Metal availability and uptake by sorghum plants grown in soils amended with sludge from different treatments

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Abstract

Several factors depending on the sludge, the soil, or the combination of both substrates, may affect element availability to plants. In this study, an assessment was done of the effect of two sludges obtained by different processes (activated sludge and facultative stabilization pond) on heavy-metal availability and uptake by sorghum plants in soils with high and low copper contents. Results obtained for DTPA-extractable metal indicated higher metal availability in sludge-amended soils. In addition, sludges caused changes in copper and zinc distribution in soil, indicating in most cases a discrete increase in the more labile metal forms. However, observed changes did not increase heavy metal concentration in plant leaves, indicating that assessment of metal availability by a chemical procedure (single extraction or metal fractionation) would not permit a good prediction of metal bioavailability. On the other hand, sludge application at a rate of 100 t ha^{-1} to high-copper agricultural soils would not imply greater mobility of this metal on account of a greater sorbing capacity provided by the sludges. Such results would indicate that sludges from wastewater treatment plants, meeting the standards of heavy metal contents, regardless of the process by which they were obtained, may be applied to several kinds of soil, even to high-copper soils, with no risk of increasing heavy metal bioavailability to phytotoxic levels in the short range.

Keywords: Sewage sludge; Metal uptake; Sequential extraction; Sludge-amended soil; Single extraction; DTPA-extractable metal

1. Introduction

Sludges resulting from wastewater treatment constitute a valuable source of essential nutrients for agricultural cultivation. In addition, organic matter from sludges improves some physical and chemical properties of the soil, leading to better plant growth. Along with this, sludge application to soils is considered a useful method for their final disposition (Han et al., 2001). However, sludges may contain high amounts of potentially toxic trace elements which may exceed soil natural concentration by two or more orders of magnitude (Epstein, 2003; Oliver et al., 2005). In countries where sludge application and irrigation with recycled residual water have been carried out for a long time, considerable heavy metal accumulation has been reported in soils (Nicholson et al., 2003; Ozores-Hampton et al., 2005) and, in some cases in the crops grown in them (Wei and Liu, 2005).

At present, about 66% of the population in Chile discharges wastewater to systems that count on treatment and it is expected that by 2010 the whole of urban wastewaters of the country will be treated (Ramirez et al., 2002). Following a worldwide trend, a large share of the resulting sludges will probably be applied to the soil. To this end, a series of regulations have been prepared in order to set the requirements concerning sanitary quality, heavy metal

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Abbreviations: DTPA, diethylenetriaminepentaacetic acid; AS, activated sludge; FSP, facultative stabilization ponds; DM, dry-matter; HOAc, acetic acid; CEC, cation exchange capacity; OM, organic matter.

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content, and specific rates of application for treated and stabilized sludges (NCh-2952). According to this standard, the elements and their maximum allowed concentration in sludge are: arsenic, 20; cadmium, 8; copper, 1000; mercury, 10; nickel, 80; lead, 300; selenium, 50; and zinc, 2000; expressed as mg kg^{-1} dry weight. The existing relationship between availability and bioavailability of trace metals in soils amended with biosolids has been a recurring subject in the field of reutilization of organic wastes resulting from wastewater treatment. Thus, attempts have been made to relate the metal uptaken by plants with total metal in soil (Henning et al., 2001), or with the metal extractable with different chemical agents by single extraction (Pascual et al., 2004) and, especially in the last few years, with the metal forms in soils and in sludges through sequential extraction procedures (Qiao et al., 2003; Fuentes et al., 2004; Amir et al., 2005). However, plant bioassays constitute the best method to measure the effect of residues (biosolids) on phytoavailability (Basta et al., 2005).

According to the process used with wastewaters, the resulting sludges have different chemical characteristics. Thus, Merrington et al. (2003) have reported that metal associated to organic matter differs in aerobically and anaerobically digested sludges. Fuentes et al. (2004) report that the stabilizing treatment used on the sludges has a strong influence on distribution of heavy metals and the phases they were associated with and their level of bioavailability. On the other hand, Warman and Termeer (2005) analyzed the effect of different amendments on the content of elements in corn and grass forage plants and found that applying the wastes from aerobic septic ponds resulted in the highest levels of Fe, Cu, and Zn in forage; compost produced the highest levels of Ca in forage and of Zn in corn, while aerobically digested sludge produced the highest Mn levels in corn. Seyhan and Erdincler (2003) found that heavy metal availability in plants grown in unstabilized sludge-amended soils is comparable to that obtained in control soils, while lime stabilization decreases heavy metal availability to plants.

