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Soil cadmium and lead affecting biochemical properties of *Matricaria chamomilla* L. at different growth stages in the greenhouse and field

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Abstract Heavy metals bioremediation by medicinal plants is an important research issue, which has yet to be investigated. *Matricaria chamomilla* accumulation of soil cadmium (Cd, 0, 10 and 40 mg/kg) and lead (Pb, 0, 60 and 180 mg/kg) affecting plant biochemical properties L. at different growth stages in the greenhouse and field was investigated. The 10-kg experimental pots (located in the greenhouse and field with 80% of field capacity moisture) were filled with the treated soils, and were planted with *M. chamomilla* L. seeds (three replicates). Plants were sampled to determine their biochemical properties including Cd and Pb contents, pigments, proline (Pro), leaf relative water (LRW), lipid peroxidation (LX), and superoxide dismutase (SOD, EC 1.15. 1.1), and catalase (CAT, EC 1.11.1.6) activities. Soil final concentration of Cd and Pb was also determined. Heavy metal stress significantly decreased plant pigment contents; however, it significantly increased plant PRO, LRW, LX and SOD, and not CAT. Heavy metal, growth stage, growth location, and their interactions significantly affected plant heavy metal concentrations. Interestingly, although significantly higher concentration of Cd was observed in plant aerial part under greenhouse conditions, plant roots had significantly higher concentrations of Cd under field conditions, and it was reverse for Pb. Increased concentration of Cd and Pb significantly enhanced plant Pro content and the highest one was resulted by Pb3 (913.46 mg/g fresh weight) significantly higher than other treatments including Cd3 (595.34 mg/g fresh weight). *M. chamomilla* is a suitable species for the bioremediation of soils polluted with Cd and Pb.

Keywords Chlorophyll · Carotenoid · Enzymatic activities · Leaf relative water · Lipid peroxidation · Medicinal plants · Proline · Soil pollution

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Introduction

Due to globe industrialization, the concentrations of heavy metals are considerably increasing affecting people health and the environment including plants. The use of medicinal plants, for the promotion of people health and treatment of different diseases, is also increasing (Melkegna and Jonah 2020). However, at the same time medicinal plants, especially in the industrialized areas are subjected to the contamination of different heavy metals including cadmium (Cd) and lead (Pb) (Doostikhah et al. 2020; Georgieva et al. 2020).

Accordingly, investigating the accumulation of heavy metals in medicinal plants, affecting their biochemical properties, is of outmost significance

(Miransari 2011). The plant *M. chamomilla* L. or German chamomile, from the *Asteraceae*/*Compositae* family, as an important medicinal plant. It is an annual herb from the composite family with: (1) a height of 50–80 cm, (2) pyramid-like roots, which are mostly accumulated on the soil surface, (3) high number of root hairs, and (4) thin and alternated leaves. The plant is grown by seed and is tolerant in heavy metal polluted soils (Pirzad et al. 2011).

Different methods including physical, chemical and biological ones have been used for the treatment of the contaminated areas. At the same time the use of tolerant plants is one of the healthiest and most efficient methods for the bioremediation of the contaminated environment. Plants used for the process of phytoremediation can act as hyperaccumulators, metal indicators and metal excluders (Pandey et al. 2019).

Plants, which are suitable for bioremediation, must be able to absorb heavy metals by their roots at the rates higher than 1000 mg/kg, with the translocation factor (shoot/root concentration) and bioconcentration factor (BCF, shoot/soil) higher than 1. However, up to now, there is not any plant with all the above-mentioned properties, and without the use of amendments it is not possible fulfilling all such characteristics. Tolerant plants are able to sequester Pb in and around their roots, and hence prevent the environment from being contaminated. Research has indicated, medical plants can be used for the process of phytoremediation, which is due to their biochemical properties (Egendorf et al. 2020).

Heavy metals may negatively affect plant growth and physiology by binding to the cellular proteins and replacing the essential cations for plant growth, which results in the malfunctioning of cellular organelles by oxidative stress (Asghari et al. 2017). Accordingly, the inactivation of enzymes and production of reactive oxygen species, may lead to the peroxidation of cellular membrane lipid, and subsequent damage of cellular RNA and DNA, amino acids and proteins. Plants alleviate oxidative stress by the production of enzymatic (i.e. superoxide dismutase, glutathione peroxidase, and catalase) and non-enzymatic (i.e. carotenoids) antioxidants (Sajedi et al. 2010, 2011).

However, the interesting point is the increased production of secondary biochemicals in heavy metal stress, which is a function of plant species, and the type and concentrations of heavy metals. The production of reactive oxygen species, under heavy metal stress, results in the production of polyunsaturated fatty acids hydroperoxide (PUFA-OOH) by the oxidation of poly unsaturated fatty acids (PUFA). The produced PUFA-OOH is ultimately converted to oxylipins, which results in the expression of the genes, required for the production and accumulation of secondary metabolites in plant cells (Maleki et al. 2017).

The present research is noticeable from two different aspects: (1) how the growth and biochemical properties of *M. chamomilla* L., as an important medicinal plant, may be affected under heavy metal stress of Cd and Pb, and (2) if bioremediation of heavy metal polluted areas with *M. chamomilla* L. negatively affect the biochemical properties including the secondary metabolites production (medicinal properties) of *M. chamomilla* L. One important method to increase plant tolerance under heavy metal stress is to induce plant production of antioxidants and secondary metabolites (Haque et al. 2020; Nabaei and Amooaghaie 2020).

With respect to the above-mentioned details, and because, to our knowledge, there is not any data on the accumulation of Cd and Pb affecting *M. chamomilla* L. biochemical properties, this research was conducted. The objective was to investigate soil Cd and Pb affecting heavy metal accumulation of *M. chamomilla*, and plant biochemical properties at different growth stages, in the greenhouse and in the field.

