J.Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 7(10):729-737, 2016

Impact of EDTA, Citric Acid and Humic Acid on Phytoremedation of Metal Contaminated Soil by Indian Mustard (*Brassica juncea*)

El-Agrodi, M. W. M.[1]; F. I. Zein[2]; G. Labeeb[1] and M. S. A. Ramadan [2]
[1] Soils Dept. Fac. of Agric., Mansoura Univ., Egypt.
[2]Soil, Water and Environment Res. Inst., A.R.C., Egypt.

ABSTRACT

A pot experiment was conducted during winter season of (2013/2014) at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate. The experiment aimed to investigate the effect of disodium ethylenediaminetetraacetic acid (EDTA) (0.0, 1.5, 3.0 and 4.5 mmol kg^{-1} soil), citric acid (CA) (0, 3, 6 and 9 mmol kg^{-1} soil) and humic acid (HA) (0.0, 0.2, 0.4 and 0.6 g kg^{-1} soil) on the phytoextraction of Cu, Zn and Pb from Al-Gabal Al-Asfar contaminated soil using indian mustard (*Brassica juncea*) and the leaching behavior of these metals from soil. The obtained results can be summarised in the following:- EDTA severely reduced indian mustard dry weight and visual symptoms of toxicity were observed, especially at 4.5 mmol EDTA kg^{-1} soil. Whilst, application of CA and HA ameliorated indian mustard growth.- EDTA was found to be the most efficient chelator in increasing concentrations of Cu and Zn in different indian mustard plant parts.- Increasing EDTA application rate up to 4.5 mmol kg^{-1} soil increased the concentrations of Cu by 1.91, 2.88, 3.04 and 1.56 folds and Zn by 1.43, 1.91, 1.83and 1.11 folds for roots, stem, leaves and seeds, respectively, compared to control treatment (0.0 mmol EDTA kg^{-1} soil).- Application of CA also increased Cu and Zn concentrations by different indian mustard plant parts, especially at 9 mmol kg^{-1} soil, but its efficacy was less than EDTA.- HA application increased Zn concentrations in different indian mustard plant parts with increasing its addition up to 0.6 g kg^{-1} soil, but its efficacy was less than that of EDTA and CA.- HA application did not affect Cu concentrations in indian mustard.- There were neither any perceptible concentrations of Pb in different plant parts of indian mustard except for roots clarifying that phytoextraction of Pb failed even after application of tested chelators. - Application of EDTA and CA to the soil increased the leaching of Cu, Zn and Pb under indian mustard cultivation with increasing application rate up to 4.5 and 9 mmol kg^{-1} soil, respectively. However, the effect of EDTA was many times higher than that of CA. Whilst, HA application did not affect the leaching of studied metals.- Using CA for the phytoremediation of Cu and CA and HA for the phytoremediation of Zn is favorable than EDTA despite the high efficiency of EDTA, due to the harmful effects of EDTA on plant growth and the increased risk of groundwater contamination via metal leaching.

Keywords: phytoremediation, phytoextraction, Zn, Cu, Pb contaminated soils and indian mustard plant.

INTRODUCTION

Nowadays soil pollution is getting great public attention where the magnitude of this serious problem is growing rapidly. One of the most serious pollutants in the environment, especially agricultural environments, is heavy metals because of their elevated level of durability and toxicity to the biota (Alkorta *et al.,* 2004). In Egypt, which located in arid and semi-arid regions, water is becoming scarce resource. Usage low quality water such as wastewater for irrigation causing soil pollution. Primary treated wastewater has been used in agriculture to irrigate about 1260 ha in Al Gabal Al Asfar since 1911 (Mahjoub, 2016).

The cleanup of contaminated soils is one of the most difficult tasks for environmental engineering, where, soil washing, excavation and land-filling can be used at highly contaminated sites but are not applicable to large areas and are very expensive. Immobilization of heavy metals has the advantage of immediately reducing risk of metals, but it considered as temporary alternative. Phytoremediation is a promising new approach, direct use of plants to clean up the soil from heavy metals in situ, friendly environment, economically cost-effective.

High-biomass and metal-hyper accumulator plant species have a high potential and promising to be used in phytoremediation, among them the *Brassicaceae* (mustard) family (Robinson *et al.,* 2009).

Availability of heavy metals to plant roots is considered as key factor limiting the phytoextraction efficiency. In the last decade the use of persistent amino-polycarboxylic acids such as ethylene diaminetetraacetic acid (EDTA) was suggested as a chelating agent to assist the phytoextraction processes (Evangelou *et al.*, 2007).Natural low molecular weight organic acids (LMWOA) such as citric acid (CA), oxalic acid, or malic acid play a significant role in heavy metal solubility (Nigam *et al.,* 2001).Humic acids contain carboxylic groups which play a prominent role on heavy metals availability (Hofrichter and Steinbüchel, 2001). Zhang *et al.,* (2003) stated that the lower stability constant of HA, compared to other synthetic chelates for metals, makes HA an ideal soil amendment for phytoextraction.

Therefore, the objectives of this study are to evaluate the efficiency of indian mustard (*Brassica juncea*) in phytoextraction of heavy metal from contaminated soil. Moreover, investigating the effect of synthetic and organic chelators on bioavailability and leachability of heavy metals.

MATERIALS AND METHODS

A pot experiment was conducted in a wired greenhouse at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate during winter season of (2013/2014), aimed to investigate the phytoextraction efficiency of Cu, Zn and Pb from Al-Gabal Al-Asfar contaminated soil using indian mustard (*Brassica juncea*) plant under chelators disodium ethylenediaminetetraacetic acid (EDTA), citric acid (CA) and humic acid (HA) addition.

Contaminated soil was collected from the surface layer (0-30 cm) of Al-Gabal Al-Asfar farm in 2013's early spring. Collected soil samples were air-dried, ground to pass through a 4 mm diameter sieve and mixed thoroughly to achieve homogeneity. Representative sample was ground to pass through a 2 mm sieve; some physical and chemical characteristics of the studied soil were determined and shown in Table (1).

