The African Journal of Plant Science and Biotechnology ©2010 Global Science Books

Hyperaccumulation: An Aspect of EDTA Chelate-Assisted Phytoextraction by Water Hyacinth

Luke N. Ukiwe* • Ndubuisi J. Aneke

[1] Department of Chemistry, Federal University of Technology, P.M.B 1526, Owerri, Nigeria

Corresponding author: * luggil2002@yahoo.com

ABSTRACT

The hyperaccumulation capacity of water hyacinth (*Eicchornia crassipes*) using chelate assistance with ethylenediaminetetracetic acid (EDTA) was studied. Results obtained with water hyacinth samples planted in salt solutions of Pb, Ni, Fe, Zn, Cu, Cr, Co and Cd spiked with EDTA and digested using HCl and HNO_3 showed that Cu was the highest metal absorbed (12.8395 mg/l) while Ni was the least (3.4420 mg/l) at all weights (3.0, 4.0, 5.0, and 6.0 g) of the salts for all the elements tested. Water hyacinth plants could only absorb 13% Cu in the control experiment (without EDTA) containing salts of the above metals but could absorb as much as 99, 79, 62, and 60% at 3.0, 4.0, 5.0, and 6.0 g, respectively of Cu. For Ni, these values were 27, 24, 19, 12% at 3.0, 4.0, 5.0 and 6.0 g, respectively but 0% in the control experiment. This result indicates that for Ni and Cu, as the weight of salt increased, the amount of metal absorbed decreased.

Keywords: biomass, heavy metals, phytoaccumulation, sewage, toxic

INTRODUCTION

Hyperaccumulation is a phytoremediation process where a plant accumulates a metal from metal substrates. Phytoremediation is also the process of using plants to remove pollutants from soil or waste waters (Zang *et al.* 2002). Phytoremediation includes phytoextraction, which is a hyperaccumulation method that involves contaminant uptake by roots with subsequent accumulation in the ground portion of the plant followed by harvesting and further disposal of the plant biomass (Ukiwe *et al.* 2008). To aid the ability for metal phytoextraction hybrid plants have been generated between hyperaccumulators. These hybrids accumulate high levels of metals that would normally have been toxic to ordinary plants thus illustrating the essence of biotechnology in enhancing the effectiveness of phytoremediation (Mitch 2002).

It has been suggested that phytoextraction is encouraged when metal availability to plant roots is aided through the addition of acidifying agents to soil solution or wastewaters (Prasad and Freitas 2003).

Water hyacinth (*Eichhornia crassipes*) is a free-floating aquatic weed that has the ability to remove metals from wastewaters. Studies have shown that the weed is very effective at hyperaccumulating arsenic (As) from contaminated water (Misbahuddin and Fariduddin 2002). This assertion was debunked by Zhu *et al*. (1999) who showed that water hyacinth does not have very high As removal capabilities and converts a large portion of the arsenate it removes to the more toxic As form within the plant itself. The ability of water hyacinth to absorb and translocate Cd, Pb, Cu, Zn, and Ni was studied in the Erh-Cheng wetlands, Taiwan. Results indicate that the plant highly bioconcentrated these trace elements thus showing that the weed is a promising candidate for hyperphytoremediation of wastewater polluted with these trace elements (Liao and Chang 2004). The metals can be solubilized by the addition of chelating agents to allow uptake of the contaminant by the plant. Metal solubilization and absorption by the plant is liable to metals transportation in the root system. Complexing agents such as ethylene diamine disuccinic acid (EDDS) have been used to enhance metal (Pb, Cd, Ni, Zn, Cu) solubility (Raskin and Ensley 2000) although Egli (2001) reported that certain drawbacks exist in the phytoremediation process, including toxicity to the soil biology. Other studies have revealed that chelating agents such as nitrilotriacetic acid (NTA), citric and oxalic acids have been applied successfully in metal solubilization (Polettini *et al*. 2008). Results obtained after analyses indicated that for given experimental conditions (pH, temperature), the remediation efficiency was strongly dependent on the specific contaminant under study with contaminant speciation and distribution in the solid matrix as well as affinity for the extracting agent playing a major role in the decontamination process. Liu *et al.* (2008) used pot and leaching column experiment for optimization of chelator-assisted phytoextraction using EDTA, lead and *Sedum alfredii* as a model system. Optimum phytoextraction occurred at added EDTA concentration of 5 mM in single dose treatment for 10 days in low Pb soil. A revealing study by Pigozzo *et al*. (2006) on the effects of agricultural recycling of sewage sludge demonstrated that through the extraction of transition metals using diethylenetriaminepentacetic acid (DTPA) in a dystrophic latosol medium, Cd, Ni, Co, Pb and Cr were not detected. The study concluded that due to low concentrations of soil samples, the extractor medium had a restricted capacity for evaluation of its phytoavailability. Baeza *et al*. (2007) also reported the degradation of EDTA in a total chlorine-free cellulose pulp bleaching effluent by uv/H_2O_2 treatment. Their study noted that depending on the initial hydrogen peroxide concentration and iron content, EDTA degradation followed first order kinetics and UV treatment without peroxide yielded effective degradation and removal of Fe (111)-EDTA complex.

