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ORIGINAL PAPER

EFFECTS OF HEAVY METAL (Pb) STRESS ON SOME GROWTH PARAMETERS AND CHEMİCAL CHANGES IN THE SOYBEAN PLANT (*Glycine max* L.)*

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Abstract

Heavy metals are very important abiotic stress factors that can induce different response mechanisms in plant bodies. These response mechanisms include modifying membrane compositions, generating small molecules and free radicals, and altering antioxidant enzyme activities. In this study, we aimed to determine the effect of lead (Pb), an important heavy metal, on some growth parameters and some important enzyme levels. This study was launched to determine the effects of lead (0, 25, 50, 75, 100 mg L^{-1}) on soybean (*Glycine max* L.) growth parameters and biochemical responses in a fully controlled aeroponic climate chamber. Growth and biochemical enzyme activity parameters of soybean plants changed under heavy metal stress. The lead content of soybeans increased with high concentrations of the metal in the environment. Lead stress negatively affected plant growth, photosynthetic activity, and chlorophyll content. The negative effect of the heavy metal was greater with increasing Pb doses. Lead application significantly increased antioxidant enzyme activities. In addition, more malondialdehyde (MDA), ascorbate peroxidase (APX), and proline were observed in plants experiencing lead stress compared to control plants. In this study, conditions of tolerance of this species to lead were determined and enzyme activity values were determined.

Keywords: soybean, Pb stress, MDA, antioxidant enzyme, chlorophyll ratio.

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INTRODUCTION

Environmental pollution, which started with the onset of industrialization in the world, is considered as one of the most important issues faced globally nowadays, causing damage to natural resources, especially to soil and water. Pollution has reached hazardous levels in numerous countries. Pollution by heavy metals, considered to be the main environmental pollutants (Waisberg et al. 2003) originating mainly from industrial wastes, is defined as the presence of a metallic element in a relatively high concentration and toxic even at lower concentrations (Leentech 2004). Heavy metals are considered as the most hazardous materials (Vanli, Yazgan 2006). Toxic heavy metals entering the body of plants, animals, and humans from the food chain can cause genetic material changes due to their mutagenicity and consequently lead to carcinogenic consequences (Yang et al. 2005). The yield of plants that grow under heavy metal stress is negatively affected. These adverse effects in plants occur through the influence on their metabolism, physiology, and biochemical properties. Heavy metal stress reduces the photosynthetic activity of plants and accelerates aging. Heavy metals usually cause plant roots to develop shorter, thinner or fewer. When a heavy metal reaches very high concentrations, it causes plant death (Sakli 2011).

Free radical formation of plants is promoted under severe metal stress, resulting in some damage to plant tissues and oxidative damage to plants. This leads to the generation of excess reactive oxygen species (ROS). Excessive production of ROS damages lipids, nucleic acids, and proteins. Plants have diverse antioxidant molecules (ascorbate, glutathione, a-tocopherol) and enzymes (SOD, POD, and CAT) that preserve themselves against oxidative harm. Antioxidant enzymes play a big role in the defensive mechanism that is triggered by heavy metal stress in plants (Unalan 2010, Ahmad et al. 2017).

Lead (Pb) has been reported to be the largest environmental pollutant of concern worldwide (Salt et al. 1995), and one of the most severe toxic heavy metals (Okcu et al. 2009). The toxic effects of Pb are primarily caused by its ability to react with sulfhydryl, carboxyl, and amine groups, thereby interfering with the activity of many enzymes significant for cell tasks, and leading to a reduction in yield and loss of microbial activity in the soil (Salt et al. 1995, Majer et al. 2002).

Other than these, more lead is retained by the roots than exerted from them. This reduces root development, while the imbalance in the intake of cations and anions negatively affects the nutrient uptake (Sharma, Dubey 2005). In order to combat these destructive effects caused by oxidative damage, plants take advantage of oil-soluble and membrane-bound antioxidants, water-soluble antioxidants (glutathione and ascorbate which play a role in the detoxification of O_2 and H_2O_2), and enzymatic antioxidants (superoxide dismutase SOD, peroxidase POX and ascorbate peroxidase APX).

The aim of this study was to determine the effect of lead stress on some growth parameters and some important enzyme levels of two common soybean varieties in Turkey.

