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Original Research

Effects of Humic Acid and EDTA on Phytoremediation, Growth and Antioxidant Activity in Rapeseed (*Brassica napus* L.) Grown under Heavy Metal Stress

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Abstract

Rapeseed has been cultivated to produce non-edible and edible oil for thousands of years. It is known as the second-largest oilseed plant in the world with 24.6 million tons of oil production in 2021. The interventions that can be carried out during the cultivation of a plant with such a high production value are quite significant. Growth, enzymatic activities, and phytoremediation of rapeseed grown under heavy metal stress supported by humic acid (HA) and ethylenediaminetetraacetic acid (EDTA) applications were investigated for the first time in this study. Three doses of EDTA (EDTA1:5 mmol/kg, EDTA2:10 mmol/kg, EDTA3:15 mmol/kg) and three doses of HA (HA1:500 mg/kg, HA2:1000 mg/kg, HA3:2000 mg/kg) were applied in heavy metal treated pots. According to experiment results, HA1 and HA2 applications increased plant dry and fresh weights, root dry and fresh weights. However, EDTA applications caused a decrease in shoot length, a number of leaves, shoot fresh and dry weights, root fresh and dry weights. Bioconcentration factor (BCF) values for Zn, Cr and Cd we found higher than in both shoots and roots of rapeseed. For all levels of EDTA, the values of BCF (shoot) and BCF (root), transfer factor (TF) and translocation factor (TLF) increased compared to HA applications. On the other hand, in comparison to heavy metal polluted soils alone (PS), all levels of HA resulted in significantly reduced APX and CAT enzyme activity, and hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents. We concluded that humic substances exert a significant influence on plant growth and reduce heavy metal toxicity in polluted soils. At the same time, HA could be more effective than EDTA in terms of phytoremediation of Cr. HA can increase phytoremediation in polluted soils as it improves plant growth and oxidative stress

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due to its organic nature. The results provide remarkable information for rapeseed cultivation in polluted regions.

Keywords: antioxidative activity, EDTA, humic acid, phytoremediation, rapeseed

Introduction

The heavy metals are required in small amounts and have the greatest toxicity effects above the concentration limits, particularly chromium (Cr), cobalt (Co), cadmium (Cd), chromium (Cr), lead (Pb) and nickel (Ni). The total concentration of heavy metals along with metallic properties and bonding states of heavy metals as well as soil properties such as pH, organic matter, redox conditions, and chelating determine mobility and toxicity in polluted soils [1, 2]. Industrial production, agricultural activities, and extensive mining release heavy metals into the ecosystem that cause serious environmental hazards and health risks for plants, animals, and human beings. For this reason, remediation of heavy metal-contaminated soils is critical for the protection of the environment and human health. One of the effective methods to remove heavy metal pollutants from the soil is phytoremediation, using special plants to accumulate pollutants in their tissues [3]. Interaction between plant species and metal characteristics causally involves in the heavy metal intake, translocation, and accumulation of plants [4, 5]. The process of phytoremediation implicates various mechanisms that involve distinct parts of plants such as uptake of heavy metals by roots, translocation to tissues, and bioaccumulation of toxic metal content with in the plant [6]. These mechanisms provide immobilization and reduction of heavy metal content in the soils [7, 8].

