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Short-term usage of sewage sludge as organic fertilizer to sugarcane in a tropical soil bears little threat of heavy metal contamination

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ABSTRACT

A field experiment was carried out to study the effect of application rates of sewage sludge and mineral nitrogen and phosphate fertilizers on As, Ba, Cd, Cr, Cu, Ni, Pb, Se, and Zn concentration in soil, cane plant, and first ratoon (residual effect) in a Typic Hapludult soil. To allow an analysis by means of response surface modeling, four rates of sewage sludge (0, 3.6, 7.2 and 10.8 t ha^{-1}, dry base), of N (0, 30, 60 and 90 kg ha^{-1}) and of P_2O_5 (0, 60, 120 and 180 kg ha^{-1}) were applied in randomized block design, in a 4 × 4 × 4 factorial scheme, with confounded degrees of freedom for triple interaction, with two replications. To evaluate the residual effect of the sludge applied to cane plant on the cane ratoon growth, mineral NK fertilizers were applied at the rates of 120 kg ha^{-1} N and 140 kg ha^{-1} of K_2O, on all treatments. The application rates of mineral nitrogen and phosphate fertilizers did not affect statistically the heavy metal concentration in the soil and in the sugarcane plants. Sewage sludge application increased As, Cd, Cu, Ni, Pb, and Zn concentrations in soil, but values did not exceed the quality standard established by legislation for agricultural soils. Although the concentrations of metals in the plants were very low, the uptake of heavy metal by sugarcane plants was generally increased by sewage sludge doses. The use of sewage sludge based on N criteria introduces a small amount of heavy metal into the agricultural system, however it poses no hazard to the environment.

1. Introduction

Sewage sludge is a residue from domestic wastewater treatment. 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).

Sludge application to agricultural soils improves soil physical and chemical properties, such as porosity, aggregate stability, bulk density, water movement and retention (Silveira et al., 2003), and soil fertility by increasing organic matter and nutrients content (Chiba et al., 2008; Alcantara et al., 2009; Franco et al., 2010). The use of sewage sludge in Brazilian agriculture is controlled by Resolution 375, issued in 2006 by the Brazilian National Environment Council (CONAMA, 2006).

The use of sewage sludge in agriculture represents one of the most viable alternatives for its final disposal. However, the presence of heavy metals may limit its application because of the risk of soil contamination and metal transfer via the food chain potentially causing metabolic disorders and chronic diseases in humans (Nogueira et al., 2009). "Heavy metal" is a general collective term, which applies to the group of metals and metalloids with high specific weight and they are natural components of the earth's crust (Hashima et al., 2011). This definition is not acceptable and also inconsistent in use as already stressed in literature. However, in Plant Sciences, the term is so widely used that it is hardly possible to eliminate it (Appenroth, 2010). In the present study, the heavy metal term indicates metals and metalloids.

Heavy metals most commonly found in sewage sludge are arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn), and the metal concentrations are governed by the nature and the intensity of the industrial activity, as well as the type of process employed during the sewage sludge treatment (Basta et al., 2005; Alvarez et al., 2008). Longer-term field studies have demonstrated consistent evidence that sewage sludge application increases heavy metal concentrations in soil (Udom et al., 2004; Melo et al., 2007; Nogueira et al., 2008, 2010). The

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levels of heavy metal in tropical soils may increase considerably even in a short-time after sewage sludge application (Oliveira and Matiazzo, 2001; Marques et al., 2007).

Heavy metal uptake by plants depends on the form in the soil and on the plant species. Moreover, most of vegetative parts of plants, especially leaves, have higher heavy metal contents than seeds, nuts and fruits. However, the accumulation of heavy metals is potentially most harmful in cereals and vegetables rather than to sugarcane which is not used for immediate human consumption, reducing the risk to human health. Consequently, sewage sludge has already been applied on sugarcane fields in the state of São Paulo (Brazil's leading cane-growing state). Studies have been conducted to investigate the effects of sewage sludge application on uptake of heavy metals by sugarcane (Marques et al., 2007; Nogueira et al., 2007; Camilotti et al., 2009), but most of them focused on the rates above those permitted by the technical criteria of Resolution 375 (CONAMA, 2006).

With increasing disposal of sewage sludge on agricultural land, the risk of soil and food contamination by heavy metals must be considered. However, field studies under tropical conditions to evaluate the effect of sewage sludge on the accumulation and availability of heavy metals in soils and uptake by sugarcane plants are inconclusive (Abreu Junior et al., 2008). Considering that Brazilian soils are quite different from temperate soils, the objective of this research was to evaluate the effect of application rates of sewage sludge and mineral nitrogen and phosphate fertilizers on heavy metal concentration in a tropical soil and its potential availability to sugarcane plants using a field experiment.

2. Materials and methods

2.1. Field procedures

The experiment was carried out in a commercial cane field in the municipality of Capivari, State of São Paulo, Brazil (22°55′45″ S and 47°33′58″ W, altitude 550 m). This area (Fig. 1) has been cultivated exclusively with sugarcane crop for near 30 years, and was chosen in this study because it was included in a project on sludge use on sugarcane, although the field had not been treated with sewage sludge until 2005.

The local climate is moist tropical (Cwa on the Köppen scale), with relatively dry winters and hot and humid summers. Annual rainfall was 1565 and 1615 mm, respectively, in the period from September 2005 to September 2006 (first growth, cane plant) and from September 2006 to October 2007 (second growth, cane ratoon).

The tropical soil (classified as a Typic Hapludult, sandy clay loam texture) was sampled at 0–20 cm deep (clay, 28%; silt, 12%; sand, 60%) before setting up the experiment for fertility analysis according to procedures described by van Raij et al. (2001) and characterizing potentially toxic elements by USEPA-3051A method (USEPA, 2007) (Table 1).

The sewage sludge (Table 2) was obtained from the Jundiaí Wastewater Treatment Plant, in the municipality of Jundiaí, State of São Paulo, Brazil. The sludge was generated in an aerated biological system, stabilized in the sedimentation ponds for about 12 months, treated with polymers, centrifuged and air-dried for at least 120 days.

The sugarcane cultivar used was RB 85-5536, which is a medium/late cycle variety, very responsive to the application of mineral fertilizers, with high stalk and sugar yields when grown under favorable conditions for its development. Prior to the experiment, in August 2005, lime was applied at the rate of 500 kg ha^{-1} in the entire area, to raise base saturation to 60% (Spironello et al., 1997).

