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Cadmium, copper, and chromium levels in maize plants and soil fertilized with sewage sludge

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Abstract

The use of sewage sludge as fertilizer is a widespread practice. However, the presence of heavy metals and pathogens in sludge are a cause for concern. The objective of this study was to determine the levels of cadmium (Cd), copper (Cu), and chromium (Cr) in leaves, aerial parts, and kernels of maize grown in red latosol that was fertilized with sewage sludge for 15 consecutive years. Metal concentrations in soil and kernel yield were also determined. A randomized block design was used, with five replicates and four treatments: T_1, mineral fertilization (control); T_2, application of sewage sludge at 5 Mg ha^{-1}; T_3, application of sewage sludge at 10 Mg ha^{-1}; and T_4, application of sewage sludge at 20 Mg ha^{-1}. All plots received potassium (K) supplementation. Fertilization with sewage sludge was as efficient as mineral fertilization. Sewage sludge had no negative effect on kernel yield. A negative correlation was observed between metal concentrations in soil and plant samples. Cd was detected at ≤0.04 mg kg^{-1} in kernels and Cu at ≤2.34 mg kg^{-1}, whereas Cr was not detected in any sample. These levels are considered safe for human consumption, according to the Brazilian Health Regulatory Agency. Fertilization with sewage sludge for 15 years did not increase Cu, Cd, and Cr levels in the soil and had no negative effect on maize, which shows the potential of sewage sludge for agricultural use.

Keywords: Bioaccumulation; Heavy metal; Pollution; Productivity; Soil science.

Introduction

The agricultural use of sewage sludge is a widespread, feasible practice. It allows for sustainable management of sludge and increases the content of organic matter and nutrients, such as phosphorus (P) and nitrogen (N), in the soil (Merlino et al., 2010; Nogueira et al., 2010; Bueno et al., 2011; Silva et al., 2016, Costa et al., 2017; Melo et al., 2018). However, there are concerns regarding the presence of pathogens and heavy metals in sludge, which can leach into the water table or be absorbed by plants and transferred through the trophic chain, resulting in serious damage to human health (Mcbride, 1995; Lee et al., 2006; Costa et al., 2017). Not only the presence but also the accumulation of heavy metals, such as cadmium (Cd), chromium (Cr), copper, (Cu), nickel (Ni), lead, and zinc, in soils may result from fertilization with sewage sludge, especially that obtained from industrial sewage (Krebs et al., 1998).

The concentration of chemical elements in plants depends on the type of soil, plant species, plant maturation stage, crop management practices, and climate (McDowell et al., 1993). Exposure time, metal species, and absorption potential of the plant are important factors that also influence the level of contamination by heavy metals in crops (Mengel and Kirkby, 1987; Alloway, 1995).

Plants take up heavy metals via soil solution (Ali et al., 2013) and may distribute them to various organs (Dowswell et al., 1996; Amusan et al., 2005; Singh et al., 2010). The composition of this soil fraction depends on soil management practices and physicochemical characteristics of the soil (Gupta et al., 2014). Trace concentrations of heavy metals are naturally present in soil and do not pose a risk to the environment or to living beings. However, the same cannot be said for high concentrations of heavy metals, which are increased by anthropogenic actions, such as mining, waste disposal, deposition, and intense agricultural activity (Peris et al., 2007).

Various studies in Brazil and in other countries reported an association between successive applications of sewage sludge and the presence of heavy metals in the soil and in crops, especially maize (Oliveira et al., 2005; Merlino et al., 2010; Nogueira et al., 2010; McBride, 1995; Kabata-Pendias and Pendias, 2001). Although accumulation of heavy metals in plants may occur without apparent toxicity symptoms or

yield loss (Jeevan Rao and Shantaran, 1996), it can affect the environment, food quality, and human health (Lee et al., 2006, Luo et al., 2007). Thus, it is important to evaluate the environmental impact of the use of sewage sludge in agricultural soils.
In this study, we determined the contents of Cd, Cu, and Cr in diagnostic leaves, aerial parts, and kernels of maize grown in red latosol fertilized with sewage sludge for 15 years. Kernel yield and heavy metal concentrations in soil were also determined.

