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Humic acid ameliorates phytoremediation, plant growth and antioxidative enzymes in forage turnip (*Brassica rapa* L.)

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Abstract: In this study, the effects of ethylenediaminetetraacetic acid (EDTA) and humic acid (HA) chelate applied to soils contaminated with heavy metals on the development, antioxidant defence system, and phytoremediation of forage turnip (*Brassica rapa* L.) were investigated for the first. Three doses of EDTA (E_1: 5 mmol/kg, E_2: 10 mmol/kg, E_3: 15 mmol/kg) and three doses of HA (HA_1: 500 mg/kg, HA_2: 1 000 mg/kg, HA_3: 2 000 mg/kg) were applied to soils contaminated with heavy metals (Cd, Pb, Zn, and Cr) in the pot. According to experiment results, HA application as chelate to the polluted soil caused a significant increase in the growth of forage turnip. Phytoremediation values of the plant for Cd heavy metal were found to be BCF_{shoot}, $BCF_{root} > 1$, and translocation factor > 1. This result proved that forage turnip has Cd accumulating properties. Also, HA application caused a decrease in H_2O_2 (46%) and malondialdehyde (6%) levels and antioxidative enzyme activity in polluted soil. It has been concluded that humic acid improves the oxidative stress conditions in the plant and is more effective in the development and growth of the plant than EDTA, so that it can be used effectively in phytoremediation studies.

Keywords: soil pollution; chelating substances; plant defence system; metal ions

Soil pollution, regarded as a severe problem in the world as well as in our country in recent years, is the deterioration of the chemical and physical features of the soil caused by solid and liquid wastes. Heavy metals are the main factors causing this deterioration. Suppose heavy metals exceed the average percentage in the soil. In that case, it causes the deterioration of the soil quality and decreases the yield and quality of products, leading to big problems for all living (Blaylock and Huang 2000). Heavy metals such as cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) are released from a variety of sources, including industrial activities, fossil fuel burning, mine dumps, insecticides, fertilisation (Seven et al. 2018). Expensive and mostly inefficient technologies such as excavation, electrokinetic remediation, soil washing, solidification, and the like have rehabilitated heavily polluted areas (Ali et al. 2017). One of the most essential techniques for cleaning these areas is phytoremediation. Using cost-effective plants for the remediation of polluted soils with heavy metals is an environmentally friendly alternative. In phytoremediation, chelating substances help absorb heavy metals from the soil, increase accumulation in the plant, and transport metals to the above-ground parts of plants (Lee and Sung 2014, Boysan et al. 2022). Hyperaccumulators usually only accumulate a specific element, and, as a rule, their agronomic characteristics, known as slow-growing, low-biomass-producing plants, limit their use for

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phytoremediation (Grčman et al. 2001). However, chelating agents directly and indirectly affect heavy metal concentration in soil and plants, healthy plant growth, and soil microbial properties (Saifullah et al. 2009). Ethylenediaminetetraacetic acid (EDTA) can enhance the mobility of heavy metals and their uptake by plants. Humic acid (HA) decreases the plant's metal forms and soluble and extractable forms of heavy metals. Hyperaccumulator plants are distinguished from other plants by taking more metals from the soil, transporting them to their upper organs, and detoxifying them in the leaves. Almost 25% of the plant species known for their hyperaccumulator property belong to the Brassicaceae family (Doğru et al. 2021). The aim of the study that we carried out with forage turnip (*Brassica rapa* L.), a member of the Brassica family, is as follows: (1) evaluation of the effects of chelates (EDTA and HA) on plant growth and development; (2) evaluation of the effects of chelate on heavy metal uptake of the forage turnip, and (3) evaluation of the effect of chelates on oxidative stress caused by heavy metal content of forage turnip.

MATERIAL AND METHOD

Characteristics of experimental soil. The soil material was taken from the Faculty of Agriculture of the Van Yuzuncu Yıl University (YYÜ) study area. The physical properties of the trial soil are given in detail in Table 1. The soil's electrical conductivity (EC) was measured using an EC meter (Thermo Scientific, Waltham, USA). Lime content was calculated using a Scheibler Calcimeter (Hızalan and Ünal 1966). Soil organic carbon was determined using the Walkley-Black method (Walkley 1947). Total N (%) was measured in soil using the Kjeldahl method (Kacar 1994). Fe, Cu, Zn, Mn, and Cd were extracted with DTPA and then measured using the atomic absorption spectroscopy (AAS) technique (Lindsay and Norvell 1978).

