

The Combined Effect of EDTA and Vermicompost on Removal of Lead from Soil by *Ocimum basilicum*

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Abstract: Among the chemical pollutants, lead is one of great importance ecologically and hygienically, because it threatens the health of the communities by penetrating into the food cycle. Phytoremediation is required along with modifying chelates to improve the efficiency of metal remediation. The aim of this study was to investigate the effect of vermicompost and EDTA on the absorption of various Pb concentrations from the soil and determine their accumulation level in the roots and shoots of *Ocimum basilicum* in the form of pot experiment in a completely randomized design with three replications. We applied vermicompost 3 mg/kg soil, EDTA 8 mg/kg soil, and lead nitrate at the concentrations of 50 and 100 mg/kg soil. AAS was employed to read the amount of Pb in the extracts. The results showed that the use of amendment materials increases significantly the Pb accumulation in the plant shoots. The results of calculating bioconcentration factor and transfer factor showed that this plant is unsuitable for the phytostabilization and phytoextraction of Pb.

Key words: Phytoremediation, *Ocimum basilicum*, EDTA, vermicompost, lead.

Introduction

In the global perspective, soil is considered the third most important component of the environment, after water and air. The soil pollution usually occurs due to unsanitary habits, various agricultural activities, improper methods for the disposal of solid and liquid wastes, deposition of airborne contaminants due to precipitation, and chemicals such as heavy metals and oil products (Dabiri and Bashiribod, 2014; Singh et al., 2011).

The heavy metals are elements with atomic mass greater than 55.8 g/mol, with features such as bioaccumulation, high toxicity, chemical stability,

weak degradability and high water solubility (Forte and Mutiti, 2017).

Lead is a toxin that is accumulated in the body and is excreted slowly, so that its half-life is 7 years in the kidney and 32 years in the bones (Capar, 1999). The symptoms of lead poisoning in adults include abdominal pain, vomiting, weakness, mental anomalies, diarrhea, fatigue, anorexia and insomnia (Papanikolaou et al., 2005).

The phytoremediation is a biological method for eliminating heavy metal pollution from the contaminated soils, where plants are used to reduce, eliminate, decompose and stabilize pollutants. This method has attracted further attention in recent years due to its

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lower cost, environmental compatibility, soil fertility, in situ operations and maintaining the area beautification (Wang et al., 2011). To improve the efficiency of metal remediation and increase the solubility of metal elements by plants, the modifying compounds such as citric acid, EDTA, CDTA and compost are used in soil (Barbafieri, 2017; Mahmood-ul-Hassan et al., 2017). In this case, the produced dry matter increases and causes the absorption of a large amount of metal elements by the plant using chemical treatments (Wallace, 1974). Phytoremediation of heavy metals can be divided into five main types: phytoextraction, phytostabilization, rhizofiltration, phytovolatilization and phytodegradation (Forte and Mutiti, 2017).

Due to the fact that soil loss in Iran is so high (Sadeghi, 2017), in this research, the phytoremediation ability of *Ocimum basilicum* has been studied to remove the lead from soil and to investigate its accumulation in different organs.

Materials and Methods

The *Ocimum basilicum* seeds, soil and vermicompost were obtained from Golbaranesabz Co. in Iran. Three soil and vermicompost samples were sent to the laboratory to evaluate the physicochemical properties such as soil texture, pH and EC as well as to determine the initial lead concentration (Khalid et al., 2017). The seeds were planted in plastic pots containing one kilogram of soil. To contaminate the soil to different lead concentrations, powdered lead (II) nitrate was poured into the soil samples with the concentrations of 50 and 100 mg/kg soil. At the beginning of the experiment, some pots were spike by vermicompost (3 mg/kg). Due to the anti-growth effect of EDTA, this substance (8 mg/kg) was added to some pots 15 days before the plant harvesting. This experiment was conducted in the completely randomized design with three replications, in total of 27 pots and 81 samples. Irrigation was done every day with urban water based on the needs of the plant, so that there was no drainage. After two months, the plants were cut off from the crown and the roots and shoots were packed in separate packages and transferred to the laboratory for digestion and concentration of heavy metal.

