Common basil (*Ocimum basilicum* L.) responses to lead ($Pb(NO_3)_2$) stress: Germination, morpho-physiological, and phytochemical

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Abstract

The negative impact of contaminated soil with heavy metals on plant and human health is an important global concern. To evaluate the effect of lead ($Pb(NO_3)_2$) stress (0, 25, 75, 100, and 150 µM) on the germination, growth, and physiological and biochemical properties of sweet basil (*Ocimum basilicum* L.), a pot experiment based on completely randomized design (CRD) with three replicates was conducted at the Department of Horticultural Science, Shahed University of Tehran, in 2018. The contaminated soil with Pb had a negative impact on germination indices (percentage and rate), growth and morphological parameters (shoot and root length and dry weight), and physiological parameters (LAI, photosynthetic pigments, and number of leaf secretory glands). Pb stress (150 µM) led to a reduction in the mean germination percentage (43.33%), germination rate (62.92%), shoot and root dry weight (60.22 and 77.43%, respectively), LAI (64.68%), total chlorophyll content (73.10%), and number of leaf secretory glands (33.3%) compared to the control treatment (without Pb) while increased peroxidase activity (62.3%), proline content (70.14%), and the root and shoot Pb content (92.10 and 97.6%, respectively). Two components of oil, Linalool and Estragole (Methyl Chavicol) composed more than 50% of the oil. On the other hand, Pb stress led to a change in the content of essential oil compounds. In general, low levels of Pb (25 µM) appear to increase the predominance of oil compounds. In conclusion, common basil cultivation in the Pb-contaminated soil could cause undesirable effects on the germination indices, growth and morphological traits, and physiological attributes but might have a positive influence under low level (25 µM) on the essential oil composition.

Keywords: Essential oil; linalool; methyl-chavicol; photosynthetic pigment; proline content

Poursaeid, M., A. Iranbakhsh, M. Ebadi, and M. H., Fotokian. 2021. Common basil (*Ocimum basilicum* L.) responses to lead ($Pb(NO_3)_2$) stress: Germination, morpho-physiological, and phytochemical*'. Iranian Journal of Plant Physiology 11* (2),3561-3570.

Introduction

Medicinal and aromatic plants are valuable sources of secondary metabolites and are used in various industries such as food and drink flavor and pharmaceutical products. Common basil (*Ocimum basilicum* L.) is one of the largest genera in Lamiaceae family which consists of 65 species native to Africa and South America, and Basil is the major crop in many countries which is used as a culinary herb (Farsaraei et al., 2020; Zolfaghari et al., 2013). As a food ingredient, this

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Received: June, 2020
Accepted: January, 2021

plant is appetizing and is used to treat bloating and gastrointestinal augmentation (Padash et al., 2019). The raw material and essential oil of basil are used greatly in folk medicines as antispasmodic, stomachic, carminative, antiulcerogenic, anti-inflammatory, anti-carcinogenic, analgesic, stimulant, radioprotective, and febrifuge agent (Farsaraei et al., 2020).

Essential oils are secondary metabolites with isoprenoid structure. Accumulation of essential oils in plants is usually limited to specialized secretory structures which are present in several different types, namely glandular trichomes (Avetisyan et al., 2017). The content and chemical composition of the basil essential oils has been the subject of many studies. The yield from different plant parts varies between 0.2-1.9% with the main components being linalool, methyl chavicol, eugenol, and methyl cinnamate, as well as 1,8-cineole, methyl eugenol, geraniol, geranial, neral, and α-bergamotene (Antić et al., 2019).

The contamination of soil by heavy metals is one of the main environmental problems in human societies as a result of industrial and agricultural activities (Padash et al., 2019; Pirzadah et al., 2019). Also, smelting facilities emit heavy metals that can be deposited on soils and in waters in the vicinity of the emission source. Following relatively long-time deposition, elevated heavy metal soil and water concentrations may exert a negative effect on animal and human health (Xing et al., 2020). Among the heavy metals, lead (Pb) is one of the toxic heavy metals with unknown biological effects that easily accumulate in sediments and soils (Padash et al., 2019).

