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Dynamics of zinc (Zn) and nickel (Ni) in a Cerrado Oxisol treated with sewage sludge for a long period

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Abstract

Sewage sludge can be a source of micronutrients, such as zinc (Zn) and nickel (Ni), for Cerrado soils; however, since they are heavy metals, continuous use of this residue requires an evaluation of the dynamics of these elements in the soil/plant system. This study aimed to evaluate the dynamics of Zn and Ni in a soil/plant system regarding extraction methods to determine the quantity of plant nutrients in the soil as well as the soil enzymatic activity in an area treated with sewage sludge for eleven years. Sewage sludge was applied annually in an Oxisol and a crop was sown. The accumulated sewage sludge doses throughout the eleven years of the experiment were 0 as control, 55, 110 and 167.5 Mg ha^{-1}, and corn was the test crop. At 60 days after sowing, corn soil samples were collected from the 0–10 cm layer (for analysis of enzymatic activity) and 0-20cm, 20-40 cm (for analysis of Zn and Ni). The activities of the enzymes urease and dehydrogenase and the extractors Mehlich-1, Mehlich-3 and DTPA were evaluated. The increments of Zn and Ni in the Cerrado Oxisol as a function of sewage sludge application over a long time were small. The Mehlich-3 extractant was the best correlated with Zn and Ni extraction by the corn plants. In the eleventh year of application, sewage sludge was not efficient at supplying Zn to plants. The application of high doses of sewage sludge for a long time affects soil enzymatic activity.

Keywords: biosolids; environmental pollution; extractors; heavy metals; plant nutrition.
Abbreviations: DAPE_days after plant emergence; DKB_Dekalb; SP_São Paulo; SS_sewage sludge.

Introduction

Cerrado (savanna) is the second largest biome in Brazil and is predominant in the Central and Northeastern portions of the country (Pavinato et al., 2009). Cerrado are composed of thin, scarce vegetation due to low fertility soils, frequently very poor in phosphorus (P) and zinc (Zn) and with high aluminium (Al) concentrations. However, the flat topography of Cerrado soils facilitates mechanization; therefore, it is considered the region with the highest agricultural expansion potential in the country. Occupying around 65% of the Cerrado biome, Oxisols are very weathered, poor soils from a chemical perspective, requiring the use of soil correctors and fertilizers for their agricultural use (Souza and Lobato, 2004). As it presents both macro and micronutrients capable of correcting soil fertility in its composition, sewage sludge emerges as an alternative for agricultural use in this scenario.

Sewage sludge is a residue from domestic wastewater treatment (Nogueira et al., 2013). Increasing costs of commercial fertilizers and large amounts of sewage sludge produced worldwide have made land application of this residue an attractive disposal option (Melo et al., 2007). Nevertheless, the final disposal of this residue demands continuous monitoring, especially for the presence of potentially toxic elements in its constitution, requiring special attention regarding the possible contamination of plants by heavy metals, primarily cadmium (Cd), chromium (Cr) lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn), of general occurrence in sewage sludge (Mosquera-Losada et al., 2009).

The use of sewage sludge in agriculture has demanded great effort in the attempt to evaluate the dynamics of potentially toxic elements it contains, because soil and plants accumulate such elements in different ways and linear responses are not always observed in the availability and absorption of elements from added sewage sludge (Nascimento et al., 2014). Nonessential elements or ones required in small amounts are often found in high concentrations in sewage sludge (Nogueira et al., 2008). When an element is classified as both a nutrient and a heavy metal at the same time, its relevance must also be evaluated regarding environmental and nutritional aspects. The elements Zn and Ni are in this category. The former is, in general, the heavy metal with the second highest concentration in sewage sludge from Brazil, behind only iron (Fe); while the latter is found in much higher amounts than those required by plants. In addition, Brazilian regulations recommend sewage sludge doses as a function of the amount of nitrogen to be applied (CONAMA, 2006), which causes micronutrients such as Zn and Ni to be applied in concentrations far beyond those needed by crops.

The fact that a heavy metal is present in soil does not mean it is in a form readily assimilable by plants (Macedo et al., 2012).