The soil samples of each pot after air-drying were placed in an oven at 30°C for 24 hours to obtain a constant weight, and then passed through a 2-mm sieve. Next, 2 cc of HF and 5 cc of HNO₃ were added to 0.2 g of the sample to be digested in a microwave oven for one hour. In the next step, 0.8 g of boric acid

was added to the samples. The final volume of samples reached 50 cc with deionized water, which was placed in an ultrasonic bath at 60°C for 30 minutes. Atomic absorption spectroscopy (AAS) was used to read the amount of Pb in the extracts. The metal concentration in the dry weight of the solid sample was calculated (EPA, 2007).

The roots and shoots of the plant were washed with distilled water, placed in oven at 70°C for 48 hours, and powdered. Next, 5 cc of HNO₃ were added to 0.2 g of the sample to be digested in the microwave oven for one hour. The final volume of samples reached 50 cc with deionized water. The AAS read the amount of Pb in the extracts. The metal concentration in the dry weight of the samples was calculated (EPA, 2007).

Equations (1) and (2) were used to calculate the BCF for each organ and the TF for any species (Zacchini et al., 2008).

$$\text{BCF} = \frac{\text{metal concentration in the plant roots or shoots (mg/kg dw)}}{\text{metal concentration in contaminated soil (mg/kg dw)}} \quad (1)$$

$$\text{TF} = \frac{\text{metal concentration in the plant shoots (mg/kg dw)}}{\text{metal concentration in the plant roots (mg/kg dw)}} \quad (2)$$

The AAS data were analyzed by Excel 2010 and SPSS23 software using Duncan's test (at 5% significance level) to compare the mean data.

Results and Discussion

Due to the fact that the soil lead is immobile at high pH, increased electrical conductivity (EC) enhances the solubility of heavy metals and, consequently, increases the absorption of metal by the plant and the soil organic matters have high affinity to the heavy metal cations (Kabata and Pendias, 1994), factors such as pH, organic matter content, texture and EC were tested in the baseline soil and vermicompost in addition to the level of Pb accumulation. As Table 1 shows, the study soil and vermicompost are not so saline soils and their acidity is suitable for plant growth.

Comparison of the Pollutant Accumulation Level in the Roots and Shoots of *Ocimum basilicum*

Our results based on one way ANOVA presented that there was significant difference ($P < 0.05$) between the Pb uptake rates in roots of control (C₀E₀Pb₀) and in those treated with 50 and 100 mg/kg. Pb accumulation level in roots of *Ocimum basilicum* is increased when

Table 1: Primary soil characteristics

Parameter	Texture	pH	EC	OC	Pb
Unit	—	—	mS/cm	%	mg kg ⁻¹
Soil	Sandy clay loam	7.4	2	8.402	0.51
Vermicompost	Sandy clay loam	7.03	3.93	13.861	0.65

Values are mean ($n = 3$)

plants were exposed to 50 and 100 mg/kg Pb relative to control plants ($C_0E_0Pb_0$).

Our results based on one way ANOVA presented that there was significant increase in the shoot accumulation of C_0E_0 , C_3 , E_8 and C_3E_8 across the Pb concentration. Pb accumulation level in shoots of *Ocimum basilicum* is increased when plants were exposed to 50 and 100 mg/kg Pb relative to control plants ($C_0E_0Pb_0$).

Our results revealed that shoots of *Ocimum basilicum* species accumulated significantly more Pb relative to roots for all treatments ($P < 0.05$). According to Figure 1, the highest amount of accumulated lead was at the concentration of 100 mg/kg in shoots of *Ocimum basilicum* in the C_3E_8 treatment (1.089 mg/kg). Raising metal accumulation by vermicompost may be attributed to the formation of N and K in soil made by earthworms that reduced the solubility of Pb and boost its mobility in *Ocimum basilicum* (Jadia and Fulekar, 2008; Sidhu et al., 2017a). Transporting Pb from roots to shoots by EDTA may be attributed to the formation of ATPases and carrier protein that changed ion transporter that freely passed through the membrane (Hong-qi et al., 2007).