One of the most notable effects of the Pb is the induction of oxidative stress in plants and the formation of reactive oxygen radicals. The different types of Reactive Oxygen Species (ROS) as the main causes of oxidative stress have a high oxidizing capacity, threaten bioluminescence and different cellular processes, interrupt normal metabolism, and ultimately lead to cell death (Padash et al., 2019). Antioxidant systems are considered a tolerance mechanism to reduce the toxicity of heavy metals in plants. These antioxidant systems include enzymes such as catalase, peroxidases, and superoxide dismutase and non-enzymatic antioxidants such as ascorbates, glutathione, flavonoids, α-Tocopherol, and carotenoids (Apel et al., 2004). In adaptive response to heavy metals, reception, and signal transduction are followed by changes in transcriptions of genes (Safari et al., 2018), organ development (Melato et al., 2012), and metabolism (Babajani et al., 2019). Scientists reported that lead contamination reduced growth, physiology, and yield of sunflower at all levels of lead stress (Saleem et al., 2018). Cultivation of sweet basil in the Cd and Pb contaminated soils could cause undesirable effects on seed germination and morphological traits but might have a positive influence on the essential oil yield, composition, and phytoremediation of the soil (Fattahi et al., 2019). The objective of this study was to evaluate the effect of lead concentration on germination, growth, physiological, and biochemical characteristics as well as lead uptake in basil.

Materials and Methods

Plant material and experimental design

To investigate germination, morpho-physiological, and phytochemical responses of common basil to lead ($Pb(NO_3)_2$) stress, an experiment was conducted under greenhouse conditions at the Department of Horticultural Science, Shahed University of Tehran, Iran, in 2018. Common basil (*Ocimum basilicum* L.) seeds of a local population were purchased from Pakan Seed Co., Isfahan, Iran. The seeds were sterilized using sodium hypochlorite and were sown in a pot containing soil with traits described in Table 1. Seedlings were kept in a greenhouse under

Table 1
Physicochemical characteristics of the applied soil

Soil	Clay (%)	Silt (%)	Sand (%)	Pb mg/kg	Cd mg/kg	Mg mg/kg	O.C (%)	pH	EC (dS/m)
Sand-loam	15	32	53	0.2	0.1	15	0.7	8	3.5

controlled temperature (26 ℃ ± 4 ℃), humidity (50 ± 10%), photoperiod (16/8 h light/dark), and light intensity (160 µmol/m-2/s-1). Following this, each pot was irrigated every 2 days (ten turns) with half strength of Hoagland's nutrient solution containing various levels of lead nitrate $Pb(NO_3)_2$ (0, 25, 75, 100, and 150 µM). Experiments were designed in a completely randomized design (CRD) with three replicates. Four-week-old plants were harvested to measure all germination, growth, morphological, and physiological parameters.

Germination percentage and rate

Germinated seeds were counted on the second day and were done daily and finally at the end of sprouting (12 days) germination percentage and germination rate were calculated according to the following equations (Aghighi Shahverdi et al., 2017):

Germination percentage (GP) = (N × 100) / M

Germination rate (GR) = ∑Ni/Ti

whereas N = sum of germinated seeds at the end of the experiment, M = total planted seeds, and Ti = number of days after germination.

Peroxidase activity

To measure the enzyme activity, 0.1 g of fresh tissue of the seedlings was used. To extract protein, 0.2 g of fresh tissue was pulverized in a mortar using liquid nitrogen and then one mL of buffer Tris-HCl (0.05 M, pH = 7.5) was added. The obtained mixture was centrifuged for 21 min at 13,000 rpm, at 4 ℃ and the supernatant was used for enzyme activity measurements (Aghighi Shahverdi et al., 2017). The peroxidase activity was determined in a reaction of the mixture, which consisted of a suitable amount of 28 mM guaiacol, 5 mM H_2O_2, 25 mM Na-phosphate buffer (pH 6.8), and enzyme (Ghanati et al., 2002).

