

## Kurşunla Kirlenmiş Topraklarda Soğanın (*Allium Cepa* L.) Şelat ve Mikrobiyal Gübre Destekli Fitoremediasyon Etkinliğinin Araştırılması

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### ÖZ

Ağır metal kirliliği son yıllarda artan çevre kirliliğini oluşturan etmenlerin başında gelmektedir. Kurşun, sanayide en fazla kullanılan elementlerden biri olduğu için toprak kirliliğinde tespit edilmektedir. Toprak kirliliğinin giderilmesinde son yıllarda alternatif bir yöntem olarak fitoremediasyon tercih edilmektedir ve fitoremediasyon toprağın biyolojik özelliklerini ve fiziksel yapısını korumaktadır. Bu çalışmada, EDTA (etilendiamin tetraasetik asit) ve hümkik Asit şelatlayıcı ajanlarının ve mikrobiyal gübrelemenin kurşunla kirlenmiş topraklarda *Allium cepa* L. (soğan)'nın fitoremediasyon etkinliği ve bitki büyüme parametreleri üzerindeki etkileri araştırılmıştır. Buna göre EDTA + mikrobiyal gübre kombinasyonu uygulanan saksılarda kök tolerans indeksi Tİ değerlerinin daha yüksek olduğu tespit edilmiştir. Hümkik asit uygulanan saksılarda ise kök Tİ değerleri daha yüksektir. Yine EDTA + mikrobiyal gübre kombinasyonu uygulanan saksılarda yaprak yaş ağırlık ve kuru ağırlık Tİ değerleri yüksektir. Araştırmada mikrobiyal gübrenin türün tolerans indeksini arttırdığı tespit edilmiştir. *A. cepa*'da Pb değerlerine göre 5 mmol kg<sup>-1</sup> EDTA ilaveli saksılarda sırası ile kök, soğan, yaprak ve toplam bitkide (28.93 mg kg<sup>-1</sup>, 36.16 mg kg<sup>-1</sup>, 262,56 mg kg<sup>-1</sup>, 327.65 mg kg<sup>-1</sup>) bulunmuştur. Hümkik asit ilaveli saksılarda toplam bitkide 10 mmol kg<sup>-1</sup>'da yüksek akümülyasyon (16.19 mg kg<sup>-1</sup>) bulunmuştur. Bitkinin Pb'yi yaprağında daha çok biriktirme eğilimi gösterdiği tespit edilmiştir. EDTA ve hümkik Asit şelatlarında BCF değerlerinin 1'den büyük olduğu tespit edilmiştir. Şelat+mikrobiyal gübre kombinasyonunda yetiştirilen *A. cepa* biyokonsantrasyon fakötürü (BCF) değerlerinin 1'den küçük olduğu tespit edilmiştir. Sonuç olarak mikrobiyal gübre ilavesinin türün Pb alımını düşürdüğü söylenebilir.

## Investigation of Chelate and Microbial Fertilizer Assisted Phytoremediation Efficiency of Onion (*Allium Cepa* L.) in Lead Contaminated Soils

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### ABSTRACT

Heavy metal pollution is one of the leading environmental pollution increasing in recent years. Since lead is one of the most widely used elements in industry, it is detected in soil pollution. In recent years, phytoremediation has been preferred as an alternative method for the removal of soil pollution and phytoremediation protects the biological properties and physical structure of the soil. In this study, the effects of EDTA (ethylenediamine tetraacetic acid) and humic acid chelating agents and microbial fertilization on phytoremediation efficiency and plant

growth parameters of *Allium cepa* L. (onion) in lead contaminated soils were investigated. Accordingly, it was determined that root TI values were higher in the pots where EDTA+ microbial fertilizer combination was applied. In the pots where humic acid was applied, root) tolerance index (TI) values were higher. Again, leaf wet weight and dry weight TI values were higher in the pots where the EDTA+microbial fertilizer combination was applied. It was determined that microbial fertilizer increased the tolerance index of the species. According to Pb values in *A. cepa*, it was found in root, bulb, leaf and total plant (28.93 mg kg<sup>-1</sup>, 36,16 mg kg<sup>-1</sup>, 262.56 mg kg<sup>-1</sup>, 327. 65 mg kg<sup>-1</sup>) in pots with 5 mmol kg<sup>-1</sup> EDTA addition, respectively. High accumulation (16.19 mg kg<sup>-1</sup>) was found at 10 mmol kg<sup>-1</sup> in total plant in pots with humic acid addition. It was found that the plant tended to accumulate Pb more in its leaves. BCF values of EDTA and humic acid chelates were found to be greater than 1. Bioconcentration factor (BCF) values of *Allium cepa* grown in chelate + microbial fertilizer combination was found to be less than 1. As a result, it can be said that the addition of microbial fertilizer decreased the Pb uptake of the species.

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## Introduction

With the development of industry, environmental pollution has become one of the most important problems of the last century. Chemical substances, petroleum products, thermal and nuclear power plants, solid wastes accumulated in the soil, metals, paper, leather, textile, cement, factory wastes, accumulation or burial of some solid wastes in inappropriate places, fertilizers and growth regulators (pesticides, fungicides, herbicides) are among the factors of soil pollution. Heavy metals are the leading cause of soil pollution. Heavy metals in the soil, in addition to reducing soil fertility, cause the microorganisms in the soil to be adversely affected and the yield and quality of the grown products to decrease. Moreover, heavy metals harm humans and animals through the food chain. Therefore, it has become inevitable to conduct studies and research for the elimination of heavy metal pollution in the ecosystem (Pinto et al., 2011; Lu et al., 2012; Zhou et al., 2020). Heavy metals cause inadequate development of vegetative and generative organs of plants, disruption of physiological activities such as photosynthesis, germination, stomatal functions, enzyme, protein synthesis, production and activity of hormones. Lead is a group IV A element of the periodic table with atomic number 82, atomic weight 207.19 g/mol and symbol Pb. Lead is the most widespread of the heavy metals, widely used in industry and easily processed. Above 550 °C, it evaporates in the ambient air and the condensed lead is emitted as lead oxide particles. Lead is one of the leading heavy metals that cause environmental pollution. Lead can be transferred to plants through air, water and soil and to other living organisms through the nutrient cycle (Wang et al., 2009). Pb, which is naturally present in all soils, poses a potential problem for plant and human health when its concentration exceeds 100 mg kg<sup>-1</sup> and its extractable amount exceeds 4 mg kg<sup>-1</sup> in agricultural areas (Chapman, 1971; Pak, 2011).

