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ROLE OF EDTA ON HYPER ACCUMULATION OF Cd WITH DIFFERENT SOLUBILITY BY *OCIMUM BASILICUM (L.)*

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ABSTRACT

This study can professionally discuss the ability of *Ocimum basilicum* to the accumulation of one heavy metal like Cd but has a different solubility. Phytoremediation is required along with modifying chelates to enhance the elimination capacity of the metal. The study was conducted to determine the effect of amendment materials like EDTA on Cd uptake from the soil and translocation to roots and shoots of *O. basilicum.* Pot experiment in randomized design was applied 5 and 10 mg/kg soil in two forms, CdCl2 and CdCO3, and the effect of EDTA. The results revealed that the concentration of Cd in *O. basilicum* was higher in case of Cd with high solubility (CdCl2) and EDTA significantly increase the ability of the plant to accumulation of various Cd forms especially with low solubility (CdCO3). The calculating translocation factor and enrichment factor were more than 1 indicating *O. basilicumis* hyperaccumulator and good enough for phyto extraction of Cd.

Keywords: Cd, EDTA, hyper accumulator, *Ocimum basilicum,* phytoextraction.

INTRODUCTION

Phytoremediation is an organic strategy for the disposal of overwhelming metals contamination from polluted soil. As of late, this strategy turns out to be increasingly alluring because of its lower coast, ecological similarity, soil richness, and keeping up the territory beautification (Wang *et al.,* 2011). There are five fundamental methods, phytoextraction, phytostabilization, rhizofilteration, phytovolatilization, and phytodegradation used in metal elimination (Forte and Mutiti, 2017). The plants with TF and EF more than one, these plants can be considered as accumulators. Hyperaccumulator is a plant which used to reduce, eliminate, decompose, and stabilize pollutants from soil (Van der Ent *et al.,* 2013; Wei and Zhou, 2008). *Phytoremediation resources of heavy metals – contaminated soil* (Xu *et al.,* 2018) is a new book containing more than 100 plant species concerned the ability of many plants for the accumulation of various heavy metals but did not illustrate the ability of a definite plant for the same heavy metal even with different forms.

During the last years, many researchers studied the ability of some plants to hyperaccumulation of heavy metals (Bianconi *et al.,* 2013; Deniau *et al.,* 2006). However, many hyperaccumulator plants have moderate development and low biomass and are difficult to grow and harvest (Zhuang *et al.,* 2007). Basil (*Ocimum basilicum* L.), is an aromatic plant belonging to the family Lamiaceae, described as an annual, herbaceous, and white-purple flowering plant (Ozcan *et al.,* 2005). The impacts of metal application on nutritional and non-nutritional crop yields have been widely examined. Additionally, a couple of exams showed *O. basilicum* capacity to withstand and gather high measurements of overwhelming metals in its tissues (Siddiqui *et al.,* 2013; Prasad *et al.,* 2011), and have also shown that some positive effects of soil amended with microelements on the growth and yield of medicinal plants (Aziz *et al.,* 2007; Pande *et al.,* 2007). Various materials can be attempted to enhance the elimination proficiency of metal and can rise the solubility of metal elements by plants, these materials are added to the soil like citric acid, EDTA, CDTA, and compost (Barbafieri, 2017; Mahmood-ul-Hassan *et al.,* 2017).

The point of this exploration was to research the role of EDTA on the uptake of different Cd forms from the soil and investigating the ability of *Ocimum basilicum* to accumulation of Cd in its parts, roots, and shoots.

MATERIALS AND METHODS

A pot experiment was conducted with soil collected from the top 30-cm soil layer of agricultural land situated 2 km west of Zagazig University, Egypt. Immediately after collection, soil samples were air-dried, crushed, ground to pass through a 2-mm sieve and mixed uniformly. Physical and chemical properties and concentration of the elements in samples were measured according to (Carter and Gregorich, 2008). The result of soil analysis was presented in (Table 1). Cadmium with high and low solubility, $CdCl_2$, and $CdCO_3$ were added to the soil at two concentrations 5 and 10 mg/kg soil. These concentrations were selected according to previous studies (Tang *et al.,* 2016). The soil was mixed thoroughly and put in plastic pots each filled with 2 kg pretreated soil.