The associated problems of scarcity, small biomass, slow growth rate, uncertain and specialized growing conditions of some hyperaccumulators and lack of hyperaccumulators for metals such as Cr, and the effectiveness of hyperaccumulators for phytoextraction remains precarious more so when their ability for metal uptake is limited (Pivetz 2001). Plant breeding, genetic development, genetic transfer of accumulating ability to higher-biomass plants, fertiliza-

Received: 13 December, 2008. Accepted: 4 February, 2010.

Original Research Paper

tion options to increase hyperaccumulator biomass are possible options that are being tested (Pivetz 2001) to enhance hyperaccumulating efficiency.

This research aimed to investigate the role of water hyacinth in hyperaccumulating metals from industrial sewage using ethylenediaminetetracetic acid (EDTA) chelate-assisted phytoextraction.

MATERIALS AND METHODS

The water hyacinth samples used in this study were obtained from a fish pond in Imerienwe, Ngor Okpala LGA, while the sewage sample was also obtained from the Aluminium Extrusion Industry, Inyishi, Nigeria. The plant samples were treated and prepared as described by Ukiwe *et al*. (2008). The sewage samples were collected from a sewage discharged point in the industry with two 100-L plastic containers previously washed and rinsed with deionised water and transported to the chemistry laboratory of the Federal University of Technology, Owerri, Nigeria. Into eight 2-L plastic containers, each previously washed and rinsed with deionised water, were poured 1-L each of the sewage which had been treated by filtration using a mesh sieve (1000 mm) to remove debris and further centrifuged (Micro Centrifuge Model 5415C) at 150 rpm. About 0.1 g each of EDTA was added to each of the eight containers and stirred while 3 g each of the salts of lead nitrate, nickel sulphate, iron sulphate, zinc sulphate, copper sulphate, chromium chloride, cobalt chloride, and cadmium nitrate respectively were also weighed and added separately to each of the eight containers and stirred. Eight separate water hyacinth plants were then planted in each of the eight containers and these containers were placed under laboratory conditions for six weeks. The samples were placed at room temperature (28°C), and light intensity 390 lux, and at 75% relative humidity. At the end of this period the plants were harvested and processed for heavy metal content analysis by acid digestion using the method described by Ukiwe and Ogukwe (2007). The concentration of the above metals within each plant was determined using absorption optimum working range, wavelengths and flame type as described by Ukiwe and Ogukwe (2007) and Ukiwe *et al*. (2008). Three treatments were processed for each of the metals 3.0 g salts and these procedures were repeated for salts at 4.0, 5.0, and 6.0 g, respectively while a control experiment of eight containers of plants with sewage sludge containing 6.0 g each of salts of the above heavy metals without EDTA was set up and the same procedure as described above to determine the concentration of heavy metals in the plants. The results obtained are shown in **Table 1**.

Data analysis

Data are given as arithmetic mean, standard error of the mean and standard deviation. The Analysis of Variance (ANOVA) was used to measure differences between mean concentrations of absorbed metals. The standard error of the difference between mean concentration of metal absorbed and control experiment and the generalized *t*-test were used to estimate the significance of values obtained.

