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Determination of the Effects of Heavy Metal Stress on Plant Development and Physiology in Feed Soybean (*Glycine Max* L.)

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HIGHLIGHTS

- With increasing doses of plants under cadmium and lead stress, a decrease was observed in stem diameter and fresh and dry weights of plants and roots.
- Seedling height and number of leaves per plant increase with the increase in metal applications. showed a decreasing trend. However, all of the applications are in the same statistical group and are not affected by the increase in metal. It was not affected as negatively as other parameters.
- It has been observed that as the concentration of heavy metals (Cd, Pb) increases, chlorophyll (SPAD) value and leaf area are suppressed.
- Increased cadmium and lead compared to the electrical conductivity control group has increased with its applications. It has been determined that metal applications have a negative effect on the RWC value. Enzyme activity is generally increased due to metal stress.

Abstract

This study was carried out to determine the tolerance level of cadmium and lead applications at different concentrations on physiological and morphological parameters in soybean (*Glycine max* L.) The experiment was carried out in Atatürk University Plant Production Application and Research Center greenhouses. Yeşilsoy variety of feed soybean (*Glycine max* L.) was used as plant material. Plant growth (seedling height, stem diameter, fresh and dry weight, etc.), physiological properties (tissue proportional water content, tissue electrical conductivity) and biochemical parameters (chlorophyll amount, superoxide distumase (SOD), catalase (CAT), heavy metal applications) peroxidase (POD) enzyme activities, etc.) were investigated. All treatments had negative effects on all parameters compared to the control group. Growth retardation was experienced at the highest concentrations applied, but plant deaths were not observed. Although it is known that cadmium metal is more toxic than lead metal, the wet weight values of the plants to which the highest dose of both metals were applied were included in the same statistical group.

Keywords: Soybean; Heavy Metal Stress; Cadmium; Lead; Phytoremediation

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1. Introduction

Environment; It is a physical, chemical, biological, social, economic and cultural environment in which all living things maintain their relationships and interact with each other throughout their lives. Environmental pollution basically exists in nature in the form of air, soil and water pollution, and as a result, it affects the entire ecosystem, including humans. While protecting the environment and natural resources from pollution is important in terms of preventing environmental pollution, the purification of polluted areas is also an important issue in the solution of existing environmental pollution (Özay & Mammadov 2013).

Industrialization and urbanization are the leading factors that cause environmental pollution (Bayçu 1997). The energy that emerged as a result of rapid population growth has led to rapid progress in industry and industrialization (Cihangir & Sağlam 1999). This situation results in the very rapid consumption of natural resources, and intensive agricultural practices cause heavy metal accumulation in the soil and in the environment (Çağlarrmak & Hepçimen 2010; Mikhailenko et al 2020). Earthquakes, floods and volcanic eruptions are the first ones that come to mind as sources of heavy metals in nature. Its spread to the environment occurs from human-based activities rather than natural resources (Kahvecioğlu et al 2010). Many factors such as the use of fossil fuels, mineral deposits, melting of metal ores, motor vehicles, urban wastes, wastewater, fertilizers, pesticides and sewage have paved the way for human-induced heavy metal pollution (Kabata-Pendias & Pendias 1999; Yerli et al 2020). It is stated that this situation has reached critical levels in many countries (Robinson et al 2001).

Heavy metals

Heavy metals are explained as metals with a density of more than 5 g/cm3, atomic number greater than 20, causing toxicity and pollution (Sönmez et al 2021). In plants, low stimulant concentrations of some heavy metals are necessary for normal and healthy plant growth (Bera et al. 2005). Metals such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), cobalt (Co) are micronutrients that play an active role in the growth and development of plants and animals. In addition, some heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) are elements that are not important for the development of living things (Niess 1999).

Heavy metals have a high relative density and even at low concentrations, they have a toxic effect on living things. All plants have the ability to collect the heavy metals necessary for them from soil and water. These metals are magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo) and nickel (Ni) (Langille and Maclean 1976). Some plants also have the ability to accumulate heavy metals whose biological functions are unknown. These heavy metals are cadmium (Cd), chromium (Cr), lead (Pb), cobalt (Co), silver (Ag), selenium (Se) and mercury (Hg) (Hanna & Grant 1962; Baker & Brooks 1989).

Reaching a certain level of heavy metal concentration in the atmosphere, water and soil also causes great problems for living things (Özay 2013). Filtering soil with its buffering feature can protect itself against various pollutants (Yerli et al 2020). However, high doses of heavy metals disrupt the physical, chemical and biological structure of the soil and cause a decrease in product yield and quality (Long et al 2002). Reaching a certain level of heavy metals directly affects the development and physiology of plants negatively and serious yield losses.

Lead (Pb)

It is the first metal of human origin to cause serious damage to the ecological system. Lead can enter the soil and atmosphere from a wide variety of sources. These sources include chimney and exhaust gases, wastes from different industries (paint, electricity, oil) and pesticides (Saygıdeğer 1995; Kalinowska 1984; Aksoy 1995). A large part of lead, which constitutes a large share in environmental pollution, is caused by the emission of gases into the environment by the combustion of gasoline used in motor vehicles (De Jonghe & Adams 1986). As a result of pollution, lead (Pb), copper (Cu), zinc (Zn) etc., which strengthens its place in the soil and atmosphere. heavy metals are becoming dangerous for plants, animals and humans. Lead has been reported to be the largest environmental pollutant. It is one of the heavy metals that cause worldwide concern

(Salt et al 1999) and the most severe toxic effect (Okçu et al 2009). It causes morphological, physiological and biochemical dysfunctions in plants. Although it is not essential for plants, it is rapidly absorbed by plants and accumulates in different parts of the plant (Fahr et al 2013). It has an inhibitory effect on activities such as seed germination, root growth, stem development, transpiration, chlorophyll synthesis and cell division in plants. However, these effects vary according to the amount of lead exposed, the duration of the effect, the developmental stage of the plant and the exposed plant tissue (Doğru 2020). Lead affects plants through their roots (Fahr et al 2013). The uptake of Pb by the roots of plants prevents its development by reducing nutrient intake (Öktüren & Sönmez 2006). Lead accumulation in plants causes stunted growth, chlorosis and darkening of the root system (Sharma & Dubey 2005). In plants under extreme lead stress, the number of leaves decreases, their length becomes smaller and their leaves become more fragile (Gupta et al 2009).