The availability of elements in soil is driven by a series of factors such as parent material, soil pH values, soil redox reactions, microbial activity, soil moisture and temperature as well (Kabata-Pendias and Mukherjee 2007). As for plants, they absorb elements according to their capacity to make exchanges with the environment. Therefore, a change in any of these variables directly influences the dynamics of the element, which makes diagnosing its availability difficult.

Studies in the field, in the long term, provide information for the establishment of regulations that standardize sewage sludge use in Brazilian soils, since they are scarce in the climatic conditions prevailing the country, where there is great potential to use sewage sludge in agricultural areas (Rangel et al., 2004).

In general, in studies under tropical conditions using sewage sludge as a source of Zn and Ni, it has supplied the needs of corn plants (Galdos et al., 2004; Rangel et al., 2006; Gomes et al., 2006); however, these studies represent short-term researches, mostly from one to three years of cultivation.

Keeping these aspects in view, the present study was conducted to assess the effect of the dynamics of zinc and nickel on the soil/plant system in a soil of Brazilian Cerrado treated with sewage sludge over a long time.

Results and Discussion

Nutrient balance

Compared to the fifth year of the experiment there was an increase in Zn-soil content above 65 % in all treatments before the eleventh year of the experimental installation and above 85 % to 60 DAPE in the eleventh year of the experiment. For Ni, compared to the fifth year of the experiment the balance of the element before beginning the eleventh year of the experiment was negative for treatments 0 (control), 55, and 110 Mg ha^{-1}. 60 DAPE in the eleventh year there were increases of around 39% (treatment 55 Mg ha^{-1}) to 98% at the highest dose of Ni applied.

These results indicate there are increased concentrations of Zn and Ni in the soil shortly after the application of sewage sludge, which over time tends to decrease. This becomes clear when evaluating the concentration of Ni in the fifth year and before the eleventh year installation. From an agricultural point of view this effect is beneficial because there is the possibility of increased availability of the element during the crop cycle.

Regarding the balance of the elements in the eleventh year of experimentation (Table 4), the values obtained immediately before the experimental installation in the 11th year showed an increasing trend for Zn as a function of the dose. Besides, soil Zn content was kept after the sewage sludge application, although the increment was proportionally higher in the Control treatment. As for Ni, values found in the Control treatment were very similar to those found for the dose of 55 Mg ha^{-1}, both before and after sewage sludge application, but the highest Ni concentrations also occurred with the highest sludge doses applied. There was a greater increase in elements in the treatments with no sewage sludge application. The 167.5 Mg ha^{-1} treatment showed the second highest Zn increase, followed by the treatment with 55 Mg ha^{-1} and then the treatment of 110 Mg ha^{-1}. For Ni, the opposite occurred; the highest sludge dose caused the smallest increase.

Zn and Ni concentrations in soil, even after 11 years of sewage sludge application, remained below the limits established by the European Community (Zn = 150 to 300 mg kg^{-1}; Ni = 30 to 75 mg kg^{-1}) (CEC, 1986), USA (Zn = 1400 mg kg^{-1}; Ni = 210 mg kg^{-1}) (USEPA, 1993), and Brazil (Zn = 450; Ni = 70 mg kg^{-1}) (CONAMA, 2009), indicating for these elements that they are within the values considered as safe according to these regulations on sewage sludge in agricultural soils of Cerrado. Considering Ni extracted by the USEPA method and the 20-40 cm layer as the natural concentration of this element in soil, one can infer that the increase in the element through sewage sludge application even after eleven years was still low.