A logistic aspect to consider in the use of sludges in agriculture is the distance between the place where sludges are produced and the agricultural zones where these wastes could be potentially applied. Many times, treatment plants in large cities are far from the zones where soils are fit to receive this kind of amendment, a fact that results in higher economic cost of application (Epstein, 2003). The great variety of climatic conditions and characteristics of the different Chilean regions have created a fairly particular array of technologies used according to the regional needs and geographical location. Thus, for example, in the north of the country it is fairly common to find plants using ponds as a system of secondary treatment, while in the southern end 100% of the plants use activated-sludge technology (Ramirez et al., 2002).

On the other hand, soils of important agricultural zones in the Metropolitan Region and Region VI exhibit a high content of metal, particularly copper (Badilla-Ohlbaum et al., 2001). In some zones, a geochemical origin is acknowledged for this metal and it occurs in forms little available to plants (Ahumada et al., 1999). In other zones the origin is anthropic, and activity at large mining centers processing copper in situ in the mountain has contaminated downstream soils (Ahumada et al., 2004). This is the background that leads to the question of the actual effect of systematic biosolid application on copper availability in this kind of soils. The purpose of this study was to investigate the effect of applying two kinds of sludge on heavy metal availability and metal uptake by sorghum (*Sorghum bicolor* L. Moench) plants grown in soils with high and low copper content.

2. Materials and methods

2.1. Soils and sludges

The VI region is located in the central valley of Chile. It is characterized by a mild weather and important agricultural and mining activities are developed there. This study included three soils representing three agricultural zones (Pichilemu, Rapel, and Rancagua), about 50 km apart from one another (Table 1). Clay loam soil (Pichilemu) is annually cultivated with wheat but its low pH results in low productivity, thus requiring annual application of liming materials. Sandy loam (Rapel) and Loam (Rancagua) soils correspond to productive soils, mainly cultivated with corn, but differing in copper content. Clay loam and Sandy loam soils were chosen because of their low contents of heavy metals as stipulated by the Chilean standard regulating biosolid application to soils. Loam soil, instead, was chosen as a model of soil not meeting the standards as regards copper content.

Sludges were obtained from the treatment of household wastewaters in two small cities, with a population of <45 000. One of them was obtained from a plant operating with the activated sludge (AS) system, located near above mentioned agricultural zones. The other sludge was from a treatment plant operating with facultative stabilization ponds (FSP), located in the north of Chile; about 700 km from the soils under study (Table 1).

The soils were taken from the surface horizon (0–20 cm deep) and they were air-dried and passed through a 2 mm sieve. The sludges were dehydrated in drying beds at their respective treatment plants and sieved in the laboratory (2 mm). Chemical analysis of sludges and soils were performed by standardized methods for soils as described by Page et al. (1982). The analyses included were: pH, organic matter, conductivity, texture, cation-exchange capacity, and contents of N, P, and heavy metals.

2.2. Plant growth

The assay with sorghum plants considered unamended (control) soil and soil amended with 100 t ha^{-1} AS or FSP sludge on a dry basis. The plants were grown in pots containing 1.5 kg, either soil or soil–sludge mixture on a

Table 1
Physical–chemical characteristics and content of heavy metals in sludges and soils

	AS sludge	FSP sludge	Metal limit in sludge	Clay loam soil	Sandy loam soil	Loam soil	Metal limit in soil
Origin	–	–	–	Pichilemu	Rapel	Rancagua	–
Coordinates	34°38′15″ S 71°21′50″ W	28°34′15″ S 70°45′29″ W	–	34°11′52″ S 71°57′52″ W	34°08′32″ S 71°22′27″ W	34°11′59″ S 70°48′19″ W	–
UTM	283314 E 6164608 N	328051 E 6838313 N	–	227903 E 6174947 N	281098 E 6219450 N	333670 E 6214211 N	–
Altitude (m)	172	424		108	138	475	
pH (water)	6.3	6.3	–	5.0	6.2	7.3	–
Conductivity (dS m^{-1})	31.7	31.3	–	4.6	4.1	3.5	–
Organic matter (%)	nd[a]	nd	–	2.8	2.0	3.2	–
Total carbon (%)	30.7	23.5	–	nd	nd	nd	–
CEC (cmol kg^{-1})	72.5	47.9	–	8.8	11.2	16.1	–
N (mg kg^{-1})$_{\text{extractable}}$	560	715	–	5	40	157	–
P (mg kg^{-1})$_{\text{extractable}}$	236	173	–	16	27	40	–
Sand (%)	nd	nd	–	45.0	64.0	38.0	–
Silt (%)	nd	nd	–	22.7	19.7	39.5	–
Clay (%)	nd	nd	–	32.3	16.3	22.5	–
Cu (mg kg^{-1})	672	831	1000	34.2	82.1	582	150
Fe (%)	2.4	2.7	nr[b]	4.0	3.5	4.6	nr
Mn (mg kg^{-1})	442	363	nr	899	677	693	nr
Ni (mg kg^{-1})	29.6	35.6	80	21	15.1	30.1	112
Pb (mg kg^{-1})	80.6	137	300	18.1	22.6	30.9	75
Zn (mg kg^{-1})	757	1444	2000	60.8	103	109	175

