# Pb-PHYTOEXTRACTION BY MAIZE IN A Pb-EDTA TREATED OXISOL

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ABSTRACT: One of the most viable strategies to restore metal contaminated soils is the introduction of plants specialized in their accumulation or able to tolerate very high metal concentrations. This research evaluated: i. the maize as a Pb-accumulator plant; ii. the effects of EDTA-chelating agent for Pb-uptake by maize; iii. amending effect of EDTA on the soil Pb-availability using different extracts. Treatments consisted of Pb rates (100; 200; 350; 1,200 and 2,400 mg kg$^{-1}$) applied to a Rhodic Hapludox in the form of $Pb_3(NO)_2$ with (0.5 g kg$^{-1}$) and without EDTA. Lead concentrations were determined in maize plant shoots. Soil available Pb was obtained using DTPA, Mehlich-3 and saturation solutions methods. Ionic speciation in the soil solution was performed using the software Visual-Minteq. Although a low *t* value was found (*t* <0.70), Pb concentration were high (>1,500 mg kg$^{-1}$ of Pb) in maize shoots regardless of EDTA addition. Maize plants treated with EDTA had lower dry matter yield, mainly due to toxic levels of Fe and Al of the Oxisol. All extracting solutions were effective to determine available Pb in soil samples, but the saturation extract is a more difficult and time consuming procedure. At low and medium Pb levels, the plants grew less on EDTA, therefore the phytoextration process was less efficient. The addition of EDTA to the soil is not recommended with the purpose of increasing Pb absorption by maize plants.
Key words: Mehlich-3, DTPA, phytoremediation, available Pb, ionic speciation

# FITOEXTRAÇÃO DE Pb POR MILHO EM UM LATOSSOLO TRATADO COM Pb E EDTA

RESUMO: Uma das estratégias mais viáveis para a descontaminação de solos é a introdução de plantas especializadas na acumulação ou que toleram altas concentrações de metais. Nesse estudo avaliaram-se: i. o potencial acumulador de Pb de plantas de milho; ii. os efeitos do EDTA na absorção de Pb pelo milho; iii. o efeito do EDTA na disponibilidade de Pb utilizando diferentes extratores. Os tratamentos consistiram em doses de Pb (100; 200; 350; 1.200 and 2.400 mg kg$^{-1}$) aplicados em um Latossolo na forma de $Pb_3(NO)_2$ com (0.5 g kg$^{-1}$) e sem EDTA. A concentração de Pb foi determinada na parte aérea das plantas de milho, e no solo determinou-se o Pb disponível pelos métodos DTPA, Mehlich-3 e pasta de saturação. A especiação iônica da solução do solo foi determinada utilizando-se o *software Visual*-Minteq. Embora tenha sido encontrado um baixo valor *t* (*t* <0.70), a concentração de Pb na parte aérea do milho foi alta (>1.500 mg kg$^{-1}$), independente da adição de EDTA. As plantas de milho que receberam EDTA apresentaram redução na massa seca, principalmente devido aos níveis tóxicos de Fe e Al do Latossolo. Todas as soluções extratoras utilizadas foram eficientes em determinar o Pb disponível nas amostras de solo, mas a pasta de saturação foi o processo mais trabalhoso. Em baixa e média concentração de Pb no solo as plantas cresceram menos com EDTA, portanto a fitoextração foi menos eficiente. Não é recomendada a adição de EDTA no solo buscando aumentar a absorção de Pb pelas plantas de milho.
Palavras-chave: Mehlich-3, DTPA, fitorremediação, Pb disponível, especiação iônica

## INTRODUCTION

Lead is a metal found in several products and materials worldwide used on a daily basis, such pigments, munitions, medical and electrical equipments, batteries, and is one of the most persistent contaminants in the soil (Kumar et al., 1995). Only in the State of São Paulo, Brazil, 217 heavy metal (HM) contaminated locations have been registered, including Pb contaminated sites (CETESB, 2006).

Phytoextraction is a technique that uses plants able to accumulate high quantities of HM in their tissues. Such low cost technology has a great potential for *in situ* remediation of large areas with low or medium level of contamination. It has the advantage of being publicly well accepted since it is a ‘green technology’ that uses sunlight as the main source of energy (Chaney et al., 2000; Robinson et al., 2003 and USEPA, 2004). One of the major difficulties about this technique is to maintain high available Pb concentrations in soil solution. To correct this problem, the use of chelating agents might help Pb desorption from the soil matrix by forming a soluble complex, therefore increasing plant absorption (Shen et al., 2002). Among these, the addition of EDTA is considered the most effective technique, specially for Pb phytoextraction (Tandy et al., 2004; Huang et al., 1997). Many previous studies have shown the increase of Pb uptake by shoots using EDTA for numerous crops: maize, pea, cabbage, bean, wheat, indian mustard and rye (Huang et al., 1997; Blaylock, 1997; Shen et al., 2002; Schmidt, 2003; Hovsepyan & Greipsson, 2005).

