Nutritional Relationships and Accumulation Capacity of Broccoli (*Brassica Oleracea* var. Italica) Grown Under the Stress Caused by Some Heavy Metals Seen in Agricultural Areas

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Abstract

Agricultural soils lose their properties as a result of pollution caused by traffic, industry, agricultural activities, and urban activities. Among various techniques for the remediation of agricultural soils, phytoremediation is the most cost effective and applicable technique. Phyto extraction technique of phytoremediation has been applied with broccoli (*Brassica oleracea* var. italica) in this study to remediate some heavy metals (Cd, Cr, and Pb). The study was carried out in controlled conditions in pots according to randomized blocks design using three replicates. Cadmium was applied as only cadmium (30 mg/kg), and together with a chelator as Cd (30 mg/kg) + EDTA, in triplicates. Chromium was applied as only chromium (10 mg/kg), and together with a chelator as Cr (10 mg/kg) + EDTA. Similarly, lead was applied as only lead (100 mg/kg), and together with a chelator as Pb (100 mg/kg) + EDTA. After 30 days of incubation, one broccoli was cultivated in each pot. Plants were harvested after 2 months of experimental period. Agro-morphological traits of the plants were measured. Some macro and micro nutrient elements and Cd, Cr and Pb elemental analyses were carried out from root and shoot of the plants. Among the agro-morphological traits, plant wet weight was the highest in lead applied pots. This was statistically significant at 5% and they fell into different groups with control pots according to Duncan test. The highest root and shoot dry weights were detected in lead and chromium applied pots. Changes in chlorophyll contents were found to be statistically insignificant. The highest lead, cadmium and chromium contents in the body of broccoli (*Brassica oleracea* var. italica) were found to be 14.0 mg/kg, 6.67 mg/kg and, 5.17 mg/kg, respectively, in the chelated applications. The order of accumulation in the root was Cr>Pb>Cd. The results revealed that this plant played a role in the remediation of lead>cadmium>chromium as a hyper accumulator plant, and broccoli grown in neutral soils could be used as a hyper accumulator plant in the phytoremediation technology.

Introduction

Pollution of soil and water sources by toxic heavy metals usually occur as a result of human activities. Technologies for cleaning up soils contaminated by heavy metals require a big capital (Gupta et al. 2013). Phytoremediation appears as an economical new technology which can be practically applied for the elimination of problems caused by pollutants. Phytoremediation has been derived from the words “phyto” which means plant, and “remediation” which means cleaning up; it entered the terminology in 1991 and is defined as “bioremediation”, “botanical remediation”, and “green remediation”. Phytoremediation is an easily applied technology to clean up the environment whose basis is formed by accumulator/hyper accumulator plants (Sadowsky 1999; EPA 2000). Hyper accumulator plants are able to accumulate high number of pollutants in their bodies and can accumulate 10-500 times more pollutants compared to other plants (Ow 1996; Adiloğlu et al. 2016; Adiloğlu 2018; Adiloğlu and Pamay 2021).

The heavy metal cadmium (Cd) is a serious pollutant that affects human health. It can be found in different forms depending on the chemical interaction in soil, bioavailability, variability and toxicity (Jinadasa et al. 2016; Tanget al. 2016). Cadmium can enter the soil via natural and anthropogenic sources. Volcanic eruptions, forest fires, and rock weatherings are the natural Cd sources (Khan et al. 2017). There is high amount of Cd in mafic and ultramafic rocks. Smelting, wastes of cement production, use of fertilizers with phosphate, ash wastes of fossil fuels are the main anthropological reasons of Cd pollution in soil (Järup 2003; Panet al. 2016). Other Cd sources are plastic stabilizers, batteries, fungicides, wastes of latex and textile productions, motor oil, solar panels and pigments (Khan et al. 2017; Mahmood et al. 2019). High Cd concentrations causes problems in physiological and biochemical processes in plants. Cd intensity was reported to cause reduction in plant wet weight, leaf length, chlorophyll content, stomatal conductivity, seed germination and ATP content (Khan et al. 2017; Rizwan et al. 2017). Cadmium inhibits photosynthesis in plants and causes mitochondrial degradation, therefore inhibits food uptake, delays shoot growth, and causes cell death and chlorosis (Khan et al. 2017). It also interferes with the metabolic processes of the plant, inhibits proton pumps, decreases root growth and harms photosynthetic processes (Mahmood et al. 2019; Rafiq et al. 2014).

