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Phytoremediation efficiencies of *Brassica napus* and *Chenopodium quinoa* in soils contaminated with Pb using chelator complexes

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Şelatör kompleksleri kullanılarak Pb ile kirlenmiş topraklarda *Brassica napus* ve *Chenopodium quinoa*'nın fitoremediasyon etkinlikleri

Abstract: Heavy metal pollution is one of the essential pollutions, and phytoremediation is one of the preferred methods to eliminate this pollution. The use of the degradable chelating agent for phytoremediation efficiency is a promising and low-cost method for removing soil contaminated with heavy metals. In this study, it was investigated whether phenanthroline and humic acid increase phytoremediation activities for *Brassica napus* L. and *Chenopodium quinoa* Wild. species and their applicability. The study was carried out under greenhouse conditions with 3 replications according to a complete random block trial design by applying 4 doses of each of the (i) control (without chelate), (ii) EDTA, (iii) nitro, (iv) pyridine, (v) 1-10 phenanthroline and (vi) humic acid treatments (0, 2.5, 5 and 10 mmol kg^{-1} The obtained results showed that the highest tolerance indices (TI) for *B. napus* was found at 2.5 mmol kg^{-1} nitro chelate. TI of *C. quinoa* was highest at 5 mmol kg^{-1} pyridine chelate. Maximum Pb accumulations were found at 5 mmol kg^{-1} 1-10 phenanthroline and 5 mmol kg^{-1} pyridine chelates in *B. napus* and *C. quinoa*, respectively. In both species, while Pb accumulations were high in roots, they were low in stems and leaves. Bioconcentration factors (BCF) were calculated highest at 2.5 mmol kg^{-1} nitro and 1-10 phenanthroline for *B. napus* and *C. quinoa*, respectively. These species were used as hyperaccumulator plants in many studies. Increasing the performance of hyperaccumulator plants to be used in cleaning the habitats exposed to heavy metal pollution will increase the efficiency of phytoremediation.

Key words: Heavy metal, pollution, accumulator plant, agricultural plants

Özet: Ağır metal kirliliği önemli kirliliklerden olup, fitoremediasyon bu kirliliği ortadan kaldırmak için tercih edilen yöntemlerdendir. Fitoremediasyon verimliliği için bozunabilir şelatlama maddesinin kullanımı, ağır metallerin topraktan uzaklaştırılması için için umut verici ve düşük maliyetli bir yöntemdir. Bu çalışmada, EDTA (etilendiamintetraasetik asit), nitro (4-nitrobenzaldehit), piridin, 1-10 fenantrolin ve hümik asidin *Brassica napus* L. ve *Chenopodium quinoa* Willd. türleri için fitoremediasyon etkinliklerini arttırıp arttırmadığı ve uygulanabilirliği araştırılmıştır. Çalışma (i) kontrol (şelat ilavesiz), (ii) EDTA, (iii) nitro, (iv) piridine, (v) 1-10 fenantrolin ve (vi) hümik asit uygulamalarının her birinden 4 doz (0, 2.5, 5 ve 10 mmol kg^{-1}) uygulayarak tam şansa bağlı blok deneme desenine göre 3 tekerrürlü olarak sera şartlarında yürütülmüştür. Elde edilen sonuçlara göre, *B. napus* için en yüksek tolerans indeksinin (TI) 2.5 mmol kg^{-1} nitro şelatta bulunmuştur. *C. quinoa*'nın TI değeri 5 mmol kg^{-1} piridin şelatta en yüksek bulunmuştur. Maksimum Pb birikimleri sırasıyla *B. napus* ve *C. quinoa*'da 5 mmol kg^{-1} 1-10 fenantrolin ve 5 mmol kg^{-1} piridin şelatlarında bulunmuştur. Her iki türde de köklerde Pb birikimleri yüksek iken, gövde ve yapraklarda düşük düzeydeydir. Biyokonsantrasyon faktörleri (BCF) en yüksek sırasıyla *B. napus* ve *C. quinoa* için 2.5 mmol kg^{-1} nitro ve 1-10 fenantrolinde hesaplanmıştır. Bu türler birçok çalışmada hiperakümülatör bitkiler olarak kullanılmıştır. Çalışma sonunda, türlerin en yüksek hiperakümülatör kapasiteleri 2.5 mmol kg^{-1} nitro ve 1-10 fenantrolin uygulamasından elde edildiği bulunmuştur. Ağır metal kirliliğine maruz habitatların temizlenmesinde kullanılacak hiperakümülatör bitkiler kadar onların performansının artırılması da fitoremediasyonun etkinliğini artıracaktır.