Materials and methods

Research site

The experiment was conducted in 2018–2019 in the research greenhouse (G) and research field (F) of Isfahan (Khorasgan) Islamic Azad University, Isfahan, Iran, with the eastern longitude of 51° and 23′ and northern latitude of 32°32′, and the altitude of 1590 m. The climate of the region is dry and semi dry with warm summers, the average yearly rainfall and temperature are equal to 130 mm and 14 °C, respectively. The physical and chemical properties of the soil (0–30 cm) were determined using the standard methods (Miransari et al. 2008) including EC = 4.5 dS/m, pH = 7.9, OC = 0.68%, $CaSO_4$ = 0.05%, total N = 0.007%, available P = 34 mg/kg, SP = 52%, sand = 18%, silt = 51%, and clay = 31%.

Table 1 The concentrations of Cd and Pb in the treated soil, and the soil used for the analyses, before and after treating with Cd and Pb

Characteristics	Pb (mg/kg)	Cd (mg/kg)
Control (not treated) before the experiment	45.75	2.5
Control (not treated) after the experiment	35.25	2.25
The composite soil sample used for the experiment	34.50	2
Cd2 before the experiment		8
Cd2 after the experiment		2.5
Cd3 before the experiment		24.5
Cd3 after the experiment		18
Pb2 before the experiment	73.75	
Pb2 after the experiment	45.25	
Pb3 before the experiment	183	
Pb3 after the experiment	110	

Experimental design

The experiment is a three-way factorial including heavy metal concertation, plant growth stage, and plant growth site on the basis of a completely randomized block design with three replicates. The experimental treatments of lead (Pb) (0, 60 and 180 mg/kg) as Pb $(NO3)_2$, and cadmium (Cd) (0, 10 and 40 mg/kg) as Cd $(NO3)_2$, $4H_2O$ were sprayed to pollute the experimental soil. The sprayed soils were stored in plastic bags for two weeks at 20 °C, and 53% humidity to achieve the equilibrium (Lindsay 1979). The plastic pots (10-kg) with the diameter and height of 25 cm were filled with the treated soils and used in the greenhouse and in the field. The soils of the pots were then examined for the concentration of heavy metals in the Laboratory of Soil and Water, Isfahan's Research and Education Center for Agriculture and Natural Resources (Bremner and Mulvaney 1982) (Table 1).

Seeds of *M. camomilla* L. were obtained from Seed and Plant Research Improvement Institute, Karaj, Iran, and were planted in the pots. The pots were planted in the March of 2018 and 2019, each experiment was one year long. The amount of seeds per pot was 0.002 g, which was mixed with silt and planted, with the final number of 15 plants in each pot. The pots were irrigated to the soil moisture of 80% of field capacity, determined by weighing the collected soils samples. The plants were sampled to determine plant [aerial part (A) and roots (R)] Cd and Pb and biochemical properties at different growth stages including tillering (Ti), stemming (St), and flowering (Fl). The plants were then completely collected at harvest, and the roots were washed with distilled water.

Antioxidant enzymes

The activity of antioxidant enzymes was determined by the following: 100 mg of the plant seedling was treated with one milliliter of the mixed bufferic compound (polyvinylpyrrolidone 1%, triton X-100, K_3PO_4 with the pH of 7) and completely homogenized. The solution was centrifuged at 15,000*g* at 4 °C for 20 min. The supernatant was used to determine the activity of antioxidant enzymes.

Catalase

The bufferic compound (2.95 mL) including K_3PO_4 (50 mL, pH = 7), and H_2O_2 (15 mM) was mixed with 0.05 mL of enzyme extract. The specific activity of catalase was determined using the following formula (volumetric activity of catalase divided by protein concentration of extract)

$$\text{Catalse activity} = \frac{\Delta A \times TV \times D}{\varepsilon \times EV} \quad (1)$$

$$\text{Volumetric activity of catalase} = \text{Catalase activity/volume unit} \quad (2)$$

$$\text{Catalase specific activity} = \text{Catalase volumetric activity (U/ml)/protein concentration of extract (mg/ml)} \quad (3)$$

U is a one unit of catalase activity, which is equal to the amount of the enzyme, which catalyses 1 μM of H_2O_2, and converts it to oxygen and water in 1 min, Δ is the absorbance difference at the wave length of 240 nm in 1 min, TV is the total volume (3 mL), EV is the extract volume (0.05 mL), ε is the catalase coefficient (39.4 mM^{-1} cm^{-1}), D is the coefficient of dilution, for example, if during extracting, 1 mL of bufferic compound is mixed with 0.1 mL homogenized plant sample, D is equal to 10.

Superoxide dismutase

The activity of superoxide dismutase was measured according to the following: 50 μL of enzyme extract was treated with 3 mL of the bufferic solution of 50 mM buffereic phosphate (pH = 7.8), EDTA 75 nM, methionine 13 mM, Nitro blue tetrazolium chloride 63 μM and riboflavin 1.3 mM. The samples were subjected to light for 15 min and then their absorbance was determined at 560 nM using spectrophotometer. A control sample, which had not been subjected to light and another control sample containing all the components of the bufferic solution except the enzymatic extract were also used. The activity of SOD was determined using the following formula:

$$\text{SOD activity} = \left[\left(\frac{\Delta Ac}{\Delta As}\right) - 1\right] \times D$$

U is a one unit of SOD activity is the amount of enzyme, inhibiting 50% of photoreduction of nitro blue tetrazolium chloride, ΔAc is the difference of light absorbance (560 nm) of control sample (without enzymatic extract) in 1 min, ΔAc is the difference of light absorbance (560 nm) of main sample (with enzymatic extract) in one minute, D is the coefficient of dilution.