MATERIAL AND METHOD

The research was carried out according to the Factorial Trial in a Completely Randomized Experimental Design with six replications in a fully controlled aeroponic climate chamber in the Department of Field Crops laboratory, Faculty of Agriculture, Van Yuzuncu Yil University, Turkey. In the study, Arısoy and Atakişi varieties, which are the most grown soybean varieties with high yield performance and resistance to stress conditions, were used as material. The Atakisi cultivar, one of the cultivars analyzed in the study, is genotypically more resistant to stress conditions than other soybean cultivars. The seeds were treated with 5% sodium hypochlorite for 10 min and then sterilized by washing them 3 times with de-ionized water (dl-H_2O). The seed material was then washed with sterile water and sterilized, then placed in a glass pot with 50% super large perlite (0-0.5 mm) and 50% peat and allowed to germinate at 26°C for 4-6 days. Seeds were watered by a Hoagland solution of 50-100 ml of half-strength (1:1 diluted Hoagland solution with deionized water) every two days from the beginning of germination. For every treatment, six seedlings were transplanted in an aeroponic cabinet that contained six replications (pots). Each pot was 8 cm in diameter. Thus, the experiment was carried on with 10 aeroponic cabinets in a fully controlled laboratory. The plants were irrigated with 1/5 Half Hoagland solution in the aeroponic system containing lead ($PbSO_4$) at doses of 0 (C), 25 (D1), 50 (D2), 75 (D3), 100 (D4) mg L^{-1} for 10 days after 25 days of planting. Ten days after the start of heavy metal applications, soybean plants were removed from the pots to determine the fresh root and shoot weights, dry root and shoot weights, leaf area index, chlorophyll, MDA (Malon Di Aldehyde), and levels of enzymes (SOD, CAT, APX (ascorbate peroxidase). Four leaves taken randomly from each plant were stored at -80°C until analysis. Then, 0.5 g samples were taken from each plant for analysis.

Analysis of chlorophyll

The chlorophyll content was determined with a portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan), which indirectly assessed the amount of chlorophyll in leaves, with 4 replications per plant.

Lipid peroxidation

The method described by Lutts et al. (1996) was used to define the proportion of malondialdehyde (MDA), a product of lipid peroxidation, which can

be perceived as an indicator of cell membrane damage. According to this method, 5 ml of 0.1% trichloroacetic acid (TCA) was added to a 0.5 g of leaf sample immersed in liquid nitrogen, and the mixture was centrifuged at 12 500 rpm for 20 minutes. 3 ml of the supernatant was taken from the 5 ml extract, and 3 ml of 0.1% TCA containing 20% thiobarbituric acid (TBA) was added. The mixture was allowed to stand in a hot water bath at 95°C for 30 min, after which the reading of absorbance values at A532 and A600 nm on a PC T-90 model spectrophotometer was carried out.

Spectrophotometric enzyme activities

To examine the change in enzyme activities that might occur in the plants under lead stress, an 0.5 g leaf sample was ground in a porcelain mortar containing fluid nitrogen and then homogenized with 10 mM phosphate buffer solution (pH:7.6) containing 50 mM of 0.1 mM Na-EDTA. The homogenized samples were centrifuged at 15 000 rpm for 15 min, and then the fluids obtained were used for enzyme analysis. The samples in which the enzyme activities were to be determined were kept at +4°C until measurement. Measurements were carried out on a spectrophotometer. Enzymatic activity analyses were performed in four replications. Superoxide dismutase (SOD) activity was performed according to the method of reducing NBT (nitro blue tetrazolium chloride) by light O_2. Accordingly, ascorbate peroxidase (APX) activity was based on the cleavage rate of H_2O_2 at 290 nm (E=2.8 mM cm^{-1}), catalase activity (CAT) at 240 nm (E=39.4 mM cm^{-1}) (Cakmak, Marschner 1992, Cakmak 1994).

Analysis of proline

An 0.5 g of the fresh plant sample was dissolved in 3% sulfosalicylic acid and then centrifuged. 2 ml of acetic acid and 2 ml of ninhydrin reagent (ninhydrin, acetic acid, and orthophosphoric acid) were added to the sample taken from the centrifuged sample. Then, the samples were placed in tubes and held in a water bath at 100°C for 1 h and the reaction was terminated in ice. The cooled samples were vortexed with 4 ml toluene and read at a spectrophotometer at 520 nm. It was then analyzed using proline standards (Bates et al. 1973).