Anahtar Kelimeler: Ağır metal, kirlilik, akümülatör bitki, tarımsal bitkiler

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1. Introduction

Heavy metal density in the soil decreases the fertility quality of the soil. It also creates dangers for organisms due to its toxic effect (Quartacci et al., 2006). Lead (Pb), which is highly toxic, is transported to organisms by air, water and soil (Kabata-Pendias, 2004). When the Pb naturally found in soils exceeds the limit value, it causes excessive heavy metal accumulation in plants (Dürüst et al., 2004).

Phytoremediation is a method that removes heavy metal pollution from the environment using plants (Raskin et al., 1997). Phytoremediation preserves the soil's biological properties and physical structure (Khan et al., 2008; Gong et al., 2018). Plant species that can accumulate 50 to 500 times more metal than the metal concentration in the soil are used in the phytoremediation method, and these species are called hyperaccumulative plants (Clemens, 2006; Özay and Mammadov, 2013). Approximately 450 plant species (only 0.2 % of angiosperms) have been defined as hyperaccumulator (Reeves, 2006).

In phytoremediation studies, chelate addition facilitates the plant's uptake by increasing the mobility of metals in the soil and so increases the accumulator capacity of plants (Adiloğlu et al., 2015). EDTA (ethylenediaminetetraacetic acid), EGTA (ethylenglutaric acid), DTPA (diethyltriaminpentaacetic acid), SDS (sodiumdodecylsulfate), NTA (nitrilotriacetate), [S, S] -EDDS (S, S-ethylenediamindiscinucin acid), humic acid

(HA), boric acid are the most commonly used chelates (Ladislas et al., 2012). This study used five chelates which are humic acid, EDTA, 1-10 phenanthroline, nitro and pyridine.

Brassica napus and *C. quinoa* are well known as hyperaccumulator species. However, previously unused chelates (nitro, pyridine, 1-10 phenanthroline) were used in this study for studied species and Pb. We aimed to determine the phytoremediation efficiencies of species in a pot experiment.

2. Materials and Method

The soil was collected from the rural village of Suluova (Akören; 40° 52' 29.1756" N and 35° 27' 42.3144" E) without traffic and industrial in Amasya Province in Türkiye. The soil was a clay loam paddy soil with a pH (in water) of 7.76 and had high organic matter content and medium calcareous. Collected soil had aqua regia-soluble concentrations of Pb of 0.12 mg kg^{-1} (Table 1).

Table 1. Chemical properties of the soil used in the experiment.

Parameters	SoilValue
Saturation (%)	62.00
pH	7.76
EC (ds/m)	572
Lime (%)	14
Organic matter (%)	7.99
Phosphorus (kg/da)	6.73
Potassium (kg/da)	7.12
Pb (mg/kg)	0.12

The collected soil was dried in the air, crushed and passed through a coarse sieve. 3 kg portions of the soil were transferred to 20 cm diameter pots. The lead was added at the rate of 100 mg kg^{-1} as $Pb(NO_3)_2$ form to each pot. One month was waited for the soil to become homogeneous after the lead addition. We added the necessary amount of nutrients (NPK fertiliser) to the pots before planting. *B. napus* and *C. quinoa* seeds were previously surface sterilised with a 10 % sodium hypochlorite solution. 20 seeds of each species were sown in each pot.

The treatments were as follows (1) control without chelates; (2) EDTA; (3) nitro; (4) pyridine; (5) 1-10 phenanthroline and (6) humic acid at doses of 0-2.5-5.0-10 mmol kg^{-1}. Plot experiment was conducted at the greenhouse conditions with natural light, 14 hours (24.2° C) in a day and 10 hours (16.7° C) in a night and about 50 % relative humidity. There were three replicates of each treatment in a randomised block design.