The heavy metal lead (Pb) can contaminate by many ways, and it has been found to be in a toxic level in the near-road agricultural areas due to traffic. When it enters the cells, it causes toxicity by changing the permeability of the cell membrane, reacting with active metabolic enzyme groups, changing essential ions, and forming complex compounds with ADP and ATP. Lead toxicity causes inhibited enzyme activities, disrupted mineral nutrition, water imbalance, hormonal disturbance, inhibited ATP production, lipid peroxidation, changes in membrane permeability, and DNA damage due to overproduction of reactive oxygen species (Kumar et al. 2019; Pourrut et al. 2011; Sethy and Ghosh 2013; Sharma and Dubey 2005). Moreover, it reduces photosynthetic activity by reducing Calvin cycle enzyme activities, inhibits opening and closing of stomata (Dogan 2019; Adiloğlu ve Sağlam 2015; Adiloğlu 2013; Liu et al. 2008; Mishra et al. 2006; Romanowska et al. 2006; Adiloğlu and Adiloğlu 2003).

Chromium toxicity appears in photosynthesis as inhibition of pigment biosynthesis, photosynthetic electron transfer and Calvin cycle, accelerating lipid peroxidation, and disruption of thylakoid membrane. An in vivo examination of inhibition effects of different doses of chromium (Cr^{+6}) on light reactions of photosynthesis of *Pisum sativum* L. was carried out. The results revealed inhibition of electron carriage between systems, and affecting the level of b6f complex levels. The inhibitory effects of Cr on PSI were more significant than those on PSII. The sensitivity of used kinetic parameters on the functions of photosynthetic reactions was thought to be suitable for the early diagnosis of toxic effects of pollutants on the plants. Many hyperaccumulator plants have been detected for chromium remediation (Todorenko et al. 2020; Dökmeci and Adiloğlu 2020; Adiloğlu and Göker 2021; Adiloğlu et al. 2021).

The family Brassicaceae is an economically important family with its 372 genus and 4060 species. Some members of Brassicaceae are well known hyper accumulators. They have become good candidates for remediation of areas contaminated by various metals/metalloids as they can translocate high amounts of metals from roots to the shoots without a phytotoxic symptom. These plants tolerate by partitioning higher amounts of heavy metals in the vacuoles of the above soil parts. This is carried out by excess expression of some metal carriers in different tissues and affects the storage in the leaves. Studies to understand the hyperaccumulation and hyper tolerance characteristics related with Brassicaceae, gathering of metals and detoxification have been carried out (Daud et al. 2018). The usage of hyperaccumulators results in low biomass rich in metals, which is an easy and economic way of metal recovery and safe elimination. On the other hand, use of non accumulators result in an expensive to safely eradicate, non economical processing to recover metals, large biomass with poor metal accumulation. Many accumulator plants used in the remediation of heavy metals are members of Brassicaceae family (Adiloğlu 2016; Adiloğlu 2018; Adiloğlu and Gürkan 2020; Adiloğlu and Pamay 2021).

There is a need for inexpensive and efficient biological solutions for the problem of heavy metal contamination of agricultural soils. Phytoremediation is the leading of such solutions. The choice of hyperaccumulator plant is very important for this method. That is why in this study the accumulator capacity of Broccoli (*Brassica oleracea* var. *italica*) for the remediation of soil contaminated by chromium, lead and cadmium was evaluated.

Materials And Methods

The soil was taken from 0-30 cm depth. It was air dried, minced and sifted through a 4 mm sieve, and put into the pots. The trial was carried out in the labs of Soil Science and Plant Nutrition Department, Faculty of Agriculture Tekirdağ Namık Kemal University, according to Randomized Blocks Design with triplicates. Three contaminants were used as CdSO_4 , $\text{Cr}(\text{NO}_3)_3$ and $\text{Pb}(\text{NO}_3)_2$ in the amounts of 30 mg kg^{-1} Cd, 10 mg kg^{-1} Cr (IV) and 100 mg kg^{-1} Pb, respectively. Constant amount of chelator (10 mmol/kg EDTA) were applied to each contaminant dose in order to increase the solubility. The plants were kept at controlled conditions at 22°C with an availability of sun light. After 30 days of incubation to naturally pollute the soil and absorption of pollutants by soil colloids, soil samples were taken from each pot and extractable Cd, Cr and Pb analyses were done. A picture from the experiment is given in Figure 1.