In recent years, phytoremediation has been preferred as an alternative method for the removal of soil pollution and phytoremediation protects the biological properties and physical structure of the soil (Khan et al., 2000; Kılıç and İpek, 2019, Tanyıldız et al., 2022, İpek 2019). Phytoremediation is the

removal of contaminants from contaminated areas using plants. Phytoremediation of contaminated areas helps to clean and protect the soil from pollution. It prevents the spread of pollution and enables soil recovery (Nair et al., 2019). Phytoremediation is classified as phytoextraction, phytostabilization, phytovolatilization, rhizodegradation, phytodegradation, rhizofiltration according to the way the pollution is separated (Er et al., 2021). Plants are called hyperaccumulators when they can accumulate 50 to 500 times more heavy metals in their organs (Elkiran, 2016; Clemens, 2006; Baker and Brooks, 1989). Although these plants accumulate 100 to 1000 times heavy metals in their organs, they do not show toxicity (Elkiran, 2016; Özay and Mammadov, 2013). The use of chelates to increase the accumulation efficiency of plants has been an increasing practice in recent years (Romkens et al., 2002; Zhang et al., 2016). Among the most widely used chelating agents in the literature, ethylenediaminetetraacetic acid, ethylenegluaric acid, diethyltriaminepentaacetic acid, sodium dodecyl sulfate, nitrilotriacetate, humic acid, boric acid, etc. can be given as examples (Ladillas, 2012). Ethylenediaminetetraacetic acid (EDTA) forms strong complexes with metal ions and is an effective chelate that affects and increases the solubility, mobility and bioavailability of heavy metals in soil (Shahid et al., 2014; Oh and Yoon, 2014; Guo et al., 2020). In various studies, EDTA application to soil has been reported to increase phytoextraction and bioaccumulation of Cd, Zn and Pb in plants (Farid et al., 2013; Hadi et al., 2014; Ali et al., 2020). However, there are also some disadvantages of applying different chemicals to soil and plants. Applied chemicals can leach into groundwater, causing toxicity to plants and disrupting of translocation of heavy metals. In addition, these chemicals may not biodegrade and may form complexes with other heavy metals, which can cause a secondary source of pollution (Nowack et al., 2006; Ali et al., 2020). Humic acids are macromolecules containing humic substances, which are organic substances distributed in nature (Haworth, 1971). Due to their amphiphilic character, humic acids form micelle-like structures under neutral or acidic conditions. Due to these properties, they are used in many fields such as agriculture, phytoremediation, medicine and pharmaceuticals (de Melo et al., 2016). Humic acids can retain pollutants and promote the natural breakdown of these substances by promoting the activity of microorganisms. The most important issue in the phytoremediation method is to select fast-growing hyperaccumulator plants that can accumulate heavy metals without being affected by specific soil contamination conditions (Sheoran et al., 2016). However, the immobility of some metals in the soil creates a limitation in the uptake of the metal by the roots of the species even if suitable hyperaccumulator species are selected (Kayser et al., 2000; Padmavathiamma and Li, 2007; Park and Sung, 2020).

*Allium cepa* is a widely cultivated vegetable species. Onion contains three different phytochemicals: organosulfur, flavonoids and fructan. In addition to vitamins C, E, B6, B2 and B1, it contains proteins such as lectin, pectin adenosine, fatty acids, essential amino acids, glycolipids and phospholipids (Pareek et al., 2017).

Research on phytoremediation has been conducted on the genus *Allium*. One of them is the study on *Allium sativum* L. species (Liu et al., 2009). It was reported that this species gave successful results on Cd uptake from soil. No other study on the hyperaccumulator property of *A. cepa* was found in the literature. The fact that this plant grows well and fast in Amasya conditions was an important factor in its selection for phytoremediation study.

In this study; *Allium cepa* L. (onion), an agricultural plant, was used in soils synthetically contaminated with Pb ions;

1. Effect of EDTA (Ethylenediamine tetraacetic acid) and Humic Acid on plant growth and development
2. The ability of onion plant to accumulate Pb heavy metal and chelate effect on heavy metal uptake
3. The hyperaccumulator property of the onion plant was investigated.

## **Materials and Method**

### **Characteristics of the Experimental Area**

The experimental greenhouse was established in the Merzifon district of Amasya province. The synthetically contaminated soil was taken at a depth of 20 cm from the rural area of Akören Village, 37 km away from Merzifon district, where there is no industry and traffic.

### **Soil Preparation and Lead Used in the Experiment**

Based on the literature information, lead was added to the soil in the form of  $Pb(NO_3)_2$ ; 100 mg  $kg^{-1}$  and the soil was homogenized and kept for one month. The soil was then placed in 20 cm diameter plastic pots at a rate of 3000 g/pot. The experiment was carried out according to the factorial experimental design in randomized blocks with three replications. The experiment was conducted under greenhouse conditions with natural light, 14 h (24.2 °C) / 10 h (16.7 °C) day/night cycle and about 50% relative humidity. The moisture content of the soil in the pots was maintained at about 75% of the field capacity. The pots were watered twice a week according to the soil water holding capacity. The chemical properties of the soil used in the experiment and the methods were as follows. Saturation 62, pH 7.76, EC 572 ds/cm (Richards, 1954); lime 14% (Çağlar, 1949); organic matter 7.99% (Walkley and Black, 1934); phosphorus 6.726 kg/da (Olsen et al., 1954); potassium 7.116 kg/da (Bower and Gschwend, 1952).