Plant material and growth condition. The cv. Lenox used in the study was obtained from the "ÜÇLER TOHUM" seed company (Bursa, Turkey). This research was conducted at the Department of Soil Science and Plant Breeding, Faculty of Agriculture, Van YYÜ University. The seeds were sown in pots containing 2.5 kg of soil and thinned to leave three plants in each pot after sowing. The plants were grown in the climate chamber at 20 ± 2 °C with 70% humidity under a 16-h light/8-h dark cycle and a 400 μmol/m^2/s light intensity. Heavy metals were added to the potting soil in liquid form and incubated for one month. As mineral fertiliser, 0.2 g/kg ammonium nitrate (NH_4NO_3), 0.08 g/kg triple super phosphate, and 0.05 g/kg potassium sulfate (K_2SO_4) were applied. A completely random design was applied in this trial with three repetitive trials. The information on all applications made in the experiment is given in Table 2. EDTA "Titriplex III (Cas No: 6381-92-6)" used in the study was obtained from Merck. "HUMAS-15," purchased from GÜBRETAŞ company (İstanbul, Turkey), was used as the humic acid source. HUMAS-15 contains 15% humic and fulvic acid. All applications were made before seed planting, and the plants were grown for 45 days. The samples harvested for antioxidant analysis were immediately treated with liquid nitrogen and stored at –80 °C.

Physical analysis. As physical analysis, the number of leaves (pieces/plant) of each plant in the experiment was taken, shoot (cm) and root length were measured, and fresh shoot weight (g/plant), dry

Table 1. Properties of experimental soil

Physical properties		Extractable with DTPA (mg/kg)		Total heavy metal (mg/kg)		Exchangeable cations (mmol$_+$/100 g)	
Texture	sandy loam	Zn	0.16	Zn	45.1	K	0.94
Organic carbon (%)	1.02	Cr	0.06	Cr	95.0	Ca	19.79
pH (1/2.5)	8.15	Cd	0.08	Cd	0.65	Mg	1.51
Salt (dS/m)	0.35	Pb	0.30	Pb	9.03	Na	2.57
Lime (%)	6.6						
P (ppm)	7.0						
N (%)	0.051						
CEC (mmol$_+$/100 g)	16.0						

DTPA – ethylenediaminetetraacetic acid; CEC – cation exchange capacity

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Table 2. Pot experiment applications

Application	Ingredient
Control	only experimental soil
Polluted soil (PS)	50 mg/kg Cr $(NO_3)_3$, 50 mg/kg $CdSO_4 \cdot 8\,H_2O$, 50 mg/kg Pb $(NO_3)_2$, 200 mg/kg Zn $ZnSO_4 \cdot 7\,H_2O$
PS + E_1	5 mmol/kg EDTA (ethylenediaminetetraacetic acid)
PS + E_2	10 mmol/kg EDTA
PS + E_3	15 mmol/kg EDTA
PS + HA_1	0.5 g/kg HA (humic acid)
PS + HA_2	1.0 g/kg HA
PS + HA_3	2.0 g/kg HA

shoot weight (g/plant), dry and fresh root weight (g/plant) was determined on a precision scale. Fresh plant parts (root and shoot) were dried in the oven at 70 °C until constant weight.

Phytoremediation parameters. Bioconcentration factor (BCF) and translocation factor (TLF) (Esringü et al. 2014) were calculated as follows: BCF = [(metal concentration in plant tissue (root or shoot), mg/kg)/DTPA concentration of soil mg/kg)]. Translocation factor (TLF) = [(metal concentration in the shoots, mg/kg)/metal concentration in the roots, mg/kg)].