Phytoremediation of heavy metal contaminated soils is an emerging technology aimed at removing heavy metals from the soil and has attracted a lot of attention as it is an environmentally friendly and relatively inexpensive technique [9, 10]. In this technique, chelates are added to the soil to increase the accumulator properties of the plants [11, 12]. Humic acid (HA), the main component of humic substances, is a ligand that interacts readily with metals [13]. Humic acid (HA) and ethylenediaminetetraacetic acid (EDTA) chelators have a significant effect on the uptake and solubility of metals in heavy metal polluted soils by plants. EDTA is applied to metal-contaminated soil to increase the bioavailability and mobility of the metals in the soil. It is worth noting that, EDTA application influences metal uptake by plants and soil solubility of metals [14]. However, EDTA-heavy metal compounds have a toxic effect on the microbiological structure of the soil. Due to the low biodegradability of EDTA, it is absorbed by the solid complexes of the soil and remains in the soil for a long time that results in groundwater pollution through washing [15]. Natural sources such as HA can be used as an alternative to this synthetic chelator EDTA [16]. Humic substances have a healing effect on soil pH and organic matter content, which increase the development of aboveground and underground parts of plants in agricultural areas [17]. In a greenhouse experiment, a commercial liquid seaweed extract derived from Ascophyllum nodosum (Stimplex), was applied to the *Capsicum annuum* L. as either a soil drench or foliar spray. Applications of Stimplex to pepper plants improved stem diameter, plant height, number of leaves and leaf area, leaf chlorophyll content, shoot fresh weight, shoot dry weight, root fresh weight and dry weight compared to the control plants [18]. HA, containing acidic phenolic and hydroxyl groups, forms compounds with heavy metals. These compounds significantly affect the solubility, usefulness, and transport of heavy metals [19]. Heavy metals cause the production of reactive oxygen radicals (ROS; H_2O_2, OH, and O^{2-}) as a toxic response to stress in the plant. Since these radicals contain unpaired electrons, they easily enter the metabolic reactions in the plant and have a detrimental effect [20]. Against this destructive effect, the plant activates antioxidative enzymes, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione (GSH), and peroxidase (POX), which implicate in the reduction of oxidative stress in plants [21-24].

The *Brassicaceae* is the largest family that includes 11 species out of 87 kinds of hyperaccumulator plants [25]. As a member of the *Brassicaceae* family, rapeseed (*Brassica napus* L.) is beneficial in the improvement of contaminated soils, and the rapeseed oil can be used as a biodiesel source as well. These features make the rapeseed the most favourite plant in the *Brassicaceae* family [26, 27]. Among the reclamation techniques of contaminated soils, plant-based remediation techniques are becoming more common today as they are environmentally friendly and cost-effective [28]. Many families, including *Brassicaceae* members, can accumulate different amounts of various heavy metals in their shoots [29].

This study aimed at investigating the effects of HA and EDTA applications on rapeseed in heavy metal contaminated soils with Pb, Cd, Cr and Zn. Firstly, it was hypothesized that EDTA applications would increase the intake of heavy metals in the rapeseed plant that may be beneficial in the phytoremediation of multi-polluted soils. It was predicted that HA would have a healthy effect on growth and development as well as antioxidant defence mechanisms of rapeseed, which is a hyper accumulator plant that may be more effective in HA applications under heavy metal stress than EDTA application.

Table 1. Characteristics of soil.

Texture	Sandy Loam	Extractable with DTPA (mg kg^{-1})		Total heavy metal (mg kg^{-1})	
pH (1/2.5)	8.15	Pb	0.30	Pb	9.03
Salt (dS m^{-1})	0.35	Cd	0.08	Cd	0.65
Lime (%)	6.6	Cr	0.06	Cr	95.0
Organic Material (%)	1.02	Zn	0.16	Zn	45.1

Materials and Methods

Soil Characterization

The experiment soil was taken from the study areas of the Faculty of Agriculture of Van Yüzüncü Yıl University, Turkey. This soil is characterized by medium calcareous, alkaline pH, low organic matter and low nitrogen (N) (Table 1).