### **Plant cultivation and chelate addition**

Shallots of *A. cepa* of equal size and weight were used in the experiment. Shallots were previously sterilized with a 10% sodium hypochlorite solution. They were then immersed in sterile distilled water for 2 hours and then immersed in 3.5% hydrogen peroxide solution for 5 minutes and finally rinsed with sterile distilled water. Experimental pots were grouped as chelate and chelate+microbial fertilizer combinations. Shallots were planted in 20 pieces per pot. Then, BM-MegaFlu branded fertilizer,

which is a mixed microbial fertilizer prepared as 1 liter per 100 mL, was added to the experimental pots selected as chelate + microbial fertilizer. After the vegetative growth of the plants (8 weeks), EDTA and humic acid chelates were added to the soil at doses of 0-2.5-5.0-10 mmol kg<sup>-1</sup>. Plants were harvested four weeks after chelate addition. The pots without the addition of heavy metal, chelate, chelate + microbial fertilizer were determined as the control group. After harvesting, all plants were washed to remove sludge and other objects from the root zone and rinsed three times with deionized water.

### **Chemical Analyses**

The plants were divided into three parts: root, bulb and leaf and all plant parts were dried in an oven at 110 °C for 30 minutes and then at 70 °C for 96 hours until constant weight. Lead heavy metal analyses were carried out using a microwave analysis system for mineralization. For this purpose, the sample was placed in a glass container, added enough 65% nitric acid and disintegrated by stirring at 50 °C for 2 hours. Metal amounts were determined by the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) method. For leaf dry matter content, the leaves of 4 plants selected during harvesting were weighed on a precision balance and their wet weights were determined in grams then dried in an oven at 65 °C for 72 hours and their dry weights were determined in g (Kacar and İnal, 2008).

### **Calculations and Statistical Analyses**

Tolerance index (TI) as plant growth index is based on plant growth parameters such as root and green parts length, root and green parts wet and dry matter and is calculated as follows (Wilkins, 1978). Root and leaf lengths were measured with a ruler.

$$TI(\%) = (\text{Metal treated plant growth parameters} / \text{Control plant growth parameters}) \times 100$$

The Bioconcentration factor (BCF) used in the selection of plants to be grown for phytoremediation was calculated as follows (Padmavathiamma and Li, 2007).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Heavy metal concentration in harvested plant}}{\text{Soil heavy metal concentration}}$$

The translocation factor (TF) of each metal in plants was calculated as the total metal content in shoots divided by the total metal content in roots. Both factors were calculated on a dry mass basis (Brooks, 1994; Gurajala et al., 2019). In this study, root and green leaf parts were used as plant growth parameters.

Heavy metal concentrations in each sample were determined by making 5 series of measurements with 3 repetitions and statistical analysis of the data was performed using SPSS (Version 18) package.

## Results and Discussions

Plant growth parameters of *A. cepa* species are given in Table 1. According to this, higher root TI values (120%, 100%, 120%) were obtained in pots applied with EDTA + microbial fertilizer combination. In the pots where humic acid was applied, root TI values were higher. Again, leaf wet weight and dry weight TI values were higher in the pots where the EDTA + microbial fertilizer combination was applied. Thanks to the microbial fertilizer, the tolerance index of the species has increased. In the study, it was found that EDTA and humic acid decreased the negative effects of Pb on the growth factors of onion plants and increased lead accumulation. Mousavi et al., (2021) reported that EDTA increased the tolerance index and alleviated the harmful effects of Cd on okra plants in their experiments with *Abelmoschus esculentus*. Gul et al., (2020) reported that EDTA increased Pb concentration in shoots, roots and uptake of *Pelargonium × hortorum* L.H. Bailey (synonym of *Pelargonium × hybridum* (L.) L'Hér. ) but showed phytotoxicity by decreasing biomass and tolerance index. In a study conducted on *Brassica napus* L. species, it was reported that humic acid application caused an increase in shoot and root TI values (Canal et al., 2022). Contrasting results were obtained in studies with chelate application. The increase in heavy metal intake together with the intake of beneficial nutrients in chelated soils causes a decrease in the amount of root, onion and leaf dry matter (Nascimento et al., 2006). The addition of chelating agents increases the availability of Pb in the soil, thus negatively affecting plant growth (Yang et al., 2022). In another study, it was found that EDTA inhibited plant growth in EDTA treated soils (Ali and Chaudhury, 2016; Tanyıldız et al., 2022; İpek, 2019). In the study, *A. cepa* species exposed to high EDTA levels showed toxic effects manifested as necrosis and chlorosis. A similar situation was reported in a study with *Zea mays* (Hovsepyan and Greipsson, 2005).

