

CHELATING AGENTS TO SOLUBILIZE HEAVY METALS FROM OXISOLS CONTAMINATED BY THE ADDITION OF ORGANIC AND INORGANIC RESIDUES

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ABSTRACT: Phytoremediation is an attractive technique for soils contaminated with heavy metals, especially in conjunction with chelating agents to assist metal phytoextraction. Nevertheless, their studies in Brazil are rare. Thus, the objective of the present work was to evaluate the efficiency of the chelating agents EDDS and EDTA for the solubilization of heavy metals from two Oxisols contaminated by organic sources in Jaguariúna (LVJ) and inorganic sources in Paulínia (LVP), São Paulo State, Southeastern Brazil. First, the soil samples were fractionated and the DTPA method was used to quantify heavy metals available forms. The results indicated that the metals were highly available in the soil fractions and could be solubilized by the chelating agents. The soil was suspended for 24 h in a chelating agent solution (EDTA or EDDS) at rates of 0, 250, 500 and 750 mg kg⁻¹ of soil. The concentration of solubilized heavy metals was determined in the resulting solution. The extent of metal solubilization varied according to soil type, the chelating agent added and the specific metal. The amount of iron solubilized, as compared to the total iron (LVJ) was 11% (EDTA) and 19% (EDDS). EDDS solubilized more Cu than EDTA in both soils but more Ni in LVJ, while EDTA solubilized more Zn in both soils but more Cd in LVP. Both EDTA and EDDS may be useful for phytoextraction from soils, although the iron content is an important factor regarding the phytoextraction of heavy metals with chelating agents in Oxisols.

Key words: soil pollution, chelating agents, tropical soils, fractionation

USO DE QUELANTES NA SOLUBILIZAÇÃO DE METAIS PESADOS DE LATOSSOLOS CONTAMINADOS PELA ADIÇÃO DE RESÍDUOS ORGÂNICO E INORGÂNICO

RESUMO: A fitoextração tem sido uma opção atrativa para remediar solos contaminados com metais pesados, principalmente quando associada à aplicação de quelantes ao solo, embora no Brasil seus estudos sejam muito incipientes. Portanto, o objetivo deste trabalho foi avaliar a eficiência dos quelantes EDTA e EDDS na solubilização de metais pesados em Latossolos contaminados por Cu, Zn, Cd e Ni, cujas fontes de contaminação foram: orgânica em Jaguariúna (LVJ) e inorgânica em Paulínia (LVP). Antes da solubilização dos metais com os quelantes as amostras de solo foram submetidas ao fracionamento seqüencial e à extração dos metais pesados pelo método do DTPA. Ainda, as amostras dos dois solos foram agitadas com as soluções dos quelantes, por 24 h, usando as seguintes doses: 0, 250, 500 e 750 mg kg⁻¹ de solo, realizando-se a determinação dos metais pesados no sobrenadante. Tanto a extração com DTPA como o fracionamento seqüencial indicaram que os metais estavam nas frações disponíveis que poderiam ser solubilizados pelos quelantes. As quantidades solubilizadas de Cu, Fe, Zn e Ni variaram em função do tipo de solo, dos quelantes e das doses aplicadas. A solubilização do Fe em relação ao seu teor total no LVJ foi de 11% (EDTA) e 19% (EDDS). O EDDS solubilizou mais Cu em ambos os solos e Ni no LVJ, enquanto o EDTA apresentou maior solubilização do Zn também em ambos os solos e do Cd no LVP. Portanto, a utilização de tais complexantes pode ser promissora na fitoextração de metais em solos, mas a avaliação da concentração de Fe no solo torna-se importante quando se utiliza a técnica de fitoextração assistida por quelantes para remediar Latossolos contaminados com metais.

Palavras-chave: poluição do solo, quelantes, solos tropicais, fracionamento

INTRODUCTION

In the state of São Paulo, Brazil, at least 15% of the land-contaminated sites are due exclusively to heavy metal addition to the soils (Cetesb, 2007). Metals such as Pb, Cd, Ni, Zn and Cu are important since high quantities of them can decrease crop production due to the risk of biomagnification and bioaccumulation in the food chain. There is also the risk of underground and superficial water contamination (Schmidt, 2003; Nowack et al., 2006).