In September 2005, the sludge was applied in the furrows, just before cane planting, at the rates of 0, 3.6, 7.2, and 10.8 t ha^{-1} (dry basis), equivalent to 0, 33, 66, and 100% of the recommended N supply (CONAMA, 2006). Nitrogen (as urea, 45% N) was applied at the rates of 0, 30, 60, and 90 kg ha^{-1} of N, equivalent to 0, 33, 66, and 100% of the recommendation for the experimental area (Spironello et al., 1997), in proportions of 1/3 applied at planting and 2/3 applied 30 days later as side dressing. Phosphorous (as triple superphosphate, 45% P_20_5) was applied, at planting only, at the rates of 0, 45, 90, and 180 kg ha^{-1} of P_2O_5, equivalent to 0, 33, 66, and 100% of the recommended supply. Since sewage sludge is poor in potassium (Table 2), this nutrient was supplied at 160 kg ha^{-1} of K_2O at planting (as potassium chloride, 58% K_2O), on all plots. To evaluate the residual effect of the sludge applied to cane

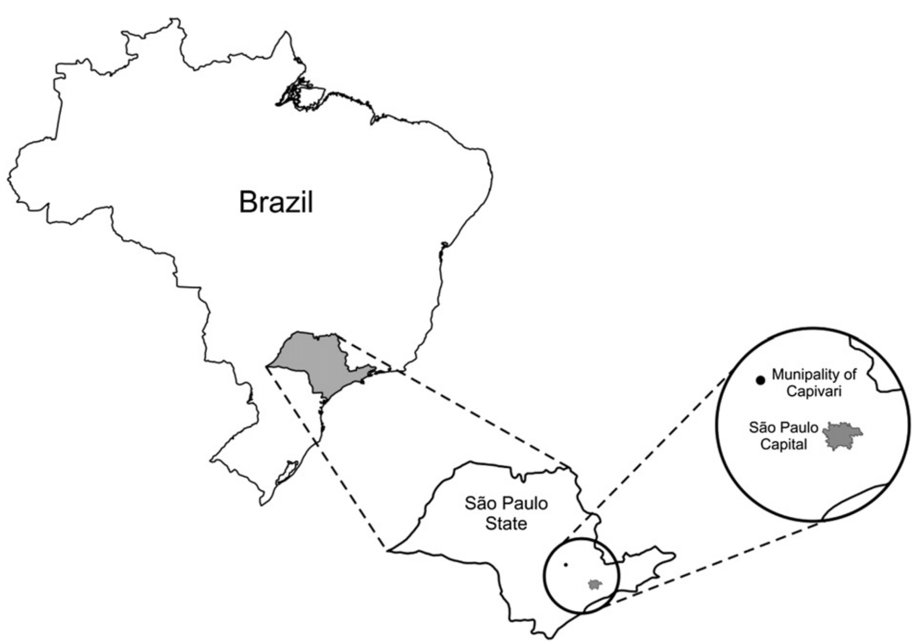

Fig. 1. Location of experimental site (not in scale), in the State of São Paulo, Brazil.

Table 1
Chemical attributes of the soil in the study area (mean ± SE, $n = 6$). Limits for metal concentration are also indicated.

Attributes	Unit	Typic Hapludult	Cetesb limits[a]	Conama limits[b]
pH $CaCl_2$ 0.01 mol L^{-1}	–	4.6 ± 0.3	–	–
Soil organic matter	g kg^{-1}	9.1 ± 2.2	–	–
P_{resin}	mg kg^{-1}	4 ± 0.3	–	–
K	$mmol_c$ kg^{-1}	1.1 ± 0.1	–	–
Ca^{2+}	$mmol_c$ kg^{-1}	19 ± 1.7	–	–
Mg^{2+}	$mmol_c$ kg^{-1}	8 ± 1.1	–	–
Al^{3+}	$mmol_c$ kg^{-1}	2 ± 0.1	–	–
H + Al	$mmol_c$ kg^{-1}	22 ± 0.7	–	–
Sum of bases	$mmol_c$ kg^{-1}	28 ± 1.3	–	–
Cation exchange capacity	$mmol_c$ kg^{-1}	50.2 ± 4.0	–	–
Base saturation	%	56 ± 2.1	–	–
Total recoverable metals				
As	mg kg^{-1}	1.92 ± 0.2	3.5	15
Ba	mg kg^{-1}	83.81 ± 4.5	75	150
Cd	mg kg^{-1}	0.03 ± 0.0	<0.5	1.3
Cr	mg kg^{-1}	12.0 ± 0.7	40	75
Cu	mg kg^{-1}	4.51 ± 1.2	35	60
Hg	mg kg^{-1}	0.04 ± 0.0	0.05	0.5
Mo	mg kg^{-1}	0.14 ± 0.1	<4	30
Ni	mg kg^{-1}	5.90 ± 0.9	13	30
Pb	mg kg^{-1}	6.19 ± 1.4	17	72
Se	mg kg^{-1}	0.07 ± 0.0	0.25	5
Zn	mg kg^{-1}	23.42 ± 2.3	60	300

[a] Quality value for agricultural soils in the São Paulo State established by the Environmental Agency of the State of Sao Paulo (CETESB, 2005).
[b] Prevention value set by the Brazilian National Environment Council for Brazilian soils (CONAMA, 2009).

plant on the cane ratoon growth, in October 2006, mineral NK fertilizers were applied at the rates of 120 kg ha^{-1} N (as urea) and 140 kg ha^{-1} of K_2O (as potassium chloride), on all treatments.

2.2. Sampling and chemical analyses

Chemical characteristics of the sewage sludge (Table 2) were determined as follows: pH and electrical conductivity (EC) were measured in deionized water at a sludge:water ratio of 1:2.5 and 1:1, respectively using a pH/ion/conductivity meter (Model 220, Denver Instrument Inc., Denver, CO, USA) (van Raij et al., 2001); total C was determined in a CN automatic analyzer (Vario Max CN, Elemental Analysensystem GmbH) (Nelson and Sommers, 1996); total N by a microkjeldahl method (Bremner, 1996); total P was determined as described by Kuo (1996); total K and the As, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, and Zn by inductively coupled plasma mass spectrometry (ICP–MS) (Model Agilent 7500ce, Agilent Technologies, Tokyo, Japan), in extracts obtained by digestion with $HNO_3 + H_2O_2 + HCl$ (USEPA, 1996).

Table 2
Chemical composition of sewage sludge,[a] on a dry basis, used in the experiment and the limits[b] for heavy metals in sewage sludge (mean ± SE, $n = 6$).

Attributes	Value	Conama limits
pH water	5.8 ± 0.25	–
Moisture (%)	78 ± 7.41	–
Electrical conductivity (dS m^{-1})	3.5 ± 0.75	–
Total C (g kg^{-1})	322 ± 42.32	–
Total N (g kg^{-1})	29.7 ± 1.18	–
Total P (g kg^{-1})	10 ± 0.91	–
Total K (g kg^{-1})	2.75 ± 0.21	–
As (mg kg^{-1})	4.22 ± 0.52	41
Ba (mg kg^{-1})	599.85 ± 42.55	1300
Cd (mg kg^{-1})	13.95 ± 1.12	39
Cr (mg kg^{-1})	277.71 ± 34.00	1000
Cu (mg kg^{-1})	304.09 ± 63.18	1500
Hg (mg kg^{-1})	1.05 ± 0.10	17
Mo (mg kg^{-1})	9.75 ± 1.12	50
Ni (mg kg^{-1})	65.55 ± 5.33	420
Pb (mg kg^{-1})	201.56 ± 30.01	300
Se (mg kg^{-1})	1.84 ± 0.22	100
Zn (mg kg^{-1})	1869.94 ± 251.47	2800

[a] Samples obtained from the Wastewater Treatment Plant of Jundiaí (Company of Basic Sanitation of the Jundiaí), State of São Paulo, Brazil.
[b] Limits to sewage sludge agricultural use established by CONAMA (2006).

