Effects of Mobilizing Agents and Organic Manure on the Phytoextraction of Pb by Maize in Clay and Calcareous Sandy Soils

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Abstract:

A greenhouse pot experiment was conducted to investigate the application effects of ethylene diamine tetra acetic acid (EDTA) and low molecular weight organic acids (LMWOA: oxalic and citric acids) on the uptake of lead (Pb) by maize grown on clay and calcareous sandy soils treated and non-treated with farmyard manure (FYM). The results showed that the shoot and root dry matter of maize were lower for EDTA treatments than those of the control or LMWOA treatments. However, EDTA was more effective than LMWOA in increasing the Pb uptake by and translocation within maize, with more Pb accumulation by plants from the clay soil than from the calcareous sandy one. Applying EDTA in conjunction with organic manure (FYM) resulted in the less negative impact on the plant growth and the highest shoot Pb accumulation. Thus, FYM may be a suitable manure for increasing the performance of chelators to enhance the phytoextraction capacity and alleviate the toxicity of the metal and/or chelators.

Keywords: EDTA, LMWOA, Farmyard manure, Zea mays, Pb uptake.

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Introduction:

Heavy metal contaminated soils are of great concern because of its adverse environmental and health effects. Therefore, the clean of metal-contaminated sites is an urgent procedure to minimize adverse impacts and reuse polluted soils safely for food production. Current technologies to remediate heavy metal contaminated soils usually depend on in situ capping or excavation, removal and disposal of the contaminated material (Vangronsveld and Cunningham, 1998). However, there are several economic and logistic restrictions that face these techniques. To avoid these restrictions, new techniques are being developed to remediate heavy metal contaminated sites such as phytoremediation (Mench *et al*., 1994; Chaney *et al*., 1997; Lasat *et al*., 2002; Oste *et al*., 2002; Friesl *et al*., 2003; Illera *et al*., 2004; Usman *et al*., 2012). Phytorextraction is considered an applied phytoremediation technique that is a promising procedure to remove heavy metals from contaminated sites.

The efficiency of phytoextraction in the removal of heavy metals by plants is mainly depending on plant growth status, tolerance of heavy metals and metal availability (Usman and Mohamed, 2009). The plants used for phytorextraction can be classified into three types: hyperaccumulators, indicators and excluders (Ghosh and Singh, 2005; Usman and Mohamed, 2009). It has been reported that the hyperaccumulating plants are tolerant to toxic levels of heavy metals and can accumulate concentrations that are greater than: 10 mg/kg Hg, 100 mg/kg Cd, 1000 mg/ kg Cu, Cr, Co and Pb, and 10,000 mg/kg Ni and Zn in their above ground tissues tissues (Baker and Brooks, 1989; Usman *et al*., 2012).

Due to the slow growth and low biomass production of hyperaccumulators, agronomic crops with high biomass yields has been recently investigated for phytoremediation purposes (Luo *et al*., 2005; Meers *et al*., 2005; Neugschwandtner *et al*., 2008; Usman and Mohamed, 2009). However, using plants in phtoremediation without a chemical assistance cannot efficiently extract heavy metals from contaminated soils. In phytoextraction technique, synthetic chelates such as EDTA, CDTA, DTPA, EGTA, EDDHA and NTA as well as low molecular weight organic acids are usually used for enhancing heavy metals uptake by plants and hence, their phytorextraction capacity (Greman *et al*., 2001; Hovsepyan and Greipsson 2005; Usman and Mohamed, 2009). However, some synthetic chelators, particularly EDTA, may have a low biodegradability so that their application to metal contaminated soils can cause water pollution through the solubilization and leaching of heavy metals. In addition, they may be responsible for plant stress (Chen and Cutright, 2001; Neugschwandtner *et al*., 2008).

Plant growth can be considered one of the most important characteristics in terms of increasing the phytorextraction capacity. The application of organic amendments is a common practice to improve soil fertility and increasing plant biomass. However, these amendment may immobilize the metals and reduce their availability to plants (Walker *et* *al.*, 2004; Wei *et al.*, 2010). The effect of organic amendments on the availability of metal to plants depends upon several factors including organic matter nature, microbial degradability, composition and their impacts on biochemical soil properties (Walker *et al.*, 2003 and 2004). Recently, it has been speculated that the application of organic manure to the metal-contaminated soil may create a good environmental condition for mitigating the toxicity that is induced by high concentrations of soluble metal-ligand complexes in the soil solution and/or the toxicity of free chelators (Usman *et al.*, 2013). Few available literatures show effects of organic amendments on the chelator performance in enhancing the metal phytoextraction.