Antioxidative enzymes, MDA, H_2O_2 and heavy metal analysis in plants. The catalase (CAT) (EC 1.11.1.6) enzyme activity was analysed according to the method reported by Cakmak et al. (1993), and the decrease in the absorbance value was determined at 240 nm wavelength within 1 min due to H_2O_2 reduction. The APX (EC 1.11.1.11) activity was analysed using the method reported by Nakano and Asada (1981). The decrease in the ascorbate and the absorbance value were measured at a wavelength of 290 nm in 1 min. The amount of lipid peroxidation was determined by the accumulation of malondialdehyde (MDA) in the tissues (Yilmaz et al. 2023). The amount of MDA accumulated in tissues was determined at 532 nm and 600 nm wavelengths, according to the report by Hodges et al. (1999). H_2O_2 was made according to the protocol reported by Velikova et al. (2000) and read at 290 nm wavelength. Dried plant leaf and root samples were burned in a mixture of hydrogen peroxide-nitric acid (HNO_3-$HClO_4$). Pb, Cd, Cr, and Zn concentrations were analysed in an atomic absorption spectrophotometer (Thermo Scientific/ICE 3000) (İbrikçi et al. 1994).

Statistical analysis. A one-way analysis of variance was performed for the differences between applications. Significant differences between implementations were tested using Duncan's multiple comparison tests. The SPSS software package version 13.0 was used in the analysis (Hilbe 2005). The least significant differences (*LSD*) were calculated at $P \leq 0.05$.

RESULTS AND DISCUSSION

HA applications significantly increased the shoot length, number of leaves, dry and fresh weight of the plant, root length, and root dry and fresh weight of forage turnip (*Brassica rapa* L.) grown in polluted soil. However, EDTA applications further reduced leaf number, shoot length, fresh and dry weight of the plant, root length, and fresh and dry weight of the root in forage turnip. HA application was observed, the leaf width was increased morphologically, and the plant leaves were dark green in this study. However, EDTA-treated forage turnips were smaller than usual and had a light green colour (Figure 1).

Together with heavy metals, increasing levels of HA application on forage turnip caused an increase of 42, 85, and 47%, respectively, in the shoot fresh weight of the plant compared to polluted soil. It caused a shoot dry weight rise of 16, 28, and 19%, respectively. However, EDTA applied at increasing levels caused a 49, 57, and 35% reduction in the shoot fresh weight of the plant, respectively, compared to PS (Figure 2). It caused a decrease in shoot dry weight by 47, 56, and 34%, respectively. Similarly, in the study conducted with the Brasicca family, HA application decreased the toxic effect caused by heavy metals on the leaves of *Brassica campestris* and *Brassica juncea*. For *B. campestris*, *B. juncea*, and *Sorghum bicolour*, EDTA application caused a decrease in plant fresh weight (Lee and Sung 2014). EDTA (5 mmol) caused a significant decrease in the root and shoot weight of mustard (*Brassica juncea* Coss) grown in soil contaminated with cadmium and zinc.

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Figure 1. The effect of humic acid (HA) and ethylenediaminetetraacetic acid (EDTA) applications on forage turnip (*Brassica rapa* L.) plant grown under heavy metal conditions. PS – polluted soil

However, EDTA + organic acid applications increased plant growth significantly (Guo et al. 2019). Plant dry weight was increased by citric acid application in *Pelargonium hortorum* (Gul et al. 2019). EDTA (10 mmol/kg) caused a decrease in the height and weight of the Maso Bamboo plant. Nevertheless, citric acid (5 mmol/kg) caused a significant increase in root dry weight (Zhang et al. 2018). Organic acids such as malic acid, citric acid, acetic acid, and humic acid have low molecular weight. These substances with low molecular weight can increase the resistance of plants under stress. Especially in phytoremediation studies on the development of the brassica plant, the humic acid heavy metal complex promotes improvement in plant growth (Yildirim et al. 2021, Canal et al. 2022). The humic substance has been found to stimulate growth in *Brassica napus* L. (Ehsan et al. 2014). Humic acid applied to soil polluted with heavy metals causes a significant increase in the uptake of mineral nutrients that play a role in plant development. It also causes a significant increase in the root development of the plant (Clapp et al. 2001). Humic acid supports plant growth by indirectly contributing to the activity of microorganisms in the soil, the cation exchange of the soil, the structure of the soil, the moisture level of the soil, and the solubility of soil ions. Humic acid forms compounds with heavy metals thanks to their phenolic and carboxyl groups. Organic acids in the soil facilitate the transport and solubility of heavy metal ions (Ebrahimian and Bybordi 2014, Sembiring et al. 2016). Humic acid immobilises Pb, Cu, and Mo in the soil (Hattab et al. 2014). The carboxylic (COOH) and phenolic (OH) groups included in the humic acid added to the soil can provide natural chelation in the soil, and due to this feature, it can regulate the availability of heavy metals in the environment (Lagier et al. 2000, Wiszniewska et al. 2016). In addition, different humic substances can exhibit various reducing and complexing properties, and fulvic acids, which have a higher carboxylic content than humic acid, have a similar effect on heavy metals (Kalčíková et al. 2016). EDTA applications are significantly practical in plant extraction in phytoremediation studies. As a synthetic chelate, EDTA disrupts the balance between plant minerals such as zinc, copper, iron, and calcium. The high level of heavy metal uptake by EDTA in plants causes phytotoxic effects (Shahid et al. 2014). It also causes damage to cell membranes by disrupting cell metabolism.