The sugarcane leaves (top visible dewlap) were sampled in January 2006 for cane plant and in January 2007 for cane ratoon, taking 20 leaves from each plot. Five stalks per plot were harvested in September 2006 for cane plant and in October 2007 for cane ratoon. Samples of stalks were weighed and then split into two subsamples: one for determining heavy metal concentration and the other for extracting the sugarcane juice.

The leaf and stalk samples were washed with water and 1:1 HCl, dried in an oven at 40 °C for 72 h, ground in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) and packed in polyethylene bags. The separation of the juice from fibers was made by squeezing 500 g of stalks for 1 min in a hydraulic press (24.5 MPa). The stalks, leaves and juice were digested in a microwave (Model TC plus labstation, Milestone, Sorisole, Italy) oven using a mixture of $HNO_3 + H_2O_2$ (for juice was added HCl) following USEPA-3051A method (USEPA, 2007) for determination of As, Ba, Cd, Cr, Cu, Ni, Pb, Se, and Zn by the ICP–MS.

Soil samples (0–20 cm depth) for heavy metal analysis were collected just after harvesting 360 (cane plant) and 730 d (cane ratoon) application of sewage sludge and mineral fertilizers. In the area reserved for soil sampling of each plot, 10 subsamples were collected at the 0–10 cm depth from the planting furrow. Soil samples were air dried and sieved (0.5 mm) prior to chemical analysis. Total recoverable metals were determined following USEPA-3051A method (USEPA, 2007). DTPA-extractable fractions of heavy metals were measured by extracting 10 g soil with 20 mL of extracting solution (0.005 mol L^{-1} DTPA, 0.01 mol L^{-1} $CaCl_2$ and 0.1 mol L^{-1} TEA adjusted to pH 7.3) according to Lindsay and Norvell (1978) procedure. The concentration of As, Ba, Cd, Cr, Cu, Ni, Pb, Se, and Zn in the digested solution and DTPA-extract was determined using the ICP–MS.

2.3. Quality control

All acids, reagents and water were ICP–MS compatible grade to achieve very low limits of detection (LOD), as shown in Table 3. The accuracy and precision of the analytical method were assured by the use of the standard reference materials (SRM 1515 – Apple Leaves and SRM 2710 – Montana Soil). The elemental recoveries from SRM ranged from 87% to 109%. Analytical quantification limits for soil and parts of sugarcane were also determined.

2.4. Statistical analysis

The experiment design was in randomized block, in a 4 × 4 × 4 factorial scheme (four rates of sludge, N and P, respectively) with confounded degrees of freedom for triple interaction, with two replications distributed in eight blocks (16 treatments per block), for a total of 64 treatments and 128 field plots. It was formerly arranged to evaluate the effects of the rates of sludge and mineral N and P fertilizers on the crop sugarcane yield and technological characteristics by means of response surface modeling, to obtain an equation of the following type: $Y = a + bS + cN + dP + eSN + fSP + gPN + hS^2 + iN^2 + jP^2$, where S is the sludge rate (t ha^{-1}), N is the N rate (kg ha^{-1}) and P is P_2O_5 rate (kg ha^{-1}) (Franco et al., 2010). However, in our work the application rates of mineral

Table 3
Validation of heavy metals concentration in standard reference materials (SRM 1515 – Apple Leaves and SRM 2710 – Montana Soil, from National Institute of Standards and Technology) and the limit of detection (LOD) in parts of sugarcane and soil by ICP–MS (mean ± SE, $n = 6$).

Element[a]	SRM 1515		SRM 2710			LOD		
	Certified (mg kg^{-1})	Determined[a] (mg kg^{-1})	Certified (mg kg^{-1})	Determined[a] (mg kg^{-1})	Leach recovery (%)	Juice (μg kg^{-1})	Leaf and stalk (μg kg^{-1})	Soil (μg kg^{-1})
As	0.038 ± 0.007	0.031 ± 0.008	590	610.9 ± 30.2	103.5 ± 5.1	0.2	3	29
Ba	49 ± 2	47 ± 5	360	395.2 ± 14.5	109.8 ± 4.0	0.3	3	28
Cd	0.013 ± 0.002	0.012 ± 0.003	20	19.4 ± 0.8	96.8 ± 3.8	0.1	1	11
Cr	0.3[b]	0.5 ± 0.2	19	16.6 ± 1.3	87.5 ± 6.8	0.2	2	19
Cu	5.64 ± 0.24	4.85 ± 0.85	2700	2501.4 ± 105.1	92.6 ± 3.9	0.4	5	44
Ni	0.91 ± 0.12	0.91 ± 0.08	10	9.7 ± 1.0	97.1 ± 9.6	0.3	3	30
Pb	0.470 ± 0.024	0.447 ± 0.044	5100	5053.9 ± 157.3	99.1 ± 3.1	0.2	3	21
Se	0.050 ± 0.009	0.073 ± 0.014	nd	nd	nd	0.4	5	44
Zn	12.5 ± 0.3	11.9 ± 0.5	5900	6250.8 ± 217.4	105.9 ± 3.7	0.7	10	80

nd: not determined.
[a] Total recoverable metal concentration was measured by USEPA-3051A method (USEPA, 2007).
[b] Noncertified values.

NP fertilizers and all interactions did not affect statistically the heavy metal concentration in the soil and in the sugarcane plants. Consequently, the response surface models were represented in their reduced linear or quadratic forms in function of sewage sludge application rates. Statistical analyses were performed using SAS procedures (SAS, 2002).

3. Results and discussion

3.1. Soil chemical properties and sewage sludge composition

The Typic Hapludult soil (Table 1) was selected for this study because it occurred in the area that included a project of sludge use on sugarcane. The chemical properties of the soil before sewage sludge and limestone application indicate a medium fertility (van Raij et al., 1997) and low concentration of heavy metals (CETESB, 2005; CONAMA, 2009).

Although the doses of sludge were applied on the N criteria it is important to control the conductivity evolution considering that the EC value determined in the sewage sludge was 3.5 dS m^{-1} (Table 2). The sewage sludge used in the experiment was rich in plant nutrients (except K) and also in heavy metals (Table 2). The relatively high concentration of heavy metals such as Ba, Cd, Pb, and Zn was due to the fact that the treatment plant collected sludge from a highly industrialized region. However, the results presented in Table 2 show that the concentrations of heavy metals were below the safety limits (CONAMA, 2006), enabling sludge applications in this area. On the other hand, since this limit was not fully established based on data of tropical soils, it becomes important to understand the behavior of heavy metals in tropical soils amended with sewage sludge, including forms and availability to plants, and translocation to plant parts in order to improve guidelines for metals-containing sludge applications and to minimize the risk for the environment and food chain.