[a] Not determined.
[b] Not regulated.

dry basis. The soil in the pots was moistened to field capacity and incubated for 30 days at room temperature (20 ± 4 °C) in order to allow the processes of chemical degradation, biodegradation and volatilization of toxic compound in sludge to reach equilibrium in soil and thus reduce the risk of phytotoxicity by the high rate of sludge applied (Epstein, 2003). *Sorghum bicolor* L. Moench (cv. sucrosorgo) seeds were germinated in sand at room temperature for 10 days. Three plants of similar size and appearance were chosen to be transplanted on the previously incubated moist soil in each pot. One week later two plantules were discarded from each pot. The plants were grown for 60 days in a plant-growth chamber. They were irradiated with artificial light at 400 μEinstein m^2 s, with a 14-h photoperiod, at day/night temperature of 24/18 °C and 50% relative humidity. They were periodically watered with distilled water to keep the initial soil moisture. The pots were distributed in the chamber following a completely random design with five repetitions per treatment. At the end of the growth period, the plants were carefully removed from the soil, the shoot was separated from the root and both parts were thoroughly washed with distilled water and oven-dried at 60 °C for 48 h. After determining the dry-matter (DM) yield of shoot and root for each repetition, the dry plant material was ground in a mill.

2.3. Sequential extraction

For sequential extraction of Cu and Zn in four different fractions, the BCR procedure was applied as described by Ure et al. (1993), just as used by Luo and Christie (1998). This procedure was applied to soils before plant growth, after plant growth, and to sludges. In every case sequential extraction was applied to 1 g of material on a dry basis. In the first step, the water-soluble and exchangeable fraction (HOAc-soluble fraction) was obtained. In the second step, the forms bound to manganese and iron oxides (reducible fraction) were obtained. In the third step, organically bound metals and sulfides were extracted (oxidizable fraction). Finally, the fraction obtained in the fourth step contained metals occluded in the crystalline structures of primary and secondary solids (residual fraction). In order to ensure the analytical quality of the results obtained in the sequential extraction of sludges, a certified reference material was used, which was SRM-2781 household sludge from NIST.

2.4. Determination of total and DTPA-extractable elements

In order to determine the elements uptaken by plants, plant material was digested using a 2:1 NHO_3:H_2O_2 mixture, following the same procedure as the fourth step of sequential extraction. Metal extraction with diethylenetriaminepentaacetic acid (DTPA) was carried out as described by Lindsay and Norvell (1978). 2.5 g of soil was shaken for 2 h with 25 ml of a solution containing 0.05 M DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine at pH 7.3. The elements present in all the solutions obtained by digestion or DTPA extraction were determined by AAS, a standard method to determine the levels at which elements in soils and sludges are found (Page et al., 1982).

2.5. Statistical analysis

One-way analysis of variance was carried out to compare the means of different treatments. Where significant

F-values were obtained, the individual means were compared by Duncan's test to a level of 5%.

3. Results and discussion

3.1. Chemical characterization of soils and sludges

The contents of heavy metals present in the sludges were below the limits set up by the Chilean standard and most of the existing international standards in this respect. Of the elements analyzed in these substrates, most of them were found in greater concentration in FSP sludge (Table 1), probably because this sludge was produced in a city located in a semi-arid zone in northern Chile, surrounded by small mining centers, where soils and water exhibit a higher content of salts. On the contrary, AS sludge was obtained from a city in the central zone, where agriculture predominates. The lower content of total carbon obtained in FSP sludge compared with AS sludge may be accounted for by the fact that this sludge stayed for over a year in the bottom of the stabilization pond and thus its organic matter is more mineralized than that of AS sludge, the processing of which lasted only a few days.

The increase in organic matter of soils after sludge application is a thoroughly established effect (Epstein, 2003). In this study, sludge application at a rate of 100 t ha^{-1} before plant growth increased the content of organic matter in about 1–2% points (Table 2). However, no significant changes were observed in this parameter after plant growth, which would imply that possible transformations affecting organic matter such as has been elsewhere described (Merrington et al., 2003) would be of a rather qualitative nature.

