EDTA ASSISTED PHYTORREMEDIATION OF A Pb CONTAMINATED SOIL: METAL LEACHING AND UPTAKE BY JACK BEANS

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ABSTRACT: Lead (Pb) is one of the main soil contaminants. It is also of difficult phytoremediation due to its low solubility and high retention on soil particles. EDTA application to soil is a strategy to increase heavy metal phytoextraction, but such chelants usually cause phytotoxicity and metal leaching side effects. Therefore, these research work objectives were to evaluate the effects of single (0.5 g kg^{-1}) and split (0.25 + 0.25 g kg^{-1}) EDTA application on Pb uptake by jack beans (*Canavalia ensiformis L.)* as well as on Pb vertical movement in a Pb contaminated soil material. Two sets of experiments were carried out under greenhouse conditions: in the first one, plants were grown in 3L-pots filled with a Pb-contaminated soil to evaluate Pb uptake by plants; for the second experiment, PVC-columns (42 cm height) were used to evaluate soil Pb leaching: the upper half-column (20 cm) was filled up with Pb-contaminated soil (1800 mg kg^{-1}) whereas the lower half-column (20 cm) was filled with clean soil. Ten 60 mm-rainfalls with a duration of five hours were simulated by dropping distilled water on the top of columns, and leachates were collected for chemical analysis. Plants did not show any visual Pb toxicity symptoms or reduction in dry matter yield. Nevertheless, Pb uptake by jack beans regarded as total plant Pb accumulation was higher in EDTA-treated plants. Vertical Pb movement was observed mostly for the single EDTA application. EDTA addition to the soil favor Pb-phytoextraction by jack beans and the split EDTA application decrease the metal leaching, indicating less risk of environmental contamination.
Key words: *Canavalia ensiformis* L., lead, phytoremediation, chelant

LIXIVIAÇÃO E ABSORÇÃO DE Pb PELO FEIJÃO-DE-PORCO ASSISTIDO PELA APLICAÇÃO DE EDTA NO SOLO

RESUMO: O chumbo (Pb) é um dos principais contaminantes de solo. Os processos de remediação são dificultados devido à alta retenção do elemento às partículas do solo. A utilização do EDTA para aumentar a fitoextração dos metais do solo tem apresentado bons resultados. Contudo, os quelantes podem causar efeitos indesejáveis como a fitotoxidez e a lixiviação do metal. Nesse sentido, avaliou-se o efeito da aplicação única (0,5 g kg^{-1}) e parcelada de EDTA (0,25 + 0,25 g kg^{-1}) na absorção de Pb pelas plantas de feijão-de-porco (*Canavalia ensiformis* L.) e na movimentação vertical de Pb em solo contaminado, sob condições de casa de vegetação. Em um experimento, as plantas foram crescidas em vasos contendo 3 L de solo contaminado por Pb. No outro, utilizaram-se colunas de PVC com 42 cm de altura, preenchendo-se os primeiros 20 cm com solo contaminado com 1800 mg kg^{-1} de Pb, seguido de outros 20 cm, com amostra de solo não contaminado. Nesse experimento, foram feitas dez lixiviações com água em volume equivalente a uma chuva de 60 mm cada. As plantas não apresentaram sintomas visuais de toxicidade de Pb, nem diferença na produção de massa seca do feijão em função dos tratamentos aplicados. No entanto, a absorção de Pb foi superior para as plantas que receberam EDTA. Houve movimentação vertical de Pb no solo, principalmente devido à aplicação única de EDTA. A adição de EDTA ao solo auxilia a fitoextração de Pb por plantas de feijão-de-porco e que o parcelamento do EDTA diminui a lixiviação do metal, mostrando se menos agressivo ao ambiente.
Palavras-chave: *Canavalia ensiformis* L., chumbo, fitorremediação, quelantes

INTRODUCTION

Metal contaminated soils occur associated with industrial and/or high density population areas. There is a great concern about soil Pb-contamination because of its occurrence, toxicity and exposition potential aspects (ATSDAR, 2005). In general, soils present low natural Pb concentration, which is inherent to the soil parent rock. At São Paulo State (Brazil), natural Pb concentrations vary between 0.01 and 1.44 mg kg^{-1} (Cancela, 2002). Soil Pb enrichment due to anthropogenic activities, such as atmospheric depositions, mining and industrial wastes occur mainly on the more superficial soil layers (Miller et al., 1983). This can be attributed to the Pb low solubility, strong soil adsorption and formation of Pb-stable compounds (Wu et al., 2000). Soil Pb is most frequently found as a bivalent cation (Pb^{2+}), which is the available form to the plants (Kabata-Pendias & Pendias, 2000).