Plant analyses

Some agro morphological traits of Broccoli (*Brassica oleracea var. italica*) harvested the trial were determined (Jones et al. 1991). Plants were dried at 65 °C for 48 hours to determine the plant dry weight (Kacar and İnal, 2010). Extraction was carried out at lab conditions according to EPA 3052 wet burning method with microwave (EPA, 1996), and elemental analyses were carried out by ICP-OES (Inductively Couple Plasma Spectrophotometer).

Soil analyses

Soil samples brought to lab were air dried and sieved with 2 mm sieve before analyses.

- pH of soil samples was measured in 1/2.5 soil/water mixture according to Jackson (1967).
- Lime content of the soils was determined volumetrically with Scheibler calcimeter (Sağlam 2012).
- Electrical conductivity was measured in 1/2.5 soil/water mixture with EC meter (Sağlam 2012).
- Texture fractions of the study soil were done according to Bouyoucos Hydrometer method (Bouyoucos 1955).
- Organic matter content was determined according to Walkley-Black method (Kacar 1995).
- Available phosphorus was determined according to Olsen method (Olsen 1982).
- Changeable potassium content was determined with 1 N Ammonium Acetate (pH 7) method (Kacar 1995).
- Extractable Fe, Zn, Cu and Mn analyses were carried out according to DTPA method (0.005 M DTPA+ 0.1 M TEA+ 0.01 M CaCl₂) (Lindsay and Norwell 1978).

Some extractable heavy metals

For the analysis of some extractable heavy metals 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA (pH 7.3) was used (Lindsay ve Norvell, 1978) and Cr, Cd and Pb amounts were detected by ICP-OES.

Statistical analyses

Variance analyses were carried out for plant agro morphological traits, nutrition elements and heavy metals in plants and soils, and significant mean values were subjected to Duncan multiple comparison tests in SPSS-17 package program.

Results And Discussion

Some chemical data of the experimental soil showed that the soil pH was neutral. The soil was insufficient in terms of organic matter. This situation is positive for this experiment. Because high organic matter in the soil can inactivate contaminants and can inhibit the uptake of them by the plants (Adiloğlu and Sağlam 2015). The lime content was medium, phosphorus and potassium were sufficient in the soil. Available Fe and Mn contents were found to be low while Zn and Cu contents were sufficient. Extractable Cr, Cd and Pb contents were lower than the toxic level. Some chemical and physical properties of the research soil are given in Table 1.

Table 1
Some physical and chemical datas of the
experimental soil

Soil properties	Unit	Value
pH	***	6.78
Electrical Conductivity	*	0.23
CaCO ₃	*	8.89
Organic matter	*	1.85
Available P ₂ O ₅	**	41.48
Exchangeable K ₂ O	**	230.25
Fe	**	0.20
Available Cu	**	0.69
AvailableZn	**	0.70
Available Mn	**	1.65
Cr content	**	0.09
Cd content	**	0.05
Pb content	**	0.37
Clay	*	20
Silt	*	29
Sand	*	51
Texture Class	-	Sandy Loam (SL)
*:%, **: mgkg ⁻¹ , ***(1:2.5 soil: water)		

Change in some agro-morphological traits of Broccoli (*Brassica oleracea* var. *italica*)

Some agro morphological traits of *Broccoli* (*Brassica oleracea* var. *Italica*) used in the experiments and grown on soils contaminated by 30 mgkg⁻¹ Cd, 10 mgkg⁻¹ Cr IV and 100 mgkg⁻¹ Pb are given in Table 2.

Table 2
Some agro-morphological measurements* of Broccoli (*Brassica oleracea* var. *italica*)