Air-dried soil equivalent to 6.5 kg oven dry soil was placed in crockery pots (25 cm diameter × 40 cm height) lined with polyethylene bags which were used to coat the inner walls of crockery pots in order to avoid water preferential flow. The polyethylene bags and pots were ended with a central hole at the bottom to collect the leached solution. A filter paper and a fine mesh tissue were used in order to prevent soil losses from the ended hole.

Table 1. Some physical and chemical characteristics of the tested soils.

Soil characteristics		
pH (soil-paste)		6.88
EC_e (soil-paste extract), dSm^{-1} at 25˚C		1.59
OM (organic matter), %		2.03
Total carbonates, %		0.22
Water holding capacity (WHC), %		35.55
CEC (cation exchange capacity), meq/100g soil.		17.92
Particle size distribution	Sand (%)	82.55
	Silt (%)	5.02
	Clay (%)	12.43
Texture class		loamy sand
Soluble cations (meq L^{-1})	Ca^{++}	5.19
	Mg^{++}	3.21
	Na^{+}	6.37
	K^{+}	0.63
Soluble anions (meq L^{-1})	$CO_3^{=}$	--
	HCO_3^{-}	6.88
	Cl^{-}	5.76
	$SO_4^{=}$	2.76
Available Macro-nutrients (mg kg^{-1})	N	35
	P	44.39
	K	147.50
DTPA-extractable(mg kg^{-1})	Pb	36.00
	Cu	27.00
	Zn	67.00
Total (mg kg^{-1})	Pb	210.00
	Cu	107.00
	Zn	380.00

The experiment was done under natural daylight in a wired greenhouse. Split plot design with three replicates was used. The main plots were assigned to chelator type, whereas, the sub-plots were subjected to chelator addition rates (C_0, C_1, C_2 and C_3) as following:

(i) EDTA (0.0, 1.5, 3.0 and 4.5 mmol kg^{-1} soil)

(ii) CA(0.0, 3, 6 and 9 mmol kg^{-1} soil)

(iii) HA (0.0, 0.2, 0.4 and 0.6 g kg^{-1} soil)

Four seeds of indian mustard plant were sown on October 20th, 2013. At fourth plant leaf stage, plants were thinned to one plant per pot. The irrigation water was added to all plants as needed in the beginning. After chelators addition, the irrigation water was added at 100% of water holding capacity (WHC) plus 5%in order to receive the leachate. NPK fertilization was done as 2.0 fold of Egyptian Ministry of Agriculture recommendation (Potassium fertilizer 25 kg K_2O fed^{-1} was applied in the form of potassium sulphate, phosphorus fertilizer 30 kg (P_2O_5) fed^{-1} was applied in the form of phosphoric acid, and nitrogen fertilizer 45 kg (N) fed^{-1} was applied in the form of Urea).

Chelating agents with selected levels were added to the soil on two equal doses with irrigation water through rapid growth stage. The first dose was added 45 days of cultivation of indian mustard plant and the second dose was added after 30 days of the first dose.

The leachate solutions collection was started from the bottom of each pot after addition of chelating agents up to the end of growing seasons.

Dropped leaves of each plant were collected during growing season. On 20th of March (2014), full maturity stage, plants were totally removed from pots and plant organs were separated to roots, stem, leaves and seeds.

Method of analysis:

Soil analysis

- Soil reaction (pH), Electrical conductivity (ECe) and soluble cations and anions according to Page (1982).
- Total organic matter (OM) was determined by the wet combustion of Walkely-Black method (Hesse, 1971).
- Available N, P, K and cation exchange capacity were determined according to Jackson (1973).
- Soil mechanical analysis was determined according to the international pipette method (Piper, 1950).
- Total $CaCO_3$ % was measured volumetrically by Collins calcimeter and water holding capacity (WHC) was measured according to Richards (1954).
- Available contents of heavy metals were extracted by diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978).
- Total contents of heavy metals were determined using aqua regia digestion according to Cottenie *et al.*, (1982).
- Heavy metals were measured using the Atomic Absorption Spectrophotometer (AAS), PERKIN ELMER 3300.
- The collected leachate solutions were filtered through Whatman No. 42 filter paper and analyzed for heavy metals using (AAS), PERKIN ELMER 3300.

Plant analysis

- Separated plant parts were gently brushed using a brush pen, washed with tap water then distilled water three times, oven dried at 70 °C for 72 h, weighed, milled in a stainless steel mill, finally analyzed for heavy metals using (AAS), PERKIN ELMER 3300, after wet digestion by ($HNO_3/HClO_4$) mixture according to Chapman and Pratt (1961).

All collected data were statistically analyzed according to the method described by Snedecor and Cochran (1980) using the CoStat package program, version 6.311 (cohort software, USA). To compare between treatment means, the Least Significant Differences (LSD) at probability 5% was used.

RESULTS AND DISCUSSION

Effect of chelator types, rates and their interaction on indian mustard dry weight (g $plant^{-1}$):

Data of Table (2) represent the effect of chelator types, rates and their interaction on indian mustard roots, stem, leaves, seeds and total dry weight production. Data reveal that chelator types significantly affected the dry weight of indian mustard different plant parts. All plant parts recorded the highest values due to HA application. EDTA reduced plant organs dry weight to a large extent compared with CA and HA.

In this respect, application of HA increased the mean values of roots, stem, leaves, seeds and total dry weight as compared to the mean values of EDTA by 18.80, 15.41, 17.42, 16.22 and 16.63%, respectively. Whilst, CA treatment increased the mean values of root, stem, leaves, seeds and total dry weight by 15.79, 10.61, 16.67, 10.81 and 12.89%, respectively, as compared to EDTA means.