After sludge application, an increase of about 0.8 pH unit was recorded for Clay loam soil. Sandy loam and Loam soils, instead, recorded only slight changes, with +0.3 and −0.3 units, respectively. After plant growth, the soils treated with AS sludge showed a decrease of 0.8–1.3 pH units. No relevant changes were recorded in FSP-amended soils. These results would indicate on the one hand the presence of a buffering effect of the pH produced by the FSP sludge, which was more evident in the soil with a lower pH (Clay loam). According to Richards et al. (1997) and Chefetz et al. (1998), such effect would be controlled by the inorganic sludge components. On the other hand, the decrease in the pH values observed in this experiment agrees with the findings of Hooda and Alloway (1993), who describe an initial increase of 1.5 pH units after amending soil with 50 t ha^{-1} sludge and a further pH decrease to lower values than the control soil. This effect would be attributed to the presence of significant amounts of mineralizable N and degradable organic matter in sludges. In this study, the effect observed with both sludges is consistent with the previously mentioned finding and is closely related to the content of nonmineralized organic matter present in both substrates.

3.2. Sequential extraction

Heavy metals in sludges show variable concentrations, but copper and zinc are usually found at a higher concentration than other elements (Epstein, 2003). A similar finding occurred in the sludges under study, thus the sequential

Table 2
pH, organic matter (%), and DTPA-extractable heavy metals (mg kg^{-1}) in soils amended with sludge, before and after cultivation

	Clay loam soil			Sandy loam soil			Loam soil		
	Control	+AS sludge	+FSP sludge	Control	+AS sludge	+FSP sludge	Control	+AS sludge	+FSP sludge
Before cultivation									
OM	2.8[a]bA[b]	4.1aA	4.7aA	2.0cA	2.7bA	3.1bA	3.2bA	4.3aA	4.5aA
pH	5.3fA	6.0eA	6.1dA	6.3dA	6.6cA	6.6cA	7.3aA	7.0bA	7.0bA
Cu	2.8dA	9.0cA	17bA	12cA	17bA	20bA	186aA	185aA	190aA
Zn	1.1eA	11cA	39aA	2.2eA	7.4dB	25bA	6.9dA	11cA	27bA
Ni	0.21bB	0.43aA	0.50aA	0.20bA	0.31abA	0.30abA	0.28abA	0.33abA	0.32abA
Pb	0.41fB	1.2dA	2.7bA	0.76eA	0.92deA	2.3bcA	2.1cA	2.4bcA	3.3aA
Mn	191bA	256aA	254aA	22dA	35cB	34cA	27dA	34cA	39cA
Fe	45eB	58dB	94aA	48eB	58dB	89bA	40fA	42fB	66cA
After cultivation									
OM	2.9bA	3.9aA	4.5aA	2.1cA	2.7bA	3.2bA	3.0bA	4.1aA	4.2aA
pH	5.2eA	4.7fB	5.8dA	6.4bcA	5.8dB	6.8bA	7.4aA	6.1cB	7.1aA
Cu	3.0fA	10eA	9.9eB	14dA	17dA	17dA	144cB	187aA	162bB
Zn	1.3eA	12bA	20aB	2.4dA	11bA	18aB	2.1dB	9.5cA	17aB
Ni	0.53aA	0.60aA	0.42abA	0.23bA	0.30bA	0.31bA	0.25bA	0.55aA	0.32bA
Pb	1.2bA	0.85bcB	0.9bcB	0.64cA	0.76cA	1.2bB	1.2bB	1.2bB	2.0aB
Mn	155bB	227aB	73dB	14eB	103cA	13eB	9.1eB	25dB	12eB
Fe	121aA	92cA	61dB	80cA	102bA	62dB	30fB	68dA	48eB

[a] Mean ± s ($n = 3$).

[b] Different small letters in each line represent significant differences ($P < 0.05$). Different capital letters in each column, for each parameter, represent significant differences ($P < 0.05$) comparing before and after cultivation.

Table 3
Sequential extraction of Cu and Zn (mg kg^{-1}) from AS, FSP, and SRM-2781 sludge

Fraction	AS	FSP	SRM-2781
Copper			
Soluble	20.8 ± 1.4[a]	11.4 ± 0.59	30.1 ± 1.3
Reducible	3.56 ± 0.11	1.90 ± 0.45	1.92 ± 0.17
Oxidizable	444 ± 9.4	303 ± 24	410 ± 3.9
Residual	163 ± 8.6	490 ± 24	167 ± 3.3
Sum	631.4	806.3	609.0
Certified value	–	–	627.4
Recovery (%)	94	97	97
Zinc			
Soluble	128 ± 1.8	47.9 ± 1.6	117 ± 1.1
Reducible	41.4 ± 1.3	77.1 ± 2.7	238 ± 4.9
Oxidizable	5.31 ± 0.53	362 ± 32	292 ± 19
Residual	552 ± 3.4	885 ± 35	601 ± 15
Sum	726.7	1372	1248
Certified value	–	–	1273
Recovery (%)	96	95	98