Data	Control	+Cd+EDTA	Cd	++Pb+EDTA	Pb	+++Cr+EDTA	Cr
Shoot WW g	22.3±1.2b	19.0±6.2b	28.4±7.5ab	40.2±10.9ab	48.3±6.9a	36.2±4.8a	39.8±3.9ab
Root WW g	2.03±0.2abc	1.20±0.2c	1.63±0.3c	2.0±0.4abc	3.6±0.2ab	1.83±0.2bc	3.23±0.2a
Plant length cm	35.33±0.8ns	30.30±1.3ns	33.33±3.7ns	39.6±0.8ns	39.7±3.5ns	36.30±1.2ns	39.6±0.8ns
Root length cm	13±2,0bc	11±0,1c	15±2.3abc	13±0.0bc	17±0.5ab	12.66±1.6bc	18.66±2.3a
SPAD	52.4±2.5ns	49.56±2.3ns	49.66±4.6ns	57.41±1.6ns	57.43±3.1ns	52.33±3.9ns	58.6±4.2ns
Shoot DW g	8.15±0.01c	7.49±0.7c	8.19±0.5c	9.5±1.01abc	10.82±0.9ab	8.46±0.4bc	11.3±1.1a
Root DW g	2.03±0.2abc	1.2±0.2c	5.17±0.3c	5.2±0.4abc	5.5±0.2ab	1.83±0.2bc	3.23±0.7a
Number leaves	17.3±1.45ab	14.00±2.00b	17.00±2.3	17.3±0.8a	20.6±1.6ab	18.66±1.2ab	19.6±2.2ab
*: values are the mean of three replicates +Cd: 30 mgkg ⁻¹ ++Pb: 100 mgkg ⁻¹ +++Cr: 10 mgkg ⁻¹ WW: wet weight DW: dry weight							

The applied contaminants did not negatively affect the chlorophyll contents of the plant (Table 2). It is seen that the plant physiologically defended itself by accumulating heavy metals in its body and also changed the central Mg atom in the chlorophyll so that heavy metals could not affect the chlorophyll (Karaman et al. 2012). Negative effects of Pb, Cd and Cr were shown on many plants in the literature. Cadmium was shown to inhibit photosynthesis in *Brassica napus*, *Helianthus annuus*, *Thlaspi erulescens*, *Zea mays*, *Pisum sativum*, *Hordeum vulgare*, *Vigna radiata* and *Triticum* which were exposed to Cd toxicity (Barylá et al. 2001; DiCagno et al. 2001; Küpper et al. 2007; Moussa and El-Gamal 2010; Popova et al. 2008; Wahid et al. 2008). The accumulation of lead in the plants has many direct or indirect effects on morphological, physiological and biochemical functions of the plants. The typical symptoms of lead toxicity are inhibition of photosynthetic pigment content and photosynthetic activity (Singh et al. 2010). Increasing doses of chromium application were shown to decrease dry weight in corn (*Zea mays* L.) (Adiloğlu and Göker 2021).

Accumulation of some contaminants (Cr, Cd, Pb) in the roots and shoot of Broccoli (*Brassica oleracea* var. *italica*) and contents of contaminants in the soil after the harvest

Remediation of Cd, Pb and Cr pollutants and accumulation in Broccoli (*Brassica oleracea* var. *italica*) are given in Table 3. The accumulation of Pb and Cd in plant body is higher in pots treated with chelator compared to the control pots. This is the result of increased available form of pollutants in the soil solution. Although there is not a significant difference between control and Cr applied with chelator pots, there is a great numerical difference. Broccoli was therefore shown to accumulate chromium pollutants in its body depending on the concentration.

Table 3
Some heavy metal (Cr, Cd, Pb) measurements of Broccoli
(*Brassica oleracea* var. *Italica*) and soil, mgkg⁻¹

Doses	Plant shoot	Plant root	Soil
Control- Cd	0.28±0.2c	0.81±0.01c	0.05±0.01c
*Cd	4.53±0.9b	5.41±0.09cd	17.7±0.03a
*Cd + EDTA	6.67±1.9a	7.8±0.08a	6.30±0.02b
Control- Pb	0.97±0.08cd	1.43±0.15d	0.37±0.1e
**Pb	6.80±0.09d	5.91±0.10b	13.10±0.1g
**Pb+ EDTA	14.0±0.09ab	10.68±0.29a	19.08±0.2f
Control- Cr	0.56±0.5d	3.43±0.03d	0.09±0.1c
***Cr	5.17±0.3b	15.79±0.45b	0.78±0.3b
***Cr + EDTA	5.75±0.2a	17.74±3.37a	1.10±0.2a
*Cd: 30 mgkg ⁻¹ **Pb: 100 mgkg ⁻¹ ***Cr: 10 mgkg ⁻¹			

The accumulation of lead pollutant in plant shoots rather than roots in the pots where lead was applied drew attention in Table 3. The other pollutants accumulated more in the plant roots as expected. The plants which can accumulate pollutants in their above ground parts are more preferred in phytoremediation, therefore broccoli (*Brassica oleracea* var. *italica*) was revealed to be employed in the remediation of lead.

