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ORIGINAL PAPER

EFFECTS OF LEAD DOSES ON THE MINERAL CONTENT AND ANTIOXIDANT CAPACİTY OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.)

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Abstract

Heavy metals are a very important abiotic stress factors that can induce different response mechanisms in plants. 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, enzymes, and the content of macro- and micro-nutrients. This study was launched to determine the effects of lead (0, 25, 50, 75, 100 mg L^{-1}) on fenugreek's (*Trigonella foenum-graecum* L.) growth parameters and biochemical responses in a fully controlled aeroponic climate chamber. Growth, biochemical enzyme activities and the content of macro- and micro-nutrients of fenugreek plants changed under heavy metal stress. In the study, a decrease occurred in parameters such as the plant height (cm), root length (cm), root and stem fresh weight (g), leaf weight (g) and leaf number (number). There were increases in malondialdehyde (MDA) and ascorbate peroxidase (APX) enzymes. There was a decrease in the content of minerals (Ca, K, Mg, Na, Cu, Ni, Fe, Zn, Mn, Cr, Ni, Se and Cd) in leaves, stem and root parts of fenugreek plants. In this study, lead tolerance conditions, the plant content of macro- and micro-nutrients and enzyme activity values of this plant were determined.

Keywords: ascorbate peroxidase, Pb stress, fenugreek, malondialdehyde.

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INTRODUCTİON

Turkey has quite a wealth of plant diversity. There are 10 754 plant taxa in the natural flora, of which 34% are endemic plants (VURAL 2003). Approximately 1000 of these plants are used for aromatic purposes in medicine. Plants are especially used in modern medicine and folk medicine for making medicines. Today, they are also processed in the food industry, perfumery and cosmetic industry, to make toothpaste, soap, leather, or paints, or grown as ornamental plants; they are also used to make a wide range of insecticides. The production of these plants is largely based on collection from nature (Tunçtürk et al. 2020). About 100 medicinal and aromatic plant species (such as thyme, laurel, sage cumin, coriander, carob, capers, cumin, fennel, licorice, oğulotu) are exported. The exports of these products from Turkey have been increasing considerably in recent years.

Fenugreek (*Trigonella foenum-graecum* L.) is an example of a plant that is widely used in alternative medicine and for aromatic purposes, but too little is known about it yet. It is used in many countries in Asia for its health benefits. Fenugreek is native to Asia Minor (Anatolia) and Iran, but it grows in the wild all over the world. Fenugreek seeds are very rich in mineral substances, proteins and vitamins. Its seeds also contain linoleic acid and oleic acid (40% and 52% of fatty acids, respectively), essential oils, nitrogen compounds, choline, nicotine amide, rutine, ash (3-4%), trigonelline (1%), flavonoids, mucilage (30%), sentionine, and also alkaloids and a phytosteroid sapogenin diosgenin, the product of hydrolysis (0.8-2.2%), and vitamins A, B and C, as well as calcium, and minerals such as iron. Steroidal saponins found in fenugreek seeds have been reported to be used for medicinal purposes (Mebey et al. 1988, Acharya et al. 2008). Fenugreek (seeds and leaves) is a well-recognized component of human food, owing to its prohealthy properties and its culinary effect on food flavor, color and texture. There are also numerous non-food uses of fenugreek. The research on the effect of abiotic stress conditions on plant growth and development contributes to the overall knowledge on plant defense or tolerance mechanisms when grown in stress conditions.

In many countries, serious contamination of soil and water resources has occurred. Heavy metals are at the forefront as the main pollutants (Waisberg et al. 2003). The presence of a high or low concentration of an element in this type of contamination is likely to occurdue to industrial waste (Yang et al. 2005). Among these elements, heavy metals are considered the most dangerous. They enter the human body through plant and animal products in the food chain.

In plants, physiological and biochemical activity is negatively affected under heavy metal stress. By reducing photosynthesis, it stmulates the growth and development of stems but results in shorter and weaker roots. Heavy metals in high concentrations cause death of plants (Yang et al.

2005). The formation of free radicals in plants under heavy metal stress leads to oxidative damage. In consequence, lipids, nucleic acids and proteins are damaged, which is caused by reactive oxygen species.

In another study, it has been determined that lead (Pb) is the most dangerous and widespread pollutant on earth (Kulaz et al. 2021). Due to its ability to react with sulfidryl, carboxyl and amine groups, lead disrupts the structure of many enzymes used in the cosmetic industry and in such plant-based products as herbal teas, spices, toothpaste, soap, leather, painting, ornamental plants and a wide range of insecticides. Lead causes yield losses by disrupting microbial activity in soil (Majer et al. 2002). Lead is one of the most abundant heavy metals in the roots of plants. It inhibits root development and distorts anion and cation exchange (Sharma, Dubey 2005).

The aim of this study has been to determine the effect of lead stress on some growth parameters, content of macro- and micro-nutrients and levels of some important enzymes in the Güraslan fenugreek variety, which is common in Turkey.

MATERIAL AND METHOD

The research was carried out in 2019, in a fully controlled plant growth room belonging to the Department of Field Crops, Faculty of Agriculture, Van Yüzüncü Yıl University. In the study, the Gurarslan fenugreek variety was used.