Phytoextraction was developed in the framework of an intense research effort for more efficient, cheaper and less hazardous techniques to remediate contaminated soils. It consists in the removal of metals by plants through uptake and accumulation into biomass (Nascimento & Xing, 2006). A phytoextracting species presents high metal uptake and tolerance, translocation to shoots and biomass yield (Lasat, 2002). Most studies on phytoextracting species were accomplished under temperate climate conditions. Under tropical conditions, jack beans have been reported as effective Pb phytoextractors (Romeiro, 2006; Pereira, 2005).

Phytoextraction might be improved via a chelant application to the soil, because it increases metal availability, and consequently, the metal uptake and accumulation by plants (Pereira, 2005; Komárek, 2007). The chelant effectiveness has been related to the increase in Pb translocation from roots to shoots (Blaylock & Huang, 2005). On this regard, EDTA has been the most succesfull chelant tested so far, despite its high phytotoxicity and low soil degradation, what might cause groundwater metal-chelant contamination (Tandy et al., 2004; Lombi et al., 2001; Wenzel, 2003 and Nascimento et al., 2006). In order to avoid such drawbacks, it has been proposed to split the EDTA application to the soil or to use a polymer-coated EDTA (Wenzel et al., 2003; Shibata et al., 2006).

The objective of this study was to evaluate the effect of split and single EDTA application to a Pb-contaminated soil, monitoring the metal leaching and its uptake by jack bean plants.

MATERIAL AND METHODS

The experiments were carried out in a greenhouse in Campinas, State of São Paulo, Brazil, using samples of a Rhodic Hapludox collected in the locality. This soil presents a sandy-clayey texture: 355 g kg^{-1} of clay, 28 g kg^{-1} of silt and 617 g kg^{-1} of sand. Soil samples were separately collected from the 0-20 and 20-40 cm depth layers, air-dried, sieved to pass a 2.0 mm screen and analyzed for chemical attributes before liming. Both soil samples had a pH of 4.1. The chemical analysis for the 0-20 and 20-40 cm soil samples, before liming, showed an organic matter content of 26 and 19 g dm^{-3}, 4 and 2 mg dm^{-3} of P; 1.1 and 0.6 mmol$_c$ dm^{-3} of K, 5 and 2 mmol$_c$ dm^{-3} of Ca; 2 and <1 mmol$_c$ dm^{-3} of Mg; 0.24 and 0.19 mg dm^{-3} of B; 1.6 and 1.5 mg dm^{-3} of Cu; 34 and 14 mg dm^{-3} of Fe; 1.7 and 1.0 mg dm^{-3} of Mn, and 0.5 Zn, respectively. Based on the chemical analysis, soil samples from the 0-20 cm depth layer were adjusted for acidity – using $Ca(OH)_2$ and $MgCO_3{\cdot}Mg(OH)_2.H_2O$ (p.a.) in the proportion 4:1 mol /Ca: Mg – to rise the base saturation to 70%, and then, incubated during 30 d. The soil moisture was kept close to fulfill 70% of soil pores with water. After liming incubation, the soil samples were contaminated with 1,800 mg kg^{-1} of Pb (8.7 mmol kg^{-1}) in the form of lead acetate, and again incubated for a period of five months, under the same soil moisture.

After the incubation period, the soil samples were fertilized with N, P, K, B, Cu, Mn and Zn, at the rates (mg kg^{-1}): 30 N (ammonium nitrate); 350 P (triple-superphosphate); 100 K (KCl p.a); 0.5 B (H_3BO_3 p.a.); 1.0 Cu ($CuSO_4$ p.a.); 4.0 Mn ($MnSO_4$ p.a.); 3.0 Zn ($ZnSO_4$ p.a.).

First set of experiments - Effect of split EDTA application upon the Pb-uptake by jack bean plants

This experiment was carried out according to a 3 × 2 factorial design with five replications, in a completely randomized arrangement. The experimental unity consisted of a 3 L-pot containing 3 kg of soil collected from the 0-20 cm depth layer, previously amended for acidity, fertilized and contaminated with Pb as described above. Jack beans (*Canavalia ensirmis*) was used as a test plant. Each pot was sown with ten jack bean seeds, and one week after emergence the four best seedlings were left. Pots received nitrogen (ammonium nitrate) in two split shootsdressing applications of 35 mg kg^{-1}, at the 7th and 21st days after emergence.