RESULTS AND DISCUSSION

Chelates are used to encourage the phytoextraction of metals. EDTA-assisted phytoremediation has been developed to clean up lead (Pb)-contaminated soil (Xu *et al*. 2007). A study on EDTA-assisted phytoremediation of heavy metals in biosolids to compare the effect of EDTA on uptake of heavy metals by hybrid poplar such as the Eastern cottonwood (*Populus deltoides*) in composted biosolids and in soil that had received injected biosolids showed that EDTA had little impact on phytoremediation of heavy metals in the composted biosolids, although the study showed that Ni was higher in the composted biosolids than in soil with injected biosolids (Liphadzi and Kirkham 2006a, 2006b). Chelating agents are added to soil to solubilize metals for enhanced phytoextraction. A recent study of the mobility of heavy metals in biosolids in a column of soil that had a plant and another column without plants after these columns have been irrigated with EDTA and drainage water. Results obtained from these columns analyzed for Cd, Cu, Fe, Zn, and Pb showed that EDTA mobilize all heavy metals and increase their concentration in drainage water (Kirkham and Liphadzi 2005; Liphadzi and Kirkham 2006c, 2009). Chelate-assisted phytoremediation has become an attractive soil remediation solution. However, metal absorption by plants is linked to metal solubility and transportation into the root system as such. Complexing agents such as EDDS and EDTA have received more attention although EDDS is preferred over EDTA due to its greater rate of degradation and strong chelating characteristics (Coscione *et al*. 2009). The result of an investigation to study the effects of adding different rates of various organic complexing agents (OCA) including EDTA, diethylenetriamine penta acetic acid (DTPA), citric acid (CA), and humic acid (HA) on heavy metal availability in contaminated soils revealed that the capacity of OCA to release B, Cd, Mo, and Pb in soil planted with corn is in the order HA > CA > EDTA > DTPA (Turan and Angin 2004). Manipulating heavy metal availability with chelating agents is a way to accelerate natural phytoremediation of contaminated soils (Diaz and Kirkham 2008). Nevertheless, increasing metal availability also increases the risk of metal movement through the soil profile, and consequently the contamination of ground water. Interaction experiments have shown that EDTA and nitrilotriacetic acid (NTA) were more efficient than malate and citrate acids in solubilizing metals (Fe, Mn, Cu, Zn, and Cd). However, NTA treatment promoted an increase in toxic elements concentration, especially As, Cd, and Pb (Penalosa *et al*. 2007). Metals in the aqueous phase of the substance are made readily bio-available for absorption by plant roots. Use of soil amendments such as synthetics (ammonium thiocyanate) and natural zeolites have yielded promising results (Prasad and Freitas 2003). EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate, acetate, etc. have been used as chelators for rapid mobility and uptake of metals from contaminated soils and wastewaters (Blaylock *et al*. 1997). Though controversial, adding chelating substance to soil or aqueous phase substances appears to be the most efficient approach to liberate

Table 1 Concentration [mean (mg/l)] of heavy metals in water hyacinth samples.