Though there are several studies showing effects of the chelating agents on the metal phytoextraction, few researchers have investigated their behavior with organic manure in different soils in this respect (Saifullah *et al.*, 2010). Therefore, the objective of this study is to investigate effects of EDTA and LMWOA in combination with farmyard manure on lead (Pb) uptake by maize grown in both calcareous sandy and clay soils.

Materials and Methods:

Sampling and characterization of soil and farmyard manure:

Surface soil samples (0-30 cm) were collected from two different soils (clay and calcareous sandy soils) at Assiut city, Egypt. All samples were air-dried, crushed and sieved through a 2-mm mesh prior to soil characterization and sorption studies. Table1 summarizes the main physico-chemical characteristics of the studied soils.

Soil pH was measured using a digital pH meter in a 1:1 suspension of soil-to-water ratio. Total soluble salts were measured in a 1:1 of soil to water extract by measuring the electrical conductivity (EC) using a digital EC meter. Calcium carbonate content was determined using the calcimeter method. Soil organic matter (OM) was determined by the Walkley and Black method (Jackson, 1973). The particle-size distribution of the soil was measured by the hydrometer method. The cation exchange capacity (CEC) of soils was determined using 1M NaOAC at pH 8.2 as a saturating solution and Na^+ ions were replaced by NH_4^+ ions using 1M NH_4OAC at pH 7. Sodium ions were measured by flame photometer (Jackson, 1973).

The pH and organic matter content of the used farmyard manure were 7.15 and 32.60%, respectively.

Table 1. Some physico-chemical characteristics of the studied soils.

Property	Unit	Claysoil	Calcareous sandy soil
Sand	g kg^{-1}	143	825
Silt	g kg^{-1}	331	111
Clay	g kg^{-1}	526	64
Textur		Clay	Sand
pH (1:1)		7.06	8.13
EC (1:1)	dS/m	0.99	2.67
$CaCO_3$	g kg^{-1}	4.2	143
Organic C	g kg^{-1}	3.7	0.60
CEC	cmol kg^{-1}	47.86	6.91
Total Pb	mg kg^{-1}	4.58	1.83

Pot experiment:

A greenhouse pot experiment was conducted to investigate the interactive effects of EDTA and organic acids as well as farmyard manure (FYM) on the availability of soil Pb and its uptake by maize grown on clay and calcareous sandy soils. The soil samples were artificially contaminated with 1000 mg Pb kg^{-1} of soil as $Pb(NO_3)_2$ and amended. Plastic pots were filled with 4 kg of the artificially Pb-contaminated clay and calcareous sandy soil samples treated and untreated with 30 g FYM kg^{-1}. Four seeds of corn were planted in each pot. The plants were thinned to two plants per pot after germination. Treatments of EDTA, citric acid and oxalic acid at 10 mmol kg^{-1} were applied to the soil samples after three weeks from planting. Basal fertilization of NPK at levels of 120 mg N kg^{-1}, 100 mg K kg^{-1} and 50 mg P kg^{-1} was added. All pots were maintained at a soil moisture content of field capacity. The experiment design was a randomized complete block with three replications.

Soil and plant analysis:

Maize plants were harvested after 55 days of planting. Shoots and roots were separated, washed with distilled water and then oven-dried at 70^oC to a constant weight to determine roots and shoots weights. Dried roots and shoots were ground and digested using a 2:1 mixture of HNO_3:$HClO_4$ acids. The digests were analysed for Pb by atomic absorption spectrophotometer (AAS).

In order to extract the readily available form of soil Pb, 10 g of each soil sample were reacted with 25 mL of 1 M NH_4NO_3 (Wang *et al.*, 2007). The suspension was then shaken and centrifuged. The supernatant was filtered through a Whatman No.42 filter paper. Lead concentrations in the filtered solutions were determined using AAS.

Statistical analysis

The mean values and standard deviation (±SD) of three replications are reported. The analysis of variance was performed using the statistic a computer program (Statsoft, 1995).

Results and Discussions:

Treatment effects on NH_4NO_3-extractable soil Pb

Figure 1 shows the soil Pb concentrations extracted by NH_4NO_3. Though a high total Pb concentration (1000 mg kg^{-1}) was added to the soil, only a low portion of Pb was extracted by NH_4NO_3 in the absence of EDTA. This is due to the redistribution of most added Pb among different fractions of the soil. The results showed also that the concentration range of NH_4NO_3-extractable Pb was wider in the clay soil (1.54-230.4 $mgkg^{-1}$) than in the calcareous sandy soil (1.71-89.3 $mgkg^{-1}$). Contrary to these findings, several authors found that fine textured soils exhibited more metal retention than coarse textured ones (Hooda and Alloway, 1994; Saifullah *et al.*, 2010). In the current study, the differences in the concentrations of NH_4NO_3-extractable Pb of both soils can be explained by the variation in the $CaCO_3$ content and pH in both soils. The high values of $CaCO_3$ content and pH of the calcareous sandy soil favor a high retention of Pb, resulting in low extractable Pb.