**Table 1.** Plant parameters, root and leaf data and tolerance index (TI) values for chelate and chelate + microbial fertilizer doses used in the experiment

	Chelate Dose	Root Length (cm)	Root (Tİ)	Leaf Length (cm)	Leaf (%Tİ)	Leaf Wet weight (g)	Leaf Wet weight (%Tİ)	Leaf Dry weight (g)	Leaf Dry weight (%Tİ)
EDTA	0*	6±0.08		31±0.12		10.59±0.4		2.2±0.1	
(mmol kg <sup>-1</sup> )	2.5	5±0.07	83	30±0.11	91	10.45±0.1	99	2.0±0.1	91
	5	5±0.07	83	32±0.13	109	8.80±0.3	83	1.11±0.1	50
	10	6±0.08	100	30±0.12	91	20.69±0.8	195	6.2±0.6	282
EDTA	0	5±0.10	100	42±0.16	100	10.30±0.4		2.5±0.3	
+ Microbial Fertiliser	2.5	6±0.11	120	40±0.15	83	10.80±0.1	105	3.3±0.4	132
	5	5±0.10	100	40±0.15	83	20.12±0.9	195	4.2±0.4	168
	10	6±0.11	120	41±0.14	92	20.85±0.9	202	5.7±0.5	228
Humic acid	0	6±0.08		31±0.13		20.83±0.5		2.5±0.5	
(mmol kg <sup>-1</sup> )	2.5	7±0.08	117	32±0.13	109	20.46±0.5	98	4.8±0.5	83

	5	8±0.10	133	32±0.13	109	10.08±0.1	48	2.5±0.3	43
	10	5±0.07	83	30±0.13	91	10.03±0.1	48	2.2±0.3	38
Humic acid	0	7±0.12		40±0.11		10.37±0.4		2.3±0.2	
+ Microbial	2.5	7±0.12	100	41±0.11	110	20.07±0.6	194	4.3±0.5	187
Fertiliser	5	5±0.10	71	41±0.12	110	20.11±0.4	194	4.4±0.5	191
	10	6±0.10	86	40±0.11	100	20.17±0.5	195	3.9±0.4	170

\* Control group

The root length of the species did not show a significant difference in the trials ( $p>1$ ). Leaf length was lower in EDTA trials and higher in EDTA+microbial fertilizer combinations. Leaf dry weight was higher in EDTA and EDTA+microbial fertilizer combinations with a TI value of 10 mmol kg<sup>-1</sup>. Humic acid increased at a dose of 2.5 mmol kg<sup>-1</sup>, while other doses were not affected. Humic acid and microbial fertilizer combination increased the amount of leaf dry matter. Angin et al., (2008) reported that humic acid positively affected leaf dry matter weight and increased Pb uptake.

In *Allium cepa*, Pb values were found in root, bulb, leaf and total plant (28.93 mg kg<sup>-1</sup>, 36.16 mg kg<sup>-1</sup>, 262.56 mg kg<sup>-1</sup>, 327.65 mg kg<sup>-1</sup>) in pots with 5 mmol kg<sup>-1</sup> EDTA addition, respectively (Figure 1 A,B,C,D).

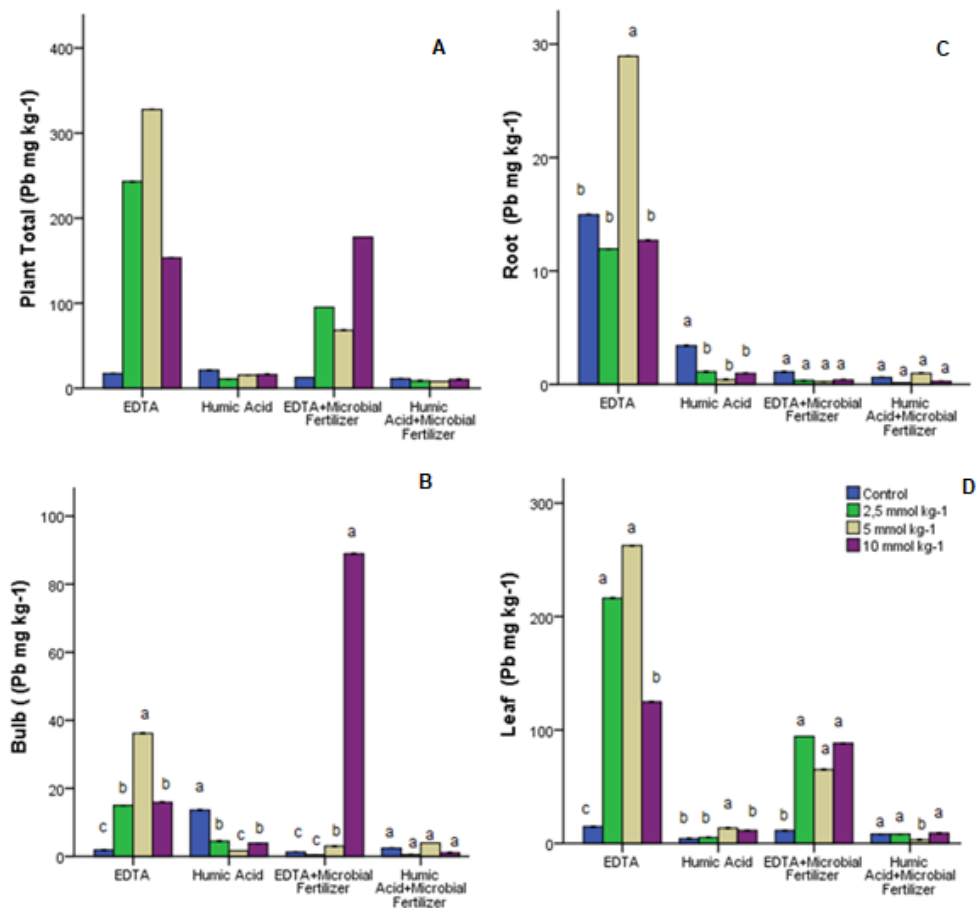


Figure 1. Pb uptake in plant organs and total plant of *A. cepa* species at chelate and chelate + microbial fertilizer combination doses (A; Pb uptake in the total plant, B; Pb uptake in the root, C; Pb uptake in the bulb, D; Pb uptake in leaf)

High accumulation (16.19 mg kg<sup>-1</sup>) was found at 10 mmol kg<sup>-1</sup> in total plant in pots with humic acid addition. It was found that the plant tended to accumulate Pb more in its leaves (Table 2). In both chelates, Pb was found to be accumulated in lower amounts in pots with microbial fertilizer addition. Biochar was reported to increase Pb uptake by *Brassica juncea* L. species in a study (Rathika et al., 2021). Humic acid increased plant growth parameters in trials with *Brassica napus* L. species (Canal et al., 2022).