Application of EDTA levels significantly reduced indian mustard dry weight yield as compared to control, 0.0 EDTA. Moreover, the reduction was increased with increasing EDTA application rate up to 4.5 mmol kg^{-1} soil. The reduction in roots, stem, leaves, seeds and total dry weight as a result of applying EDTA at a rate of 4.5 mmol kg^{-1} soil were 25.00, 13.41, 23.31, 17.07 and 18.09%, respectively, as compared to control.

Table 2. Effect of chelator types, rates and their interaction on plant parts dry weight (g $plant^{-1}$) of indian mustard.

Treatments		Roots	Stem	Leaves	Seeds	Total
EDTA mmol kg^{-1} soil	0	30.0	73.8	29.6	16.4	149.8
	1.5	26.9	69.6	26.3	14.0	136.8
	3.0	27.1	67.8	26.9	15.0	136.8
	4.5	22.5	63.9	22.7	13.6	122.7
Mean		26.6	68.8	26.4	14.8	136.5
F-test		**	**	**	ns	**
$LSD_{0.05}$		2.5	4.8	2.7		10.2
$LSD_{0.01}$		3.9	7.5	4.2		15.9
CA mmol kg^{-1} soil	0	30.0	73.8	29.6	16.4	149.8
	3.0	29.7	74.0	30.2	15.3	149.2
	6.0	33.2	81.0	33.3	17.4	164.9
	9.0	30.3	75.5	30.0	16.6	152.4
Mean		30.8	76.1	30.8	16.4	154.1
F-test		ns	ns	ns	ns	ns
$LSD_{0.05}$						
$LSD_{0.01}$						
HA g kg^{-1} soil	0	30.0	73.8	29.6	16.4	149.8
	0.2	29.2	77.2	29.5	17.6	153.5
	0.4	32.0	80.6	31.3	16.7	160.6
	0.6	35.1	86.0	33.7	17.9	172.7
Mean		31.6	79.4	31.0	17.2	159.2
F-test		ns	ns	ns	ns	ns
$LSD_{0.05}$						
$LSD_{0.01}$						
F-test (treatments)		**	**	**	*	**
$LSD_{0.05}$		1.7	2.7	2.1	1.9	6.5
$LSD_{0.01}$		2.6	3.5	3.3		9.9
F-test (Interaction)		**	ns	ns	ns	ns
$LSD_{0.05}$		1.5				
$LSD_{0.01}$		2.4				

Visual symptoms of toxicity were observed with EDTA treatment, especially at high level (4.5 mmol kg^{-1} soil), as shown in (fig. 1), such as some chlorosis and necrosis (brown dots) on leaves, particularly on old ones, and the leave became yellow and died slowly. Similar results were found by Taalab *et al.*, (2009). This could be attributed to either increasing EDTA itself and its metal complexes in root media as suggested by Chen and Cutright (2001) or heavy metal accumulation in plant parts causing toxicity as discussed later.

Regarding the effect of CA application rates, increasing CA applied rate up to 6.0 mmol kg^{-1} soil in Al-Gabal Al-Asfar soil increased plant organs and totaldry weight but the increment values in each trait was insignificant. However, the corresponding values for roots, stem, leaves and seeds were increased by 10.67, 9.76, 12.50, 6.10 and 10.08% compared with control. These increases in plant growth and biomass might be due to increased nutrient uptake and CA-induced chelation of metals decreasing free metal ions in plants as suggested by Najeeb *et al.*, (2011). CA application increased plant growth, biomass, and chlorophyll content by reducing oxidative stress and enhancing antioxidant enzyme activities (Zaheer *et al.*, 2015).

Fig 1. visual symptoms of toxicity on indian mustard leaves due to application of 4.5 mmol EDTA kg^{-1}soil.

Increasing HA applied rates increased plant dry weight, but this increase was insignificant as a result of their non-significant effects on plant organs dry weight. The highest level used of HA (0.6 g HA kg^{-1} soil) recorded the highest values of plant parts dry weight as compared to control, where the increment values of roots, stem, leaves, seeds and total dry weight were 17.00, 16.53, 13.85, 8.9.15 and 15.29% as compared to control.

Data of Table (2) illustrate that no significant effects were found due to the interaction between chelator types and addition rates on plant dry weight, except for roots where applying 0.6 g HA kg^{-1} soil achieved the highest roots dry weight, 35.10 g.

Effect of chelator types, rates and their interaction on Cu concentration (mg kg^{-1}DW) of indian mustard plant:

Data of Table (3) show the effect of chelator types, rates and their interaction on Cu concentrations of roots, stem, leaves and seeds.

Regarding chelator types effect, results reveal that applied chelators significantly affect Cu concentrations in plant parts. Where, EDTA gave the highest Cu concentration of indian mustard plant parts. HA application recorded the lowest Cu concentration in plant parts. In this respect, the concentrations of Cu in roots, stem, leaves and seeds was increased due to EDTA application compared to HA by 46.78, 100.00, 110.20 and 37.40%, respectively. Whereas, CA treatment increased that trait of roots, stem, leaves and seeds compared to HA by 25.71, 49.02, 48.98 and 3.25%, respectively. The high efficacy of EDTA could be attributed to that addition of EDTA to soil transfers metal sorption and precipitation equilibrium toward enhanced heavy metals dissolution due to forming metal-EDTA complexes as reported by Hadi *et al.*, (2010).

Table 3. Effect of chelator types, rates and their interaction on Cu concentration (mg kg^{-1}) of roots, stem, leaves and seeds of indian mustard.