EDTA (solution) treatments were applied onto the soil as follows: (i) Control – 0.0 of disodium EDTA; (ii) 0.5 g kg^{-1} of disodium-EDTA, in a single application, 20 days after seed germination; (iii) split of 0.5 g kg^{-1} of disodium-EDTA in two applications of 0.25 g kg^{-1}, 20 and 40 days after seed germination. Pot soil moisture was daily monitored by pot weighting and adding distilled water whenever necessary to keep 70% of soil pores filled.

Plant shoots and roots were harvested in two growth stages: 40 days after emergence, before the second EDTA application (0.25 mg kg^{-1}) and 60 days after emergence, at flowering start. Plant shoots were first rinsed in tap water, then in 1% HCl solution and finally in distilled water. After excess water flowed off, each sample was put in paper bags and dried in a forced air oven at 70 ºC until constant weight, and then, weighed and ground in a *Wiley* type grinder. Roots were sieved to separate them from soil, rinsed in tap water, immersed in a 0.02 mmol L^{-1} disodium EDTA solution during 90 minutes, thoroughly rinsed in distilled water, bagged, dried at 70 ºC and ground as described for plant shoots. All ground vegetal samples were submitted to HNO_3/H_2O_2 digestion in a microwave oven and analyzed for heavy metals, macro and micronutrients by plasma emission spectroscopy (ICP-OES) (Abreu, 1997).

After plant material collection, soil samples were also collected from each experimental unity and submitted to chemical analysis for heavy metals availability using DTPA (pH 7.3) and total element concentrations using the USEPA 3051 method (USEPA, 2004).

The Pb transport from soil to the shoots was evaluated using the transfer factor (F) = TC (mg kg^{-1}) + RC (mg kg^{-1}) / SC (mg kg^{-1}) where, TC = Pb shoots concentration; RC = Pb root concentration; SC = Pb soil concentration obtained with USEPA 3051 method (Lubben & Sauerbeck, 1991). The species ability in Pb translocation from roots to shoots was estimated using the translocation index (TI) suggested by Bichequer & Bohrlen cited by Paiva et al. (2002): TI (%) = TQ (mg per pot)/ WPQ (mg per pot) × 100, where, TQ = element accumulation in the shoots; WPQ = element accumulation in the whole plant (shoots + roots). These indicators allowed estimating the plant efficiency in Pb removal and also the period of time necessary to remove all soil Pb, considering four annual cultivation cycles.

Second set of experiments - Effect of split EDTA application on the vertical movement of Pb through the soil column

The experimental design was a completely randomized 3 × 10 factorial (three EDTA application types and ten Pb leaching periods), with four replications.

EDTA treatments consisted of: (i) control – 0.0 of disodium-EDTA; (ii) a single application of 0.5 g kg^{-1} of disodium-EDTA in the beginning (zero time); (iii) split of 0.5 g kg^{-1} of disodium-EDTA in two applications of 0.25 g kg^{-1}, at 0 and 20 days after the EDTA single application. The ten leaching times corresponded to 4; 11; 18; 32; 46; 60; 74; 86; 100; and 114 d after zero-time EDTA application, when simulated rainfalls were performed in order to induce leaching.

The experimental units consisted of rigid PVC columns (10 cm diameter × 42 cm height) to which bottom was added a PVC cap with a center hole and a plastic hose used to collect the leached solution. Paraffin was used to coat the walls in order to avoid water preferential flow, while a filter paper and a fine mesh acrylic manta was used in order to prevent soil losses.

The upper half-column (20 cm) was filled up with Pb-contaminated soil whereas the lower half-column (20 cm) contained a non-contaminated soil, prepared as described above. The soil samples were slightly wetted and transferred to the column followed by slightly compactation at 10 cm height portions, in order to reduce differences among the experimental unities and guarantee more homogeneous soil density inside the columns (Amaral Sobrinho et al., 1999).

Afterwards, the columns were saturated with distilled water, and soon after, EDTA solution was added onto the soil surface both at zero time (single and split EDTA treatments) and at 20 days after (split EDTA treatment). At each of the ten leaching times, 471 mL of deionized water was dropped onto the soil surface, during 5 hours, simulating a 60 mm rainfall. Drained solution (leachate) was collected, weighed and analyzed for Pb by ICP-OES. After the last percolate sampling, soil samples from the column layers were separately collected for Pb analysis as described in the first experiment.

Data was submitted to analyses of variance and the mean values were compared according the Tukey test ($p < 0.05$), using the software SISVAR 4.6 (Ferreira, 1999).