Photosynthetic pigments

For measuring the content of photosynthetic pigments, 0.25 g fresh tissue was extracted using 5 mL 80% acetone. The extract was centrifuged at 11,000 rpm for 10 min. The optical density (O.D.) of the extract was measured at wavelengths 646.8 and 663.2 nm to estimate Chl. a and Chl. b respectively, using a spectrophotometer. The amount of pigment present in each sample was calculated according to the following equations (Lichtenthaler, 1987):

Chl. a (mg/g FW) = 12.7 (O.D of 663) – 2.69 (O.D of 645) × (v/(w×1000)

Chl. b (mg/g FW) = 22.9 (O.D of 645) – 4.68 (O.D of 663) × (v/(w×1000)

Total Chl. (mg/g FW) = Chl a + Chl b

where W is the fresh weight by grams for extracted tissue, V is the final size of the extract in 80% acetone, and O.D is the optical density at a specific wavelength.

Proline content

Approximately 0.5 g fresh seedling tissue was homogenized in 10 ml 3% aqueous sulfosalicylic acid. Then, the aqueous solution was filtered through Whatman paper No. 2 and finally, 2 ml of the filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 ℃. The reaction mixture was extracted with 4 mL toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrometer (Bates et al., 1973).

Essential oil analysis

Essential oil samples were analyzed using GC–MS (GC-2010 SHIMADZU), according to the method described in detail for the type, column, etc. (Fattahi et al., 2016). Basil leaves were collected and hydrodistilled using a Clevenger apparatus. Gas chromatography/mass spectrometry (GCMS) analysis was equipped with a split–splitless injector (split ratio; 30:1), scan time 1s, ionization energy 70 eV, and mass range of 40-300 amu. a column (60 m × 0.25 mm i.d., film thickness 0.25 m); oven temperature was 60-230 ℃ at a rate of 7 ℃/min, transfer line temperature 260 ℃. The carrier gas was helium, with a linear velocity of 31.5 cm/s. The oils were diluted in

dichloromethane (2 µl of oil in the 2 ml solvent), the next 2µl oil of each treatment was injected to GC/MS manually. The retention indices were calculated, for all volatile constituents using a homologous series of n-alkanes C8-C22 on the HB-5 column. The essential oil constituents were determined by comparing their GC retention indices, mass spectra with data published in the literature. Compounds were further identified using their mass spectra data compared to National Institute of Standards and Technology mass spectra library data provided by the software of the GC-MS system. Essential oil components were shown as a relative percentage of the total oil.

Number of leaf secretory glands

Leaf secretory glands consist of several cells providing different functions. The Pb-mediated changes in the distribution of leaf secretory glands were monitored by a Scanning Electron Microscope (SEM) (Hazzoumi et al., 2017). To measure the number of leaf stomata, Wang and Clark (1993) method was used. For each leaf sample, 8 observations of a light microscope (Leica Galen III, magnification: 200) were prepared. The number of leaf stomata was determined by GSA Image Analyzer V3.8.6 Software.

Leaves area index (LAI)

The total leaf area per plant was measured using a Li-Cor LI-3100 leaf area meter (LI-COR Inc., Lincoln, Nebraska, USA) (Alizadeh et al., 2010).

Determination of Pb content of shoot and root

Plants were dried in an oven at 65 ℃. The dried samples were ground and digested using HNO_3, H_2SO_4, HCl. The Pb content of the solutions was determined by Inductively Coupled Plasma Mass Spectrometry ICP- OES [Perkin Elmer ELAN 6100DRC-e]) (Akpinar-Bayizit et al., 2010).

Statistical Analysis

Normality of distribution for the obtained data was calculated according to the Kolmogorov-Smirnov and Shapiro-Wilk test. Then the studied traits were statistically analyzed by the Statistical Analysis System software (SAS Institute, Cary, NC, USA, and Version 9.2). The differences among means were separated using LSD test (least significant difference) at 0.05 statistical probability level. The Pearson correlation coefficient was used to measure relationships between growth, morpho-physiological, and biochemical traits using SAS software vr.9.2.

