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Chelate-Assisted Phytoremediation of Cu-Pyrene-Contaminated Soil Using *Z. mays*

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Abstract This study compares the efficiency of a synthetic chelate (ethylenediaminetetraacetic acid-EDTA), a natural low-molecular-weight organic acid (citric acid), and their combination for phytoremediation of Cu-pyrene co-contaminated soils. *Zea mays* was grown in each soil and amended with citric acid and/or EDTA to understand the effect of chelates during phytoremediation of contaminated soils. In Cu or pyrene-contaminated soil, plant growth was negatively affected by EDTA (43 %) and citric acid (44 %), respectively, while EDTA + citric acid promoted (41 %) plant growth in co-contaminated soil. EDTA and EDTA + citric acid increased the phytoextraction of Cu in Cu-contaminated and co-contaminated soils, respectively. In pyrene-contaminated soil, all tested chelates increased the dissipation of pyrene reaching 90.4 % for citric acid, while in co-contaminated soil, only citric acid or EDTA + citric acid enhanced pyrene dissipation. These results show that *Z. mays* can be effective with the help of chelates in phytoextraction of Cu and dissipation of pyrene in co-contaminated soil.

Keywords EDTA · Citric acid · *Z. mays* · Phytoremediation

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1 Introduction

Heavy metals including Cu can affect the way land is used in the future because of their non-biodegradable nature. They can cause varying toxicities to plants and as such could affect vegetation growth (Chigbo and Batty 2013). Polycyclic aromatic hydrocarbons (PAHs) including pyrene have also become a problem to the soil environment as a result of processes including wastewater irrigation and industrial activities (Shi et al. 2005). High concentrations of Cu in the environment pose a risk to plant species by reducing plant growth and photosynthesis as well as by inducing oxidative stress (Schill et al. 2003; Gunawardana et al. 2011). Pyrene, on the other hand, is photomutagenic; and since the simultaneous exposure to light and pyrene by humans is inevitable, there is a threat to human health (Yan et al. 2004). Therefore, a robust and economical technology for treatment of these pollutants is required, and phytoremediation may have the potential to fully remediate the soils contaminated with Cu and pyrene.

Various studies have shown the role that plants play in the uptake of metals (Ebbs and Kochian 1998; Chen and Cutright 2001) as well as in the remediation of the soil contaminated with organic contaminants (Binet et al. 2000; Wang et al. 2012). However, the ability of plants to remediate PAH has been low, partly due to their recalcitrant nature or low solubility in the soil (Ke et al. 2003). On one hand, this could be beneficial as the toxicity of PAHs to plants is decreased; while on the other hand, it could pose a long-term problem, since PAH will remain in soil and not biodegradable.

A large number of studies have been carried out on the uptake of metals with the help of chelates such as ethylenediaminetetraacetic acid (EDTA), citric acid, nitriliotriacetic acid (NTA), or their combinations (Nowack et al. 2006; Jean et al. 2008). EDTA is a synthetic chelating agent that is not biodegradable in the soil (Wasay et al. 1998). EDTA plays an important role in phytoextraction of metals from the soil by complexing the metals and increasing their concentration in shoot of plants. Citric acid, on the other hand, is a natural low-molecular-weight organic acid that is biodegradable (Jean et al. 2008). These chelates have high affinity for metals and are able to increase their bioavailability in the soil. This helps to increase the uptake of these metals to the upper part of plants during phytoremediation. Similarly, various studies have shown the role of chelates including humic acid in facilitating the degradation of PAHs in the soil directly or indirectly by stimulating microbial activity (Ke et al. 2003). However, very few studies have investigated the role of chelates during phytoremediation of PAH and heavy metal in co-contaminated soils.

The aim of this study is to understand the role of two chelating agents—a synthetic chelate (EDTA) and a naturally occurring organic acid (citric acid)—and their combination in the dissipation of pyrene and the concurrent phytoextraction of Cu by *Z. mays* in a co-contaminated soil. Although there are many Cu hyperaccumulators due to their less successful in situ application, e.g., low biomass, *Z. mays* was chosen because of its high biomass production and ability to tolerate higher concentrations of heavy metals (Wuana and Okieimen 2010).