RESULTS AND DISCUSSION

Effect of split EDTA application upon the Pb-uptake by jack beans (*Canavalia ensiformis*)

Pb concentrations varied between 320 and 463 mg kg^{-1} in the shoots and from 102 to 802 mg kg^{-1} in the roots. No visual symptoms of Pb toxicity were observed in the shoots of jack bean plants during the entire experiment. Pb shoots symptoms are usually characterized by dark green and weak leaves, old leaf wilting and low shoots dry matter yield (Kabata-Pendias & Pendias, 2000). On the other hand, plant roots showed visual Pb toxicity symptoms characterized by dark brown fragile roots with low number of radicles.

Jack beans shoots presented 444 mg kg^{-1} (dw) on the single applied EDTA while for split EDTA application shoots reached 463 mg kg^{-1} of Pb. The toxic Pb concentration range in shoots of most plants, reported in literature, is between 30 and 300 mg kg^{-1} of Pb (Kabata Pendias & Pendias, 2000). Thus, jack bean shoots showed Pb concentrations values above the highest known limits for plant toxicity and no visual symptoms of Pb toxicity were observed. Neither the single nor the split EDTA application affected the shoots dry matter yield (Figure 1).

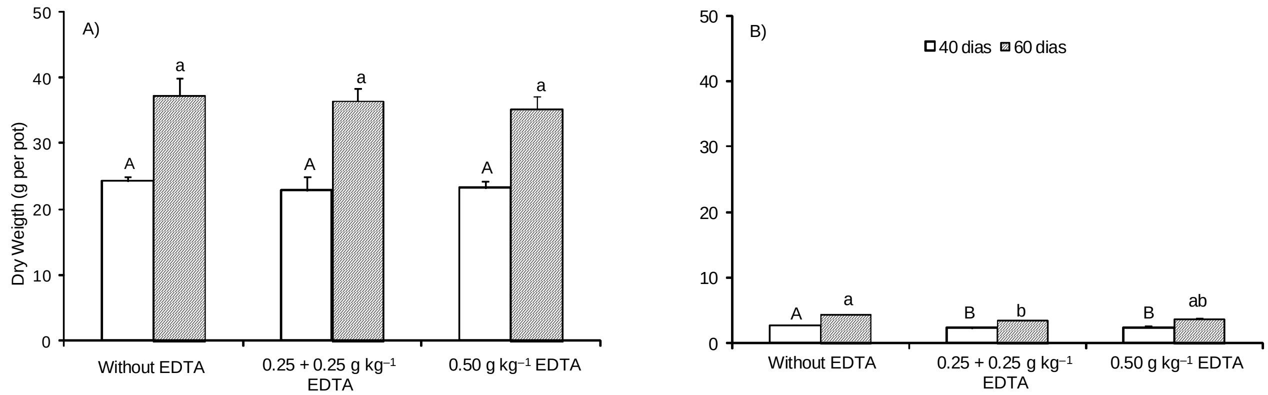

Figure 1 - Single and split EDTA application effects on dry matter yields of shoots (A) and roots (B) of jack bean plants grown in a Pb-contaminated soil at 40 days (capital letters) and 60 days (small letters) after seed germination. Means followed by the same letters in each plant part do not differ (Tukey test, $p < 0.05$).

Maize and *Populus* sp. plants grown in soil containing 1360 mg kg^{-1} of Pb did not show any difference in shoots biomass among EDTA treatments (3 - 6 and 9 mmol kg^{-1} of EDTA) (Komarek et al., 2007). The lowest EDTA dose was almost twice the one used in the present experiment. Conversely, different toxicity susceptibilities among species have been observed for ten plant species grown in metal-contaminated soil treated with 2.5 to 5 mmol kg^{-1} of EDTA. All species showed visual symptoms of chlorosis and necrosis, but the plants of Chinese mung bean (*Vigna radiata*) and common buckwheat (*Fagopyrum esculentum*) died two days after 5 mmol kg^{-1} of EDTA application, while barley plants showed higher tolerance to heavy metals (Chen et al., 2004). Such metal tolerance differences among species have been also reported by several authors. In a study with maize, jack beans and sunflower grown in a Pb contaminated Oxisol treated with a single EDTA application (0.5 g kg^{-1}), severe Pb toxicity symptoms were observed only in sunflower plants (Pereira, 2005). For turnip (*Brassica rapa* L.) plants grown in a Pb, Zn and Cd contaminated soil treated with EDTA were very susceptible and showed visual toxicity symptoms even with the split EDTA application (Greman et al., 2003).