Leaf area index (LAI)

Four leaves were selected from each pot. Selected leaves were measured and averaged with the Easy Leaf Area software. Total counts of green leaf pixels and red calibration pixels are used to estimate the leaf area from: leaf area = (green pixel count) × (calibration area/red pixel count). And then, the leaf area index was calculated as a function of net leaf area per plant and the population density/unit area with the following formula suggested by Board (2000).

$$\text{LAI} = \frac{\textit{total net leaf area of the plant (cm?)}}{\textit{total area covered by the plant (cm?)}}.$$

Analysis of statistics

Statistical analyses of the data variance analyses of the data obtained from the study were performed according to the Factorial Trial in a Randomized Experimental Design with six replications. The results of the analysis of variance were evaluated according to the *F* test (IBM SPSS 22.0) and the significant differences were compared and grouped according to the LSD (Least Significant Difference) multiple comparison test (Costat v: 6.3).

RESULTS AND DISCUSSION

Fresh root and shoot weight

Table 1 shows the data obtained from the study conducted to determine the response of soybean varieties to different lead doses. As seen from Table 1, in terms of the fresh root and shoot weights, there was no statistically significant ($P>0.05$) difference between the varieties. Moreover, the differences in fresh root and shoot weight values obtained after lead application were statistically nonsignificant ($P>0.05$). Therefore, the fresh root and shoot weight values were ranged within 2.84 - 3.08 g in roots, and 5.04 - 5.46 g in shoots (Table 1). In some previous studies on lead dose application to different plants, Çolak, Dogan (2011) reported that wheat had the lowest fresh stem at the highest Pb dose application. Ayhan et al. (2007) stated that Pb concentrations had a negative effect on the growth of corn plants. Güler (2011) reported that Pb in increasing doses reduced root and leaf growth on corn and sunflower plants. Kiran et al. (2015) declared that there was a decrease in the amount of stem, leaves, and root weight after lead application to curly salad plant compared to the control. Contrary to our findings, Çolak (2009) reported that the highest Pb dose applied to wheat plants resulted in the lowest plant fresh weight. This is thought to be due to the fact that different plants or even genotypes tend to respond differently to the applications of this metal.

Dry root and shoot weight

As a result of the measurements, it was found that there were no statistically significant ($P>0.05$) differences between the varieties in terms of dry root and shoot weights (Table 1). Moreover, the differences between Pb doses in terms of dry root and shoot weights were not statistically significant ($P>0.05$). In the study, it was observed that the interaction of varieties and lead doses had an effect on dry root weight. Although, when the results obtained were evaluated statistically, it was determined that the effect of the

Table 1

Fresh and dry root and shoot weights (g), leaf area index (g), and chlorophyll ratio of soybean varieties treated with lead at different doses

Variation Sources		Pb doses	Fresh root	Dry root[I]	Fresh shoot	Dry shoot	Leaf area[II]	Chlorophyll[II]
			(g)	(g)	(g)	(g)	index (LAI)	(μmol m^{-2})
Varieties (V)	Arısoy	control	2.83	0.230	4.96	0.910	4.72	46.57
		D1	2.73	0.230	5.48	0.940	5.24	45.94
		D2	2.93	0.250	5.02	0.860	4.18	46.12
		D3	2.89	0.260	5.29	0.970	5.30	46.67
		D4	2.83	0.190	5.41	0.920	5.08	45.90
		mean	**2.84**	**0.230**	**5.23**	**0.920**	**4.90B**	**46.24A**
	Atakişi	control	3.01	0.230	5.13	0.970	5.68	44.42
		D1	3.22	0.250	5.44	0.960	5.50	44.50
		D2	2.74	0.220	5.40	0.940	5.57	44.22
		D3	2.85	0.230	5.53	1.000	5.37	44.12
		D4	3.33	0.270	5.32	0.910	5.40	43.98
		mean	**3.03**	**0.240**	**5.36**	**0.960**	**5.50A**	**44.25B**
LSD for V (0.05)			**ns**	**ns**	**ns**	**ns**	**0.426**	**0.370**
Pb doses		control	2.92	0.230*c*	5.04	0.940	5.20	45.50
		D1	2.97	0.240*b*	5.46	0.950	5.37	45.22
		D2	2.84	0.240*b*	5.21	0.900	4.88	45.17
		D3	2.87	0.250*a*	5.41	0.980	5.33	45.39
		D4	3.08	0.230*c*	5.36	0.910	5.24	44.94
		mean	**2.94**	**0.240**	**5.30**	**0.940**	**18.22**	**45.25**
LSD for Pb (0.05)			**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
MS for V			0.797	0.003	0.385	0.031	8.106**	89.421**
MS for Pb			0.163	5.989	0.519	0.020	0.696	0.818
MS for V x Pb			0.436	0.009**	0.171	0.005	1.411	0.730

MS – mean square, ** 1% significant level

[I] There is no statistically significant (1%) difference between the means shown with the same *italic* and lowercase letters in the same column.