Results and discussion

Kernel yield

Table 1 shows that soils treated with mineral fertilizer or different doses of sewage sludge showed similar physicochemical characteristics, differing only in pH, which had no effect on yield. Kernel yield (9.04–10.21 Mg ha^{-1}) did not differ significantly among treatments (Fig. 1) and was higher than the 2011/2012 national average (CONAB, 2013).
In previous studies carried out in the same experimental area, the yield of maize crops fertilized with sewage sludge was similar to that of crops treated with mineral fertilizer (Nogueira et al., 2010).
Sewage sludge increases organic matter and N availability in the soil, which can explain why its efficiency was similar to that of mineral fertilizer. In the present study, sewage sludge was enriched with potassium (K), as sludge is a poor source of this nutrient (Barbosa et al., 2007).
Soil physical, chemical, and biological attributes in agricultural, forest, recreation, and restoration areas were improved by sewage sludge application (Nogueira et al., 2010; Zornoza et al., 2012). These improvements were attributed to the increase in organic matter. Antolin et al. (2005) also observed an improvement in soil chemical quality with sewage sludge application.
The agricultural use of sewage sludge increased the productivity of several crops. Behling et al. (2009) observed an increase of 1.224 kg ha^{-1} in the yield of soybean fertilized with sewage sludge. Albuquerque et al. (2015) reported that sunflower yield increased with sewage sludge dose. Higgins (1994) and Rappaport et al. (1988) treated red latosol with 20 to 80 Mg ha^{-1} of sewage sludge for 4 years and observed a linear increase in maize yield with sewage sludge fertilization. In a study by Merlino et al. (2010), higher maize productivity was achieved with long-term use of sewage sludge in comparison with mineral fertilizer.

Cd, Cu, and Cr concentrations in leaves, aerial parts, and kernels

Cd concentrations in leaves were low (0.11 to 0.13 mg kg^{-1}) and did not differ between treatments (Table 2). As Cd is highly mobile, a low concentration of this metal in leaves suggests low soil availability. Similar results were reported by Merlino et al. (2010), who found no increase in Cd leaf concentrations in maize crops grown in soil treated with sewage sludge. Junio et al. (2011) reported that Cd levels were low in soils fertilized with sewage sludge or natural phosphate and undetectable in leaves of maize grown in these soils.
Cu contents in leaves were also low (3.55 to 4.58 mg kg^{-1}) and showed no differences between treatments (Table 2). Maize plants were, in fact, moderately deficient in Cu, which may be justified by the low mobility of this element (Seidel et al., 2009). Similar results were reported by Merlino et al. (2010) and Junio et al. (2011).
Concentrations of Cd and Cu in aerial parts did not differ between treatments. Cd levels varied from 0.03 to 0.04 mg kg^{-1}, and Cu levels from 2.98 to 3.23 mg kg^{-1}. Merlino et al. (2010) found similar whole-plant concentrations of these metals.
Cd is not essential for plant growth and can be toxic at high concentrations (Silva et al. 2016). For instance, Cunha et al. (2008) observed that soil Cd levels of 8.7 to 13.1 mg kg^{-1} were toxic to plants.
In maize kernels, Cd concentrations ranged from 0.01 to 0.03 mg kg^{-1} and Cu concentrations ranged from 1.12 to 2.34 mg kg^{-1}. There were no significant differences between treatments. According to the standards of the Brazilian Health Regulatory Agency (ANVISA, 1965), Cd and Cu contents in kernels were within the acceptable limits for human consumption. The values were also within the recommended limits for silage and hay (Nogueira et al., 2010).
The analytical method used in the current study was not sensitive enough to detect low concentrations of Cr, neither in leaves nor in kernels. Therefore, Cr concentrations are expressed as the limit of detection, 0.18 mg kg^{-1}. It is important to note that this value is above the safe levels for human consumption (0.10 mg kg^{-1}) (ANVISA, 1965). Merlino et al. (2010) also failed to detect Cr in maize kernels.
Cd and Cu accumulation was higher in diagnostic leaves than in kernels. Similar results were found by Wang et al. (1997) in their study of Ni accumulation in winter wheat crops following application of sewage sludge at 60 Mg ha^{-1}.