Pot Experiment

Some modifications were made to the protocol used by Turan and Esringü [34] for the pot experiments. Before the experiment, heavy metals in the doses of 50 mg kg^{-1} Cr as chromium nitrate $Cr(NO_3)_3$ 50 mg kg^{-1} Cd as cadmium sulphate ($CdSO_4.8H_2O$) and 50 mg kg^{-1} Pb as lead nitrate ($Pb(NO_3)_2$ and 200 mg kg^{-1} Zn as zinc sulphate ($ZnSO_4.7H_2O$) were applied to the pots that each was 2.5 kg. The soil contaminated with heavy metals was added to the potting soil in the liquid form which was left to incubate for one month. Then, the pot experiment was carried out in the climate room. The experiment conditions were adjusted to 20±2ºC temperature, 60% humidity, 16:8 hours photoperiod since rapeseed is a cool climate plant. Chemical fertilizers of 80 mg kg^{-1} phosphorus (P) as triple superphosphate, 200 mg kg^{-1} nitrogen (N) as ammonium nitrate, and 50 mg kg^{-1} potassium (K) as potassium sulphate were added. In this study, a completely randomized design with the three-replication trial was implemented. In our experiment, the purpose of applying two different chelates is to increase the mobility of heavy metals with low mobility. Eight applications of EDTA and HA were as follows: 1-Control; 2- Polluted soil with heavy metals (PS); 3-PS+HA$_1$ (500 mg kg^{-1}); 4- PS +HA$_2$ (1000 mg kg^{-1}); 5-PS+HA$_3$ (2000 mg kg^{-1}); 6- PS+EDTA$_1$ (5 mmol kg^{-1}); 7-PS+EDTA$_2$ (10 mmol kg^{-1}); and 8- PS+EDTA$_3$ (15 mmol kg^{-1}).

Chemical and Physical Analysis of the Soil

Soil samples were air-dried in an unlit area and passed through a 2 mm sieve. The soil texture was identified by the Bouyoucous hydrometer method [30]. The pH was measured using a 1: 2.5 soil-water mixture [31]. Lime content was determined using the Scheiblercalcimeter [32]. Soil organic matter was determined using the Walkley-Black method [33]. The total N was measured by using the Kjeldahl method [34]. The extractable Pb, Cr, Cd and Zn amounts were determined by the diethylenetriaminepentaacetic acid (DTPA) method [35]. The total Pb, Cr, Cd and Zn in the soil were determined using the method developed by Khan and Frankland [36].

Phytoremediation Parameters

Bioconcentration factor (BCF), transfer factor (TF) and translocation factor (TLF) [37] were calculated as follow:

$$\text{BCF} = [(\text{Metal concentration in plant tissue (root or shoot), mg kg}^{-1})/\text{DTPA concentration of soil mg kg}^{-1})]$$

$$\text{TF} = [(\text{Metal concentration (root+shoot), mg kg}^{-1})/(\text{Metal concentration of soil, mg kg}^{-1})]$$

$$\text{TLF} = [(\text{Metal concentration in the shoots, mg kg}^{-1})/(\text{Metal concentration in the roots, mg kg}^{-1})]$$

Antioxidative Enzymes, MDA, H_2O_2 and Heavy Metals Analyses in Plant

Enzymatic measurements were carried out at 0-4ºC. The supernatant was used as a crude enzyme extract for catalase (CAT) enzyme analysis. CAT (EC 1.11.1.6) activity was determined as a decrease in absorbance at 240 nm for 1-min, following the decomposition of H_2O_2 [38]. Ascorbate peroxidase (APX) enzyme (EC 1.11.1.11) activity was determined following the decrease of ascorbate by measuring the change in absorbance at 290 nm for 1 min in 2 ml of a reaction mixture containing 50 mM KH_2PO_4 (pH 7.0), 1 mM EDTA-Na$_2$, 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 50 ml of crude enzyme extract [39]. The levels of lipid peroxidation were measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation. To a 1.0 ml aliquot of the supernatant 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. After centrifugation at 10,000g for 10 mins, the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA equivalent was calculated [40]. Leaf sample (0.25 g) was homogenized in 2.5 ml of 1%

TCA. 1 ml, 10 mM KH_2PO_4 (pH = 7) phosphate buffer, and 1 ml, 1 M KI were added on 0.5 ml supernatant. The mixture of absorbance was determined at 390 nm. The value obtained from the mixture was compared with the graphic value. The graphic value obtained from the reading values of 50, 100, 200, 300, 400, 500, and 700 µl H_2O_2 standards [41]. Dried plant shoot and root samples were digested with a mixture of HNO_3-$HClO_4$ acids and analysed for the concentration of Pb, Cd, Cr and Zn by using atomic absorption spectrophotometer [42].