The addition of EDTA significantly increased the concentrations of the NH_4NO_3-extractable soil Pb, which were 24-150 fold in the clay soil and 27-44 fold in the calcareous sandy soil higher than those of the control soils. Citric acid was the only organic acid that significantly increased the concentration of NH_4NO_3-extractable Pb of the FYM treated and non-treated clay soil and the non-treated FYM calcareous sandy soil, which exhibited a small increase that was accounted by 1.48-3.76 fold higher than that of the control soil. The low or non-significant effect of LMWOA on increasing Pb mobility is mainly attributed to their fast biodegradation. Based on these findings, EDTA was more effective in mobilizing soil Pb than the low molecular weight organic acids. EDTA has been widely used as a mobilizing agent to release the heavy metal from insoluble solid phases by forming dissolved complexes due to its very high binding affinity with metals (Nowack, 2002; Saifullah *et al*., 2010; Almaroai *et al*., 2012). The log K of EDTA, citric acid and oxalic acid is in the order of EDTA (17.8) > citrate (7.98) > oxalate (6.16). As the ability of chelating the agent to chelate Pb is parallel with the log K, the more stable complex is (Qin *et al*., 2004; Yang *et al*., 2006). Therefore, the greater the stability of the EDTA-Mo^{+2} complex is the greater the effect of EDTA on increasing Pb solubilization and mobilization (compared to citrate and oxalate acids). The data suggested that the complex formation of the chelating agent such as EDTA and Pb could affect the metal precipitation and/or the metal retention to negatively charged soil surfaces (Dong-Mei *et al*., 2003; Shahid *et al*., 2012), resulting in increases in Pb solubility of the soil. These results showed that EDTA resulted in more NH_4NO_3-extractabile Pb in the clay soil than in the calcareous sandy one. Some researchers have suggested lower complexation of Pb by EDTA in the calcareous soils than that in the non-calcareous ones (Shahid *et al*., 2012). Soil pH may be the main factor that affects the efficiency of EDTA to mobilize the soil metal (Manouchehri *et al*., 2006; Shahid *et al*., 2012). It has been speculated that EDTA is the most effective at low soil pH and its metal binding efficiency tended to decrease with increasing the soil pH (Shahid *et al*., 2010).

Generally, the soil samples treated with FYM produced a lower Pb extractability compared to the non-treated ones. These reductions were found in the absence of EDTA as well as in the soil samples treated with oxalic and citric acids. The effect of organic amendment on increasing Pb sorption is mainly due to the highest affinity of Pb to organic matter, which reduces the metal solubility and mobility. By contrast, the present results showed that a higher concentration of NH_4NO_3-extractale Pb was found in the soil samples treated with EDTA in the presence of FYM. According to many reports, EDTA can release Pb from several soil compartments, especially Pb bound to the organic fraction (Shahid *et al*., 2012).