Cadmium (Cd)

Today, it is one of the heavy metals, which is an important factor in environmental pollution. Among the heavy metals, it is an element with high solubility in water. Since it is soluble in water, it is taken into biological systems by plants and sea creatures, and it accumulates and spreads rapidly in nature (Özkan 2009). Cadmium is found in phosphate fertilizers, detergents and refined petroleum derivatives in compound form, and cadmium pollution occurs with their widespread use. It is used industrially in nickel/cadmium batteries, coating of steel in the ship industry, paint industry, alloys and electronics industry due to its resistance to corrosion, especially in sea conditions (Kahvecioğlu et al 2007). Cadmium is an element that is toxic to plants (Çatak et al 2000). Some of the cadmium sources that affect the respiration of plants; water pipes, burning coal, fertilizers used in the seed stage and flue gases released from factories (Kahvecioğlu et al 2007).

There is no biological activity of cadmium, which is not an absolutely necessary metal in the nutrition of living things (Marschner 2008). Its mobility in the soil facilitates its inclusion in the food chain. Cadmium taken by plants causes disruption of many activities such as protein synthesis, nitrogen and carbohydrate metabolism, enzyme activation, photosynthesis and chlorophyll synthesis (Mengel 2001). Cadmium prevents the growth and development of plant roots, making it difficult to take water and ions, and also reduces the yield and quality of the plant because it inhibits the plant photosynthesis rate and enzyme activity (Asri & Sönmez 2007). In a study on legumes (alfalfa, soybean), it was reported that cadmium inhibits photosynthesis by respiration (Huang et al 1974).

Some of the plants can carry high levels of metal in their bodies. These types of plants, which can accumulate many metals in a hundred times more than other plants without any damage, are called hyperaccumulator (metal accumulator) plants (Özbek 2015). Plants take the heavy metal from the soil into their body at high rates through their roots, transmit it to the stem and store it in other tissues and organs of the plant (stem and leaf). Studies to investigate the usability of these tolerant plants in the remediation of heavy metal-containing soils or waters are increasing.

Phytoremediation is the use of hyperaccumulator plants (Dushenkov et al 1997) to bring the ecological pollution caused by heavy metals to a controllable level or to neutralize this pollution (Dushenkov et al 1997), a method of making nature cleaner and more useful through plants (Clemens et al 2002; Pulford & Watson 2003; Gardea-Torresdey et al 2005). In the phytoremediation technique, hyperaccumulator plants are selected that can absorb the heavy metals contained in the pollutants in the environment, have a high ability to accumulate in their tissues and passivate the heavy metals they have accumulated after different stages. Green breeding is a breeding method that achieves positive results by choosing genetically adjusted plants, especially selected, in the breeding of low and medium risk polluted environments. Compared with different breeding methods, it has many advantages such as low input cost, convenience in application and time saving (Glass et al 1999).

Soybean (*Glycine max* L.)

The first country where the soybean (*Glycine max* L.) plant, which is known to have emerged in the lands of East Asia about 5000 years ago, is accepted as China (Öner 2006). Soybean, which has been noticed in the Far East countries, has increased its importance in the region by creating an important food and livelihood for the people living in these regions. Nutritionists underline that the use of soybean in food and animal feed use has increased rapidly in many countries in the last 30 years, and that it should increase even more in terms of good nutrition (Thuzar et al 2010).

Soybean is one of the plants with the largest cultivation area in the world (Turhan 2019). According to 2018 data, 34.68% of the world's oilseed production belongs to soybeans and ranks sixth among the most produced plants in the world (FAO 2019). According to 2022 data, world soybean cultivation area is 126.951.517 ha and production is 353.463.735 million tons. In our country, according to the agricultural statistics of 2021; soybean cultivation area was 438.917 da and soybean production was 182 thousand tons (TUIK 2021). Since our country cannot meet the demand for soybean, it is an importer country, and according to the data of 2021, it imported 3.0 million tons of soybean annually (Anonymous 2021). Soybean plant grows successfully in many parts of the world, as it adapts to various climatic conditions. The highest yield is obtained in climates where the temperature is 25°C between May and September. Temperatures lower than 18 °C and higher than 40 °C adversely affect the growth and development of soybean plant (Tüfekçi 2019).

80% of soybean (Glycine max L.) cultivation in Turkey is grown as a second crop in Çukurova (Metin and İlker 2016). Considering its place in human and animal nutrition (poultry, small cattle, dairy and beef cattle) and its workability in the industrial field, it is understood that the value of soybean has increased in our country (Nazlıcan 2010). Soybean, which is one of the few important plants in the world, takes its place as a protein source in the rations of poultry, sheep and cattle, dairy and beef cattle (Öner 2006). It is rich in linolenic acid, also known as omega-3 fatty acid. The amount of omega-3, which cannot be made by the body and is one of the essential fatty acids, varies by 5-11% in soybean (Arıoğlu 2007). Soybean, which has a wide place in the industry, is used as a variety of foodstuffs due to the excess protein content (in the production of flour, milk, yogurt and cheese in the production of soybean meat); It is used in the manufacture of many industrial products (paint, linoleum, glue, etc.). In summary, soybean, which is beneficial in all respects, is one of the most valuable industrial plants in the world (Turhan 2019).