Hovsepyan and Greipsson (2005) reported that plant species exposed to moderate levels of EDTA showed the highest level of heavy metal transport from roots to leaves in their study with lead heavy metal. High doses of EDTA have been found to cause the dissolution of mineral components in the soil, degrading soil properties and increasing heavy metal uptake by plants (Guan et al., 2022). In *A. cepa*, Pb uptake was found to be high in pots treated with 5 mmol kg<sup>-1</sup> EDTA. In pots with humic acid addition, Pb uptake was lower than in pots with EDTA addition. In a study conducted with *Allium sativum* species, it was found that Cd-treated soil was able to accumulate approximately 1.826 times more Cd than the control group (Jiang et al., 2001). Many studies have also suggested that the addition of EDTA to soil increases the translocation of Pb in plants (Huang et al., 1997; Gleba et al., 1999; Deram et al., 2000; Hovsepyan and Greipsson, 2005, Miao et al., 2012; Shahid et al., 2014). The effect of EDTA on heavy metal uptake ranges from 200-fold higher accumulations depending on plant, metal and soil type (Evangelou et al., 2007). When applied with NPK fertilizer, EDTA has been reported to increase Cd uptake by *Amaranthus hypochondriacus* L. (Li et al., 2012; Shahid et al., 2014). De La Rosa et al. (2007) showed that EDTA application had a negative effect on Pb uptake by *Salsola tragus* L. plants. It has been reported that heavy metal accumulation in plant roots decreases as a result of the binding of metals in the soil with EDTA chelate, but this increases metal translocation in the above-ground parts (Zhivotovsky et al., 2011; Shahid, et al., 2014).

BCF value is used to determine the phytoremediation potential of plants. The higher the BCF value, the higher the accumulation capacity of the species. In *A. cepa*, BCF values were found to be greater than 1 for EDTA and humic acid chelates. In *A. cepa* in the soil to which EDTA chelate was added, the value at the dose of 5 mmol kg<sup>-1</sup> was high. In humic acid, 10 mmol kg<sup>-1</sup> dose was found to be the highest value (Table 2).

BCF values of *A. cepa* grown in chelate + microbial fertilizer combination were found to be less than 1 (Table 2). BCF value is used to determine the phytoremediation potential of plants. The higher the BCF value, the higher the accumulation capacity of the species. In *A. cepa*, BCF values were found to be greater than 1 for EDTA and humic acid chelates. In *A. cepa* in the soil to which EDTA chelate was added, the value at the dose of 5 mmol kg<sup>-1</sup> was high. In humic acid, 10 mmol kg<sup>-1</sup> dose was found to be the highest value (Table 2). BCF values of *A. cepa* grown in chelate + microbial fertilizer combination were found to be less than 1 (Table 2).



**Table 2.** BCF and TF values in *Allium cepa*

		BCF	TF
	0	0.89±0.02	9.28±0.34
EDTA (mmol kg <sup>-1</sup> )	2.5	4.86±0.02	15.48±0.19
	5	5.25±0.01	8.26±0.08
	10	2.38±0.01	88.32±0.76
EDTA + Microbial Fertiliser	0	3.24±0.06	5.23±0.01
	2.5	0.15±0.01	8.65±0.09
	5	0.25±0.01	37.20±0.64
	10	0.27±0.01	15.69±0.26
Humic acid (mmol kg <sup>-1</sup> )	0	0.60±0.09	9.00±0.51
	2.5	3.54±0.02	10.53±0.05
	5	2.10±0.06	24.47±8.27
	10	8.17±0.09	19.92±0.01
Humic acid+ Microbial Fertiliser	0	3.73±0.08	1.39±0.15
	2.5	0.31±0.01	64.44±1.02
	5	0.21±0.01	7.28±0.01
	10	0.10±0.01	37.64±0.32

As a result, it can be said that the addition of microbial fertilizer decreased the Pb uptake of the species. Table 2 shows that  $TF > 1$  in *A. cepa* at all chelate doses. Zayed et al., (1998) classified BCF as  $< 0.01$  non-accumulator plants, 0.01-0.1 low accumulator plants, 0.1-1.0 medium accumulator plants, 1-10 high accumulator or hyperaccumulator plants. By using this ratio, the absorption of elements in the soil by the plant can be shown and the magnitude of elemental transfer from soil to plant can be quantitatively estimated (Kalender and Alçiçek, 2016). Accordingly, it can be said that *A. cepa* is a high accumulator in all chelates. In *A. cepa*,  $TF > 1$  was found at all doses of all chelates and chelates added to the species. Plants with TF values  $> 1$  were classified as highly efficient plants for metal translocation. In addition, EDTA has been found to have a high binding capacity for Pb in many studies (Blaylock et al., 1997; Tai et al., 2007; Najeep et al., 2017; García et al., 2017). It was determined that EDTA chelate increased Pb uptake with increasing application doses (Lai and Chen, 2005). The addition of humic acids to soil has been reported to increase the uptake of heavy metals from soil by plants (Li and Shuman, 1996; Halim et al., 2003; Evangelou et al., 2004). Similar results were obtained in this study. It is thought that humic acid improves the ability of plants to accumulate heavy metals because it decreases soil pH. Humic acid application has been reported to increase the BCF value of plants (Rasouli-Sadaghiani et al. 2019, Canal et al., (2022)). In trials with *Zea mays* L., humic acid and microbial fertilizer application was found to increase Cu, Zn and Pb uptake and humic

acid and bacterial fertilizer application has been reported as a way to increase heavy metal uptake (Li et al., 2014).