Weights of heavy metals	3.0 g		4.0 g		5.0 g		6.0 g		Control experiment	
Heavy metals	**Mean (mg/l) ± SD × 10^{-2}**	**SEM × 10^{-2}**	**Mean (mg/l) ± SD × 10^{-2}**	**SEM × 10^{-2}**	**Mean (mg/l) ± SD × 10^{-2}**	**SEM × 10^{-2}**	**Mean (mg/l) ± SD × 10^{-2}**	**SEM × 10^{-2}**	**Mean (mg/l) ± SD × 10^{-2}**	**SEM × 10^{-2}**
Pb	2.9078 ± 9.8	5.6	2.0980 ± 0.2	1.1	2.9544 ± 0.2	1.1	2.0950 ± 0.2	1.1	0.5372 ± 4.9	2.8
Ni	0.8146 ± 0.2	1.1	0.9420 ± 0.2	1.1	0.9672 ± 0.2	1.1	0.7182 ± 3.1	1.2	0.0000 ± 0.0	0.0
Fe	1.7182 ± 0.2	1.1	2.5833 ± 0.2	1.1	2.9682 ± 0.2	1.1	2.4962 ± 0.2	1.1	0.2205 ± 1.8	1.0
Zn	2.8248 ± 0.3	1.7	2.5881 ± 9.9	5.7	2.0197 ± 0.2	1.1	4.7682 ± 0.2	1.1	0.0197 ± 0.0	0.0
Cu	2.9733 ± 0.2	1.1	3.1890 ± 3.9	2.2	3.1086 ± 2.9	1.6	3.5686 ± 9.9	5.7	0.8248 ± 2.9	1.6
Cr	2.9622 ± 0.2	1.1	2.8146 ± 0.0	0.0	3.7124 ± 0.2	0.1	3.6286 ± 0.2	1.1	0.2499 ± 1.8	1.0
Co	2.0843 ± 0.2	1.1	2.8812 ± 0.0	0.0	2.5793 ± 0.4	0.2	2.9510 ± 0.0	0.0	0.3852 ± 1.8	1.0
Cd	2.0345 ± 0.3	1.7	2.9148 ± 0.1	0.0	2.5910 ± 0.0	0.0	3.3037 ± 9.9	5.7	0.0583 ± 0.0	0.0

SEM: Standard Error of the Mean

labile metal contaminants in the solution. The principle of chelating is such that free metal ions in the solution are complexed allowing further dissolution of the absorbed or precipitated phases until an equilibrium is reached between the complexed metal, free metal, and insoluble metal fractions (Prasad and Freitas 2003; Manoucheri and Bermond 2009). Chelate-assisted (induced) hyperaccumulation is a situation where metals on site are initially immobilized to allow for rapid establishment and growth of plants. When sufficient biomass of the plants has been obtained, chelating materials are applied to the aqueous substrate resulting in the liberation of large quantities of the metal into the solution. Large amounts of metal are absorbed by plant roots and are translocated to the shoot tissue where they accumulate to toxic levels. Plants are harvested after death and removed from site. This process is in contrast to the normal phenomenon of phytoextraction where plants are given a gradual exposure to non-toxic quantities of metals in solution and accumulation occurs gradually over time as the plants grow. Though novel, chelate-assisted hyperaccumulation has its drawbacks. After metal absorption occurs, residual chelate in the soil becomes a lingering problem. Since large amount of chelate-bound metals are liberated into the soil or aqueous solution they are thus leached into deeper soil layers and pose serious problems when attempted to be recovered through phytoremediation and may require the use of more expensive conventional remediation procedure as the liberated metals have the ability to migrate into uncontaminated areas, possibly groundwater reservoirs (Cunningham *et al.* 1997).

EDTA chelating is widely accepted as an effective treatment for metal toxicity. It attaches itself to heavy metals and carries the metals from the soil or sewage to the root structures. As already indicated, chelating agents are used to solubilize heavy metals prior to uptake by the root system. Water hyacinth together with EDTA is being used to aid purification of wastewaters and sewage systems. The plant has the property to accumulate heavy metals in the root tissues. Harvesting the plant then removes these nutrients from the system. **Table 1** shows values obtained on the hyperaccumulating ability of water hyacinth. It is observed from **Table 1** that the degree of hyperaccumulation decreased as the weight of salts increased notably with Ni and Cu. Cu was the most absorbed metal totaling 12.8395 mg/l overall for all salts weights followed by Zn with 12.2008 mg/l. Ni was the least absorbed metal totaling 3.4420 mg/l overall at all weights. Going through the values in the control experiment the amount of Cu absorbed was 13% as opposed to 99, 79, 62, and 60% Cu absorbed at 3.0, 4.0, 5.0 and 6.0 g, respectively. Zero percent of Ni was absorbed in the control experiment but 27, 24, 19, and 12% Ni was absorbed at 3.0, 4.0, 5.0 and 6.0 g, respectively. During the 6-week planting period, the survival period of plants in higher salts weights was shorter than those of lower salts; this could explain why plants in the higher salts weights absorbed fewer metals than their lower weight counterparts.