The metal availability to the plants may be determined by using saline, acid and/or chelating extracting solutions, or quantifying the element in the soil solution (Abreu et al., 2002). In non-contaminated soils, the use of extracting solutions for the evaluation of Pb availability to the plants, as well as its determination in the soil solution has not been successful because of its low concentration in soil extracts, very close to the equipment detection limits, which results in analytical errors (Bataglia et al., 1983).

Few studies have been found to date in the literature involving Pb phytoextraction and availability in Oxisols. Therefore, this research aimed at evaluating: (a) the maize as a Pb-accumulator plant on an Oxisol, (b) the effects of EDTA-chelating agent on the Pb-uptake by maize plants and (c) the EDTA amendment effect on soil Pb-availability using the procedures of DTPA, Mehlich-3 and saturation extracting solutions.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse, at Campinas, State of São Paulo, Brazil (22º53’ S; 47º03’ W), in 3 $dm^3$ plastic pots filled with soil samples of a Rhodic Hapludox, during 2004. Maize (*Zea mays* L., cv. IAC-8333) was used as test plant.

### Soil sampling and handling

Oxisol soil samples were collected from the 0-20 cm soil layer, at Campinas, SP, Brazil, from which subsamples were used to analyze physical and chemical attributes (Table 1). Air-dried soil samples were sieved through a 2 mm-mesh screen and amended for acidity with $Ca(OH)_2$ and $MgCO_3 Mg(OH)_2 H_2O$, at the ratio 4:1 (Ca:Mg), to elevate the pH to 5.5. After liming, soil samples were incubated for 30 days with the soil moisture maintained close to field capacity with distilled water, to provide complete soil-lime reaction.

### Trial procedures and experimental design

The experiment was carried out in a completely randomized design, arranged in a (6 × 2) factorial (six Pb rates, with and without EDTA) with three replications. The Pb rates consisted of $Pb(NO_3)_2$ in mg $kg^{-1}$: 0 (control); 100; 200; 350; 1,200 and 2,400. These five rates are equivalent to levels of: (a) alert; (b) intervention in agricultural areas; (c) intervention in residential areas; (d) intervention in industrial areas; and (e) twice the level of intervention in industrial areas, respectively, according to values of reference informed by CETESB (2001). The treated soil samples remained incubated for 5 months and soil moisture was monitored and maintained close to field capacity.

After the incubation period, $NH_4NO_3$ was added to soil samples to compensate the N from $Pb(NO_3)_2$, taking the highest rate as reference. Thus,

Table 1- Chemical and physical attributes of a Rhodic Hapludox before treatments.†

| Liming | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OM | P | pH | C.E.C | K | Ca | Mg | Base Saturation | B | Cu | Fe | Mn | Zn | Cd | Cr | Ni | Pb |
| g $kg^{-1}$ | mg $kg^{-1}$ | | ------$mmol_c$ $kg^{-1}$------ | | | | % | ----------------------------mg $kg^{-1}$---------------------------- | | | | | | | | |
| 24 | 3.0 | 4.2 | 48.5 | 1.2 | 3.0 | 2.0 | 13 | 0.29 | 2.0 | 33.0 | 6.5 | 0.8 | 0.1 | <0.01 | <0.01 | 0.78 |

| Granulometric analysis | | | | | |
|---|---|---|---|---|---|
| Clay | Silt | Coarse sand | Fine sand | Total sand | Soil texture |
| ----------------------------------------g $kg^{-1}$---------------------------------------- | | | | | |
| 355 | 28 | 453 | 164 | 617 | Sandy-clayey |

†Raij & Quaggio (1983) and Camargo et al. (1986).

$NH_4NO_3$ was added at rates of 324, 310, 297, 276, 162 and 0 mg kg$^{-1}$, for the increasing rates of Pb, respectively (0, 100, 200, 350, 1,200 and 2,400 mg kg$^{-1}$). After that, soil samples were fertilized with P, K, S, B, Cu, Mn and Zn, as follows (mg kg$^{-1}$): 360 (P-$P_2O_5$); 100 (K-KCl); 30 (Sº); 0.5 (B-$Na_2B_4O_7$); 1.0 (Cu-$CuSO_4$); 8.0 (Mn-$MnCl_2$) and 2.0 (Zn-$ZnSO_4$). Phosphate (supertriple - granules) and sulfur (elemental - powder) were applied in the solid form and the other elements, in solution. Following, each treated soil sample was split in two parts; and one received disodium-EDTA applied at the rate of 0.5 g kg$^{-1}$ soil (Huang et al., 1997) and the other was not treated.

Maize (cv. IAC-8333) was seeded in 3 dm$^3$ plastic pots filled with the treated soil samples (six Pb rates × two EDTA rates × three replications = 36 pots). Each pot received 24 seeds and after seedling emergence (one week after sowing), six plants per pot were left. The pot soil moisture was daily monitored by pot weighing and adding distilled water whenever necessary to keep 70% of total soil pores filled with water. Some applications of natural insecticide *'Neem' (Azadirachta indica)* were made to control maize leaf caterpillars. Shoots were harvested 45 days after seeding.