The experiment was conducted in 2-liter plastic pots filled with as a mixture of 1/3 sand, 1/3 perlite and 1/3 soil, and set up according to a random plot trial pattern with 3 replications. In the study, five different lead doses were tested (0, 25, 50, 75, 100 mg L^{-1} $PbSO_4$). The seeds were first sterilized for 15 min in a 5% sodium hypochlorite (NaClO) solution. Then, they were washed several times with pure water and brought to readiness for sowing in October. In the study, 3 seeds were planted in each pot and the best seedling was left in every pot (Tunçtürk et al. 2020) Afterwards, 5 weeks after sowing the seeds (31th October), the pots are placed in a climate-controlled chamber with a temp set at 25°C and humidity at 65%, with the 16/8 h light/ dark photoperiod. Pb stress was applied to plants. Hoagland solution was prepared in a 1:5 ratio. The pH of this solution was adjusted to 7.0. Except for the control plots, the other objects were administered doses of this solution at 3-day intervals. In total, 200 ml of solution was given to the plants during five applications. The experiment was terminated by sampling the plants on the 50th day (7th week). The plants were removed from the pots together with their roots, after that the roots were separated, and the aerial parts (after taking samples necessary for mineral substance analysis) were stored in a deep freezer at -80°C to be used in biochemical analyses (malondialdehit and ascorbate peroxidase). Other basic growth parameters such as

the plant height, root length, root and stem fresh weight, number of leaves and leaf weight were measured, while the content of several elements (Ca, K, Mg, Na, Cu, Ni, Fe, Zn, Mn, Pb, Cr, Ni, Se and Cd) was determined in root, leaf and stem parts. For the mineral element analysis, samples of the first three leaves from the tip were taken, placed in lidded glass jars and stored in a deep freezer at -40°C until analysis. For the analysis of ions, an amount 200 mg of each leaf sample stored in the freezer was weighed and 10 mL of 0.1 N nitric acid (HNO_3) was added to it. The samples, which were kept in lidded plastic boxes in a dark environment at room temperature for one week, were then shaken for 24 h in a shaker, and the K^+ and Ca^{+2} ions in the prepared extracts were determined by the flame photometric method (an Eppendof flame photometer), and the ion concentrations in the wet leaf sample were determined (Taleisnik et al. 1997). Samples for analyses of Na, Mg, Fe, Zn, Mn, Cu, Pb, Cr, Cu, Ni, Pb and Se were prepared by wet digestion and the readings were determined with the help of an atomic absorption spectrometer (AAS). This was done according to the following method (Lutts et al. 1996): a portion of 200 mg was weighed from the leaf samples stored in the deep freezer, 5 mL of 0.1% trichloroacetic acid (TCA) was added to the plant mass and centrifuged at 12 500 rpm for 20 minutes. 3 mL of filtrate were taken from the extract and 3 mL of 0.1% TCA containing 20% thiobarbutyric acid (TBA) were added. After the mixture was kept in a hot water bath at 95°C for 30 min, absorbance values were read on a spectrophotometer adjusted to 532 and 600 nanometer (nm) wavelengths. Ascorbate peroxidase activity, that is the reduction of hydrogen peroxide (H_2O_2) bound to ascorbic acid, was measured at a wavelength of 290 nm. A mixture of 50 mM phosphate buffer (KH_2PO_4), 0.5 mM ascorbic acid, 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1.5 mM H_2O_2 were used as the reaction solution (pH 7.0). 3 mL of the reaction solution were mixed with 0.2 mL of plant extract. 0^{th} and 60^{th} seconds readings were taken at 290 nm wavelength on the spectrophotometer. The reaction was initiated by the addition of 0.2 mL of enzyme extract. Evaluation was made by considering the change in absorbance within 1 min (Sairam et al. 2005).

Statistical processing of the variance analyses of the data obtained from the study was performed according to the Factorial Trial in a Randomized Experimental Design with six replications. The results 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

Plant height (cm)

In Table 1, values of the plant height of fenugreek plant exposed to different lead (Pb) doses are given. According to the results, the difference between the plant height values relative to the increasing lead (Pb) doses was found to be statistically significant at the level of 5%. The highest plants

Table 1

The effect of Pb doses on changes in some morphological and biochemical properties of fenugreek

Pb Doses	Plant height (cm)	Root length (cm)	Root fresh weight (g)	Stem fresh weight (g)	Leaf fresh weight (g)	Leaves number (number)	MDA (nmol g^{-1})	APX (µmol g^{-1})
Control (0)	14.6 *ab*	20.4 *a*	0.665	0. 84	2.283 *a*	24.16 *a*	3.05 *e*	0.196 *d*
Pb-25	15.8 *a*	18.6 *b*	0.646	0.78	2.246 *b*	23.25 *ab*	3.75 *cde*	0.223 *c*
Pb-50	13.2 *b*	18.2 *b*	0.613	0.70	2.245 *b*	22.58 *b*	4.00 *b*	0.544 *b*
Pb-75	13.3 *b*	17.7 *c*	0.594	0.73	2.339 *bc*	20.75 *b*	4.43 *ab*	0.583 *b*
Pb-100	11.8 *c*	16.7 *d*	0.537	0.68	1.916 *c*	19.08 *c*	4.34 *a*	0.776 *a*
Average	13.6	18.3	0.611	0.75	2.205	21.96	3.91	0.464
Pb doses (Pb)	*	*	ns	ns	**	**	**	**
CV (%)	11.6	9.8	23.5	19.8	16.22	19.6	16.3	13.4

MDA – malondialdehit; APX – ascorbate peroxidase, ns – not significant, * significant at $P<0.05$ level, ** significant at $P<0.01$ level, and there is no statistical difference between the means indicated by letters.