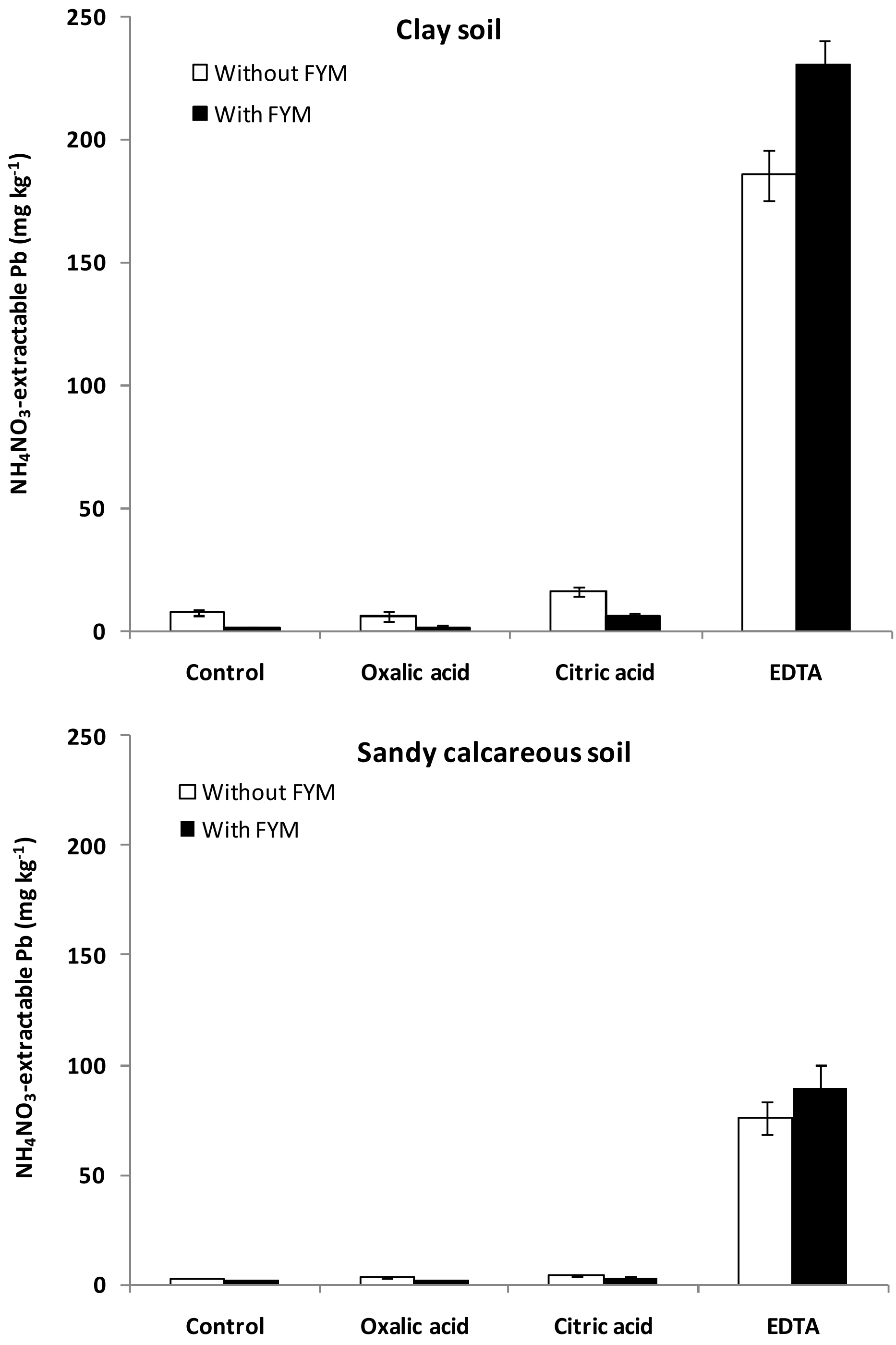

Figure 1. Effect of oxalic acid, citric acid and EDTA on NH_4NO_3-exractable Pb of FYM amended and un-amended soils; ± SD

Treatment effects on plant growth:

The dry matter of maize shoots and roots was higher in the clay soil than in the calcareous sandy soil (Fig. 2). It was generally lower in the EDTA treated soils than either in the control or in the LMWOA treated ones. The growth of maize plants was affected by FYM application. Applying FYM significantly simulated the biomass production of the plants compared to the control soil. In the FYM treated calcareous sandy and clay soils, , the dry matter of maize shoots significantly increased by 78 and 39%, respectively, for the control, 101 and 42%, respectively, using oxalic acid, 95 and 61%, respectively, using citric acid and 157 and 98%, respectively, using EDTA as compared to the FYM treated ones. These results confirm the beneficial effect of the organic amendment on the growth of maize plants even in the presence of EDTA, mainly due to its effect as an organic fertilizer.

