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Research

The Effect of Humic Acid on Plant Growth, Phytoremediation and Oxidative Stress in Rapeseed (*Brassica napus* L.) Grown Under Heavy Metal Stress

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Abstract: The aim of this study was to investigate the effects of humic acid (HA) applications on rapeseed (Brassica napus L.) growth, heavy metal uptake, bioconcentration factor (BCF), translocation factor (TF), tolerance index (TI), catalase (CAT), ascorbate peroxidase (APX) enzyme activities and hydrogen peroxide (H2O2) content in polluted soil with lead (Pb), chromium (Cr), cadmium (Cd), and zinc (Zn). Three doses of HA (Control, HA1:500 mg kg-1, HA2:1000 mg kg-1, HA3:2000 mg kg-1) were applied in pots. HA1, HA2, and HA3 applications increased plant growth parameters compared to polluted soil. Compared to the control, HA applications in polluted soil increased the Pb, Cr, Cd, and Zn concentrations in the plant. However, HA applications in polluted soil significantly decreased the heavy metal content in roots and shoots of the plant compared to polluted soil. BCF in both roots and shoots of the plants were greater than 1 for Pb, Cr, Cd, and Zn. However, specifically HA2 application decreased the shoot and root BCF values in polluted soil. TF was smaller than 1 in Pb, Cr, Cd, and Zn in polluted soil. On the other hand, HA applications for Cd increased TF values. Shoot TI decreased 17.37 %, and root TI decreased 9.09% in polluted soil. CAT and APX enzyme activities and H2O2 increased significantly in polluted soil. However, HA applications decreased CAT and APX enzyme activities and H2O2 content in rapeseed. It is concluded that HA application in Pb, Cr, Cd, and Zn polluted soil has a remedial effect on the development of rapeseed by reducing heavy metal content and oxidative stress.

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1. Introduction

The quality of life on earth and the quality of the environment depend on each other. With the increase of urbanization and the development of industry, the use of human-oriented heavy metals has increased. Heavy metals are released into the environment in significant quantities because of industrial activities such as mining, energy and fuel production, and excessive use of pesticides and fertilizer (Okcu et al., 2009). Especially in developed countries, this situation emerges as a more serious problem with each passing day. The most important effect of soil pollution in terms of environmental health is the transfer of pollutants in the soil to the human body due to plants consumed directly or as food for animals that feed on these plants (Singh and Kalamdhad, 2011). Some plants can tolerate heavy metals that can be toxic to most organisms. Such plants are called “hyperaccumulator plants”. It is reported that there are approximately 400 plant species that accumulate metal in their parts. The dominant families with this feature are Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae. The Brassicaceae family is the largest family with this feature, with 11 genera and 87 species (Özbek, 2015). Hyperaccumulator plants can absorb one or more heavy metals from the soil. Hyperaccumulator plants can grow easily in exceeding levels of heavy metal contaminated soils and can accumulate metal ions in their different organs (Kürşat, 1999; Rascioa and Navari-Izzo, 2011).

Humic acid (HA) is a component of mixed organic matters formed as a by-product of a certain decomposition process of plants, animals, and microbial substances. HA is an organic compound soluble in alkaline medium and insoluble in acidic medium. Humic-based substances consist of functional groups, namely carboxyl, alcoholic and phenolic hydroxyl, carbonyl, and methoxyl groups containing oxygen (Cozzolino et al. 2016). The main heteroatoms in humic substances are oxygen. There are carboxylic (COOH), phenolic-alcoholic (OH), ketonic-quinoid (C=O), and OCH3 (ether and ester) functional groups within humic substances (Kwiatkowska-Malina, 2018). The molecular weight of HA is 50000 g/mol, and the surface area is determined as 2000 m^2/g. HA cation exchange capacity varies between 500 and 1200 me/100g (Alpay, 2013). In the presence of metals, HA has chelating properties. In other words, it can complex well with heavy metals. When HA is decomposed at high pH values, complex reactions and chelation occur between metals and HA molecules. These organic macro molecules also increase the solubility of heavy metals, which significantly affects their biological availability and transportation (Lagier et al., 2000; Alım, 2020). HA interact not only with metals but also with toxic substances in terms of environmental pollution, such as many polluting hydrocarbons, pesticides, and oil. It is asserted that HA forms a strong compound with heavy metals, reducing the stress effect of heavy metals on plant development (Özkay et al., 2016). It has been found that HA increases the intake of nutrients because of the development of the root zone (Çimrin et al., 2001; Gülser and Ayaş, 2016). Rastghalam et al., (2011) investigated the phytoextraction effect of HA in the experiment soils in which various levels of lead (Pb) were applied in their study with rapeseed plants. They reported that HA application was associated with the increase in Pb accumulation in roots and the transport to the shoots in concert with the levels of Pb applications to soils.