Damage to the soybean plant during its developmental stages can create stress in the plant, which can negatively affect plant growth and development. The ability of soybean plant to compensate for these negativities is quite low. In the studies carried out to eliminate the damage caused by heavy metals in agricultural lands, it has been focused on preventing heavy metals from reaching people directly or indirectly. Studies conducted with many plant varieties also show differences in the reactions of plants to heavy metal stress. As a result of our literature research, it is seen that there are few studies of heavy metal stress in soybean plant. This study was carried out to determine the effects of soybean plant on some parameters against heavy metal stress, which has become an important agricultural problem in the world and in our country.

2. Materials and Methods

The study was carried out as pot work in the greenhouses of Atatürk University Plant Production Application and Research Center in 2021. In the study, 'Yeşilsoy' forage soybean variety, which was registered by the Eastern Mediterranean Agricultural Research Institute, was used as plant material. In pots with a volume of 2 liters; Garden soil, sand, peat mixture was filled and planted at a depth of 2-3 cm, with 5 seeds in each pot. The plants that reached the seedling stage were thinned so that three plants with homogeneous appearance were left in each pot.

Heavy Metal Application

Attempt; cadmium 3 doses ($CdSO_4.8H_2O$ (100, 200 and 300 mg/kg), lead 3 doses ($PbNO_3$ (1000, 2000 and 3000 mg/kg) and 1 control group (no application), 7 applications, 3 replications) and 5 pots from each replication, a total of 105 (7x3x5=105) pots were conducted in a randomized plot design. To pollute the soil, different concentrations of cadmium (Cd) (100, 200 and 300 mg/kg) and lead (Pb) (1000, 2000 and 3000 mg/kg) metals were mixed into the trial soil and watered in the amount of field capacity and the incubation period of 3 weeks was started. At the end of the incubation period, soybean seeds were planted. The pot trial was completed in 50 days and at the end of the trial, the measurements, observations, weighing and analyzes stated below were performed while the plants were in or out of the pots. has been made.

Body Diameter (mm)

The stem (stalk) part of the above-ground parts of the plants at the harvest stage was measured in mm using a digital caliper (Güllap et al 2022).

Above Ground Fresh Weight (g/plant)

It was calculated by removing the above-ground part of the plant, weighing each plant on a sensitive scale and taking the average (Güllap et al 2022).

Above Ground Dry Weight (g/plant)

It was calculated by drying in an oven at 68°C for 48 hours (Güllap et al 2022).

Root Fresh Weight (g/plant)

The roots were removed from the soil, washed and cleaned of soil particles, and then the water was removed with blotting papers. Its weight was determined by weighing it on a precision scale (Güllap et al 2022).

Root Dry Weight (g/plant)

The measurement was made by drying in an oven at 68°C for 48 hours (Güllap et al 2022).

Seedling Length (cm)

It was measured in cm with a ruler (Güllap et al 2022).

Number of Leaves (piece/plant)

The number of leaves in each pot and each plant was taken as a number and the average was calculated (Güllap et al 2022).

Amount of Chlorophyll (as SPAD Value by Chlorophyll Meter)

Chlorophyll content of plant leaves (SPAD-502, Konica Minolta Sensing, Inc., Japan brand) was determined with the SPAD-502 meter device (Lichtenthaler & Wellburm 1983).

Leaf Area (cm^2 /plant)

Leaf areas of the plants in each heavy metal application were determined with a leaf area meter (LICOR, Model: LI-3100, Lincoln, USA) (Güllap et al 2022).

Tissue Electrical Conductivity

An indication of the damage caused by the stress on the leaf tissue and especially in the cell membrane is the electrical conductivity measurements taken from the wet leaf tissues. For this analysis, discs (1cm in diameter) taken from the last grown true leaves of 2 plants taken randomly from each of the replications were placed in glass bottles filled with 20 ml of distilled water, shaken for 24 hours in the shaker, and then the electrical conductivity of the soaking water was measured with a device, and the permeability of the cell membrane was determined. (damage rate) was determined (EC1). The samples were placed in an autoclave and kept at 121 °C for 20 minutes, and the second measurement was taken (EC2). Relative electrical conductivity values were determined by calculating the EC1/EC2 ratio (Kaya et al 2003).

Tissue Proportional Water Content (RWC)

The leaf discs (1 cm in diameter) taken from 2 randomly selected plants among the plants in the treatments were immediately weighed and their fresh weights were determined (TA). After weighing, the discs were taken into petri dishes containing 20 ml of distilled water and kept for 5 hours, then the discs were wiped with the help of blotting paper, the excess water was removed and weighed again. With this method, turgorous weights (TU) were determined. Afterwards, these discs were taken into petri dishes, dried in an oven at 72°C for 48 hours, and their dry weights were determined (KA). Tissue water content (RWC) values were calculated according to the following formula (Kaya et al 2003).

(RWC) = [(TA – KA) / (TU – KA)] x 100

Determination of Catalase (CAT - EC: 1.11.1.6) Activity (EU/Gta)

The method used to determine catalase (CAT) activity is the method used by researchers named Havir & Mchale (1987) on the principles in the literature of Luck (1965). According to this method, the activity determination was made on the basis of monitoring the absorbance decrease at 240 nm while providing the conversion of H_2O_2 to O2 and H2O for catalase (CAT) activity measurement (Havir & Mchale 1987).

Determination of Peroxidase (POD - EC: 1.11.1.7) Activity (EU/gTA)

In order to determine the peroxidase (POD) activity assay, guaicol was made by monitoring the absorbance increase of the colored compound, which is the result of the reaction in which H_2O_2 is the substrate, at 470 nm (Angelini et al 1990).

Determination of Superoxide Dismutase (SOD – EC: 1.15.1.1) Activity (EU/Gta)

Superoxide dismutase (SOD) activity, inhibition of photochemical reduction of nitro blue tetrazolium (NBT) was determined on the basis of spectrophotometric detection (Agarwal and Pandey 2004; Yordanova et al 2004).

