403
Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 25(2), 403- 409, 2017

EFFICIENCY OF EDTA ON ZN AND CU PHYTOREMEDIATION

[30]

Maram, M. Mohammed[1]; T.F. Mohammaden[1]; S.S. Eisa[2] and Kawthar, A.E. Rabie[2]
1- Isotopes Geology Dept., Nuclear Materials Authority, Cairo, Egypt
2- Agricultural Botany Dept., Fac. Agric., Ain Shams Univ., Cairo, Egypt

Keywords: Phytoremediation; zinc; copper; hyper-accumulation; Ryegrass; Indian mustard; EDTA

ABSTRACT

Phytoextraction of heavy metal from contaminated soils is promising remediation technology. In the present study, hyper-accumulator plants, indian mustard (*Brassica juncea*.(L) *czern*) and ryegrass (*Lolium multiflorum* Lam) have been used to remove the excess undesirable concentrations of zinc and copper from contaminated soil. Zinc and copper uptake have been enhanced by adding EDTA to the contaminated soil using two concentrations (2.5 and 7.5 mmol/Kg soil). Accumulation of Zn by the indian mustard shoots and roots under the effect of EDTA recorded 4 to 6 times as adsorbed by the control while less enhancement of Zn uptake was recorded by the ryegrass shoots and roots. On the other hand, Cu accumulation showed significant enhancing by the ryegrass shoot comparing to the indian mustard shoot at the both employed EDTA concentrations. The ryegrass roots gave enhanced Cu uptake at the EDTA conc. 7.5 mmol/Kg soil only while the indian mustard roots recorded an increasing in the Cu-uptake with the two EDTA concentrations

INTRODUCTION

The excessive using of fertilizers, pesticides, fungicides, sewage sludge and bad watering practices cause contamination of agricultural soils with various pollutants particularly the heavy metals (**Passariello et al 2002).** Although their trace amounts are essential for the plants grown but the high concentration of zinc and copper have potential toxic effect on plants **(Yadav, 2010).**

Phytoextraction (also known as phytoaccumulation or phytoabsorption) refers to the uptake of contaminants from soil or water by plant roots and their translocation and accumulation in the above ground biomass i.e. shoots (**Ghosh & Singh, 2005; Kotrba et al 2009; Garbisu and Alkorta, 2003**). Metal translocation to shoots is a crucial biochemical process desirable for the effective phytoremediation where harvesting of the root biomass is generally not feasible (**Halim et al 2003; Yang et al 2005 and McIntyre, 2003**).

The hyper-accumulator plants have the ability to uptake heavy metals in 50–500 times greater than normal plants (**Baker and Brooks,1989**) where they accumulate the heavy metals in their shoots to levels far greater than non -accumulating plants (**Memon et al 2001; McGrath et al 2001 and Memon & Schroder, 2009).** Several decades ago, about 400 hyper-accumulator plants were known such as; Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, Flacourtaceae, Lamiaceae, Poaceae, Violaceae and Euphorbiaceae families (**Bolan et al 2014 and Prasad, 2003**). Adding of synthetic chelates (e.g. EDTA) to the soil cause significant increasing of Cd, Cu, Ni, Pb, and Zn accumulation by plants **(Huang and Cunningham, 1996; Blaylock et al 1997 and Huang et al 1997).**

MATERIAL AND METHODS

The hyper-accumulator plants Indian mustard and ryegrass were selected to testify their uptake efficiency for Zn and Cu ions under effect of different EDTA concentrations.

The Indian mustard seeds were purchased from Siegers seed Co., while the ryegrass seeds were obtained from The Improved Seeds Production Unit, Agriculture Research Center, Ministry of Agriculture, Dokki, Cairo, Egypt. On the other hand, the used soil sample was collected from agricultural field in El-Sharkia Governorate, Egypt. Physical and chemical properties of the used soil are determined (**Table 1).**

(Received 12 April, 2017)
(Revised 3 May, 2017)
(Accepted 10 May, 2017)

Two concentrations (2.5 and 7.5 mmol/Kg) of the Ethylenediaminetetraacetic acid (EDTA) were prepared by dissolving the proper weight of the analytical grade EDTA salt (MERCK Co.) in distilled water to obtain the desired concentrations.