3.2. Heavy metals in soil

Total recoverable As in the soil increased with increasing rate of sewage sludge and followed a quadratic model ($p < 0.01$) for both plant and ratoon cane crops (Tables 4 and 5). Total recoverable As in the soil after 360 days of sewage sludge application (direct effect) ranged from 1.74 (control) to 2.87 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment), while the total recoverable As in the soil after 720 days of sludge application (residual effect) ranged from 1.74 (control) to 2.62 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). Nevertheless, the concentration of soil-extractable As

Table 4
Concentration of heavy metals (mg kg^{-1}, dry basis) in the soil after 360 days (direct effect in the cane plant) application of sewage sludge ($n = 32$).

Heavy metal/method	Sewage sludge (t ha^{-1}) 0	3.6	7.2	10.8	Equation	r^2
Arsenic						
Total recoverable	1.741	1.847	1.990	2.867	$y = 0.015x^2 - 0.065x + 1.78$	0.88**
DTPA-extractable	0.039	0.045	0.033	0.088	NS	NS
Barium						
Total recoverable	80.68	64.96	62.49	94.48	NS	NS
DTPA-extractable	0.53	0.47	0.41	0.42	NS	NS
Cadmium						
Total recoverable	0.044	0.082	0.190	0.225	$y = 0.057x + 0.04$	0.95**
DTPA-extractable	0.012	0.037	0.080	0.112	$y = 0.009x + 0.01$	0.88**
Chromium						
Total recoverable	21.32	13.53	17.42	18.36	NS	NS
DTPA-extractable	0.012	0.012	0.011	0.011	NS	NS
Copper						
Total recoverable	6.00	6.83	8.39	10.54	$y = 0.437x + 5.63$	0.68**
DTPA-extractable	0.65	1.36	1.72	2.64	$y = 0.181x + 0.63$	0.86*
Nickel						
Total recoverable	3.71	5.60	5.12	8.55	$y = 0.424x + 3.56$	0.66**
DTPA-extractable	0.21	0.25	0.30	0.40	$y = 0.018x + 0.19$	0.71**
Lead						
Total recoverable	5.93	6.58	6.83	9.66	$y = 0.097x + 5.46$	0.62**
DTPA-extractable	0.73	0.89	1.73	1.57	$y = 0.085x + 0.75$	0.71**
Selenium						
Total recoverable	0.071	0.063	0.065	0.083	NS	NS
DTPA-extractable	0.007	0.007	0.004	0.004	NS	NS
Zinc						
Total recoverable	8.83	14.71	19.53	31.60	$y = 2.127x + 7.47$	0.88**
DTPA-extractable	0.73	1.34	3.64	7.61	$y = 0.678x - 0.20$	0.92**

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
NS: not significant.
n = number of analyzed samples.

Table 5
Concentration of heavy metals (mg kg^{-1}, dry basis) in the soil after 720 days (residual effect in the cane ratoon) application of sewage sludge ($n = 32$).

Heavy metal/ method	Sewage sludge (t ha^{-1})				Equation	r^2
	0	3.6	7.2	10.8		
Arsenic						
Total recoverable	1.746	1.829	1.920	2.620	$y = 0.012x^2 - 0.055x + 1.77$	0.94**
DTPA-extractable	0.033	0.036	0.035	0.040	NS	NS
Barium						
Total recoverable	80.43	63.15	78.97	91.02	NS	NS
DTPA-extractable	0.51	0.42	0.37	0.42	NS	NS
Cadmium						
Total recoverable	0.035	0.051	0.151	0.187	$y = 0.0153x + 0.023$	0.87**
DTPA-extractable	0.014	0.021	0.065	0.093	$y = 0.0015x + 0.012$	0.76**
Chromium						
Total recoverable	22.56	22.17	20.06	21.95	NS	NS
DTPA-extractable	0.009	0.010	0.010	0.011	NS	NS
Copper						
Total recoverable	4.50	6.58	12.48	12.42	$y = 0.773x + 4.67$	0.88**
DTPA-extractable	0.86	1.04	1.92	2.20	$y = 0.135x + 0.77$	0.74**
Nickel						
Total recoverable	4.64	5.29	5.04	9.90	$y = 0.497x + 3.73$	0.43*
DTPA-extractable	0.19	0.21	0.24	0.47	$y = 0.027x + 0.14$	0.77**
Lead						
Total recoverable	5.59	6.39	9.21	11.01	$y = 0.535x + 5.18$	0.84**
DTPA-extractable	0.70	1.06	2.09	1.58	$y = -0.018x^2 + 0.291x + 0.58$	0.72*
Selenium						
Total recoverable	0.068	0.071	0.078	0.092	$y = 0.0002x + 0.003$	0.41*
DTPA-extractable	0.003	0.003	0.005	0.005	NS	NS
Zinc						
Total recoverable	8.87	13.07	18.63	25.94	$y = 1.616x + 8.01$	0.89**
DTPA-extractable	0.75	1.29	2.73	6.57	$y = 0.569x - 0.10$	0.88**

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
NS: not significant.
n = number of analyzed samples.

after 360 days (cane plant) and even 720 days (cane ratoon) did not increased with the application of sewage sludge rates. This results agree with those obtained by Corrêa et al. (2008) after applying two sewage sludge sources (from the Wastewater Treatment Plant of São José dos Campos and Barueri cities, Sao Paulo State, Brazil) in a Oxisol under no-tillage system cropped from 2002 up to 2004 with pigeon pea (*Cajanus cajan*), soybean (*Glycine max* L.), and black oat (*Avena strigosa* Schreb.). According to the authors, the concentration of As in soil estimated by DTPA method ranged from 0.003 to 0.011 mg kg^{-1}.

The Environmental Agency of the State of Sao Paulo (Cetesb) established 3.5 mg As kg^{-1} as a reference value for soils quality of the São Paulo State (CETESB, 2005) and the Brazilian National Environment Council (Conama) establishes the value 15 mg As kg^{-1} as a prevention value for agricultural soils in Brazil (CONAMA, 2009) (Table 1), our data verify that soil As concentration was far below those limit values (Tables 4 and 5).

The addition of Ba through the sewage sludge application was not sufficient to increase total recoverable Ba and extractable Ba in the soil for both crops (Tables 4 and 5). The Brazilian National Environment Council published maximum levels of Ba (1300 mg Ba kg^{-1}, dry basis) in sewage sludge for safe use in agriculture (Table 2) (CONAMA, 2006) and recently established the prevention value of Ba (150 mg Ba kg^{-1}) in agricultural soils (CONAMA, 2009). Thus, the values of Ba in the sludge (mean concentration of 600 mg Ba kg^{-1}, dry basis) and in the soil (ranging from 62.5 to 94.5 mg Ba kg^{-1}) were below the allowed by Conama. However, soil Ba concentration was slightly higher than the quality value established by Cetesb (Table 1). There is minimal information available in the literature regarding Ba contamination of soil. A long-term field study of Ba behavior in sewage sludge was reported by Nogueira et al. (2010). In this work, the accumulated rates of sewage sludge applied for nine consecutive years increased soil Ba concentration as estimated by Jackson's method (total Ba) by the USEPA's method 3050B (total recoverable Ba). In another study with eleven consecutive years of sludge application, the soil Ba concentration also increased with increasing rates of sewage sludge (Merlino et al., 2010).