Usually more severe visual symptoms are observed in jack bean plants' roots when grown in a soil with a single EDTA application (Paiva et al. 2000; Kabata Pendias & Pendias, 2000). In the present study, higher concentrations of Pb, up to 962 mg kg^{-1}, were found in plants collected 40 days after seed germination, which may explain the toxicity symptoms observed. Similar behavior has been reported in literature and attributed to the damaging of the mitosis process in the roots and water and nutrient transport to the shoots (Geebelen et al., 2002; Horng et al., 2001; Kabata Pendias & Pendias, 2000).

No differences were found among shoots' dry matter between single or split EDTA treatments. However, root's dry matter of plants grown with split EDTA application were different (lower) than the ones from the control, both at 40 and 60 days, while for EDTA single application this effect was observed only at the end of the experiment (60 days) (Figure 1).

Twenty days after the first EDTA application to the soil, no differences were found between the Pb concentrations in shoots and roots and the control and split EDTA application: both were lower than the Pb concentrations in plants from the single EDTA application treatment (Figure 2). These results were expected since, according to this treatment, at that sampling moment (at 40 days), only half EDTA dose (0.25 g kg^{-1}) had been applied. The same was observed for the total amount of Pb accumulated per pot in jack bean plant parts (Figure 2): that is, Pb in plants from the single EDTA application was almost twice the Pb found in the control plants, probably because of the Pb-EDTA greater availability to the plants. On the other hand, in the split EDTA application (0.25 + 0.25 g kg^{-1}), lower Pb-EDTA complex might be formed since the soil used is rich in Fe and Al and these elements strongly compete with Pb and are preferentially bound by EDTA. This hypothesis is corroborated by Pereira et al.(2007) that observed an increase Fe concentration in the soil solution when 0.5 g kg^{-1} EDTA was applied to a Pb-contaminate soil

At the end of the experiment jack bean shoots from EDTA treatments, irrespective of the EDTA application form, presented higher Pb concentrations and accumulation values up to 460 mg kg^{-1} (Figure 2). Similar results were reported for maize and common bean plants grown in Pb contaminated soil treated with 5 mmol kg^{-1} of EDTA (Luo et al., 2005). At the end of 14 days, common beans showed higher Pb accumulation (487 mg kg^{-1}) than maize plants (270 mg kg^{-1}).