Basil seeds (*Ocimum basilicum* L.) were provided from the agricultural research center of Egypt and cultivated in pots. To avoid the negative effect of EDTA on plant growth, EDTA was added to some soil pots twice after germination and growing of the plant. Three replicates were performed for each treatment (Cd_5, E + Cd_5, Cd_{10} and E + Cd_{10}). Pots were placed in a complete randomized block design. Plant and soil samples in pots were collected after 12 weeks from cultivation and mature growth.

Cadmium availability in soil was determined by the DTPA extraction procedure. 20 ml of DTPA extraction solution (0.005 M DTPA, 0.01 M $CaCl_2.2H_2O$, and 0.1 M TEA at pH adjusted to 7.3 using HCL) added to 10 g of soil sample. The concentration was determined by flame atomic absorption spectroscopy according to (Simmons *et al.,* 2005).

Plant samples were separated into roots and shoots then rinsed with distilled water, oven-dried, and powdered. Samples were digested in 2:1 v/v HNO_3:$HClO_4$, cadmium concentration was determined by atomic absorption spectrophotometer according to (Rania *et al.,* 2015). The results of Cd concentrations in plants were expressed as (mg/kg).

The translocation factor (TF) was calculated by dividing the concentration of metal in shoots by the concentration of metal in roots. Enrichment factor (EF) was calculated with the concentration ratio in plant shoots to soil (Yang *et al.,* 2019).

Data were analyzed by variance analysis (ANOVA) depending on a completely randomized design (CRD) with three replicates (n=3). Means values were compared at (P) level of < 0.05 by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results showed that phytoremediation was with wide convenience for different Cd forms in soil. Cd has different solubility in water, $CdCl_2$ with high solubility while $CdCO_3$ is low solubility. The concentration of Cd in *Ocimum basilicum* under the effect of high solubility Cd treatment was higher than under low solubility Cd treatment. The results are consistent with the findings of (Xuekai *et al.,* 2019) which showed Cd accumulation in *Bidens pilosa* under the effect of EDTA.

Determination of *Ocimum basilicum* biomass

The mean values of *Ocimum basilicum* shoot and root biomass in control were 12.16 g/plantand 4.2 g/plantrespectively. The shoot biomass in treatments ranged from 12 g/plantto 13.26 g/plant while the root biomass is ranged from 3.33 g/plantto 4.8 g/plant. This means that there was no significant difference ($p > 0.05$) in *O. basilicum*s hoot and root biomass between treatments and control (Table 2), indicating that *O. basilicum* showed very strong tolerance to different Cd forms and biomass of the plant was not affected by Cd. Biomass of plants is considered as a significant factor reflecting phytoremediation productivity and resistance to trace metals. Some plants show tolerance to high levels of metals in soil and this appears when the biomass of plants was not affected. Root development hindrance under the effect of Cd might be because of lignifying of the cell wall, which controls the extension of cell and supplement take-up, and the reaction of the shoot can emerge from photosynthetic response restraint, that forestalls natural gathering (Baycu *et al.,* 2017).

Determination of extractable Cd

Results showed that there was a significant difference ($p < 0.05$) between extractable Cd concentrations in Cd addition ($CdCl_2$ and $CdCO_3$) and with adding EDTA or not (Table 3). At concentration 5 mg/kg of treatment $CdCl_2$, the average extractable Cd concentration was 1.56 mg/kg while of treatment $CdCO_3$ was 0.90 mg/kg. When EDTA was added, these averages were 2.33 mg/kg and 1.33 mg/kg respectively (Table 3). At concentration 10 mg/kg of treatment $CdCl_2$, the average extractable Cd concentration was 4.23 mg/kg while of treatment $CdCO_3$ was 2.56 mg/kg. When EDTA was added, these averages were 4.83 mg/kg and 3.13 mg/kg respectively (Table 3). The results showed that the high and low solubility forms of Cd were not much different on extractable Cd concentrations, Kabata-Pendias (2011) found that the Cd availability in soil not only controlled by the initial form of Cd but also by its transformation through the chemical reactions occurred in soil. The metal transport can be stimulated by diffusion and convection, Lin *et al.,* 2016 found that the most persuasive parameter was the underlying Cd^{2+} concentration, then the power of soil buffer for Cd^{2+}, the field capacity of the soil, and the impedance factor. The effect of heavy metals contamination on soil can be determined not only by the amount of heavy metals available in soils but also by the total amount of heavy metals in these soils (Lin *et al.,* 2016).