It can cause a significant decrease in chlorophyll content (Saifullah et al. 2009, Eissa 2017). EDTA (10 mmol/kg) applied to soil contaminated with Pb, Cd, and Zn caused leaf necrosis, rapid ageing of shoots, and decreased yield in the *Brassica rapa* L. (Grčman et al. 2001). EDTA toxicity symptoms observed in *Lolium perenne* and *Brassica juncea* caused an essential decrease in plant fresh weight (Johnson et al. 2010). Kos et al. (2003) showed that EDDS application has toxic effects, such as necrosis and rapid senescence in *Amaranthus* sp., *Brassica napus*, *B. rapa*, and *Cannabis sativa*. Rashid et al. (2014) investigated chelate-assisted uptake of metals and reported that DTPA was associated with a significant biomass decrease in *Lactuca sativa*. The EDTA-metal complex, which enters the plant roots, decomposes after entering the plant roots and increases the free ionic heavy metals, which can cause a toxic effect (Wu et al. 2021). Heavy metals application caused an increase in the plant's antioxidative enzymes catalase (CAT) and ascorbate peroxidase (APX) levels, H_2O_2, and MDA levels compared to the control application (Figure 3). H_2O_2, MDA, and APX increased by 63, 17, and 21%, respectively, in PS + E_3 applications compared to PS. However, CAT, APX, H_2O_2, and MDA levels decreased by 60, 27, 46,

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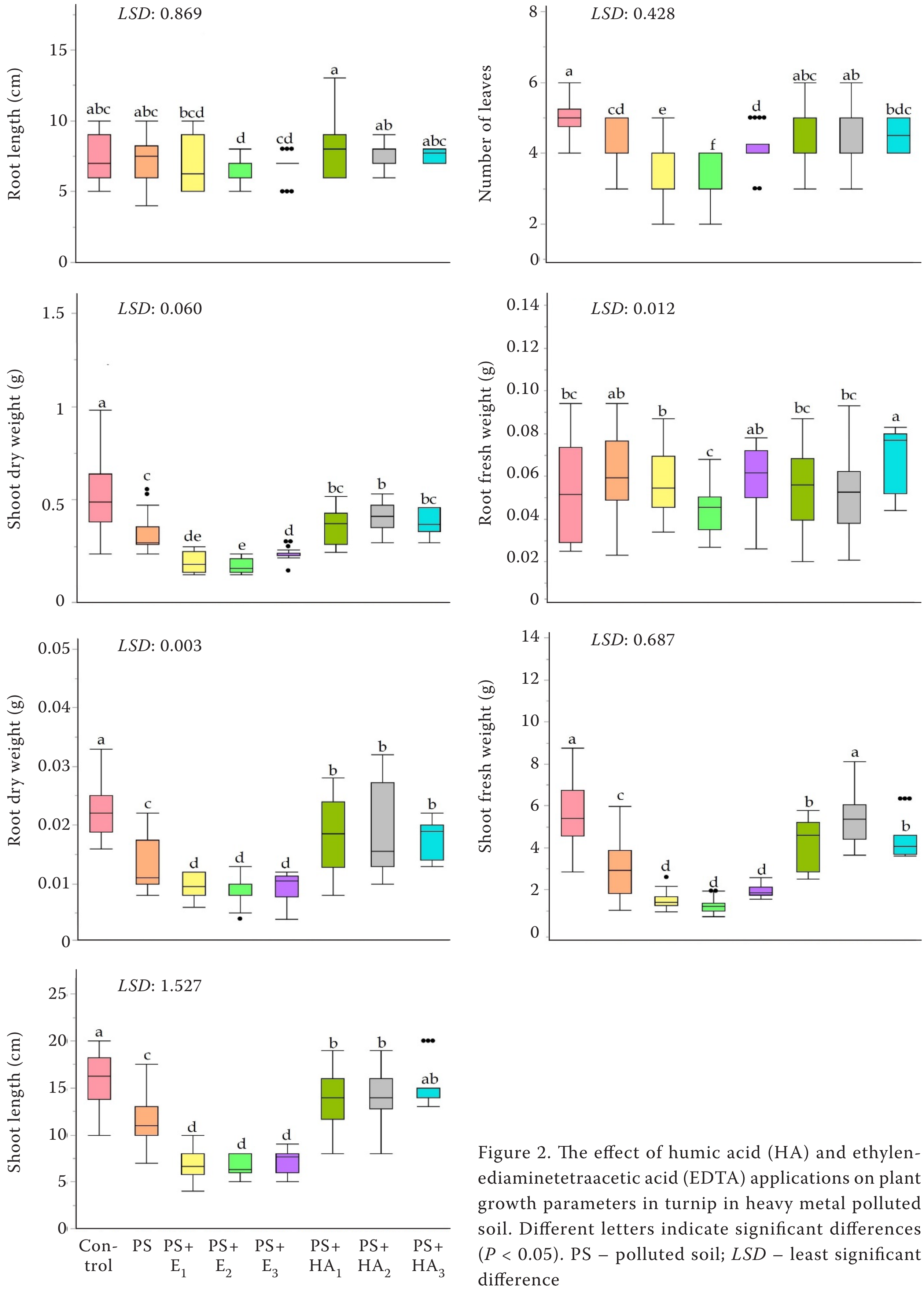