Treatments		Roots	Stem	Leaves	Seeds
EDTA mmol kg^{-1} soil	0	163.3	20.8	20.0	13.3
	1.5	202.5	31.7	35.8	15.8
	3.0	251.7	50.8	48.3	17.5
	4.5	312.5	60.0	60.8	20.8
Mean		232.5	40.8	41.2	16.9
F-test		**	**	**	**
$LSD_{0.05}$		12.8	3.3	4.2	2.7
$LSD_{0.01}$		20.0	5.1	6.6	4.2
CA mmol kg^{-1} soil	0	163.3	20.8	20.0	13.3
	3.0	174.2	27.5	26.7	11.7
	6.0	202.5	33.3	31.7	11.7
	9.0	255.8	40.0	38.3	14.2
Mean		199.0	30.4	29.2	12.7
F-test		**	**	**	ns
$LSD_{0.05}$		11.7	4.5	4.7	
$LSD_{0.01}$		18.2	7.0	7.2	
HA g kg^{-1} soil	0	163.3	20.8	20.0	13.3
	0.2	169.2	20.8	20.8	11.7
	0.4	156.7	20.8	18.3	11.7
	0.6	144.2	19.2	19.2	12.5
Mean		158.3	20.4	19.6	12.3
F-test		*	ns	ns	ns
$LSD_{0.05}$		19.2			
$LSD_{0.01}$					
F-test (treatments)		**	**	**	**
$LSD_{0.05}$		9.8	3.3	4.9	2.1
$LSD_{0.01}$		14.9	5.0	7.5	3.2
F-test (Interaction)		**	**	**	ns
$LSD_{0.05}$		8.5	2.8	4.3	
$LSD_{0.01}$		12.9	4.3	6.5	

Regarding chelator rates effect, increasing of EDTA levels up to 4.5 mmol kg^{-1} soil high significantly increased Cu concentration in roots, stem, leaves and seeds by 1.91, 2.88, 3.04 and 1.56 folds compared to control, respectively. Similar trend was found by Wu *et al.*, (2004), where 3 mmol EDTA kg^{-1} soil elevated Cu concentration from 15.3 to 39.8 mg kg^{-1} in indian mustard plant grown on a paddy soil polluted with Cu. The neutral charge, metal-EDTA complex are not blocked or attached by carboxyl groups or polysaccharides of rhizoderm cell surface. In this way, EDTA causes the metal to enter directly to the plant roots (Shahid *et al.*, 2012).

In case of CA, similar trend to EDTA was found, where Cu concentrations in roots, stem and leaves were significantly increased with increasing CA application levels up to 9 mmol kg^{-1} soil. While no significant effect on Cu concnetrations in seeds was found due to application of different CA levels. In this respect, the values of Cu concentration in roots, stem and leaves increased as compared with control by 1.57, 1.92 and 1.92 folds, respectively, when CA was applied at a level of 9 mmol kg^{-1} soil. These results are in harmony with that of Evangelou *et al.*, (2006) who revealed that application of 62.5 mmol kg^{-1} CA had 2-folds increase of Cu concentrations in tobacco (*N. tabacum*) shoots. Also, Zaheer*et al.*, (2015) found that addition of CA increased Cu uptake by *B. napus* plants and the negative effects of Cu toxicity can be overturned by CA application.

Concerning the effect of HA on Cu concentrations of indian mustard plant, the obtained results point out that different applied rates of HA decreased concentrations of Cu in plant parts. However, this decrease in Cu concentration in indian mustard was insignificant, except for roots was significant. The highest reduction of Cu concentrations in roots and stem (mg kg^{-1}DW) were obtained using 0.6 g HA kg^{-1} soil and the corresponding reductions were 11.69 and 7.69% as compared to control, respectively. While application of 0.4 g HA kg^{-1} soil achieved the highest reduction of Cu concentration in leaves and seeds and these reductions were amounted by 8.50 and 12.03% as compared to control, respectively. These results are in parallel trend with that of Wang *et al.,* (2010). They found that increasing HA addition from 3.09 to 7.89 g kg^{-1} soil to Cu and Cd contaminated sediment reduced Cd concentrations in *Vallisneria spiralis* roots and shoots by 26.4–50.3% and 14.3–33.0%, whereas, Cu accumulation was decreased much more with 44.0–77.0% and 35.0-62.7%, respectively. Humic acid clearly reduced Cu and Cd bioavailability and toxicity in *V. speralis* due to forming complexes of HA with metal ions.

Regarding the interaction between chelator types and their addition rates, significant effects on Cu concentration (mg kg^{-1}) in roots, stem, leaves and seeds were found. While no significant effect on Cu concentrations in seeds was found. However, application of 4.5 mmol EDTA kg^{-1} soil achieved the highest concentrations of Cu in plant organs.

Effect of chelator types, rates and their interaction on Zn concentration (mg kg^{-1}DW) of indian mustard plant:

Data in Table (4) represent the effect of chelator type, rates and their interaction on Zn concentrations of roots, stem, leaves and seeds of indian mustard.

Concerning the effect of chelator types used, it was noticed that Zn concentrations in different plant parts were significantly differed due to chelators types application. In this respect, EDTA increased roots, stem, leaves and seeds Zn concentration (mg kg^{-1} DW) as compared to HA by 14.90, 37.59, 18.25 and 4.11%, respectively. Whereas, application of CA treatment increased the mean values of Zn concentration (mg kg^{-1}DW) in roots, stem, leaves and seeds by 5.24, 8.69, 1.67 and 11.92% as compared to HA, respectively.

Table 4. Effect of chelator types, rates and their interaction on Zn concentration (mg kg^{-1} DW) of roots, stem, leaves and seeds of indian mustard.