Bradford assay

The method of Bradford (1976) is a colorimetric method using spectrophotometer to determine sample protein. Bradford indicator was prepared according to the following: 100 mg Coomassie blue (G250) was dissolved in 50 mL ethanol 95%, and was then treated with 85% phosphoric acid. The solution was brought up to the volume (1 L) using distilled deionized water. The solution was then filtered using Whatman (#1) filter paper. The indicator was stored in the dark glass tubes.

Standard protein solution

One milligram of bovine serum albumin (BSA) was dissolved in 1 mL of distilled deionized water to get the standard protein solution of 1 mg/mL. The solution was stored at − 20 °C.

Standard curves

The standard curves were prepared according to the following: 10, 20, 40, 60, 80, and 100 μL of standard protein solutions were poured into autoclaved glass tubes and were brought up to the volume of 100 μL by distilled deionized water. A blank sample for the spectrophotometer was also prepared using 100 μL of distilled deionized water. Each glass tube was treated with 5 mL of Bradford indicator and was mixed thoroughly. The samples were determined by the spectrophotometer after a maximum of 1 h at the wave length of 595 nm, and using the standard curves, the samples were measured for protein concentration (mg/mL) of plant samples. The protein concentration (mg/mL) was calculated by multiplying the instrument value by the dilution factor.

Measurement of plant pigment contents

Using an acetone solution (80%), 0.2 g of leaf fresh sample was smashed in a mortar, followed by the extraction of a 20-mL solution, which was used to determine chlorophyll *a*, *b*, total and carotenoid by the spectrophotometer instrument (Sherwood model) and the following equations:

$$\text{Chlorophyll } a = (12.7 \times \text{A663} - 2.69 \times \text{A645})\ \text{V}/1000$$

$$\text{Chlorophyll } b = (22.9 \times \text{A645} - 4.68 \times \text{A663})\ \text{V}/1000\text{W}$$

$$\text{Chlorophyll total} = \text{Chlorophyll } a + \text{chlorophyll } b$$

$$\text{Carotenoid} = 100\ (\text{A470}) - 1.82\ (\text{mg chl } a) - 85.02(\text{mg chl } b)/198$$

Table 2 Analysis of variance indicating the effects of the experimental treatments on the Pb and Cd contents of plant aerial and root

	d.f	Pr > F			
		PbA	PbR	CdA	CdR
H1	5	< 0.0001	< 0.0001	< 0.0001	< 0.0001
S	2	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Si	1	< 0.0001	< 0.0001	0.1127	< 0.0001
H1*S	10	< 0.0001	< 0.0001	< 0.0001	< 0.0001
H1*Si	5	< 0.0001	< 0.0001	0.1122	< 0.0001
S*Si	2	< 0.0001	0.0006	< 0.0001	0.0005
H1*S*Si	10	< 0.0001	< 0.0001	< 0.0001	< 0.0001

H1 the initial concentration of Pb and Cd, *S* plant growth stage, and *Si* plant growth site, *A* aerial part, *R* roots

V is the volume of the filtered solution (the centrifuge supernatant), A is the light absorption at the wavelengths of 663, 645 and 470 nm, W is the sample fresh weight (g).

Measurement of heavy metal concentration

The concentrations of heavy metals in the plant samples were determined by the wet oxidation method using nitric acid (Bremner and Mulvaney 1982). One milligram of plant sample was treated with 10 mL of nitric acid 65% and 2-mL oxygen peroxide for 24 h and then using a heater (85–100 °C) the samples were heated until the samples became colorless and the brown steams were evaporated. The samples were cooled down and then filtered with Whatman filter paper (#42) and brought up to the volume of 50 mL and the concentration of heavy metals were determined using atomic absorption spectrophotometer (Perkin Elmer AAnalyst 800).

Plant proline, relative water content and sugar were measured according to Noein and Soleymani (2021), Arzanesh et al. (2011), and Askarnejad et al. (2021), respectively.

Statistical analysis

The results were subjected to analysis of variance to indicate the significant differences between the treatments using SAS. Means and their corresponding standard deviations were determined and compared using least significant difference (LSD) at P = 0.05. Graphs were plotted using SAS Proc Plot.

Results

Analysis of variance indicated the significant effects of heavy metals (Cd and Pb), growth stage, growth location, and their interactions on the heavy metal concentrations of plant aerial parts and roots. However, the single effect of growth location and its interaction with heavy metal treatment were not significant on the Cd concentration of aerial part (Table 2).

Cd concentration of plant aerial parts and roots

Increasing Cd concentration, increased plant concentrations of Cd, with significant differences between the aerial part (42.22 mg/kg) and the roots (92.76 mg/kg). The highest concentration of Cd in the aerial part was resulted by treatment Cd3StG (78.25 mg/kg) and the least one was resulted by Pb2TiG (0.32 mg/kg); however, in the case of root Cd concentration the corresponding values were related to Cd3StF (143.56 mg/kg) and Cd1TiG (0.31 mg/kg) treatments, respectively (Table 3).