Results

Germination parameters

Results indicated that the effect of Pb stress was significant on germination percentage and germination rate ($p \leq 0.01$). The highest germination percentage (100%) and germination rate (4.56 seed/day) were achieved in the control treatment (without Pb) and the lowest means of these traits (56.67% and 1.69 seed/day, respectively) in 150 µM treatment. In other words, Pb stress (150 µM) led to 43.33% and 62.92% reduction in the average germination percentage

Table 2
Effect of $Pb(NO_3)_2$ stress on germination and morphological characteristics of basil (*Ocimum basilicum* L.)

$Pb(NO_3)_2$ levels (µM)	Germination percentage (%)	Germination rate (seed/day)	Shoot length (cm)	Root length (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Control	100.0±0.00 a	4.56±0.13 a	55.63±0.35 a	6.73±0.25 a	2.737±0.05 a	0.257±0.006 a
25	70.0±2.86 b	3.23±0.15 b	52.07±0.26 b	4.77±0.13 b	2.081±0.01 b	0.168±0.006 b
75	68.33±1.66 bc	2.64±0.24 bc	50.20±0.58 c	3.73±0.11 c	1.962±0.006 c	0.137±0.005 c
100	63.33±1.66 c	2.21±0.26 cd	45.63±0.40 d	3.22±0.06 d	1.553±0.008 d	0.071±0.001d
150	56.67±1.66 d	1.69±0.25 d	42.40±0.56 e	2.90±0.05 d	1.088±0.03 e	0.058±0.003 d
LSD ($p \leq 0.05$)	4.11	1.03	2.11	0.58	0.11	0.035
Significance level	**	**	**	**	**	**

Average and standard deviation values with different letters in the same column are statistically different (LSD, $p \leq 0.05$); **= $p \leq 0.01$

and germination rate, respectively compared to the control treatment (Table 2).

Shoot and root length

The effect of Pb stress was significant on the shoot and root length. The results of the comparisons of means showed that Pb stress led to a decrease in root and shoot length such that the highest shoot and root length were related to the treatment without Pb (control) by 55.63 and 6.37 cm, respectively and the lowest shoot length was related to 150 µM (42.40 cm) and root length to 100 and 150 µM (3.22 and 2.90 cm, respectively). Furthermore, 150 µM Pb concentration led to 23.78% and 56.90% reduction in the average of the shoot and root length of basil, respectively compared to the control group without Pb (Table 2).

Shoot and root dry weight

The shoot and root dry weights were significantly affected by Pb stress ($p \leq 0.01$). Lead stress significantly decreased shoot and root dry weights. The highest shoot (20737 g/plant) and root (0.257 g/plant) dry weights were observed in the control treatment (without Pb) and the lowest shoot (1.088 g/plant) and root (0.058 g/plant) dry weights were recorded under 150 µM Pb treatment. In 150 µM treatment, 60.22% and 77.43% decreases were observed in mean shoot and root dry weights, respectively compared to the control treatment (Table 2).

Leaf area index (LAI)

The effect of Pb stress was significant on LAI ($p \leq 0.01$). The results of the comparisons of means showed that the highest (3.97) and lowest (1.41) means of LAI were achieved in 0 and 150 µM treatments, respectively. Increasing Pb concentration up to 150 µM caused significant decrease in LAI by 64.48% compared to the non-stress conditions (Table 3).

Photosynthetic pigments

Results indicated that the effect of Pb stress on photosynthetic pigments such as chlorophyll (Chl.) a, b, and total Chl. was significant at $p \leq 0.01$ level. According to the results of comparisons of means, the highest Chl. a (0.95 mg/g FW), Chl. b (0.50 mg/g FW), and total Chl. (1.45 mg/g FW) were found in no-Pb treatment (control). By increasing Pb concentration, Chl. a, b, and total Chl. contents decreased. Under 150 µM treatment, 72.63%, 74.0%, and 73.10% reductions were observed in Chl. a, b, and total contents, respectively compared to the control treatment (without Pb). The lowest Chl a (0.25 mg/g FW). Chl b (0.13 mg/g FW), and total Chl (0.39 mg/g FW) were related to 150 µM treatment (Table 3).