2 Materials and Methods

2.1 Soil Preparation

A standard commercial top soil (Travis, UK) was sieved (2 mm sieve) for removal of coarse particles. Air-dried soil of 250 g was initially spiked with pyrene by dissolving 100 mg of pyrene in 25 mL of acetone, and then mixed with 750 g of soil once the acetone had volatilized completely in the fume hood. Acetone of 25 mL was also added to control and other soil treatments. Cu of 50 mg kg^{-1} was prepared by dissolving 0.126 g of $CuSO_4$ and was added singly to pyrene-spiked soils and fresh soils. The spiked soil was thoroughly mixed by sieving and stored in a dark room for equilibration for 28 days before planting.

2.2 Experimental Set-Up

The experimental layout was designed in a randomized block of 16 treatments with three replicates of each. Pots with no plants were included in order to observe non-plant-facilitated dissipation of pyrene. Each spiked soil of 1 kg was placed in plastic pots (12.5 cm in height). One 3-week-old seedling of *Z. mays*, with a uniform size of about 3–4 cm with three leaves, was transferred into a pot. The chelates used in the present study were EDTA and citric acid, which were applied after 15 days of transplanting the *Z. mays* in order to allow for acclimatization. Treatments included the control soil (without application of chelate), 0.146 g kg^{-1} of EDTA, 3 g kg^{-1} of citric acid, and 0.146 g kg^{-1} EDTA+3 g kg^{-1} citric acid applied as solutions to each soil surface at doses of 48.6 mg kg^{-1} and 1 g kg^{-1} for EDTA and citric acid, respectively, for 3 weeks to reduce the effect of the chelates on plant growth and as suggested by Wenzel et al. (2003), split applications were more effective. After 60 days of growth, plants were harvested by cutting the shoots just above the soil surface and washed with deionized water. Each pot was then emptied, and the roots were separated from the soil by washing with running tap water. The roots were then rinsed with deionized water three times to remove all soil particles. All samples were oven-dried to constant weight at 65 °C for 72 h, and then weighed for biomass calculations and plant analysis.

2.3 Analysis of Plants and Soil Samples

Approximately 0.2 and 0.1 g of ground shoot and root dry matter, respectively, were digested using 5 mL of 30 % HNO_3 at 90 °C for 8 h. Digested plant samples were then analyzed for total Cu using flame atomic absorption spectroscopy (FAAS). For soluble Cu in the soil, approximately 5 g of soil was mixed with 50 mL of deionized water and shaken for 2 h. The solution was filtered and analyzed using FAAS. The metal translocation ration was calculated as the ration of the concentration of Cu in root to Cu concentration in root. For determination of pyrene concentration in the soil, approximately 5 g of fresh soil sample spiked with p-terphynyl-d14 was extracted with 15 mL of 2:1 hexane:acetone mixture and 5 mL of 4:1 acetone:triethylamine mix, after approximately 7 g of sodium sulfate was added to remove any moisture and extracted using the microwave extraction unit (CEM MARS). The supernatant of 1 mL was filtered through 2 g of silica gel column with 10 mL elution of 1:1 hexane:dichloromethane. The extract was concentrated by evaporation of the dichloromethane under a stream of nitrogen, and the residue was dissolved in hexane with a final volume of 1.0 mL for GC analysis. Separation was achieved according to the following program: the initial oven temperature was 40 °C (held for 4 min) and increased to 320 °C at 40 °C/min (held for 2 min). Helium was used as the carrier gas. Extracts were analyzed by a capillary GC/mass spectrometer (Agilent GC-MS) in selective ion mode (SIM), and quantification was done by comparison against an established 6-point calibration curve. The average percentage recovery for surrogate was 86.9 %.

2.4 Statistical Analysis

All treatments were replicated three times. The mean and standard error (SE) of each treatment was calculated using Microsoft Office Excel 2007. The comparisons of the normally distributed shoot dry matter, Cu concentration, and accumulation, as well as of the soil residual pyrene, were carried out by one-way analysis of variance using Minitab 16.0. When a significant difference was observed between treatments, multiple comparisons were made by the Tukey HSD test.