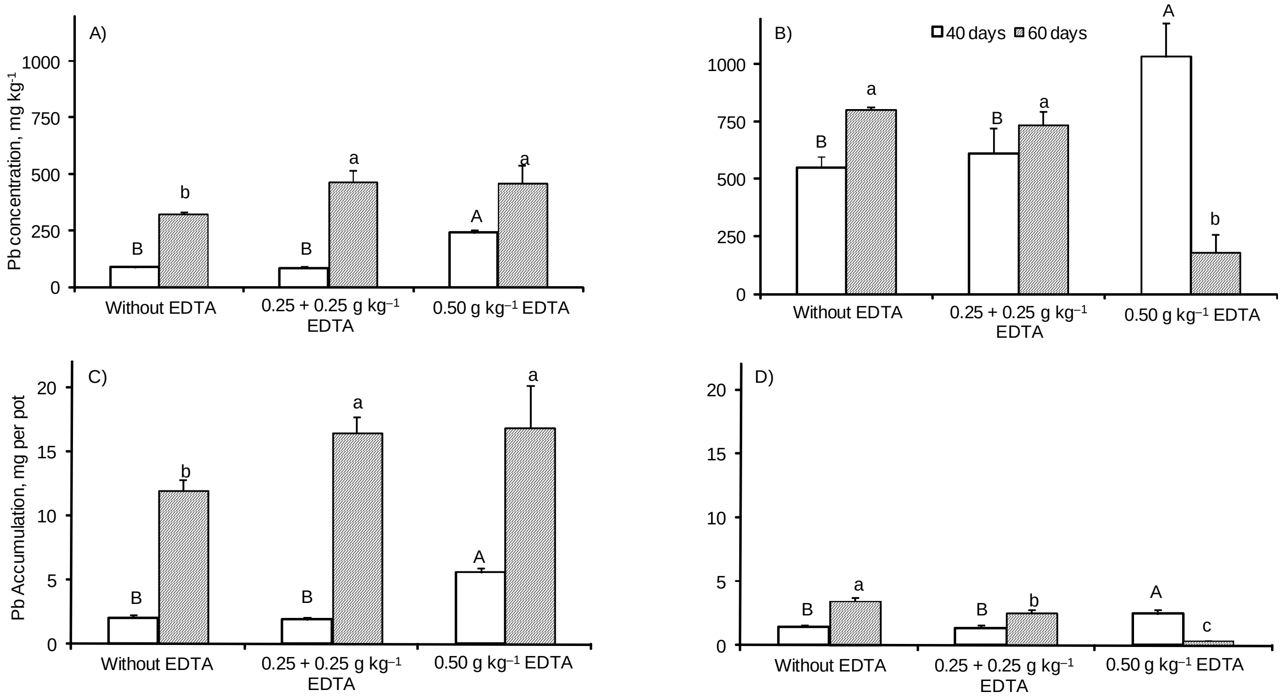

Figure 2 - Single and split EDTA application effects on the Pb concentration and content in shoots (A and C) and roots (B and D) of jack bean plants grown in a Pb-contaminated soil at 40 days (capital letters) and 60 days (small letters) after seed germination. Means followed by the same letters in each plant part do not differ (Tukey test, $p < 0.05$).

Also, increased Pb concentrations were found in maize shoots (415.4 mg kg^{-1}) and roots (5047.7 mg kg^{-1}) when 10 mmol kg^{-1} of EDTA was applied to a Pb-treated (500 mg kg^{-1}) Ultisol ten days before harvest (Melo, 2006). A increase by 290% in Pb accumulation was observed by sunflower, tobacco and castor bean plants grown in a Pb contaminated soil (1800 mg kg^{-1}) treated also with 1 mmol kg^{-1} of EDTA (Zeitouni, 2007). In addition, a 7-fold increase in jack bean shoots Pb concentration of plants grown in contaminated soils treated with 0.5 g kg^{-1} of EDTA was also reported (Pereira, 2005). These results evidenced the EDTA enhancing effect on the Pb availability and accumulation by plants. It has been proposed that EDTA can desorb the heavy metal from soil, forming a soluble metal-EDTA complex in the soil solution, available to the plants (Vassil et al., 1998). According to such authors, the physiological bases of the complex uptake and accumulation by plants are not known, and it is supposed that this synthetic chelant affects the root membrane barriers that usually control acquisition and translocation of solutes.

The EDTA effect on the Pb concentration and accumulation in jack bean roots at the end of the experiment was inversely related to the one observed in the plant shoots. These values were higher in the control plants, intermediary in the ones from the split EDTA application and lower in the ones from the single EDTA application (Figure 2). The roots Pb accumulation was 2.2% of the plant Pb in plants from the single EDTA application, in comparison to 22.5% root Pb accumulation in the control plants.

A three time increase in Pb translocation in *Brassica juncea* plants growing in nutrient solution with 0.2 mmol kg^{-1} EDTA compared to control plants was observed, independently of the pH (3.5 and 5.5) by Blaylock et al. (1997). On the other hand, some Pb translocation from roots to shoots of sunflower plants grown in a Pb contaminated soil treated with 2 g kg^{-1} of EDTA have also been reported (Liphadzi & Kirkham, 2006). Furthermore, there are studies reporting higher metal mobility in plants grown in EDTA treated soil and relating this to the chelant entry into the plant. The Pb-EDTA complex was found in xylem exudates of Indian mustard plants grown in pH 4 nutrient solution containing 0.5 mmol L^{-1} of $Pb(NO_3)_2$ and 1 mmol L^{-1} of K_2EDTA (Vassil et al., 1998). The analysis by EXAFS (Extended X Ray Absorption Fine Structure) spectral comparisons indicated the presence of Pb, EDTA and Pb-EDTA in the leaves when Pb and Zn forms accumulated in the leaves of common bean plants grown in nutrient solution containing 125 μmol L^{-1} of $Pb(NO_3)_2$ and 125 μmol L^{-1} of Na_2H_2EDTA or only 1,666 μmol L^{-1} of $Pb(NO_3)_2$ (Sarret et al., 2001).

In this experiment, Pb concentration in shoot and roots of jack bean plants grown in soil without EDTA addition and treated with split EDTA application did not differ at 40 days after germination (Figure 2). However, when grown on soil treated with the single EDTA application, either shoots or roots, showed a Pb concentration 225 % higher in comparison to the other treatments. At 60 days after germination, Pb accumulation in plants from the split EDTA application was higher than in the control but did not differ from the single application treatment.

The translocation index (TI) was suggested by Bichequer & Bohrlen cited by Paiva et al. (2002) and indicates the species ability in metal translocation from roots to shoots. Jack bean plants from the single EDTA application presented a 98% TI (Table 1). This can be considered an excellent index for phytoextracting plants and can be used as evaluation criterion for selecting plants for higher metal acquisition from contaminated soils.