Sewage sludge application to the soil linearly increased total recoverable Cd ($p < 0.01$) and extractable Cd ($p < 0.01$) in the soil for both crops (Tables 4 and 5). There were positive correlations between total recoverable Cd and extractable Cd in soil for cane plant ($r^2 = 0.91$; $p < 0.01$) and cane ratoon ($r^2 = 0.93$; $p < 0.01$). Therefore, for cane plant the total recoverable Cd in the soil ranged from 0.044 (control) to 0.225 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment), while extractable Cd ranged from 0.012 (control) to 0.112 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). In the cane ratoon the total recoverable Cd in the soil ranged from 0.035 (control) to 0.187 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment), while extractable Cd ranged from 0.014 (control) to 0.093 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). Despite this, the levels of Cd in soil were well below the prevention value of Cd in agricultural soils but near to the quality value of Cd in soils (Table 1). Cadmium has been exhaustively studied in sewage sludge contaminated soils (Bertoncini et al., 2004; Udom et al., 2004; Chaudri et al., 2007; Nogueira et al., 2008). Increasing soil Cd concentration occurs because sewage sludge often contains relatively high Cd concentration and thus repeated sludge application increases the soil metal concentration. The high concentrations of Cd in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake. Also, Cd is highly mobile in soil and plant (Tyler and McBride, 1982).

As shown in Tables 4 and 5, there was no effect of the sewage sludge application on total recoverable Cr and extractable Cr in the soil for both crops. The same trend was reported by other studying sewage sludge application to sugarcane crop (Silva et al., 1998; Camilotti et al., 2007). In contrast, Oliveira and Matiazzo (2001), in a field study evaluating the application of sewage sludge from Barueri (mean concentration of 385.0 mg Cr kg^{-1}, dry basis), a very industrialized city inserted in the big São Paulo megalopolis, in cane plant and cane ratoon, verified that soil Cr increased with the applications of sludge, ranging from 15.55 to 27.19 mg kg^{-1}. Similar results were observed by Marques et al. (2007) and Merlino et al. (2010). In the present study, the concentration of Cr in the soil was below the prevention and quality values of Cr for Brazilian soils (Table 1).

At the occasion of cane plant and cane ratoon harvesting, total recoverable Cu and extractable Cu in the soil linearly increased with increasing rates of sewage sludge. By 360 days after sludge application, total recoverable Cu ranged from 6.00 (control) to 10.54 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment), while extractable Cu ranged from 0.65 (control) to 2.64 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). In the cane ratoon, 720 days after sludge application, total recoverable Cu ranged from 4.50 (control) to 12.48 mg kg^{-1} (in the 7.2 t ha^{-1} sewage sludge treatment), while extractable Cu ranged from 0.86 (control) to 2.20 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). There were positive correlations between total recoverable Cu and extractable Cu in the soil for cane plant ($r^2 = 0.78$; $p < 0.01$) and cane ratoon ($r^2 = 0.88$; $p < 0.01$). Similar results were reported by Oliveira and Matiazzo (2001). Soil total Cu was far below the prevention and quality values of Cu for Brazilian soils (Table 1).

Analogous to Cu, the rates of sewage sludge applied to soil increased ($p < 0.01$ or $p < 0.05$) total recoverable and extractable of Ni, Pb and Zn in soil for cane plant and cane ratoon crops (Tables 4 and 5). Martins et al. (2003) observed that increasing sewage sludge rates induced a linear increase in the Ni, Cu and Zn concentrations in soil extracted with DTPA and Mehlich 3.

By 360 (direct effect in the cane plant) and 720 days (residual effect in the cane ratoon) after application of sludge, the highest value of total recoverable Ni in the soil was 8.55 and 9.90 mg kg^{-1} for the treatment with 10.8 t ha^{-1} of sewage sludge, respectively. The same trend was observed for extractable Ni, the highest values were 0.40 mg kg^{-1} (cane plant) and 0.47 mg kg^{-1} (cane ratoon) in the treatment with highest rate of sludge. Moreover, the relationship between total recoverable Ni and extractable Ni in the soil and sewage sludge application rates followed a linear model ($p < 0.01$ and $p < 0.05$) for both crops.

A positive correlation ($r^2 = 0.70$; $p < 0.05$) was observed between total recoverable Ni and extractable Ni in soil at 720 days (residual effect in the cane ratoon). Martins et al. (2003) found that soil DTPA-extractable Ni concentration increased linearly with increasing sewage sludge rates. Melo et al. (2007), in an experiment with maize, observed that sewage sludge application at the rate of 20 t ha^{-1} $year^{-1}$, for six successively years, increased the concentration of Ni extracted by USEPA-3050B ($HNO_3 + H_2O_2 + HCl$) and by Jackson method ($HClO_4 + HF$). Rangel et al. (2004) and Oliveira et al. (2005) found increasing values of total Ni concentration in the soil when sewage sludge from Sabesp–Barueri was applied (rates of sludge ranging from 8 to 65 t ha^{-1}, dry basis) to Brazilian Oxisols. However, other authors (Martins et al., 2003; Nogueira et al., 2009) found no effect from the addition of sewage sludge (rates of sludge ranging from 20 to 127.5 t ha^{-1}, dry basis) on the concentration of total Ni. Total recoverable Ni in soil was well below the prevention and quality values of Cu for Brazilian soils (Table 1).

The relationship between total recoverable Pb in soil and sewage sludge application rate fitted a linear model ($p < 0.01$) for both crops (Tables 4 and 5). However, the correlation between extractable Pb in soil and sludge application rate followed a linear model ($p < 0.01$) for the cane plant and a quadratic model for the cane ratoon. For cane plant crop, total recoverable Pb ranged from 5.93 (control) to 9.66 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment) while extractable Pb ranged from 0.73 (control) to 1.73 mg kg^{-1} (in the 7.2 t ha^{-1} sewage sludge treatment). In the cane ratoon crop, total recoverable Pb ranged from 5.59 (control) to 11.01 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment) while extractable Pb ranged from 0.70 (control) to 2.09 mg kg^{-1} (in the 7.2 t ha^{-1} sewage sludge treatment). The rate of 8.1 t ha^{-1} sewage sludge resulted in the greatest level of extractable Pb for the cane ratoon crop. There was positive correlation ($r^2 = 0.65$; $p < 0.05$) of total recoverable Pb with extractable Pb for cane ratoon (soil samples collected after 720 days of sewage sludge application). Other reports also showed increases in soil Pb concentration with increasing rates of sewage sludge (Marques et al., 2007; Merlino et al., 2010). However, Nogueira et al. (2008) showed that soil Pb concentration was not affected by successive sewage sludge application (rate of sludge ranging from 45 to 127.5 t ha^{-1}, dry basis). Soil total Pb was well below the limit values of 17 mg Pb kg^{-1} (Pb quality value for agricultural soils in the São Paulo State) and of 72 mg Pb kg^{-1} (Pb prevention value for Brazilian soils) (Table 1).