Phytoremediation has become an attractive soil remediation solution due to advantages including the conservation of the soil structure and lesser impact than the usual engineering alternatives as well as favorable economic aspects, among others (Raskin & Ensley, 2000). However, metal absorption by plants is limited by low metal solubility and transportation in to the root system. In such case, the use of complexing agents to assist phytoextraction of metals has been proposed to enhance metal solubility. In spite of that, some major drawbacks exists such as the toxicity to the soil biology, sometimes even for plant species, and the slow rate of degradation, (Egli, 2001).

In the past few years EDDS ([S,S] ethylene-diamine disuccinic acid) has received some attention due to its greater rate of degradation and its strong chelating characteristics comparable to EDTA, although its biodegradation rate varies largely depending upon the examined conditions (Evangelou et al., 2007; Schowanek et al., 1997). Several EDDS phytoremediation studies have been conducted mainly with a soil pH close to neutral (Hauser et al., 2005; Meers et al., 2005; Luo et al., 2005). Under those conditions, EDDS has been more effective than EDTA since the Ca competition for the EDDS is lower. On the other hand, there are no studies addressing this issue in oxidic soils, where Fe should be the major com-

petitor for the chelating agent (Tandy et al., 2004; Hauser et al., 2005). Thus, the objective of the present work was to evaluate the efficiency of the chelating agents EDDS and EDTA for the solubilization of heavy metals from Oxisols contaminated by organic sources in Jaguariúna (LVJ) and inorganic sources in Paulínia (LVP) located in São Paulo State, Southeast Brazil.

MATERIAL AND METHODS

Samples of Rhodic Hapludox soils were collected from the 0 – 0.20 m depth in two locations: Paulínia (22°54'20'' S; 47°3'39'' W) and Jaguariúna (22°46'25'' S; 47°06'99'' W), SP. The samples were air-dried and sieved to 2 mm. The soil from Paulínia (LVP) was sampled in an area where an accidental leaching of micronutrient raw fertilizer's material occurred, approximately 20 years ago. The area is being monitored by the São Paulo State Environmental Agency (Cetesb, 2007). This soil has a loam texture and high concentrations of available Cu, Mn and Zn (Table 1). The soil from Jaguariúna (LVJ) has a clayey texture and high concentrations of Cu and Zn (Table 1). It was sampled in an experimental agricultural corn plot that had previously received sewage sludge yearly (from 1999 to 2005). This site received sewage sludge from the Barueri Wastewater Treatment Facility which has a high metal content. The application rate for the sewage sludge was based on the amount of nitrogen being eight times higher than the nitrogen needed for the corn crop, assuming a 30% nitrogen mineralization rate in the added sewage sludge (Bettiol & Camargo, 2006).

The total metal content was evaluated using a microwave nitric acid digestion procedure (Table 2) (US-EPA, 2008). In order to access the "pool" of metals, the soil samples were fractionated in four fractions, namely soluble/exchangeable (S+E), organic

Table 1 - Chemical and physical characterization of the soils tested.

Soil	OM g dm ⁻³	pH CaCl ₂	DTPA							Granulometric analysis		
			Cu	Fe	Mn	Zn	Cd	Ni	Pb	Silt	Clay	Sand
			----- mg dm ⁻³ -----							----- g dm ⁻³ -----		
LVP	37	5.7	62	14	60	287	0.7	0.3	9.7	320	250	430
LVJ	47	5.6	20	159	6.0	70	0.3	6.0	2.0	470	89	441

*Chemical analysis according to Raij et al. (2001); Physical analysis according to Camargo et al. (1989).

Table 2 - Total metal content (SW 846- 3051) in the soils tested.

Soil	Ca	Cu	Mn	Zn	Cd	Ni	Pb	Fe
								mg kg ⁻¹
----- mg kg ⁻¹ -----								
LVP	1800	225	481	646	8	8	50	468.4
LVJ	2633	86	110	210	3	30	23	169.7

matter (OM), oxidable/reducible (OX) and residual (RES) (Anhstrom & Parker, 1999). The soluble/exchangeable fraction was obtained by addition of 0.1 mol L⁻¹ Sr(NO₃)₂ solution followed by a 2 h agitation and 10 min centrifugation at 6,500 g; the organic fraction was extracted with a 5% NaOCl solution at pH 8.5. The tubes were kept in a 90°C water bath for 30 min prior to the centrifugation for extraction of the oxidable/reducible fraction using a solution containing ammonium oxalate 0.2 mol L⁻¹, ascorbic acid 0.1 mol L⁻¹ and oxalic acid 0.2 mol L⁻¹ with the pH adjusted to 3.0. For the residual fraction the samples were oven dried and digested with nitric acid in a microwave oven. The fractionation (in duplicate) was repeated for each extraction step, followed by washing with a NaCl solution and corrected for soil losses.