(approximately 15.8 cm) were found after applying the 25 mg L^{-1} lead dose, and the lowest 11.8 cm ones grew at 100 mg Pb L^{-1}. Along with increasing lead (Pb) doses, a decrease in the plant height of fenugreek was observed. Simirality, permanent damage may occur in every plant species exposed to lead depending on the metal's dose. After exposure to 15 mM Pb, a 28% and 29% reduction in fresh and dry weight in wheat and spinach plants, respectively, was observed (Lamhamdi et al. 2013). In the study in which branched millet was exposed to 5 different lead doses (0, 30, 60, 90, 120 mg kg^{-1}), it was determined that the plant height was between 15.2-23.7 cm, and the increasing lead doses decreased the plant height (Alacabey, Çelebi 2020). In similar studies, it was stated that some heavy metals, such as lead, have effects on plants even at low doses, while causing growth and metabolic disorders in many plants when present in high concentrations (Claire et al. 1991, Fernandes, Henriques 1991).

Root length (cm)

The effect of different lead (Pb) doses on the root length of fenugreek was found to be statistically different at the level of 5% (Table 1). Root length

values varied between 16.7-20.4 cm. The largest root length of 20.4 cm was obtained in the control group, while the lowest one, 16.7 cm, was measured after the application of 100 mg L^{-1}. It was determined that lead doses the root length of fenugreek plants.

In a study conducted by Dere, Doğan (2020) involving different lead doses (0, 10, 100 and 1000 mg L^{-1}), it was stated that the root length of peanuts decreased by 10.7- 35.2%. Ayhan et al. (2007) stated that different Pb concentrations in maize plant showed significant differences in the coleoptile and root growth of the experiental plants compared to the control, and heavy metal applications negatively affected the plant's growth. In similar studies, depending on a plant species and variety, the toxicity of Pb at certain concentrations was found to inhibit seed germination and decrease the root-stem length and weight (Peng et al. 2005, Kıran et al. 2014). Eun et al. (2000) stated that heavy metals, such as lead (Pb), disrupt cell division in plants and negatively affect the growth and development of plants.

Root fresh weight (g)

It was determined that the effect of lead (Pb) doses on root fresh weight in fenugreek was statistically insignificant. Depending on the increased lead doses in fenugreek, the fresh root weight varied between 0.537-0.665 g (Table 1). Kulaz et al. (2021), in their study on soybean, stated that the root fresh weight ranged between 2.84 and 3.08 g depending on the lead doses. In a study on curly lettuce, lead doses decreased the plant's root fresh weight (Kıran et al. 2014). Çolak (2009) reported that lead doses applied to wheat significantly reduced root fresh weight compared to the control group. It is thought that differences between the findings is due to the different responses of different genotypes under different ecologies conditions.

Stem fresh weight (g)

The effect of different doses of lead (Pb) on the stem fresh weight of fenugreek was found to be statistically insignificant. Depending on the increased lead doses in fenugreek, the fresh stem weight varied between 0.68-0.84 g (Table 1).

According to the findings obtained in this study, it was determined that increasing doses of lead (Pb) decreased fresh weight of stems compared to the control group. In the study on peanuts conducted by Dere, Doğan (2020), it was stated that increasing lead (Pb) doses reduced the stem and leaf fresh weights. Weight gain in plants is generally accepted as growth. Many researchers have reported that stress caused by heavy metals leads to a decrease in plant growth in general, depending on a plant species and variety (Ouzounidou et al. 1997, Vitoria et al. 2001). Permanent damage may occur in every plant species exposed to lead depending on the metal's dose. After exposure to 15 mM Pb, there was a 28% and 29% reduction in fresh

and dry weight in wheat and spinach plants, respectively (Lamhamdi et al. 2013).

Leaf fresh weight (g) and number of leaves (number)

As seen in Table 1, the effect of different lead (Pb) doses on leaf fresh weight and number of leaves in fenugreek was found to be statistically significant at the 1% level. The fresh leaf weight of fenugreek plant varied between 1.916-2.283 g, and the number of leaves varied between 19.08-24.16. The highest fresh leaf weight and number (2.283 g, 24.16 units respectvly) were obtained from the control group, and the lowest value (1.916 g, 19.08 number) was obtained from the application of 100 mg L^{-1} lead (Pb). It was determined that increasing lead doses in both parameters significantly decreased leaf fresh weight values and number. Güler (2011) reported that increasing doses of lead (Pb) applied to corn and sunflower plants reduced leaf growth. It was determined that increasing lead doses in lentils decreased leaf formation. In addition, lead caused losses in leaf fresh and dry weights caused by chlorosis, necrosis and wilting (Jana, Barua 1987).