Statistical Analyses

One-way analysis of variance was conducted to explore differences in applications. Significant differences across applications were tested using Duncan's Multiple Range Test. The SPSS software was utilized in the analyses [43].

Result

Plant Growth

HA_1 and HA_2 applications in soil polluted with heavy metals did not cause a significant difference in shoot length, shoot dry weight, root length, and root fresh weight from the control application. On the other hand, HA_1 and HA_2 applications in soil polluted with heavy metals (PS) increased shoot fresh and dry weight, root fresh and dry weight in the plant compared to PS application (Table 2). Other chelate applications ($EDTA_1$, $EDTA_2$ and $EDTA_3$) in soil polluted with heavy metals decreased shoot length, number of leaves, shoot fresh and dry weight, and root length in rapeseed plant compared to the control and PS applications (Table 2).

Effect of EDTA and HA Applications on Phytoremediation Parameters (BCF,TF and TLF) in Rapeseed

The BCF in the shoot and root of the plant were calculated to predict the rate of heavy metal accumulation by rapeseed under different treatments (Table 3). In $EDTA_1$, $EDTA_2$, and $EDTA_3$ applications, BCF(shoot), BCF(root), TF and TLF values increased compared to PS application for Pb, Cd, Cr and Zn. TF and TLK values in EDTA applications increased compared with HA doses for Cd and Pb in the plant. However, TLF values in HA applications caused an increase compared with EDTA applications for Zn. TF values were higher than 1 for Pb, Cd and Zn. $BCF_{(shoot)}$ and $BCF_{(root)}$ values were higher than 1 in plants for Cd, Cr and Zn (Table 3). $BCF_{(shoot)}$ value was lower than 1 for Pb in HA applications. On the other hand, EDTA applications caused an increase in $BCF_{(shoot)}$ above 1 for Pb (Table 3).

Effects of EDTA and HA Applications on Antioxidative Activity in Rapeseed

CAT activity was decreased with HA_1 dose in soil polluted with heavy metals compared to the PS application. APX activity was decreased with HA_1 and HA_3 doses in soil polluted with heavy metals compared to the PS application. CAT activity was increased with $EDTA_2$ and $EDTA_3$ doses in soil polluted with heavy metals compared to PS application. APX activity was increased with $EDTA_1$, $EDTA_2$ and $EDTA_3$ doses in soil polluted with heavy metals compared to PS application (Fig. 1, Table 1).

HA_2 and HA_3 applications decreased MDA activity in the plant compared to PS applications. HA_1, HA_2 and HA_3 applications decreased H_2O_2 activity compared to PS applications. However, MDA activity increased in response to $EDTA_1$, $EDTA_2$ and $EDTA_3$ doses in soil

Table 2. The Effect of HA and EDTA applications on growth parameters of rapeseed in soil contaminated with Pb, Cd, Cr and Zn.

Applications	Shoot length (cm)	Number of leaves (per plant^{-1})	Shoot fresh weight (g pot^{-1})	Shoot dry weight (g pot^{-1})	Root length (cm)	Root fresh weight (g pot^{-1})	Root dry weight (g pot^{-1})
Control	18.12a*	5.06ab	5.22a	0.504a	12.33a	0.252a	0.055a
PS	17.69a	4.61bcd	3.98c	0.293b	10.06bc	0.151c	0.019c
PS+HA_1	17.75a	5.00ab	4.12b	0.401a	11.00ab	0.204b	0.037b
PS+HA_2	18.28a	4.56bc	4.39b	0.413a	10.81ab	0.219b	0.044b
PS+HA_3	14.81b	4.67abc	3.64c	0.316b	8.83cd	0.124c	0.027c
PS+$EDTA_1$	12.95c	4.22d	2.05d	0.187c	9.31bcd	0.128d	0.024c
PS+$EDTA_2$	9.89d	3.55e	1.65de	0.147cd	8.17d	0.124d	0.023c
PS+$EDTA_3$	9.06d	3.44e	1.24e	0.112e	8.64cd	0.123d	0.022c