Peroxidase activity

The peroxidase activity was significantly affected by Pb stress ($p \leq 0.01$). The activity of this enzyme significantly increased by Pb treatment.

Table 3
Effect of $Pb(NO_3)_2$ stress on some physiological characteristics of basil (*Ocimum basilicum* L.) seedlings

$Pb(NO_3)_2$ levels (µM)	LAI	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Peroxidase activity (U/mg protein.min)	Proline content (µmol/g FW)	Number of secretory glands	Root Pb content (mg/kg FW)	Shoot Pb content (mg/kg FW)
Control	3.97±0.11 a	0.95±0.11 a	0.50±0.02 a	1.45±0.02 a	0.070±0.001 e	7.30±0.20 e	15±0.88 a	26.6±0.75 e	4.95±0.14 e
25	3.17±0.08 b	0.58±0.02 b	0.32±0.04 b	0.91±0.0671b	0.093±0.003 d	9.21±0.13 d	13±0.57 b	97.4±0.57 d	11.87±0.34 d
75	2.44±0.18 c	0.45±0.02 c	0.28±0.04 b	0.73±0.07 bc	0.120±0.003 c	13.06±0.10 c	12±0.51 bc	156.8±2.71 c	66.90±1.59 c
100	1.88±0.14 d	0.36±0.03 d	0.21±0.04 bc	0.57±0.08 cd	0.156±0.005 b	18.19±0.13 b	11±0.52 bc	262.1±3.46 b	124.27±2.35 b
150	1.41±0.04 e	0.26±0.03 e	0.13±0.02 c	0.39±0.06d	0.186±0.006 a	24.45±0.15 a	10±0.57 c	337.1±3.0 a	187.60±3.71 a
LSD ($p \leq 0.05$)	0.05	0.09	0.12	0.11	0.015	1.85	1.01	22.74	6.06
Significance level	**	**	**	**	**	**	**	**	**

Average and standard deviation values with different letters in the same column are statistically different (LSD, p = 0.05); **= $p \leq 0.01$

Table 4
Correlation coefficients among morpho-physiological traits of basil (*Ocimum basilicum* L.) under $Pb(NO_3)_2$ stress

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.90													
3	0.90	0.86												
4	0.93	0.95	0.9											
5	0.93	0.92	0.97	0.94										
6	0.93	0.93	0.96	0.97	0.96									
7	0.93	0.88	0.94	0.94	0.95	0.96								
8	0.93	0.95	0.93	0.98	0.95	0.97	0.93							
9	0.87	0.87	0.91	0.87	0.89	0.88	0.84	0.94						
10	0.93	0.94	0.94	0.95	0.94	0.95	0.91	0.99	0.98					
11	-0.91	-0.83	-0.99	-0.9	-0.97	-0.95	-0.95	-0.92	-0.87	-0.91				
12	-0.87	-0.78	-0.97	-0.85	-0.95	-0.91	-0.94	-0.86	-0.83	-0.86	0.98			
13	0.81	0.87	0.83	0.87	0.86	0.9	0.89	0.83	0.7	0.79	-0.82	-0.83		
14	-0.9	-0.85	-0.99	-0.91	-0.98	-0.96	-0.96	-0.92	-0.87	-0.91	0.99	0.99	-0.86	
15	-0.84	-0.75	-0.97	-0.84	-0.93	-0.9	-0.93	-0.84	-0.81	-0.84	0.98	0.99	-0.81	0.98

1. Germination percentage; 2. Germination rate; 3. Shoot length; 4. Root length; 5. Shoot dry weight; 6. Root dry weight; 7. LAI; 8. Chlorophyll a; 9. Chlorophyll b; 10. Total chlorophyll; 11. Peroxidase activity; 12. Proline content; 13. Number of secretory glands, 14. Root Pb content; 15. Shoot Pb content

-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

High negative correlation — Non-correlation — High positive correlation

The highest activity (0.186 U/mg protein.min) was related to 150 µM and lowest activity (0.070 U/mg protein.min) was recorded in control. In 150 µM Pb level, 62.3% increase was observed in the mean trait compared to the control treatment (without Pb).