Malondialdehyde (MDA) and ascorbate peroxidase (APX) activity

The effect of lead (Pb) doses on lipid peroxidation (malondialdehiyde) and activity of ascorbate peroxidase (APX) in fenugreek was found to be statistically significant at the 1% level (Table 1). According to the findings, increases in both parameters were detected in parallel with the increasing lead (Pb) doses. The amount of lipid peroxidation, which is an indicator of membrane-induced damage in plant cells, ranged from 3.05 to 4.34 nmol g^{-1}. While the lowest malondialdehyde value was obtained from the control group and the highest level was measured in 100 mg L^{-1} Pb application (4.34 nmol g^{-1}). Heavy metals have been proven to cause physiological regression in plants as well as an increase in malondialdehyde and in the activity of antioxidative enzymes (superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase) – Kıran et al. (2014), Tunçtürk et al. (2020). In a study testing 0, 25, 50, 75, 100 mg L^{-1} $PbSO_4$ lead doses on soybeans, it was reported that malondialdehyde values ranged from 22.22 to 22.03 nmol g^{-1} (Kulaz et al. 2021). It has been determined that lead (Pb) doses increase the amount of malondialdehyde in wheat and rice (Çolak Doğan, 2011, Verma Dubey, 2003). Kıran et al. (2015) reported the highest malondialdehyde value was measured as 150 mg kg^{-1} in the lettuce plant to which lead doses (0, 150 and 300 mg kg^{-1}) had been applied. Ascorbate peroxide activity increased in parallel with increasing lead (Pb) doses. According to the results obtained in this study, the highest value was obtained as 0.776 μmol g^{-1} from 100 mg L^{-1} Pb application, and the lowest were obtained from control applications (0.196 μmol g^{-1}). In terms of the activity of ascorbate peroxidase, it is seen in Table 1 that 50 and 75 mg L^{-1} Pb applications are in the same Duncan group. Kulaz et al. (2021) reported

that ascorbate peroxidase values in soybeans ranged between 2.490 and 2.660 μmol g^{-1}, depending on increasing doses of lead (Pb). It is certain that excessive metal intake affects the activities of antioxidant enzymes. In a similar study on rice plant, the response to stress caused by lead (Pb) doses was manifested as an increase in ascorbate peroxidase activity (Verma Dubey 2003). In another study, similarly to our findings, an increase in the ascorbate peroxidase level in response to increasing lead (Pb) doses was found in lettuce (Boysan 2015, Tunçtürk et al. 2020).

Macro and micro-nutrients in different plant parts of fenugreek

The average values of macro- and micro-nutrients accumulated in leaves, stems and roots of fenugreek at different lead (Pb) doses are given in Table 2.

Statistically significant differences at the level of 1% were found between the interaction of lead doses, plant parts and plant parts x lead doses in terms of the calcium content relative to the gradually increasing doses of lead (Pb) applied to fenugreek plants. Depending on the increased lead (Pb) doses in fenugreek, the lowest average Ca content was 6.49 g kg^{-1} TA from the Pb-75 dose, and the highest one was 27.09 g kg^{-1} from the control group. It was stated that there were ionic similarities between Pb^{2+} and Ca^{2+} ion accumulation. In fact, it has been shown that lead may displace Ca during specific physiological processes (Azmat et al. 2009).

As a result of lead application, the highest average Ca content of 19.77 g kg^{-1} among the plant parts was obtained in the leaves, while the lowest one, equal 11.46 g kg^{-1}, was accumulated in the roots. In another study, similar results were obtained, as it was stated that the Ca level in the root tips decreased after exposure to lead doses (Eun et al. 2000). In the PP x Pb interaction, the highest average Ca content (36.37 and 35.49 g kg^{-1}) was obtained from the leaves in the control and 25 mg L^{-1} Pb application treatments, while the lowest Ca content (3.23 g kg^{-1}) was detected in the roots after 75 mg Pb L^{-1} application. As a result of lead application, Ca^{2+} levels decreased in Norway spruce, which is similar to our finding of the regression in growth (Rout, Das 2003). In a study conducted on tomato seedlings exposed to lead doses (0, 75, 150 and 300 mg t^{-1}), Ca, Mg, K decreases in the presence of elements such as P, Na, Fe, Zn, Cu and Mn caused nutrient deficiency (Akıncı, Çalışkan 2010). It has been stated that gradually increasing doses of lead (Pb) increase the Ca content in the corn plant (Güler 2011). In another study, they reported that increasing Pb doses positively affected the rate of Ca accumulated in rye leaves (Akinci et al. 2010, İğdelioğlu 2014).