[a] Mean ± s ($n = 3$).

extraction procedure was performed on the two sludges and on the certified sludge. Mass balance at the end of the extracting procedure indicated good recovery, in all cases being over 94%. Finding both metals in all of the fractions obtained with the certified material assured the analytical quality of the results. In AS and SRM-2781 sludges copper was mainly found in the oxidizable fraction with about 65–70% (Table 3 and Fig. 1). For these sludges, the metal bound to the residual fraction was also important, with about 25%. In FSP sludge, copper was mainly found in the residual fraction, followed by the oxidizable fraction. In the three sludges, the metal in the HOAc-soluble and reducible fractions was <5%. As regards Zn, the metal bound to the residual fraction predominates in the three sludges, ranging from 48% to 76%. Zn bound to the oxidizable fraction was important for FSP and SRM-2781 sludges while the available fraction was important for AS sludge.

Sequential extraction of Cu and Zn in sludge-amended and unamended soils showed good percent of recovery, with values ranging from 89% to 102% (Table 4 and Fig. 1). The distribution of both metals in the different forms before plant growth was similar to that obtained after plant growth. In general, the order of importance of Cu forms was: $\text{Cu}_{\text{Residual}} > \text{Cu}_{\text{Oxidizable}} > \text{Cu}_{\text{Reducible}} > \text{Cu}_{\text{HOAc-soluble}}$. In several cases, over 50% of Cu was associated to the residual fraction, whereas the HOAc-soluble fraction was in all cases lower than 15%. Instead, in Loam soil the fraction of available Cu ranged from 69.9 to 79 mg kg^{-1}, an amount far higher than found in Clay loam and Sandy loam soils, where this fraction was never over 4 mg kg^{-1}.

The distribution of the metal in sludges was fairly different from the distribution obtained in the sludge-amended soils. Considering that copper in sludges was mainly found in the residual and oxidizable fraction, these fractions would be expected to increase more in sludge-amended soils. This was true in Clay loam soil and in Sandy loam soil, but not in Loam soils. It has been postulated that once the sludge is applied to the soil, the sludge properties would predominate in the metal chemistry in the short range and that these properties would have a smaller influence along time, the control of the soil characteristics becoming stronger (Smith, 1996). This hypothesis is supported by the results herein obtained, since on the one hand the sludges in Loam soil produce a discrete decrease of the metal in the available fraction, which agrees with the fact that sludge increases the sorption capacity of soil, on the other hand in sludge-amended Clay loam and Sandy loam soils there was a slight increase in the available fraction, which could be attributed to association of the metal with soluble organic matter supplied by sludges.

In unamended soils, Zn fractionation showed the following order: $\text{Zn}_{\text{Residual}} \gg \text{Zn}_{\text{Oxidizable}} > \text{Zn}_{\text{Reducible}} > \text{Zn}_{\text{HOAc-soluble}}$, ranging from 69% to 83% in the residual fraction (Table 4 and Fig. 1). In amended soils, the residual fraction is still the most important, in most of the cases being above 60%. However, the available fraction became second in importance, with increases of 2–11 times in relation to the control, this effect being more evident with FSP sludge. The reducible and oxidizable fractions came last.

Even though a large amount of Zn in sludges was found in the residual form, soil amendment with these sludges caused an increase in Zn concentration in most of the fractions, which points to a redistribution of the metal. In Clay loam soil, however, sludge did not increase Zn in the residual fraction, and the metal was redistributed in the other fractions, an effect that could be related to the lower pH exhibited by this soil. In this sense, Qiao et al. (2003), having observed similar changes, account for this effect by the greater sensitivity of Zn to the soil pH.

3.3. Plant biomass

DM yield in the aerial part of unamended soils was in the order Sandy loam > Loam > Clay loam (Table 5). The better yields observing in Sandy loam and Loam soils would be accounted for the conditions of pH and contents of available macronutrients favoring for sorghum growth (Table 1), the same as reported by Bennett et al. (1990). This reason would also account for the low yield of plants grown in Clay loam soil. The conditions shown by this soil were markedly improved by sludge addition, a fact that caused a DM increase of 5 and 9 times with AS and FSP sludges, respectively. However, it was not possible to separate the effect of sludge on pH and on the level of macronutrients. Considering that Clay loam soil has a historical record of low productivity on account of its acidity, which is annually improved by addition of liming materials and that it has been fully demonstrated that sorghum yield decreases in soil with a pH below 6 (Bennett et al. (1990)), pH seems to be the most probable limiting factor in this case. Sandy loam soil exhibited good conditions of pH and macronutrients, which would explain the lack of effect on DM by addition of FSP sludge. However, AS sludge application caused a decrease probably due to a