Hydrogen Peroxide (H_2O_2) Analysis (mmol/kg)

Hydrogen peroxide (H_2O_2) analysis, Velikova et al (2000) was carried out according to the method reported. (H_2O_2) contents were calculated using a pre-made standard calibration curve using different concentrations of (H_2O_2)

Malondialdehyde (MDA) Analysis(nmol/g)

Thiobarbituric acid ($C_4H_2N_2O_2S$)-reactive substances are formed as a byproduct of lipid peroxidation (ie degradation products of fats). Therefore, TBARS values were measured as mal ondialdehyde (MDA), which is a degraded product of lipid and determines lipid peroxidation. Lipid peroxidation analysis, Şahin et al. It is based on the transaction steps reported in (2018). MDA concentration was determined from the absorbance curve using an extinction coefficient of 155 mmol/L.

Statistical Evaluation

The experiment was set up in a randomized plot design with three replications. All the data obtained at the end of the study were subjected to the variance analysis test with the SPSS 18 package program, and the comparison of the averages was made according to the Duncan multiple comparison test (Yıldız & Bircan 1991).

Intervention ARY studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

3. Results

The differences in the mean stem diameter (mm), plant fresh and plant dry weight (g), root fresh and root dry weight (g) of lead (Pb) and cadmium (Cd) applications are presented in Table 1. It is known that heavy metal accumulation in the soil negatively affects plant production and reduces the yield and quality obtained from the unit area (Yerli 2020). In our study, the application without Pb and Cd pollution in all parameters (control application) had the highest values. With the increase of metal concentration, the amount of decrease in the values accelerated. For example, the difference between the lowest doses (Pb1000-Cd100) of the control application and Pb-Cd applications was 0.04-0.05 mm, while the difference between the highest doses (Pb3000-Cd300) was 0.36-0.28 mm. Similarly, in the plant fresh weight parameter, the control group has a higher weight than the plants in all treatments, but the decrease in the first applications (Pb1000-Cd100) was 0.52-0.83 g, while in the highest concentration application (Pb3000-Cd300) it was 2.52-2 compared to the control. There was a weight loss of .39 g. The lowest value (1.45 g) in plant dry weight measurements was obtained from Cd 300 application. The negative effects of increasing doses of cadmium on plant weight have been supported by many studies (Bachir et al 2004; Tiryakioğlu et al 2006; John et al 2009; Safarzadeh et al 2013). The increase in metal concentration in root fresh and dry weights had a reducing effect on root mass. Researchers have reported that root inhibition is an important factor in determining metal toxicity (Lyu et al 2018). In our study, Pb (3000) and Cd (300) applications caused a 52-70% decrease in root fresh weight and 63-76% decrease in root dry weight compared to the control application (Table 2). According to Groppa et al (2008) determined as a result of his studies that heavy metals negatively affect root growth, development and new root formation in plants.

Table 1. Effects of Applications on Plant Growth in Soybean[1]

Treatments (mg kg^{-1})	Stem Diameter (mm)	Plant Fresh weight (g)	Plant Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Control	2.35 a	8.41 a	2.29 a	1.66 a	0.62 a
Pb 1000	2.31 a	7.89 a	1.96 ab	1.38 ab	0.35 b
Pb 2000	2.25 ab	7.64 a	1.87 abc	1.11 abc	0.28 bc
Pb 3000	1.99 c	5.89 b	1.61 bc	0.79 bc	0.23 bc
Cd 100	2.30 a	7.58 a	1.95 ab	1.02 abc	0.19 bc
Cd 200	2.19 ab	7.15 ab	1.91 ab	0.69 bc	0.16 c
Cd 300	2.07 bc	6.02 b	1.45 c	0.50 c	0.1 c

[1]Means marked with different letters are statistically different.

The average seedling length, which is one of the basic growth parameters, was determined as 20.06 cm in the control application (Table.2). Plant height averages of lead (Pb) and cadmium (Cd) metal applications were behind the average seedling height of the control group. Plant heights in lead applications were measured as 19.93 cm, 18.06 cm and 16.53 cm. A 17% height loss was observed between the seedling height of the control group and the highest concentration of lead application (3000 mg/kg). The average seedling height in Cd applications is 19.13 cm, 17.26 cm and 16.26 cm. The average height of the plants grown under the conditions of the highest Cd application (300 mg/kg) was determined as 19% shorter than the control group. In the stress studies on the subject, Siyah et al (2013) found that Cd applications in cotton plant, Aksu (2019) lettuce, Yılmaz & Kökten (2019) sorghum reduced plant height, root-stem dry and wet mass growth parameters compared to control.

Table 2. Effects of Applications on Seedling Height, Number of Leaves, Chlorophyll Value (SPAD) and Leaf Area in Soybean1

Treatments (mg kg^{-1})	Seedling height (cm)	Number of leaves (pcs/plant)	Chlorophyll SPAD	Leaf area (cm^2/plant)
Control	20.06 a	13.60 a	37.50 a	112.75 a
Pb 1000	19.93 a	11.13 b	37.10 ab	101.48 ab
Pb 2000	18.06 bc	11.06 b	35.46 bc	90.98 bcd
Pb 3000	16.53 cd	10.80 b	34.89 c	87.46 bcd
Cd 100	19.13 ab	11.66 b	35.96 abc	99.51 abc
Cd 200	17.26 cd	11.03 b	35.03 c	86.69 cd
Cd 300	16.26 d	11.53 b	34.66 c	83.99 d

[1]Means marked with different letters are statistically different.