The effect of lead (Pb) application on the plant parts and PP x Pb interaction in terms of K was statistically significant at the 1% level. As a result of the lead (Pb) application, among all tested plant parts, the highest average K content was 34.01 g kg^{-1} in the stem and the lowest one was 9.93 g kg^{-1} in the roots part. In this study, the highest average lead (Pb) content was obtained from the control group (24.73 g kg^{-1}), while the lowest value was

Table 2

The effect of Pb doses on the content of macro- and micro-nutrients of different plant parts of fenugreek

Pb Doses	Plant part	Ca (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})	Cu (g kg^{-1})	Ni (g kg^{-1})
Control (0)	leaf	35.49 *a*	33.5 *ab*	6.74 *bc*	6.31 *ab*	23.43 *ab*	0.65 *de*
	stem	30.50 *ab*	22.85 *bc*	15.71 *a*	2.52 *d*	15.58 *c*	1.16 *cd*
	root	15.30 *cd*	17.84 *bcd*	3.41 *d*	3.17 *cd*	5.47 *ef*	2.56 *c*
Average		27.09 *A*	24.73 *A*	8.62 *A*	4.00 *AB*	14.82 *B*	1.46 *BC*
Pb-25	leaf	36.37 *a*	33.75 *ab*	6.82 *bc*	7.51 *a*	29.50 *a*	0.41 *de*
	stem	15.85 *cd*	25.19 *b*	2.68 *e*	3.30 *c*	13.41 *cd*	1.17 *cd*
	root	17.24 *bcd*	5.66 *d*	3.86 *cd*	1.77 *e*	11.69 *d*	2.59 *c*
Average		23.15 *B*	21.53 *BC*	4.45 *B*	4.19 *A*	18.20 *A*	1.39 *C*
Pb-50	leaf	16.96 *b*	25.43 *b*	3.20 *d*	2.20 *de*	17.21 *bc*	0.37 *e*
	stem	11.23 *de*	27.90 *ab*	1.63 *ef*	5.76 *b*	7.46 *de*	0.90 *d*
	root	15.70 *cd*	13.03 *cd*	4.03 *c*	2.31 *de*	0.86 *fgh*	1.59 *c*
Average		14.63 *C*	22.12 *AB*	2.95 *C*	3.42 *BC*	8.51 *D*	0.95 *D*
Pb-75	leaf	4.52 *f*	4.45 *def*	1.37 *efg*	0.93 *ef*	19.03 *b*	0.23 *ef*
	stem	11.71 *d*	45.94 *a*	2.91 *e*	6.19 *ab*	6.70 *e*	0.76 *d*
	root	3.23 *g*	9.88 *d*	3.16 *d*	1.00 *e*	18.41 *b*	14.93 *a*
Average		6.49 *E*	20.09 *BC*	2.48 *C*	2.71 *D*	14.71 *B*	5.31 *A*
Pb-100	leaf	5.49 *efg*	5.36 *de*	1.28 *fg*	1.04 *e*	19.83 *b*	0.76 *d*
	stem	14.82 *d*	48.21 *a*	3.65 *d*	7.06 *ab*	5.44 *ef*	1.40 *cd*
	root	5.83 *ef*	3.27 *ef*	1.38 *ef*	1.02 *e*	12.68 *d*	5.98 *abc*
Average		8.53 *D*	18.94 *C*	2.13 *C*	3.04 *C*	12.64 *C*	2.71 *B*
Average of PP	leaf	19.77 *A*	20.49 *B*	3.90 *B*	3.59 *B*	21.80 *A*	0.96 *B*
	stem	16.82 *B*	34.01 *A*	5.31 *A*	4.97 *A*	9.71 *C*	0.58 *C*
	root	11.46 *C*	9.93 *C*	3.16 *C*	1.85 *C*	10.02 *B*	2.82 *A*
Plant part (PP)		**	**	**	**	**	**
Pb doses (Pb)		**	**	**	**	**	**
PP x Pb		**	**	**	**	**	**
CV (%)		9.6	10.1	15.6	16.1	7.2	9.9

* It is significant at the $P<0.05$ level, ** at the $P<0.01$ level, and there is no statistical difference between the means indicated by letters;
CV – coefficient of variation

18.94 g kg^{-1}, both at Pb-100 mg L^{-1} doses. As a result of this study, the highest mean cadmium (Cd) content in the PP x Pb interaction was detected in the stem of 48.21 and 45.94 g kg^{-1} at the Pb-100, Pb-75 mg L^{-1} applications. The lowest K content (3.27 g kg^{-1}) was obtained in the root part

as a result of Pb-100 mg L^{-1} Pb application. In a similar study, it was reported that the cadmium (Cd) content of lettuce increased under the influence of 5 different lead doses (0, 50, 100, 150, 200 µM Pb) compared to the control group (Aksu 2019). It has been reported that lead doses have an important inhibitory antagonistic effect on K^+ uptake in plants (Haussling et al. 1998). It has been stated that lead (Pb) doses cause inhibition of the acumulation of macro-nutrients (especially K, P, Ca and Mg) and cause regression in the plant's growth and development (Akıncı et al. 2010).