The toxic effects of heavy metals exert influence on both physiological and morphological characteristics of plants and cause oxidative stress. The excessive increase of reactive oxygen radicals in plant cells, which is the main cause of oxidative stress, may lead to the deterioration of metabolic functions. However, antioxidative enzymes, which are the secondary defense mechanism in plant cells, may reduce the toxic effects of oxidative stress damage in the plant. (Hamilton et al., 2012).

The present study aimed to determine the effects of applied HA levels on plant growth, heavy metal concentrations, phytoremediation properties, and antioxidative enzyme activity of rapeseed (*Brassica napus* L.) plant grown in soil polluted with Pb, Cr, Cd, and Zn. It was predicted that HA would have a healthy effect on growth and development as well as antioxidant defense mechanisms of rapeseed.

2. Material and Methods

The experiment soil was taken from the study areas of the Faculty of Agriculture of Van Yüzüncü Yıl University. This soil is characterized by low nitrogen, medium calcareous, low organic matter, alkaline pH, and without salt (Table1). For total nitrogen N, according to the Kjeldahl method, the limit values specified are % 0.045-0.090, degree: low nitrogen (Kacar, 1994). For lime ($CaCO_3$), according to the Scheibler calcimeter method, the limit values are % 5-15, degree: medium calcareous (Kacar, 1994). For O.M., according to Walkley- Black wet burning method, the limit values are % 1-2, degree: low organic matter (Müftüoğlu et al., 2014). Salt and pH were measured in 1/2.5 soil-water mixture, with the limit values for pH: >8.5, degree: alkaline, the limit values for salt: <4 dS m^{-1}, degree: without salt (Müftüoğlu et al., 2014). DTPA-Fe, Zn, Mn are at low level DTPA-Cu is at sufficient level (Müftüoğlu et al., 2014). Total Cd, Pb, Zn, and Cr are under toxic levels in the soil. The toxic level for Cd is 1 mg kg^{-1}. Toxic levels for Pb and Cr are100 mg kg^{-1} Toxic level for Zn is 150 mg kg^{-1} (Schachtschabel et al., 1993).

Table1. Experimental soil and humic acid properties

	Experimental Soil	Humic Acid
Texture	Sandy Loam	
pH (1/2.5)	8.15	8-10
Salt, dS m^{-1}	0.35	
Lime, %	6.6	
Organic Material, %	1.02	25
Total N, %	0.056	
Total humic acid+fulvic acid %		65
Extractable with DTPA		
mg kg^{-1}		
Fe	0.90	
Cu	1.40	
Mn	1.24	
Cr	0.06	
Zn	0.60	
Cd	0.08	
Pb	0.30	
Total heavy metal		
mg kg^{-1}		
Zn	45.13	
Cd	0.65	
Pb	9.03	
Cr	95.0	

2.1. Pot experiment

Using the method used by Turan and Esringü (2007) for potting experiments. In the experiment, heavy metals in doses of 100 mg kg^{-1} Cr as chromium nitrate (Cr $(NO_3)_3$), 100 mg kg^{-1} Cd as cadmium sulphate ($CdSO_4.8H_2O$), and 100 mg kg^{-1} Pb as lead nitrate (Pb $(NO_3)_2$ and 250 mg kg^{-1} Zn as zinc sulphate ($ZnSO_4.7H_2O$) were applied to the pots that each was 2.5 kg. The soil polluted with heavy metals added to the potting soil as the liquid was left to incubate for a month. Then, the pot experiment was carried out in the growth chamber. Eight of the rapeseed seeds were planted in each pot. Thinning was done immediately after germination so that three plants were left in each pot. The temperature was adjusted to 20 ± 2°C since rapeseed is a cool weather crop. Chemical fertilizers of 80 mg kg^{-1} phosphorus (P) as triple superphosphate (TSP), 200 mg kg^{-1} nitrogen (N) as ammonium nitrate (NH_4NO_3), and 50 mg kg^{-1} potassium (K) as potassium sulphate (K_2SO_4) were added. A completely randomized experimental design with the three-replication trial was implemented in the trial. Five applications of HA were as follows follows: 1-Control, 2- Polluted soil (PS) with heavy metals, 3- PS+HA_1 (500 mg kg^{-1}), 4- PS +HA_2 (1000 mg kg^{-1}), 5- PS +HA_3 (2000 mg kg^{-1}).