Except for extractable Se at the occasion of cane ratoon harvesting, the addition of Se through the sewage sludge application was not sufficient to increase the concentration of Se in both crops (Tables 4 and 5). The concentration of Se was well below the limit value (5 mg Se kg^{-1}) established by CONAMA (2009). The total recoverable Se linearly ($p < 0.05$) increased with increasing rate of sewage sludge, ranging from 0.068 (control) to 0.092 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). Nevertheless, there are few data in the literature about Se in sewage sludge (Heninger et al., 1997). In Brazil, no detailed observations of the environmental consequences of long-term application of sewage with regards to Se have yet been reported. In the present state of knowledge there is no serious contamination risk in the short term concerning the disposal of sludges on cane fields, but the accumulation of Se in soils through the addition of sludge to land can have in the long run an impact on groundwater, animals and human beings.

The sewage sludge used in this work had a relatively high concentration of Zn (Table 2). This element is a micronutrient to higher plants, but its high concentration in the sludge may limit its application because of the risk of soil contamination and transfer via the food chain by root absorption and accumulation in aerial part of plants (Nogueira et al., 2008). In this aspect, we observed that the loadings of sewage sludge to soil linearly ($p < 0.01$) increased total recoverable Zn in the soil, ranging from 8.83 to 31.60 and 8.87–25.94 mg kg^{-1}, respectively, at 360 (cane plant) and 720 days (cane ratoon) of sewage sludge application rates. Also, extractable Zn linearly related with external loading rate of sludge, ranging from 0.73 to 7.61 mg Zn kg^{-1} in the cane plant and from 0.75 to 6.57 mg Zn kg^{-1} in the cane ratoon. Similar correlation coefficients were obtained between total recoverable Zn with extractable Zn in the soil for cane plant ($r^2 = 0.95$; $p < 0.01$) and cane ratoon ($r^2 = 0.93$; $p < 0.01$). Others investigations yielded similar results (Silva et al., 1998; Oliveira and Matiazzo, 2001; Martins et al., 2003; Camilotti et al., 2007; Nogueira et al., 2008). In general, the concentrations of Zn in soil were affected by sewage sludge application. Although soil Zn concentration increased as a function of sewage sludge applications rates, the levels of Zn in soil were well below the quality value for agricultural soils in the São Paulo State of 60 mg Zn kg^{-1} and the prevention value for Brazilian soils of 300 mg Zn kg^{-1} (Table 1).

3.3. Heavy metals in sugarcane plant

Sewage sludge application to the soil did not affect the concentration of As in the stalk and juice in both crops, but quadratically (ranging from 0.017 to 0.022 mg kg^{-1}) and linearly (ranging from 0.028 to 0.053 mg kg^{-1}) increased leaf As concentration, respectively, for cane plant and cane ratoon crop (Tables 6 and 7). Due to the paucity of information on the levels of As in sugarcane plants, as well in other crops, it is difficult to evaluate the results. Studies have been conducted on plant As uptake and metabolism (Zhao et al., 2009), but minimal information is available on the toxic effects of As on sugarcane, especially from sewage sludge applications. Our results indicate that no harmful effects occur when sludge is applied based on nitrogen criteria according to the Resolution 375 (CONAMA, 2006).

Sewage sludge application linearly ($p < 0.01$ and $p < 0.05$) reduced the concentration of Ba in the leaf, stalk and juice for both crops (Tables 6 and 7). There is limited information available in the literature regarding Ba contamination of plants, included sugarcane. Similar to As, few investigations have been conducted in field to examine the effects of Ba on plants and there is also limited information on the toxic effects of Ba on sugarcane. In a long-term study, with maize plants grown in a tropical soil (Typic Eutrorthox), it was observed that with increasing sewage sludge application rates from

Table 6
Heavy metal concentration (mg kg^{-1}, dry basis) in different parts of cane plant under varying sewage sludge rates applied ($n = 32$).

Cane plant	Sewage sludge (t ha^{-1})				Equation	r^2
	0	3.6	7.2	10.8		
Arsenic						
Leaf	0.018	0.017	0.019	0.022	$y = 7.11 \times 10^{-5}x^2 - 0.0004x + 0.01$	0.86**
Stalk	0.018	0.018	0.018	0.015	NS	NS
Juice	0.002	0.001	0.002	0.002	NS	NS
Barium						
Leaf	39.45	38.03	33.75	31.10	$y = -0.814x + 39.98$	0.88**
Stalk	13.92	11.23	11.72	8.55	$y = -0.360x + 13.07$	0.61*
Juice	0.99	0.98	0.84	0.80	$y = -0.016x + 0.97$	0.59*
Cadmium						
Leaf	0.009	0.017	0.023	0.027	$y = 0.0017x + 0.010$	0.97**
Stalk	0.028	0.039	0.066	0.074	$y = 0.0047x + 0.026$	0.96**
Juice	0.003	0.005	0.009	0.010	$y = 0.0007x + 0.003$	0.93**
Chromium						
Leaf	0.476	0.494	0.493	0.444	NS	NS
Stalk	0.311	0.363	0.292	0.190	$y = -0.003x^2 + 0.025x + 0.30$	0.88**
Juice	0.014	0.016	0.016	0.011	$y = -0.0001x^2 + 0.001x + 0.01$	0.92**
Copper						
Leaf	5.66	5.83	5.63	5.62	NS	NS
Stalk	4.48	5.01	4.83	3.92	$y = -0.032x^2 + 0.311x + 4.27$	0.72*
Juice	0.50	0.54	0.52	0.48	NS	NS
Nickel						
Leaf	0.616	0.627	0.604	0.602	NS	NS
Stalk	0.310	0.369	0.518	0.350	$y = -0.005x^2 + 0.059x + 0.27$	0.68*
Juice	0.039	0.035	0.039	0.037	NS	NS
Lead						
Leaf	1.007	1.009	1.011	1.028	NS	NS
Stalk	0.470	0.471	0.477	0.439	NS	NS
Juice	0.024	0.027	0.026	0.025	NS	NS
Selenium						
Leaf	0.0082	0.0086	0.0088	0.0090	$y = 6.63 \times 10^{-5}x + 0.008$	0.74**
Stalk	0.0037	0.0040	0.0040	0.0035	NS	NS
Juice	0.0006	0.0004	0.0005	0.0004	NS	NS
Zinc						
Leaf	21.79	28.94	30.01	33.24	$y = 0.984x + 23.18$	0.88**
Stalk	13.39	17.73	23.61	27.93	$y = 1.375x + 13.23$	0.81**
Juice	1.86	2.61	3.39	3.79	$y = 0.182x + 1.93$	0.94**

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
NS: not significant.
n = number of analyzed samples.

Table 7
Heavy metal concentration (mg kg^{-1}, dry basis) in different parts of cane ratoon[a] under varying sewage sludge rates applied ($n = 32$).