Cadmium (Cd) toxicity affects the photosystem II (PS II) (Baker 1991) and stress damage can be easily detected by chlorophyll change in fluorescent structures (Maxwell and Johnson 2000). Ribulose-1,5 diphosphate carboxylase (RuBisCo) and phosphoenol pyruvate carboxylase, the two enzymes that take place in CO₂ fixation, are the main targets of Cd. It reduces the activity of RuBisCo by changing its structure, by changing the Mg ion which is the vital cofactor of carboxylation reactions, and by directing the Mg ions to oxidation reactions (Shanmugaraj et al. 2019). Water oxidation complex of photosystem II is affected by the modifications of Qb binding sites and replacement of Ca²⁺ in Ca/Mn clusters. Stimulation of calmodulin like proteins by structural changes to regulate mechanisms such as gene regulations, stress tolerance, ion exchange by interacting with Ca²⁺ ions significantly increase during Cd stress. Concentration significantly increases during Cd stress (Geiken et al. 1998; Yang and Poovaiah 2003; Sigfridsson et al. 2004; DalCorso et al. 2008; Shanmugaraj et al. 2019).

Various physiological of plants such as the transpiration ratio, stomatal movements, plant water absorption, enzyme activity, seed germination, protein synthesis in plant, cell membrane stability, hormonal balance of the plant are negatively affected by chromium contamination (Asri and Sönmez 2006).

Salido et al. (2003) employed Chinese fern (*Pteris vittata* L.) and Indian mustard (*Brassica juncea* L.) as the accumulator plants in a study to investigate the phytoremediation of arsenic and lead contamination. The absorption of arsenic and lead were shown to increase by the application of EDTA. As a result, they revealed that As and Pb pollution in soil can be inhibited by the phytoremediation method.

Effects of some contaminants (Cr, Cd, Pb) accumulated in the shoots of Broccoli (*Brassica oleracea* var. *italica*) on some macro and micro nutrient elements

Change in the macro and micro nutrient elements which are vital for plant development in broccoli plant (*Brassica oleracea* var. italica) during the remediation of Cd, Pb and Cr heavy metals from the soil used in the research are given in Table 4. A significant decrease in macro and micro nutrient elements in the pots where chromium was applied together with the chelator was observed. Copper and zinc among micro nutrient elements were especially found to increase in the case of lead pollution. The reason of this situation is thought to be increased solubility of these micro elements due to EDTA chelator application. Moreover, a synergistic effect might have occurred between these elements. The other pollutant cadmium positively affected all plant macro elements except Mg significantly at 5% significance level, and the elements fell into different groups in Duncan test. A synergistic effect among all the micro nutrient elements was observed in plants grew in both cadmium and Cd+EDTA applied pots. That is why the plants continued to develop and were not affected from the contaminants that took up into their bodies.

Calcium is absolutely required for synthesis of cell wall and development of the plants. Approximately 90% of Ca is present in cell walls. It behaves as the cohesion factor for cell binding and keeping the structures together in plant tissues. In case of Ca insufficiency, new tissue generation in roots and shoots slow down. As a result, the productivity of the plant is adversely affected. Calcium is also one of the keystones of plant defense mechanism and helps the recognition and reaction to stress elements. Since calcium is not mobile in plants, the insufficiency symptoms first appear in young leaves and and young tissues are the first to be damaged by insufficiency of calcium (Karaman et al. 2012).

Table 4
Effects of some contaminants (Cr, Cd, Pb) accumulated in the shoots of Broccoli (*Brassica oleracea* var. italica) on some macro and micro nutrient elements