The metal transference from soil to plants can be evaluated by the transference factor (F) and this index is supposed to be as high as possible when used as criterion to select phytoextracting species. Jack bean plants from the single EDTA application presented a F = 0.23. This is considered low when compared to phytoextracting reference species like the Indian mustard plants showing F = 1.7 (Henry, 2000). All plants from EDTA treatments showed the same F value (0.23) that were superior to the control F value (0.15) (Table 1). Similar transference factor values have been found for kenaf (*Hisbiscus cannabinus*. var. aokawa) plants (0.1) and turnip (0.30) (Santos, 2005). Higher F values (0.6) were obtained for maize plants grown in Oxisol samples treated with 2400 mg kg^{-1} de Pb (with and without EDTA) although for jack bean plants grown with increasing Pb rates (100 - 400 µmol L^{-1}) in the nutrient solution did not observe significant differences for F values (average 0.04) among treatments (Pereira et al., 2007; Romeiro, 2006).

The translocation (TI) and transference (F) indexes allow estimating the Pb removing plant efficacy and the time in years necessary to completely clean the soil. For the conditions of this experiment, it would be necessary 39 years of jack beans cultivation without EDTA to remove all the Pb applied (1800 mg kg^{-1}) to the soil (Table 1). With EDTA application the remediation period of time would drop to 28 years, an 11-year saving time. Zeitouni et al. (2007) evaluated the effects of EDTA rates on the metal uptake (Pb, Cd, Cu, Ni and Zn) by several species (tobacco, castor bean, sunflower, pepper plants) and observed that EDTA application reduced the cultivation period necessary to remediate contaminated soils. In the case of sunflower, 1431 cropping cycles would be necessary to get the soil completely Pb-free, but with 1 mmol kg^{-1} EDTA application the number of cycles would drop to 251. This result was attributed to the species and EDTA dose.

In this experiment, soil Pb availability (evaluated in DTPA pH 7.3 extracts) increased in response to the single EDTA application, at 40 days after seed germination (Table 2). However, at 60 days, both EDTA application systems showed the same soil Pb availability expressed by the Pb-EDTA complex formed (in DTPA extracts), once the second dose of the split EDTA had been already applied.

Table 1 - Translocation index (TI), transference factor (F), treatment efficacy in soil Pb removing and remediation period of time necessary to clean the soil using jack beans as phytoextractor plants.

EDTA treatment	Translocation index - TI	Transference actor - F	Efficacy[1]	Time period[2]
	%		%	years
Without EDTA	77.5	0.15	0.63	39.6
0.25 + 0.25 g kg^{-1} EDTA	86.7	0.23	0.86	28.9
0.5 g kg^{-1} EDTA	97.8	0.23	0.89	28.1

[1]Efficacy = Pb content in shoots /total Pb to remove from soil *100. [2]Considering four cropping cycles per year.

Table 2 - Pb concentration in pot soil samples at 40 and 60 days after seed germination (soil extracts obtained by DTPA and USEPA 3051 methods).

EDTA treatment	Pb - DTPA		Pb - USEPA 3051	
	40 dias	60 dias	40 dias	60 dias
	mg kg^{-1}			
Without EDTA	847.4 b	913.8 a	1,888.4 a	1,889.8 a
0.25 + 0.25 g kg^{-1} EDTA	868.0 b	946.4 a	1,959.4 a	1,826.6 a
0.5 g kg^{-1} EDTA	931.8 a	943.0 a	1,947.4 a	1,824.8 a

*Means followed by the same letters in each plant part do not differ (Tukey test, $p < 0.05$).

Effect of EDTA application on the soil Pb vertical movement

Althougt Pb is one of the slowest moving metals in the soil compared to other metals' mobility (Alloway, 1990), the soil EDTA application significantly increased the soil Pb vertical movement, independently of the single or split EDTA application (Figure 3). For metal leaching evaluation in soils treated with 10 mmol kg^{-1} of EDTA higher Pb mobility was observed with EDTA application (Sun et al., 2001). Similar results were also reported by Greman et al. (2003), Liphadzi et al. (2003), Tandy et al. (2004), Wu et al. (2004) and Hauser et al. (2005). Studying the phytoremediation of a sewage-sludge metal-contaminated soil, some authors observed high Pb leaching in the soil profile when 0.5 g kg^{-1} of EDTA was applied. Soil Pb accumulated 30 cm below the surface layer reaching up to 0.4 mg L^{-1} concentration. Besides, several other metals were also leached in the drainage water (Madrid et al., 2003).