Correlations between Cd, Cu, and Cr concentrations in soil, leaves, and kernels

Soil concentrations of Cr, Cu, and Cd were more strongly correlated with leaf concentrations than with kernel concentrations (Table 2). Negative correlations were observed between Cr concentrations in soil and those in leaves and kernels, Cd concentrations in soil and in leaves, and Cu concentrations in soil and in kernels. These results support that Cr and Cd are present in sewage sludge in forms not readily available for plant uptake (Gomes et al., 2006; Silva et al., 2016) or are immobilized in soil by the formation of organic complexes (Soares et al., 2001). Gomes et al. (2006) observed that Cu contents in maize kernels decreased with increasing sludge doses.
Correlations between Cu levels in soil and in leaves were positive, probably because of the high mobility of the metal.
No correlations were found between Cd concentration in soil and kernels (Table 2). The lack of significant correlations is attributable to the slow transfer of Cd from roots and leaves to kernels (Corguinha et al. 2015), which is a beneficial property of maize crops because it prevents Cd from accumulating to toxic levels in edible parts of the plant.

Table 1. Chemical characteristics of red latosol treated with sewage sludge for 14 consecutive years.

Treatment	Resin P (mg dm^{-3})	OM (g dm^{-3})	pH($CaCl_2$)	K^+	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	CV (%)
				(mmol$_c$ dm^{-3})						
T_1	100^a	26^a	5.4^a	4^a	40^a	17^{ab}	34^b	61.0^b	95^a	64^a
T_2	34^c	22^b	5.1^a	2.6^b	23^c	15^b	38^b	40.6^b	78.6^d	52^c
T_3	86^b	26^a	5.2^a	3.1^b	28^b	16^a	38^b	47.1^a	85.1^c	55^b
T_4	88^b	26^a	4.7^a	2.3^b	21^d	13^c	52^a	36.3^{ab}	88.3^b	41^d
CV	3.97	4.87	4.09	19.67	6.26	8.13	4.91	9.75	2.04	2.34

[a, b, c, d] Means followed by the same letter do not differ significantly by Tukey's test at $P < 0.05$. T_1, control (fertilized with NPK); T_2, fertilized with sewage sludge at 5 Mg ha^{-1}; T_3, fertilized with sewage sludge at 10 Mg ha^{-1}; and T_4, fertilized with sewage sludge at 20 Mg ha^{-1}. OM, organic matter; SB, sum of bases; CEC; cation-exchange capacity; and CV, coefficient of variation.

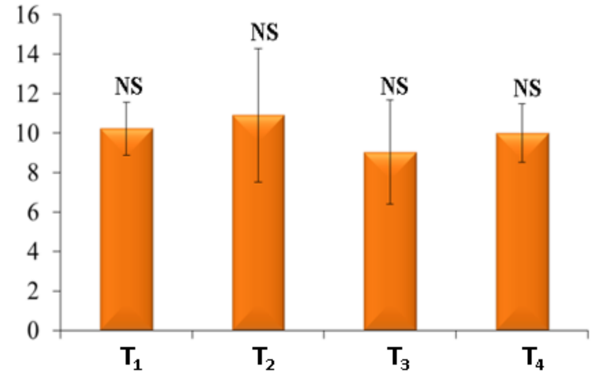

Fig 1. Kernel yield (Mg ha^{-1}) of maize grown in soil fertilized with sewage sludge. T_1, control (fertilized with NPK); T_2, fertilized with sewage sludge at 5 Mg ha^{-1}; T_3, fertilized with sewage sludge at 10 Mg ha^{-1}; and T_4, fertilized with sewage sludge at 20 Mg ha^{-1}. NS; not significantly different from control (Tukey's test; $P \geq 0.05$).