Note. HA = Humic acid; EDTA = ethylenediaminetetraacetic acid; *Different letters in the same column indicate significant differences ($p<0.05$)

Table 3. The effects of HA and EDTA applications on BCF$_{(shoot)}$, BCF$_{(root)}$, TF, TLF in soil contaminated with Pb, Cd, Cr and Zn.

Pb	BCF (shoot)	BCF (root)	TF	TLF (shoot/root)
Control	0.770de*	2.66e	3.23 c	0.083d
PS	0.919d	7.43c	1.90 d	0.124c
PS+HA$_1$	0.532e	4.26d	1.23 e	0.126c
PS+HA$_2$	0.753de	9.13b	1.98 d	0.081d
PS+HA$_3$	0.63e	7.42c	1.99 d	0.084d
PS+EDTA$_1$	1.99c	8.41bc	4.12 b	0.236b
PS+EDTA$_2$	2.55b	11.19a	6.51 a	0.226b
PS+EDTA$_3$	3.66a	11.34a	6.60 a	0.323a
Cd	BCF (shoot)	BCF (root)	TF	TLF (shoot/root)
Control	7.11d*	9.39ab	3.07 d	0.65d
PS	6.80d	10.23ab	10.34 c	0.67d
PS+HA$_1$	8.90c	8.96b	7.41 c	0.86c
PS+HA$_2$	6.15d	8.98b	14.32 b	0.69cd
PS+HA$_3$	8.57c	6.72c	10.35 c	1.28a
PS+EDTA$_1$	13.59a	10.93ab	19.91a	1.27a
PS+EDTA$_2$	10.98b	9.86ab	20.63 a	1.13ab
PS+EDTA$_3$	10.73b	11.49a	16.16 b	1.04b
Cr	BCF (shoot)	BCF (root)	TF	TLF (shoot/root)
Control	7.49de*	176 d	0.12 f	0.048d
PS	12.22c	206bc	0.39 d	0.062c
PS+HA$_1$	7.05e	142d	0.25 e	0.052d
PS+HA$_2$	10.77c	203bc	0.42 cd	0.052d
PS+HA$_3$	10.51cd	192bc	0.41 d	0.054d
PS+EDTA$_1$	17.36b	232ab	0.50 b	0.075cd
PS+EDTA$_2$	19.37b	203 bc	0.48 bc	0.094b
PS+EDTA$_3$	25.23a	260 a	0.67 a	0.098a
Zn	BCF (shoot)	BCF (root)	TF	TLF (shoot/root)
Control	14.95c*	18.20d	0.51 e	0.53d
PS	10.31d	12.30d	2.51 c	0.847b
PS+HA$_1$	9.01d	8.25e	2.05 d	1.18a
PS+HA$_2$	9.96d	9.18e	2.31 cd	1.09a
PS+HA$_3$	9.27d	9.02e	2.28 c	1.05a
PS+EDTA$_1$	12.74c	23.38c	4.68 b	0.548c
PS+EDTA$_2$	19.14b	34.06b	7.31 a	0.562c
PS+EDTA$_3$	21.24a	36.34a	7.35 a	0.583c

Note. HA = Humic acid; EDTA = ethylenediamine tetraacetic acid; BCF= Bio-concentration factors; TF = transfer factor; TLF = translocation factor;* Different letters in the same column indicate significant differences ($p<0.05$).