These results are consistent with the findings of Salimi et al. (2015) which showed that Pb and Cd accumulation levels in the shoots increased compared to root and seed in *Brassica napus*. Taheripur et al.

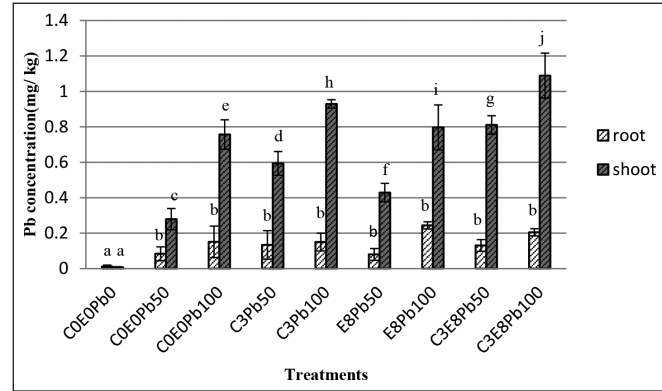


Figure 1: Compares the Pb accumulation level in the roots and shoots of *Ocimum basilicum* ($n = 3$). Different letters are significantly different in each contaminant group according to the Duncan's test ($p \leq 0.05$) (C_0E_0 : unamendment, C_3 : 3 mg vermicompost kg⁻¹ soil, E_8 : 8 mg EDTA kg⁻¹ soil and C_3E_8 : 3 mg vermicompost kg⁻¹ soil with 8 mg EDTA kg⁻¹ soil).

(2016) found the highest absorption of Cu and Zn by shoots affected by EDTA and citric acid in *Zea mays* L.

Determination of BCF and TF Levels

The BCF and TF indicate the ability of plants to tolerate and accumulate heavy metals in their organs as well as their ability to phytoextraction and phytostabilization (Barbafieri et al., 2017). The results of Table 2 show

Table 2: Bioconcentration factor (BCF) and Translocation factor (TF) values of Pb in *Ocimum basilicum*

Samples	Element	Dose (mg/kg)	BCF		TF
			Root	Shoot	
C_0E_0	Pb	0	0.064	0.040	0.636
C_0E_0	Pb	50	0.009	0.030	3.321
C_0E_0	Pb	100	0.006	0.034	5.013
C_3	Pb	50	0.008	0.037	4.432
C_3	Pb	100	0.005	0.035	6.193
E_8	Pb	50	0.018	0.098	5.362
E_8	Pb	100	0.017	0.056	3.266
C_3E_8	Pb	50	0.017	0.056	6.190
C_3E_8	Pb	100	0.015	0.067	5.312

Values are averages of three replicates

that the BCF was not greater than 1 in the root and shoots of *Ocimum basilicum* at none of the studied lead concentrations, but the TF was more than 1 in the examined samples, with the exception of the control. Therefore, this species is unsuitable for lead phytostabilization due to a root $BCF < 1$ and $TF > 1$ (Sidhu et al., 2017b).

The maximum lead TF is in C_3Pb_{100} , which is consistent with the findings of Chorom et al. (2009) who found the highest lead TF for canola in treatment with high concentration of the compost.

The factor indicating the species ability for phytoextraction is BCF in the shoots. In this species, this coefficient was less than 1 for Pb in all tested treatments; hence, *Ocimum basilicum* is improper for phytoextraction of lead at the examined concentrations.

Conclusion

The analysis results show that Pb was observed in all plant organs of *Ocimum basilicum*. The comparison of plant organs showed a higher amount of lead in the shoots compared with the root of the plant so that it can be said that the shoots of the *Ocimum basilicum* are able to accumulate further lead. Due to the higher concentrations of this pollutant in the shoot and consumption of this plant by humans, this amount of contaminants may enter the body and lead to health risks. The highest Pb concentration in the plant shoots was observed in the C_3E_8 treatment. The BCF and TF findings after applying the treatments and adding lead showed that *Ocimum basilicum* is neither phytoextraction nor phytostabilization.

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