Regarding the effect of EDTA application mode on the soil Pb vertical movement, it was observed that the split EDTA application (2 × 0.25 g kg^{-1}) reduced the Pb leaching compared to the single application of 0.5 g kg^{-1} (Figure 3). In studies of phytoextraction, Greman et al. (2001) and Wenzel et al. (2003) have recommended the split EDTA application aiming at decreasing or avoiding metal leaching in the soil profile.

Soil Pb leaching was gradual and lower with the soil split EDTA application (Figure 4). The soil leachate analysis showed maximum Pb concentration of 275 mg L^{-1} in leachates from the single EDTA application treatment, and of 150 mg L^{-1}, in the split application, after 1.1 and 1.86 L of water leaching, respectively. However, as evidenced in the present study, there is a great concern even with the split EDTA application because it caused significant Pb leaching. If such Pb quantities reach the underground waters, they might represent serious hazard to the environment and

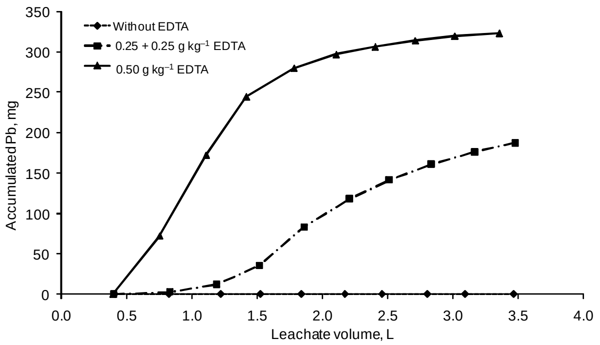

Figure 3 - Cumulative amount of Pb recovered with soil leachates as a function of the collected volume and of the mode oh EDTA application.

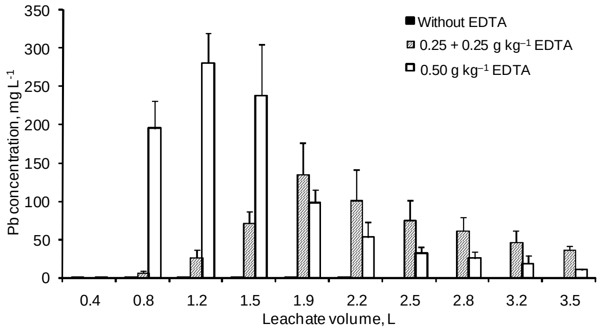

Figure 4 - Pb concentration in each collected leachate.

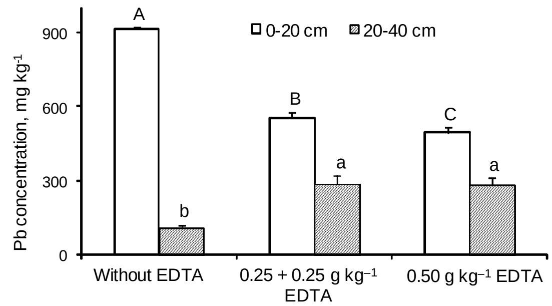

Figure 5 - Pb concentrations in the 0-20 cm (capital letters) and 20-40 cm (small letters) soil depth layers, in soil extracts obtained with DTPA, after ten leaching procedures. Means followed by the same letters do not differ by (Tukey test, $p < 0.05$).

human health. Such results are corroborated by Chen et al. (2004) when studying the EDTA application to improve phytoremediation of heavy-metal-contaminated soils, including Pb. According to the São Paulo State Environment Sanitation Technology Company (CETESB, 2006), Pb concentration values above 10 μg L^{-1} in underground waters are considered cases of intervention that present direct or indirect potential risks to human health.

Pb tended to remain in the top layer (0-20 cm) in soil samples not treated with EDTA (Figure 5), thus confirming the low mobility of this metal, and it reached a concentration of 2110 mg kg^{-1} (total Pb) against 211 mg kg^{-1} in the 20-40 cm depth soil layer. In the soil samples with EDTA, Pb levels reached, in average, 1200 and 520 mg kg^{-1} in the 0-20 cm and 20-40 cm depth soil layers, respectively, which corresponds to a significant increase in Pb vertical movement in the soil.

CONCLUSIONS

The phytoextraction potential of jack beans assisted by EDTA addition was maintained independently of EDTA mode of application to the soil, either in a single or split EDTA dose.

The split EDTA application might cause less environment damage to underground water.

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