In this study, the effect of (Pb) doses on lead doses, plant parts and PP x Pb interaction in terms of Mg content of the plant was statistically significant at the level of 1%. The highest Mg content (8.65 g kg^{-1}) was obtained from the control group. The lowest Mg content was found at 50, 75 and 100 mg L^{-1} Pb doses (2.95, 2.48 and 2.13 g kg^{-1}). The Mg contents obtained from these three doses were included in the same group. As a result of lead (Pb) dose application, it was determined that the highest average Mg content among the plant parts was accumulated in the stem with 5.31 g kg^{-1} , and the lowest in the root with 3.16 g kg^{-1}. The results of the study by Karakaş (2013), in which they stated that the Pb application in the canola plant, the Ca, K, Mg and Na accumulation in the stem is higher than the root, are in accordance with our research findings. In the PP x Pb interaction, the highest average Mg content (15.71 g kg^{-1}) was obtained from the stem part in the control application, and the lowest Mg content (1.28 g kg^{-1}) was obtained from the leaf part after 100 mg Pb L^{-1} application (Table 1). Aksu, Yıldız (2007) reported that the magnesium level in tomato increased due to the increase in Pb doses (0, 0.05, 0.1, 0.5, 1, 2, 3, 5, 10 and 20 mg kg^{-1} Pb) compared to the control. It has been reported that lead doses have a significant antagonistic effect on Mg^{2+} uptake in plants (Haussling et al. 1998). It has been stated that Mg is the element most affected by lead doses among macro and micro nutrients (Lamhamdi et al. 2013).

The effect of Pb doses on Na content was significant at the 1% level, on lead doses, plant parts and PP x Pb interaction. The highest Na content (4.00 g kg^{-1}) was obtained from Pb-25 mg L^{-1} dose, while the lowest 2.71 g kg^{-1} was obtained from Pb-75 application. In a study conducted on cucumber, it was determined that Pb doses increased the Na content compared to the control (Erdal et al. 2000). In different parts of the plant, the Na content was measured at the highest with 4.97 g kg^{-1} in the stem, and the lowest in the roots with 1.85 g kg^{-1}. In the PP x Pb interaction, the highest Na ratio was determined with 7.51 g kg^{-1} in leaves and Pb-25 mg L^{-1} applications. The lowest value was found at 0.93 g kg^{-1} and Pb-75 mg L^{-1} dose in the leaf. It has been reported that lead doses have a significant antagonistic effect on Na+ uptake in plants (Haussling et al. 1998).

The effect of lead doses on Cu and Ni contents of fenugreek, plant parts and PP x Pb interaction was found to be statistically significant at the 1% level. The highest Cu (18.20 mg kg^{-1}) and Ni (5.31 mg kg^{-1}) contents were

obtained from Pb-25 and Pb-75 mg L^{-1} Pb doses, respectively, and the lowest Cu (12.64 g kg^{-1}) and Ni contents. Respectvly were tated at (1.39 mg kg^{-1}) Pb-100 and Pb-25. The highest Cu and Ni contents (21.8 and 2.82 mg kg^{-1}) were detected in leaves and roots, respectively, while the lowest Cu and Ni contents were detected in the stem with 10.02 and 0.58 mg kg^{-1}. In the PP x Pb interaction, the highest Cu content (29.50 mg kg^{-1}) was determined from the Pb-25 dose in the leaf part, and the Ni content (14.93 mg kg^{-1}) from the Pb-75 applications in the root. The lowest Cu and Ni values were detected in roots and leaves at 0.86 mg kg^{-1} and 0.23 mg kg^{-1} at Pb-50 and Pb-75 doses. Other heavy metal ions may have the same effects as lead. It is known that some heavy metals prevent the inhibition of other elements, especially lead. For example, in a study conducted in cabbage, it was stated that Ni^{2+} or Cd^{2+} accumulation increased growth by reducing the negative effect of lead doses (Pandy, Sharma 2002). In similar studies, it has been stated that lead stress causes inhibition of elements such as Mg, Fe and Cu in the chloroplast, chlorophyll, thylakoids and grana structure of plants, thereby disrupting ion exchange (Haider et al. 2006, Akıncı et al. 2010).

Heavy metal content in different plant parts in fenugreek

At the end of the experiment, the effect of lead (Pb) doses, plant parts and PP x Pb interaction on some heavy metal contents such as Fe, Zn, Mn, Pb, Cr, Pb and Se in fenugreek was found to be statistically significant at the 1% level (Table 3).

In this study, the highest Fe content was determined with 202.21 mg kg^{-1} from the control group, and the lowest Fe content was determined with 127.31 mg kg^{-1} from the Pb-75 dose application. Increasing lead (Pb) doses caused a decrease in Fe content in fenugreek compared to the control group. Considering the Fe content of lead doses in plant tissues, the highest value was found in the root with 289.12 mg kg^{-1}, and the lowest value was determined in the stem with 66.82 mg kg^{-1}. As a result of the lead dose application, the maximum Fe content in the plant parts was found to be 369.13 and 360.57 mg kg^{-1}, and the Pb-0 and Pb-25 lead doses in the root. The lowest value was obtained from the P-100 dose in the stemswith 28.06 mg kg^{-1}. In another study, it was found that Fe levels decreased in root tips after exposure to lead (EUN et al. 2000). Environment x genotype interaction is thought to be effective in the emergence of this difference (Tuçtürk et al. 2020).