In the study carried out under greenhouse conditions, it was determined that Cd and Pb applications caused a decrease in the number of leaves, leaf area and chlorophyll values in soybean (Table 2). While the lowest value (10.80 pieces/plant) was observed in the application of lead (3000 mg/kg), it was determined that all doses of Cd and Pb were in the same group statistically in terms of the number of leaves. Chlorophyll (SPAD) value decreased statistically in all treatments of Pb and Cd compared to the control treatment. One of the negative effects of cadmium toxicity on plants is that it affects the chlorophyll biosynthesis process (Sheoran et al. 1990). It has also been reported that all heavy metals increase chlorophyll destruction and inhibit its synthesis (Zengin & Munzuroğlu 2005). In terms of leaf area, the highest value was observed in the control application with 112.75 cm², while the lowest value was found in the Cd 300 application with 83.99 cm². In general, applied heavy metals decreased the leaf area compared to the control application (Table 2). It is known that the essential nutrients of plants exposed to metal toxicity are less than plants under control conditions. Therefore, plant height, number of leaves and leaf area have lower values (Mengoni et al 2000; Jayakumar et al 2007)

The effects of different doses of heavy metal applications on tissue electrical conductivity (DEI) and tissue proportional water content (RWC) values in soybean are shown in Table 3. As Pb and Cd stress increased, it was observed that DEI value increased in soybean compared to the control application. The lowest value was seen in Cd 100 application as 16.25%. In RWC values, heavy metal applications showed a decreasing trend, although there was not much difference compared to the control, and the closest average value to the control was seen in Cd 100 application as 83.54%

Table 3. Effects of Applications on Electrical Conductivity and RWC in Soybean[1]

Treatments mg/kg	MP (%)	RWC (%)
Control	15.91 c	84.42 a
Pb 1000	16.3 bc	82.95 a
Pb 2000	18.7ab	80.8 bc
Pb 3000	19.9 a	78.81 d
Cd 100	16.5 c	83.54 a
Cd 200	17.3 bc	81.18 b
Cd 300	19.16 a	79.01 cd

[1] Means marked with different letters are statistically different.

Metal applications create toxicity in the plant, affect the plasma membrane permeability and cause a decrease in water content. Cadmium has been reported to interact specifically with water balance (Barceló et al 1986; Poschenrieder et al 1989; Costa & Morel 1994). The decrease in RWC values may be due to the decrease in hydraulic conductivity caused by metal applications (Ehlert et al 2009). Previous studies have shown that metal toxicity causes a decrease in RWC values in some plant species (Manousaki & Kalogerakis 2009; Ahmad et al 2011).

Table 4. Effects of treatments on antioxidant enzyme activities (CAT, POD and SOD) in Soybean[1]

Treatments (mg/kg)	CAT-(EU/Gta)	POD-(EU/gTA)	SOD-(EU/Gta)	H_2O_2-(mmol/kg)	MDA- (nmol/g)
Control	0.017 b	27.84 a	77.20 cd	241.66 e	157 d
Pb 1000	0.014 cd	21.52 bc	80.86 bc	310.04 c	2.04 c
Pb 2000	0.036 b	12.06 e	83.82 b	313.63 c	4.54 a
Pb 3000	0.077 a	18.29 cd	73.38 d	366.67 a	4.45 a
Cd 100	0.008 e	14.17 de	90.24 a	272.68 d	1.9 c
Cd 200	0.012 de	23.10 b	92.59a	266.04 d	2.35 b
Cd 300	0.013 cde	14.05 de	92.05 a	331.73 b	2.30 b

[1] Means marked with different letters are statistically different.

In the phytoromediation technique, antioxidant enzymes play a major role in the process of heavy metal uptake by plants or in the process of heavy metal stress (Bhaduri & Fulekar 2012). This role is to allow the plant to survive in case of heavy metal overload in plant organs. Numerous studies have reported that the working principle of enzyme activity depends on a combination of parameters such as the type of stress conditions, its functioning in the plant, and the type of plant. Table 4 shows the effects of heavy metal applications on antioxidant enzyme activities in soybean. It was observed that the activation of catalase enzyme increased as the cadmium concentration increased. In the lead application, it was concluded that the Pb1000 concentration decreased below the control group and increased in other applications. There was a decrease in POD value compared to the control application. The highest POD value was 23.10 in Cd 200 application. There was a regular increase in SOD values except for Pb 3000 application. It was observed that there was an increase in SOD enzyme activity in parallel with the increasing concentration in cadmium application. The highest increase was observed in the Cd 200 application as 92.59, followed by the statistically similar Cd 100 (90.24) and Cd 300 (92.05) applications. Cadmium-induced increases in enzyme activities in soybean are in agreement with other studies (Melo et al 2011; Alyemeni et al 2017; Finger-Teixeira et al 2010).

In Table 4, it was observed that hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) activity increased in the soybean plant treated with Pb and Cd compared to the control application. The highest peroxidase

(H2O2) values were observed in Pb 3000 and Cd 300 applications as 366.67 and 331.73 mmol/kg, respectively. Again, the highest malondialdehyde (MDA) value was determined as 4.54 nmol/g in the Pb 2000 dose, which increased compared to the control application, and it was found to be in the same group statistically with the subsequent Pb 3000 (4.46) application. Malondialdehyde (MDA) is a value that varies depending on the type and severity of the stress source (Tunçtürk et al 2021). In a study on quinoa, it was stated that root length, root fresh-dry weight and chlorophyll amount decreased due to the increase in drought stress, but the MDA value increased by 82% (Aslam et al 2020). In a thesis study examining the effects of cadmium applications on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) activities in cress, it was observed that the values increased due to the increase in metal application (Alım 2020).

Reducing the existing pollution through in situ plants has been one of the research topics of recent years. Knowing the metal uptake removal system of the plant to be used is a very important factor for success in removing pollution. In this study, it was tried to understand the reactions of soybean to heavy metal applications in greenhouse conditions through some parameters. It was observed that lead and cadmium metals significantly suppressed plant growth. However, since the results of the research are based on one-year data, it may be recommended to repeat the research by using more genotypes and metal concentrations, and to conduct similar studies in field conditions where soil depth and climate factors are important in order to reveal more reliable and precise results for the purpose of the research.

4. Discussion

Reducing existing pollution through plants has been one of the research topics of recent years. Knowing the metal uptake and removal system of the plant to be used in the environment, It is a very important factor for success in eliminating pollution. In this study conducted. The tolerance of soybean to heavy metal applications under greenhouse conditions was tried to be understood through some parameters. Lead and cadmium metals have important effects on plant growth. It was observed that it suppressed the research results consist of one year's data. Research using more genotypes and metal concentrations It may be recommended to repeat.