Cane ratoon	Sewage sludge (t ha^{-1})				Equation	r^2
	0	3.6	7.2	10.8		
Arsenic						
Leaf	0.028	0.033	0.040	0.053	$y = 0.0024x + 0.026$	0.82**
Stalk	0.012	0.013	0.012	0.014	NS	NS
Juice	0.001	0.001	0.001	0.001	NS	NS
Barium						
Leaf	65.00	63.10	46.60	48.50	$y = -1.76x + 65.2$	0.47*
Stalk	11.61	8.62	7.33	6.80	$y = -0.41x + 10.88$	0.55*
Juice	0.91	0.79	0.68	0.59	$y = -0.029x + 0.90$	0.75**
Cadmium						
Leaf	0.005	0.012	0.016	0.022	$y = 0.001x + 0.005$	0.95*
Stalk	0.013	0.020	0.031	0.052	$y = 0.004x + 0.009$	0.81**
Juice	0.002	0.005	0.007	0.011	$y = 0.001x + 0.002$	0.79**
Chromium						
Leaf	0.42	0.32	0.45	0.50	NS	NS
Stalk	0.14	0.14	0.13	0.13	NS	NS
Juice	0.008	0.008	0.009	0.008	NS	NS
Copper						
Leaf	4.73	6.00	6.32	6.04	$y = -0.0295x^2 + 0.4376x + 4.75$	0.68*
Stalk	2.49	3.05	2.98	2.67	NS	NS
Juice	0.40	0.45	0.48	0.43	NS	NS
Nickel						
Leaf	0.43	0.44	0.41	0.51	NS	
Stalk	0.17	0.14	0.11	0.11	$y = -0.006x + 0.16$	0.64**
Juice	0.034	0.031	0.030	0.031	NS	
Lead						
Leaf	1.03	1.32	1.31	1.54	NS	NS
Stalk	0.22	0.24	0.23	0.20	NS	NS
Juice	0.015	0.014	0.019	0.016	NS	NS
Selenium						
Leaf	0.012	0.012	0.013	0.011	NS	NS
Stalk	0.0023	0.0024	0.0031	0.0025	NS	NS
Juice	0.0004	0.0003	0.0004	0.0003	NS	NS
Zinc						
Leaf	21.66	30.90	36.08	39.94	$y = 1.601x + 23.28$	0.89**
Stalk	11.56	14.46	16.15	18.40	$y = 0.614x + 11.81$	0.77**
Juice	1.80	2.28	2.98	3.33	$y = 0.144x + 1.81$	0.79**

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
NS: not significant.
n = number of analyzed samples.
[a] Residual effect of sewage sludge application.

45 to 167.5 t ha^{-1} (dry basis) the concentration of Ba in the stem, leaf, and straw decreased (Nogueira et al., 2010; Merlino et al., 2010). These data agree with those obtained in the present study. This may be attributed to organic matter and phosphorus added in the sludge, which may reduce the availability of soil Ba to plants.

The application sewage sludge rates linearly ($p < 0.01$ and $p < 0.05$) increased the concentration of Cd in the leaf, stalk and juice for both crops (Tables 6 and 7). However, the sugarcane plants did not display any symptoms of Cd toxicity or of deficiency in other nutrients which might be caused by the presence of Cd in the soil (Tables 6 and 7). Some authors found that *Saccharum* spp. is a specimen with potential tolerance to high Cd concentrations (Fornazier et al., 2002; Sereno, 2004). In the present work, the minimum and maximum concentrations of Cd in the sugarcane plants ranged from 0.002 to 0.074 mg kg^{-1} for both crops. Although Cd levels increased and the accumulation occurred in stalk (edible part), the concentration of Cd was very low. As this part of the plant is commercially used for sugar production, it is essential to officially establish maximum limit for Cd in sugarcane, which is currently not available. The results from this study demonstrated that the Cd concentration in stalk of the sugarcane was largely unaltered and appeared to be safe for sugar consumption when sludge is applied based on the N criterion (CONAMA, 2006).

As shown in Tables 6 and 7, there was no effect of the sewage sludge application on leaf Cr concentration of cane plant and in all the parts of cane ratoon. The application rates of sludge were quadratically correlated with the concentration of Cr in the stalk and juice of the cane plant. The highest level of Cr in the stalk and juice occurred at the application rates of 4.1 and 5.0 t ha^{-1}. In a pot experiment carried out using two Brazilian Oxisols amended with one limed-digested sewage sludge and cultivated with sugarcane, Bertoncini et al. (2004) showed that leaf Cr concentration ranged from 2.3 to 7.7 mg kg^{-1} and juice Cr concentration ranged from 0.33 to 1.1 mg kg^{-1}. The low Cr concentration in the aerial part of sugarcane may be attributed to the accumulation of Cr in roots as Cr^{3+} is mostly bound to cell walls (Kabata-Pendias and Mukherjee, 2007).

Sewage sludge application quadratically ($p < 0.05$) increased stalk Cu concentration in the cane plant, from 3.92 to 5.01 mg Cu kg^{-1} (Table 6), and leaf Cu concentration in the cane ratoon reached the highest at the sludge rate of 7.40 t ha^{-1} (Table 7). However, there was no effect of the sewage sludge application on the leaf and juice in the cane plant and on the stalk and juice in the cane ratoon. Oliveira et al. (2002) showed that Cd, Cr, Ni and Pb concentrations in sugarcane leaves, stalks and juice were below the detection limit of the analytical method used, and that Cu and Zn contents in plants were not affected by successive waste compost applications. When Cu levels are higher than 150 mg kg^{-1}, some agricultural species may show adverse effects (Segura-Muñoz et al., 2006). However, in the present study the sugarcane plants did not display any symptoms of Cu toxicity. The Cu leaf concentrations are considered adequate for sugarcane, which ranges from 6 to 15 mg Cu kg^{-1} (Spironello et al., 1997).

A significant effect of sewage sludge rates on the concentration of Ni in the stalk for cane plant was observed with a quadratic adjustment ($p < 0.05$) as the sludge rates increased. The sludge rate that resulted in the highest value of stalk Ni concentration in the cane plant was 6.34 t ha^{-1} (Table 6). Loadings of sewage sludge to soil linearly decreased ($p < 0.01$) the stalk Ni for cane ratoon, with a concentration ranging from 0.11 to 0.17 mg kg^{-1} (Table 7). The decrease in stalk Ni concentration may be attributed to the dilution effect due to gain of dry mass (Franco et al., 2010) from sludge application since the Ni concentration in the leaf and juice was not affected for both crops.

The application of sewage sludge did not affect the concentration of Pb in the sugarcane plants for both crops (Tables 6 and 7). Some authors reported that absorbed Pb by plants is mainly retained in their roots with a small amount being transported to the aerial part of the plant (Malavolta, 2006; Kabata-Pendias and Mukherjee, 2007). Our results indicated that the leaf Pb concentration in both crops showed normal values, considering that typical Pb levels vary between 2 and 10 mg kg^{-1} in plants (Türkan et al., 1995).