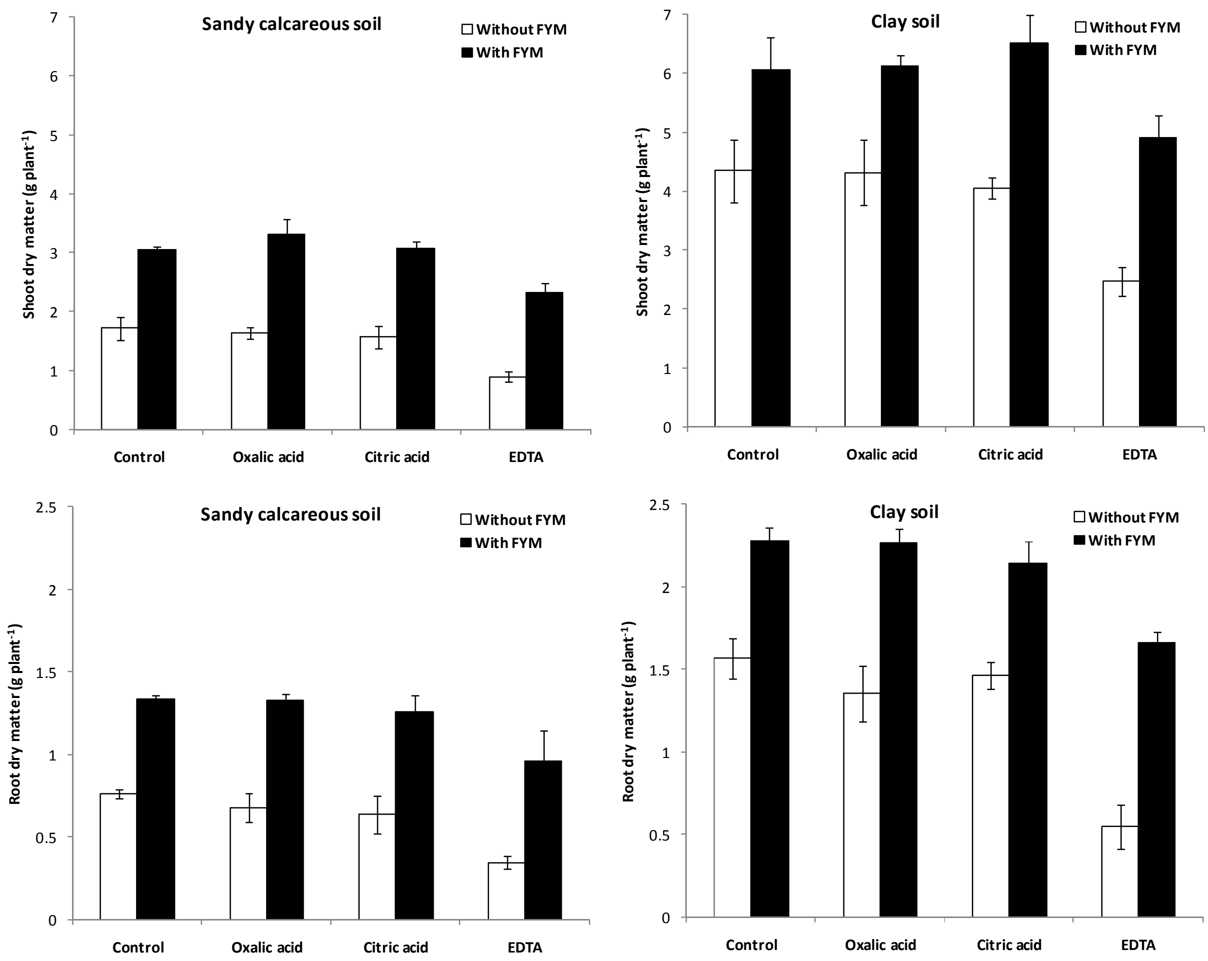

Figure 2. Effect of oxalic acid, citric acid and EDTA on shoot and root dry matter of maize plant grown in FYM amended and un-amended soils; ± SD.

Treatment effects on concentrations and accumulation of Pb by plants:

The concentrations of Pb in the roots and shoots of maize plants were affected by the applied treatments (Table 2). In the calcareous sandy soil, regardless the organic amendment, applying LMWOA showed small insignificant increases in the concentrations of Pb in both shoots and roots of maize plants. However, citric acid that was applied to the clay soil showed a significant increase in the Pb concentrations of the shoots. Overall, EDTA was the most effective mobilizing agent in increasing Pb concentrations in the shoots and the roots of maize. With applying EDTA, concentrations of Pb in the shoots of maize significantly increased from 6.51-7.62 mg kg^{-1} to 211-239 mg kg^{-1} in the calcareous sandy soil. Meanwhile, in the clay soil, shoot Pb concentrations increased from 12.7-16.6 to 468-492 mg kg^{-1} with EDTA apptication. It has been reported that enhancing Pb solubility and mobility following the application of EDTA is mainly responsible for the increase of Pb concentrations plant shoots (Saifullah *et al*., 2010; Luo *et al*., 2005). Previous studies have speculated that the increased Pb accumulation by plants occurs by the formation of the Pb-EDTA complex, which is the major form of Pb being extracted and translocated by plants (Vassil *et al*., 1998; Epstein *et al*., 1999; Luo *et al*., 2005; Zhao *et al*., 2010). However, the increase of the metal solubility in soils following the application of EDTA may result in increasing the metal toxicity to soil microorganisms and plants (Evangelou *et al*., 2007). In this context, the results of the current study showed that potential decreases in the root and shoot dry matter of maize plants were observed when the soil was treated by EDTA (especially in the absence of FYM). By contrast, oxalic acid and citric acid treatments did not significantly decrease the plant growth of maize. However, they were less effective than EDTA in enhancing metal concentration in shoot biomass.

Without using EDTA or LMWOA, shoot and root Pb concentrations of the plants grown in the soil treated with FYM were lower than those of the plants grown in the FYM non-treated soil. It may be explained by the lower soil Pb bioavailability induced by the application of organic amendment. Soil metal availability can be decreased by the organic amendment due to the transformation of metal from more readily available forms to some less or unavailable forms such as fractions associated with organic materials, carbonates or oxides resulting in a reduced metal content in the plants (Walker *et al*., 2004; Wei *et al*., 2010).