EDTA is the most common chelator used to enhance the ability of plants to accumulate Cd in soil (Eissa, 2016; Tananonchai *et al.,* 2019). EDTA improves the Cd availability in soil especially with Cd of low solubility ($CdCO_3$) (Table 3), so that EDTA is used especially in this research due to its important role in enhancing the accumulation of Cd in *O. basilicum* (Tananonchai *et al.,* 2019; Eissa, 2016).

Level of accumulated Cd in shoots and roots of *O. basilicum*

Results showed that Cd accumulated in shoots at treatment 10 mg/kg Cd more than that at 5 mg/kg Cd (Table 3), this occurred in the 2 forms of Cd ($CdCl_2$ and $CdCO_3$). The mean value of Cd concentration in shoots was 20.1 mg/kg at treatment 5 mg/kg $CdCl_2$ while at treatment 5 mg/kg $CdCO_3$ was 5.70 mg/kg. At treatment 10 mg/kg $CdCl_2$, the mean value of Cd concentration in shoots was 27.13 mg/kg while at 10 mg/kg $CdCO_3$ was 13 mg/kg. Results showed that the addition of EDTA leads to rising in Cd accumulation of shoots of *Ocimum basilicum* in all treatments (Table 3). The mean value of Cd concentration increased by 19 % in the treatment of $CdCl_2$ with EDTA and increased by 51 % in the treatment of $CdCO_3$ with EDTA. Also, this occurred at 10 mg/kg of Cd where the mean value of Cd concentration in shoots increased by 9 % in the treatment of $CdCl_2$ with EDTA and increased by 22 % in the treatment of $CdCO_3$ with EDTA. EDTA can transport heavy metals to different parts of plants and this might be ascribed to the arrangement of ATPases and bearer protein that changed particle transporter that can pass freely through the membrane (Hong-qi *et al.,* 2007).

The changes in Cd accumulation in roots of *Ocimum basilicum* under the use of EDTA addition and the effect of the high and low solubility of Cd in soil were the same that in shoots (Table 3). Salimi *et al.,* (2015) mentioned that the shoots of Brassica napus had higher absorption levels of Pb and Cd more than that were in seeds and roots of the same plant and this is consistent with the findings of this research. Taheripur *et al.,* 2016 found that Zea mays L. can accumulate high amounts of Cu and Zn, especially in shoots under the effect of EDTA and citric acid. The distribution of Cd forms in *O. Basilicum* organs defines successful phytoextraction of the Cd. The amounts of Cd in roots and shoots of *O. Basilicum* made the plant grow efficiently in soils polluted with Cd. The ability of *O. Basilicum* for accumulation of heavy metals might be attributed to high content of secondary metabolites, Selma *et al.,* 2017 found that Cd application improved the quality of all secondary metabolites indicating that flavonoids, and other phenolics play an significant role in plant survival under stress. Therefore, after increased phenolscontent, plants are able to respond to heavy metal toxicity, eliminate reactive oxygencompounds and boost plant metabolism (Manan *et al.,* 2015).

Levels of EF and TF

All treated samples showed enrichment factor (EF) more than one (Table 4). The results of EF of all samples changed similar to the results of Cd concentrations in shoots. The treatments 5 and 10 mg/kg Cd with adding EDTA or not give similar results of translocation factor (TF) ($p < 0.05$). TF was significantly higher ($p < 0.05$) in treatments of 5 mg/kg Cd than that in treatment of 10 mg/kg Cd (Table 4). The tolerance capacity of plants can be determined by the results of EF and TF. Barbafieri *et al.,* 2017 found that plants have the ability of phytoextraction and phytostabilization of metals from soil in addition to their abilities to accumulate heavy metals in plant parts. Wei and Zhou (2008) reported that some Chinese weed species had translocation factor (TF) and enrichment factor (EF) more than one even for Cd form with low solubility. The same result was confirmed by Van der Ent *et al*., (2013) on many hyper accumulators under the effect of metal and metalloid trace elements. These findings were consistent with the results of this research, *O. basilicum* showed the same characteristics of both EF and TF.

Due to the results mentioned previously, *O. basilicum* can be considered as a hyper accumulator of Cd with the two solubility, high and low and this confirms the high ability of *O. basilicum* to accumulate different forms of Cd in soil.