2.2. Chemical and physical analysis of soil

Soil samples were taken from a depth of 0-30 cm. Soil samples were air-dried in a shaded area and passed through a 2 mm sieve. Before filling the pots, the soil was mixed with a liquid-heavy metal mixture. Then the soil was left to incubate for one month. In the soil samples taken before and after the plant harvest, respectively, the soil texture was determined by the Bouyoucos hydrometer method (Bouyoucos, 1951). The pH was measured using in 1:2.5 soil-water mixture (Jackson, 1958). Lime content was determined using Scheibler calcimeter (Hızalan and Ünal, 1966). Soil organic matter was determined using the Walkley Black method (Walkley, 1947). Total N was measured using the Kjeldahl method (Kacar, 1994). The extractable Pb, Cr, Cd and Zn concentrations were determined by the DTPA method (Lindsay and Norvell, 1978). The total Pb, Cr, Cd, and Zn in the soil were determined according to Khan and Frankland (1983).

2.3. Phytoremediation parameters

Bioconcentration factor (BCF), Translocation factor (TF), and Tolerance Index (TI) parameters were calculated based on studies on phytoremediation. BCF and TF (Esringü et al. 2014) were calculated. TI was calculated (Çifçi, 2020) as follows:

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

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

$$TI\ (\%) = \frac{[(\text{Metal Applied Plant Growth Parameters})]}{[(\text{Control Plant Growth Parameters})]} \times 100$$

2.4. Antioxidative enzymes, H_2O_2, and heavy metal analyses in plant

Enzymatic measurements were carried out at 0-4^{0}C. The supernatant was used as a crude enzyme extract for CAT enzyme analyzes. 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 (Çakmak et al., 1993). APX (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 (Nakano and Asada, 1981). 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 (Potassium iodide) were added on 0.5 ml supernatant. The mixture of absorbance was determined at 390 nm (Velikova et al., 2000). Dried plant shoot and root samples were digested with a mixture of HNO_3-$HClO_4$ acids and analyzed for the concentration of Pb, Cd, Cr, and Zn (İbrikçi et al., 1994).

2.5. Statistical analysis

The descriptive statistics were computed for experiment soil and humic acid properties. One-way analysis of variance was performed to compare growth parameters, heavy metal content in the plant, phytoremediation parameters, and antioxidative activity. Significant differences among applications were determined by Duncan's Multiple Range Tests using the 13.0 SPSS software package (Düzgüneş et al., 1987).

3. Results

3.1. Plant growth

The effect of HA applications on growth parameters is given in Table 2 as variance analysis results. The HA applications were statistically significant at all growth parameters (P <0.01). In the study conducted with rapeseed plants, a significant decrease was found in plant height, plant fresh weight, plant dry weight, leaf number, root length, root fresh, and dry weight of rapeseed grown in PS application compared to the control application. However, PS+HA_1, PS+HA_2, and PS+HA_3 caused a greater increase in shoot length, shoot fresh and dry weight, the number of leaves, root length, and root fresh and dry weight compared to the PS application (Table 3).

Table 2. The results of variance analysis on the effects of HA applications on the growth parameters in polluted soil

Variation Source	F(*value*)	P(*significant*)
Shoot length	72.38	0.000
Shoot fresh weight	53.63	0.000
Shoot dry weight	47.76	0.000
Leaf of number	15.38	0.000
Root length	16.37	0.000
Root fresh weight	55.31	0.000
Root dry weight	32.00	0.000

Table 3. The Effect of HA applications on the growth parameters of the rapeseed plants in soil polluted with Pb, Cd, Cr, and Zn

Applications	Shoot **length (cm)**	Shoot fresh weight**(g pot^{-1})**	Shoot dry weight **(g pot^{-1})**	Leaf of number **(per plant^{-1})**	Root **length (cm)**	Root fresh weight **(g pot^{-1})**	Root dry weight **(g pot^{-1})**
Control	22.34 ± 2.51**a***	9.49 ±2.31**a**	0.90 ±0.35**a**	5.61±0.61**a**	10.61±1.9 **a**	0.57±0.16**a**	0.087± 0.04**a**
PS	7.70 ±0.93**c**	1.58 ± 0.15**d**	0.04± 0.01**d**	3.44 ±1.38**b**	4.76±2.06 **d**	0.048± 0.02**c**	0.009± 0.01**c**
PS + HA$_1$	17.70 ± 2.62**b**	4.45±0.58**c**	0.41 ±0.05 **c**	6.17±1.47 **a**	7.22± 3.40**c**	0.19±0.13**b**	0.033± 0.02**b**
PS + HA$_2$	17.19 ±2.18**b**	4.66±1.74**bc**	0.48±0.05**bc**	6.17±1.47**a**	8.76±1.83 **b**	0.21±0.08 **b**	0.032 ±0.02**b**
PS + HA$_3$	18.31±4.18**b**	6.09 ±2.28 **b**	0.61±0.25 **b**	5.78±1.06**a**	8.90± 1.87**b**	0.24±0.10**b**	0.034 ±0.02**b**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn (p<0.05), Polluted Soil:PS.