3 Results

3.1 Effects of EDTA and Citric Acid on Growth of *Z. mays*

In the absence of chelates, *Z. mays* planted in Cu-contaminated soil showed normal development and no visual symptoms of toxicity to Cu. With the application of 3-mmol kg^{-1} soil, EDTA significantly inhibited ($p<0.05$) plant growth in the soil contaminated with Cu only (Figs. 1a, b). A 43 % significant reduction ($p<0.05$) in shoot dry matter with the addition of EDTA was observed. Citric acid or a combination of citric acid and EDTA did not significantly reduce shoot dry matter yield. Plants were slightly chlorotic and visibly stunted with EDTA application at the end of the experiment. They also appeared to wilt on day 1 and 2 when EDTA was added.

Co-contamination with Cu and pyrene caused significant inhibitory effects in *Z. mays* (Fig. 1a). Shoot dry matter yield significantly decreased by 47 % relative to control treatments (no contamination and no chelates).

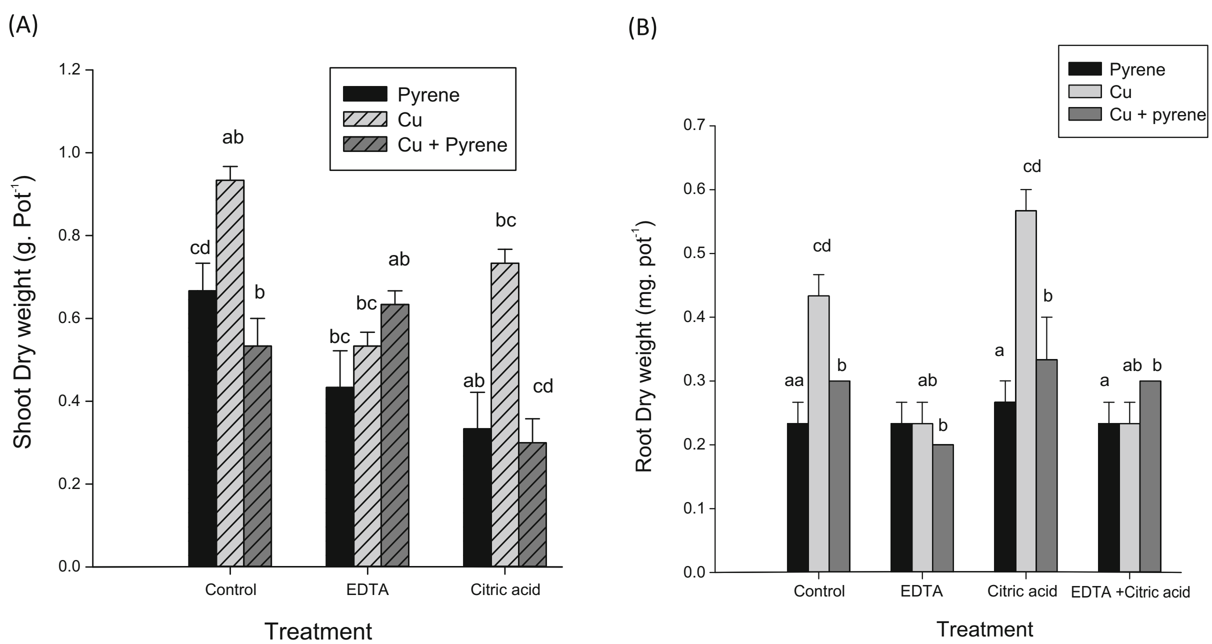

Fig. 1 Effects of chemical amendments and pollutant combination on shoot and root dry weight of *Z. mays* after 60 days. *Bars* (means±SE, $n=3$) with *different letters* are significantly different in each contaminant group based on Tukey HSD ($p\leq0.05$)

The combined application of EDTA and citric acid to co-contaminated soils promoted (41 %) the growth of *Z. mays*, while the single application of either EDTA or citric acid did not seem to have an effect on the shoot dry matter yield of *Z. mays.*

The application of Cu, pyrene, or Cu + pyrene did not affect the root biomass of *Z. mays* (Fig. 1b). In addition, the application of chelates including EDTA, citric acid, and EDTA + citric acid to pyrene and Cu + pyrene co-contaminated soil did not significantly ($p>0.05$) affect the root biomass of *Z. mays* relative to control treatments. However, EDTA and EDTA + citric acid significantly reduced the root biomass of *Z. mays* from 0.43 to 0.23 g pot^{-1} in single Cu-contaminated soil.