Doses	Control	*Cd	*Cd + EDTA	**Pb	**Pb+ EDTA	***Cr	***Cr + EDTA
P ⁺	0.11±2.7e	0.09±3.5f	0.16±12.5b	0.15±8.7d	0.21±11.5a	0.20±3.5a	0.16±3.07c
K ⁺	1.66±0.1d	1.56±2.6e	2.02±2.8a	1.75±2.6c	2.47±0.3a	1.84±2.6b	1.68±4.9d
Ca ⁺	1.65±0.6c	1.57±2.0d	1.89±2.7b	1.97±2.5a	1.17±1.1g	1.50±1.1e	1.25±0.6f
Mg ⁺	0.20±2.6a	0.20±2.1ab	0.19±2.9c	0.19±1.3bc	0.17±1.6e	0.17±1.4d	0.15±1.1f
Fe ⁺⁺	19.05±0.1e	22.6±0.3b	34.15±0.4a	29.75±0.3b	18.55±0.2e	24.3±0.2e	9.05±0.1f
Cu ⁺⁺	2.85±0.04f	2.68±0.03g	4.28±0.02a	3.57±0.03d	4.14±0.03b	3.98±0.03c	3.14±0.01e
Zn ⁺⁺	16.8±0.02e	13.56±0.1f	23.8±0.2b	22.8±0.2c	24.1±0.08b	29.4±0.2a	22.3±0.1d
Mn ⁺⁺	22.6±0.01e	13.8±0.02g	46.8±0.05b	41.5±0.1c	19.6±0.3f	51.6±0.1a	26.9±0.2d
B ⁺⁺	19.3±0.6f	14.2±0.5g	35.06±0.3a	31.4±0.1b	22.6±0.04e	28.6±0.1c	23.5±0.1d
*Cd: 30 mgkg ⁻¹ **Pb: 100 mgkg ⁻¹ ***Cr: 10 mgkg ⁻¹ +: % ++: mgkg ⁻¹							

Cadmium (Cd) affects the transport functions of macro and micro nutrient elements by reducing absorption. Cd harms absorption, transport and distribution of macro and micro nutrient elements such as Ca, Mg, P, K, S, Fe, Mo, Zn, B, and Cu in plants like sugar beet, pea and barley (Hernández et al. 1996; Chang et al. 2003; Metwally et al. 2005; Guo et al. 2007; Adiloğlu 2017; Shanmugaraj et al. 2019; Adiloğlu 2020; Adiloğlu 2021).

The reduction in the photosynthetic activity in plants exposed to lead is a result of destruction of chlorophyll subunits, inhibition of plastoquinone and carotenoid synthesis, deficiency in basic elements such as Mn and Fe, substitution of bivalent cations with Pb, and destruction of electron transport system (Pourrut et al. 2011; Sharma and Dubey 2005).

According to Wallace et al. (1976) Cr contents in chromium accumulating plants are really high. For instance, Cr content in the leaves of Cr toxicated plants were 1-4 mg/kg, and even more in the roots.

Effects of some contaminants (Cr, Cd, Pb) accumulated in the roots of Broccoli (*Brassica oleracea* var. *italica*) on some macro and micro nutrient elements

The interactions and accumulation of heavy metals in the Broccoli (*Brassica oleracea* var. *italica*) in roots with the absolutely required macro and micro nutrient elements are given in Table 5.

Table 5
Accumulation of Cd, Pb and Cr pollutants in the roots of broccoli and effects on some nutrient elements

Doses	Control	*Cd	*Cd + EDTA	**Pb	**Pb+ EDTA	***Cr	***Cr + EDTA
P ⁺	0.07±2.4f	0.07±3.0e	0.84±3.9d	0.10±2.4b	0.09±2.3b	0.14±1.9a	0.10±1.27c
K ⁺	1.03±0.5d	1.12±6.5c	1.11±5.0c	1.30±1.3b	1.28±1.3b	1.43±1.4a	0.90±0.5e
Ca ⁺	0.42±0.4e	0.36±1.8f	0.79±0.1b	0.36±3.6f	0.47±0.5d	0.52±0.3c	1.27±1.3a
Mg ⁺	0.14±0.10c	0.15±1.8b	0.12±0.1d	0.10±0.17e	0.12±8.4d	0.09±0.9b	0.16±1.3a
Fe ⁺⁺	570±0.4g	1179±0.36c	1406±0.9	635±2.7f	1041±5.08d	966±8.6e	3895±31.2a
Cu ⁺⁺	5.36±0.04f	6.4±0.01d	10.9±0.10b	5.97±0.07e	6.14±0.01e	7.15±0.04c	15.1±0.07a
Zn ⁺⁺	13.6±0.01d	10.4±0.09e	19.7±0.06b	17.5±0.08c	17.6±0.05c	19.8±0.1b	28.06±0.2a
Mn ⁺⁺	32.1±0.08f	47.7±0.3d	85.9±0.3b	42.3±0.3e	43.4±0.1e	67.8±0.3c	20.0±1.7a
B ⁺⁺	12.6±0.5e	12.8±0.11e	19.7±0.06b	14.7±0.03d	15.6±0.1c	15.0±0.2d	23.2±0.04a
*Cd: 30 mgkg ⁻¹ **Pb: 100 mgkg ⁻¹ ***Cr: 10 mgkg ⁻¹ +: % ++: mgkg ⁻¹							