3.2. Effects of HA applications on heavy metal content in shoot and root of rapeseed (*Brassica napus* L) plant

The effect of HA applications on heavy metal content in shoot and root of rapeseed plants are given in Table 4 as variance analysis results. The HA applications were statistically significant at heavy metal content of shoot and root in the plant ($P < 0.01$). The highest concentrations of Pb, Cr, Cd, and Zn were determined in PS application in both roots and shoot parts of the rapeseed plant. However, PS+HA$_1$, PS+HA$_2$, PS+HA$_3$ applications caused a statistically significant decrease in Pb, Cr, Cd, and Zn contents in both roots and shoots of rapeseed compared to the PS application (Table 5 and Figure 1,2).

Table 4. The results of variance analysis on the effects of HA applications on heavy metal content in polluted soil

Variation Source	F(*value*)	P(*significant*)
Shoot(Pb)	157	0.000
Root(Pb)	784	0.000
Shoot(Cr)	692	0.000
Root(Cr)	200	0.000
Shoot(Cd)	1020	0.000
Root(Cd)	71.41	0.000
Shoot(Zn)	1134	0.000
Root(Zn)	120	0.000

Table 5. The Effect of HA applications on the heavy metal contents in the shoots and the roots of the rapeseed plants in soil polluted with Pb, Cd, Cr, and Zn (mg kg-1)

Applications	Pb		Cr		Cd		Zn	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	3.81± 0.41c*	27.20±4.78d	0.66 ±0.11d	10.70± 1.19d	0.22±0.04d	1.73±0.04d	10.43±0.84 e	13.01±0.88e
PS	113± 18.69a	280 ±8.70a	14.57±1.0a	140± 8.02a	112±3.21a	439± 71.64a	117± 3.91a	263±26.09a
PS + HA_1	9.38±0.69b	174±9.27b	3.05± 0.19b	74.02±12.58b	103±3.01b	278 ±40.06b	72.51±1.96b	236±33.b
PS + HA_2	8.90± 0.27b	141±3.96c	1.88 ±0.20c	40.57±4.32c	73.92±4.43d	135 ±17.62c	67.59±2.82c	146±16.0d
PS + HA_3	8.93±0.35b	150 ±7.67c	2.28 ±0.25c	69.65±6.67b	84.07±3.00c	258±48.48b	56.29±2.02d	205± 4.32c

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn (p<0.05), Polluted Soil: PS.

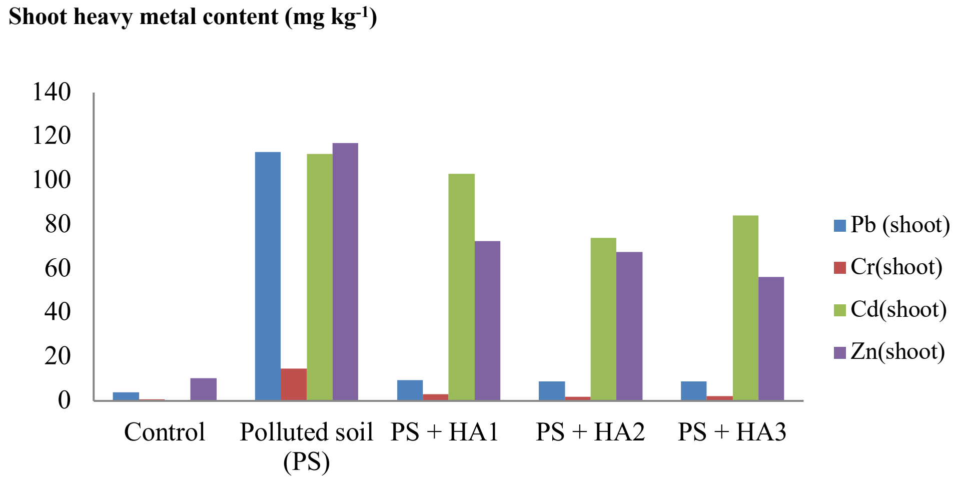

Figure 1. The effect of HA applications on the heavy metal contents in shoots of rapeseed plants in polluted soil.