3.2 Effects of EDTA and Citric Acid on Solubility of Cu in Soil

The addition of EDTA significantly increased the water extractable Cu in single Cu-contaminated soil relative to contaminated soil with no chelates (Table 2). This significant increase was not observed in soil co-contaminated with Cu and pyrene. The present result showed that EDTA increased the water extractable Cu from 0.73 to 1.84 mg kg^{-1} in single Cu-contaminated soil. Citric acid did not significantly ($p<0.05$) affect the concentration of soluble Cu in single Cu-contaminated soil or co-contamination. The Cu mobilized by EDTA in single Cu-contaminated soil was to a significant ($p<0.05$) extent higher than that of citric acid or combination of citric acid and EDTA. The combined application of EDTA and citric acid to single Cu-contaminated soil did not significantly ($p>0.05$) affect the extractable Cu. However, when the soil was co-contaminated with pyrene, the addition of EDTA and citric acid significantly ($p<0.05$) increased the concentration of soluble Cu from 0.40–2.12 mg kg^{-1}.

3.3 Effect of EDTA and Citric Acid on Shoot and Root Cu Accumulation

For single Cu soil contamination, the mean accumulation of Cu in the shoot of *Z. mays* increased with the application of EDTA and decreased with the combined application of EDTA and citric acid (Table 1). Without chelates, *Z. mays* accumulated 18.6 μg pot^{-1} of Cu. The increase in Cu accumulation in *Z. mays* shoot as compared to control pots where EDTA was applied was significant ($P<0.05$), increasing by 35 %. Although EDTA was more effective in enhancing shoot accumulation of Cu under single soil Cu contamination, citric acid did not affect Cu shoot accumulation. Results showed that Cu accumulation in the shoot of *Z. mays* remained at 12.4 μg pot^{-1}. When EDTA and citric acid were combined, the shoot accumulation of Cu significantly reduced 1.66 fold when compared to control treatments (no chelates). Under co-contamination of Cu and pyrene, the combined application of EDTA and citric acid affected shoot accumulation of Cu. Results showed a significant ($p<0.05$) 2.77-fold increase in Cu accumulation.

The root accumulation was affected in a different way. For example, under single Cu contamination, the combination of EDTA and citric acid significantly ($p<0.05$) decreased the root accumulation of Cu from 25.65 to 18.09 μg pot^{-1}. In co-contaminated soils, the application of chelates showed a significant reduction with the addition of EDTA or citric acid. The effect of EDTA on root accumulation of Cu was less prominent, but nevertheless was significant when compared to combined treatment of EDTA + citric acid or treatments with no chelates.

Table 1 Shoot and root accumulation and TF per treatment (mean values±SE, $n=3$)

Treatments	50 mg kg^{-1} Cu			50 mg kg^{-1} Cu+100 mg kg^{-1} pyrene		
	Shoot accumulation (μg kg^{-1})	Root accumulation (μg kg^{-1})	TF	Shoot accumulation (μg kg^{-1})	Root accumulation (μg kg^{-1})	TF
0.146 g kg^{-1} EDTA	28.58±0.85	25.41±3.97	0.50±0.02	16.57±1.53	20.23±0.27	0.26±0.09
3 g kg^{-1} citric acid	12.39±0.74	26.44±2.04	0.36±0.01	8.60±1.39	29.59±4.76	0.32±0.03
0.146 g kg^{-1} EDTA + 3 g kg^{-1} citric acid	11.18±0.75	18.09±2.57	0.23±0.02	44.05±1.92	54.85±2.86	0.27±0.02
No chelates	18.60±1.05	25.66±1.44	0.34±0.02	15.88±2.00	53.88±0.87	0.17±0.02

Table 2 Soluble soil Cu and total plant removal of Cu per treatment (mean values±SE, n=3)

Treatments	50 mg kg^{-1} Cu		50 mg kg^{-1} Cu+10 mg kg^{-1} pyrene	
	Soluble Cu (mg kg^{-1})	Total Cu removal (μg pot^{-1})	Soluble Cu (mg kg^{-1})	Total Cu removal (μg pot^{-1})
0.146 g kg^{-1} EDTA	1.84±0.02	53.99±2.36	0.528±0.03	36.81±1.68
3 g kg^{-1} citric acid	0.64±0.03	38.84±2.36	0.426±0.009	38.19±6.16
0.146 g kg^{-1} EDTA + 3 g kg^{-1} citric acid	0.61±0.007	29.28±3.11	2.12±0.04	98.90±1.15
No chelates	0.73±0.01	44.26±2.29	0.462±0.014	69±2.31