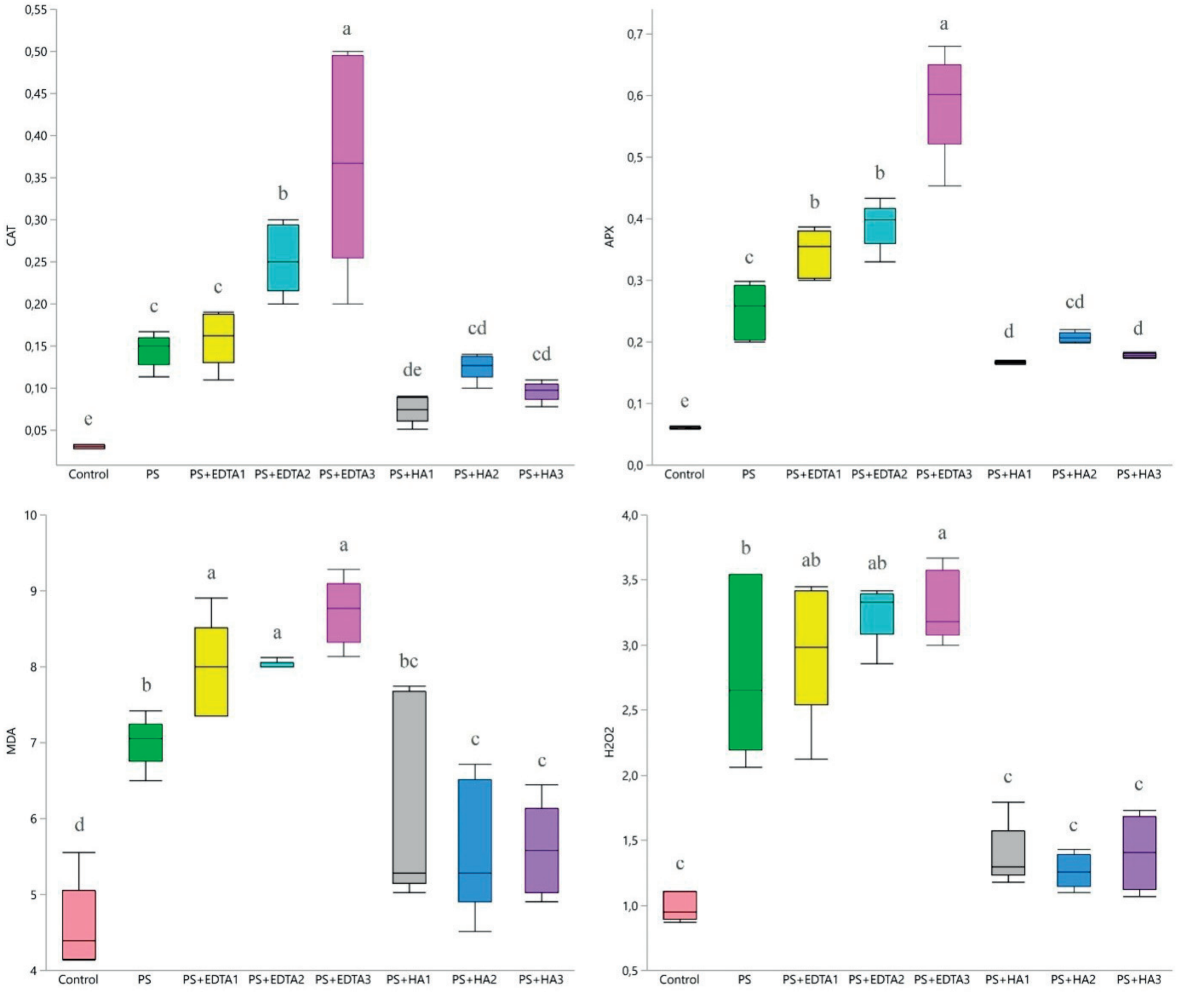

Fig. 1. The Effect of HA and EDTA applications on antioxidative activity in rapeseed.

polluted with heavy metals compared to PS application. H_2O_2 activity increased $EDTA_3$ application compared to PS application (Fig. 1; Supp. Table 1).