Plant materials for heavy metal analysis were harvested after mature (four weeks). The harvested plants were classified as root, stem and leaf. They were rinsed with tap water and then with distilled water, dried at 65° C for 48 h, and their dry weights were measured. Plant materials were analyzed for Pb concentrations using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) method.

Growth parameters such as root, stem and leaf length, and fresh and dry matter were used for tolerance index (TI) calculations (Wilkings, 1978).

$$\mathrm{TI} = \frac{\text{Metal applied plant growth parameters}}{\text{Control plant growth parameters}}$$

Bioconcentration Factor (BCF): The ratio between the total heavy metal concentration in the plant and soil (Mertens et al., 2005; Ladislas et al., 2012).

$$\mathrm{BCF} = \frac{\text{Heavy metal concentration in the plant biomass}}{\text{Soil heavy metal concentration}}$$

Translocation factor (TF) is the ratio of the metal concentration in the plant stem to the root's metal concentration. It was used to give an idea about the plant's heavy metal carrying ability (Padmavathiamma and Li, 2007; Badr et al., 2012; Alaribe and Agamuthu, 2015).

$$\mathrm{TF} = \frac{\text{Shoot heavy metal concentration}}{\text{Root heavy metal concentration}}$$

Data were tested statistically by analysis of variance and Tukey's honestly significant difference (HSD) tests were carried out by using SPSS 22.0 version.

3. Results

The highest tolerance indices was calculated for *B. napus* (126 %) and *C. quinoa* (165 %) at 2.5 mmol kg^{-1} nitro and 5 mmol kg^{-1} pyridine chelates, respectively. Many chelate treatments of *C. quinoa* have been found to higher tolerance indices than the *B. napus* (Figure 1).

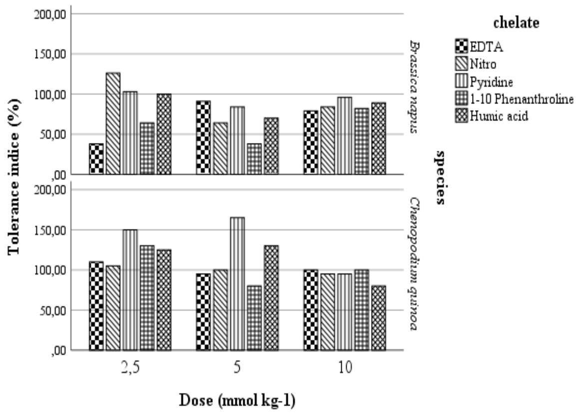

Figure 1. Tolerance Index (TI) values of *B. napus* and *C. quinoa* plant at the different chelate treatments.

We found that Pb accumulation was highest (252.13 ± 3.80) in *B. napus* at 5 mmol kg^{-1} 1-10 phenanthroline and lowest accumulation (63.80 ± 0.50) was found at 10 mmol kg^{-1} humic acid chelate. For *C. quinoa*, highest accumulation (606.00 ± 9.60) was found at 10 mmol kg^{-1} pyridine and lowest accumulation (131.46 ± 0.25) was found at 5 mmol kg^{-1} in EDTA (Table 2).

Comparing lead accumulation in plant organs of species, the accumulation capacity of the roots is higher (Figure 2).

According to the repeated measures ANOVA (RMANOVA) results, heavy metal accumulations were significantly among roots, stems, leaves and whole plants for each species considering chelate, chelate dose and chelate * chelate dose parameters (Table 3).

BCF values is higher than 1 at all chelates treatments in *B napus* and *C. quinoa*. We found the highest BCF (6.15) at 2.5 mmol kg^{-1} in nitro chelate for *B. napus* and the highest

BCF (23.31) at 2.5 mmol kg^{-1} 1-10 phenanthroline for *C. quinoa*. TF values of species were generally found to be less than 1 (Table 4).

Table 2. Pb concentration of total plant at the different chelate treatments.