Table 2. Cadmium (Cd), copper (Cu), and chromium (Cr) contents (mg kg^{-1}) in diagnostic leaves, aerial parts, and kernels of maize grown in red latosol treated with sewage sludge.

Treatment	Diagnostic leaf			Aerial parts			Kernel		
	Cd	Cu	Cr*	Cd	Cu	Cr*	Cd	Cu	Cr*
T_1	0.11^a	4.58^a	<0.18	0.04^a	2.98^a	<0.18	0.01^b	1.11^a	<0.18
T_2	0.12^a	4.39^a	<0.18	0.03^a	3.22^a	<0.18	0.01^b	2.34^a	<0.18
T_3	0.13^a	3.75^a	<0.18	0.03^a	3.21^a	<0.18	0.02^{ab}	1.16^a	<0.18
T_4	0.13^a	3.55^a	<0.18	0.04^a	3.13^a	<0.18	0.03^a	2.34^a	<0.18
CV (%)	7.18	11.76	–	40.31	18.11	–	18.16	52.22	–

[a, b] Means followed by the same letter do not differ significantly by Tukey's test at $P < 0.05$. *Cr levels were considered equal to the limit of detection (0.18 mg kg^{-1}) for all samples. T_1, control (fertilized with NPK); T_2, fertilized with sewage sludge at 5 Mg ha^{-1}; T_3, fertilized with sewage sludge at 10 Mg ha^{-1}; and T_4, fertilized with sewage sludge at 20 Mg ha^{-1}. OM, organic matter; SB, sum of bases; CEC; cation-exchange capacity; and CV, coefficient of variation.

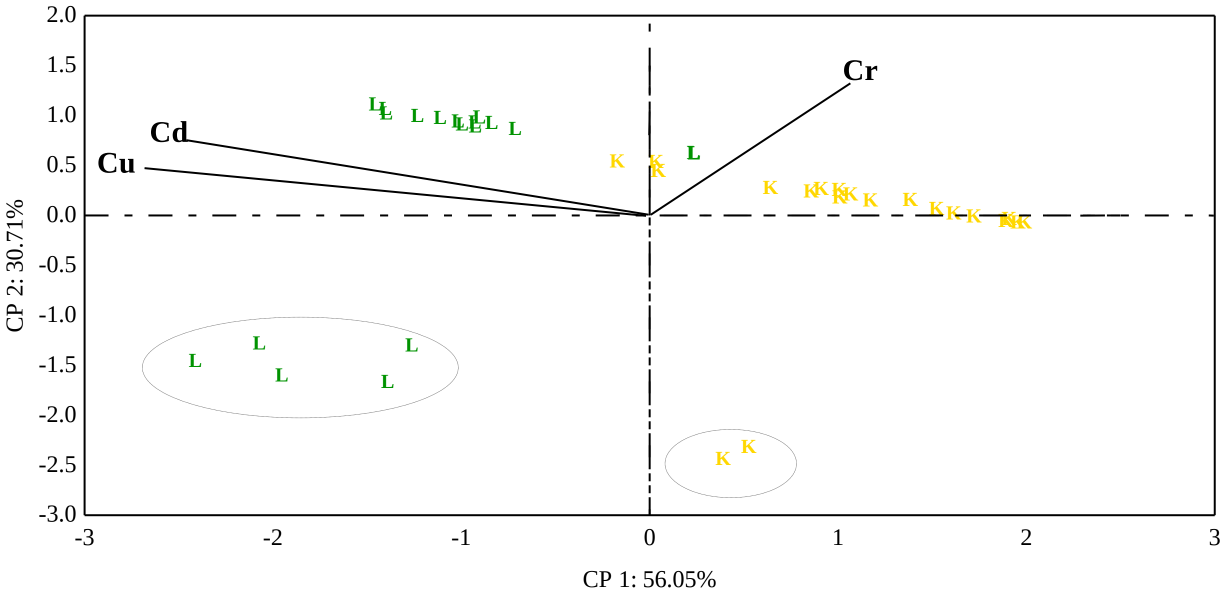

Fig 2. Principal component analysis (PCA) of cadmium (Cd), copper (Cu), and chromium (Cr) concentrations in maize leaves (L) and kernels (K).