Figure 2. The effect of humic acid (HA) and ethylenediaminetetraacetic acid (EDTA) applications on plant growth parameters in turnip in heavy metal polluted soil. Different letters indicate significant differences ($P < 0.05$). PS – polluted soil; *LSD* – least significant difference

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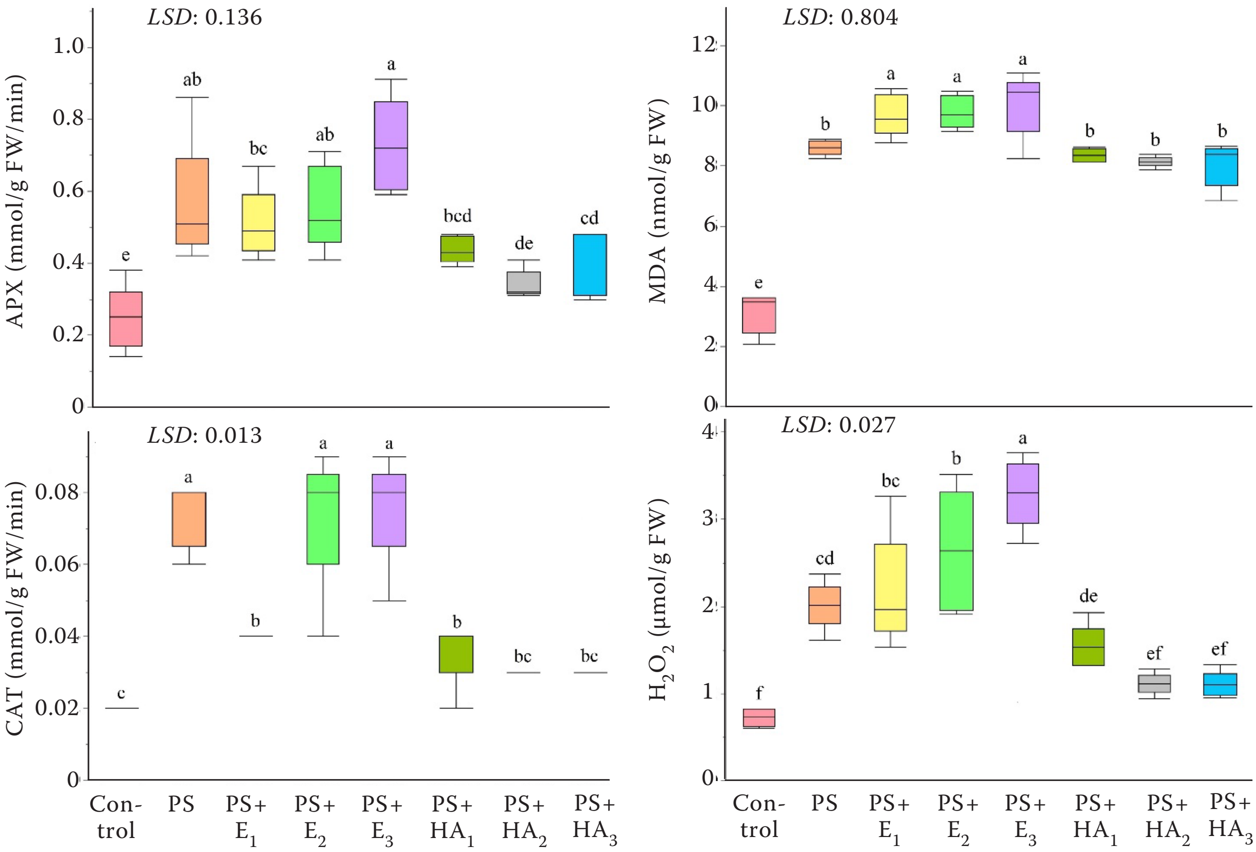