The control treatment showed greater increases in Zn and Ni compared to the treatments receiving sewage sludge. The hypothesis for this effect is that the sewage sludge treatments received, consequently, large amounts of Zn and Ni, causing an excess of free Ni^{2+} and Zn^{2+} ions in the soil solution. Considering this excess, there may be a displacement of these elements to the soil solid fraction, causing a more stable bond between them and the clay minerals, obeying a mass balance. This effect was reported by Nogueira et al. (2008) when determining Zn with the extractors $HNO_3 + H_2O_2 + HCl$ (USEPA, 1993) and $HClO_4 + HF$ (Jackson, 1958) in the ninth year of his experiment, where a higher Zn extraction by the second method was found. Also, according to Melo et al., (2007) the USEPA method extracted about 47% of the Ni extracted by the Jackson method. Thus, the Ni extracted by the USEPA method is really not the total Ni. Nogueirol et al. (2013) stated that trace elements show great affinity for oxides and hydroxides, which are the predominant minerals in this type of soil, and cannot be extracted by the semi-total method (USEPA, 1993) applied in this study. This also explains why the treatments with sludge application did not produce increments proportional to the applied doses. Jalali and Moharrami (2007) described Zn with the second and Ni with the third highest adsorption force to Fe, Al and Mn oxides and hydroxides, while Fontes (1992) detailed this phenomenon for tropical conditions and Abat et al. (2012) confirmed this effect for Zn and Soares et al., (2011) for Ni. From the environmental perspective, heavy metal complexation by soil particles is beneficial, because it neutralizes their toxic effects.

Bioavailability of Zn and Ni

Zn and Ni values determined through different extractants oscillated only in thesurface soil layers (0–10 and 10–20 cm), not differing in the 20–40 cm layer except for Zn content determined through the Mehlich-1 extractor, according to which values decreased from the Control treatment to the 110 Mg ha^{-1} dose, beyond which point they no longer differed (Table 5). The fact that the elements changed their concentrations only in the 0–20 cm soil layer is also a positive aspect from the environmental perspective, and values found for the 20–40 cm layer can be attributed to the natural concentration of each element in the soil, considering leaching negligible. This effect was also verified by Macedo et al. (2012) for the elements Cr, Cd and Pb in a study conducted under the same conditions.

The coefficients of variation showed higher values for Ni when extractions simulating phytoavailability were used – Mehlich-1, Mehlich-3 and DTPA (Table 5). As for the semi-total concentration, the highest coefficient of variation occurred for Zn. Evaluating phytoavailability throughout the soil profile and in each one of the studied layers, based on mean comparisons, the results found with Mehlich-3 and DTPA were similar (Table 5). Regarding Ni, the extractors Mehlich-1, Mehlich-3 and DTPA showed increasing extraction values as a function of the dose, changing only the capacity of each one to extract the element. The high coefficients of variation indicated the greater difficulty of

Table 1. Crop and treatments used in eleven years of experiment.

Year	Crop	Sewage sludge Mg ha^{-1}					
		0^a Control			55^a	110^a	167.5^a
		N$^{(MF)}$	$P_2O_5$$^{(MF)}$	$K_2O$$^{(MF)}$			
1997-98	Corn	0	0	0	5	10	2.5
1998-99	Corn	76	30	30	5	10	2.5
1999-00	Corn	140	50	90	5	10	2.5
2000-01	Corn	150	50	90	5	10	20
2001-02	Corn	170	50	90	5	10	20
2002-03	Corn	170	50	90	5	10	20
2003-04	Sunflower	50	20	20	5	10	20
2004-05	Sunhemp	0	18	18	5	10	20
2005-06	Corn	170	50	90	5	10	20
2006-07	Corn	170	50	90	5	10	20
2007-08	Corn	170	50	90	5	10	20

^{a}Sewage sludge accumulated in 11 years of experiment, on a dry basis. ^{MF} – mineral fertilizer