[II] There is a statistically significant (1%) difference between the means shown with the same capital **bold** letters in the same column.

varieties x Pb dose interactions on the dry root was very important (P<0.01) because of the different responses of the cultivars to Pb doses due to genetic differences (Figure 1). As a result, the observed dry root and shoot weights were determined at 0.230 g and 0.240 g for roots, and 0.920 g and 0.960 g for shoots of the Arısoy and Atakişi varieties, respectively. Since the effect of lead doses on dry root weight is significant, it was determined that the

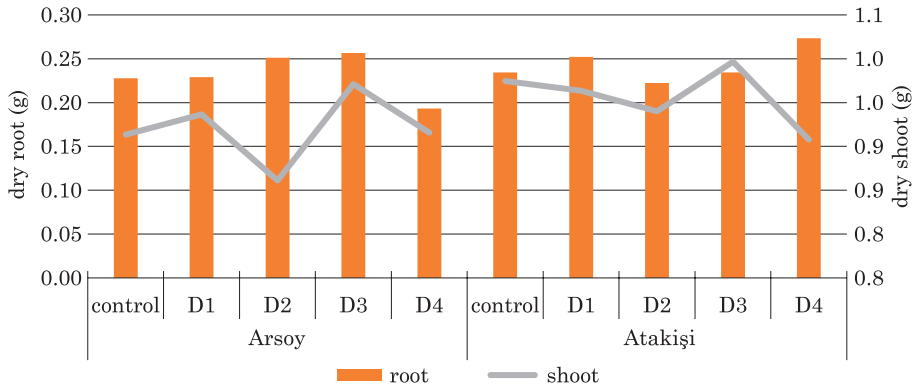

Fig. 1. Variety x lead interaction of dry root and shoot mean weight values of soybean varieties applied lead at different doses

highest dry root weight (0.250 g) was observed from the D3 lead dose, while the lowest was observed from control (0.230 g) and D4 (0.230 g) applications. Based on the averages according to the lead doses, it was determined that the dry shoot weights ranged from 0.900 to 0.980 g. Lead is known to inhibit plant growth and development, causing changes in physiological and morphological characteristics of plants under lead stress. Non-specific symptoms of lead toxicity are inhibition of root growth, dwarf growth, and chlorosis (Burton et al. 1984). However, lead concentrations do not pose hazard to many plant species unless they exceed 150 mg kg^{-1} in soil (Durust et al. 2004).

The results are consistent with the results of Porter, Sheridan (1981), who reported that there was no physical evidence of lead toxicity from the application of 100 mg mL^{-1} Pb to alfalfa, and the results of Taylor, Allinson (1981), who reported that lead doses had no significant effect on the yield of the plant. However, application of lead doses exceeding 300 mg kg^{-1} significantly harmed plant health.

Leaf area index (LAI)

Table 1 indicates that there was a statistically significant difference between the varieties used in the experiment at the level of 1%, while there was no statistically significant (P>0.05) difference between lead doses. In the study, the interacting effect of lead doses and varieties on the leaf area index was irregular and insignificant (P>0.05). In the study, the highest leaf area index was calculated at 5.50 from the Atakişi variety. Based on the averages according to the lead doses, it was determined that the calculated leaf area index ranged from 4.88 to 5.37. The results are in line with the values of leaf area index determined before pod tying previously reported in soybean by Kizilgeçi et al. (2021).

Chlorophyll (SPAD) measurement

In this study, chlorophyll amounts were determined by spectrophotometric measurements in homogenates of two soybean cultivars treated with lead at 0, 25, 50, 75, and 100 mg L^{-1} doses. As a result of the measurements, it was found that there were significant ($P<0.01$) differences between the varieties in terms of chlorophyll amounts in homogenates, and it was observed that they formed different groups (Table 1). But there were no statistically significant ($P>0.05$) differences between lead doses and between the interactions of lead doses and varieties.