Interestingly, although significantly higher concentration of Cd was observed in plant aerial part under greenhouse conditions, plant roots had significantly higher concentrations of Cd under field conditions, compared with the greenhouse conditions (Table 2). The stemming growth stage resulted in the highest Cd concentration of plant aerial part (14.14 mg/kg) and plant roots (30.57 mg/kg). There was not a significant difference between the field and greenhouse conditions in terms of Cd in plant aerial part (10.85 and 9.91 mg/kg), however roots contained significantly

Table 3 The final concentrations of plant Cd and Pb at different growth stages, affected by the experimental treatments

H1	S	Si	CdA		CdR		PbA		PbR	
			(mg/kg)							
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cd1	Fl	F	0.39h	0.08	2.64h	1.60	0.65nop	0.29	5.63mn	0.52
Cd1	Fl	G	0.97h	0.11	2.67h	0.33	3.91ghijklm	0.33	18.29ghijklm	1.29
Cd1	St	F	0.46h	0.14	7.06gh	1.93	22.59b	2.19	10.96jklmn	1.00
Cd1	St	G	1.58h	0.60	3.62gh	0.89	4.82fghi	0.64	23.59efghij	8.20
Cd1	Ti	F	0.37h	0.15	10.81g	2.67	5.87fg	1.09	18.06ghijklm	2.08
Cd1	Ti	G	3.09h	1.21	0.31h	0.03	1.07mnop	0.35	110.32b	19.80
Cd2	Fl	F	18.04ef	2.82	25.24ef	2.27	0.37p	0.04	3.40n	1.05
Cd2	Fl	G	21.41de	1.00	31.02e	3.90	3.52ghijklmn	1.25	18.16ghijklm	1.40
Cd2	St	F	12.66g	2.40	61.47d	9.86	17.85c	2.45	26.16efghi	8.21
Cd2	St	G	26.13d	6.21	29.98e	5.99	4.93fgh	0.63	7.54klmn	1.72
Cd2	Ti	F	13.63fg	1.95	30.47e	1.31	10.32e	1.87	11.96ijklmn	1.65
Cd2	Ti	G	10.66g	0.41	19.31f	3.48	2.67hijklmnop	1.33	14.72hijklmn ijklmn ijklmn ijklmn ijklmn ijklmn	2.69
Cd3	Fl	F	37.35c	0.47	102.66b	5.57	0.52po	0.20	14.16hijklmnhijklmn	2.06
Cd3	Fl	G	44.45b	5.43	64.00d	1.28	4.30fghijk	0.69	20.01fghijkl	2.89
Cd3	St	F	45.08b	11.57	143.56a	10.29	12.92de	1.90	16.23ghijklmn	4.26
Cd3	St	G	78.25a	9.53	104.64b	3.52	4.42fghij	2.45	24.98efghij	1.84
Cd3	Ti	F	45.23b	3.86	73.62c	14.24	6.87f	1.63	14.54efghij	1.88
Cd3	Ti	G	2.93h	0.45	68.08cd	14.56	3.33ghijklmno	1.54	37.27e	8.03
Pb1	Fl	F	0.61h	0.10	2.74h	1.47	1.61jklmnop	0.21	6.44lmn	1.14
Pb1	Fl	G	0.42h	0.08	2.82h	0.86	1.91ijklmnop	0.46	16.48ghijklmn	0.65
Pb1	St	F	1.02h	0.43	3.25h	0.51	22.06b	1.21	21.02fghijk	5.26
Pb1	St	G	1.97h	0.03	1.84h	0.18	5.33fgh	0.47	12.13ijklmn	6.51
Pb1	Ti	F	0.37h	0.01	3.21h	0.61	11.47e	3.20	25.61efghi	6.28
Pb1	Ti	G	0.33h	0.02	3.52gh	0.73	1.78jklmnop	0.17	27.88efgh	2.59
Pb2	Fl	F	0.70h	0.15	1.58h	0.24	1.42klmnop	0.38	18.53ghijklm	1.39
Pb2	Fl	G	0.36h	0.10	2.20h	0.76	1.27lmnop	0.09	34.18ef	1.71
Pb2	St	F	0.39h	0.28	1.99h	1.48	18.89c	0.87	6.34lmn	3.92
Pb2	St	G	0.78h	0.20	1.52h	0.80	14.81d	1.36	18.79ghijklm	0.22
Pb2	Ti	F	0.34h	0.06	3.27h	1.03	5.24fgh	0.56	77.29c	12.25
Pb2	Ti	G	0.32h	0.02	4.00gh	1.96	3.77ghijklm	0.47	76.51c	9.20
Pb3	Fl	F	0.80h	0.11	3.48gh	0.64	3.56 ghijklmn	1.61	15.20hijklmn	4.97
Pb3	Fl	G	0.46h	0.07	2.09h	0.55	1.48klmnop	0.19	68.48cd	20.85
Pb3	St	F	0.55h	0.04	4.07gh	0.70	29.06a	7.55	2.47n	0.62
Pb3	St	G	0.84h	0.15	3.89gh	0.80	6.93f	1.66	56.88d	10.98
Pb3	Ti	F	0.49h	0.08	2.23h	0.37	10.71e	1.35	137.51a	33.02
Pb3	Ti	G	0.33h	0.04	2.45h	0.25	4.15fghijk	1.36	30.15efg	7.24

H1 the initial concentration of Pb and Cd, *S* plant growth stage, and *Si* plant growth site

PbA, PbR, CdA and CdR stand for Pb and Cd of the aerial and root, respectively, Cd1, Cd2 and Cd3 stand for control, 10 and 40 mg/kg Cd, respectively. Pb1, Pb2, Pb3 stand for control, 60 and 180 mg/kg Cd, respectively. *Fl*, *St* and *Ti* stand for flowering, stemming and tillering, respectively. *F* and *G* stand for field and greenhouse, respectively

Mean values followed by different letters are statistically different at $P \leq 0.05$ using least significant difference (LSD)