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References

Agarwal S, Pandey V (2004). Antioxidant enzyme responses to NaCl stress in Cassia angustifolia. *Biologia Pantarum* 48(4): 555-560.

Ahmad P, Nabi G, Ashraf M (2011). Cadmium-induced oxidative damage in mustard [(Brassica juncea L.) Czern.& Coss.] plants can be alleviated by salicylic acid. *South African Journal of Botany* 77(1): 36-44.

Aksoy A (1995). Kayseri-Kırşehir karayolu kenarında yetişen bitkilerde ağır metal kirlenmesi. II. *Ulusal Ekoloji Ve Çevre Kongresi* 1-8.

Aksu G (2019). Kadmiyum ile kirli alanlarda bitki besin elementlerinin alınımı üzerine indol asetik asitin etkisi. *Toprak Bilimi ve Bitki Besleme Dergisi* 7 (2): 80- 85.

Alım Z (2020). Hümik Asit Uygulamalarının Ağır Metal Stresi Altında Yetiştirilen Terede Bitki Gelişimi ile Bazı Fizyolojik ve Biyokimyasal Özllikler Üzerine Etkileri. *Yüksek Lisans Tezi*, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Erzurum.

Alyemeni MN, Ahanger MA, Wijaya L, Alam P, Ahmad P (2017). Contrasting tolerance among soybean genotypes subjected to different levels of cadmium stress. *Pak. J. Bot.* 49(3): 903-911.

Angelini R, Manes F, Federico R (1990). Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. *Planta* 182(1): 89-96.

Anonim (2021). Web Adresi:https://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Piyasalar%C4%B1/2021-Ocak%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Raporu/Soya,%20Ocak-2021,%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Piyasa%20Raporu--.pdf, Erişim Tarihi: 16.08.2022.

Arıoğlu HH (2007). Yağ bitkileri yetiştirme ve ıslahı ders kitabı. Çukurova Üniversitesi Ziraat Fakültesi ofset atölyesi, Adana.

Aslam MU, Raza MAS, Saleem MF, Wagas M, Iqbal R, Ahmad S, Haider I (2020). Improving strategic growth stage-based drought tolerance in quinoa by rhizobacterial inoculation. *Community Soil Science Plant Anal*. 51 (5): 1-16.

Asri FÖ, Sönmez S (2007). Kadmiyumun çevre ve insan sağlığı üzerine etkileri. Derim, 24(1): 32-39.

Bachir LD, Wu F, Zhang G, Wu H (2004). Genotypic difference in effect of cadmium on development and mineral concentrations of cotton. *Communications in Soil Science and Plant Analysis* 35(1-2): 285-299.

Baker AJ, Brook R (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecover*, 1(2): 81-126.

Balcı T (2019). Ağır metal stresinin roka (Eruca sativa L.)'da bitki gelişimi ve fizyolojisi üzerine etkisi. Yüksek Lisans Tezi, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Erzurum.

Barcelo J, Poschenrieder C, Andreu I, Gunse B (1986). Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. *J. Plant Physiol*, 125: 17-25.

Bayçu G (1997). Picea abies'te Kadmiyum Toksisitesi ve Köklerde Kadmiyum Birikimi. XIII. Ulusal Biyoloji Kongresi, 17(20): 433-442.

Bera AK, Bera A, Samadrita BR (2005). Impact of heavy metal pollution in plants. Developments in physiology biochemstry and molecular biology of plants 1: 105-124.

Bhaduri AM, Fulekar MH (2012). Antioxidant enzyme responses of plants to heavy metal stress. *Reviews in Environmental Science and Bio-Technology* 11: 55–69.

Cihangir N, Sağlam N (1999). Removal of cadmium by Pleurotus sajor-caju basidiomycetes. *Acta Biotechnologica*, 19(2): 171-177.

Clemens S, Palmgren MG, Kramer U (2002). A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science*, 7(7): 309-315.

Costa G, Morel JL (1994). Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiol, Biochem* 32: 561-570.

Çağlarırmak N, Hepçimen AZ (2010). Ağır metal toprak kirliliğinin gıda zinciri ve insan sağlığına etkisi. *Akademik Gıda* 8(2): 31-35.

Çatak E, Çolak G, Tokur S, Bilgiç O (2000). Bazı domates ve tütün genotiplerinde kadmiyum etkilerini inceleyen istatiksel bir çalışma. *Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 2(1): 13-41.

De Jhonge WR, Adams FC (1986). Biogeochemical cycling of organic lead compounds. Toxic metals in the atmosphere 561-594.

Doğru A (2020). Bitkilerde kurşın toksisitesi ve kurşun toleransı. *Black Sea Journal of Agriculture* 3(4): 329-339.

Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B (1997). Phytoremediation: a novel approach to an old problem. *In Studiesin Environmental Science* 66: 563-572.

Ehlert C, Maurel C, Tardieu F, Simonneau T (2009). Aquaporin mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology* 150(2): 1093-1104.

Fahr M, Laplaze L, Bendaou N, Hocher V, Mzibri ME, Bogusz D (2013). Effect of lead on root growt. *Frontiers in Plant Science* 4: 175.

FAO (2019). Crop description and climate. FAO Stat. Food and agriculture organization of the United Nations.

Finger-Teixeira A, Ferrarese MDLL, Soares AR, Da Silva D, Ferrarese-Filho O (2010). Cadmium-induced lignification restricts soybean root growth. *Ecotoxicology and Environmental Safety* 73(8): 1959-1964.

Gardea-Torresdey JL, Peralta-Videa JR., De La Rosa G, Parsons JG (2005). Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectrosopy. *Coordination Chemistry Reviews* 249(17-18): 1797-1810.

Glass DJ, Raskin I, Ensley BD (1999). Economic patential of phytoremediation. Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. New York, USA: John Wiley, Sans.

