the sludge was applied.

Acknowledgement

We are grateful to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for financial support.

References

Abreu, C.A., Abreu, M.F., Raij, B., van Santos, W.R., 1995. Comparação de métodos de análise para avaliar a disponibilidade de metais pesados em solos. Revista Brasileira de Ciência do Solo 19, 463–468.

Anderson, A., Nilson, K.O., 1972. Enrichment of trace elements from sewage sludge fertilizer in soils and plants. Ambio 1, 176–179.

Anjos, A.R.M., Mattiazzo, M.E., 2000. Metais pesados em plantas de milho cultivadas em Latossolos repetidamente tratados com biossólido. Scientia Agricola 57, 769–776.

Bataglia, O.C., Furlani, A.M.C., Teixeira, J.P.F., Furlani, P.R., Gallo, J.R., 1983. Métodos de análises químicas de plantas. Instituto Agronômico, Campinas, p. 48.

Bremner, J.M., Keeney, D.R., 1965. Steam-distillation methods for determination of ammonium, nitrate and nitrite. Analytica Chimica Acta 32, 485–495.

CETESB, 1999. Aplicação de lodos de sistema de tratamento biológico em áreas agrícolas. Critérios para projeto e operação. ABIA, São Paulo, 32 p. (p. 4.230, Manual Técnico).

Chaney, R.L., Giordano, P.M., 1977. Microelements as related to plant deficiencies and toxicities. In: Elliot, L.F., Stevenson, F.J. (Eds.), Soils for Management of Organic Wastes and Wastewaters. SSSA, Madison, Wisconsin, pp. 234–279.

Dabin, B., 1971. Étude d'une méthode d'extraction de la matiére humique des sols. Science du Sol 1, 47–63.

Haq, A.U., Bates, T.E., Soon, Y.K., 1980. Comparison of extractors for plant-available zinc, cadmium and copper in contaminated soils. Soil Science Society of America Journal 44, 772–777.

Hue, N.V., 1988. Residual effects of sewage sludge application on plant and soil-profile chemical composition. Communications in Soil Science and Plant Analysis 19, 1633–1643.

Jackson, M.L., 1958. Soil Chemical Analysis. Prentise-Hall, Inc., Engelwood Cliffs, NJ, pp. 283-284.

Longo, R.M., Melo, W.J., 2005a. Urea hydrolysis in Oxisols: effects of substrate concentration, temperature, pH, incubation time and storage conditions. Revista Brasileira de Ciência do Solo 29, 851–857.

Longo, R.M., Melo, W.J., 2005b. Urease activity in Oxisols as influenced by vegetation cover and sampling time. Revista Brasileira de Ciência do Solo 29, 645–650.

Malavolta, E., 1994. Fertilizantes e seu Impacto Ambiental: micronutrientes e Metais Pesados, Mitos, Mistificação e Fatos. Produquímica, São Paulo, 153 pp.

Martins, A.L.C., Bataglia, O.C., Camargo, O.A., 2003. Copper, nickel and zinc phytoavailability in an Oxisol amended with sewage sludge and liming. Scientia Agricola 60, 747–754.

Mattigod, S.V., Page, A.L., 1983. Assessment of metal pollution in soil. In: Thornton, I. (Ed.), Applied Environmental Geochemistry. Academic Press, London, pp. 355–394.

May, P.B., Douglas, L.A., 1976. Assay for soil urease activity. Plant and Soil 45, 760–764.

McBride, M.B., 1995. Toxic metal accumulation from agricultural use of sludge: are USEPA regulations protective? Journal of Environmental Quality 24, 5–18.

McGrath, S.P., Lane, P.W., 1989. An explanation for the apparent losses of metal in long-term field experiments with sewage sludge. Environmental Pollution 60, 235–256.

Mehlich, A., 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis 15, 1409–1416.

Melo, W.J., Marques, M.O., Ferreira, M.E., Melo, G.M.P., Melo, V.P., 2002. Chemical properties and enzyme activity in a sewage sludge-treated soil. Communications in Soil Science and Plant Analysis 33, 1643–1650.

Miller, R.W., Azzari, D.T., Gardiner, D.T., 1995. Heavy metals in crops as affected by soil types and sewage sludge rates. Communications in Soil Science and Plant Analysis 26, 703–711.

Paiva, H.N., Carvalho, J.G., Siqueira, J.O., 2002. Nutrient content in cedro (*Cedrela fissilis* Vell.) seedlings submitted to increasing levels of nickel in nutrient solution. Revista Árvore 26, 279–284.

Pimentel Gomes, F., 1966. Estatística Experimental, Second ed. Nobel, São Paulo, 404 pp.

Pires, A.M.M., Mattiazzo, M.E., 2003. Biosolid conditioning and the availability of Cu and Zn for rice. Scientia Agrícola 60, 161–166.

Raij, B., van Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendações de Adubação e Calagem para o Estado de São Paulo. Instituto Agronômico, Campinas, 285 pp. (Boletim Técnico 100).

Sidla, R.C., Hook, J.E., Kardos, L.T., 2003. Heavy metals application and plant uptake in a land disposal system for wastewater. Journal of Environmental Quality 5, 97–102.

Silveira, M.L.A., Alleoni, L.R.F., Guilhermje, L.R.G., 2003. Sewage sludge and heavy metals in soils. Scientia Agricola 60, 793–806.

USEPA (United States Environmental Protection Agency), 1986. Standards for the use and disposal of sewage sludge. Washington. (Code of Federal Regulations 40 CRF Part 503).