Green house experiment

Two thousand grams of soil was transferred to each plastic pot (25 cm diameter and 20 cm depth). For the indian mustard plant, 40 seeds were sown in each pot and after appearing of the first pair of true leaves the seedlings were thinned to 20 plants/pot. On the other hand, the ryegrass seeds were sown without thinning (20 seeds/ pot). Two weeks later, all the pots were fertilized every week with Hoagland's solution **(Hoagland and Arnon, 1950)** .

For two months, the pots were weighted daily and irrigated with de-ionized water to compensate the water loss due to the evapotranspiration and to adjust its content in the soil as 70% of field capacity. Two days before the soil amendment, the irrigation process was stopped to allow decreasing of the water content. After two months of seeds sowing, the pots of each plant were divided into 3 sets (the first is used as control while the second and third sets were treated by 2.5 and 7.5 mmol/kg respectively). Experiments were conducted in completely randomized design with six replicates . All the plants were harvested two weeks after amendment.

Growth parameters and plant digestion

The harvested plants were carefully washed by distilled water to remove the adhered soil particles. After this the shoot and root lengths were recorded in the fresh plants. The plants were completely dried at 70^oC in the dryness oven for 48 hours. The total weight of the dried plants was determined then 0.5 g of each dried plant was digested using an acid mixture of H_2SO_4 and HNO_3 in a ratio of 2:1 respectively (v/v) as well as drops of $HClO_4$. The mixture was put into a glass beaker and heated at 220^oC till completed dryness. The residues were dissolved in 10 ml HCl (2N) then up to volume of 50 ml by adding distilled water **(Homer and Parker, 1961).**

Table 1. Physical and chemical properties of the soil

Parameters	Value
pH	7.05
EC	1.838 dSm^{-1}
Saturation soluble extract (meq /L)	
Ca^{+2}	6.8
Mg^{+2}	3.6
Na^{+}	6.3
K^{+}	1.3
Cl^{-}	13.6
CO_3^{-2}	Nill
HCO_3^{-}	1.2
SO_4^{-}	3.2
heavy metals (ppm)	
Zn	858
Cu	112

Statistical analysis

All data were subjected to statistical analysis for the computation of differences between control and amended pots. Mean values (based on six replicates) were calculated and subjected to statistical analysis through the Duncan's Studentized Range Test using SAS statistical software.

Zinc and copper determination

Zinc and copper ions were determined in the plant solution using the atomic absorption spectrometer (model: Unicam 969, England).

Bioconcentration factor (BCF) and translocation factor (TF) calculation

The BCF refers to the shoot and root efficiency for metal absorption from the soil and calculated according to the following equation:

$$BCF_{shoot} = \frac{metal\ concentration\ in\ shoot}{metal\ concentration\ in\ soil} \times 100$$

$$BCF_{root} = \frac{metal\ concentration\ in\ root}{metal\ concentration\ in\ soil} \times 100$$

On the other hand, the TF records the plant's ability for metal translocation from root to shoot and calculated as:

$$TF = \frac{metal\ concentration\ in\ shoot}{metal\ concentration\ in\ root}$$

RESULTS AND DISCUSSION

Zinc and copper uptake

The obtained data **(Tables 2 & 3)** showed the following:

- Increasing the metals uptake by both plants as the EDTA concentration increases with notable higher metals accumulation in the shoot comparing to the root. It is worth to mention that the indian mustard showed better Zn-uptake efficiency than the ryegrass while the vice versa was in the Cu-uptake efficiency.
- The translocation factor (TF) of Zn-uptake is positively affected by both the EDTA concentrations in the ryegrass while it is affected only by the EDTA concentration of 2.5 mmol/Kg soil in the case of indian mustard. On the other hand, the TF of Cu-uptake showed different behavior where it increased only at 2.5 mmol of EDTA concentration in case of the ryegrass and at EDTA concentration of 7.5 mmol with the indian mustard.
- The bioconcentration factor (BCF) always revealed an increasing trend at the all employed EDTA concentrations for both the used plants either in their shoots or roots.