Groppa MD, Rosales EP, Lannone MF, Benavides MP (2008). Nitric oxide, polyamines and Cd-induced phytotoxicity in wheat roots. *Phytochemistry* 69(14): 2609-2615.

Gupta D, Nicoloso F, Schetinger M, Rossato L, Pereira L, Castro G, Srivastava S, Tripathi R (2009). Antioxidant defense mechanism in hydroponically grown Zea mays seedlings under moderate lead stress. *J Hazard Mater* 172(1): 479-484.

Gullap MK, Severoglu S, Karabacak T, Yazici A, Ekinci M, Turan M, Yildirim E (2022). Biochar derived from hazelnut shells mitigates the impact of drought stress on soybean seedlings. *New Zealand Journal of Crop and Horticultural Science* 1-19.

Hanna WJ, Grant CL (1962). Spectrochemical analysis of the foliage of certain trees and ornamentals for 23 elements. *Bulletin of the Torrey Botanical Club* 293-302.

Havir EA, McHale NA (1987). Biochemical and developmental characterization of multiple forms of catalese in tobacco leaves. *Plant physiology* 84(2): 450-455.

Huang CY, Bazzaz FA, Vanderhoef LN (1974). The inhibition of soybean metabolism by cadmium and lead. *Plant physiology* 54(1): 122-124.

Jayakumar K, Jaleel CA, Vijayarengan P (2007). Changes in growth, biochemical constituents, and antioxidant potentials in radish (*Raphanus sativus* L.) under cobalt stress. *Turkish Journal of Biology* 31(3):127–136.

John R, Ahmad P, Gadgil K, Sharma S (2009). Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by Brassica juncea L. *International journal of plant production* 3(3): 66-75.

Kabata-Pendias A, Pendias H (1999). Biogeochemistry of trace elements. Pwn, Warszava, 400.

Kahvecioğlu Ö, Kartal G, Güven A, Timur S (2007). Metallerin çevresel etkileri-I. Metalurji dergisi 136: 47-53.

Kahvecioğlu Ö, Kartal G, Güven A, Timur S (2010). Metallerin çevresel etkileri- 1. İTÜ Metalürji ve Malzeme Mühendisliği Bölümü.

Kalinowska A (1984). Lead concentrations in the slug Arion rufus from sites at different distances from a tourist road. *Ecolocigal bulletins*, 46-49.

Karanlık S Ergün N, Tiryakioğlu M (2013). Farklı kadmiyum düzeylerinin pamuk bitkisinde (Gossipium hirsutum L.) büyüme, Cd, Fe, Zn konsantrasyonu ve antioksidatif enzim aktiviteleri üzerine etkisi. *Tarım Bilimleri Araştırma Dergisi* 6 (2): 83-88.

Kaya C, Higgs D, Ince F, Amador BM, Cakir A, Sakar E (2003). Ameliorative effects of potassium phosphate on salt-stressed pepper and cucumber. *Journal of Plant Nutrition* 26: 807-820.

Langille WM, Maclean KS (1976). Some essential nutrient elements in forest plants as related to species, plant part, season and location. *Plant And Soil* 45(1): 17-26.

Lichtenthaler H, Wellburm AR (1983). Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11(5):591–593.

Long SP, Ainsworth EA, Davey PA, Bernacchi CJ, Dermody OC (2002). A meta-analysis of elevated [CO2] effects on soybean (Glycine max.) physiology, growth and yield. *Global Change Biology* 8(8): 695-709.

Luck H (1965). Catalase. In Bergmeyer, H.U., Ed., Method of Enzymatic Analysis, Academic Press, New York and London 885-894.

Lyu J, Park J, Pandey KL, Choid S, Leed H, Saeger JD, Depuydt S, Han T (2018). Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in Lactuca sativa L., *Ecotoxicology and Environmental Safety* 149: 225-232.

Manousaki E, Kalogerakis N (2009). Phytoextraction of Pb and Cd by the Mediterranean saltbush (Atriplex halimus L.). Metal Uptake in Relation to Salinity, Environ. *Sci. Pollut. R.* 16:844-854.

Marschner H (2008). Mineral nutrition of higher plants. Academic press, Second edition. London.

Melo LCA, Alleoni LRF, Carvalho G, Azevedo RA (2011). Cadmium and barium toxicity effects on growth and antioxidant capacity of soybean (Glycine max L.) plants, grown in two soil types with different physicochemical properties. *Journal of Plant Nurition and Soil Science* 174(5): 847-859.

Mengel KK (2001). Principles of plant nutrition.Dortrecht: Kluwer academic publishers.

Mengoni A, Gonnelli C, Galardi F, Gabbrielli R, Bazzicalupo M (2000). Genetic diversity and heavy metal tolerance in populations of Silene paradoxa L. (Caryophyllaceae): a random amplified polymorphic DNA analysis. *Molecular Ecology* 9:1319-1324.

Metin M, İlker E (2016). Ana ürün koşullarında bazı soya (*Glycine max*. L. Merill) hat ve çeşitlerinin Aksaray bölgesine adaptasyonu üzerine çalışmalar. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi* 25(2): 176-181.

Mikhailenko AV, Ruban DA, Ermolaev VA, Van Loon AJ (2020). Cadmium pollution in the tourism environment: a literature review. Geoscienses 6(10): 242.

Munzuroğlu Ö, Gür N (2000). Ağır Metallerin Elma (Malus sylvestris Miller cv. Golden)'da Polen Çimlenmesi ve Polen Tüpü Gelişimi Üzerine Etkileri. Türk Biyoloji Dergisi 24: 677-684.

Nazlıcan AN (2010). Soya yetiştiriciliği. www.cukurovataem.gov.tr/upload/ 2010/.../soyayetistiriciligi_ l.pdf. (Erişim Tarihi:13.10.2017).

Niess DH (1999). Microbial heavy-metal resistance. *Applied Microbiology And Biotechnology* 51(6):730-750.