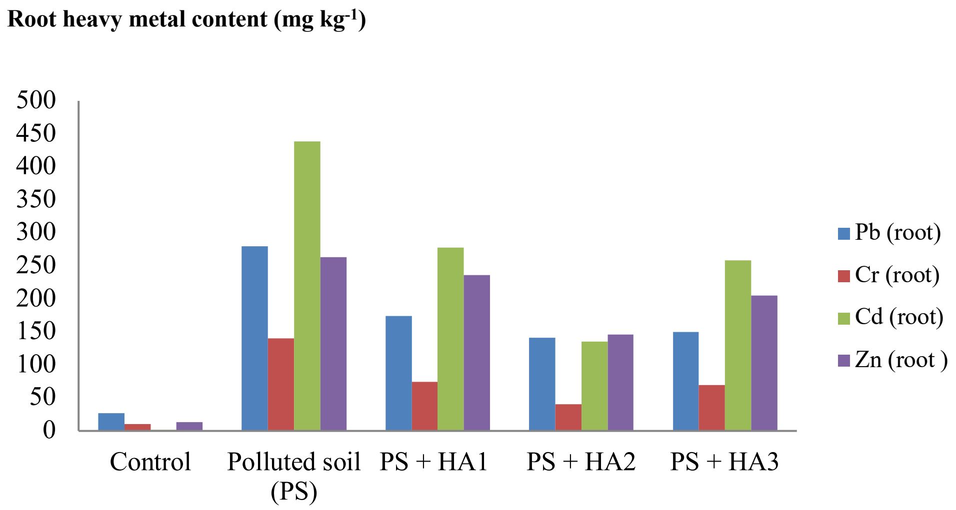

Figure 2. The effect of HA applications on heavy metal content in the root of rapeseed in polluted soil.

3.3. The Effects of HA applications on phytoremediation parameters (BCF, TF, and TI %) of rapeseed (*Brassica napus* L) plant

The effects of HA applications on phytoremediation parameters of rapeseed plants are given in Table 6 as variance analysis results. The HA applications were statistically significant at all phytoremediation parameters (P <0.01). In the present study conducted with rapeseed plants, shoot and root BCF values were higher than 1 for Pb, Cr, Cd, and Zn. Nevertheless, PS+HA_1, PS+HA_2, and

PS+HA$_3$ applications caused a decrease in the shoot BCF values for Pb, Cr, Cd, and Zn compared to the PS application. PS+HA$_1$ and PS+HA$_2$ applications caused a decrease in the root BCF values for Pb, Cr, Cd and Zn compared to the PS application. TF values were lower than 1 for Pb, Cr, Cd, and Zn. However, PS+HA$_1$, and PS+HA$_2$ applications for Cd caused an increase in the TF values compared to the PS application (Table 7). In this case, it was determined that both HA applications decreased Pb, Cr, Cd, and Zn accumulation and increased the transport of Cd to the upper organs in the rapeseed plant. While shoot and root TI values were 100 % in the control application, shoot and root TI values were 17.37 % and 9.09 % in PS application. In comparison to PS applications, PS+HA$_1$, PS+HA$_2$, and PS+HA$_3$ applications caused increases in the shoot and root TI values (Table 8).

Table 6. The results of variance analysis on the effects of HA applications on phytoremediation parameters in polluted soil

Variation Source	F(*value*)	P(*significant*)
BCF$_{(shoot)}$ Pb	86.14	0.000
BCF$_{(shoot)}$ Cr	689	0.000
BCF$_{(shoot)}$ Cd	125	0.000
BCF$_{(shoot)}$ Zn	285	0.000
BCF$_{(root)}$ Pb	343	0.000
BCF$_{(root)}$ Cr	56.14	0.000
BCF$_{(root)}$ Cd	24.65	0.000
BCF$_{(root)}$ Zn	23.93	0.000
TLF (Pb)	107	0.000
TLF (Cr)	34.73	0.000
TLF (Cd)	39.03	0.000
TLF (Zn)	21.86	0.000
TI $_{(shoot)}$	43.21	0.000
TI $_{(root)}$	60,626	0.000

Table 7. The Effect of HA applications on the phytoremediation parameters (BCF and TF) of the rapeseed plants in soil polluted with Pb, Cr, Cd, and Zn