* < 0.05; ** < 0.01

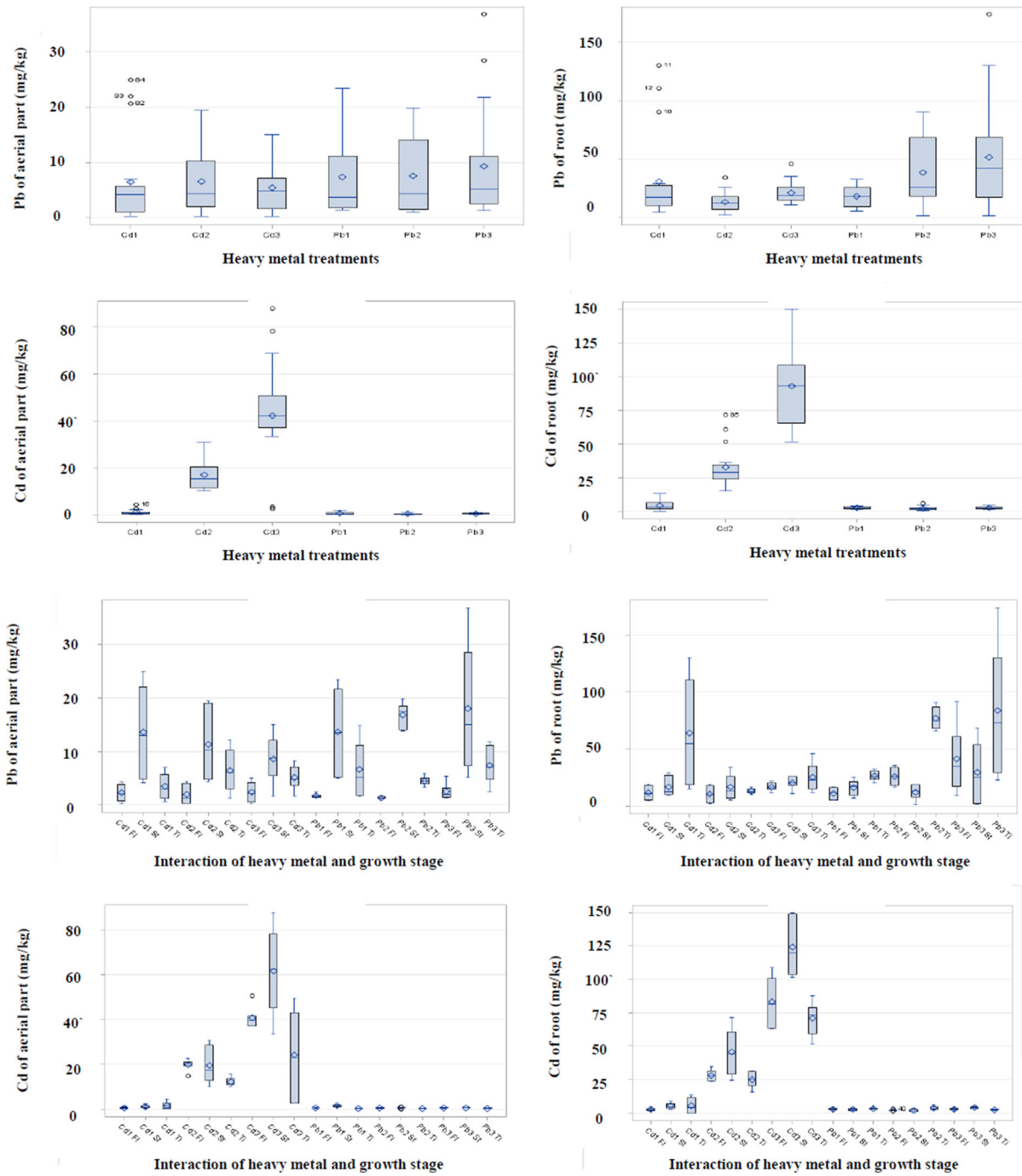
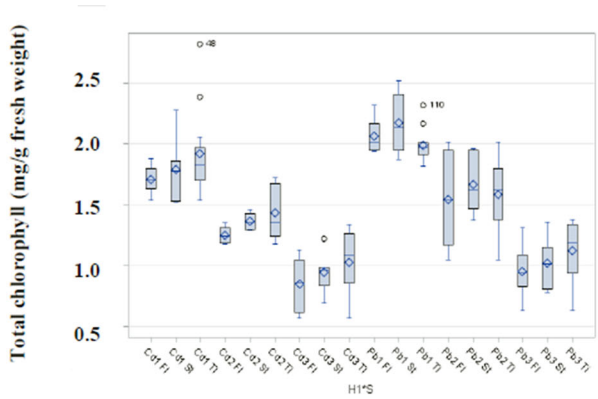
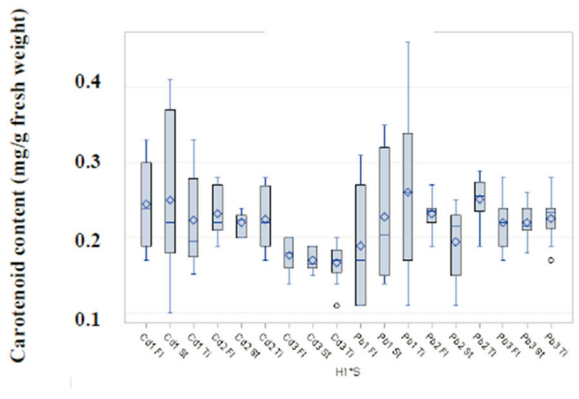
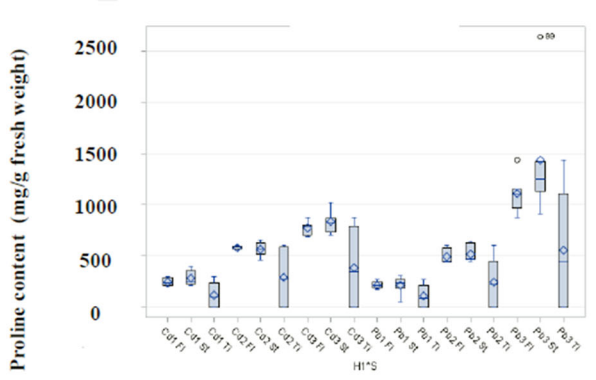
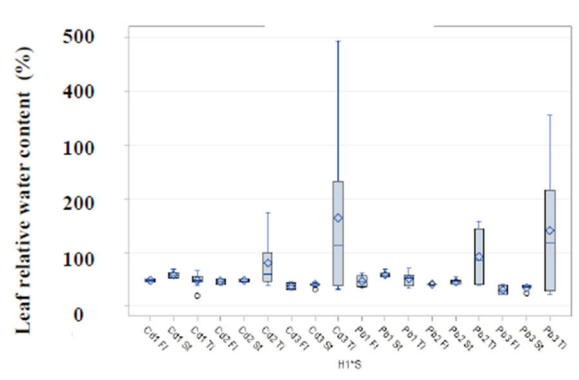