As seen in Table 3, the highest value in terms of Zn content was obtained from the P-25 lead dose with 41.68 mg kg^{-1}, and the lowest value was obtained from the P-100 dose with 17.06 mg/kg. In terms of plant parts, the lowest Zn content was 6.89 mg kg^{-1} in the stem and the highest 46.65 mg kg^{-1} in the leaf part. In another study, the lowest lead doses were found in the root part (Eun et al. 2000). Considering the Zn contents in terms of PP x Pb interaction, the highest value was obtained from the control group in the leaf

Table 3

Effect of Pb doses on heavy metal content of different plant parts of fenugreek

Pb doses	Plant parts	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Pb (mg kg^{-1})	Cr (mg kg^{-1})	Se (mg kg^{-1})	Cd (mg kg^{-1})
Pb-0	leaf	109.83 *c*	87.33 *a*	81.77 *a*	10.34 *bc*	0.58 *d*	1.47 *ab*	23.54 *ab*
	stem	145.13 *bc*	10.60 *d*	6.28 *e*	3.78 *d*	0.42 *e*	1.23 *b*	4.35 *e*
	root	360.57 *a*	15.77 *cd*	34.87 *bc*	5.46 *c*	4.40 *ab*	1.19 *b*	3.67 *ef*
Average		202.21 *A*	37.90 *B*	40.97 *AB*	6.52 *D*	1.80 *C*	1.30 *BC*	10.52 *E*
Pb-25	leaf	86.05 *d*	73.93 *ab*	62.47 *a*	15.76 *ab*	0.71 *bcd*	2.97 *a*	19.10 *b*
	stem	58.50 *efg*	10.35 *d*	9.12 *d*	16.22 *ab*	0.49 *d*	0.95 *c*	25.12 *ab*
	root	369.13 *a*	40.77 *ab*	54.74 *ab*	5.69 *c*	5.25 *a*	0.57 *cd*	15.53 *bc*
Average		171.23 *B*	41.68 *A*	42.11 *A*	12.56 *B*	2.15 *A*	1.49 *A*	19.92 *B*
Pb-50	leaf	106.17 *c*	26.17 *bc*	23.51 *c*	13.05 *b*	0.65 *bc*	1.62 *ab*	22.79 *ab*
	stem	68.21 *ef*	5.98 *de*	15.68 *cd*	15.43 *ab*	0.47 *d*	1.05 *b*	6.68 *de*
	root	134.23 *c*	12.04 *d*	41.21 *b*	4.93 *c*	3.26 *ab*	1.24 *b*	23.18 *ab*
Average		102.87 *E*	14.73 *E*	26.80 *B*	11.14 *C*	1.46 *B*	1.30 *BC*	17.55 *C*
Pb-75	leaf	82.99 *de*	15.41 *cd*	9.62 *d*	18.77 *a*	0.29 *f*	1.48 *ab*	15.24 *bc*
	stem	34.23 *gh*	3.84 *e*	14.60 *cd*	14.50 *b*	0.38 *e*	0.57 *cd*	6.74 *cde*
	root	264.73 *b*	42.09 *ab*	24.09 *c*	20.45 *a*	1.05 *b*	1.15 *b*	25.85 *ab*
Average		127.31 *D*	20.45 *C*	16.10 *C*	17.91 *A*	0.57 *D*	1.06 *C*	15.94 *D*
Pb-100	leaf	102.40 *cd*	30.40 *b*	10.91 *d*	17.62 *ab*	0.38 *e*	0.97 *c*	14.02 *c*
	stem	28.06 *hı*	3.69 *ef*	15.88 *c*	14.18 *b*	0.73 *bcd*	0.39 *cde*	14.60 *c*
	root	316.93 *ab*	17.10 *c*	17.36 *c*	9.69 *bc*	1.32 *b*	1.03 *b*	36.03 *a*
Average		149.12 *C*	17.06 *D*	14.72 *C*	13.83 *B*	0.81 *D*	0.79 *D*	21.55 *A*
Average of PP	leaf	97.48 *B*	46.65 *A*	37.65 *A*	15.10 *A*	0.52 *B*	1.70 *A*	18.93 *B*
	stem	66.82 *C*	6.89 *C*	12.13 *C*	12.82 *B*	0.49 *B*	0.83 *C*	11.49 *C*
	root	289.12 *A*	25.55 *B*	34.45 *A*	9.24 *C*	3.05 *A*	1.03 *B*	20.85 *A*
Plant part (PP)		**	**	**	**	**	**	**
Pb doses (Pb)		**	**	**	**	**	**	**
PP x Pb		**	**	**	**	**	**	**
CV (%)		7.2	6.6	8.9	8.7	17.5	14.2	7.2

It is significant at the * P<0.05 level, ** at the P<0.01 level, and there is no statistical difference between the means indicated by letters;
CV – coefficient of variation

with 87.33 mg kg^{-1}. Aksu (2007), the lowest value was determined as 3.69 mg kg^{-1} at the dose of Pb-100 in the stem part. In a study conducted on tomatoes, they reported that there were irregular increases and decreases in zinc content compared to the control, depending on the increase in Pb doses (0, 0.05, 0.1, 0.5, 1, 2, 3, 5, 10 and 20 mg kg^{-1} Pb).