### **Conclusion**

In this study, the lead heavy metal removal performance of *A. cepa* species from the soil with the help of EDTA and humic acid chelating agents of Pb heavy metal ions was investigated. The sensitivity and resistance of *A. cepa* species to heavy metals were determined by measuring the biomass of these plants and in general, the species showed resistance to heavy metals and there was no significant change in their biomass. It was found that the hyperaccumulator properties of the species increased when EDTA and humic acid chelates were used, while the BCF value decreased when microbial fertilizer was added. It is thought that the onion plant can be used in biogas production after heavy metal uptake in the soil is ensured.

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**Conflict of Interest Declaration:** There is no conflict of interest between the authors.

Contributions of the researchers are equal.

### **Contribution Rate Statement Summary of Researchers**

The authors declare that they contributed to the article to a similar extent.

### **References**

- Ali S., Abbas Z., Rizwan M., Zaheer IE., Yavaş İ., Ünay A., Daim MMA., Jumah MB., Hasanuzzaman M., Kalderis D. Application of floating aquatic plants in phytoremediation of heavy metals polluted water: A review. *Sustainability* 2020; 12(5): 1927.
- Ali SY., Chaudhury S. EDTA-enhanced phytoextraction by *Tagetes* sp. and effect on bioconcentration and translocation of heavy metals. *Environmental Processes* 2016; 3(4): 735-746.
- Angin I., Turan M., Ketterings QM., Cakici A. Humic acid addition enhances B and Pb phytoextraction by vetiver grass (*Vetiveria zizanioides* (L.) Nash. *Water, Air, and Soil Pollution* 2008; 188: 335-343.
- Baker AJM., Brooks RR. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* 1989; 1: 81–126.
- Blaylock MJ., Salt DE., Dushenkov S., Zakharova O., Gussman C., Kapulnik Y., Ensley BD., Raskin I. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science and Technology* 1997; 31(3): 860-865.

- Bower CA., Gschwend FB. Ethylene glycol retention by soils as a measure of surface area and interlayer swelling. *Soil Science Society of America Journal*, 1952; 16(4): 342-345.
- Brooks RR. Plants that hyperaccumulate heavy metals. *Plants and the chemical elements: biochemistry, uptake, tolerance and toxicity*, 1994; 87-105.
- Canal SB., Bozkurt MA., Yılmaz H. The effect of humic acid on rapeseed (*Brassica napus* L.) plant growth, heavy metal uptake, phytoremediation parameters (BCF, TF and TI), and antioxidant activity in heavy metal polluted soil. *Yuzuncu Yıl University Journal of Agricultural Sciences* 2022; 32(2): 237-248.
- Chapman HD. Evaluation of the micronutrient status of soil. In *Int Symp Soil Fert Eval New Delhi Proc* 1971; (1): 927-947.
- Clemens S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* 2006; 88(11): 1707-1719.
- Çağlar KÖ. Toprak Bilgisi. A.Ü.Yayın1949; No:10
- De la Rosa G., Peralta-Videa JR., Cruz-Jimenez, G., Duarte-Gardea M., Martinez-Martinez A., Cano-Aguilera I., Sharma NC., Sahi SV., Gardea-Torresdey JL. Role of ethylenediaminetetraacetic acid on lead uptake and translocation by tumbleweed (*Salsola kali* L.). *Environmental Toxicology and Chemistry: An International Journal* 2007; 26(5): 1033-1039.
- de Melo BAG., Motta FL., Santana MHA. Humic acids: Structural properties and multiple functionalities for novel technological developments. *Materials Science and Engineering* 2016; (62): 967-974.
- Deram A., Petit, D., Robinson B., Brooks R., Gregg P., Van Halluwyn C. Natural and induced heavy-metal accumulation by *Arrhenatherum elatius*: Implications for phytoremediation. *Communications in soil science and plant analysis* 2000; 31(3-4): 413-421.
- Elkıran Ö. Removal of heavy metals from the environment via phytoremediation green reclamation method. *Hacettepe Journal of Biology and Chemistry* 2016; 44(4): 525-533
- Er H., Meral R., Kuşlu Y. Tuzlu toprakların halofit bitkiler kullanarak fitoremediasyon yöntemiyle iyileştirilmesi olanaklarının değerlendirilmesi. *Turkish Journal of Scientific Reviews* 2021; 14(2): 101-110.
- Evangelou MW., Ebel M., Schaeffer A. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere* 2007; 68(6): 989-1003.
- Farid M., Ali S., Shakoor MB., Bharwana SA., Rizvi H., Ehsan S., Hannan F. EDTA assisted phytoremediation of cadmium, lead and zinc. *Int J Agron Plant Prod* 2013; 4(11): 2833-2846.
- García S., Zornoza P., Hernández LE., Esteban E., Carpena RO. Response of *Lupinus albus* to Pb-EDTA indicates relatively high tolerance. *Toxicological & Environmental Chemistry* 2017; 99(9-10): 1378-1388.