**Analytical procedures**

**Determination of Pb concentrations in plant shoots**

After harvest, maize shoots were rinsed first in tap water and thereafter in 1% HCl solution and distilled water, and dried in a forced-air oven at 70ºC until constant weight. In sequence the shoot dry matter yields were determined, ground in a *Wiley* type grinder and submitted to oven digestion (incineration) according to Bataglia et al. (1983).

**Oven digestion -** One gram of dry and ground plant tissue was oven digested at 500ºC for 3 hours. After that, ashes were suspended in 5 mL of a 6 mol L$^{-1}$ HCl solution and heated on an electrical plate until dry (complete evaporation). The residues were suspended in (2 mol L$^{-1}$) HCl, transferred to 50 mL volumetric flask and volumes were adjusted with distilled water and filtered through blue-ribbon paper filter (Bataglia et al., 1983). The plant extracts were analysed for P, Ca, Mg, Cu, Fe, Mn, Zn, Al, Cd, Cr, Ni and Pb by induced coupled plasma emission spectrometry (ICP-OES) (Jobin Yvon 40P, Longjumeau, France).

**Determination of available Pb in soil samples**

After soil incubation and before maize seeding, soil samples were thoroughly revolved and 0.2 kg of soil was collected from every pot. The samples were sieved through a 2 mm-mesh screen and submitted to the following the chemical procedures for the determination of available Pb:

**DTPA -** Extracting solution = 0.005 mol L$^{-1}$ diethylenetriaminepentaacetate (DTPA) + 0.1 mol L$^{-1}$ triethanolamine (TEA) + 0.01 mol L$^{-1}$ calcium chloride ($CaCl_2$) at pH 7.3. Procedure according to Lindsay & Norvel (1978) – 10 cm$^3$ of soil + 20 mL of extracting solution and 2 hour-shaking. The extracting solution was analyzed for Pb by ICP-OES.

**Mehlich-3 -** Extracting solution = 0.2 mol L$^{-1}$ $CH_3COOH$ + 0.25 mol L$^{-1}$ $NH_4NO_3$ + 0.015 mol L$^{-1}$ $NH_4F$ + 0.015 mol L$^{-1}$ $HNO_3$ + 0.001 mol L$^{-1}$ EDTA at pH 2.5. Procedure described by Mehlich (1984) – 5 cm$^3$ of soil + 20 mL of extracting solution and 5 min-shaking. The extracting solution was analyzed for Pb by ICP-OES.

**Saturation Extract -** Procedure described by Wolt (1994): deionized water was added to 0.5 kg of soil until the saturation point; the soil paste was then filtered through Buchner funnel with slow-filtering paper-filter, using vacuum, and the extract was collected in an Erlenmeyer flask.

The soil extracts were analyzed for Pb by ICP-OES. The soil extracts were also analyzed for $Cl^-$, $NO_3^-$, $NH_4^+$, $SO_4^{2-}$, $PO_4^{2-}$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Fe^{3+}$, $Pb^{2+}$, $Al^{3+}$, electrical conductivity (EC) and pH, as follows: $Cl^-$ using a selective electrode (3 mL soil solution + 9 mL buffer at pH 4.7) (Orion 710-A); $NO_3^-$ and $NH_4^+$, by Kjeldahl method, as described by Cantarella & Trivelin (2001); $SO_4^{2-}$, $PO_4^{2-}$, $Zn^{2+}$, $Cu^{2+}$, $Fe^{2+}$, $Pb^{2+}$ and $Al^{3+}$ by ICP-OES (respective estequiometric calculations for P and S were made); $Ca^{2+}$ and $Mg^{2+}$ by atomic absorption spectrometry (Perkin Elmer 5100); $K^+$ by flame photometry (Micronal B262); EC using a electrical conductivity meter (Orion C708) and pH using a pH meter (Metrohm 692 pH/ionmeter).

All the applied EDTA (0.5 mg kg$^{-1}$) was considered dissolved in the soil solution. All data obtained was submitted to the geochemistry modeling *visual*-MINTEQ 2.30 (Gustafsson, 2004) to obtain the percent distribution of ionic species in the soil solution.

Results were submitted to analysis of variance and regression with linear or polynomial models (*$P$ < 0.05 or **$P$ < 0.001) using the *software* ESTAT v.2.0. (1992).

## RESULTS AND DISCUSSION

**Maize phytoextractor potential**

The EDTA chelant applied to the soil caused a decrease in shoot dry matter yield of maize plants (Figure 1). Such effect might be explained by the EDTA increase on the availability of heavy metals (HM) or due to EDTA toxicity (Cui et al., 2004).