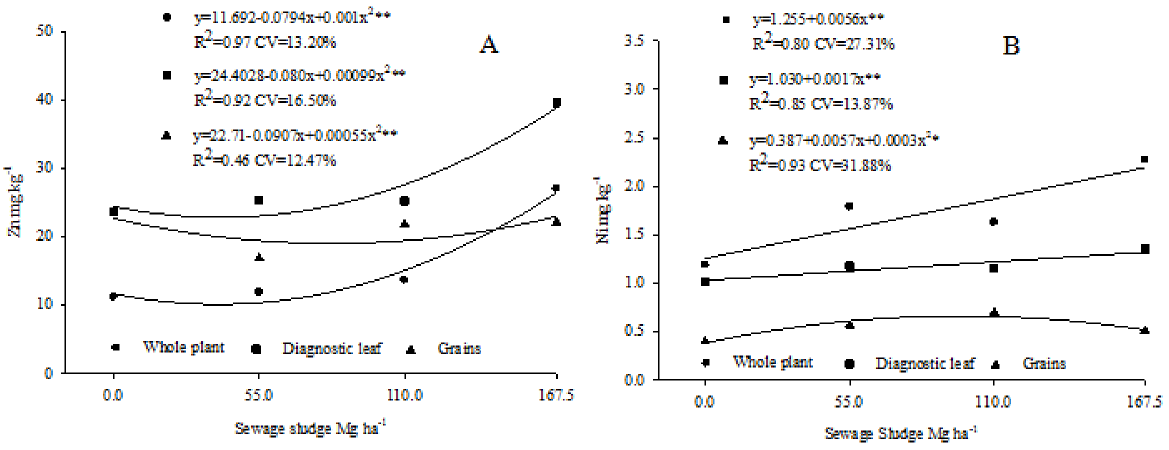

Fig 1. Regression equation expressing the effects of sewage sludge doses on the Zn (A) and Ni (B) concentrations in corn plants. * significant at 5% of probability. ** significant at 1% of probability. CV = coefficient of variation.

these extractors at simulating the phytoextraction capacity. The Mehlich-1 extractor was the one that recovered the most Ni and the least Zn.

Experiments determining heavy metal concentrations in soil, especially coming from residues like sewage sludge, commonly show large coefficients of variation, due to the uneven distribution of residues, affinity for specific clay minerals and complexation by organic matter. This variation is even higher when the soil type, management, plant or plant parts (roots, leaves and stem) are different, or even when extractors are used for multi-element evaluation. Studies such as those by Degryse et al. (2009) and Takeda et al. (2006), aiming to verify the available fraction of heavy metals in soil, reported the same difficulty. Diesing et al. (2008), evaluating Zn speciation in soils contaminated with heavy metals, also verified higher soil/plant correlation coefficients when extractors evaluating exchangeable and soluble forms were used.

With the treatments used in the experiment, only the highest dose of sewage sludge supplied sufficient Zn to the crop. That occurred even with the minimum annual dose applied (5 Mg ha^{-1}), surpassing the Zn dose of 4 kg ha^{-1} recommended for corn (Raij et al., 1997). This allows the conclusion that throughout the years, areas receiving sewage sludge do not show Zn in a phyto-available form.

The excessive concentration of heavy metals in soil, as well as their transformations and interactions with other elements throughout the years, seems to limit the capacity of extractors to simulate their availability to plants. This effect becomes evident when the Mehlich-1 extractor is used, which recovered small amounts of Zn at the maximum sewage sludge dose, at which concentration the plants extracted the most Zn from the soil. The effect of time also seems to interfere with the availability and absorption capacity of elements by plants. In tropical regions, the absorption of Zn by plants in areas with no sewage sludge application has always caused concern due to the excess of this element. In the case of this study, if the critical Zn level between 15 and 50 mg kg^{-1} established for corn (Malavolta et al., 1997) is considered, only the dose of 167.5 Mg ha^{-1} met the needs of the crop. In the same area, in a study referring to the ninth year of the experiment, Nogueira et al. (2008) verified that, at the first used doses (accumulated) of sewage sludge (0, 45 and 90 Mg ha^{-1}), corn plants reached the critical interval considered ideal for Zn, while the highest dose of sludge (127.5 Mg ha^{-1}) surpassed the critical level, indicating luxury consumption or toxicity.

Zn and Ni in plants

Zn concentration in the whole corn plants showed a quadratic trend as a function of the dose, dropping from the Control treatment until the dose of 42.68 Mg ha^{-1}, and increasing again after that (Figure 1). A similar effect was observed for

Table 2. Soil chemical characterization (0-20 cm) before the experiment installation in the eleventh agricultural year.