The potential effectiveness of the plant for phytoextraction is dependent on both shoot biomass and shoot metal concentration (Usman and Mohamed, 2009; Almaroai *et al*., 2012). Significant differences in shoot Pb uptake were found among the applied treatments (Table 2).. Lead uptake by maize plants grown in the FYM untreated calcareous sandy soil was 13.0, 14.8, 12.1, 216 µg $plant^{-1}$ for control, oxalic acid, citric acid and EDTA treatments. However, the respective Pb uptake by the plants grown in this soil that was amended by FYM was 19.8, 19.3, 25.3 and 491 µg $plant^{-1}$. Meanwhile, in the FYM un-amended and amended clay soil, the shoot Pb uptake was 72.1 and 76.8µg $plant^{-1}$, respectively, for the control, 81.7 and 81.0µg $plant^{-1}$, respectively, for oxalic acid, 213 and 290 µg $plant^{-1}$, respectively, for citric acid, and 1164 and 2502 µg $plant^{-1}$, respectively, for EDTA. These results indicate that both citric acid and EDTA significantly enhanced Pb uptake by maize grown in the soil. EDTA was the most effective chelating agent in enhancing the uptake of Pb by maize plants, mainly due to its effect on increasing shoot metal concentrations (Usman and Mohamed, 2009; Saifullah *et al*., 2010). EDTA is superior in solubilising the soil Pb for root uptake and translocation into shoots, mainly due to its strong chemical affinity for Pb (log K = 17.88) (Epstein *et al*., 1999; Kos and Lestan, 2004; Meers *et al*., 2005; Luo *et al*., 2006; Luo *et al*., 2005).

Applying EDTA in conjunction with FYM showed higher shoot dry matter compared to the EDTA treatment in the absence of FYM. However, there was not any significant difference in the shoot Pb concentration between them. Compared to the soil that was untreated by FYM, EDTA showed higher Pb shoot uptake in the presence of FYM. EDTA may increase the shoot Pb concentration and at the same time the organic manure may enhance the shoot dry matter of maize plants. Wei *et al.* (2010) found that the soil available metal and shoot metal concentrations were significantly reduced by applying chicken manure, but the metal extraction ability of plants increased due to the increased plant biomass. The results of the present study also showed that citric acid increased Pb uptake in most cases, but it was less effective than EDTA. This result is probably due to the lower soil Pb availability with citric acid application in comparison to that with adding EDTA. Precipitation and/or re-adsorption of the metal may be the main reason for the resulted lower efficiency of the single application of LMWOA in enhancing the metal bioavailability. In addition, the relatively low level of LMWOA (10 mmol kg^{-1}) that was applied in the current study may also be responsible for the low Pb availability to plants. However, application levels that are higher than 10 mmol kg^{-1}may be phytotoxic.

Table 2. Effect of oxalic acid, citric acid and EDTA in combination with FYM on the concentration and uptake of Pb by maize plants in calcareous sandy and clay soils; ± SD.

		Pb concentrations (mg kg^{-1})		Shoot Pb uptake	Pb concentrations (mg kg^{-1})		Shoot Pb uptake
		shoot	root	(µg $plant^{-1}$)	shoot	root	(µg $plant^{-1}$)
		Calcareous sandy soil			Clay soil		
Without FYM	Control	7.62±1.2	59.0±8.0	13.0±0.7	16.6±1.8	73.2±11	72.1±13
	Oxalic acid	8.90±2.4	56.1±9.6	14.8±4.8	18.8±4.5	86.4±10	81.7±23
	Citric acid	7.76±2.2	67.3±12	12.1±2.8	52.5±7.2	132±21	213±26
	EDTA	239±19	395±49	216±21	468±30	486±57	1164±192
With FYM	Control	6.51±1.8	53.0±10	19.8±5.1	12.7±1.2	54.4±7.7	76.8±10
	Oxalic acid	6.41±1.6	64.8±6.8	19.3±5.8	13.2±3.1	63.8±12	81.0±21
	Citric acid	8.27±2.1	62.0±11	25.3±5.6	44.8±7.4	104±10	290±28
	EDTA	211±26	425±41	491±66	507±89	443±73	2502±546