	Pb	**Cr**	**Cd**	**Zn**
Applications	**BCF$_{shoot}$**	**BCF $_{shoot}$**	**BCF $_{shoot}$**	**BCF $_{shoot}$**
Control	0.78± 0.26**b***	7.49±0.33**d**	1.84±0.33**c**	14.95±1.28**a**
PS	3.26±0.59**a**	77.62± 3.81**a**	4.29±0.11**a**	6.66± 0.18**b**
PS + HA$_1$	0.41±0.01**c**	22.45±0.93**b**	4.22±0.12**a**	4.38± 0.13**c**
PS + HA$_2$	0.42± 0.01**c**	13.51±1.86**c**	3.25±0.29**b**	4.55±0.41**c**
PS + HA$_3$	0.76±0.04**b**	21.57± 3.13**b**	4.47±0.15**a**	4.09± 0.04**c**
	Pb	**Cr**	**Cd**	**Zn**
Applications	**BCF(root)**	**BCF (root)**	**BCF (root)**	**BCF (root)**
Control	2.66±0.31**d***	160±17.36**d**	9.39±0.12**b**	18.21±0.67**a**
PS	8.10±0.52**b**	748±96.55**a**	16.75±2.80**a**	15.04±1.55**b**
PS + HA$_1$	7.61±0.43**b**	547±109**b**	11.42±1.55**bc**	14.30±2.10**b**
PS + HA$_2$	6.70±0.34**c**	289±17.28**c**	5.92±0.68**d**	9.81±1.37**c**
PS + HA$_3$	13.63±0.51**a**	659±74.01**a**	13.70±2.55**b**	14.91±0.57**b**
	Pb	**Cr**	**Cd**	**Zn**
Applications	**TF**	**TF**	**TF**	**TF**
Control	0.14±0.02**b***	0.062±0.02**b**	0.13±0.02**c**	0.80±0.08**a**
PS	0.41±0.07**a**	0.104±0.01**a**	0.26±0.05**c**	0.45±0.05**b**
PS + HA$_1$	0.054±0.001**b**	0.043±0.01**c**	0.37± 0.05**b**	0.18±0.04**c**
PS + HA$_2$	0.064±0.001**b**	0.047±0.01**c**	0.56±0.08**a**	0.47±0.05**b**
PS + HA$_3$	0.058±0.001**b**	0.033±0.01**c**	0.34±0.06**bc**	0.28±0.01**c**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn ($p<0.05$), Polluted Soil: PS.

Table 8. The Effects of HA applications on phytoremediation parameter (TI) of rapeseed in soil polluted with Pb, Cr, Cd, and Zn (%)

Applications	TI (shoot)	TI (root)
Control	100±0.001**a***	100±0.001 **a***
PS	17.37±3.73**d**	9.09±4.72 **c**
PS + HA$_1$	49.21±12.92**c**	37.92±25.21 **b**
PS + HA$_2$	52.80±25.89**c**	40.58±21.24**b**
PS + HA$_3$	68.15±32.16**b**	44.19±21.24**b**

*a,b,c,d,e: Statistically significant mean differences are indicated with different letters in the same column ($p<0.05$), TI: Calculated according to the fresh weight of the plant. Polluted Soil: PS

3.4. The Effects of HA applications on antioxidant activity of rapeseed (*Brassica napus* L) plant

The effects of HA applications on the antioxidant activity of rapeseed plants are given in Table 9 as variance analysis results. The HA applications were statistically significant at CAT, APX enzyme activities, and H_2O_2 content ($P<0.01$). In the study conducted with rapeseed plants, the CAT and APX enzyme activities from the antioxidative enzymes and H_2O_2 content in PS application were higher than in the control application. However, PS+HA$_1$, PS+HA$_2$, and PS+HA$_3$ applications caused a decrease in CAT and APX enzyme activities and H_2O_2 content compared to the PS application (Table 10). Accordingly, the application of HA to the heavy metal contaminated soils resulted in a decrease in oxidative stress.

Table 9. The results of variance analysis on the effects of HA applications on antioxidant activity of rapeseed in polluted soil

Variation Source	F(*value*)	P(*significant*)
CAT	90,971	0.000
APX	49,465	0.000
H_2O_2	92,801	0.000

Table 10. The Effects of HA applications on antioxidative activity in rapeseed in soil polluted with Pb, Cr, Cd, and Zn

Applications	CAT(mmolg^{-1}FWMin^{-1})	APX(mmolg-^{1}FW Min^{-1})	H_2O_2(μmol g^{-1} FW)
Control	0.0088±0.001**d***	3.03±0.33**b**	1.58±0.14**c**
PS	0.044±0.001**a**	8.43±1.85**a**	9.49±1.31**a**
PS + HA$_1$	0.026±0.001**b**	1.97±0.84**c**	3.91±0.47**b**
PS + HA$_2$	0.031±0.001**b**	2.06±0.21**b**	4.00±0.10**b**
PS + HA$_3$	0.024±0.001**b**	2.83±0.38**b**	4.15±0.88**b**

*a,b,c,d,e:Statisticallysignificantmeandifferencesareindicatedwithdifferentletters in thesamecolumn ($p<0.05$), Polluted Soil: PS.