3.4 Effect of EDTA and Citric Acid on Translocation of Cu and Total Removal of Cu by *Z. mays* from Contaminated Soils

Cu translocation from the root to the shoot of *Z. mays* was affected by chelates. The addition of EDTA resulted significantly (p<0.05) higher translocation ratios of Cu after 60 days of planting in single Cu-contaminated soil. The present result showed that the translocation of Cu from root to the shoot of *Z. mays* reached 0.50 with EDTA application and had increased by 2.36 fold when compared to control treatments (metal with no chelates). The translocation of Cu with the application of citric acid was less efficient when compared to EDTA but nevertheless had significantly increased from 0.34–0.36.

In co-contaminated soils, the translocation factor for Cu reached 0.17 without chelate application. The combined application of EDTA and citric acid dramatically increased Cu translocation without any severe toxicity symptoms being observed. The net removal of Cu from the soil increased from 69.0–98.90 μg pot^{-1} with EDTA + citric acid application in co-contaminated soils (Table 2), while single application of EDTA or citric acid were ineffective for the removal of Cu in the soil.

3.5 Effect of EDTA or Citric Acid on Residual Pyrene Concentration in Soil

When the soil was contaminated with pyrene only, all the chelates applied significantly (p<0.05) decreased the residual pyrene in soil when compared to soils with no chelate application. Results showed that the application of citric acid significantly decreased the residual pyrene from 20.09–7.46 mg kg^{-1}. Correspondingly, EDTA and EDTA + citric acid also significantly decreased the residual pyrene concentration from 20.09–13.06 and 12.61 mg kg^{-1}**,** respectively.

In Cu + pyrene co-contaminated soil, the effect of applied chelates varied. EDTA did not seem to enhance the dissipation of pyrene when compared to planted soil without the application of chelates (Fig. 2). The soil residual pyrene concentration remained at 23.25 mg kg^{-1} representing a 69 % dissipation of pyrene in soil over 60 days of planting. Interestingly, the application of citric acid and EDTA + citric acid significantly decreased the residual pyrene concentration from 15.5–7.69 and 10.61 mg kg^{-1}, respectively, when compared to the planted soil without the application of chelates.

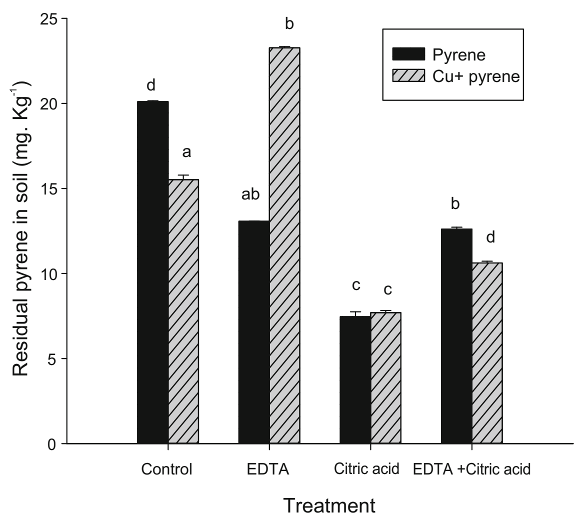

Fig. 2 Effects of chemical amendments and pollutant combination on residual pyrene concentration in soil after 60 days. *Bars* (means±SE, n=3) with *different letters* are significantly different in each contaminant group based on Tukey HSD (p≤0.05)