The effect of lead (Pb) doses on Mn content was found to be significant. The highest Mn content was measured at 42.11 mg kg^{-1}. Pb-25 lead dose, and the lowest value was measured at 14.72 mg kg^{-1} Pb-100 dose. In parallel with the increasing lead doses, the Mn content of the plant decreased. In a similar study, it was determined that lead doses in Norway spruce caused growth retardation by reducing Mn^{2+} levels (Rout, Das 2003) obtained in the sky. The lowest Mn content was found in the stem with 12.13 mg kg^{-1}. Considering the PP x Pb interaction in terms of Mn content, the highest value was found in the leaf part of the control group with 81.77 mg kg^{-1}, and the lowest value was found in the stem with 6.28 mg kg^{-1} (Table 3). Manganese is involved in the production of oxygen from water in photosynthesis. It is one of the most affected elements depending on the plant species and variety and the intensity of the dose (Haider et al. 2006).

Considering the effect of increasing amounts of lead doses on the Pb content of fenugreek, the lowest value was obtained from Pb-0 dose with 6.52 mg kg^{-1}, and the highest value was obtained from Pb-75 application with 17.91 mg kg^{-1}. In terms of lead (Pb) content in plant parts, the highest value was found in the leaf with 15.10 mg kg^{-1}, and the lowest value in the root part with 9.24 mg kg^{-1}. In a study conducted with corn and sunflower, the highest Pb content was obtained from the control group (Gül 2013). Similar to our findings, in a study conducted on tomatoes, it was stated that there was an increase in lead content compared to the control, depending on the increase in Pb doses (0, 0.05, 0.1, 0.5, 1, 2, 3, 5, 10 and 20 mg kg^{-1} Pb). The lead (Pb) contents of plants vary according to plant species and varieties. For example, it has been suggested that rhizobium bacteria found in the roots of leguminous plants inhibit lead uptake (Mesmar, Jaber 1991).

In this study, as seen in Table 3, the highest value of the Cd content was obtained in response to the dose of 2.15 mg kg^{-1} Pb-25, while the lowest value (0.57 mg kg^{-1}) was determined at the dose of P-75. The highest Cr content in the plant parts was measured in the root (3.05 mg kg^{-1}), and the lowest values (0.52-0.49 mg kg^{-1}) were measured in the stem and leaves in the same experimental group. The highest level of the interaction of plant parts x lead doses was determined as 5.25 mg kg^{-1} in the Pb-25 dose in the root part, and the lowest value (0.29 mg kg^{-1}) in the leaf part was determined in the Pb-75 dose variant.

As for the Se content, the highest value (1.49 mg kg^{-1}) was obtained in response to the P-25 lead dose, and the lowest value (0.79 mg kg^{-1}) was obtained at the P-100 dose. In terms of plant parts, the highest Zn content was measured at 1.70 mg kg^{-1} in the leaf and the lowest equalled 0.83 mg kg^{-1} in the stem part. Considering the Se contents in terms of PP x Pb interaction, the highest value was obtained in the leaf (2.97 mg kg^{-1}) from the Pb-25 lead variant. The lowest value was detected in the stem at the dose of Pb-100, where it equalled 0.39 mg kg^{-1} (Table 3). It is known that lead doses stop the uptake of elements such as selenium in plants and

therefore cause regression in growth and development (Tunçtürk et al. 2020, Kulaz et al. 2021).

As a result of this study, the highest value of the Cd content (21.55 mg kg^{-1}) was obtained following the application of the Pb-100 dose, while the lowest value (10.52 mg kg^{-1}) was found in the control. The highest Cr content in the plant parts was measured in the root with 3.05 mg kg^{-1}, and the lowest values (0.52-0.49 mg kg^{-1}) were measured in the stem and leaves and took place in the same group. The highest level of interaction of plant parts x lead doses was determined as 5.25 mg kg^{-1} in the Pb-25 dose in the root part, and the lowest value (0.29 mg kg^{-1}) in the leaf part was determined in the Pb-75 dose. In a study conducted in cabbage, it was stated that Ni^{2+} or Cd^{2+} accumulation increased growth by reducing the negative effects of lead doses (Pandy, Sharma 2002).

CONCLUSION

In this study, the effects of lead, a heavy metal that is an important source of abiotic stress in many plants, on some growth parameters, the content of macro- and micro-nutrients and levels of some important enzymes in fenugreek were investigated. As a result of increasing Pb application doses, morphological features such as the plant height, root length, leaf fresh weight, number of leaves, fresh and dry roots decreased at varying rates. In addition, there was a decrease in the elemental content of Ca, K, Mg, Na, Cu, Ni, Fe, Zn, Mn, Cr, Ni, Se and Cd in the leaf stem and root parts of the plant. Increases in ascorbate peroxidase (APX) enzymes and malondialdehyde (MDA) were observed. In this study, the effect of the heavy metal lead on fenugreek was evident because the tolerance limits were exceeded.

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