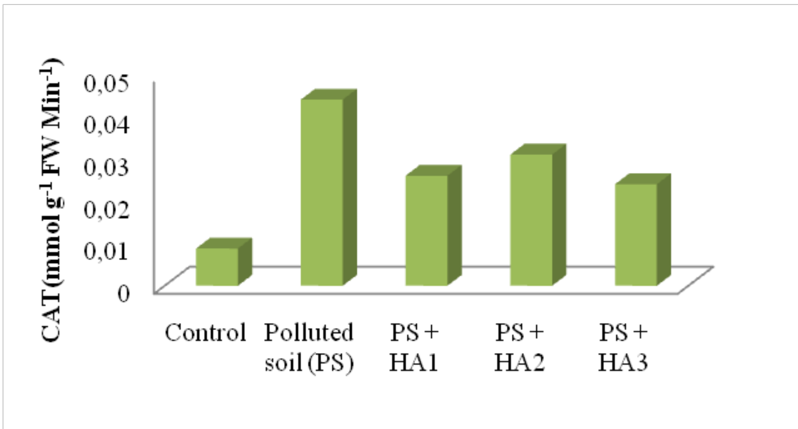

Figure 3. The effects of HA applications on CAT enzyme activities in the rapeseed plants in polluted soil.

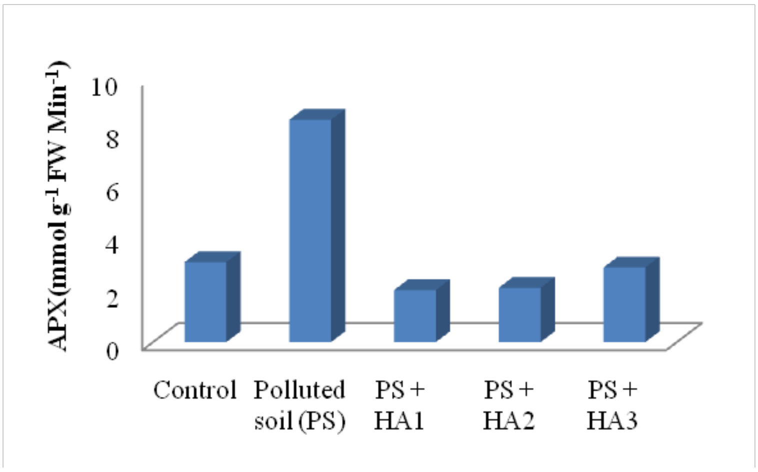

Figure 4. The effects of HA applications on APX enzyme activities in the rapeseed plants in polluted soil.

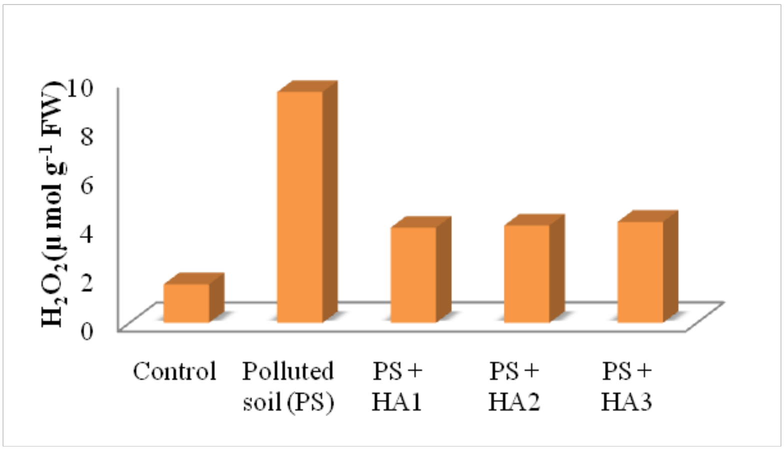

Figure 5. The effects of HA applications on H_2O_2 contents in the rapeseed plants in polluted soil.

According to all these results, HA applications increased some growth characteristics by reducing the inhibitory effects of soil contaminated with Cd, Cr, Pb, and Zn. In addition, HA decreased the effect of oxidative stress due to the decrease of heavy metal concentration in the plant.