Table 3. Correlation coefficients between chromium (Cr), copper (Cu), and cadmium (Cd) contents in maize leaves, kernels, and soil sampled at different depths.

	Leaf Cr	Leaf Cu	Leaf Cd	Kernel Cr	Kernel Cu	Kernel Cd
Soil depth of 0.0 to 0.5 m						
Soil Cr	−0.41**	0.92**	0.74**	−0.48**	0.48**	−0.52**
Soil Cu	−0.41**	0.85**	0.62**	−0.46**	−0.03**	−0.13**
Soil Cd	−0.66**	−0.45**	−0.72**	−0.61**	−0.76**	0.89^{ns}
Soil depth of 0.5 to 0.10 m						
Soil Cr	−0.61**	0.69**	0.48**	−0.67**	0.65**	−0.57**
Soil Cu	−0.92**	0.39**	0.04**	−0.94**	−0.02**	0.13**
Soil Cd	−0.67**	−0.52**	−0.78**	−0.61**	−0.73**	0.88^{ns}
Soil depth of 0.10 to 0.20 m						
Soil Cr	−0.51**	0.87**	0.65**	−0.58**	0.49**	−0.49**
Soil Cu	−0.64**	0.31**	−0.01**	−0.64**	−0.53**	0.52**
Soil Cd	−0.56**	−0.68**	−0.89**	−0.49**	−0.74**	0.90^{ns}

Cr levels were considered equal to the limit of detection (0.18 mg kg^{-1}) for all samples. ns, not significant. ** significant 1%.

Principal component analysis (PCA) of Cd, Cu, and Cr levels in leaves and kernels

The results of Cd, Cu, and Cr accumulation in leaves and kernels were subjected to PCA (Fig. 2). Multivariate statistical techniques such as PCA are useful to interpret environmental data. For instance, they can help distinguish the presence of naturally occurring metals from the presence of metals brought about by anthropogenic activities (Micó et al., 2006; Navee Dullah et al., 2013; Sun et al., 2013; Parelho et al., 2014; Silva et al., 2016). This differentiation is important to compare metal concentrations present in sludge, soil, and plant samples with reference levels for groundwater and agricultural plants.

Principal component 1 (PC1) and principal component 2 (PC2) explained together 86.76% of the total variance of the dataset (PC1, 56.05%; PC2, 30.71%). PC1 showed that diagnostic leaves had the highest Cd and Cu concentrations, followed by aerial parts and kernels. Cr concentrations in all samples were considered equal to the detection limit and therefore cannot be compared.

PC2 showed that Cd and Cu were prone to accumulate in leaves. This is in agreement with the findings of Costa et al. (2007) regarding the transfer of heavy metals from soil to soybean and rice grains. However, in the current study, Cd and Cu accumulation was not sufficient to reach toxic levels, indicating that use of K-enriched sewage sludge does not lead to unacceptable concentrations of Cd, Cu, and Cr in plants for human consumption or other purposes (ANVISA, 1965).

Materials and methods

Experiment location

The experiment was performed at the Experimental Farm of the São Paulo State University, Jaboticabal, Brazil (21°15′22″S, 48°15′18″W, 618 m above sea level). In 1997/98, an experimental plot with eutrophic red latosol began to be treated with sewage sludge. The treatment was maintained for 15 consecutive years.

In the first year, treatments consisted of fertilization with 2.5, 5.0, and 10.0 Mg ha^{-1} of sewage sludge on a dry basis. The control, T_1, consisted of mineral fertilization.

From the second year onward, mineral fertilization (T_1) was determined on the basis of soil analysis and recommendations for maize cultivation, according to Raij et al. (1996). From the fourth year onwards, plots receiving 2.5 Mg ha^{-1} began to receive 20 Mg ha^{-1} of sewage sludge. A total of 0, 75, 150, and 247.5 Mg ha^{-1} of sewage sludge were applied during the 15 years of experiment to plots assigned to treatments 1, 2, 3, and 4, respectively.