Treatments	pH	OM	P	K	Ca	Mg	H+Al
Mg ha^{-1} SS*	$CaCl_2$	g dm^{-3}	mg dm^{3}	——mmol$_c$ dm^{-3}——			
0 Control	5.0	27	49	4.5	27	6	38
55	5.1	27	50	2.8	33	8	34
110	5.2	30	87	3.0	40	9	34
167.5	4.7	31	107	1.8	33	7	58

OM = organic matter. *SS = sewage sludge, on a dry basis, in accumulated in eleven years of residue application

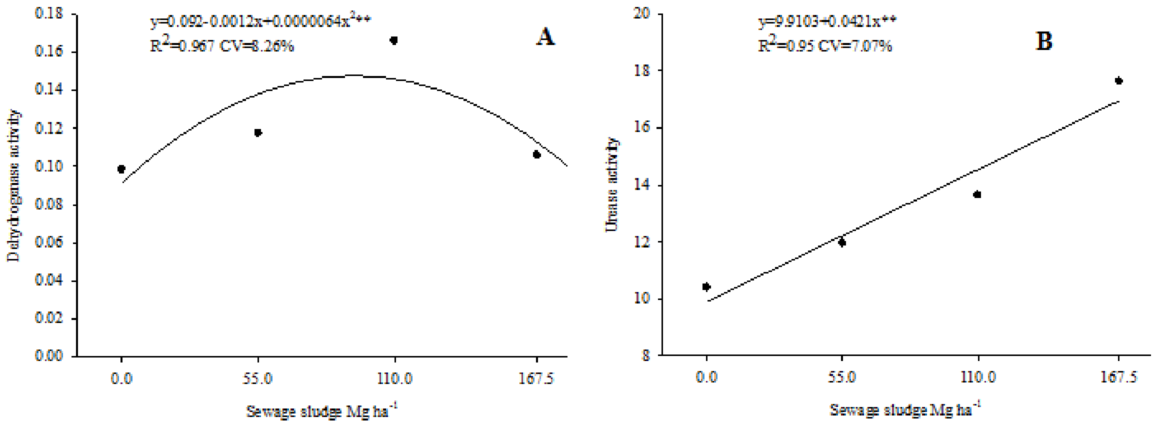

Fig 2. Regression equation expressing the effects of accumulated doses of sewage sludge on the (A) urease (mg kg^{-1} h^{-1} N-NH_4^+) and (B) dehydrogenase (µg TFF g^{-1} dia^{-1}) activities, in the soil layer of 0-10 cm. * significant at 5% of probability. ** significant at 1% of probability. CV = coefficient of variation.

diagnostic leaves, which in turn the trend line also showed the smallest Zn concentration (21.40 Mg ha^{-1}). As for the grains, the trend line was also quadratic; however, unlike the previous ones, it showed a maximum concentration of the element for the dose of 82.96 Mg ha^{-1} of sewage sludge. In fact, this range in Zn concentration as a function of sludge dose, in the opposite way as described to the whole plant and diagnostic leaves, may indicate a possible Zn redistribution from leaves to seeds, as plant Zn-use efficiency in order to guarantee seed quality, which justifies the distinct effects in the trend lines. In plants, Ni concentrations stayed close to the ones considered normal (0.1 and 0.4 mg kg^{-1}) by Malavolta et al. (1997). However, these values are general, with no specific study for corn. Ni concentrations in diagnostic leaves and whole plants, although they increased as a function of the sewage sludge dose, showed small variations from the smallest to the highest dose. A quadratic trend was found for the grains from the Control treatment, with a maximum at the estimated dose of 97.20 Mg ha^{-1}, and tending to drop after this point. Since Ni is not listed in the fertilization recommendation programs for crops in Brazil and there is no set critical level for corn, it was not possible to infer the capacity of the sewage sludge to supply this element to the plants. As for the correlations between soil and plants (Table 6), except for USEPA digestion, which did not correlate with it in the estimation of Ni, all the extractors were efficient in the attempt to estimate the amount of Zn available to plants; particularly the Mehlich-3 extractor, which showed the highest coefficient of determination both for the whole plant and the diagnostic leaf. For Ni, the extractors were efficient only in the correlation with whole plants and still with less efficiency compared to Zn. No extractor correlated with the Ni concentration in diagnostic leaves. The same occurred for Zn and Ni in grains.