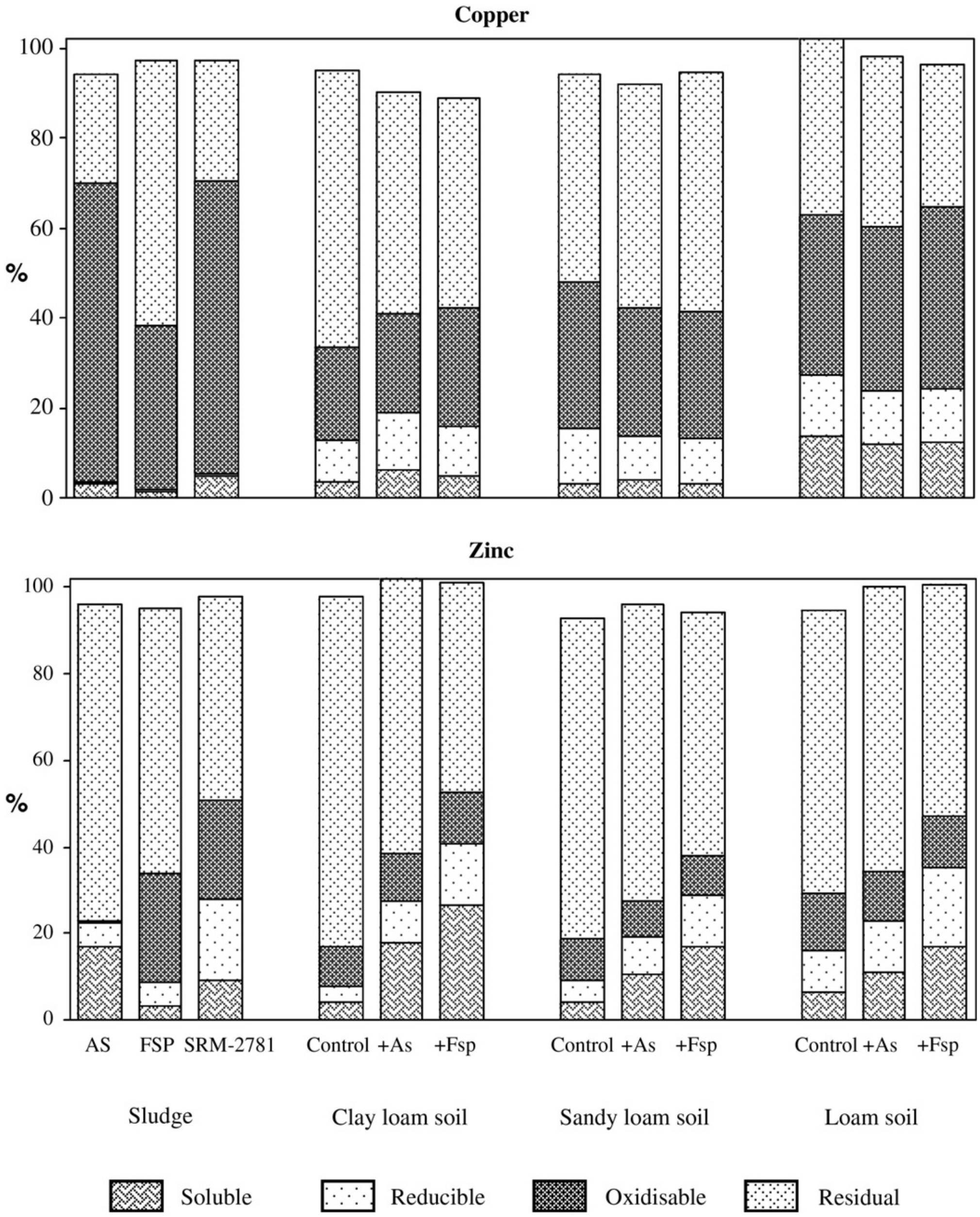

Fig. 1. Percentage of extracted metal from sludges and control soils and soils amended with sludge.

release of phytotoxic ammonium-N from the mineralization of organic N present in larger amounts in the sludge obtained by a process of activated sludge (Epstein, 2003). Sludge application to Loam soil caused slight changes in pH, staying near neutral (Table 2); nevertheless, in spite of the nutrient supply by the sludge, no increase was observed in the leaf mass (Table 5), indicating the possible existence of a physical factor that might have an effect on the moderate productivity obtained in this soil.