Discussion

Chelating agents preserve metal ions from inappropriate chemical reactions and develop their availability for plant roots. Different chelating agents are present as synthetic forms such as EDTA, ethylenediamine di-hydroxyphenyl acetic acid (EDDHA), and as natural forms such as amino acids, organic acids and phenolics. Each one has a separate task in nutrients and metal bioavailability for plant uptake [44]. In this study, we tried to examine the mechanisms by which HA and EDTA applications increase Cd, Cr, Zn and Pb uptake of rapeseed plant in contaminated soils. Our results showed that despite the high concentrations of heavy metals (Cd, Cr, Zn and Pb) in the contaminated soil, the rapeseed plant survived successfully. According to the results obtained in this study, HA treatments increased some growth characteristics by reducing the inhibitory effects of soil contaminated with Cd, Cr, Pb and Zn. On the other hand, EDTA treatments decreased the growth and development of the plant. Therefore, HA ameliorated heavy metal toxicity symptoms in rapeseed plant (Table 2). The prevailing notion asserts that HA increases the growth of the plant, and it can reduce the negative effects of toxicity by forming strong bonds with heavy metals [45]. At the highest curtailed irrigation level, HA caused an increase in plant chlorophyll, fresh weight, and K, Ca and Zn uptake. Because HA can trigger root branching and root hairs production resulting in more tolerance of plants under stressful conditions [46]. Previous studies found that foliar application of fulvic acid to wheat plants grown in a medium treated with increased levels of Cr significantly increased the number of leaves per plant as well as root and shoot lengths compared to plants treated with Cr alone [47, 48]. Another study identified that rapeseed plant caused an increase in heavy metal intake due to the increased level of EDTA applied to the soil contaminated with Pb, Cd and Co, but a decrease in the amount of root and shoot dry matter [49, 14]. In another study, EDTA applied in soils with high Cr content caused a decrease in the dry matter content of the rapeseed plant. Moreover, it was found that the Cr concentration in the plant increased. It was reported that the increase in the heavy metal concentration of the plant by EDTA was caused by the toxic effect of Cr, which prevents the uptake of some macro and micronutrient elements [50]. However, Habiba, et. al. [51] found that rapeseed (*Brassica napus* L.) with EDTA application increase plant growth, biomass, chlorophyll content, photosynthetic parameters under copper stress. It was found that *Brassica napus* L. has tolerance against copper stress. In a study by Guo, et al. [52] it was identified that there was a decrease in shoot fresh and dry weight, shoot and root length in all EDTA applications in the soils contaminated with heavy metals compared to controls. The decrease in shoot and root development in plants can be explained by exposure to EDTA, which reduces chlorophyll biosynthesis in plants. The reason for the reduced shoot development in chelates applications may be the result of plant uptake of the heavy metal concentration in the contaminated soils, exceeding the capacity of the plant's defense system [53-55].