Enzyme activity in the soil

Urease activity (figure 2) showed an increasing linear trend from the Control treatment on, while dehydrogenase activity showed a quadratic behaviour with an increase from the Control treatment onward, with a maximum at the estimated dose of 93.58 Mg ha^{-1} and a decreasing trend afterward. The enzyme analyses may be indicative of changes occurring in the environment as a function of the sewage sludge application. Urease activity increased as a function of the sewage sludge dose. At first, one could infer that the application of sewage sludge stimulated microbial activity. However, dehydrogenase activity also showed a quadratic trend. According to Tabatabai (1994), dehydrogenase is an enzyme that promotes the oxidation of a specific substrate by the subtraction of hydrogen and is strongly related to the biomass when external sources of C are added to the soil, being an enzyme with greater sensitivity to the environmental quality. Actually, there might be a selection of microorganisms producing or related to the urease activity in soil, which justifies the linear trend of this enzyme and the reduction in dehydrogenases activity from the dose of 93.58 Mg ha^{-1} onward. Studies with Ni on the effects of microbiota on soil were developed by Dalton et al. (1985), which claimed that the soil microbiota is significantly affected by heavy metals. Milosevic et al. (2002), also evaluating the effects of Ni on soil microbiota, drew conclusions similar to the ones in this study.

Materials and Methods

Area and soil description

The experiment was installed in 1997 and conducted until 2008 in the city of Jaboticabal-SP, Brazil (21º15’22” S;

Table 3. Chemical characterization of the sewage sludge used during the eleven year of experiment.

	N	P	K	Cd	Cr	Cu	Mn	Ni	Pb	Zn
	$g\ kg^{-1}$			$mg\ kg^{-1}$						
1997-98	32	17	4,8	8	290	664	228	268	152	1800
1998-99	37	11	1,7	12	1190	551	294	595	371	3810
1999-00	29	17	1,5	8	764	660	257	360	180	2328
2000-01	29	15	1,8	10	699	719	263	354	171	1745
2001-02	37	15	2,7	9	778	627	287	350	155	2345
2002-03	34	22	1,9	11	808	722	222	231	186	2159
2003-04	41	19	0,1	10	736	690	194	297	173	2930
2004-05	34	19	1,3	8	798	998	206	299	169	2474
2005-06	34	19	1,3	8	798	998	206	299	169	2474
2006-07	22	16	0,9	2	360	220	419	32	39	748
2007-08	33	38	1,5	3	284	573	727	57	77	1028

[a] Data expressed on dry basis.

Methodology of analysis: N, microKjeldhal method; P, vanado –molybdate spectrophotometric method; K, flame photometry;
heavy metals, atomic absorption spectrometry (AAS). Except for N, the other determinations were carried out in the extracts obtained by heating the samples with concentrated HNO_3, H_2O_2 and HCl.

Table 4. Balance of Zn and Ni contents in soil treated with sewage sludge (S.S.) in the eleventh of experiment.

	Treatments Mg ha^{-1}			
	0*Control	55*	110*	167.5*
	Zn (mg kg^{-1})			
Before S.S. application in the 11° year	100.01	117.34	139.87	156.25
After S.S. application in the 11° year	126.45	135.58	156.88	180.08
Balance (Δ Zn)	26.45	18.24	17.01	23.83
	Ni (mg kg^{-1})			
Before S.S. application in the 11° year	13.08	12.94	15.93	21.13
After S.S. application in the 11° year	23.08	22.02	25.90	28.41
Balance (Δ Ni)	10.00	9.08	9.97	7.28

SS = sewage sludge. [a] accumulated doses in eleven years of residue application

48º15'18" W; 610 m). The climate is classified as Cwa, according to Köppen classification. The soil in the area is an Oxisol, with 500 g kg^{-1} of clay, 290 g kg^{-1} of silt and 210 g kg^{-1} of sand.

Experimental design, treatments and cultivations

The experimental design was in randomized blocks, with 4 treatments and 5 repetitions, in plots of 60 m^2 (6 x 10 m). Treatments, crops and chemical composition of the sewage sludge used in the 11-yr experiment are found in Table 1. The values of sewage sludge (dry matter) accumulated in the 11 years of the experiment were 0 (control), 55, 110 and 167.5 Mg ha^{-1}.