Proline content

The effect of Pb treatment was significant on proline content ($p \leq 0.01$). The proline content increased under 150 µM Pb treatment by 70.14% compared to the 0 µM (control treatment). The highest (24.45 µmol/g FW) and lowest (7.30 µmol/g FW) proline contents was observed under 150 µM and 0 µM Pb treatments, respectively (Table 3).

Number of secretory glands

Based on the data analysis results, the effect of Pb stress was significant on the number of secretory glands ($p \leq 0.01$). Soil contaminated with Pb reduced the number of secretory glands as compared to plants grown in control soil without contamination. The highest (15.0) and lowest (10.0) numbers of secretory glands were found in 0 and 150 µM treatments. In 150 µM Pb level, 33.3% reduction was observed in the mean trait compared to the control treatment (without Pb) (Table 3).

Root and shoot Pb content

Based on the data analysis results, the root and shoot Pb contents were significantly affected by Pb stress ($p \leq 0.01$). The Pb contents in shoot and root parts of the plant increased by Pb treatment by 92.10 and 97.6%, respectively in 150 µM compared to the 0 µM (control treatment). This decrease in shoots was more than roots at all treatment levels. The highest root (337.1 mg/kg FW) and shoot (187.60 mg/kg FW) Pb contents were related to 150 µM while the lowest root (26.6 mg/kg FW) and shoot (4.95 mg/kg FW) contents were recorded under control treatment (Table 3).

Correlation coefficients

There were significant negative and positive correlations among morpho-physiological properties as well as among growth and germination parameters. The results of the simple correlation (Pearson) are presented in Table 4. Based on the results of this table, shoot dry weight

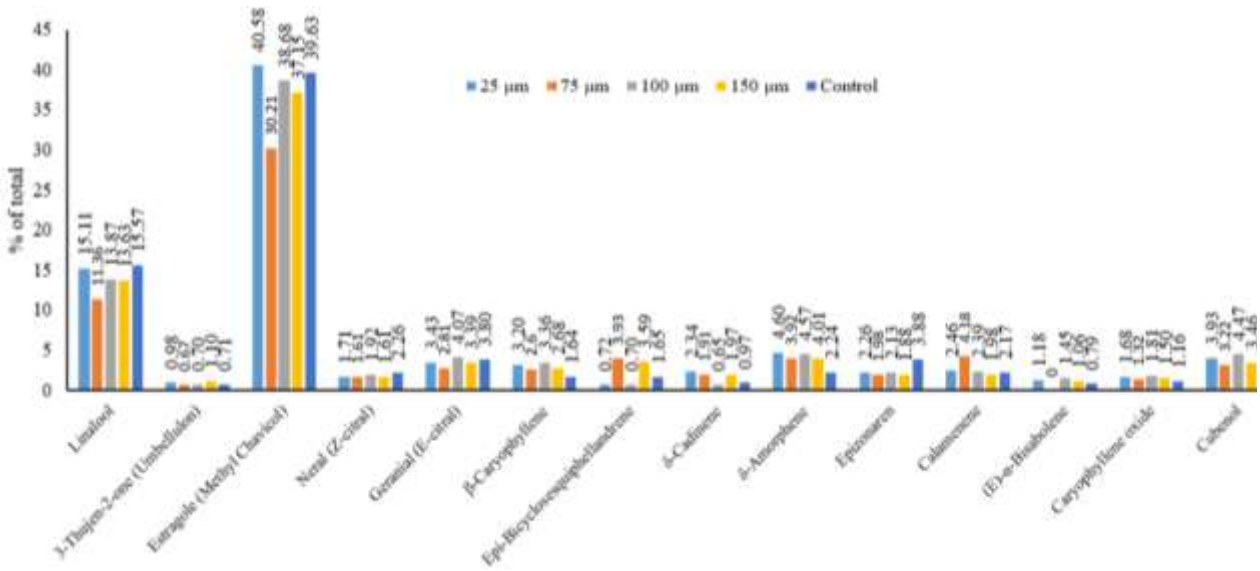

Fig. I. Effects of $Pb(NO_3)_2$ stress on essential oil component of basil (*Ocimum basilicum* L.)