4. Discussion

As a part of humus, when HA is added to the soil, it affects the availability of heavy metals in the soil to the plant. This is because it has hydroxyl, carboxylic (COOH), phenolic-alcoholic (OH), ketonic-quinoid (C=O), and OCH3 (ether and ester) functional groups (Nardi et al., 2009). HA affects heavy metal uptake by plants. The addition of HA increases the amount of soil organic matter that changes soil properties, cation exchange capacity, and pH. This change affects the mobility of heavy metals in the soil and affects their uptake by plants (Xu et al., 2018). In the present study carried out with rapeseed plants, humic levels applied to soil contaminated with Pb, Cr, Cd, and Zn caused increases in shoot length**,** shoot fresh and dry weight, leaf number, root length, root fresh and dry weight (Table 3). HA application to soil polluted with Pb, Cr, Cd, and Zn caused decreases in heavy metal content in both root and shoot parts of the rapeseed plants compared to alone polluted soil (Table 5). HA application has been found to be associated with cadmium uptake in tobacco plants when applied to Cd in soil (Evangelou, 2004). Fulvic acid is another component of the humus. Fulvic acid application, either foliar or non-foliar, inhibited Cr uptake in the wheat plant, causing an increase in the plant's biomass and photosynthetic pigments (Ali et al., 2015; Ali et al., 2018).

Shoot and root BCF values in rapeseed plants, which is one of the hyperaccumulator plants, were found as follows: Cr > Zn > Cd>Pb in Pb, Cr, Cd, and Zn polluted soils. Shoot and root BCF values were greater than 1. Zayed et al. (1998) suggested that metal-accumulating plants should have shoot and root BCF values were greater than 1. However, in the present study, it was determined that HA applications caused a decrease in shoot and root BCF values in rapeseed. TF values were lower than 1 for Pb, Cr, Cd, and Zn. This indicates that rapeseed accumulates heavy metals mostly in the roots, and their transport to the upper organs is low. However, HA applications for Cd caused an increase in the TF values. In a similar study, Pandey (2013) found BCF >1 and TF<1 in the roots of wild castor oil plants. In the present study, the TF values were determined as Zn > Cd > Pb > Cr. In another study, Turan and Esringü (2007) determined TF values in *Brassica Napus* L. as Zn > Cu > Cd > Pb in heavy metal contaminated soils. In our study shoot, TI decreased by 17.37 %, and root TI decreased by 9.09 % in soil polluted with Pb, Cr, Cd, and Zn of the rapeseed plant compared to the control application**.** However, HA application caused an increase in shoot and root TI values (Table 8). In another study conducted with wild castor oil plants in multi-contaminated soil, the TI was lower than the application of EDDS and zeolite to the soil, but the application of nano zeolite caused an increase in TI (Çiftçi, 2020). Similarly, biochar and hydrothermally treated coal gangue (HTCG) applied to rapeseed plant in copper mine tailings resulted in the lowest transfer rate (TR), the lowest root and shoot BCF values, and the lowest TF for Cu, Cd, Cr, Ni, Pb, and Zn compared to the application of (HTCG) together. In the improvement study by rapeseed in the copper mine, it was stated that the combined application of biochar and hydrothermally treated coal gangue reduces the bioavailability of heavy metals (Munir et al., 2020).

In the current study, the CAT and APX activities of the antioxidative enzymes and the H_2O_2 content as a free radical formed as a result of oxidative stress increased significantly in the rapeseed plant compared to the control. However, in comparison to polluted soil with heavy metals, PS+HA_1, PS+HA_2, and PS+HA_3 applications caused a significant decrease in CAT and APX enzyme activities as well as H_2O_2 content (Table 10 and Figures 3,4,5). Antioxidative enzymes are released to reduce the effects of radicals formed in response to oxidative stress. Another building block of humus is fulvic acid. In another study, wastewater application increased the Cr concentration in the wheat plant. On the other hand, fulvic acid application caused a decrease in H_2O_2 levels caused by Cr toxicity in the wheat plant (Ali et al., 2015; Ali et al., 2018). Garcia et al. (2016) stated that HA promotes antioxidative enzyme activity against radicals under heavy metal stress conditions. An aquaculture study conducted by Dobbss et al. (2018) showed that HA application reduces iron uptake under high iron toxicity conditions. However, when HA was treated together with iron, a decrease in antioxidant enzymes was detected compared to the treatments separately.

5. Conclusion

HA has a significant effect on the mobility of heavy metals when applied to soil polluted with heavy metals. This situation significantly affects the heavy metal content of the plant. HA can increase plant development and growth by affecting the soil properties in the growth environment and by influencing plant nutrient availability. We concluded that HA had a positive effect on rapeseed growth, HA has hyperaccumulator properties in soil contaminated with multiple heavy metals thereby reducing the heavy metal uptake and oxidative stress in the plant. HA can increase phytoremediation in polluted soils as it improves plant growth and oxidative stress due to its organic nature.

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