The standard error of the mean of the control experiment and heavy metal at 3.0 g, which is used as a standard to evaluate possible significances in other heavy metal weights, was 0.2926. The generalized t-test (t = 54) was used to test the significance difference between the means of sets observation between 3.0 g salt weights and the control experiment. This value at df = 14 show significance at the 1% confidence level.

A field survey of plants growing in metal-contaminated sites in Egypt examined their usefulness in phytoremediation and revealed that maximum Cr (64 mg/kg) and Ni (25 mg/kg) contents were observed in *Diplachne fusca*, while the highest Cu concentration (174 mg/kg) was observed in *Urtica urens* (Abou-Shanad *et al*. 2007). Single extraction with diethylene triamine pentacetic acid (DTPA) was used by Kao *et al.* (2007) to evaluate the phytotoxicity of metals with *Brassica chinensis*. Analytical results indicated that the heavy metals contents with DTPA extraction were much higher than those without DTPA extraction. *Nicotiana tabacum*, a fast growing, high biomass plant has a high tolerance for various organic and inorganic pollutants. The plant can accumulate heavy metals in relatively high levels especially Cd, in comparison to other plant species such as water hyacinth (Evangelou *et al*. 2007). *N. tabacum* is also not susceptible to various organic pollutants, such as polychlorinated biphenyls (PCB) and trinitrotoluene (TNT).

Hyperaccumulation requires different plant characteristics for optimum effectiveness. These characteristics include the ability to remove high concentrations of metals, the ability to tolerate, translocate and accumulate high concentrations of heavy metals in the shoots and leaves, rapid growth rate and high biomass production without producing toxic degradation products. Water hyacinth has been used effectively in wastewater treatment (Salt *et al*. 1995). Its extensive root system and ease to grow in water without much effort and ability of the weed to sustain itself under varying climatic conditions, makes water hyacinth the plant of choice for maximum uptake of metals. Hence, small communities or industries with very little resources can develop their own wastewater treatment systems using the weed.

REFERENCES

Abou-Shanab RA, Ghozlan HA, Ghanem KM, Moaward HA (2007) Heavy metals and plants from various metal contaminated sites in Egypt. *Terrestrial and Aquatic Environmental Toxicology* **1**, 7-12

Baeza C, Oviedo C, Zaror C, Rodriguez J, Freer J (2007) Degradation of EDTA in a total chlorine free cellulose pulp bleaching effluent by UV/H_2O_2 treatment. *Journal of the Chilean Chemical Society* **52**, 707-717

Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulouk Y, Ensley BD, Raskin I (1997) Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environmental Science and Technology* **31**, 860-865

Coscione AR, Abreu CA, Santos GC (2009) Chelating agents to solubilize heavy metals from oxisols contaminated by the addition of organic and inorganic residues. *Ciência e Agricultura (Piracicaba, Brazil)* **66**, 103-106

Cunningham SD, Shann JR, Crowley DE, Anderson TA (1997) Phytoremediation of contaminated water and soil. In: Kruger EL, Anderson TA, Coats JR (Eds) American Chemical Society Symposiums Series No. **664**, Washington DC, pp 117-121

Diaz FM, Kirkham MB (2008) Testing the manipulation of soil availability of metals. *Methods in Biotechnology* **23**, 121-129

Egli T (2001) Biodegradation of metal-complexing aminopolycarboxylic acids. *Journal of Bioscience and Bioengineering* **92**, 89-97

Evangelou MWH, Ebel M, Schaeffer A (2007) Tobacco (*Nicotiana tabacum*) a pot phytoremediator. *Terrestrial and Aquatic Environmental Toxicology* **1**, 46-53

Kao PH, Huan CC, Hseu ZY (2007) Chemical speciation and phytotoxicity of heavy metals in sewage sludge for the germination of Chinese cabbage seeds. *Terrestrial and Aquatic Environmental Toxicology* **1**, 1-6

Kirkham MB, Liphadzi MS (2005) Heavy metal displacement in chelate-assisted phytoremediation of biosolids soil. *Americam Geophysical Union Abstract* **H**43B-07

Liao SW, Chang WL (2004) Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. *Journal of Aquatic Management* **42**, 60-68