A previous study using mustard plants grown in nutrient solution containing 0.5 mmol $L^{-1}$ de $Pb(NO_3)_2$ and 2.5 mmol $L^{-1}$ of EDTA showed that EDTA caused a decrease in leaf tissue water content (Vassil et al., 1998). Similar results were obtained on decreased photosynthesis rate, transpiration and stomata conductance in artichokes (*Cynara cardunculus* L.) grown in Pb-contaminated soils, with 1.0 mmol $L^{-1}$ de EDTA (Hernandez-Allica et al., 2003).

In the present study, the decreased maize shoot dry matter yields observed might be attributed to Fe and Al concentrations found in shoots (average of 396 mg $kg^{-1}$ to 405 mg $kg^{-1}$, respectively). Adequate maize leaf Fe concentrations usually range from 30 and 250 mg $kg^{-1}$ (Raij & Camargo, 1997) and Al concentrations in leaf tissues of most plants range around 50 and 400 mg $kg^{-1}$ (average of 200 mg $kg^{-1}$) (Bergmann, 1992). The Fe and Al values found in maize shoots were above the adequate range and might be attributed to an increase in soil solution metal availability due to the presence of EDTA.

Without EDTA, the Fe and Al concentrations in the soil solution were on average, 0.43 and 1.15 mg $L^{-1}$, which are considered below hazard levels. Iron concentrations in the soil solution between 30 and 500 mg $L^{-1}$ can cause toxicity in several crops (Sousa et al., 2004) and concentrations of Al higher than 3.6 mg $L^{-1}$ usually drastically reduce maize yield (Sparks, 1995). On the other hand, in the presence of EDTA, Fe and Al concentrations in the soil solution were fairly high, 107 and 3.6 mg $L^{-1}$, respectively, being then considered in the range of hazard values.

Another possibility of the decrease in shoot dry matter is the difference of P found in shoots. The average over the treatments without EDTA was 15.94 g $kg^{-1}$ and with EDTA was 24.6 g $kg^{-1}$.

Maize shoot dry matter Pb concentrations were not affected by the soil-applied EDTA (Figure 2). The data obtained for shoot Pb concentrations in relation to the Pb rates applied to the soil were fit to linear regression equations ($R^2$ = 0.98), reaching 1,600 mg $kg^{-1}$. Shen et al. (2002); Cui et al. (2004) have reported some expressive increases in shoot Pb concentrations of several plants in function of EDTA addition to the soil, such as peas, maize, cabbage, Indian mustard and lupines. The latter authors observed a 30-fold increase in maize shoot Pb concentrations (from 100 to 3,000 mg $kg^{-1}$) in the presence of 2,500 mg $kg^{-1}$ of total soil Pb and 0.5 g $kg^{-1}$ of EDTA. A previous study has also shown the influence of five levels (0-2.5 mmol $kg^{-1}$) of EDTA on maize cultivated on an Ultissol with 500 mg $kg^{-1}$ of Pb during eight weeks (Hovsepyan & Greipsson, 2005). This study showed that EDTA also increases the Pb shoot concentration and the plants height decrease with the increase of EDTA levels.

The results obtained in the present research might indicate a superior potential for maize cv. IAC-8333 as Pb-accumulator. Besides, there is an economical advantage for phytoremediation of contaminated areas with this maize cultivar, since EDTA application is not needed.

There is no good explanation for such high maize shoot Pb concentrations obtained in treatments without EDTA. One hypothesis might be the maize plant ability to release exudates as organic acids that complex metals (phytosiderophores), favoring their

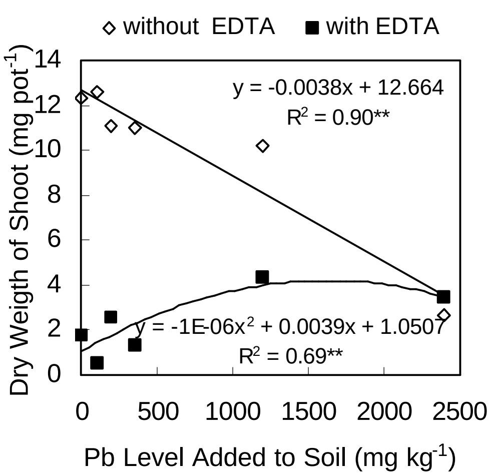


Figure 1 - Effects of increasing Pb rates and EDTA applied to the soil, on the shoot dry matter yields of 45-day-old maize plants.