Chelate	Dose (mmol kg^{-1})	Total Plant Pb (mmol kg^{-1})	
		B. napus	*C. quinoa*
Control		5.81 ± 0.03	6.01 ± 0.07
EDTA	2.5	129.93 ± 0.11	229.23 ± 2.70
	5	160.86 ± 0.95	**131.46 ± 0.25**
	10	121.83 ± 0.80	255.10 ± 0.20
Nitro	2.5	146.06 ± 0.65	215.03 ± 1.30
	5	153.06 ± 0.25	233.46 ± 1.15
	10	97.56 ± 2.15	264.80 ± 0.20
Pyridine	2.5	133.43 ± 0.05	562.70 ± 1.10
	5	132.90 ± 1.20	289.26 ± 0.35
	10	68.16 ± 0.45	**606.00 ± 9.60**
1-10 Phenanthroline	2.5	99.13 ± 0.70	516.96 ± 3.55
	5	**252.13 ± 3.80**	511.90 ± 0.70
	10	147.36 ± 0.75	225.80 ± 2.50
Humic acid	2.5	103.93 ± 0.30	472.13 ± 0.60
	5	97.26 ± 0.05	462.40 ± 16.88
	10	**63.80 ± 0.50**	290.00 ± 0.43

4. Discussions

Adding chelates to soils increases heavy metal and nutrient intake and disrupts nutrient intake balance (Chen et al., 2003; Turan and Angin, 2004; Turgut et al., 2004; Turan and Esringü, 2008). This decrease dry matter in the root, stem and leaf of the plant (Lai and Chen, 2005; Nascimento et al., 2006; Quartacci et al., 2006; Ben Rejeb et al., 2013; Zaier et al., 2014). In this study, 1-10 phenanthroline, nitro and pyridine were used for the first time. Previous studies have revealed that EDTA and humic acid increase heavy metal accumulation by plants (Ali and Chaudhury, 2016; Halim et al., 2003; Evangelou et al., 2004). EDTA has high binding capacity for Pb (Elliott et al., 1986; Quartacci et al., 2006; Safari Sinegani and Khalilikhah, 2011; Kanwal et al., 2014; Tai et al., 2018) and is used for Pb uptake at different dose treatments (Patra and Goldberg, 2003; Evangelou et al., 2004; Lai and Chen, 2005).

It has been found that EDTA application slows down the biomass increase in *Ricinus communis* L. species (Zhang et al., 2016), increases the accumulation of Pb in *Paspalum fasciculatum* and is higher in the roots than other organs (Salas-Moreno and Marrugo-Negrete, 2020). Humic acid application increase Cu accumulation and restrict Zn uptake in roots of *Chrysopogon zizanioides* (Vargas et al., 2016). Many studies have found that these chelates increase heavy metal accumulation in roots (Bhattacharya et al., 2010; Idris et al., 2016).

In the current study, chelate applications have been found to reduce growth and biomass in *B. napus* and increase growth and biomass in *C. quinoa*. For *B. napus*, Pb accumulation is highest for 5 mmol kg^{-1} 1-10 phenanthroline chelate and lowest for 10 mmol kg^{-1} humic

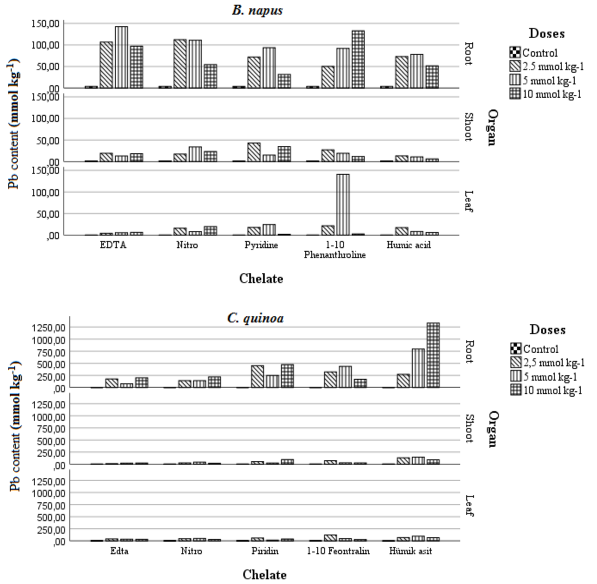

Figure 2. Pb in the plant organs at the different chelate treatments.

acid chelate. The highest concentration of Pb in *C. quinoa* was found at a dose of 10 mmol kg^{-1} pyridine, and the lowest accumulation was found in 5 mmol kg^{-1} EDTA chelate. Pb accumulation in the roots of both species was higher than in the stem and leaves. These results are similar to previous studies. Generally, high Pb uptake was found in both species in nitro, pyridine and 1-10 phenanthroline applications.