Okçu M, Tozlu E, Kumlay M, Pehluvan M (2009). Ağır metallerin bitkiler üzerine etkileri. *Alinteri Journal of Agriculture Science* 17(2): 14-26.

Özay H (2013). Comparison study of low cost fly ash supported Cu, Co and Ni metal catalyst systems for the reduction of 4-nitrophenol. *Science of Advenced Materials* 5(6): 575-582.

Öktüren FA, Sönmez S (2006). Ağır metal toksisitesinin bitki metabolizması üzerine etkileri. *Derim* 23(2): 36-45.

Öner T (2006). Soya sektör raporu. İstatistik Şubesi 5(2017): 1-84.

Özay C, Mammadov R (2013). Ağır metaller ve süs bitkilerinin fitoremediasyonda kullanılabilirliği. *Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 15(1): 68-77.

Özbek K (2015). Hiperakümülasyon Ve Türkiye Florasındaki Hiperakümülatör Türler Kürşad Özbek. *Toprak Bilimi Ve Bitki Besleme Dergisi* 3(1): 37-43.

Özkan G (2009). Endüstriyel bölge komşuluğunda kıyısal kırsal alandaki hava kalitesi; Muallimköy'de partikül maddede ve topraktaki ağır metal kirliliği (Yayımlanmamış Yüksek Lisans Tezi). GYTE Fen Bilimleri Enstitüsü, Gebze 9: 12-22.

Poschenrieder C, Guns, B, Barcelo J (1989). Influence of cadmium on water relations, stomatal resistance and abscisic acid content in expanding bean leaves. *Plant physiol* 90: 1365-1371.

Robinson B, Russell C, Hedley M, Clothier B (2001). Cadmium adsorption by rhizobacteria: implications for New Zealand pastureland. *Agriculture, Ecosystems and Environment* 87(3): 315-321.

Safarzadeh S, Ronaghi A, Karimian N (2013). Effect of cadmium toxicity on micronutrient concentration, uptake and partitioning in seven rice cultivars. *Archives Of Agronomy and Soil Science* 59(2): 231-245.

Sahin U, Ekinci M, Ors S, Turan M, Yıldız S, Yıldırım E (2018). Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea* var. capitata). *Scientia Horticulturae* 240: 196-204.

Salt DE, Benhamou N, Leszczyniecka M, Raskin I, Chet I (1999). A possible role for rhizobacteria in water treatment by plant roots. *International Journal of Phytoremediation* 1(1): 67-79.

Saygıdeğer S (1995). Lypcopersicum esculentum L. bitkisinin çimlenmesi ve gelişimi üzerine kurşunun etkileri. *II. Ulusal Ekoloji ve Çevre Kongresi* 588-597.

Sharma P, Dubey RS (2005). Lead toxicity in plants. *Brazilian Journal of Plant Physiology* 17(1): 35-52.

Sheoran IS, Aggarwal N, Singh R (1990). Effects of cadmium and nickel on in vivo carbon dioxide exchange rate of pigeon pea (Cajanus cajan L.). *Plant and Soil* 129(2): 243-249.

Sönmez O, Kılıç FN (2021). Toprakta ağır metal kirliliği ve giderim yöntemleri. *Turkish Journal of Agricultural Engineering Research* 2(2): 493-507.

Thuzar M, Puteh AB, Abdullah NAP, Lassim MBM, Jusaff K (2010). The effects of temperature stress o the quality and yield of soybean. *Journal of Agricultare Science* 2: 1-8.

Tiryakioğlu M, Eker S, Ozkutlu F, Husted S, Cakmak I (2006). Antioxidant defense system and cadmium uptake in barley genotyes differing in cadmium tolerance. *Journal of Trace Elements in Medicine and Biology* 20(3): 181-189.

Tunçtürk R, Tunçtürk M, Oral E (2021). Kuraklık stresi koşullarında yetiştirilen soya fasulyesinin (Glycine max L.) bazı fizyolojik özellikleri üzerine rizobacterium (PGPR) uygulamalarının etkisi. *ÇOMÜ Ziraat Fakültesi Dergisi* 9 (2): 359-368. DOI: 10.33202/comuagri.881226.

Turhan S (2019). Farklı humik asit dozlarının soya (Glycine max L. merrill) çeşitlerinde verim ve kalite üzerine etkisi. *Yüksek Lisans tezi*, Iğdır Üniversitesi, Tarla Bitkileri Ana Bilim Dalı.

Tüfekçi Ş (2019). Konya-Ereğli ticaret borsası 2019 AR-GE soya fasülyesi raporu, Ziraat mühendisi.

TÜİK (2021). Türkiye İstatistik Kurumu. Web Sayfası. http://www.tuik.gov.tr (Erişim Tarihi 16.08.2022)

Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant science 151(1): 59-66.

Yerli C, Çakmakcı T, Sahin U, Tüfenkçi Ş (2020). Ağır metallerin toprak, bitki, su ve insane sağlığına etkileri. *Türk Doğa ve Fen Dergisi* 9 (özel sayı): 103-114.

Yıldız N, Bircan H (1991). Araştırma ve deneme metotları. Atatürk Üniversitesi Yayınları.

Yılmaz HŞ, Kökten K (2019). Kadmiyum (Cd) uygulamasının tane sorgumda (Sorghum bicolor L.) bazı morfolojik özellikler üzerine etkisinin belirlenmesi. *Türk Tarım ve Doğa Bilimleri Dergisi* 6(3): 447-456.

Yordanova RY, Christov KN, Popova LP (2004). Antioxidative enzymes in barley plants subjected to soil flooding. *Environmental And Experimental Botany* 51(2): 93-101.

Zengin KF, Munzuroğlu Ö (2005). Fasulye fidelerinin (Phaseolus vulgaris L. Strike) klorofil ve karotenoid miktarı üzerine bazı ağır metallerin (Ni2+, Co2+, Cr3+, Zn2+) etkileri. *FÜ Fen ve Mühendislik Bilimleri Dergisi* 17(1): 164-172