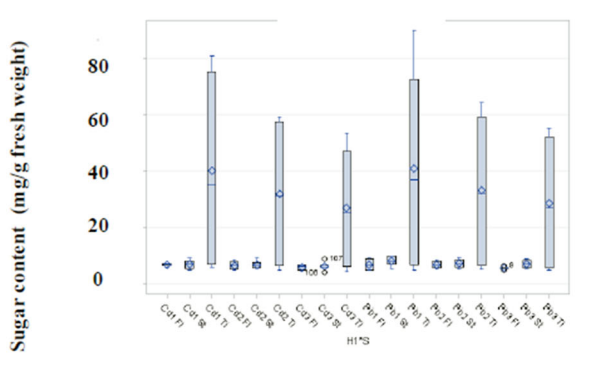
Fig. 1 Plant contents of Pb and Cd affected by the single effects of heavy metals and their interaction with plant growth stage. *A* Greenhouse, *B* field, *Cd1*, *Cd2* and *Cd3* stand for control, 10 and 40 mg/kg Cd, respectively. *Pb1*, *Pb2*, *Pb3* stand for control, 60 and 180 mg/kg Cd, respectively. *Fl*, *St* and *Ti* stand for flowering, stemming and tillering, respectively. F and G stand for field and greenhouse, respectively

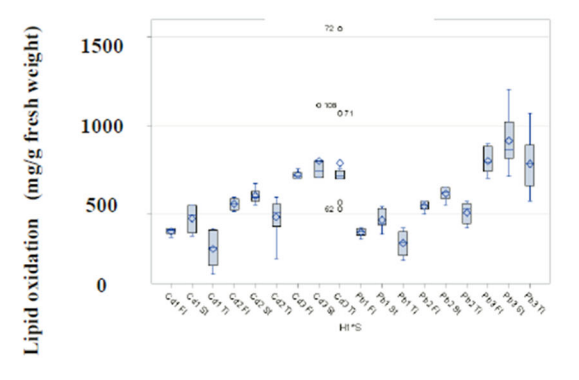

Total chlorophyll (mg/g fresh weight)
2.5
2.0
1.5
1.0
0.5
H1*S

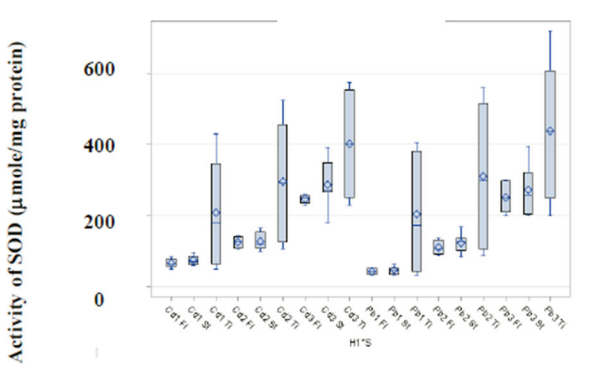

Carotenoid content (mg/g fresh weight)
0.4
0.3
0.2
0.1
H1*S

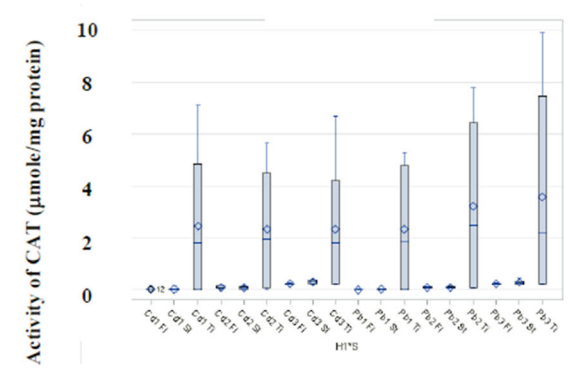

Proline content (mg/g fresh weight)
2500
2000
1500
1000
500
0
H1*S


Leaf relative water content (%)
500
400
100
200
100
0
H1*S


Sugar content (mg/g fresh weight)
80
60
40
20
0
H1*S


Lipid oxidation (mg/g fresh weight)
1500
1000
500
0
H1*S


Activity of SOD (µmole/mg protein)
600
400
200
0
H1*S


Activity of CAT (µmole/mg protein)
10
8
6
4
2
0
H1*S

◀**Fig. 2** Plant biochemical properties affected by the interaction effects of heavy metals and plant growth stage. *A* Greenhouse, *B* field, *Cd1*, *Cd2* and *Cd3* stand for control, 10 and 40 mg/kg Cd, respectively. *Pb1*, *Pb2*, *Pb3* stand for control, 60 and 180 mg/kg Cd, respectively. *Fl*, *St* and *Ti* stand for flowering, stemming and tillering, respectively. *F* and *G* stand for field and greenhouse, respectively

higher Cd concentration (26.85 mg/kg) than the aerial part (19.33 mg/kg) under field conditions (Fig. 1, Table 3).