Table 3. RMANOVA test results between the chelate and pb concentration in root, stem, leaf and plant in *B. napus* and *C. quinoa* species. ($p<0.01$** , $p<0.05$*, ns=p>0.05).

		B. napus		***C. quinoa***	
Source		**F value**	**Sig.**	**F value**	**Sig.**
Chelate	Root	4229.06	0.00**	184.42	0.00**
	Stem	13938.5	0.00**	56.77	0.00**
	Leaf	26359.8	0.00**	26.9	0.00**
	Total plant	4767.32	0.00**	5089.42	0.00**
Chelate dose	Root	38764.94	0.00**	512.27	0.00**
	Stem	37898.91	0.00**	50.45	0.00**
	Leaf	35374.2	0.00**	122.57	0.00**
	Total plant	47790.31	0.00**	20488.08	0.00**
Chelate * Chelate dose	Root	3207.4	0.00**	70.15	0.00**
	Stem	5364.1	0.00**	13.9	0.00**
	Leaf	24275.97	0.00**	15.75	0.00**
	Total plant	2531.71	0.00**	2076.16	0.00**

Zayed et al. (1998) classified the plants into four groups considering BCF. BCF <0.01; non-accumulator plant, BCF = 0.01-0.1; low accumulator plant, BCF = 0.1-1.0; medium accumulator plants, BCF = 1-10; high accumulative or hyperaccumulator plant. BCF values of *B. napus* and *C. quinoa* species are greater than 1 in all applications. BCF values of *B. napus* species in nitro chelate were the highest at 2.5 mmol kg^{-1} doses, while in *C. quinoa* species, at a dose of 2.5 mmol kg^{-1}, 1-10 phenanthroline was found to be the highest in chelate. *B. napus* and *C. quinoa* show high accumulative properties.

Table 4. BCF and TF values of species at the different chelate treatments.

Chelate	Dose (mmol kg^{-1})	BCF		TF	
		B. napus	*C. quinoa*	*B. napus*	*C. quinoa*
Control		1.57	2.98	0.57	0.60
EDTA	2.5	1.61	4.80	0.22	0.29
	5	3.82	3.00	0.13	0.73
	10	2.38	6.44	0.25	0.27
Nitro	2.5	6.15	8.76	0.30	0.51
	5	5.67	8.75	0.38	0.64
	10	1.83	7.26	0.79	0.21
Pyridine	2.5	2.52	10.31	0.84	0.25
	5	1.99	6.61	0.42	0.16
	10	1.18	21.70	1.15	0.28
1-10 Phenanthroline	2.5	4.55	23.31	0.97	0.59
	5	3.16	15.98	1.73	0.17
	10	2.16	6.09	0.10	0.31
Humic acid	2.5	2.33	12.03	0.42	0.74
	5	3.75	19.71	0.24	1.46
	10	1.18	6.35	0.23	1.19

If TF> 1, chelates interact with metals to form a metal-chelate pair. It provides faster transport of metal ions to the plant's aboveground organs (Ladislas et al., 2012). TF values of *B. napus* and *C. quinoa* species were generally less than 1. TF values for *B. napus* were higher than 1 in 10 mmol kg^{-1} dose of pyridine and 5 mmol kg^{-1} 1-10 phenanthroline. TF values of *C. quinoa* were found higher than 1 in 5 and 10 mmol kg^{-1} humic acid applications. As a result, we put forward the contributions of different chelate applications in selecting plants to be used in phytoremediation studies. Also, determining the underground and aboveground parts of the selected plants' accumulator capacity gives an idea about how environments can be used.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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