4 Discussions

4.1 Plant Growth

The presence of Cu in the soil did not affect the growth of *Z. mays*, while pyrene and a combination of pyrene and Cu significantly decreased the growth of *Z. mays* after 60 days of planting. Visual symptoms of toxicity, like wilting and chlorosis, were observed in *Z. mays* leaves growing in single Cu-contaminated soil with applied chelates. However, relative to control, Fig. 1 shows that only EDTA application had a significant effect on dry matter. The reduction in shoot dry matter after EDTA treatment is possibly due to the toxicity of EDTA itself and the metal-EDTA complexes (Chen and Cutright 2001; Vassil et al. 1998). Obviously, the comparatively low biomass reduction observed with EDTA in the present study could be due to the lower concentration of EDTA used (0.15 g kg^{-1}). The application of citric acid or a combination of citric acid and EDTA did not affect plant growth under single Cu soil contamination. Since adequate concentrations of natural low molecular weight organic acids (NLMWOA)—including citric acid—have the ability to detoxify intracellular heavy metals through binding (Lee et al. 1977), the concentration of citric acid applied to the contaminated soil was most probably sufficient to detoxify the intracellular Cu and hence limit plant growth inhibition. In this study, the application of EDTA, or citric acid alone, or in combination did not significantly affect the growth of *Z. mays* in pyrene-contaminated soil. As shown in Fig. 1a, citric acid and EDTA caused a slight but nonsignificant decrease in shoot dry matter of *Z. mays*. This nonsignificant decrease in shoot dry matter may have been as a result of the toxic effect that the pyrene-contaminated soil already had on the growth of *Z. mays*. It is also possible that the concentrations of chelates applied may not have been enough to cause a more significant toxicity effect on the *Z. mays* than the one caused by pyrene contamination. In contrast, under co-contamination of Cu and pyrene, the combined application of EDTA and citric acid promoted the growth of *Z. mays*. There was a significant ($p<0.05$) increase in shoot dry matter of *Z. mays* indicating that a combination of EDTA and citric acid at the present concentration could alleviate the growth inhibition caused by pyrene and copper co-contamination and increase plant tolerance to adverse environmental conditions. Gunawardana et al. (2011) observed that sulfate and citric acid treatments significantly increase biomass yield of *L. perenne*, although it also enhanced accumulation of Cu. It is likely that when *Z. mays* is exposed over a longer period to EDTA + citric acid, reduction in biomass of *Z. mays* could be observed. However, it is also expected that the longer *Z. mays* absorb Cu, the higher the amount of Cu is extracted.

4.2 Concentration and Accumulation of Cu

Chemically enhanced phytoextraction has been proposed as an effective approach for the removal of heavy metal from soils using plants (Baylock et al. 1997; Liphadzi et al. 2003). Several chelating agents such as EDTA, citric acid, EDDS, and salicylic acid have been tested for their ability to mobilize and increase the accumulation of heavy metals (Luo et al. 2005; Turgut et al. 2004; Yang et al. 2011). In this study, the highest concentration and accumulation of Cu reached 53.8 mg kg^{-1} DW and 28.57 μg pot^{-1}, respectively, in *Z. mays* shoots after the application of EDTA to Cu-contaminated soil. This is about three times the concentration or twice the accumulation observed in plants without the application of chelates, with the application of citric acid, or the combination of citric acid and EDTA. The level of enhancement observed could be due to the low concentration of EDTA used as well as low concentration of Cu. Similar results were observed by Wu et al. (2004) where 3 mmol kg^{-1} of EDTA significantly enhanced shoot uptake of Cu. When EDTA is applied to the soil, its initial action is to complex soluble metals in the soil solution. This reduces the activity of the free metals, while the dissolution of bound metal ions begins to compensate for the shift in equilibrium (Baylock et al. 1997). In the present study, the application of EDTA to single Cu-contaminated soil increased the H_2O extractable Cu from 0.73 to 1.84 mg kg^{-1} (Table 2). Among the chelates evaluated in this study, EDTA appeared to most effectively solubilize soil-bound Cu and maintain a high soluble Cu concentration in single Cu-contaminated soil. Under co-contamination, the combined application of EDTA and citric acid was more effective than the single application of EDTA or citric acid. Soluble metals are potentially bioavailable and can either be taken up by plants, leached or dissolved by the soil exchange sites (Kim and Li 2010). The effectiveness of EDTA in enhancing the shoot accumulation of Cu in single Cu-contaminated soil was significantly higher than that of citric acid and a combination of citric acid and EDTA (Table 1). Under single soil Cu concentration, the addition of citric acid seemed not to enhance the uptake of Cu in the shoot of *Z. mays*. Wu et al. (2003) observed that low molecular weight organic acids including citric acid had a very small effect on the concentration of Cu, Zn, Cd, and Pb in the shoot of *E. splendens* when compared to EDTA. The non-increase of Cu in the shoot of *Z. mays* with the application of citric acid could be as a result of the lower stability of the metal complexes formed, and also because citric acid is weak and biodegradable. Probably, due to the biodegradation of organic acids like citric acid, it is possible that the pH of the soil will increase as a result of consumption of H^+ from carboxylic acid and liberation of OH^- and CO_2 (Gramss et al. 2004). This results in a lack of complexing agents, and as such, the bioavailability of Cu is decreased.