Pb concentration of plant aerial parts and roots

Higher concentrations of plant Pb were resulted by increasing the concentrations of Pb. Accordingly, the highest Pb concentration of aerial part (9.32 mg/kg) and roots (51.78 mg/kg) was resulted by Pb3. The stemming growth stage resulted in the highest and significantly different Pb accumulation (13.72 mg/kg) compared with the other growth stages (Table 3). However, in the case of plant roots, the highest and significantly different root concentration (48.49 mg/kg) was resulted by the tillering growth stage. Plant aerial part contained significantly higher concentration of Pb under field conditions (10.11 mg/kg), compared with the greenhouse (4.13 mg/kg), however plant roots had higher concentrations of Pb in the greenhouse (34.24 mg/kg) related to the field (23.97 mg/kg) (Fig. 1, Table 3).

Plant biochemical properties

Analysis of variance indicated the single and the combined experimental treatments significantly affected plant biochemical properties including chl *a*, chl *b*, chl T, carotenoid, proline, leaf relative water, sugar, lipid peroxidation, superoxide dismutase and catalase (Table 4).

Pigment contents

With increasing Cd and Pb concentration, plant pigment contents including chla, chlb, chlT, and carotenoid significantly decreased. There were significant differences between tillering and flowering growth stages in terms of chl *a* and chl T. Plant chl T was also significantly different at the flowering and stemming stages. Plant pigment contents were significantly higher in the greenhouse than the field (Fig. 2, Table 5).

Proline (Pro)

Increased concentration of Cd and Pb significantly enhanced plant Pro content and the highest one was resulted by Pb3 (913.46 mg/g fresh weight) significantly higher than the other treatments including Cd3 (595.34 mg/g fresh weight). Plant growth stages were significantly different in terms of proline content, which was significantly higher in the field (498.32 mg/g fresh weight) than the greenhouse (392.04 mg/g fresh weight) (Fig. 2, Table 5).

Leaf relative water

Plant relative water significantly increased with increasing Cd and Pb concentration as the highest one was resulted by Cd3 (101.44%) significantly different from the other treatments including Pb3 (86.24%). The highest LRW was resulted by the tillering growth stage significantly higher than the other growth stages. However, there were not significant differences between the field and greenhouse conditions in terms of LRW (Fig. 2, Table 5).

Sugar

Although with increasing Cd and Pb concentration, plant sugar content decreased, the differences were not significant. The tillering growth stage resulted in the highest and significantly different sugar content, compared with the other growth stages. The sugar content in the field and greenhouse were not significantly different (Fig. 2, Table 5).

Lipid peroxidation

The increased concentration of Cd and Pb significantly increased lipid peroxidation, and the highest oxidation was resulted by Pb3 (918.75 mg/g fresh weight) significantly higher than the other treatments. There were significant differences among different growth stages in terms of lipid peroxidation. Field conditions (615.17 mg/g fresh weight) resulted in significantly higher LX than the greenhouse conditions (523.34 mg/g fresh weight) (Fig. 2, Table 5).

Table 4 Analysis of variance indicating the effects of the experimental treatments on plant biochemical properties

	d.f	Pr > F									
		Cha	Chb	ChT	Car	Pro	LRW	Sugar	LX	SOD	CAT
H1	5	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0566	0.8116	< 0.0001	< 0.0001	0.8315
S	2	0.0134	0.3796	0.0140	0.4425	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Si	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0291	0.4403	0.4018	< 0.0001	0.0507	0.5259
H1*S	10	0.1481	0.0002	0.3779	0.2404	0.0302	0.0344	0.9980	0.5430	0.9999	0.9986
H1*Si	5	0.0002	0.0014	0.0067	0.0015	0.5702	0.8349	0.9995	0.0117	0.9787	0.9962
S*Si	2	0.2875	0.3064	0.4190	0.3048	0.3044	0.2929	0.8765	0.0771	0.9343	0.7392
H1*S*Si	10	0.6114	0.1728	0.1553	0.2003	0.9829	0.9956	1.0000	0.9207	1.0000	1.0000

H1 the initial concentration of Pb and Cd, *S* plant growth stage, and *Si* plant growth site

Superoxide dismutase (SOD)

SOD level was significantly enhanced by increasing the concentration of Cd and Pb as the highest ones were related to Pb3 (350.27 µmole/mg protein) and Cd3 (335.26 µmole/mg protein) significantly higher than the other treatments. The highest SOD was resulted in the tillering growth stage significantly higher than the other growth stages. SOD in the field (252.56 µmole/mg protein) and in the greenhouse (206.06 µmole/mg protein) were not significantly different.

Catalase

Interestingly, increasing the level of Cd and Pb, did not alter plant CAT content. Catalase was also the highest at the tillering growth stage, significantly different from the other growth stages. The concentration of CAT in the field (1.30 µmole/mg protein) and in the greenhouse (1.54 µmole/mg protein) were not significantly different.

Discussion

The medicinal plant *M. chamomilla* L. was tested for its tolerance under heavy metal contamination of Cd and Pb. With increasing the concentration of Cd and Pb, plant accumulated higher rates of such heavy metals, especially by the roots. This can be of significance, because growing medicinal plants in heavy metal polluted soils is important from economic and environmental aspects. Research has indicated medicinal plants can tolerate heavy metal contamination up to some levels, however, the tolerance of some medicinal plants including *M. chamomilla*, under heavy metal stress, is higher compared with the other species of medicinal plants (Pandey et al. 2019).