Table 2.Effect of EDTA concentrations on Zn-uptake (ppm)

Treatments	Plant	Shoot	Root	TF	BCF_{shoot}	BCF_{root}
Control	Indian mustard	36.4 ±4 e	31.4 ± 3.3 d	1.16	4.3	3.65
	Ryegrass	38.6 ± 5.7 e	22.5 ± 4.2 e	1.7	4.4	2.6
EDTA 2.5 mmol/ Kg soil	Indian mustard	179.7 ± 12.9 b	120 ± 5.2 b	1.5	20.7	14
	Ryegrass	67.5 ± 5.1 d	30.02 ± 3.7 d	2.2	7.9	3.5
EDTA 7.5mmol/Kg soil	Indian mustard	208 ± 9.7a	186 ±4.9 a	1.12	24.3	21.7
	Ryegrass	89.5 ± 4.2 c	36.6 ± 2.28 c	2.4	10.4	4.3

Table 3. Effect of EDTA concentrations on Cu-uptake (ppm)

Treatments	Plant	Shoot	Root	TF	BCF_{shoot}	BCF_{root}
Control	Indian mustard	7 ± 1.01 e	5.9 ± 1.2 e	1.1	6.3	5.3
	Ryegrass	14.8 ± 1.4 d	21.6 ± 2 c	0.7	13.2	19.3
EDTA 2.5 mmol/ Kg soil	Indian mustard	16.2 ± 2 d	14.5 ± 2 d	1.1	14.5	13
	Ryegrass	50 ± 3 b	21 .7± 3 c	2.3	44.6	19.5
EDTA 7.5mmol/Kg soil	Indian mustard	41.2 ± 3 c	28.6 ± 3.5 b	1.4	36.8	25.5
	Ryegrass	63.6 ± 4.8 a	30.4 ± 3a	2.1	56.9	27.2