The laboratory experimental design was completely randomized, in a full factorial scheme of two soils × two chelating agents × four rates (including zero), in triplicate. The chelating agents were EDTA (ethylenediamine tetracetic disodium salt) and EDDS ([S, S] - ethylenediaminedisuccinic trissodium salt), at rates of 0, 250, 500 and 750 mg kg⁻¹ of soil. The chelating agent addition rate were (in mmol kg⁻¹ of soil): 0, 0.7, 1.3, 2.0 of EDTA and 0, 0.7; 1.4 and 2.1 of EDDS. The pH of the chelating agents was measured before their addition. For the EDTA solutions the pH was of 4.0 ± 0.2 and for EDDS the pH was of 7.0 ± 0.2 for all addition rates.

For each experimental parcel (centrifuge tubes) 3.0 g of soil and 30.0 mL of a CaCl₂ 10 mmol L⁻¹ solution containing the chelating agent was added. For the zero addition rates no soil was added before the addition of the CaCl₂ solution. Following that sequence, the soil was suspended for 24 h at 300 g and centrifuged at 3,000 g for 15 min (Nascimento, 2006). The upper layer was separated and Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb were determined using an axial ICP-AES, Varian Vista MPX.

The data obtained was submitted to the analysis of variance, considering soil, chelating agent, and addition rates as the source of variance.

RESULTS AND DISCUSSION

A comparison of the metal content of the studied soils with others reported in the literature confirm that they should be considered as contaminated ones (Table 2). Amounts of Cd, Cu and Zn in the LVP and of Cd and Zn in LVJ are close to or above of the maximum content commonly found in most soils (Kabata-Pendias & Pendias, 1992). Also, when one considers the local environmental agency recommended limit (Cetesb, 2001), the LVJ sample should also be con-

sidered as a Ni contaminated one. The reference limit for metal concentrations in São Paulo State soils are (in mg kg⁻¹) Cu - 35 and 60, Zn - 60 and 300, Cd - <0.5 and 3, Ni - 13 and 30.

The DTPA method for quantifying heavy metals in soil samples (Lindsay & Norvell, 1978) can be helpful in monitoring soil contamination with Cd, Cr, Pb and Ni (Vidal-Vasquez et al., 2005) in addition to the fact that this method is also useful to evaluate micronutrient availability for agricultural purposes (Abreu et al., 2005). Thus, the levels of Zn, Cu and Cd, especially those found in LVP (Table 1), should be of substantial concern due to their high availability to plants and the potential for entering the food chain.

The fractionation of heavy metals in soils is a technique extensively used to enable a better understanding of metal binding to the soil solid phase, which ultimately reflects their availability to plants. This method allows one to follow changes in metal content among different soils, according to the used management procedures (Peters, 1999). Usually, there is a general agreement that the metals found in the soluble/exchangeable fraction and in the organic matter fraction are more readily available for plant uptake, and consequently more susceptible to binding on chelating agents.

In spite of the source of contamination, inorganic (LVP) or organic (LVJ), the fractionation showed that larger amounts of Cu were found in the organic matter (OM) and reducible/oxidable (OX) fractions; larger amounts of Zn were found in the soluble/exchangeable (S+E), OM and OX fractions; and larger amounts of Cd were found in the OX fraction (Table 3).

When compared to total metal amounts found in the soil samples (Table 2) it can be noticed that results (Table 3) correspond to 33.5% of Cu, 12.5% of Ni, 60.5% of Pb and 71.4% of Zn in the more available fractions (S+E and OM) of LVP. For LVJ, in the same fractions, the results indicate 44.3% of Cu; 42.3% of Ni, 42.0% of Pb and 70.6% of Zn.

The presence of metals on highly available fractions, such as those examined in this study, should be only expected for the LVJ, the soil contaminated by the addition of sewage sludge, since in the area where the LVP was sampled no organic matter was added either from the accidental leaching or from vegetal sources (Table 1). The addition of metals to the soil due to the repetitive application of sewage sludge leads to a contamination with the metals remaining in the most labile fractions of the soil, mainly due to an organic matter introduction to the system (Bettiol & Camargo, 2006; Galdos et al., 2004).

Table 3 - Metal fractionation in the LVP and LVJ soils.