It was clear from the results that EDTA enhanced Cu uptake by *Z. mays* under single soil Cu contamination, while it had no effect on Cu and pyrene co-contaminated soil (Table 1). Also, after the application of EDTA, *Z. mays* seemed to suffer from a more severe phytotoxicity under single Cu contamination than when Cu and pyrene were co-contaminated. It is possible that the root of *Z. mays* in single Cu-contaminated soil would experience heavier physiological damages, which could lead to subsequent breakdown of the root exclusion mechanism causing indiscriminate uptake of Cu by plants. This assumption is consistent with the fact that enhancement of Cu concentrations in the shoots of *Z. mays* was more pronounced in single Cu-contaminated soil than when Cu and pyrene were combined. Under co-contamination, the increased accumulation of Cu in *Z. mays* as observed in single Cu soil contamination was not found in soils treated with EDTA treatment up to 0.15 g kg^{-1}. Probably under co-contamination of Cu and pyrene, less than 0.146 g kg^{-1} of EDTA application was insufficient to break down plant uptake barriers under the conditions of our present study. This observation was consistent with the observation that EDTA was less toxic to *Z. mays* under Cu and pyrene-mixed contaminated soil than in single Cu-contaminated soil.

The effects of combined amendments on contaminated soils can be synergistic or antagonistic (Gunawardana et al. 2010). Under co-contamination with Cu and pyrene, the enhancement of Cu accumulation with EDTA + citric acid was obvious and interesting; biomass yield was not decreased. Therefore, a combination of EDTA and citric acid could be considered as a viable amendment for enhancing Cu phytoextraction from metal-PAH-contaminated soil. The shoot Cu concentration, as well as the water extractable Cu, with the application of EDTA + citric acid was also the highest of any treatment under co-contamination at the end of the experiment (Table 2). This increase could be attributed to the synergistic effect of citric acid or EDTA, which increases ligand availability in solution through a potentially different mode of action and uptake pathway (Gunawardana et al. 2010).

4.3 Phytoremediation Potential

The success of phytoremediation is dependent on shoot biomass as well as on shoot Cu concentration (Jiang et al. 2004). The potential effectiveness of each plant with chelate application was evaluated by the total amount of Cu removed from the soil. The present results showed that EDTA was more efficient than citric acid or EDTA + citric acid when soil was spiked with Cu alone. Sinhal et al. (2010) showed in their research that although both citric acid and EDTA enhanced phytoextraction of Zn, Cu, Pb, and Cd, EDTA was more efficient during phytoextraction. In co-contaminated soils, the combined application of EDTA and citric acid was more efficient in Cu removal compared to citric acid, EDTA, or control (no chelates) treatments and is supported by Yang et al. (2011) who suggested that combined treatments of EDTA, cysteine, and tween-80 was a more promising application to improve the phytoremediation of heavy metals under Cd-PAH-mixed contaminated soil situations. In addition to total metal content, the translocation factor (TF) (Table 1) need to be considered in order to evaluate the ability of an accumulator to accumulate and transport heavy metals in plants. Metal translocation is expressed as the ratio of the metal level in the shoots to that in the roots (Gunawardana et al. 2010; Marchiol et al. 2004). In the present study, it indicates the ability of chelates to affect the transfer of Cu from root to shoot.