Characteristic	Quantity
% Clay	17
% Silt	18
% Sand	65
Soil texture	Sandy loam
pH	7.4
EC (ds/m)	2.4
% OC	6.9
% Total nitrogen	0.099
Available P (mg/kg)	8.77
Available K (mg/kg)	180

Table 1: Physical and chemical properties of soil used in this experiment before adding cadmium.

CONCLUSION

Cd with different solubility accumulated in shoots and roots of *Ocimum basilicum*, the accumulation of high solubility Cd was higher. EDTA enhances the efficiency of Cd remediation by increasing the Cd uptake from soil. Due to biomass of *Ocimum basilicum* not affected and the findings of EF and TF, *Ocimum basilicum* is suitable for phyto extraction and with hyper accumulation potential of Cd.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this article.

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Forms	Treatments	Biomass (g /plant)	
		Shoot	Root
$CdCl_2$	Cd_5	12.33 ± 0.088^{NS}	$4.8 \pm 0.057^{*}$
	$E + Cd_5$	12 ± 0.057^{NS}	4.5 ± 0.115^{NS}
	Cd_{10}	$13.26 \pm 0.145^{*}$	3.90 ± 0.057^{NS}
	$E + Cd_{10}$	$12.8 \pm 0.057^{**}$	$3.33 \pm 0.088^{*}$
$CdCO_3$	Cd_5	12.23 ± 0.088^{NS}	4.5 ± 0.115^{NS}
	$E + Cd_5$	12 ± 0.057^{NS}	$3.63 \pm 0.033^{*}$
	Cd_{10}	12.10 ± 0.10^{NS}	4.73 ± 0.088^{NS}
	$E + Cd_{10}$	12.13 ± 0.145^{NS}	3.9 ± 0.057^{NS}
Control		12.16 ± 0.120	4.2 ± 0.152

Table 2: Biomass of Shoot and root of O. basilicum (g/plant)

Means and Standard errors based on ANOVA analysis, *significant, P<0.05; **significant, P <0.05; ***significant, P<0.001 and Ns is nonsignificant difference.

Forms	Treatments	Concentration of Cd (mg/kg)		
		Shoot	Root	Extractable Cd
$CdCl_2$	Cd_5	$20.1 \pm 0.493^{***}$	$8.1 \pm 0.635^{**}$	$1.56 \pm 0.145^{**}$
	$E + Cd_5$	$24.03 \pm 0.742^{***}$	$12.93 \pm 0.409^{***}$	$2.33 \pm 0.145^{**}$
	Cd_{10}	$27.13 \pm 0.463^{***}$	$17.8 \pm 0.321^{***}$	$4.23 \pm 0.145^{***}$
	$E + Cd_{10}$	$29.76 \pm 1.134^{***}$	$25.93 \pm 0.520^{***}$	$4.83 \pm 0.088^{***}$
$CdCO_3$	Cd_5	$5.70 \pm 0.776^{**}$	$5.1 \pm 0.635^{*}$	$0.90 \pm 0.057^{**}$
	$E + Cd_5$	$11.83 \pm 0.545^{**}$	$8.93 \pm 0.409^{**}$	$1.33 \pm 0.088^{**}$
	Cd_{10}	$13 \pm 0.404^{***}$	$9.8 \pm 0.321^{***}$	$2.56 \pm 0.066^{***}$
	$E + Cd_{10}$	$15.93 \pm 0.520^{***}$	$13.76 \pm 1.134^{***}$	$3.13 \pm 0.088^{***}$
Control		0 ± 0	0 ± 0	0.176 ± 0.012

Table 3: Concentration of Cd in Shoot and root of O. basilicum and extractable Cd in soil (mg/kg)

Means and Standard errors based on ANOVA analysis, *significant, P<0.05;

**significant, P <0.05;
***significant, P<0.001

Forms	Treatments	TF	EF
$CdCl_2$	Cd_5	2.54	12.9
	$E + Cd_5$	1.86	10.8
	Cd_{10}	1.51	6.4
	$E + Cd_{10}$	1.14	6.13
$CdCO_3$	Cd_5	1.84	8.33
	$E + Cd_5$	1.32	8.90
	Cd_{10}	1.32	5.06
	$E + Cd_{10}$	1.16	5.06

Table 4: The calculated values of TF and EF of Cd in O. basilicum.

Values are average of 3 replicates.

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