There was a significant increase of the macro nutrient elements except potassium and phosphorus, and micro elements except manganese in the pots where chromium was applied together with the chelator. Increases in the amounts of all macro and micro nutrients except potassium were observed in the case of lead pollution. The reason of this situation is thought to be increased solubility of these micro elements due to EDTA chelator application. Moreover, a synergistic effect might have occurred between these elements. The other pollutant cadmium positively affected all plant macro elements except Ca significantly at 5% significance level, and the elements fell into different groups in Duncan test. A synergistic effect among all the micro nutrient elements except Zn was observed in plants grown in cadmium and Cd+EDTA applied pots. That is why the plants continued to develop and were not affected from the contaminants that took up into their bodies.

When chromium (Cr⁺⁶) enters the root cell, it is easily reduced to Cr (III) form by the Fe (III) reductase enzyme. Since chromium immobilizes in the voids of root cells, a high Cr accumulation is observed in roots (Adhikari et al. 2020; Hayat et al. 2012). Chromium (Cr⁺⁶) toxicity can change enzyme activities, reduce resistance to pathogenic organisms, causes changes in behavioural modifications, population structure and species diversity and inhibition of photosynthesis (Pradhan et al. 2019; Sethuraman and Balasubramanian 2010). If chromium (Cr⁺⁶) accumulation in plants exceeds metabolic capacity for detoxification, seed germination, root and shoot development and biomass production slow down (Hayat et al. 2012; Sharma and Dubey 2005). Moreover, chromium (Cr⁺⁶) affects nutrient uptake and interferes with antioxidant systems and intercellular membrane structures. This metal harms the photosynthetic process as it decreases lipid peroxidation in proteins and photosynthetic pigments, (Diwan et al. 2012; Panda and Choudhury 2005; Singh et al. 2013;

Vajpayee et al. 2000). Electron transport, CO₂ fixation and photophosphorylation are affected by chromium (Cr⁺⁶) (Ali et al. 2006; Diduret et al. 2013; Mathur et al. 2016; Pandey et al. 2013; Susplugas et al. 2000; van Assche and Clijsters 1983).

In root filtration method, pollutants adsorb to the roots or taken by the roots depending on the biotic and abiotic processes. During these processes, pollutants can be uptaken by the roots and can be transported. The immobilization of pollutants on or in the roots is substantial at this point. The pollutants can later be extracted from the plants by various methods. This method can be applied to underground waters, ground waters and wastewaters (Adiloğlu 2021).

Effects of some contaminants (Cr, Cd, and Pb) on some macro and micro nutrient elements in soil where Broccoli (*Brassica oleracea* var. *italica*) was grown

The effects of contaminants and EDTA chelator on some macro and micro nutrient elements in the soils are given in Table 6.

Table 6

Accumulation and effects of Cd, Pb and Cr on some plant nutrient elements in soil where broccoli was grown

Doses	Control	*Cd	*Cd + EDTA	**Pb	**Pb+ EDTA	***Cr	***Cr + EDTA
P ⁺	37.1±0.2c	31.8±0.3e	23.1±0.2f	33.4±0.4d	41.9±0.3a	38.2±0.1b	41.3±1.3a
K ⁺	1.37±0.32	1.34±0.7b	1.17±0.4d	1.24±0.7c	1.16±1.9d	1.42±1.1a	1.36±0.9b
Ca ⁺	39.4±4.1b	46.5±7.3a	37.8±8.2c	39.4±3.8b	37.5±4.9c	38.3±2.9bc	37.2±4.8c
Mg ⁺	2.92±6.8c	3.22±4.5a	2.70±3.2d	2.73±2.1d	2.8±3.5cd	2.81±1.1cd	3.12±4.1b
Fe ⁺⁺	9.3±0.5c	12.5±0.7b	7.37±0.3e	7.5±0.02e	12.5±0.4b	8.1±0.02d	15.2±0.6a
Cu ⁺⁺	1.01±0.1d	1.08±1.8a	0.9±0.1e	1.07±0.2a	1.03±0.1c	1.06±0.1b	1.03±0.2c
Zn ⁺⁺	0.95±0.1d	1.10±0.1b	0.7±0.1f	0.9±0.1e	1.16±0.2a	0.97±0.1d	1.03±0.1c
Mn ⁺⁺	13.1±0.1f	25.8±0.15a	14.9±0.1e	15.3±0.2d	20.0±0.4b	16.0±0.6c	17.8±0.9b
B ⁺⁺	0.26±0.1d	0.31±0.1bc	0.29±0.1c	0.32±0.1b	0.32±0.1b	0.36±0.1a	0.3±0.1ab
*Cd: 30 mgkg ⁻¹ **Pb: 100 mgkg ⁻¹ ***Cr: 10 mgkg ⁻¹ +: % ++: mgkg ⁻¹							