Metal	S + E	OM	OX	RES	Total calculated ²	% to total measured ³
----- mg kg ⁻¹ -----						
LVP						
Cd	<0.1	<0.1	6.0	2.5	8.4	110.8
Cu	0.23	62.55	90.0	34.2	186.9	83.2
Ni	<0.1	1.07	<0.1	7.1	8.2	101.0
Pb	<0.1	28.56	12.0	6.7	47.2	95.1
Zn	81.4	361.50	61.0	116.2	620.1	96.0
LVJ						
Cd	<0.1	<0.1	3.9	<0.1	3.9	125.8
Cu	0.2	30.2	25.3	12.9	68.6	79.6
Ni	3.2	7.2	11.5	2.8	24.6	81.1
Pb	<0.1	8.1	9.0	2.2	19.3	83.2
Zn	32.9	104.2	41.6	15.4	194.1	92.5

¹Metal found in pool samples S+E = soluble plus exchangeable, OM = organic matter; OX = oxidic, RES- residual. ²sum of fractions S+E, OM, OX and RES. ³see Table 2

Furthermore, the source of contamination does not appear to have an important role in the metal distribution in the soil's solid phase (Table 3). Among the metals analyzed, substantial Zn amount was found in the organic matter fraction of both soils. Similar results were reported by Borges & Coutinho (2004) for a soil treated with sewage sludge, although the fractionation of oxidic soils has also shown that immediately, or longer after the application of the organic matter, a large part of the zinc can be located in the oxidable and residual fractions (Hseu, 2006; Araújo & Nascimento, 2006). This would be expected by a contamination caused by the introduction of inorganic sources, which result in lower availability for biological agents.

In general, the chelating agents were efficient in the solubilization of heavy metals from the contaminated soil. The extent of metal solubilization varied according to soil characteristics, the added chelating agent and the specific metal considered (Table 4). For both studied soils, for the solubilized amounts of Cu, Fe, Zn and Ni (only for LVJ) an increase on the extracted amount could be related to the an increase in the applied rates (Figure 1). EDSS solubilized more Cu than EDTA in both soils. In spite of that, the solubilization of Fe and Ni was more affected by the kind of soil (Figure 1), with greater solubilization of Fe and Ni in LVJ by EDSS and of Zn in LVP by EDTA.

In both samples cadmium, even at low concentration, was solubilized only by EDTA. Although Cd is expected to be highly soluble, and thus should be solubilized by both chelating agents, such results can be partly explained by the constant formation of a

metal-chelating agent complex. Since the greater the log of the formation constant, the greater the rate of formation of the chelated form of the metal, Cd-EDTA (log K equals to 16.5) would be preferentially formed to Cd-EDSS (log K equals to 12.7) (Tandy et al., 2006). In addition, it should also be noted that Cd was found in the less available soil fractions (Table 3). Therefore, the formation of other complexes with higher concentrated metals, such as those of Cu and Zn, would be favored, thus reducing further the efficacy of EDSS binding to Cd. Similar results were reported before by Meers et al. (2005) and Luo et al. (2005).

None of the chelating agents tested efficiently solubilized Pb. This suggests that even when assisted with chelating agents the use of phytoextraction to the clean Pb up from soil may be limited due to its low solubilization. Pereira et al. (2007) evaluated the effect of EDTA on maize grown in a soil containing 2,400 mg kg⁻¹ of Pb and observed a transference factor from soil to shoots of 0.52 and 0.57 with and without of 0.5 mg kg⁻¹ of EDTA, respectively. In an experiment with castor bean, sunflower, pepper and tobacco Zeitouni (2003) observed that the removal of Pb in an Oxisol varied from 0.01 (sunflower) to 0.07 (tobacco) in the absence of EDTA and from 0.02 (sunflower) to 0.38 (tobacco) with the addition of 1 mmol kg⁻¹ of EDTA to the soil.

The solubilization of Fe was important for both the soils and chelating agents studied (Table 4). This can be explained by the greatest metal-chelating agent complex formation constant for EDTA and EDSS with iron (log K Fe-EDTA 25.1 and log Fe-EDSS 23.7) (Tandy et al., 2004; Tandy et al., 2006).

Table 4 - Metal solubilized by EDTA and EDDS from contaminated Oxisol, compared to the total metal content (shown in Table 2)¹.