Treatments		roots	stem	leaves	seeds
EDTA mmol kg^{-1} soil	0	533.3	127.5	235.8	74.2
	1.5	580.8	170.0	280.0	78.3
	3.0	691.7	200.0	325.0	79.2
	4.5	764.2	243.3	431.7	82.5
Mean		642.5	185.2	318.1	78.6
F-test		**	**	**	ns
$LSD_{0.05}$		24.5	16.1	19.0	
$LSD_{0.01}$		38.1	25.1	29.7	
CA mmol kg^{-1} soil	0	533.3	127.5	235.8	74.2
	3.0	560.8	138.3	253.3	78.2
	6.0	602.5	152.5	280.0	85.8
	9.0	657.5	166.7	325.0	99.9
Mean		588.5	146.3	273.5	84.5
F-test		**	**	**	**
$LSD_{0.05}$		20.9	14.2	15.6	9.5
$LSD_{0.01}$		32.6	22.1	24.3	14.7
HA g kg^{-1} soil	0	533.3	127.5	235.8	74.2
	0.2	544.2	128.3	255.0	77.3
	0.4	560.8	134.2	270.8	75.0
	0.6	598.3	148.3	314.2	75.3
Mean		559.2	134.6	269.0	75.5
F-test		**	**	**	ns
$LSD_{0.05}$		21.0	7.8	23.7	
$LSD_{0.01}$		32.7	12.2	36.9	
F-test (treatments)		**	**	**	*
$LSD_{0.05}$		16.9	8.4	16.2	8.2
$LSD_{0.01}$		25.6	12.7	24.5	
F-test (Interaction)		**	**	**	**
$LSD_{0.05}$		14.6	7.3	14.0	7.1
$LSD_{0.01}$		22.1	11.0	21.3	10.8

Tabulated data show that Zn concentration of each plant part was significantly increased with increasing EDTA addition rate up to 4.5 mmol kg^{-1} soil, except for seeds where the increases in Zn concentrations were insignificant. Accordingly, addition of 4.5 mmol EDTA kg^{-1} soil significantly increased Zn concentrations in roots, stem and leaves by 43.30, 90.82 and 83.08% as compared to control, respectively. The concentrations of Zn in seeds was insignificantly increased with increasing EDTA levels up to 4.5 mmol kg^{-1} soil by 11.19 % increase compared to control. Increasing Zn concentration of plant parts with EDTA application is a normal result due to its effect on Zn availability in soil. These results are in a consistency with that of Wu *et al.,* (2003) who outlined that 3 mmol EDTA kg^{-1} soil increased Zn concentration in soil solution.

In case of CA, the obtained results also reveal that Zn concentrations in indian mustard plants parts were increased by increasing CA levels up to 9 mmol kg^{-1} soil. Accordingly, application of 9.0 mmol CA kg^{-1} soil significantly increased Zn concentrations in roots, stem, leaves and seeds as compared to control by 1.23, 1.31, 1.38 and 1.35 folds, respectively. These results are in harmony with those of Najeeb *et al.*, (2009). They stated that CA induced chelation of metals decreasing free ions in plant, hence CA promote plant for more metal uptake with no negative effect.

Regarding to HA, application at different levels revealed significant increases in different plant parts, except for seeds. Application of 0.6 g HA kg^{-1} soil achieved the highest values of Zn concentrations in roots, stem and leaves as compared to control and lower HA application levels. The corresponding increases for roots, stem and leaves were increased as compared to control by 12, 19, 16.31 and 33.25%, respectively. A parallel results were found by Turan and Angin (2004),they found that HA (at rates of 5 and 10 mmol kg^{-1} soil) was effective natural chelator in enhancing Pb, Cd, Mo and B desorption from soil and increasing their accumulation in *Zea mays L.* and *Helianthus annus L.*. Also, Topcuoğlu (2012) found that the availability and uptake of Zn by tobacco plant was significantly raised by HA application at 1% and 2%.

In respect to the interaction between chelator types and their addition rates, a significant effect on roots, stem, leaves, seeds and total uptake of Zn (mg kg^{-1}) by indian mustard were found. Whereas, application of 4.5 mmol EDTA kg^{-1} soil revealed the highest concentrations of Zn in different plant parts.

Effect of chelator types, rates and their interaction on Pb concentrations (mg kg^{-1} DW)of indian mustard plant:

It is worthy to mention that there were not any perceptible concentrations of Pb in different plant parts except for roots. These findings are in agreement with that of Padmavathiamma and Li (2007) who reported that Pb translocation from roots to above-ground parts is very poor. The major proportion of Pb is concentrated in root tissues. Only 3% of roots Pb are translocated to shoots(Zimdahl, 1975). The utmost roots Pb is deposited, especially as Pb-pyrophosphate, along the cell walls as reported by Meyers *et al.,* (2008).

Fig (2) shows the effect of chelator types, rates and their interaction on Pb concentrations of indian mustard roots. The results prove that Pb concentrations in roots was affected by chelator types used in this study, where EDTA and CA increased Pb concentrations in roots as compared to HA treatment by 57.74% and 4.45%, respectively.

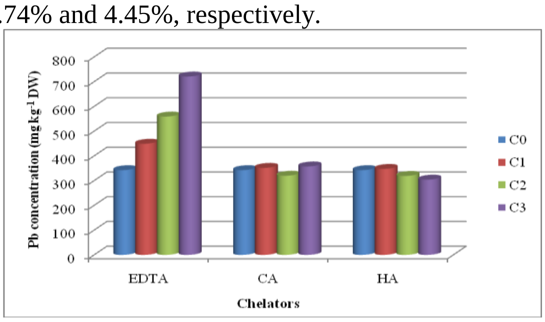

Fig 2. Effect of chelators and their application levels on Pb concentration (mg kg^{-1} DW) in indian mustard roots.

Raising EDTA levels up to the highest level used (4.5 mmol kg^{-1} soil) significantly increased Pb concentrations in plant roots by 2.10 folds than that of control. Similarly, raising CA levels up to 9 mmol kg^{-1} soil increased Pb concentration in roots as compared to control by 4.38%, but this increase was insignificant. These results are in a similar trend with those of Freitas *et al.,* (2013). They outlined that usage of CA at 40mmol kg^{-1} soil was effective in the Pb solubilization of the soil and in the induction of Pb absorption by Maize (*Zea mays*) and vetiver (*Chrysopogon zizanoides*).

On the other hand, no significant effect on Pb concentration in roots was found due to increasing HA application level to the soil. A slight decrease in Pb concentrations in roots was found with increasing HA rates. These results are in harmony with those of Wang *et al.,* (2010). They stated that HA clearly reduced Cu and Cd bioavailability and toxicity in *V. speralis* due to forming complexes of HA with metal ions.