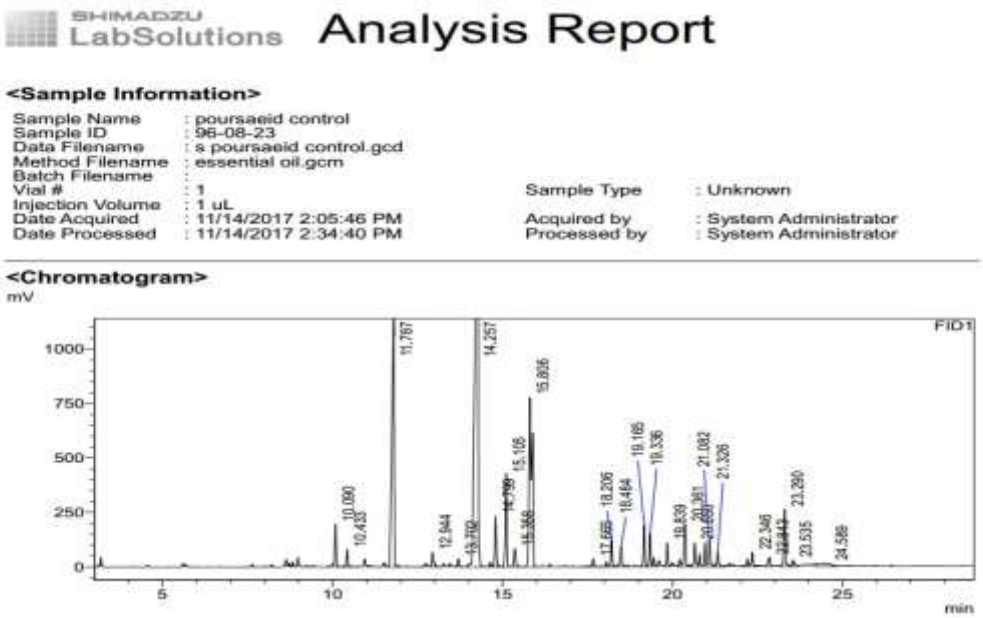

Fig. II. Chromatogram analysis report of basil essential oil component in control

positively and significantly correlated with germination percentage, germination rate, shoot, and root length, root dry weight, LAI, Chl. a, b, total Chl., and the number of secretory glands while it negatively and significantly correlated with peroxidase activity, proline content, and root and shoot Pb contents. Correlation coefficients results indicated that the peroxidase activity, proline content, and root and shoot Pb contents negatively and significantly correlated with the other germination, growth, and morpho-physiological characteristics.

Essential oil component

The results of GC–MS analyses of the essential oil are shown in Figs. (I) and (II). Results showed the essential oil of basil included Linalool, 3-Thujen-2-one (Umbellulon), Estragole (Methyl Chavicol), Neral (Z-citral), Geranial (E-citral), β-Caryophyllene, Epi-Bicyclosesquiphellandrene, δ-Cadinene, δ-Amorphene, Epizonaren, Calamenene, (E)-α-Bisabolene, Caryophyllene oxide, and Cubenol. Two components of oil, Linalool and Estragole (Methyl Chavicol) composed more than 50% of the oil. On the Other hand, Pb stress led to a change in the content of

essential oil compounds. In general, low levels of Pb (25 µM) appear to increase the predominance of oil compounds.

Discussion

The present study was conducted to evaluate the effect of Pb stress on germination, growth, physiology, and biochemical parameters of common basil plants. Our results showed a reduction in germination and growth indices, as well as a change in physiology and biochemical traits under Pb-amended soil as compared to plants grown in control soils.