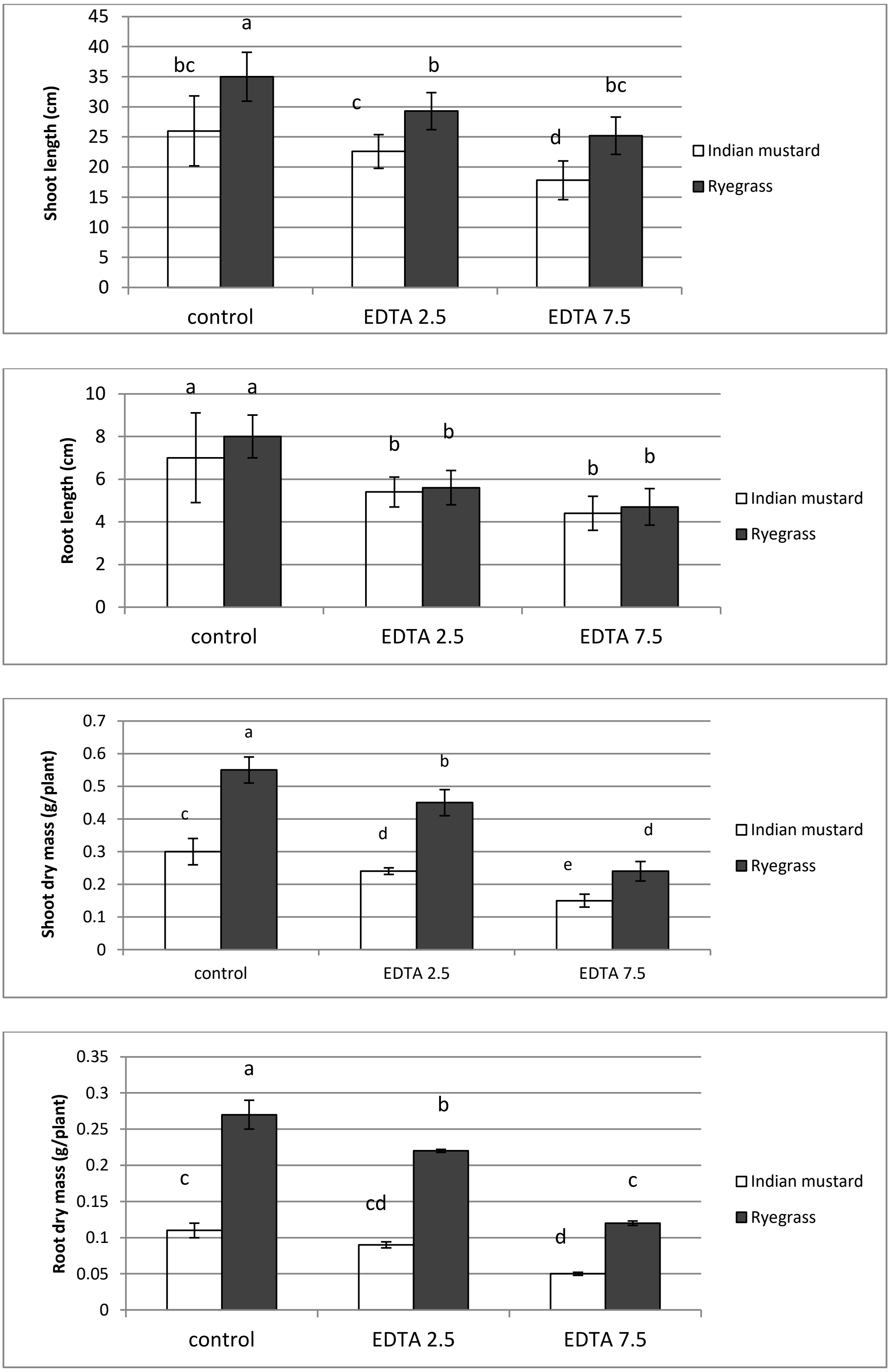

Figure 1. Effect of EDTA concentrations on plant's biomass

The enhanced metals-uptake by plants under the effect of EDTA (chelating agent) is likely to be attributed to the rapid dissolving of heavy metals and formation of the soluble metal-chelate complexes hence increasing the availability of these contaminants for the phytoremediation process **(Reddy and Chandhuri, 2009)**. Phytoremediation efficiency is usually controlled by many factors such as contaminants availability in soil, plant root development and the plant tolerance to each particular contaminant (**Pilon-smits, 2005).**

Some plant species (e.g. *Lolium perenne* L) recorded better growth in soil contaminated with Cu due to the probable antagonistic effects to the plant toxicity **(Lin et al 2006)**. Such assumption could explain the enhanced Cu-Uptake by the ryegrass relative to the indian mustard. The metals-uptake difference between the indian mustard and the ryegrass is reasonable to be ascribed to the destroying effect of EDTA on the roots physiological barriers by removing Fe^{2+}, Ca^{2+} and other divalent cations from the plasma membrane, which is thought to be important for the root selectivity properties **(Luo et al 2005).** Also, such distortion was more effective on the indian mustard causing the loss of its selectivity towards the necessary elements hence its suffering of biomass-decreasing comparing to the ryegrass.

The variance analysis of growth parameters (shoot-root length and shoot-root dry mass) showed sensible decreasing in the treated plants with various EDTA concentrations relative to the control samples **(Figure 1),** this decreasing in the plant biomass production is usually related to the toxic effect of the absorbed heavy metals (**Lim et al 2004 & 2012).**

In general, heavy metals cause a toxicity effect on plant growth resulting in less biomass production (**Lim et al 2004 & 2012).** Accordingly, application of EDTA resulted in enhancing the metals-uptake that pose a damaging effect on stability and permeability of the cell walls (**Duquene et al 2009 and Saifullah et al 2010)**, hence a significant decreasing in both the indian mustard biomass and, in less amount, the ryegrass biomass was observed.

CONCLUSION

Indian mustard and ryegrass are favorable hyperaccumulator plants for up-taking Zn and Cu contaminants from the polluted soil due to their high yielded biomass. Application of the EDTA amendment to the polluted soil significantly enhanced the cleanup process where it chelates the immobilized pollutants and increases their uptake and translocation into the harvestable plant biomass. Generally, both Cu and Zn revealed higher accumulation into the shoots relative to the roots in both the used plants with significant tendency toward Zn by the indian mustard and more tendency toward Cu by the ryegrass. The indian mustard biomass is sensibly decreased by the EDTA treatment more than decreasing in the ryegrass biomass.

REFERENCES

Baker, A.J.M and Brooks, R.R. 1989. Terrestrial higher plants which hyperaccumulate metal elements: are view of the irdistribution, ecology and phytochemistry. **Biorecovery, 1,81–126.**

Blaylock, M., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y. and Raskin, I. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. **Environ. Sci. Technol., 31, 860–865.**

Bolan, N., Kunhikrishnan, A., Thangarajana, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B. and Scheckel, K. 2014. Remediation of heavy metal (loid)s contaminated soils – to mobilize or to immobilize? **J. Hazard. Mater., 26,141–166.**

Duquene, L., Vandenhove, H., Tack, F., Meers, E., Baeten, J. and Wannijn, J. 2009. Enhanced phytoextraction of uranium and selected heavy metals by Indian mustard and ryegrass using biodegradable soil amendments. **Sci. Total Environ. 407, 1496–1505.**