Figure 3. Effects of humic acid (HA) and ethylenediaminetetraacetic acid (EDTA) applications on antioxidative activity in forage turnip (*Brassica rapa* L.). Different letters indicate significant differences (P < 0.05). APX – ascorbate peroxidase; MDA – malondialdehyde; CAT – catalase; FW – fresh weight; PS – polluted soil

and 6%, respectively, in the PS + HA_3 application. Antioxidative enzymes form a defence mechanism to reduce oxidative damage in the plant. Hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl (OH^-) are the primary free radicals that cause oxidative damage. The effectiveness of these radicals is reduced by reducing and oxidising them with antioxidative enzymes (Hasanuzzaman et al. 2012). Malondialdehyde level indicates the peroxidation of lipids secreted in the cell membrane because of oxidative damage (Ali et al. 2015). Our study found that the H_2O_2 and MDA content of forage turnip plants grown in polluted soil increased compared to the control application. Accordingly, it was found that the activity of antioxidant enzymes CAT and APX increased to reduce oxidative damage in polluted soil with heavy metals. EDTA application, which is a chelate, caused a rise in the effect of oxidative stress in the plant by promoting the solubility of heavy metals. Oxidative stress caused an enhancement in H_2O_2 and MDA levels and the activities of CAT and APX, which are among the antioxidative enzymes (Canal and Bozkurt 2018). Humic acid application as a chelate reduced the levels of H_2O_2 and MDA and in the activities of CAT and APX, which are antioxidative enzymes. APX and CAT enzyme activity at the HA_2 level was the same as the control application. Similarly, fulvic acid applied to a wheat plant grown in Cr-polluted soil caused a substantial decrease in H_2O_2 and MDA levels in the plant, resulting in a reduction in the effect of free radicals or a decrease in membrane damage (Ali et al. 2015, 2018). Under heavy metal stress conditions, amino chelates can prevent peroxidation of the cell membrane lipids due to their content's amino acid structure (Souri et al. 2019). Humic acid application caused a significant increase in the growth and development of the plant. However, since the enhancement in the intake of heavy metals was not at the same level as the growth and development of the plant, the concentration of heavy metals decreased. This effect is called the dilution effect. The dilution effect in the plant reduced the oxidative stress effect. Similarly (Rizwan et al. 2017), zinc-lysine (Zn-lysine) chelate applied to cadmium-

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polluted soil improves oxidative stress in the wheat plant, increases plant growth, and increases CAT and APX enzyme activity. In contrast, applying different amino acids decreases antioxidative enzymes in soybeans (Teixeira et al. 2017).

BCF_{shoot} and BCF_{root} were determined to estimate the capacity of the forage turnip plant to accumulate lead, cadmium, chromium, and zinc heavy metals under HA and EDTA applied conditions (Table 3).