It was observed that EDTA significantly enhanced the translocation of Cu in Cu-contaminated soil but not in co-contaminated soils containing Cu and pyrene. The combination of EDTA and citric acid significantly decreased the translocation of Cu in single Cu-contaminated soil, but the shoot to root efficiency increased in co-contaminated soil. Higher translocation as observed with EDTA in single Cu-contaminated soil could be as a result of reduced metal binding to root tissues (Baylock et al. 1997). Romkens et al. (2002) suggested that when Cu is complexed with an amendment, Cu would be more easily reallocated to harvestable plant tissues than free metal ions. Relatively stable Cu complexes are readily absorbed by roots and transported to the above ground parts due to the higher affinity of EDTA to Cu (Degryse et al. 2006). This complexation could decrease the binding of free metal ions to negatively charged carboxyl groups in the xylem cell walls (Wenger et al. 2003). In co-contaminated soils (Table 1), the enhancement of translocation with EDTA was less than in single Cu-contaminated soil. It could be that the interactions of Cu and pyrene with EDTA resulted in reduced Cu transport through the plant parts. Luo et al. (2005) showed that when metal and metal-EDTA complexes are simultaneously present in solution, they effectively compete for uptake, therefore reducing Cu transport rate to shoots. Also, pyrene has been shown to be able to accumulate in shoots of plants from direct translocation from roots (Gao and Zhu 2004). Therefore, increased competition for uptake in the presence of pyrene, Cu, and Cu-EDTA complex could have caused the slight reduction observed.

4.4 Pyrene Dissipation

High molecular weight PAHs including pyrene have often not been successfully dissipated in contaminated soil. At the end of this plant trial, the residual pyrene concentration in single or co-contaminated soil decreased and reached 57.8 and 67.7 %, respectively, even without the application of chelates or plant growth (Fig. 2). However, the dissipation of pyrene in single pyrene or Cu-pyrene co-contaminated soil was significantly higher in planted soil than in unplanted soil. This suggests that the root system of *Z. mays* and probably other physiological characteristics of *Z. mays* played an important role in pyrene dissipation in Cu-pyrene-contaminated soil.

It could be seen that in single and mixed pyrene-contaminated soils, all tested chelates significantly increased the dissipation rate of pyrene except EDTA, which did not enhance pyrene dissipation in co-contaminated soil. Also, citric acid had a more significant effect when compared to EDTA or combined application of EDTA and citric acid. The dissipation rate of pyrene with the application of citric acid reached 90 % at the end of the trial (Fig. 2). It could be that citric acid provided more nutrients for indigenous microbes to proliferate and biodegrade the pyrene in soil, thereby increasing the biodegradation rate (Andrew et al. 2007). In addition, the exudation of organic compounds by the roots of *Z. mays* can affect the activity of the microbes in soil and thus, indirectly, the solubility of PAHs (Marschner et al. 1995). The concentration of the chelates including citric acid in final soil was not analyzed, and it could be possible that co-metabolism of the citric acid with pyrene occurred to improve biodegradation of pyrene (Wei et al. 2009). The dissipation of pyrene with the application of chelates in single as well as co-contamination could be associated with the effects of chelates on physico-chemical processes including contact between microorganisms and PAHs. Wei et al. (2009) suggested that variations in pH values caused by low molecular weight organic acids (LMWOAs) hardly had any effect on PAH degradation and therefore concluded that contact between PAH and microorganisms was highly related to PAH biodegradation. This study also showed that citric acid was more effective in enhancing pyrene dissipation than EDTA or EDTA + citric acid in both single and co-contaminated soil, and this reflects the result of the previous works, which showed that organic acids influence the activities of enzymes that help in the degradation of PAHs like laccases and manganese peroxidase (Eibes et al. 2005; Ting et al. 2011).

5 Conclusions

The present study showed that *Z. mays* could be very effective in phytoextraction of Cu and dissipation of pyrene in Cu-pyrene co-contaminated soil with the help of chelates. Of all the chelates used in the present study, EDTA was more effective in the removal of Cu from single Cu-contaminated soil at the concentration used in the trial, whereas the combined application of EDTA and citric acid had the most effective improvement in Cu uptake in Cu + pyrene co-contaminated soil. The effectiveness of the applied chelates in the dissipation of pyrene varied in the present study. In single pyrene-contaminated soil, citric acid was more effective in decreasing the residual pyrene in soil with the help of *Z. mays*, while in co-contaminated soils, citric acid, and EDTA + citric acid were more effective in the dissipation of pyrene. Therefore, in co-contaminated soils, the combined treatment of EDTA + citric acid will best suit the phytoextraction of Cu as well as the dissipation of pyrene. Although citric acid was more effective than EDTA + citric acid in the dissipation of pyrene in co-contaminated soil, the difference in dissipation was only over 3 %. Also, because citric acid did not enhance the uptake of Cu in co-contaminated soil, the combined treatment of EDTA + citric acid which enhanced both the uptake of Cu as well as the dissipation of pyrene will be the preferred alternative.

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