Leaching of Cu, Zn and Pb metals (mg pot^{-1}) under indian mustard cultivation:

Data in Table (5) illustrate the effect of chelator types, their addition rates and their interaction on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard cultivation in Al-Gabal Al-Asfar soil.

Chelate types significantly affected the leached amount of Cu, Zn and Pb under indian mustard cultivation. Where, EDTA had up normal effect on Cu and Zn leaching. The leached amounts due to EDTA application were many times higher than that of CA and HA. In this respect, Application of EDTA treatment increased Cu, Zn and Pb leaching than that of HA treatment by 26.14, 17.54 and 26.33 folds. Whilst, using CA increased Cu, Zn and Pb leaching than that of HA treatment by 2.14, 2.88 and 1.11 folds. These results are in agreement with those of Nascimento *et al.*, (2006) who compared synthetic chelates (EDTA and DTPA) with LMWOA (oxalic acid, citric acid, vanillic acid and gallic acid) in enhancing heavy metals phytoextraction by indian mustard from multi-metal contaminated soils. They reported that the effectiveness of LMWOA were not accompanied by raising the risk of leaching for these metals so unlike EDTA and DTPA.

Concerning chelator levels effect on leached studied metals, increasing EDTA level up to 4.5 mmol kg^{-1} soil linearly increased the leached Cu, Zn and Pb (mg pot^{-1}) as compared to the control by 58.29, 38.36 and 62.89 folds, respectively.

Likewise EDTA, application of CA up to 9 mmol kg^{-1} soil significantly increased Cu and Zn leaching under indian mustard cultivation, while, non-significant effect regarding to Pb leaching increase. Application of 9 mmol CA kg^{-1} soil increased leached Cu, Zn and Pb (mg pot^{-1}) by 4.14, 6.20 and 1.22 folds, respectively, compared with control. Contradictory, application of HA different levels did not reveal any significant effect on leaching of tested metals.

Concerning chelator type - rates interaction effects on leached Cu, Zn and Pb, highly significant effect was found on these metals leaching (mg pot^{-1}) under indian mustard cultivation. Where, the highest values of leached Cu, Zn and Pb were obtained using EDTA at a level of 4.5 mmol kg^{-1} soil. So, application of higher level of EDTA to the soil is an inappropriate for use in phytoextraction due to its environmental impact on groundwater contamination via metal leaching. These results are in conformity with that of Meers *et al.*, (2005) who revealed that there exists a high binding constant of EDTA for metals (Pb, 19.71; Zn, 18.00 and Cu, 20.49), allowing the dissolution of heavy metals.

Table 5. Effect of chelator types, rates and their interaction on Cu, Zn and Pb leaching (mg pot^{-1}) under indian mustard cultivation.

Treatments		Metal leaching (mg pot^{-1})		
		Cu	Zn	Pb
EDTA mmol kg^{-1} soil	0	0.7	5.5	0.9
	1.5	7.0	57.1	8.1
	3.0	24.6	119.2	29.1
	4.5	40.8	211.0	56.6
Mean		18.3	98.2	23.7
F-test		**	**	**
$LSD_{0.05}$		3.9	17.6	1.4
$LSD_{0.01}$		6.0	27.4	2.2
CA mmol kg^{-1} soil	0	0.7	5.5	0.9
	3.0	0.8	9.3	1.0
	6.0	1.4	15.4	1.0
	9.0	2.9	34.1	1.1
Mean		1.5	16.1	1.0
F-test		**	**	ns
$LSD_{0.05}$		0.5	6.7	
$LSD_{0.01}$		0.7	10.4	
HA g kg^{-1} soil	0	0.7	5.5	0.9
	0.2	0.7	5.4	0.9
	0.4	0.6	5.3	0.9
	0.6	0.6	6.0	1.0
Mean		0.7	5.6	0.9
F-test		ns	ns	ns
$LSD_{0.05}$				
$LSD_{0.01}$				
F-test (treatments)		**	**	**
$LSD_{0.05}$		2.1	5.5	0.2
$LSD_{0.01}$		3.2	8.4	0.4
F-test (Interaction)		**	**	**
$LSD_{0.05}$		1.8	4.8	0.2
$LSD_{0.01}$		2.7	7.3	0.3

Compared to EDTA, CA had a small effect on metal concentrations in leachate as its effectiveness was not accompanied by increasing the risk of leaching for these metals so unlike EDTA. These results are in agreement with that of Jean-Soro *et al.*, (2012) who revealed that EDTA is more effective in enhancing heavy metals desorption from the soil solid phase. In most cases, the effect of EDTA was many times greater than that of CA.

Application of different HA levels did not affect metal leachabilty under sunflower and indian mustard cultivation in both soils. This result was in agreement with Halim *et al.*, (2003) who signified that while humic substances are potentially useful to increase plant-availability of heavy metals in soil, they concomitantly reduce the environmental mobility of these contaminants.

CONCLUSION

It can be concluded that EDTA had the highest efficacy on enhancing Cu and Zn absorption by indian mustard plants. Application of CA also increased Cu and Zn concentrations in indian mustard plant, while HA was only efficient to increase Zn absorption by indian mustard plant. The high concentrations of Cu and Zn in the plant organs due to applying EDTA compared to the other chelators induced toxicity, so reduced the dry matter of these organs.

Using CA for the phytoremediation of Cu and CA and HA for the phytoremediation of Zn is favorable than EDTA despite the high efficiency of EDTA, due to either its harmful effect of high rates on plant growth or its increment effect of groundwater contamination risk via metal leaching.

Phytoextraction of Pb was failed even with EDTA, CA and HA chelators addition, where there were neither any perceptible concentrations of Pb in the above ground portion of indian mustard plant parts.

REFERENCES

Alkorta, I.; Hernandez-Allica, J.; Becerril, J. M.; Amezaga, I.; Albizu, I. & Garbisu, C., (2004). 'Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic'. reviews in Environmental Science and Bio-technology, 3: 71-90.