The phytoremediation efficiency is highly dependent on the hyperaccumulator plant species used in the phytoremediation trial and the bioavailability of the metals [56]. The phytoremediation efficiency is highly dependent on the hyperaccumulator plant species used in the phytoremediation trial and the bioavailability of the metals [57]. In many studies, it has been stated that the rapeseed (*Brassica napus* L.) is an important hyper-accumulator plant that can be used in phytoremediation [58]. When the results of the previous studies are examined, Indian mustard showed the highest accumulation for Al, Cr, Mo and Se phytoextraction. Besides, sunflower for Cd, Ni, Pb and Zn and rapeseed for Cu performed the highest phytoextraction [59]. In addition, EDTA application in *Brassica napus* L. plant significantly increased the concentrations of DTPA extractable Cu, Ni, Pb, Cd, Co, Cr and Zn, while remarkably decreasing the concentrations of Mo, Se, Al and As [59]. The effect of HA on elemental solubility varies as a function of soil pH, organic structure, and the molecular weight of organo-elemental complexes [17, 60]. A high TLF value indicates that more heavy metals are carried from roots to shoots. A lower TLF value indicates that the plant accumulates more heavy metals in the roots [61]. The TF value is dependent on the plant species and heavy metals found in the soil. Shoots and roots that accumulate heavy metals with high TF can be easily removed from contaminated soils with the harvest [4, 61]. In this study, TLF value was found to be higher in rapeseed plants under HA application only in Zn contaminated soils compared to EDTA application. Besides, BCF, TF and TLF values were higher in the EDTA application than HA application for all heavy metals. BCF values of the roots were higher than BCF values in the shoots of the rapeseed for the EDTA applications in Cr, Zn and Pb polluted soils, indicating that EDTA induced accumulation of the heavy metals in the roots. Moreover, rapeseed as a species hinders the transportation of heavy metal content to higher parts of the plant. Similarly, a previous study also reported that heavy metals such as Zn, Cu, Pb and Cd accumulate more in the roots of and *Brassica juncea* L. and *Brassica napus* L. plants compared to the shoots of the plants with EDTA application [44, 62, 63]. It seems that EDTA chelate application increases the efficiency of the removal of heavy metals from the soil, as well as translocation efficiency of the plant's biomass [52, 54]. However, HA application decreased $BCF_{(root)}$ in *B. juncea* for Cd, Cu, Pb and Zn. However, EDTA application increased $BCF_{(root)}$ in all plants. Because soil with HA can enhance the degradation of organic contaminants, reduce toxicity and chelate more metals. Moreover, HA application increased microbial activity in the long run that was associated with the increase in heavy metal accumulation in plants [64]. Similarly, Gul et. al. [65] reported that EDTA and citric acid treatments supported Pb phytoremediation, but EDTA reduced plant biomass by 28.4%. Citric acid not only increased Pb uptake but also increased Pb accumulation efficiently without toxicity.

Antioxidant enzymes play important roles in creating resistance to ROS toxicity caused by potentially toxic metals in plants [23, 24, 38]. It was investigated that adding EDTA to the cultivation medium where 50 and 100 μM Pb was applied caused an increase in CAT and APX enzyme activities, but a decrease in MDA and H_2O_2 levels in *Brassica napus* L. [51, 66]. As a result of the study, it was reported that EDTA is especially healing in Pb stress in *Brassica napus* L. In another study, it was reported that citric acid application increased antioxidant enzymes of SOD, CAT, POD, and APX under low Cu levels. Moreover, *Brassica napus* can tolerate Cu stress to an extent by strengthening the plant defense system [51, 67]. In this study, we found that EDTA applications result in a heightened accumulation of heavy metals in plant organs. This caused a significant increase in antioxidative enzyme activity, MDA and H_2O_2 levels in concert with heightened plant stress. On the contrary, HA applications reduced heavy metal uptake that leads to a decrease in oxidative stress. This caused a significant decrease in antioxidative enzyme activity, MDA and H_2O_2 levels. Similarly, oxidative stress under heavy metal stress conditions could be considerably reduced by amino chelate treatment [68]. Amino chelates mainly due to their amino acid content can reduce lipid peroxidation that caused by heavy metal stress [44]. Gallic acid, under cold stress conditions, improved the oxidative stress in soybeans [69].

Conclusion

As a result of the study, it is identified that HA reduced heavy metal toxicity by supporting plants, grown in polluted soil with heavy metals. Moreover, HA chelate had a healing effect on plant development and organic defence mechanisms in multi-polluted soils. At the same time, HA was more effective than EDTA in phytoremediation. HA can increase phytoremediation in polluted soils as it improves plant growth and oxidative stress due to its organic nature. This study results supported the literature and provided new insights about EDTA and HA applications that further studies are needed.

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Conflict of Interest

The authors declare no conflict of interest.

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