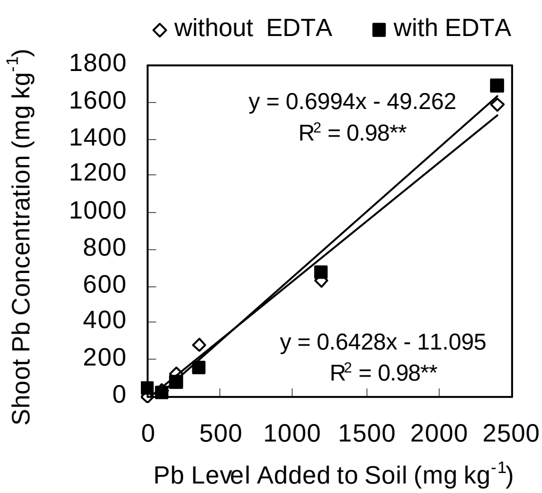


Figure 2 - Relation between Pb rates applied to the soil and Pb concentrations in the shoots of 45-day-old maize plants, in the presence and absence of EDTA.

uptake by roots. There are many evidences of such strategy of metal absorption present in grass roots; von-Wiren et al. (1996) observed Fe and Zn uptake by maize roots bound to organic acids released by the roots in the rizosphere, which are absorbed by specific proteins in the root cell membranes.

Nevertheless, EDTA caused a decrease in dry matter yield and as a consequence, affected the Pb accumulation in shoots (Figure 3). The relation between Pb rates applied to the soil and Pb contents in maize shoots fit quadratic equations, when without EDTA; but fitted linear equations with EDTA application. The maximum shoot Pb content without EDTA was 7.0 mg per pot, and with EDTA, 5.7 mg per pot (Figure 3).

A plant species potential to remediate a contaminated soil with heavy metals can be assessed using different criteria: by knowing its hyperaccumulation ability and/or calculating the transference factor (***t***). Depending on the criteria, a plant may or may not be considered a phytoremediator for heavy metals. Hyperaccumulators are those plants able to extract and accumulate in their tissues high metal concentrations, that is, values > 1,000 mg $kg^{-1}$ Pb of dry matter (Raskin et al., 1994). The transference factor (***t***) is defined as the ratio between the total element concentrations in the plant and soil (Henry, 2000). The higher the ***t*** factor the more effective the phytoextractor. Based on the former criterion the maize cultivar used in this research can be considered a Pb-hyperaccumulator, since its shoot Pb concentrations were close to 1,600 mg $kg^{-1}$ for the highest Pb rate (2,400 mg $kg^{-1}$), independently of the presence of EDTA (Figure 2). Based on the latter criterion, the *t* values calculated for the increasing Pb rates were the following: 0.36; 0.64; 0.80; 0.52; and 0.66, without EDTA; and 0.24; 0.39; 0.46; 0.57 and 0.70, with EDTA, respectively.

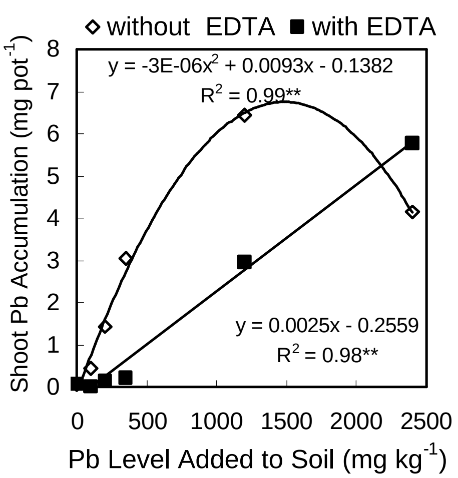


Figure 3 - Relation between Pb rates applied to the soil and Pb accumulated in shoots of 45-day-old maize plants, in the presence and absence of EDTA.

The *t* values obtained with and without EDTA were very close. However, *t* values were lower than 1.0 for all treatments. Such values were well below the ones found by Henry (2000) for mustard *(Brassica juncea)* (1.7), considered an excellent plant for Pb accumulation. Thus, according to the *t* criterion, the maize cultivar IAC 8333 is not so effective to remediate Pb-contaminated areas compared to the mustard plants. In spite of that, the cultivar showed to be a good hyperaccumulator in this experiment. Besides, other favorable characteristics such as fast growth, short plant cycle and easy crop management might encourage its recommendation for phytoremediation.

**Soil Pb availability**

In general, the relation between Pb rates applied to the soil and the Pb concentrations found in the soil extracts fitted equations, with determination coefficients very similar to each other ($R^2$ = 0.99) for all extracting solutions (DTPA, Mehlich-3 and saturation extract) (Table 2), which means that all solutions were effective to detect the Pb applied to the soil.

The extracting solution efficacy to assess soil Pb availability to the plants is usually determined through the relation between the quantities determined in the soil extracts and the quantities accumulated by the plants. With this criterion it was observed that DTPA and Mehlich-3 solutions showed similar effectiveness in assessing the available Pb to the plants, with $R^2$ > 0.96 (Figures 4a, 4b). These results are in agreement to those obtained by Abreu et al. (2002) studying lettuce (*Lactuca sativa* L.), signalgrass (*Brachiaria decumbens* Stapf), elephant grass (*Pennisetum purpureum* Schumacher), and Brazilian satintail (*Imperata brasiliensis*). It is also interesting to point out (Figures 4a, 4b) the similarity between the curves obtained with and without EDTA.