Chelating agent	Cd	Cu	Fe	Ni	Zn
mg kg ⁻¹ of soil	-----		% (m/m)	-----	
EDTA					
LVP					
0	<0.6	<0.02	<0.01	<0.6	3.6
250	0.8	3.6	0.08	<0.6	7.85
500	1.3	6.7	0.16	<0.6	11.5
750	2.0	9.2	0.20	<0.6	15.4
EDDS					
0	<0.6	<0.02	<0.01	<0.6	3.6
250	<0.6	16.1	0.01	<0.6	2.8
500	<0.6	24.7	0.31	<0.6	6.3
750	<0.6	27.7	0.66	<0.6	11.4
EDTA					
LVJ					
0	<1.7	<0.06	0.2	1.0	1.8
250	3.0	6.9	2.1	3.1	12.8
500	6.3	13.1	5.4	7.1	22.0
750	7.7	16.8	11.0	12.1	27.3
EDDS					
0	<1.7	<0.06	0.2	1.0	1.8
250	<1.7	24.0	4.5	11.5	2.2
500	<1.7	30.5	12.4	18.1	3.7
750	<1.7	32.2	18.9	19.8	6.1

¹Solubilized lead could not be quantified since it was below the instrumental detection limit.

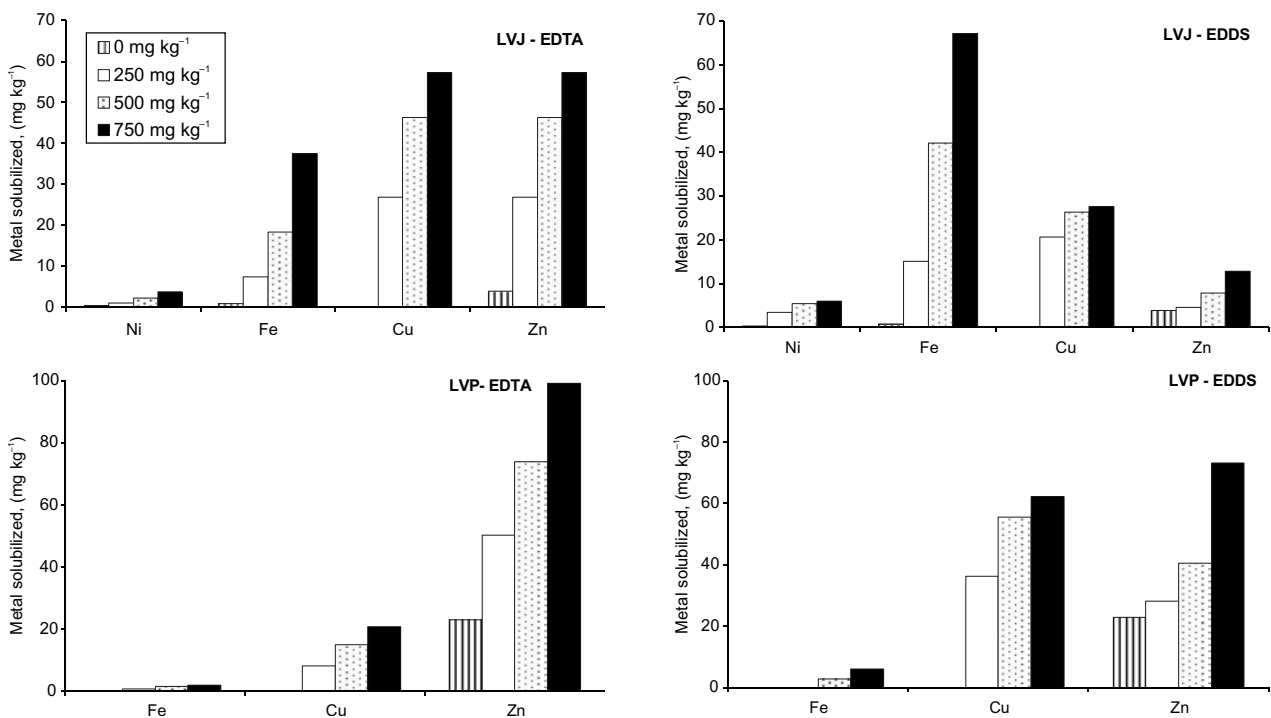


Figure 1 - Metal solubilized from contaminate Oxisols. LVP – contaminated by inorganic residue addition; LVJ contaminated by organic residue addition.