As seen in Table 1, the highest chlorophyll amount was measured as 46.24 from the Arısoy variety. In terms of the averages according to the lead doses, it was found that the measured chlorophyll amounts ranged from 44.94 to 45.50. It is known that high concentrations of lead disrupt the structure of chloroplasts, reduce the rate of photosynthesis, reduce chlorophyll synthesis, and inhibit the electron transport system and the enzymes involved in the Calvin cycle (Ewais 1997). In their study, Miranda, Ilangovan (1996) applied lead at 30, 50, 100, and 200 mg L^{-1} concentrations to *Lemna gibba* L. and no significant chlorotic symptoms were seen in the plants as a result. However, they reported significant and evident symptoms at 500 mg L^{-1} lead concentrations and also a reduction in the total chlorophyll rate. In this study, it was found that lead concentrations do not have a negative effect on chlorophyll structure in soybeans.

Malondialdehyde (MDA)

The effect of lead application on soybean cultivar MDA values was found to be statistically significant ($P<0.01$), while there was no statistically significant ($P>0.05$) difference between the varieties and between the interactions of lead doses and varieties (Table 2). In the study, the highest MDA value was determined at 23.68 from the D1 lead dose while the lowest value was obtained from the control treatment. In terms of the averages according to the varieties, it was observed that the values of MDA ranged from 22.22 to 22.03. In previous studies carried out under the conditions of lead stress, the researchers reported that MDA levels increased in wheat (Çolak, Doğan 2011) and paddy (Verma, Dubey 2003). Kiran et al. (2015) who investigated the effect of lead doses (0, 150, and 300 mg kg^{-1}) on morphological and biochemical properties of curly salad plant, and stated that the highest MDA was determined after the 150 mg kg^{-1} lead application whereas the lowest MDA value was obtained from the control plot.

Ascorbate peroxidase (APX)

APX enzyme activity values obtained from roots of the Arısoy and Atakişi soybean varieties revealed that this parameter increased significantly compared to control plants (Table 2). As the data in Table 2 concerning APX show, the differences between the doses of lead were found to be statistically

Table 2

MDA, APX, CAT, prolin, and the SOD ratio of soybean varieties treated with lead at different doses

Variation Sources		Pb doses	MDA[I]	APX[I]	CAT[I; II; III]	Proline	SOD
Varieties (V)	Arısoy	control	19.34	1.390	0.125ab	1.900	124.4
		D1	24.53	1.720	0.045b	1.230	115.0
		D2	23.91	2.040	0.047b	1.500	115.7
		D3	22.47	3.470	0.035c	2.020	116.9
		D4	20.87	3.850	0.049b	1.440	119.9
		mean	**22.22**	**2.490**	**0.060B**	**1.620**	**118.4**
	Atakişi	control	22.25	1.960	0.113b	3.100	122.3
		D1	22.82	2.500	0.089b	2.660	121.5
		D2	19.86	2.850	0.264a	1.930	118.8
		D3	22.21	2.560	0.115b	0.800	115.8
		D4	23.03	3.420	0.055b	0.710	121.5
		mean	**22.03**	**2.660**	**0.127A**	**1.840**	**120.0**
LSD for V (0.05)			**ns**	**ns**	**0.043**	**0.653**	**3.732**
Pb doses		control	20.80*b*	1.670*c*	0.119*ab*	2.500	123.4
		D1	23.68*a*	2.110*c*	0.067*b*	1.950	118.3
		D2	21.89*ab*	2.450*bc*	0.155*a*	1.710	117.3
		D3	22.34*ab*	3.020*ab*	0.075*b*	1.410	116.4
		D4	21.95*ab*	3.630*a*	0.052*b*	1.080	120.7
		mean	**22.13**	**2.580**	**0.094**	**1.730**	**119.2**
LSD for Pb (0.05)			**2.571**	**0.813**	**0.068**	**ns**	**ns**
MS for V			0.830	0.608	0.067**	0.379	49.214
MS for Pb			19.936**	10.615**	0.022*	1.760	85.093
MS for V x Pb			36.396	2.784	0.025*	2.034	30.422

MS – mean square; * 5% significant level, ** 1% significant level

[I] There is no statistically significant (1%) difference between the means shown with the same *italic* and lowercase letters in the same column.

[II] There is no statistically significant (5%) difference between the means shown with the same lowercase letters in the same column.