3.4. Extractability with DTPA and element bioavailability

Before plant growth, sludge application increased the amount of DTPA-extractable metal (Table 2). This effect is greater in Clay loam soil amended with FSP sludge. Zinc showed the highest increase, from 1 to 25 times the control concentration. Before and after plant growth, most of the elements showed higher concentration in soils with sludge. However, extractability of some elements decreased, especially in soils amended with FSP sludge.

In relation to element uptake by sorghum plants, the concentration of Cu in the leaves of control plants was similar in the three soils, with values ranging from 14 to 19 mg kg^{-1} (Table 5), which did not correlate with DTPA-extractable Cu or with total Cu found in the soils (Table 2). This low leaf concentration would be accounted for by copper homeostasis at a plant cell level, an effect that has been widely demonstrated for copper in several plant

Table 4
Sequential extraction of copper and zinc (mg kg^{-1}) from soils amended with AS or FSP (100 t ha^{-1}), after cultivation

Fraction	Clay loam soil			Sandy loam soil			Loam soil		
	Control	+AS sludge	+FSP sludge	Control	+AS sludge	+FSP sludge	Control	+AS sludge	+FSP sludge
Copper									
Soluble	1.2 ± 0.1[a]	3.3 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	4.0 ± 0.1	3.5 ± 0.1	79.5 ± 0.4	69.9 ± 0.7	72.0 ± 1.1
Reducible	3.2 ± 0.1	7.0 ± 0.4	6.5 ± 0.4	9.8 ± 0.1	9.7 ± 0.1	10.6 ± 0.3	79.4 ± 0.2	68.8 ± 0.9	70.8 ± 1.1
Oxidizable	7.0 ± 0.6	12.1 ± 0.8	15.8 ± 0.6	26.7 ± 0.2	28.8 ± 0.1	29.7 ± 0.2	206 ± 0.5	214 ± 3.1	237 ± 1.1
Residual	21.1 ± 0.8	26.7 ± 0.4	27.9 ± 0.9	38.0 ± 0.2	50.4 ± 0.2	56.9 ± 0.1	229 ± 0.7	220 ± 2.1	186 ± 3.3
Sum	32.5	49.1	53.1	77.2	92.9	101	593	572	565
Recovery (%)	95	90	89	94	92	95	102	98	96
Zinc									
Soluble	2.5 ± 0.1	15.0 ± 0.1	27.9 ± 0.4	4.2 ± 0.1	13.2 ± 0.1	24.7 ± 0.1	6.8 ± 0.2	14.5 ± 0.1	25.7 ± 0.4
Reducible	2.3 ± 0.4	7.9 ± 1.1	14.7 ± 0.3	5.4 ± 0.1	10.8 ± 0.2	17.0 ± 0.5	10.7 ± 0.4	15.4 ± 0.6	27.8 ± 3.2
Oxidizable	5.4 ± 0.2	8.9 ± 2	12.4 ± 1.7	9.7 ± 0.2	10.2 ± 0.1	13.7 ± 2.2	14.5 ± 2.0	14.6 ± 1.2	18.1 ± 2.1
Residual	49.4 ± 0.4	52.9 ± 3.4	51.1 ± 2.4	76.7 ± 0.2	84.8 ± 0.3	81.8 ± 2.6	71.6 ± 1.6	85.5 ± 1.7	81.9 ± 5.0
Sum	59.6	84.7	106	96	119	137	104	130	154
Recovery (%)	98	102	101	93	96	94	95	100	101

[a] Mean ± s ($n = 3$).

Table 5
Dry matter yield (g) and concentration (mg kg^{-1}) of heavy metals in shoot and root of sorghum grown on soils amended with sludge (100 t ha^{-1})