- Gleba D., Borisjuk NV., Borisjuk LG., Kneer R., Poulev A., Skarzhinskaya M., Dushenkov S., Logendra S., Gleba YY., Raskin I. Use of plant roots for phytoremediation and molecular farming. *Proceedings of the National Academy of Sciences* 1999; 96(11): 5973-5977.
- Guan H., Dong L., Zhang Y., Bai S., Yan L. GLDA and EDTA assisted phytoremediation potential of *Sedum hybridum* 'Immergrunchen' for Cd and Pb contaminated soil. *International Journal of Phytoremediation* 2022; 24(13): 1395-1404.
- Gul I., Manzoor M., Kallerhoff J., Arshad M. Enhanced phytoremediation of lead by soil applied organic and inorganic amendments: Pb phytoavailability, accumulation and metal recovery. *Chemosphere* 2020; 258: 127405.
- Guo J., Lv X., Jia H., Hua L., Ren X., Muhammad H., Wei T., Ding Y. Effects of EDTA and plant growth-promoting rhizobacteria on plant growth and heavy metal uptake of hyperaccumulator *Sedum alfredii* Hance. *Journal of Environmental Sciences* 2020; 88: 361-369.
- Gurajala HK., Cao X., Tang L., Ramesh TM., Lu M., Yang X. Comparative assessment of Indian mustard (*Brassica juncea* L.) genotypes for phytoremediation of Cd and Pb contaminated soils. *Environmental Pollution* 2019; 254: 113085.
- Hadi F., Ali N., Ahmad A. Enhanced phytoremediation of cadmium-contaminated soil by *Parthenium hysterophorus* plant: effect of gibberellic acid (GA3) and synthetic chelator, alone and in combinations. *Bioremediation Journal* 2014; 18(1): 46-55.
- Halim M., Conte P., Piccolo A. Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. *Chemosphere* 2003; 52(1): 265-275.
- Haworth RD. The chemical nature of humic acid. *Soil Science* 1971; 111(1): 71-79.
- Hovsepyan A., Greipsson S. EDTA-enhanced phytoremediation of lead-contaminated soil by corn. *Journal of Plant Nutrition* 2005; 28(11): 2037-2048.
- Huang JW., Chen J., Berti WR., Cunningham SD. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology* 1997; 31(3): 800-805.
- İpek A. Bazı tarım bitkileri kullanılarak kurşun kirliliğinin şelat destekli fitoremediasyon yöntemiyle giderilmesi. Amasya Üniversitesi, Fen Bilimleri Enstitüsü Yüksek Lisans Tezi. Amasya, Türkiye, 2019
- Jiang W., Liu D., Hou W. Hyperaccumulation of cadmium by roots, bulbs and shoots of garlic (*Allium sativum* L.). *Bioresource Technology* 2001; 76(1): 9-13.
- Kacar B., İnal A. Bitki Analizleri. Nobel Yayınları, Yayın No: 1241, Fen Bilimleri, 892. Nobel Yayın 2008; 892 s. Ankara, Türkiye.
- Kalender L., Alçiçek Ö. *Astragalus angustifolius*, *Artemisia* sp. ve *Juncus effusus* uranyum ve toryum için biyoakümülatör özellikleri, Fırat Üniversitesi, Mühendislik Bilimleri Dergisi 2016; 28(2): 267-273.

- Kayser A., Wenger K., Keller A., Attinger W., Felix HR., Gupta SK., Schulin R. Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: The use of NTA and sulfur amendments. *Environmental Science and Technology* 2000; (34): 1778–1783.
- Khan AG., Kuek C., Chaudhry TM., Khoo CS., Hayes WJ. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* 2000; 41(1-2): 197-207.
- Kılıç DD., İpek A. Bazı tarım bitkileri kullanılarak arıtma çamurundan kurşun kirliliğinin şelat destekli fitoremediasyon yöntemiyle giderilmesi. *Journal of the Institute of Science and Technology* 2019; 9(1): 458-467.
- Ladillas S., El-Mufleh A., Gerente C., Chazarenc F., Andres Y., Bechet B. Potential of aquatic macrophytes as bioindicators of heavy metal pollution in urban stormwater runoff. *Water Air and Soil Pollution* 2012; 223(2): 877–888.
- Lai HY., Chen, ZS. The EDTA effect on phytoextraction of single and combined metals-contaminated soils using rainbow pink (*Dianthus chinensis*). *Chemosphere* 2005; 60(8): 1062-1071.
- Li NY., Fu QL., Zhuang P., Guo B., Zou B., Li ZA. Effect of fertilizers on Cd uptake of *Amaranthus hypochondriacus*, a high biomass, fast growing and easily cultivated potential Cd hyperaccumulator. *International journal of phytoremediation* 2012; 14(2): 162-173.
- Li T., Cheng H., Oh K., Hosono S. Effect of humic acid and bacterial manure on distribution of heavy metals in different organs of maize. *International Journal of Environmental Science and Development* 2014; 5(4): 393.
- Li Z., Shuman LM. Redistribution of forms of zinc, cadmium and nickel in soils treated with EDTA. *Science of the Total Environment* 1996; 191(1-2): 95-107.
- Liu D., Zou J., Meng Q., Zou J., Jiang W. Uptake and accumulation and oxidative stress in garlic (*Allium sativum* L.) under lead phytotoxicity. *Ecotoxicology* 2009; 18: 134-143.
- Lu S., Du Y., Zhong D., Zhao B., Li X., Xu M., Wu L. Comparison of trace element emissions from thermal treatments of heavy metal hyperaccumulators. *Environmental Science and Technology* 2012; 46(9): 5025-5031.
- Miao YANG., Xiao XY., Miao XF., Guo ZH., Wang FY. Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead. *Transactions of Nonferrous Metals Society of China* 2012; 22(6): 1462-1469.
- Mousavi A., Pourakbar L., Moghaddam SS., Popović-Djordjević J. The effect of the exogenous application of EDTA and maleic acid on tolerance, phenolic compounds, and cadmium phytoremediation by okra (*Abelmoschus esculentus* L.) exposed to Cd stress. *Journal of Environmental Chemical Engineering* 2021; 9(4): 105456.
- Nair AS., Machiavelli S., Babita K. Phytoremediation of heavy metals contaminated soil using tegetes patula. *Annals of Biology* 2019; 35(2): 181-185.