[III] There is a statistically significant (1%) difference between the means shown with different capital **bold** letters in the same column.

significant ($P<0.01$), while the difference between the varieties and between the interactions of lead doses and varieties were found to be statistically nonsignificant ($P>0.05$). As a result, the highest APX value was observed at 3.630 from the D4 lead dose while the lowest value was measured from control and D1 treatments at 1.670 and 2.110, respectively. Based on the

averages according to thevarieties, it was determined that the values of APX ranged from 2.490 to 2.660. It is certain that excessive metal uptake has an effect on the activities of antioxidant enzymes. However, it cannot be generalized that this effect will be an increase or decrease in enzyme activities. Verma, Dubey (2003) investigated the effects of different lead element doses on enzyme activities in rice plants, and reported an increase in APX activity in response to Pb stress, htus supporting the results of our study.

Catalase (CAT) activity

As a result of the analyses of the homogenates prepared from the roots of two soybean varieties, significant differences ($P<0.01$) were found between the varieties in terms of CAT values, and it was determined that they formed different groups, with the highest CAT value at 0.127 determined for the Atakişi variety (Table 2).

As observed in Table 2, the differences between the doses of lead were found to be statistically significant ($P<0.05$). In terms of lead doses applied, the highest CAT value was obtained from the D2 lead dose, while the lowest values were observed in D1, D3, and D4 at 0.067, 0.075, and 0.052, respectively. In the study, in terms of CAT values, significant differences emerged between the interaction effects of the two factors, as the varieties reacted differently to lead doses due to genetic differences. In this regard, the highest CAT value was obtained from the Atakişi variety x D2 lead dose treatment at 0.264, while the lowest value was obtained from Arısoy variety x D1 lead dose treatment at 0.035. Verma, Dubey (2003) investigated the toxic effect of lead on the enzyme activity of rice plants, they reported that increased lead concentrations reduce the activity of CAT enzyme in the roots of the plant. It was determined that these results partially overlapped with the results of the study in which we determined that CAT enzyme activity decreasing after reaching the 50 mg L^{-1} concentration in soybean plants. Dey et al. (2007) reported that increased lead element concentrations decreased CAT activity in the root and stem of wheat (*Triticum aestivum*). Islam et al. (2008) reported significant decreases in CAT activity in leaves of two ecotypes of the curry plant (*Elsholtzia arginine*) with increasing lead doses.

Proline

As a component of the non-specific defense systems against lead poisoning, proline acts as a metal chelating agent and protein stabilizer to alleviate metal toxicity (Sharma, Dubey 2005). In this study, it was determined that the differences between the varieties, between the applied lead doses, and between their interaction in terms of proline values measured in the leaves of two soybean cultivars were nonsignificant ($P>0.05$). As seen in Table 2, the proline values were determined at 1.620 in Arısoy and 1.840 in Atakişi. In the study, the average values of proline obtained according to the lead doses ranged from 1.080 (D4 lead dose) to 2.500 (control treatment). It is

known that respondes to heavy metal stress vary widely in many plant species due to the genotypic structure. Therefore, contrary to our study results, Çolak (2009) reported that the values of proline in the green parts of wheat were 0.50, 1.25, and 0.93 (µmol g^{-1} T.A). He stated that the proline content increased in response to Pb applications and the highest proline content was obtained after the application of a 10 mg L^{-1} Pb dose.

Superoxide dismutase (SOD) activity

SOD, which is an antioxidant enzyme, generally has high activity at a low Pb concentration, but it has been found to exhibit different responses to increasing lead doses, first decreasing and then increasing. In the study, it was determined that the varieties, lead doses, and the interaction of the two factors did not have any statistically ($P>0.05$) significant effect on the SOD activity values of the leaves of the two soybean varieties (Table 2). As seen in Table 2, it was determined that the values of SOD were measured at 118.4 in Arısoy and 120.0 in Atakişi. Based on the averages according to the lead doses, the SOD activity values ranged from 116.4 to 123.4. Islam et al. (2008) reported significant decreases in the SOD activity in leaves of two ecotypes of the curry plant (*Elsholtzia arginine*) with increasing lead doses.

CONCLUSION

In this study, the effect of lead heavy metal, which is an important source of abiotic stress in many plants, on some growth parameters and some important enzyme levels of soybean was investigated. As a result of increasing doses of Pb applications, morphological features such as fresh and dry root weights, leaf area index, and chlorophyll contents decreased at varying rates. The results clearly showed that there were differences in superoxide dismutase (SOD), catalase activity (CAT) and ascorbate peroxidase (APX) enzymes, and malondialdehyde (MDA) contents in plants as a result of lead exposure. In addition, it was thought that the reason for the limited effect of lead heavy metal in this study was due to the dose applications of soybean within the tolerance limits.

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