Chapman, H. D. & Pratt, F., (1961). "Methods of Analysis of: Soils, Plants and Water". Calif. Univ., USA.

Chen, H. & Cutright, T., (2001). EDTA and HEDTA effects on cadmium, Cr, and Ni uptake by *Helianthus annuus*. Chemosphere, 45: 21–28.

Cottenie, A., Verloo, P. M., Kiekens, L., Velghe, G. & Camerlynck, R, (1982). "Chemical Analysis of Plants and Soils". Lab. Anal and Agrochem., State Univ., Gent Belgium. pp. 63.

Evangelou, M. W. H.; Ebel, M. & Schaeffer, A., (2006). Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. Chemosphere, 63: 996–1004.

Evangelou, M. W. H.; Ebel, M. & Schaeffer, A., (2007). Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere, 68: 989–1003.

Freitas, E. V.; Nascimento, C. W.; Souza, A. & Silva, F. B., (2013). Citric acid-assisted phytoextraction of lead: A field experiment. Chemosphere, 92: 213–217.

Hadi, F.; Bano, A.; & Fuller, M. P., (2010). The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays L.*): The role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. Chemosphere, 80: 457–462.

Halim, M.; Conte, P. & Piccolo, A., (2003). Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere, 52(1): 265–75.

Hesse, P. R., (1971). "A Text book of Soil Chemical Analysis". John Murray Ltd, London, pp. 520.

Hofrichter, M. & Steinbüchel, A., (2001). Biopolymers, vol. 1, Lignin, Humic Substances and Coal. Wiley Europe-VCH, Weinheim, New York.

Jackson, M. L. (1973). "Soil Chemical Analysis". Prentice Hall of Englewood cliffs, New Jersey, USA.

Jean-Soro, L.; Bordas, F. & Bollinger, J. C., (2012). Column leaching of chromium and nickel from a contaminated soil using EDTA and citric acid. Environ. Pollut., 164: 175–181.

Lindsay, W. L. & Norvell, W. A., (1978). Development of DTPA soil test for zinc, iron, manganese and copper. Soil, Sci. Soc. Am. J., 42:421-428.

Mahjoub, O., (2016). Wastewater use in agriculture and relevance of micropollutants in North African countries. In: Fatta-Kassinos, D., Dionysiou, D. D. &Kümmerer, K. (Editors). Wastewater Reuse and Current Challenges. 44: 193-212.

Meers, E.; Ruttens, A.; Hopgood, M.; Lesage, E. & Tack, F. M. G., (2005). Potential of *Brassica rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. Chemosphere, 61: 561–572.

Meyers, D. E. R.; Auchterlonie, G. J.; Webb, R. I. & Wood, B., (2008). Uptake and localisation of lead in the root sytem of indian mustard. Environ. Pollut., 153:323–332.

Najeeb, U.; Jilani, G.; Ali, S.; Sarwar, M.; Xu, L. & Zhou, W. J., (2011). Insight into cadmium induced physiological and ultrastructural disorders in *Juncus effusus L.* and its remediation through hexogenous citric acid. J. Hazard. Mater., 186: 565–574.

Najeeb, U.; Xua, L.; Ali, S.; Jilani, G.; Gonga, H. J.; Shenc, W. Q. & Zhou, W. J., (2009). Citric acid enhances the phytoextraction of manganese and plant growth by alleviating the ultrastructural damages in *Juncus effusus L.* J.Hazard.Mater., 170: 1156–1163.

Nascimento, C. W. A.; Amarasiriwardena, D. & Xing, B., (2006). Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environ. Poll., 140: 114-123.

Nigam, R.; Srivastava, S.; Prakash, S. & Srivastava, M. M., (2001). Cadmium mobilisation and plant availability – the impact of organic acids commonly exuded from roots. Plant Soil, 230: 107–113.

Padmavathiamma, P. K. & Li, L. Y., (2007). Phytoremediation technology: hyperaccumulation metals in plants. Water Air Soil Pollut., 184: 105–126.

Page, A. L., (ed.) (1982). "Methods of Soil Analysis", part 2: Chemical and Microbiological Properties, (2^{nd} Ed.) American Society at Agronomy, Inc. Soil. Sci Soc. Of Am. Inc., Madison. Wisconsin, USA.

Piper, C. S., (1950)."Soil and Plant Analysis", Interscience Publishing Inc. New York.

Richards, L. A., (Ed) (1954). "Diagnosis and Improvement of Saline and Alkali Soils". U. S. Salinity laboratory staff. Agric. Handbook No. 60, Washington, D. C. USA.

Robinson, B. H.; Banuelos, G.; Conesa, H. M.; Evangelon, W. H. & Schulin, R., (2009). The phytomanagement of trace elements in soil. Crit. Rev. Plant Sci., 28 (4), 240–266.

Shahid, M; Pinelli, E. & Dumat, C., (2012). Review of Pb availability and toxicity to plants in relation with metal speciation: Role of synthetic and natural organic ligands. J. Hazard. Mater., 219–220, 1–12.

Snedecor, G. W. and W. G. Cochran, (1980)."Statistical Methods", Seventh ed. The Iowa State Univ. Press, Ames. Iowa, USA, pp. 1–507.

Taalab, A. S.; Zaghloul, B. M. & Gérard, F., (2009). Effect of synthetic chelators and low molecular weight organic acid in enhancing uptake of lead and nickel by mustard (*Sinapis Alba L.*). Egypt. J. Soil Sci., 49(2): 203-214.

Topcuoğlu, B., (2012). The influence of humic acids on the metal bioavailability and phytoextraction efficiency in long-term sludge applied soil. Conference on International Research on Food Security, Natural Resource Management and Rural Development. Georg-August Universität Göttingen and University of Kassel-Witzenhausen. Göttingen, Germany, September 19-21.

Turan, M., & Angin, I. (2004). Organic chelate assisted phytoextraction of B, Cd, Mo and Pb from contaminated soils using two agricultural crop species. Acta Agriculturæ Scandinavica Section B, Soil and Plant Science, 54: 221–231.