All the macro and micro nutrient elements in the soil of cadmium applied pots were significantly lower (at 5% significance level). This shows that EDTA chelator increased the uptake of nutrient elements by the plants.

All the macro and micro nutrient elements, except Ca, in soil increased after the application of EDTA in the pots polluted with lead. This was also obvious in plant results and EDTA application to the soil polluted with Pb increased the uptake of nutrient elements from the soil. Plant available nutrient elements were found to be higher in EDTA applied soils after the experiment. These results were statistically significant at the level of 5%.

The effects of EDTA applications on some macro and micro nutrient elements of soils after the chromium polluted pots differ. Mg and P of the macro nutrient elements and Fe, Zn, and Mn micro nutrient elements were found to be higher after the application of EDTA. The solubilities of the mentioned elements were significantly (at 5% level) and positively affected.

Soil lead content depends heavily on soil pH, colloid size and cation exchange capacity. Moreover, root surface area, root excretions and transpiration degree affect the absorption and usability of Pb. Plant roots absorb Pb via Ca²⁺ channels or in an apoplastic way (Kumar et al. 2019; Pourrut et al. 2011).

Chromium (Cr) is accepted as one of the most toxic heavy metals and abounds in the earth crust. It enters the food chain via the plant absorption. It is found as chromite (FeCr_2O_4) or as a complex with other metals in ultramafic and serpentine rocks and in nature (Oliveira 2012). Moreover, Cr is released into the ecosystems as a result of anthropogenic activities such as unconscious use of chemical fertilizers, melting of metals, municipality waste waters, and wastes of different industries such as textile, ceramic, leather, steel and galvanic industries (Adhikari et al. 2020; Avudainayagam et al. 2003). Low affinity sulfate carriers mediate the absorption of chromium (Cr^{+6}) (Skeffington et al. 1976).

The relationship between Cr absorption from soil solution to the plant bodies and organic acids in the soil solution was determined, and together with the increased organic acid concentration in soil, the plants were shown to absorb more Cr from soil solution (Srivastava et al. 1999).

Conclusion And Suggestions

Health problems related with environmental pollution increase day by day. Uncontrolled polluted agricultural soils and the inability of remediation form the basis of these problems. The studies revealed that heavy metals reached significant levels in soils of our country. This study investigated the potential of phytoremediation method for cleaning up of soils and to carry out a healthy agricultural production. High quality soils will improve public health and life quality through the plants and animals. The usability of Broccoli (*Brassica oleracea* var. italica) as a hyperaccumulator plant to remediate cadmium, chromium and lead from the soils contaminated due to industrial activities, intense population and intense traffic was revealed by this study. This plant is especially important for the removal of heavy metal lead. Because this contaminant was accumulated especially in the upper parts of the plant and this plant was showed to be a hyperaccumulator to phytoextract the contaminant. Chromium and cadmium accumulated more in the roots of the plant. For these heavy metals, rhizofiltration can be suggested as a phytoremediation method.

Remediation of heavy metal contaminated agricultural soils is vital for the sustainable productivity of soils. Because, contaminated soils negatively affect the health of the organisms, especially humans. Use of hyperaccumulator plants for the remediation of such soils has become popular recently and heavy metals can therefore be cleaned up successfully from the agricultural soils. This study revealed the potential of broccoli (*Brassica oleracea* var. italica) to remediate the heavy metals Cr, Cd and Pb from the soils. The use of hyperaccumulator plants such as broccoli has been suggested to phytoremediate the ongoing heavy metal pollution in agricultural fields for healthy and quality crop production.

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Figures

Figure 1

A photo from experimental process (original).