Treatment effects on phytoextraction efficiency

The phytoextraction efficiency depends upon the ability of a plant to accumulate and transfer the metal from the roots to the aboveground comportments of the plant (Usman and Mohamed, 2009; Almaroai *et a*l., 2012). The ratio of Pb concentration to root Pb concentration or the translocation factor (TF) can be applied to assess the capacity of a plant to transfer the metal from the roots to the shoots (Usman and Mohamed, 2009; McGrath and Zhao, 2003). The results generally showed that the Pb concentrations in roots were higher than those of maize shoots as indicated by the TF value that is lower than 1 for all treatments (except for EDTA in the clay soil treated with FYM) (Table 3). EDTA and citric acid treatments significantly increased the TF of Pb. The most effective treatment in enhancing Pb translocation was EDTA. The percentage of absorbed Pb translocated from the roots to the shoots of maize plants significantly increased from 22.5% in the control to 61.1% for EDTA in the FYM un-treated sandy soil. It also increased from 21.8% in the control to 24.4% for citric acid and to 54.8% for EDTA in the FYM treated sandy soil. Meanwhile, by applying EDTA and citric acid, the percentage of absorbed Pb translocated from the roots to shoots of maize plants grown in the FYM un-treated clay soil significantly increased from 38.7% in the control to 81.1 and 52.4%, respectively, and from 38.4% in the control to 77.0 and 56.6%, respectively, in the clay soil treated with FYM. These findings indicate that with EDTA application, a high portion of Pb tends to be accumulated by shoots and to translocate from the roots to the aboveground parts of plants. EDTA is well known to enhance Pb uptake and translocation from the roots to the aboveground parts, mainly due to the reduction of Pb sequestration in the roots (Andra *et al*., 2009; Jamil *et al*., 2009; Barrutia *et al*., 2010; Shahid *et al*., 2012). This can be considered as one of the most important characteristics for applying EDTA in phytoremediation.

Table 3. Effect of oxalic acid, citric acid and EDTA in combination with FYM on shoot to root ratio (TF) and metal absorbed by shoot/metal absorbed by entire maize plants (% of absorbed Pb).

Treatments		Calcareous sandy soil		Clay soil	
		TF	% of absorbed Pb	TF	% of absorbed Pb
Without FYM	Control	0.13	22.5	0.23	38.7
	Oxalic acid	0.17	27.9	0.22	40.8
	Citric acid	0.12	22.5	0.40	52.4
	EDTA	0.61	61.1	0.97	81.1
With FYM	Control	0.12	21.8	0.24	38.4
	Oxalic acid	0.10	19.8	0.22	36.0
	Citric acid	0.13	24.4	0.43	56.6
	EDTA	0.50	54.8	1.16	77.0

The values of remediation factor (RF) for Pb with adding EDTA in both soils and adding citric acid in the clay soil were higher than that for the control soil (Fig. 3). The highest value of Pb remediation factor was obtained for EDTA treatment. The highest amount of Pb removed by maize plants after EDTA addition reached 0.123% of the total soil Pb content, suggesting a very low amount of Pb could be removed in comparison to the total amount of Pb in the soil. Based on these findings, however, EDTA that was applied in conjunction with organic manure showed less negative impact on the plant growth, mainly due to providing nutrients such as N, P, and K for plant growth. Therefore, applying FYM may create a suitable condition for alleviating the toxicity of the metal, or chelator and/or soluble metal-ligand complexe in the soil solution. These results suggest that the success of phytoextraction process is dependent upon the plant growth, soil characteristics and soil additives that maximize the removal of the metal from the contaminated soil. In the presence of FYM, the used concentration of EDTA (10 $mmolkg^{-1}$) had low negative effect on the plant growth and significantly enhanced Pb extraction. Therefore, good growth of maize plants with applying an organic manure and a high shoot metal concentration with adding EDTA may encourage the roots to remove more metal from soil and increase the metal uptake by the aboveground biomass of plants.