Liphadzi MS, Kirkham MB (2006a) EDTA assisted phytoremediation of heavy metals in biosolids: comparison of composted biosolids and soil with injected biosolids soil. In: *18th World Congress of Soil Science*, July 9-15, Philadelphia, USA, pp 158-162

Liphadzi MS, Kirkham MB (2006b) Heavy metal displacement in EDTA-assisted phytoremediation of biosolids soil. *Water Science and Technology* **54**, 147-153

Liphadzi MS, Kirkham MB (2006c) Availability and plant uptake of heavy metals in EDTA assisted phytoremediation of soil composted biosolids. *South African Journal of Botany* **72**, 370-374

Liphadzi MS, Kirkham MB (2009) Partitioning and accumulation of heavy metals in sunflower grown at biosolids farm in EDTA-facilitated phytoremediation. *Bioremediation, Biodiversity and Bioavailability* **3**, 36-42

Liu D, Islam E, Ma J, Wang X, Mahmood Q, Jin X, Li T, Yang X, Gupta D (2008) Optimization of chelator-assisted phytoextraction using EDTA, lead and *Sedum alfredii* Hance as a model system. *Bulletin of Environmental Contamination and Toxicology* **81**, 30-35

Manoucheri N, Bermond A (2009) EDTA in soil science: A review of its application in soil trace metal studies. *Terrestrial and Aquatic Environmental Toxicology* **3**, 1-15

Pigozzo AT, Lenzi E, Junior JL, Scapim CA, Costa AC (2006) Transition metal rates in latosol twice treated with sewage sludge. *Brazilian Archives of Biology and Technology* **49**, 515-526

Pivetz BE (2001) Phytoremediation of contaminated soil and ground water at hazardous waste sites. Ground Water Issue. EPA, 540. S-01, 500. Washington

DC

Polettini A, Pomi R, Calcagnoli G (2008) Assisted washing for heavy metal and metalloid removal from contaminated dredged materials. *Water, Air, and Soil Pollution* **196**, 183-198

Prasad MNV, Freitas HMO (2003) Metal hyperaccumulation in plants – Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology* **6**, 285-296

Mitch ML (2002) Phytoextraction of toxic metals: A review of biological mechanism. *Journal of Environmental Quality* **31**, 109-120

Misbahuddin M, Fariddin A (2002) Water hyacinth removes arsenic from arsenic contaminated drinking water. *Archaelogical Environmental Health* **57**, 516-519

Penalosa JM, Carpene RO, Vazques S, Agha R, Granado A, Sarra MJ, Esteban E (2007) Chelate-assisted phytoextraction of heavy metals in a soil contaminated with a pyritic sludge. *Science Total Environment* **378**, 199-204

Raskin I, Ensley BD (2000) *Phytoremediation of Toxic Metals Using Plants to Clean up the Environment* (3rd Ed), John Wiley, NY, 365 pp

Turam M, Angin I (2004) Organic chelate assisted phytoextraction of B, Cd, Mo, and Pb from contaminated soils using two agricultural crop species. *Acta Agriculture Scandinavica* **54**, 221-231

Ukiwe LN, Nwoko CIA, Enenebeaku CK (2008) Intrinsic role of pH variables on the sorption of heavy metals by water hyacinth (*Eichhornia crassipes*) on the Niger Delta Rivers, Nigeria. *The African Journal of Plant Science and Biotechnology* **2**, 112-114

Ukiwe LN, Ogukwe CE (2007) Potassium ion uptake by water hyacinth (*Eichhornia crassipes*) on the lower reaches of the Niger River, Nigeria. *The African Journal of Plant Science and Biotechnology* **1**, 36-39

Xu Y, Yamaji N, Shen R, Ma JF (2007) Sorghum roots are inefficient in uptake of EDTA chelated lead. *Annals of Botany* **99**, 869-875

Zhang W, Cai Y, Tu C, Ma LQ (2002) Arsenic speciation and distribution in an arsenic hyperaccumulating plant. *Science of the Total Environment* **300**, 167-178

Zhu YL, Zayed AM, Qian JH, Souza NT, Terry N (1999) Phytoaccumulation of trace elements by wetlands plants. 11. Water hyacinth. *Journal of Environmental Quality* **28**, 339-345