The soil solution Pb analysis (saturation water extract) is another strategy to evaluate soil Pb availability (Sparks, 1995). The results have shown a polynomial regression relation between Pb concentrations in the soil solution and Pb in maize shoots with $R^2$ = 0.90, for the treatments without EDTA. However, it was not so effective for the treatments with EDTA, with $R^2$ = 0.46 (Figure 5). For the treatments with EDTA, the saturation extract (soil solution) was the less effective in predicting the soil-Pb availability as compared to DTPA and Mehlich-3 extracts. These findings are consistent with a previous study that did not show correlation between the soil solution-Pb and the one found in wheat leaves (Lee et al., 2004).

Table 2 - Regression analysis between Pb concentrations in the extracts obtained by Mehlich-3, DTPA and saturation extracting solutions methods, and Pb rates applied to the soil, in the presence or absence of EDTA.

| Method | Regression Equation | $R^2$ |
|---|---|---|
| | ----------------------------------Without EDTA--------------------------------- | |
| Mehlich-3 | $y = -0.0001\ x^2 + 1.29\ x - 21.94$ | 0.99** |
| DTPA | $y = -0.00008\ x^2 + 1.00\ x - 16.4$ | 0.99** |
| Saturation Extract | $y = 0.00006\ x^2 - 0.06\ x + 9.10$ | 0.99** |
| | ----------------------------------With EDTA---------------------------------- | |
| Mehlich-3 | $y = -0.0001\ x^2 + 1.22\ x - 17.41$ | 0.99** |
| DTPA | $y = 0.84\ x + 3.07$ | 0.99** |
| Saturation Extract | $y = -0.0003\ x^2 + 0.99\ x + 61.90$ | 0.99** |

(y = Pb concentration, mg kg$^{-1}$, in each soil extract type and x = Pb rate applied to the soil, mg kg$^{-1}$).

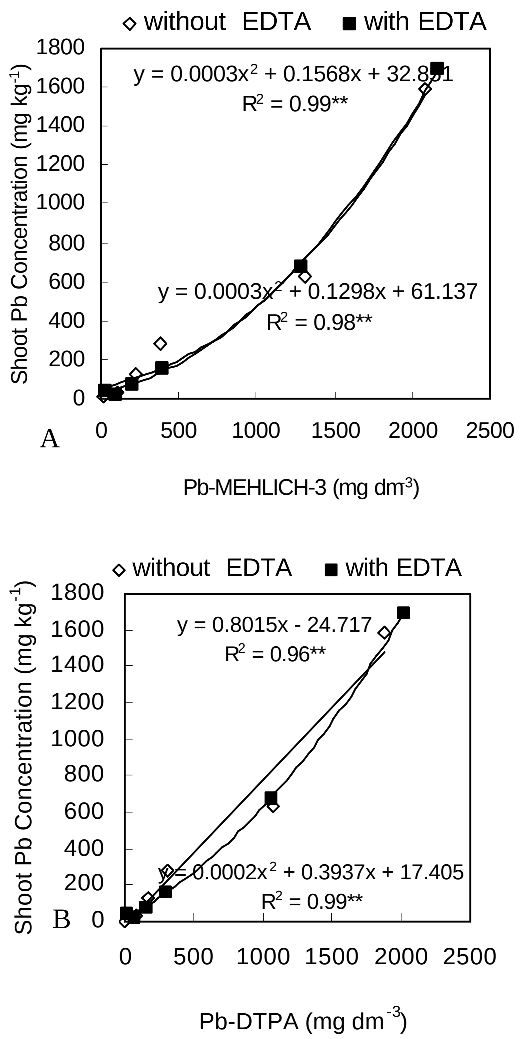


Figure 4 - Relation between Pb concentrations in the extracts obtained with (a) Mehlich-3 and (b) DTPA extracting solutions and the Pb concentrations in the shoots of 45-day-old maize plants, in the presence and absence of EDTA.

## Ionic speciation in the soil solution

Chemical analysis of the main ions present in soil solution showed that the total Pb, Fe and Al were

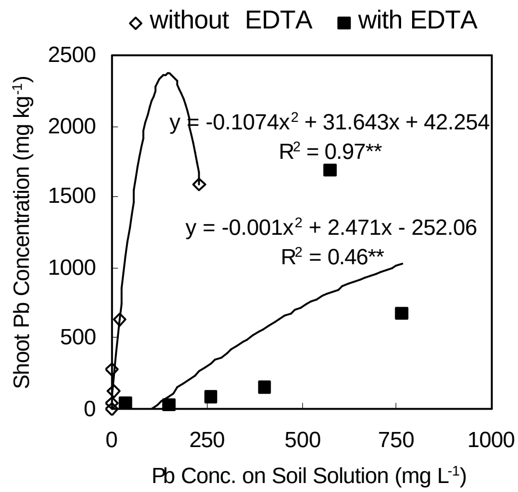


Figure 5 - Relation between Pb concentration in the soil solution and Pb concentrations in the shoots of 45-day-old maize plants, in the presence and absence of EDTA.