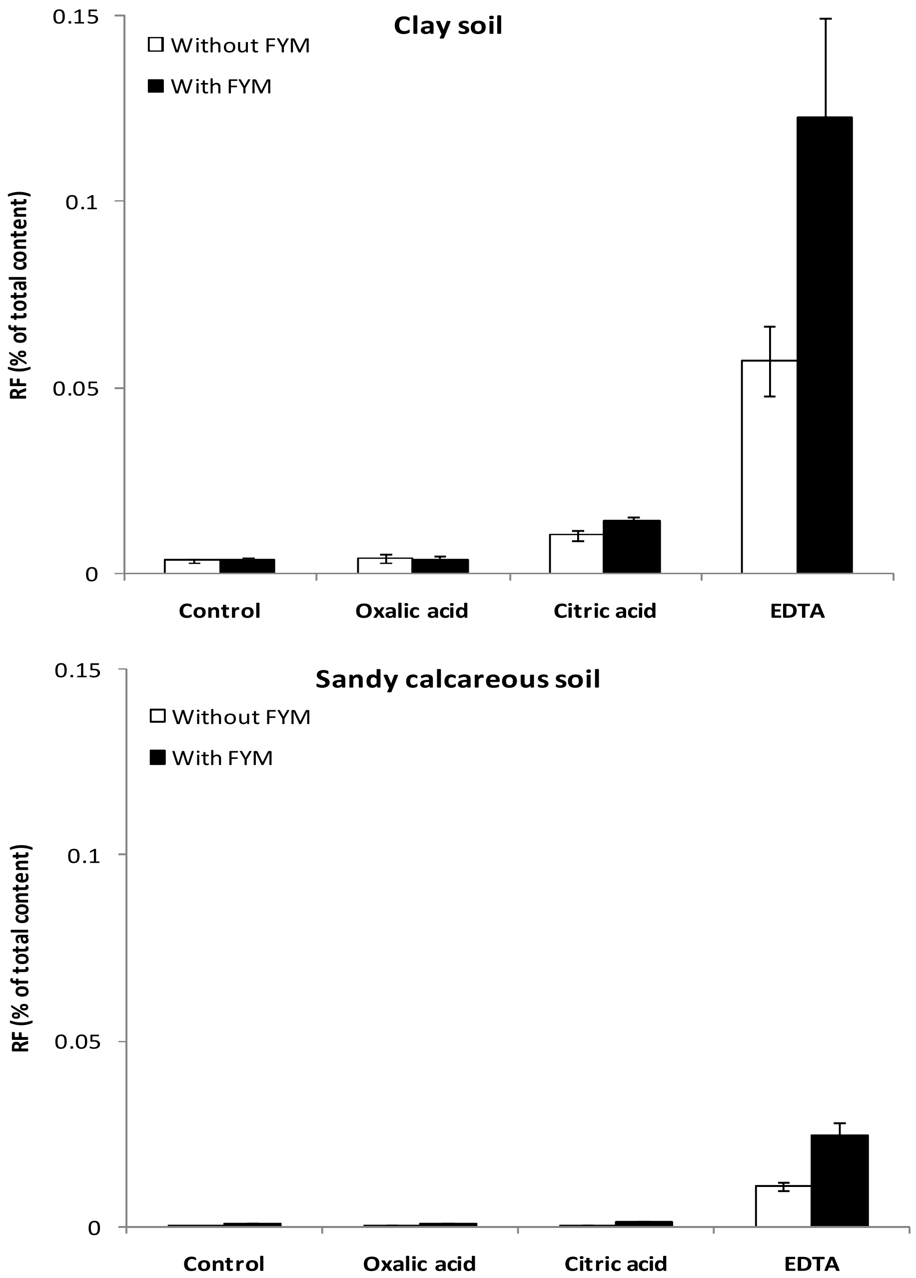

Figure 3. Effect of oxalic acid, citric acid and EDTA on the remediation factor (RF) of FYM amended and un-amended soils; ± SD.

Conclusions:

The results of this study showed that EDTA was highly effective in the enhancement of the Pb uptake by and translocation within maize plants. The results suggest that the success of phytoextraction is dependent upon the plant growth, soil characteristics and soil additives that maximize the removal of the heavy metal from the contaminated soil. Applying EDTA in conjunction with FYM showed higher shoot dry matter compared to EDTA treatment in the absence of FYM, but there were no significant differences in shoot Pb concentrations between them. However, EDTA showed higher Pb shoot uptake in the presence of FYM compared to the soil that was not amended by FYM. Thus, the application of EDTA in conjunction with organic manure to increase the metal uptake by plant shoots may be a novel approach for phytoextraction purpose. Further studies are required to evaluate the interactive effects of mobilizing agents and organic amendments derived from different sources on phytoextraction efficiency.

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تأثير عوامل محفزة للحركة والسماد العضوي على الاستخلاص الحيوي للرصاص بواسطة الذرة الشامية في الاراضى الطينية و الرملية الجيرية

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الملخص:

أجريت تجربة أصص بالصوبة لدراسة تأثير إضافة كل من EDTA وحامض الاوكساليك وحامض الستريك للتربة على استخلاص الرصاص وامتصاصه بواسطة الذرة الشامية في الاراضى الطينية والرملية الجيرية المعاملة والغير معاملة بالسماد العضوي (FYM) وقد لوضحت النتائج التالية:

١- نقص في الوزن الجاف لكل من المجموع الخضري والجذري لنباتات الذرة الشامية في الاراضى المعاملة بـ EDTA عن تلك المعاملة بحامض الاوكساليك وحامض الستريك

٢- وجود تأثير كبير لإضافة الـ EDTA للتربة على كمية الرصاص الممتص والمنتقل إلى نبات الذرة الشامية مع تراكم أكثر للرصاص في النباتات النامية في الاراضى الطينية بالمقارنة بتلك النامية في الاراضى الرملية الجيرية

٣- نقص نمو النبات وتراكم الرصاص به في الاراضى المضاف إليها الـ EDTA مع السماد العضوي (FYM)

٤- زيادة تأثير المواد المخلبية على زيادة الاستخلاص الحيوي للرصاص بواسطة نباتات الذرة الشامية وسمية المواد المخلبية بإضافة السماد العضوي للتربة الملوثة.