In the first year, the control treatment had no fertilization; from the second year on, mineral fertilizer was applied according to the recommendations in Raij et al. (1997) for each crop; and from the fourth year on, with the aim of inducing phytotoxicity, the highest dose of sewage sludge (167.5 Mg ha^{-1}) was changed from 2.5 to 20 Mg ha^{-1} (Table 1).

For the balance of Zn and Ni in the soil we used data from the fifth year of the experiment (when the metals were quantified for the first time in this study) published in Oliveira et al., (2005), to compare with the values from the eleventh year. According to the treatments in the fifth year of the experiment, the concentrations of Zn and Ni in the reference soil were, respectively: 0 (control): 56.17 and 13.34 mg kg^{-1}; 55 Mg ha^{-1}: 66.24 and 15.82 mg kg^{-1}, 110 Mg ha^{-1}: 84.07 and 16.03 mg kg^{-1}; 167.5 Mg ha^{-1}: 80.36 and 14.29 mg kg^{-1}.

Conducting the experiment

Yearly, the tested crops were sown at the beginning of the rainy season according to the respective recommendations in Raij et al. (1997). The sewage sludge was applied 15 days before sowing, manually, according to each treatment, and incorporated to a depth of 10 cm with the aid of a disc harrow.

Conducting the experiment in the eleventh year

The soil analysis and the chemical characterization of the sewage sludge used in the 11th year of the experiment are found in Tables 2 and 3, respectively. The corn hybrid used was DKB 390®, sown in furrows, with 0.9 m spacing between rows and 6 sowing lines, for a population equivalent to 60,000 plants per hectare. Sowing and top-dressing mineral fertilizations in the control treatment were performed according to Raij et al. (1997). In plots that received sewage sludge, potassium was applied in the form of KCl in the top-dressing, according to the deficit in each treatment calculated based on the recommended dose by Raij et al., (1997).

Plant sampling

At 60 days after plant emergence (DAPE), diagnostic leaf samples were collected, immediately below and opposed to the corn ear (Malavolta et al., 1997). On the same occasion, 10 soil samples per plot were collected (5 in the sowing line, 5 cm away from the plants, and 5 between the rows) in the layers at 0–10, 10–20 and 20–40 cm. At 80 DAPE three whole plants were collected from each plot through excavation to a depth of 40 cm in order to preserve the roots. At 128 DAPE corn ears were collected for grain analysis.

Laboratory analyses

Plant analysis

Diagnostic leaves, whole plants and clean grains were stored in paper bags and put in the oven with forced air circulation

Table 5. Zn and Ni contents in soil treated with sewage sludge for eleven years extracted by the methods: USEPA, Mehlich-1, Mehlich-3 and DTPA.

	Zn				Ni			
Layer (cm)	0* Control	55*	110*	167.5*	0* Control	55*	110*	167.5*
	USEPA							
0-10	133.73 bA	138.50 bA	170.74 aA	190.26 aA	24.61 cA	22.09 dA	26.88 bA	29.72 aA
10-20	119.18 bA	132.66 bA	143.03 abB	169.91 aA	21.55 cB	21.95 cA	24.92 bB	27.11 aB
20-40	131.20 aA	133.87 aA	129.51 aB	138.37 aB	23.40 aAB	23.70 aA	23.74 aB	23.73 aC
	CV%	a = 16.97		b = 10.69	CV%	a = 5.10		b = 5.13
	Mehlich-1							
0-10	4.61 cB	9.64 aA	7.04 bA	1.81 dA	1.07 cA	1.65 cA	2.91 bA	4.66 aA
10-20	5.34 bB	4.28 bB	8.26 aA	1.52 cA	0.68 bcA	1.47 bcA	2.31 bA	3.74 aB
20-40	8.54 aA	5.59 bB	1.08 cB	2.51 cA	0.29 aB	0.52 aB	0.46 aB	0.58 aC
	CV%	a = 14.17		b = 20.03	CV%	a = 44.84		b = 25.61
	Mehlich-3							
0-10	12.04 bA	8.48 bA	10.16 bA	25.70 aA	0.23 cA	0.41 bcA	0.68 bA	1.07 aA
10-20	14.57 aA	9.53 aA	12.55 aA	13.96 aB	0.25 bA	0.40 bA	0.52 bA	0.95 aA
20-40	3.74 aB	2.86 aB	2.18 aB	4.14 aC	0.12 aA	0.11 aB	0.12 aB	0.14 aB
	CV%	a = 39.31		b = 29.96	CV%	a = 49.54		b = 34.12
	DTPA							
0-10	12.25 bcA	9.96 cA	15.11 bA	22.05 aA	0.42 cA	0.81 bcA	1.40 bA	2.58 aA
10-20	9.76 aA	4.05 bB	6.72 abB	10.67 aB	0.33 bA	0.60 bAB	0.91 bA	1.99 aA
20-40	3.76 aB	3.28 aB	2.43 aC	4.43 aC	0.06 aA	0.15 aB	0.13 aB	0.19 Ab
	CV%	a = 33.60		b = 28.79	CV%	a = 92.87		b = 50.28