In addition, although LVP has a lower total iron content (Table 2) much more iron was solubilized from LVP. Concentrations were 37.5 and 64.2 mg kg⁻¹ for EDTA and EDDS, which correspond to 33.6 % and 54.8 % of the added chelants, respectively. For LVP, the amounts were 1.9 and 6.2 mg kg⁻¹, which correspond to 1.7 % and 5.3 % of the added chelants, respectively. This could be attributed to a large amount of soluble Fe in LVP, as determined by DTPA (Table 1). Pereira et al. (2007) also reported a high competition of Fe with Pb by EDTA in phytoextraction studies with maize, more than 90% of the Fe in the soil solution was complexed to EDTA applied from 0 to 2400 mg kg⁻¹ of Pb to the Oxisol. Working with heavy metals column extraction from soils and using EDDS, Hauser et al. (2005), reported that the longer the extraction time the greater the amount of iron leaching. According to the authors, this was due to the formation of Fe(III)EDDS through slow dissolution of iron oxides. Such studies suggest that the amount of chelating agent to add for assisted phytoextraction from Oxisols should be greater than that needed for the extraction of heavy metal contaminants due to the competition with iron.

Ni was solubilized only from LVP, probably due to its greater concentration in LVP as compared to LVP. For LVP the solubilization of Ni was quite low for both chelating agents. This may be due to its low concentration in the soil and its presence in soil fractions with low availability (Tables 2 and 3) on the examined conditions. For Ni found in LVP, EDTA solubilized 12.1% compared to 19.8% of EDDS. Thus, for Ni EDDS was a better solubilizing agent than EDTA.

The larger solubilization of Cu and Zn in the two soils, as tested with both chelating agents, can be attributed to factors such as their high concentration in the soils, their presence in more labile soil fractions, as indicated either by the DTPA method (Table 2) or the fractionation (Table 3), and the high formation constants of their metal-chelating agent complexes (log *k* Cu-EDTA 18.8 and log *k* Zn-EDTA 16.5; log *k* Cu-EDDS 20.5 and log *k* Zn-EDDS 15.3) (Tandy et al., 2004; Tandy et al., 2006).

At the greatest rates of EDTA used the Cu extracted was 9.2 % for LVP and 16.8% for LVP; while for EDDS the Cu extracted was 27.7% and 32.2% (Table 4). EDDS was more efficient than EDTA for Cu solubilization from soil for all the rates tested (Figure 1). This behavior is in agreement with the results obtained by Luo et al. (2005) for artificially multi metal contaminated soils, using an equimolar concentration of chelating agents and metals, for soils with a pH close to neutral. On the other hand, the largest

Zn solubilization was observed with EDTA, solubilizing 15.4% for LVP and 27.3% for LVP; compared to 11.4% and 6.1% with EDDS (Table 4). EDTA was superior to EDDS for both soils since for all rates tested it was different from the results reported by Luo et al. (2005) but in agreement with those found by Meers et al. (2005). They examined a dredged sediment derived from surface soil collected at a disposal site in Belgium, with a pH close to neutral and a high content of organic matter. According to Meers et al. (2005), EDDS promoted a larger mobilization of Cu and Ni than EDTA, while for Zn the effects were comparable for the two chelating agents.

The increase of metal found in the extracts was not in the same order of magnitude for LVP and LVP. For LVP the following order of increasing solubilization with EDTA and EDDS was found: Zn > Cu > Fe with either EDTA as EDDS. On the other hand, for LVP the solubilization was: Zn > Fe > Cu by EDTA and Fe > Cu > Zn by EDDS. The explanation of these observations is not as trivial as it might seem since the solubilization order suggested by the metal-chelating agent complex formation constants should be Fe > Cu > Zn. Thus, the three factors pointed above should be taken into account to explain this.

Several factors should be considered when comparing chelating agents for heavy metal decontamination of soils (Tandy et al, 2004). Among them, they recommend that the chelating agent/heavy metal ratios should always be larger than one. However, for phytoremediation procedures extensive use of such high concentrations of the chelating agents can damage the plants and produce heavy metal leaching and thus should be carefully done. The major disadvantage of using smaller quantities of chelating agents is the competition with other metals found in soil, which are sometimes in much larger concentrations than the metal contaminants addressed.

CONCLUSIONS

In general, both EDTA and EDDS were efficient for the solubilization of heavy metals (Cu, Ni, Zn and Cd) from cultivated Oxisols contaminated by the addition of organic and inorganic residues. The use of both EDTA and EDDS can be considered promising for phytoextraction procedures.

The evaluation of iron content in soil samples is an important factor to be considered on laboratory procedures for the phytoextraction of heavy metals with chelating agents in Oxisols.

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