the most affected elements by Pb rates and EDTA application to the soil (Figure 6). Soil samples treated with EDTA presented considerable increase in the concentrations of these elements in the soil solution. Pb concentrations in the soil solution increased up to the rate of 1,200 mg kg$^{-1}$, decreasing afterwards and the opposite occurred with the Fe and Al concentrations (Figure 6). In the soil solution, concentrations of Fe between 30 and 500 mg L$^{-1}$ may cause toxicity to the several crop species (Sousa et al., 2004) and of Al above 3.6 mg L$^{-1}$ is known to drastically reduce crop yields, mainly maize (Sparks, 1995). In the present experiment, the Fe concentrations in the soil solution (Figure 6-b), with EDTA, were within the toxicity levels according to Sousa et al. (2004) for the five Pb rates. Similar behavior was observed for Al, the concentrations of which in the soil solution were within the toxicity range for the three first Pb rates (Figure 6-c). Accordingly to the soil solution concentrations, the Fe and Al shoot concentrations were also affected. The EDTA treatments showed an average shoot con-

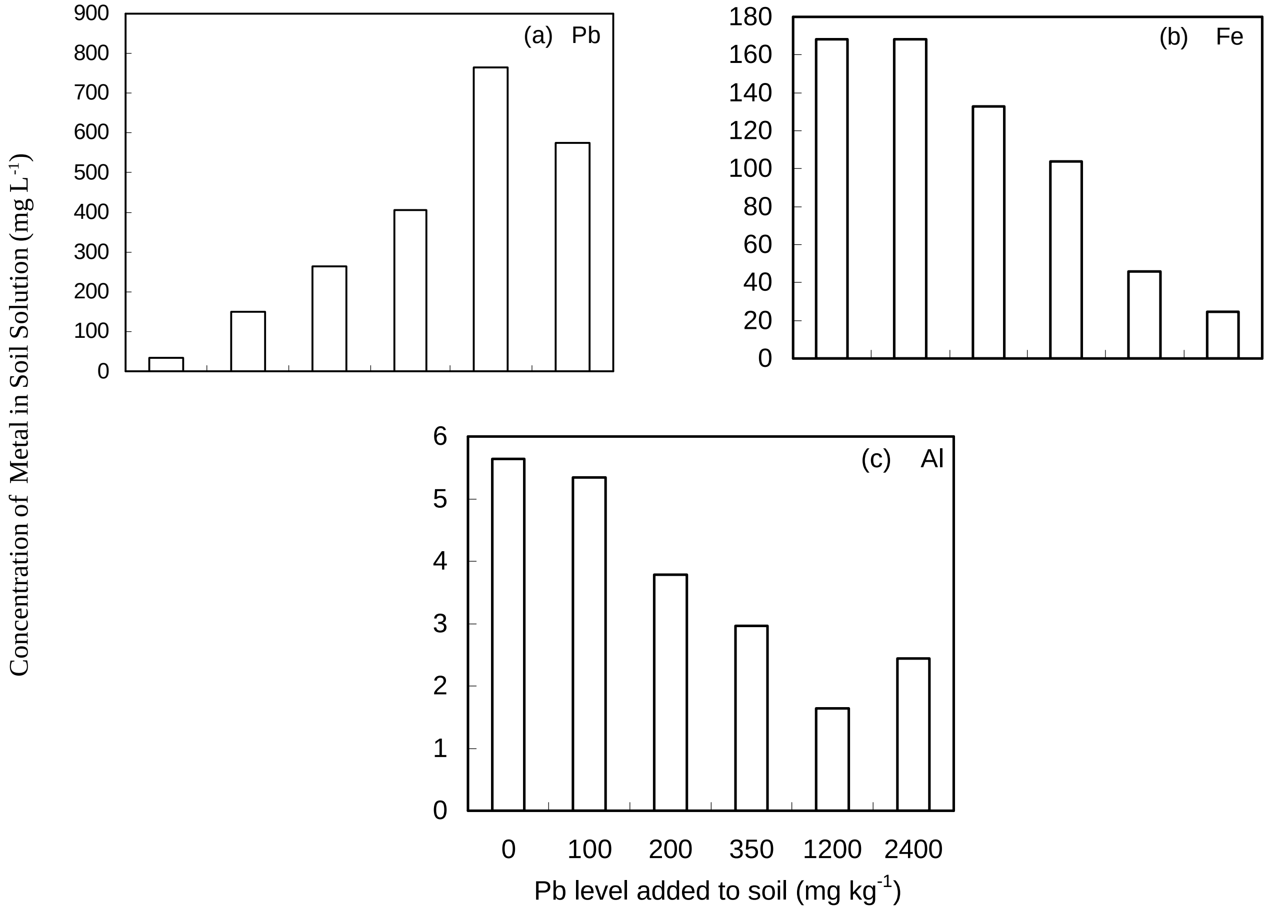


Figure 6 - Effects of Pb rates and EDTA applied to the soil on the concentrations of (a) Pb; (b) Fe; and (c) Al, in the soil solution.