Means followed by the same letter, lower case in rows and upper case in columns, do not differ by Tukey test at 5% of probability. * Sewage sludge doses, on dry basis, accumulated in eleven years of application.CV = coefficient of variation (a = row; b = column).

Table 6. Correlation between the availability of metals in soil and the concentration in corn plants, leaves and grains.

Extractor	Whole Plant	Diagnostic Leaf	Grains
	Zn		
USEPA	0.49*	0.62**	0.06^{ns}
Mehlich-1	0.67**	0.78**	0.17^{ns}
Mehlich-3	0.77*	0.89**	0.34^{ns}
DTPA	0.70**	0.70**	0.28^{ns}
	Ni		
USEPA	0.35^{ns}	0.24^{ns}	-0.10^{ns}
Mehlich-1	0.60**	0.43^{ns}	-0.02^{ns}
Mehlich-3	0.59**	0.28^{ns}	0.05^{ns}
DTPA	0.50*	0.27^{ns}	-0.02^{ns}

* significant at 5% of probability
** significant at 1% of probability
ns = not significant

at 60º–70 ºC until a constant weight was attained. After that, the material was digested with nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) subjected to temperatures of 90–100 °C (USEPA, 1996). In the obtained extracts, Zn and Ni concentrations were determined by atomic absorption spectroscopy with an air-acetylene flame.

Analysis of Zn and Ni in soil

The "semi-total" (because this method does not fully digest the soil) concentrations of Zn and Ni were determined using the 3050b method of the EPA (USEPA, 1996). For the exchangeable soil concentrations, the following extractants were used: DTPA (Lindsay and Norwell, 1978); Mehlich-1 (Delfelipo and Ribeiro, 1981) and Mehlich-3 (Mehlich, 1984).

Analysis of enzymatic activity in the soil

In the samples from the 0–10 cm layer, urea hydrolysis rate was also determined, which is a function of the urease activity, where soil is incubated in the presence of urea, which is converted into ammonia (May and Douglas 1976) through steam distillation (Bremner and Keeney, 1965). Also in the samples from the 0–10 cm layer, dehydrogenase activity was determined through the method proposed by Casida Jr. (1977), where triphenyltetrazolium chloride was used as an artificial electron acceptor reduced by the enzyme action, forming triphenyltetrazolium formazan, measured in spectrophotometer at 485 nm.

Statistical analysis

Results were subjected to variance analysis. In cases where the F test was significant at a 1 or 5% level, regression analysis was applied (diagnostic leaves, whole plant, grains, urease and dehydrogenase activities) or the Tukey's test at 5% probability, using the split-plot scheme (metals in soil profile) for means comparison. Correlations were also made between the concentrations of Zn and Ni available in soil and the concentrations of Zn and Ni in the corn plants, diagnostic leaves and grains. Statistical analyses were performed using the ASSISTAT statistical program (Silva and Azevedo 2002).

Conclusions

This study shows that for Cerrado conditions the increase of Zn and Ni concentration in soil as a function of sewage sludge application for a long time are small, and most of Zn and Ni could be bonded to other soil mineral components, making them not readily available to plants. Mehlich-3 was the most accurate extractor to estimate Zn and Ni available to corn plants. The study of enzymatic activities of ureases and dehydrogenases indicates that the use of high sewage sludge doses throughout time may affect soil microorganisms biodiversity.

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