	Clay loam soil			Sandy loam soil			Loam soil		
	Control	+AS sludge	+FSP sludge	Control	+AS sludge	+FSP sludge	Control	+AS sludge	+FSP sludge
Shoot									
DMY	1.2[a]f[b]	6.4b	10.8a	10.5a	2.8e	10.2a	4.5cd	4.1d	4.9c
Cu	19.3c	21.4bc	20.7bc	14.4d	26.9a	22.6bc	16.0c	23.3b	27.9a
Ni	11.8a	7.1b	7.0b	8.3b	BDL	BDL	BDL	BDL	BDL
Zn	119a	19.7d	27.0c	77.9b	21.2d	19.5d	63.2b	14.9e	16.2de
Mn	427c	1115a	96.0d	61.3E	624b	30.2f	63.6e	84.4d	65.5e
Pb	BDL	BDL	10.1a	BDL	9.9a	9.6a	BDL	10.4a	BDL
Fe	38.6e	151b	140b	99.6c	182a	138bc	37.7e	71.5d	105c
Root									
DMY	0.27h	0.90de	1.7c	3.1a	0.47g	2.3b	1.2d	0.50g	0.7e
Cu	34.4f	65.5d	25.1g	41.8e	236c	50.3d	277b	980a	294b
Ni	BDL	18.1ab	15.2b	11.5c	23.4a	11.5c	12.1bc	22.2a	13.1bc
Zn	115b	248a	113b	36.1e	88.7c	70.1d	46.7e	109b	123b
Mn	317c	1291a	305c	99.2e	970b	95.9e	159d	155d	88.6e
Pb	BDL	20.3bc	15.9c	15.3c	BDL	18.0c	15.6c	31.1a	22.0b
Fe	6615a	4340bc	5305b	3391d	2506e	3884cd	5446ab	3850cd	3065de

BDL: below detection limit.

[a] Mean ± s ($n = 5$).

[b] Different letters in each line represent significant differences ($P < 0.05$).

species (Cobbett and Goldsbrough, 2002). In the roots of control plants, copper concentration followed the order Clay loam < Sandy loam < Loam, which agrees with DTPA-extractable Cu ($r = 0.9997$). In Sandy loam and Loam soils, AS or FSP sludge application increased the concentration of foliar copper by 46–87%. On the other hand, the root showed an increase of 42–564% in copper concentration in the three soils treated with AS. On the contrary, for this same parameter, FSP sludge showed a contradictory behavior with a decrease of 27%, an increase of 20%, and a lack of an effect in Clay loam, Sandy loam, and Loam soils, respectively.

Zn concentration reached in the leaves of control plants had an inverse correlation with soil pH ($r = -0.97$), exhibiting the order Clay loam > Sandy loam > Loam. No correlation was observed for this parameter with DTPA-extractable Zn or with total Zn (Table 1). Although Clay loam soil has a lower Zn content, the concentration in the root was 2–3 times higher than those obtained in plants grown in Sandy loam and Loam soils.

Zn foliar concentration after sludge addition was in all the cases lower than the control, with a decrease of 73–83%. On the one hand, these results confirm Basta's report (2005) on several cases where less solubility and phytoavailability of trace elements have been observed as a result of a significant content of sorbents in the biosolids applied to the soil. On the other hand, the observed decrease agrees with the higher pH values found in amended soils, just as stated by Qiao et al. (2003). These results, however, are in opposition with the higher availability found with DTPA-extractable metal and with sequential extraction in sludge-amended soils. In this respect, Zn concentration in the root was in most of the cases higher than in the control, the increase ranging from 94% to 164%. This fact could imply the interaction of some sludge component with Zn in the root, producing a higher retention of this element in plants grown in sludge-amended soils.

Sludge application to the soil had a different effect on foliar concentration of Mn, Ni, Fe, and Pb. Thus, Fe and Pb increased their concentration compared with control plants in all of the soils with both sludges. Ni decreased its concentration in Clay loam and Sandy loam soils, but it was not detected in Loam soil. Finally, Mn decreased or kept its concentration with FSP sludge, increasing it instead in all of the soils with AS sludge. An increase in Ni, Mn, and Pb concentration was observed in the roots of plants grown in AS-amended soils, while no conclusive results were obtained with FSP sludge.

Considering that the cost of applying DTPA extraction is lower than that of applying sequential extraction, it would be convenient to know whether simple extraction would be enough to decide on the application of sludge as amendment. However the great differences in the results obtained between plant metal concentration, available metal in DTPA-treated soil and available metal as determined by sequential extraction in this study prevent us from recommending one method or another.

4. Conclusions

The results obtained for DTPA-extractable metal indicated a higher availability of elements in sludge-amended soils. However, the concentrations observed in the plant shoot permitted to conclude that this type of simple extraction was not a good predictor of element bioavailability. Similarly, the changes produced by sludge in copper and Zinc distribution in soil, which in most cases involved an increase in the more labile metal forms, did not agree with the concentration observed in the shoot. On the other hand, sludge application to agricultural soils with high copper levels would not imply a greater mobility of this metal, probably because of a greater sorbing capacity provided by the sludges, In this manner, sludges from wastewater treatment plants obtained both from stabilization ponds and from activated-sludge processing may be applied to different kinds of soils, even to high-copper soils, with no risk of increasing heavy metal bioavailability to phytotoxic levels, with further benefits to crops, whether by improving plant nutrition or by providing favorable pH conditions for plant growth in nutrient-poor or low-pH soils. In addition, the procedure can be an effective way of disposal of these residues.

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