Garbisu, C. and Alkorta, I. 2003. Basic concepts on heavy metals oil bioremediation. **Eur. J. Min. Process. Environ. Prot., 3, 58–66**

Ghosh, M. and Sing, S.O. 2005. A review on phytoremediation of heavy metals and utilization of its byproducts. **Appl. Ecol. Environ. Res., 3, 1–18.**

Halim, M., Conte, P. and Piccolo, A. 2003. Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. **Chemosphere., 52, 265–275**

Hoagland, D.R. and Arnon, D.I. 1950. **The water culture method for growing plants without soil. Circular.** California Agricultural Experiment Station., **347, 1-32.**

Homer, D. and Parker, F. 1961. Methods of Analysis for soils, plants and waters, **Agriculture & Natural Resources, pp. 61-64.**

Huang, J., Chen, J., Berti, W. and Cunningham, S. 1997. Phytoremediation of lead contaminated soils: Role of synthetic chelates in lead phytoextraction. **Environ .Sci. Technol., 31,800–805.**

Huang, J.W. and Cunningham, S.D. 1996. Lead phytoextraction: species variation in lead uptake and translocation. **New Phytol., 134, 75–84.**

Kotrba, P., Najmanova, J., Macek, T., Ruml, T. and Mackova, M. 2009. Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. **Biotechnol. Adv., 27, 799–810.**

Lim, J.M., Salido, A.L. and Butcher, D.J. 2004. Phytoremediation of lead using Indian mustard (*Brassica juncea*) with EDTA and electrodics. **Microchem. J., 76, 3–9.**

Lim, J.M., Jin, B. and Butcher, D.J. 2012. A comparison of electrical stimulation for electrodic and EDTA-enhanced phytoremediation of lead using Indian mustard (*Brassica juncea*). Bull. **Korean Chem. Soc., 33, 2737–2740.**

Lin, Q., Wang, Z., Ma, S. and Chen, Y. 2006. Evaluation of dissipation mechanisms by *Lolium perenne* L., and *Raphanus sativus* for pentachlorophenol (PCP) in copper contaminated soil. **Sci. Total Environ., 368, 814–822.**

Luo, C., Shen, Z. and Li, X. 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. **Chemosphere., 59, 1–11.**

McGrath, S.P., Zhao, F.J. and Lombi, E. 2001. Plant and rhizoshphere processes involved in phytoremediation of metal-contaminated soils. **Plant Soil., 232, 207–214.**

McIntyre, T. 2003. Phytoremediation of heavy metals from soils. **Adv. Biochem. Eng. Biotechnol., 78, 97–123.**

Memon, A.R. and Schroder, P. 2009. Implications of metal accumulation mechanisms to phytoremediation. **Environ. Sci. Pollut. Res., 16, 162–175.**

Memon, A.R., Aktoprakligil, D., Ozdemir, A. and Vertii, A. 2001. Heavy metal accumulation and detoxification mechanisms in plants. **Turk. J. Bot., 25,111–121.**

Passariello, B., Giuliano, V., Quaresima, S., Barbaro, M., Caroli, S., Forte, G., Garelli, G. and Lavicoli, I. 2002. Evaluation of the environmental contamination at an abandoned mining site. **Microchemical Journal., 73, 245-250.**

Pilon- Smits, E. 2005. Phytoremediation. **Annu. Rev. Plant Biol., 56, 15-39.**

Prasad, M.N.V. 2003. Phytoremediation of metal-polluted ecosystems :hype for commercialization. **Russ. J. Plant Physiol., 50, 686–700.**

Reddy, K.R. and Chandhuri, K.S. 2009. Fenton-like oxidation of polycyclic aromatic hydrocarbons in soils using electrokinetics. **J. Geotech. Geoenviron. Eng., 135, 1429–1439**

Saifullah, R., Zia, M.H., Meers, E., Ghafoor, A., Murtaza, G., Sabir, M., Ziaur-Rehman, M. and Tack, F.M.G. 2010. Chemically enhanced phytoextraction of Pb by wheat in texturally different soils. **Chemosphere., 79, 652–658.**

Yadav, S.K. 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metals stress tolerance of plants. **South African Journal of Botany, 76, 167-179.**

Yang, X., Feng, Y., He, Z. and Stoffella, P.J. 2005. Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. **J. Trace Elem. Med. Biol., 18, 339–353.**