- Najeeb U., Ahmad W., Zia MH., Zaffar M., Zhou W. Enhancing the lead phytostabilization in wetland plant *Juncus effusus* L. through somaclonal manipulation and EDTA enrichment. *Arabian Journal of Chemistry*, 2017; 10, S3310-S3317.
- Nascimento CW., Amarasiriwardena D., Xing B. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil, *Environmental Pollution* 2006;140: 114-123
- Nowack B., Schulin R., Robinson BH. Critical assessment of chelant-enhanced metal phytoextraction. *Environmental Science and Technology* 2006; 40(17): 5225-5232.
- Oh SY., Yoon MK. Chemical extraction of arsenic from contaminated soils in the vicinity of abandoned mines and a smelting plant under subcritical conditions. *Soil and Sediment Contamination: An International Journal*, 2014; 23(2): 166-179.
- Olsen SR. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture, 1954.
- Özay C., Mammadov R. Ağır metaller ve süs bitkilerinin fitoremediasyonda kullanılabilirliği. *BAÜ Fen Bil. Enst. Dergisi* 2013; 15(1): 67-76
- Padmavathiamma PK., Li LY. Phytoremediation technology: hyper-accumulation metals in plants. *Water Air and Soil Pollution* 2007; 184(1-4): 105-126.
- Pak O. Kırklareli sınırları içerisindeki otoban kenarlarında bulunan tarım arazilerinde bazı ağır metal kirliliğinin araştırılması. Namık Kemal Üniversitesi, Fen Bilimleri Enstitüsü, Toprak Bilimi ve Bitki Besleme ABD, Yüksek Lisans Tezi, Tekirdağ, Türkiye, 2011
- Pareek S., Sagar NA., Sharma S., Kumar V. *Onion (Allium cepa L.) Fruit and Vegetable Phytochemicals: Chemistry and Human Health*, 2nd Edition 2017; 1145-1162.
- Park S., Sung K. Leaching potential of multi-metal-contaminated soil in chelate-aided remediation. *Water, Air, Soil Pollution*, 2020; 231(2): 40.
- Pinto PX., Al-Abed SR., Reisman DJ. Biosorption of heavy metals from mining influenced water onto chitin products. *Chemical Engineering Journal* 2011; 166(3): 1002-1009.
- Rasouli-Sadaghiani MH., Karimi H., Ashrafi Saeidlou S., Khodaverdiloo H. The effect of humic acid on the phytoremediation efficiency of Pb in the contaminated soils by wormwood plant (*Artemisia absantium*). *JWSS-Isfahan University of Technology* 2019; 22(4): 261-278.
- Rathika R., Srinivasan P., Alkahtani J., Al-Humaid LA., Alwahibi MS., Mythili R., Selvankumar T. Influence of biochar and EDTA on enhanced phytoremediation of lead contaminated soil by *Brassica juncea*. *Chemosphere* 2021; 271, 129513.
- Richards LA. (Ed.). *Diagnosis and improvement of saline and alkali soils* (No. 60). US Government Printing Office 1954.
- Romkens P., Bouwman L., Japenga J., Draaisma C. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. *Environmental Pollution* 2002; 116(1): 109-121.

- Shahid M., Austruy A., Echevarria G., Arshad M., Sanaullah M., Aslam M., Nadeem M., Nasim W., Dumat C. EDTA-enhanced phytoremediation of heavy metals: a review. *Soil and Sediment Contamination: An International Journal* 2014; 23(4): 389-416.
- Sheoran V., Sheoran AS., Poonia P. Factors affecting phytoextraction: a review. *Pedosphere* 2016; 26(2): 148-166.
- Tai Y, Yang Y, Li Z, Yang Y, Wang J, Zhuang P, Zou B. Phytoextraction of 55-year-old wastewater-irrigated soil in a Zn–Pb mine district: effect of plant species and chelators. *Environmental Technology* 2017; 1-13.
- Tanyıldız İpek A., Kılıç DD., Sürmen B. Phytoremediation efficiencies of *Brassica napus* and *Chenopodium quinoa* in soils contaminated with Pb using chelator complexes. *Anatolian Journal of Botany* 2022; 6(1): 13-17.
- Walkley A., Black IA. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science*, 1934; 37(1): 29-38.
- Wang X., Wang Y., Mahmood Q., Islam E., Jin X., Li T., et al. The effect of EDDS addition on the phytoextraction efficiency from Pb contaminated soil by *Sedum alfredii* Hance. *J Hazard Mater.* 2009;168, 530–535.
- Wilkins DA. The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist* 1978; 80(3): 623-633.
- Yang Y., Jiang M., Liao J., Luo Z., Gao Y., Yu W., He R., Feng S. Effects of simultaneous application of double chelating agents to pb-contaminated soil on the phytoremediation efficiency of *Indocalamus decorus* QH Dai and the soil environment. *Toxics* 2022; 10(12): 713.
- Zayed A., Gowthaman S., Terry N. Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *Journal of Environmental Quality* 1998; 27(3): 715-721.
- Zhang H., Guo Q., Yang J., Ma J., Chen G., Chen T., Zhu G., Wang J., Zhang G., Wang X., Shao C. Comparison of chelates for enhancing *Ricinus communis* L. phytoremediation of Cd and Pb contaminated soil. *Ecotoxicology and Environmental Safety* 2016; 133, 57-62.
- Zhivotovsky OP., Kuzovkina YA., Schulthess CP., Morris T., Pettinelli, D. Lead uptake and translocation by willows in pot and field experiments. *International journal of phytoremediation* 2011; 13(8): 731-749.
- Zhou J., Chen LH., Peng L., Luo S., Zeng QR. Phytoremediation of heavy metals under an oil crop rotation and treatment of biochar from contaminated biomass for safe use. *Chemosphere* 2020; 125856.