Experimental design and treatments

In the 2011/12 agricultural year, corresponding to the 15th year since the beginning of soil treatment with sewage sludge, we carried out a randomized block design with four treatments and five repetitions. The treatments were as follows: T_1, mineral fertilization (control); T_2, 5 Mg ha^{-1} sewage sludge; T_3, 10 Mg ha^{-1} sewage sludge; and T_4, 20 Mg ha^{-1} sewage sludge.

Soil samples (0.0–0.20 m depth) were collected from all treatment plots for analysis of soil fertility, which was determined according to the methods of Raij et al. (2001) (Table 1). T_1 (control) received fertilization with NPK, according to the recommendations of Raij et al. (1996) for the production of 80 kg ha^{-1} maize.

Maize (*Zea mays*) was planted during the first 6 years of the experiment, followed by sunflower (*Helianthus annuus*) in the seventh year and crotalaria (*Crotalaria juncea*) in the eighth year. Maize was grown successively from the ninth year onwards.

The sewage sludge used during the 15th year of the experiment was obtained from the sewage treatment plant of the São Paulo State Water Sanitation Company (SABESP), Monte Alto, SP, Brazil. Sewage sludge contained 5.60, 246.65, and 546.54 mg kg^{-1} of Cd, Cu, and Cr, respectively, as determined by the United States Environmental Protection

Agency (US EPA) method 3050 B (USEPA, 1996). These levels are within the limits allowed for agricultural use of sewage sludge, according to CONAMA Resolution no. 375 (2006). Sewage sludge was broadcast and incorporated into the soil with a disc harrow. After sludge application, the field was furrowed at distances of 0.9 m, and control plots (T_1) were fertilized with ammonium sulfate and potassium chloride (KCl). T_2, T_3, and T_4 plots were fertilized with KCl only.

Maize (Bt hybrid Impacto Viptera) seeds were sown after fertilization. When seedlings were about 0.2 m high, plants were thinned to 5–7 plants per meter. Sixty days after emergence (DAE), 10 soil samples were collected at three depth ranges each (0.00–0.05, 0.05–0.10, and 0.10–0.20 m). Samples were air dried, sieved through a 2 mm mesh, and subjected to chemical analysis.

Determination of metal concentrations in soil and plant samples

Maize leaves opposite and below the primary ear (diagnostic leaves) were harvested at 60 DAE. Aerial parts of 6 plants per treatment were cut close to the soil surface from two inner rows at 90 DAE. Cobs were harvested manually at 125 DAE from two inner rows, excluding 1 m from the edges of the plot. Moisture was adjusted to 13% for the calculation of kernel yield. Prior to metal determination, soil samples were digested with HNO_3 in a microwave oven and plant samples were digested with a mixture of HNO_3, HCl, and H_2O_2 on a heating plate, according to US EPA methods 3051A and 3050B, respectively (USEPA, 1996). Cd, Cu, and Cr concentrations were determined by atomic absorption spectrophotometry using acetylene flame for Cd and Cu determination and acetylene–nitrous oxide flame for Cr determination. The limit of detection (LD) was calculated according to Giné-Rosias (1998).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and means were compared by Tukey's test at $P < 0.05$. It was necessary to transform the data to a normal distribution using $\sqrt{x}$. After standardization ($\mu = 0$; $\sigma = 1$), the data were subjected to a PCA. The number of retained components was determined using an eigenvalue of 1.00 and a cumulative variance of 70%, according to Kaiser (1958). Statistical analyses were performed using Statistica 7.0 (StatSoft Inc., 2005).

Conclusion

Application of K-enriched sewage sludge had the same effect as mineral fertilization on maize kernel yield. Correlations between Cd, Cu, and Cr levels in soil and in plants were low in most cases. Cd and Cu contents in leaves and aerial parts of the plant were considered low and were not affected by sewage sludge doses.

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