The plant's BCF (root) values were high in all lead, cadmium, chromium, and zinc applications. BCF_{shoot} and BCF_{root} values for Cd in forage turnip were found from $BCF_{shoot} > 1$, $BCF_{root} > 1$, and TLF > 1. When evaluated according to the applications made, it was determined that the BCF_{shoot}, BCF_{root}, and TLF values of the plant decreased with increasing levels of HA application compared to the PS. It was found that the EDTA forage turnip applied at increasing rates

Table 3. Effect of humic acid (HA) and ethylenediaminetetraacetic acid (EDTA) applications on phytoremediation parameters in forage turnip (*Brassica rapa* L.)

		BCF_{shoot}	BCF_{root}	$TLF_{shoot/root}$
Pb	Control	0.26 ± 0.10^{c}	1.70 ± 0.11^{e}	0.03 ± 0.05^{d}
	PS	0.52 ± 0.01^{b}	3.95 ± 0.21^{c}	0.31 ± 0.00^{b}
	PS + HA_1	0.20 ± 0.02^{c}	3.15 ± 0.20^{d}	0.06 ± 0.01^{c}
	PS + HA_2	0.20 ± 0.01^{c}	3.16 ± 0.20^{d}	0.06 ± 0.01^{c}
	PS + HA_3	0.19 ± 0.01^{c}	3.09 ± 0.15^{d}	0.06 ± 0.00^{c}
	PS + E_1	1.32 ± 0.12^{b}	8.21 ± 0.67^{b}	0.16 ± 0.03^{a}
	PS + E_2	1.52 ± 0.11^{a}	11.67 ± 0.44^{a}	0.13 ± 0.01^{b}
	PS + E_3	1.26 ± 0.11^{b}	8.05 ± 0.60^{b}	0.16 ± 0.01^{a}
Cd	Control	0.53 ± 0.05^{f}	2.88 ± 0.20^{e}	0.20 ± 0.02^{e}
	PS	3.71 ± 0.14^{b}	3.85 ± 0.05^{c}	3.76 ± 0.10^{b}
	PS + HA_1	2.30 ± 0.17^{d}	3.24 ± 0.21^{d}	2.30 ± 0.15^{c}
	PS + HA_2	2.02 ± 0.05^{e}	3.59 ± 0.06^{d}	2.02 ± 0.05^{d}
	PS + HA_3	2.60 ± 0.10^{c}	2.25 ± 0.22^{e}	2.60 ± 0.10^{c}
	PS + E_1	4.75 ± 0.14^{a}	4.81 ± 0.35^{b}	4.80 ± 0.26^{a}
	PS + E_2	3.69 ± 0.22^{b}	4.00 ± 0.19^{c}	3.82 ± 0.25^{b}
	PS + E_3	3.59 ± 0.25^{b}	5.91 ± 0.14^{a}	4.03 ± 0.11^{b}
Cr	Control	0.02 ± 0.00^{d}	0.11 ± 0.01^{f}	0.23 ± 0.04^{a}
	PS	0.04 ± 0.01^{c}	0.45 ± 0.01^{d}	0.09 ± 0.02^{c}
	PS + HA_1	0.02 ± 0.00^{d}	0.39 ± 0.03^{de}	0.05 ± 0.00^{c}
	PS + HA_2	0.02 ± 0.00^{d}	0.34 ± 0.05^{e}	0.06 ± 0.01^{c}
	PS + HA_3	0.03 ± 0.00^{d}	0.38 ± 0.05^{e}	0.18 ± 0.02^{b}
	PS + E_1	0.05 ± 0.00^{b}	0.57 ± 0.03^{c}	0.11 ± 0.01^{b}
	PS + E_2	0.05 ± 0.00^{b}	0.75 ± 0.06^{b}	0.12 ± 0.01^{b}
	PS + E_3	0.06 ± 0.00^{a}	0.98 ± 0.21^{a}	0.11 ± 0.01^{b}
Zn	Control	0.30 ± 0.04^{f}	0.88 ± 0.06^{d}	0.34 ± 0.04^{d}
	PS	0.81 ± 0.02^{d}	1.61 ± 0.05^{c}	0.52 ± 0.00^{c}
	PS + HA_1	0.91 ± 0.02^{d}	1.56 ± 0.04^{c}	0.57 ± 0.01^{c}
	PS + HA_2	0.84 ± 0.02^{d}	1.52 ± 0.04^{c}	0.55 ± 0.02^{c}
	PS + HA_3	0.61 ± 0.01^{e}	0.73 ± 0.08^{d}	0.84 ± 0.11^{a}
	PS + E_1	1.96 ± 0.26^{c}	2.78 ± 0.45^{b}	0.71 ± 0.08^{b}
	PS + E_2	2.24 ± 0.07^{b}	3.98 ± 0.22^{a}	0.56 ± 0.03^{c}
	PS + E_3	2.72 ± 0.07^{a}	4.08 ± 0.12^{a}	0.66 ± 0.01^{b}