Except for leaf Se concentration in the cane plant, the loadings of sewage sludge to soil did not affect the concentration of Se in sugarcane plants. Leaf Se concentration linearly ($p < 0.01$) increased with increasing rate of sewage sludge, ranging from 0.0082 (control) to 0.0090 mg kg^{-1} (in the 10.8 t ha^{-1} sewage sludge treatment). According to Kabata-Pendias and Mukherjee (2007), most plants contain rather low foliar Se, around 0.025 mg kg^{-1} and rarely exceed 0.1 mg kg^{-1}. Due to the paucity of information on the levels of Se in sugarcane plants it is difficult to evaluate the results. However, our results demonstrated that the Se concentration in sugarcane is very low, at sub-ppb level, and is rarely changed by sludge application based on the N criterion (CONAMA, 2006).

The concentrations of Zn in leaf, stalk or juice in the cane plant and cane ratoon were linearly ($p < 0.01$) correlated with external loading rate of sewage sludge (Tables 6 and 7). The leaf Zn concentration in both crops ranged from 21.8 to 39.9 mg kg^{-1}. These values are considered adequate for sugarcane, which ranges from 10 to 50 mg kg^{-1} (Spironello et al., 1997). The data agree with those obtained by Oliveira and Matiazzo (2001).

3.4. Heavy metals uptake correlation

Correlation was conducted between total recoverable metals or extractable metals in soil and the heavy metals uptake by sugarcane plants (Table 8). The close correlations between levels of metals in soil and their uptake by sugarcane plants were expected, since the addition of sewage sludge increased available Cd, Cu, Ni, Pb, and Zn concentrations in soil (Tables 4 and 5). The concentrations of Cd and Zn in the soil were highly correlated with these metals absorbed in sugarcane plant parts for both crops. However, no correlation of soil Cr and Se concentrations with the uptake of these metals in sugarcane plants was observed. There were only positive correlations of soil As, Cu, Ni, and Pb concentration with those in sugarcane parts. In general, total metal extractions using strong acid (e.g., USEPA's methodology for total recoverable) are not effective to indicate availability of heavy metals in soil, but in this study we verified significant correlation of total recoverable As, Cu, and Pb with these metals in the sugarcane leaves.

The efficacy of chemical extractants is evaluated mainly by the correlation degree between the metal concentrations determined in soil and those in the leaf tissue. The efficacy of the extractants also depends on the type of soil, the element under concern and the plant species (Oliveira and Matiazzo, 2001; Martins et al., 2003). In the State of São Paulo, the DTPA solution has been used to extract and determine both micronutrients and toxic metals in contaminated soils. However, most of the positive and high correlations obtained between soil and plant element concentrations were Fe, Mn, Cu and Zn (Abreu et al., 2007). In the present work, except for Cd and Zn, the DTPA extractant was not adequate to evaluate the availability of the studied heavy metals to sugarcane plants as evidenced by the low correlation (Table 8).

Table 8
Correlation coefficients of Pearson's (r) And respective statistical significances by t test, obtained between total recoverable metals or extractable metals in soil and heavy metals uptake by sugarcane plants grown in a Typic Hapludult soil treated with increasing rates of sewage sludge ($n = 12$).

Heavy metal/ method	Cane plant			First ratton[a]		
	Leaf	Stalk	Juice	Leaf	Stalk	Juice
Arsenic						
Total recoverable	0.84^{**}	-0.57^{NS}	-0.28^{NS}	0.94^{**}	0.32^{NS}	0.22^{NS}
DTPA-extractable	0.41^{NS}	-0.36^{NS}	-0.12^{NS}	0.51^{NS}	0.03^{NS}	0.38^{NS}
Barium						
Total recoverable	-0.50^{NS}	-0.41^{NS}	-0.28^{NS}	0.16^{NS}	0.15^{NS}	-0.1^{NS}
DTPA-extractable	0.39^{NS}	0.65^{NS}	0.79^{*}	-0.09^{NS}	-0.13^{NS}	0.02^{NS}
Cadmium						
Total recoverable	0.95^{**}	0.99^{**}	0.96^{**}	0.94^{**}	0.92^{**}	0.90^{**}
DTPA-extractable	0.96^{**}	0.96^{**}	0.90^{**}	0.93^{**}	0.96^{**}	0.94^{**}
Chromium						
Total recoverable	-0.51^{NS}	-0.72^{NS}	-0.85^{NS}	0.27^{NS}	-0.68^{NS}	0.42^{NS}
DTPA-extractable	0.20^{NS}	0.01^{NS}	-0.16^{NS}	0.37^{NS}	0.10^{NS}	0.07^{NS}
Copper						
Total recoverable	-0.26^{NS}	-0.44^{NS}	-0.34^{NS}	0.77^{*}	0.47^{NS}	0.68^{NS}
DTPA-extractable	-0.21^{NS}	-0.52^{NS}	-0.18^{NS}	0.51^{NS}	0.41^{NS}	0.72^{*}
Nickel						
Total recoverable	-0.45^{NS}	0.01^{NS}	0.02^{NS}	0.67^{NS}	-0.40^{NS}	0.32^{NS}
DTPA-extractable	-0.44^{NS}	0.24^{NS}	0.27^{NS}	0.71^{*}	-0.56^{NS}	0.24^{NS}
Lead						
Total recoverable	0.58^{NS}	-0.31^{NS}	0.09^{NS}	0.81^{*}	-0.07^{NS}	0.24^{NS}
DTPA-extractable	0.17^{NS}	0.26^{NS}	0.38^{NS}	0.43^{NS}	0.01^{NS}	0.41^{NS}
Selenium						
Total recoverable	0.36^{NS}	-0.63^{NS}	-0.50^{NS}	-0.63^{NS}	0.42^{NS}	-0.20^{NS}
DTPA-extractable	-0.13^{NS}	-0.68^{NS}	-0.09^{NS}	0.14^{NS}	0.63^{NS}	-0.07^{NS}
Zinc						
Total recoverable	0.87^{**}	0.89^{**}	0.88^{**}	0.88^{**}	0.91^{**}	0.86^{**}
DTPA-extractable	0.81^{*}	0.87^{**}	0.87^{**}	0.86^{**}	0.84^{**}	0.80^{**}

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
NS: not significant.
n = number of analyzed samples.
[a] Residual effect of sewage sludge application.

4. Conclusion

Sewage sludge application based on the nitrogen criterion increased soil As, Cd, Cu, Ni, Pb and Zn concentrations for both cane plant as a direct effect of sludge, and for cane ratoon as a residual effect of sludge. However, even the highest element concentrations were far below the quality and prevention values established by the legislation for agricultural soils. The uptake of heavy metals in the parts of sugarcane plants was generally increased by sewage sludge application, but the levels of metals in plants were very low for toxic elements, and adequate for micronutrients. The results from this study indicate that agronomic utilization of sewage sludge on a tropical soil do not cause heavy metal contamination in soil and sugarcane within a short term. It is suggested that an application of sewage sludge to medium fertility tropical soil may provide beneficial quantities of plant nutrients with low risks of heavy metals.

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