Wang, Q.; Li, Z.; Cheng, S. & Wu, Z., (2010). Influence of humic acids on the accumulation of copper and cadmium in *Vallisneria spiralis L.* from sediment. Environ Earth Sci., 61: 1207–1213.

Wu, L. H.; Luo, Y. M.; Christie, P. & Wong, M. H., (2003). Effects of EDTA and low molecular weight organic acids on soil solution properties of a heavy metal polluted soil. Chemosphere, 50: 819–822.

Wu, L. H.; Luo, Y. M.; Xing, X. R. & Christie, P., (2004). EDTA-enhanced phytoremediation of heavy metal contaminated soil with indian mustard and associated potential leaching risk. Agr. Ecosyst. Environ., 102: 307–318.

Zaheer, I. E.; Ali, S.; Rizwan, M.; Farid, M.; Shakoor, M. B.; Gill, R. A.; Najeeb, U.; Iqbal, N. & Ahmad, R., (2015). Citric acid assisted phytoremediation of copper by *Brassica napus L.* Ecotoxicology and Environmental Safety, 120: 310–317.

Zhang, X.; Ervin, E. H., & Schmidt, R. E. (2003). Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. Crop Science, 43: 952–956.

Zimdahl, R. L., (1975). Entry and movement in vegetation of lead derived from air and soil sources. paper presented at 68th Annu. Meeting of the Air Pollution Control Association, Boston, MA, June 15, 2, 1975.

تأثير الإديتا وحامض الستريك وحامض الهيوميك على عملية التخلص من العناصر الثقيلة بواسطة نباتات الخردل الهندي في الأراضي الملوثة

محمد وجدي محمد العجرودى[١] ، فاروق إبراهيم زين [٢] ،جمعة لبيب احمد[١] ومحمد سعد عبد الستار رمضان[٢]

[1]قسم الأراضي ـ كلية الزراعة ـجامعة المنصورة

[2]معهد بحوث الأراضي والمياه والبيئة مركز البحوث الزراعية

أجريت تجربة أصص شتاء عام 2014/2013 في محطة البحوث الزراعية بسخا - محافظة كفر الشيخ. تهدف التجربة إلى دراسة تأثير كل من الـ EDTA (0 و 1.5 و 3.0 و 4.5 ملليمول/كجم تربة) وحامض الستريك (0 و 3 و 6 و9 ملليمول/كجم تربة) وحامض الهيوميك (0 و 0.2 و 0.4 و 0.6 جم/كجم تربة) علي إستخلاص النحاس والزنك والرصاص من التربة الملوثة بالجبل الأصفر باستخدام الخردل الهندي وسلوك الغسيل لهذه المعادن من التربة. وفيما يلي أهم النتائج المتحصل عليها:ـ أوضحت النتائج ان استعمال الـ EDTA أظهر إنخفاضاً شديداً في المادة الجافة لنباتات الخردل الهندي، بالإضافة الي ظهور بعض اعراض التسمم خاصة عند إضافة الـ EDTA بمعدل 4.5 ملليمول/كجم تربه. بينما أدي استعمال حامض الستريك و حامض الهيوميك الي تحسين نمو النباتات.ـ كان للـ EDTA التأثير الأكبر علي زيادة تركيزات النحاس والزنك في أجزاء النبات خاصة عند اضافتها بمعدل 4.5 ملليمول/كجم تربه مقارنة بحامض الستريك وحامض الهيوميك. حيث ان زيادة معدلات الـ EDTA المضافة الي 4.5 ملليمول/كجم تربه أدي الي زيادة تركيزات النحاس بمقدار 1.9 و 2.88 و 3.04 و 1.56 ضعف والزنك بمقدار 1.43 و 1.91 و 1.83 و 1.11 ضعف في الجذور والسيقان والأوراق والبذور علي التوالي. ـ أدي استخدام حامض الستريك أيضا الي زيادة تركيزات النحاس والزنك في أجزاء النبات المختلفة خصوصا عند اضافته بمعدل 9 ملليمول/كجم تربه ولكنه كان اقل كفاءة من الـ EDTA. ـ أدي إضافة حامض الهيوميك الي زيادة تركيزات الزنك في الأجزاء النباتية لنباتات الخردل الهندي وذلك بزيادة معدل الإضافة الي 0.6 ملليمول/كجم تربه ولكنه كان أقل فعالية من الـ EDTA وحامض الستريك. في حين أن إضافة حامض الهيوميك لم تؤثر على امتصاص النحاس بواسطة النباتات.ـ لم يكن هناك اي تركيزات محسوسة من الرصاص في الأجزاء النباتية لنبات الخردل الهندي بإستثناء الجذور موضحا بذلك فشل إستخلاص الرصاص من التربة حتي بعد اضافة المخلبيات موضع الدراسة.ـ أدي اضافة كل من الـ EDTA وحامض الستريك الي زيادة تركيز كل من النحاس والزنك والرصاص في راشح التربة وزاد ذلك التأثير بزيادة معدل الإضافة الي 4.5 و9 ملليمول/كجم تربه على التوالي. عموما، ذلك التأثير كان أكثر بكثير في حالة الـ EDTA مقارنة بحامض الستريك. بينما لم يكن هناك اي تأثير لحامض الهيوميك علي رشح العناصر موضع الدراسة من التربة. ـ تدل النتائج على انه يفضل إستعمال حامض الستريك في استخلاص النحاس واستعمال كل من حامضي الستريك والهيوميك في استخلاص النحاس والزنك من الأراضي الملوثة عن استخدام الـ EDTAعلى الرغم من الكفاءة العالية للـ EDTA في استخلاص العناصر الثقيلة باستخدام نباتات الخردل الهندي وذلك نتيجة التأثير الضار للـ EDTAعلى نمو النباتات بالإضافة الي زيادة مخاطر تلوث المياه الارضية والجوفية بالعناصر الثقيلة في حالة استخدام الـ EDTA.