PS – polluted soil; E – ethylenediaminetetraacetic acid; TF – transfer factor; TLF – translocation factor; BCF – bioconcentration factors. *Different letters in the same column indicate significant differences ($P < 0.05$)

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caused an increase in BCF_{shoot} and BCF_{root} values compared to P. EDTA levels as a synthetic chelate in terms of Pb, Cr, Cd, and Zn in forage turnip (*Brassica rapa* L.) cause an increase in BCF_{shoot} and BCF_{root} values. The results showed that the accumulation of heavy metals was highest in the root area, followed by shoots. Similarly, increased EDTA application applied in contaminated soil in terms of Zn and Pb increased the solubility of metals, resulting in BCF_{root} > BCF_{shoot} (Ebrahimi et al. 2015). HA application was found to cause a decrease in BCF_{shoot} and BCF_{root} values in terms of Pb, Cr, Cd, and Zn in forage turnip (*Brassica rapa* L.). Lee and Sung (2014) found that HA application caused a decrease in BCF_{shoot} value for *B. juncea*, *B. campestris*, and *S. bicolour*, while EDTA reasoned an enhancement in BCF_{shoot} value in soil polluted with Pb and Cd where they applied different chelates. HA has strong adsorption and exchange effects on some anions and cations in the soil, limiting the uptake of heavy metals from the soil to the plant (Zhao et al. 2023). Also, its effect on the soil is slow; it has been stated that it can increase heavy metal accumulation when it has a long-term effect. The formation of stable complexes of humic and fulvic acids with metal ions may be due to the high content of oxygen-containing functional groups, including various carboxyl (COOH), phenolic, alcoholic, enolic-OH, and C=O structures (Dinauer 1982). However, HA may contain protein and carbohydrate residues that can constitute stable complexes with metals in the natural state (Shao et al. 2023). Our study found that the forage turnip plant (*Brassica rapa* L.) had BCF_{shoot} < 1 and TLF < 1 in Pb, Cr, and Zn heavy metals for HA. However, we found that the BCF_{shoot} and BCF_{root} values for Cd are bigger than 1. In other words, the forage turnip plant has a hyperaccumulator feature for Cd. The TLF value of more than 1 means that the forage turnip plant carries Cd more from the root region to the upper organs. Similarly, in the study conducted with the Brassica family, the highest BCF_{root} value for Cd was B. *juncea* > B. *campestris* > S. *bicolo*r > *H. annuus* (Lee and Sung 2014). Brassica cultivars effectively ameliorate polluted soils with heavy metals (Kanwal et al. 2014). It shows that the forage turnip (*Brassica rapa* L.) plant can be used for the phytoextraction of polluted soil with Cd. Cadmium uptake and accumulation can vary depending on the species. The high biomass production of Brassica species provided under various climatic and cultivation conditions can increase cadmium accumulation and uptake. Using resistant Brassica species and appropriate regulators can increase agricultural remediation for Cd (Rizwan et al. 2018). Also, due to its low biodegradability, EDTA leakage can create significant pollution, especially for groundwater (Anjum et al. 2012). EDTA also has a toxic effect on microorganisms living in the soil, especially on nematodes (Vandevivere et al. 2001, Shinta et al. 2021). Due to the toxicity of EDTA to biota, the use of natural organic chelating agents (humic acid, oxalic acid, malic acid, citric acid, etc.) has a significantly less toxic effect on plant growth (Evangelou et al. 2006).

In summary, the application of humic acid to soil contaminated with multiple heavy metals increased the growth and development of forage turnips and reduced the effect of oxidative stress caused by heavy metals on the plant. The plant's oxidative stress reduction supported growth and development, and biomass increased. Although the increase in biomass causes the concentration of heavy metals in the plant to be diluted, it can be concluded that the application of humic acid increases phytoremediation per unit area.

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