Application of Pb concentration up to 80 mg/L significantly decreased basil seed germination percentage to 9.33% (Fattahi et al., 2019) which is in line with the findings of the present study. Also, there was a general decrease in the germination rate by increasing Pb concentrations since the highest germination rate was observed in control (1.69 seed per day) (Fattahi et al., 2019). Germination indices are the most important feature of vegetative stage of a plant under stress conditions (Enteshari et al., 2013). Reduction in germination indices and growth parameters by Pb stress might be due to decrease in the uptake of nutrients by Pb, causing interference in respiration, reduction in the photosynthetic activity of plants, and disturbance in the cell membrane permeability (Saleem et al., 2018). Lead also caused interference in activities of enzymes that involved in the photosynthetic Calvin cycle and sugar and nitrogen metabolism, also causing structural damage, reduction of biochemical and physiological activities, and growth inhibition (Jayasri et al., 2017).

Growth and morphological traits such as shoot and root lengths and shoot and root dry weights decreased under Pb stress. Similarly, other researchers reported the same results in common basil (Fattahi et al., 2019). Morphological changes of Vetiver Grass (Chrysopogon zizanioides) associated with metal-induced stress were also examined (Melato et al., 2012). It has been reported that the effect of Pb in peppermint reduced leaf area and dry weight of the shoot parts under Pb treatments in comparison with control (Amirmoradi et al., 2012) which confirms the findings of the present study.

Although stress conditions lead to oxidative damage by the production of ROS, antioxidant enzymes have an important protective role in scavenging ROS and protecting plant tissues. The increased activity of peroxidase enzyme under Pb stress could be an indicator of increased ROS production and accumulation of protective mechanisms to alleviate oxidative damage. The increased activity of peroxidase and catalase enzymes under stress conditions has been indicated (Zafari et al., 2020).

In the current study, photosynthetic pigments such as Chl. a, b, and total were reduced by Pb contamination. Chlorophyll loss was shown to be accompanied by the damage of the mesophyll chloroplasts, which leads to a lower photosynthetic rate under stress. Also, the reduction in chlorophyll content under stress has been considered as a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Zafari et al., 2020). The reduction in physiological parameters might be due to averting the inclusion of iron molecule by Pb into phytoporphyrin ring of Chl. and reducing the synthesis of Chl. (Saleem et al., 2018). Lead reduces the production of Chl. molecule either by decreasing the activity of chlorophyllase or minimizing the uptake of Mg and Fe by plants (Sharma et al., 2005). Lead also degraded the chlorophyll molecule (Doğan et al., 2009).

In our study, Pb contamination increased the peroxidase activities and proline content as compared to plants grown in the soil with no Pb. To survive with heavy metals toxicity or to keep heavy metals level in the physiological range, plants employ a mechanism to control the uptake and metals detoxification. To alleviate the harmful effects of ROS, plants promote the activities of antioxidants that protect them against oxidative stress produced by lead concentration (Janmohammadi et al., 2013). In plants, Pb affects several metabolic activities in different cell components. Effects also include decreases in seed germination, disruption of nutrient translocation, reduction in cell division, inhibition of photosynthesis, and ultimately reduction in shoot and root growth (Zhong et al., 2020).

In this study, the chemical composition of basil essential oil was investigated. The contents

of the essential oils were in good agreement with the data in the existing literature (Antić et al., 2019). The composition of essential oils of the three basil cultivars cultivated in Armenia was quite different: *O. basilicum* var. purpureum essential oil contained 57.3% methyl-chavicol (estragol), *O. basilicum* var. thyrsiflora oil had 68.0% linalool, and the main constituents of *O. xcitriodorum* oil were nerol (23.0%) and citral (20.7%) (Avetisyan et al., 2017).

The finding of this study lead to the conclusion that the effect of Pb stress caused by the increase in the concentration of root and shoot Pb in the morpho-physiology of the plants is different. Reductions in germination, growth, and some physiological traits and increased enzymatic and non-enzymatic antioxidants such as peroxidase activity and proline content in the leaves indicate the toxic effects of this heavy metal and the production of free oxygen radicals. In conclusion, common basil cultivation in Pb-contaminated soils could cause undesirable effects on the germination indices, growth and morphological traits, and physiological attributes although it might have a positive influence on the essential oil composition under low levels.

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