Table 3 - Percent distribution of main ionic species in the soil solution for different Pb rates applied to the soil, in the presence and absence of EDTA.

| Ion applied | Ionic species | Pb rates, mg $kg^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 100 | 200 | 350 | 1,200 | 2,400 |
| | | % | | | | | |
| | | Without EDTA | | | | | |
| $Pb^{2+}$ | $Pb^{2+}$ | 71.3 | 66.1 | 68.5 | 67.3 | 74.2 | 77.6 |
| | $PbNO^{3+}$ | 15.1 | 22.2 | 19.1 | 20.3 | 16.4 | 18.4 |
| | $PbSO_4$ | 10.5 | 8.0 | 9.3 | 9.3 | 6.5 | 2.6 |
| $Fe^{3+}$ | $FeOH^{2+}$ | 21.0 | 21.4 | 24.9 | 30.0 | 40.2 | 60.7 |
| | $Fe(OH)^{2+}$ | 76.2 | 78.1 | 73.6 | 69.3 | 58.2 | 34.8 |
| | | With EDTA | | | | | |
| $Pb^{2+}$ | $PbEDTA^{2-}$ | 98.6 | 97.9 | 96.7 | 98.3 | 97.4 | 96.3 |
| | $PbHEDTA^{-}$ | 1.3 | 2.0 | 3.2 | 1.6 | 2.0 | 3.1 |
| $Fe^{3+}$ | $FeEDTA^{-}$ | 99.8 | 99.8 | 99.7 | 99.8 | 99.7 | 99.7 |
| $EDTA^{4-}$ | $PbEDTA^{2-}$ | 3.0 | 12.3 | 21.2 | 33.2 | 62.1 | 46.5 |
| | $AlEDTA^{-}$ | 3.4 | 3.3 | 2.8 | 1.8 | 1.0 | 1.4 |
| | $FeEDTA^{2-}$ | 52.0 | 51.9 | 41.0 | 32.1 | 14.1 | 7.5 |
| | $CaEDTA^{2-}$ | 33.3 | 23.2 | 22.0 | 24.7 | 14.0 | 27.7 |

centration of 396 mg $kg^{-1}$ of Fe and 486 mg $kg^{-1}$ of Al; the treatments without EDTA showed 294 mg $kg^{-1}$ of Fe 332 mg $kg^{-1}$ of Al. Therefore, high Fe and Al concentrations in the soil solution were the cause for maize dry matter reduction in treatments with EDTA.

The increase observed in soil solution Fe and Al concentrations, inversely related to Pb rates, is due to the high soil Fe and Al contents and their strong affinity with EDTA. Among the metals occurring in the soil, $Fe^{2+}$, $Al^{3+}$ and $Pb^{2+}$ have higher complex formation constants with EDTA (Baccan et al., 2001). Therefore, the chelant efficacy to bind Pb decreases as other high affinity elements are present, such as $Fe^{2+}$, $Cu^{2+}$, $Al^{3+}$, $Cd^{2+}$, $Zn^{2+}$ and $Co^{2+}$, drastically decreasing free $EDTA^{4-}$ in solution (Geebelen et al., 2002). When Pb is present in low concentrations, the formation of other metal-EDTA complexes is favored in relation to the $Pb\text{-}EDTA^{-2}$ complex. Under high Pb concentrations, the inverse will occur. Therefore, metals compete among each other for EDTA and the one present in higher concentration in the soil solution will prevail for the $metal\text{-}EDTA^{4-}$ complex formation. This fact is especially relevant for red Oxisols that naturally contain high Fe and Al contents.

Without EDTA application, the prevailing form was free $Pb^{2+}$, which corresponded to 71% (in average) of the total Pb-forms (Table 3). On the other hand, in treatments with EDTA, most Pb was bound to EDTA as $PbEDTA^{2-}$ (in average 97%) and no free $Pb^{2+}$ was found. Similar behavior was observed for Fe, that is, more than 90% of total Fe formed complex with EDTA ($FeEDTA^{-}$). From the total forms of $EDTA^{4-}$ complex, $PbEDTA^{2-}$ increased from 3.0 to 46.5%, corresponding to the rates of 0 to 2,400 mg $kg^{-1}$, respectively. The opposite tendency was observed for the complexes $AlEDTA^{-}$, $FeEDTA^{-}$ and $CaEDTA^{2-}$, which decreased with the increasing soil Pb rates. Chelants can desorb heavy metals from the soil matrix and form soluble complexes in the soil solution favoring metal uptake by plant roots (Vassil et al., 1998).

## CONCLUSIONS

The maize cultivar IAC-8333 was effective in accumulating Pb in the shoots presenting good potential as Pb-phytoextractor. It is not recommended to use EDTA to increase Pb extraction from Oxisol by maize plants. The extracting solutions Mehlich-3 and DTPA were effective for assessing Pb availability on soil.

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