According to our results, *M. chamomilla* was able to accumulate Cd and Pb to maximum levels of 78.25 (Cd3StG) and 143.56 mg/kg (Cd3StF), and 29.06 (Pb3StF) and 137.51 mg/kg (Pb3TiF) in plant aerial parts and roots, respectively. Research has indicated *M. chamomilla*., as a tolerant plant under heavy metal contamination, can accumulate high Cd concentration in the following order: roots > leaves > flowers, which is similar to our results. It has also been

Table 5 Plant biochemical properties affected by Cd and Pb

H1	Si	Cha		Chb		Cht		Car		Pro	
		mg/g fresh weight									
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cd1	F	0.94d	0.09	0.81c	0.11	1.76c	0.13	0.19def	0.04	222.03efg	141.04
Cd1	G	1.26a	0.19	1.08a	0.18	1.91bc	0.40	0.28a	0.08	162.56g	98.81
Cd2	F	0.80e	0.10	0.53ef	0.05	1.33de	0.14	0.20cde	0.02	454.00cd	274.39
Cd2	G	0.82e	0.15	0.55ef	0.09	1.41d	0.20	0.25bc	0.03	412.38	251.41
Cd3	F	0.51g	0.11	0.30i	0.13	0.81g	0.23	0.15f	0.02	638.71bc	390.31
Cd3	G	0.67f	0.08	0.44gh	0.05	1.12f	0.13	0.19def	0.01	551.98bcd	335.55
Pb1	F	1.05bc	0.04	0.90b	0.06	1.95b	0.06	0.19ef	0.12	190.20fg	116.79
Pb1	G	1.13ab	0.11	1.04a	0.16	2.16a	0.22	0.28ab	0.05	131.55g	89.24
Pb2	F	0.77e	0.15	0.57e	0.07	1.34de	0.20	0.24c	0.03	413.87de	258.77
Pb2	G	1.03cd	0.05	0.70d	0.13	1.85bc	0.14	0.22cd	0.05	338.00defg	204.01
Pb3	F	0.56g	0.19	0.38hi	0.09	0.94g	0.28	0.22cde	0.03	1071.14a	762.88
Pb3	G	0.74ef	0.07	0.47fg	0.05	1.18ef	0.11	0.23cd	0.03	755.77b	464.60
		LRW		Sugar		LX		SOD		CAT	
		% mg/g fresh weight µmole/mg protein									
H1	Si	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cd1	F	51.41	7.47	21.69	27.52	398.57d	111.41	161.30d	148.33	1.08	1.96
Cd1	G	49.24	12.14	25.72	32.68	341.00d	104.60	118.41d	108.93	1.42	2.59
Cd2	F	69.92	52.17	18.22	22.53	542.13c	59.95	232.71bcd	160.15	1.18	1.92
Cd2	G	55.77	10.60	20.64	23.00	516.11c	118.15	191.17 cd	150.16	1.26	2.20
Cd3	F	120.94	167.39	15.67	18.14	861.57a	257.73	350.34a	139.43	1.14	1.56
Cd3	G	81.94	70.89	17.67	19.82	685.56b	67.45	320.19ab	138.17	1.48	2.32
Pb1	F	44.77	12.40	21.23	27.15	408.43d	83.40	138.71d	155.81	1.16	2.10
Pb1	G	57.16	6.02	27.33	34.01	353.77d	69.87	110.74d	138.34	1.19	2.13
Pb2	F	66.52	47.52	18.87	22.97	561.59c	56.91	237.02bcd	185.89	1.66	2.84
Pb2	G	68.30	46.42	21.63	24.48	524.19c	65.53	190.60 cd	173.15	1.67	2.91
Pb3	F	91.46	124.78	16.87	19.66	918.75a	124.80	395.29a	162.28	1.60	2.59
Pb3	G	81.01	78.81	18.58	21.58	719.39b	85.65	305.26abc	174.78	2.24	3.70

H1 the initial concentration of Pb and Cd, *S* plant growth stage, and *Si* plant growth site, *Cha* chlorophyll *a*, *Chb* chlorophyll *b*, *Cht* chlorophyll total, *car* carotenoid, *Pro* proline, *LRW* leaf relative water, *LX* lipid peroxidation, *SOD* superoxide dismutase, *CAT* catalase

Mean values followed by different letters are statistically different at $P \leq 0.05$ using least significant difference (LSD)

* < 0.05; ** < 0.01

indicated that the mobility of Cd in plant is higher than Pb (Pandey et al. 2019; Zeng et al. 2020).

The effect of experimental place (growth conditions) was also significant on the accumulation of heavy metals in *M. chamomilla* as field results were significantly higher than the greenhouse. This might be due to the more suitable and natural conditions of the field for plant growth and physiological activities compared with the greenhouse. The results indicated less Pb was accumulated in the plant compared with Cd, which is similar to Pandey et al. (2019) and Huang et al. (2019).

Plant biochemical properties were also determined in this research to indicate how the accumulation of Cd and Pb may affect plant medicinal properties, which is of economic and environmental significance. The

results indicated plant antioxidant activities improved *M. chamomilla* tolerance in a heavy metal polluted soil. Accordingly, under heavy metal stress of Cd and Pb, the production of the antioxidant enzyme superoxide dismutase (SOD) and lipid peroxidation significantly increased in *M. chamomilla*.

Plant growth stage was also a determining factor as the highest activities of the enzyme was resulted in the tillering growth stage. However, interestingly the activity of CAT was not increased by the stress of heavy metal. The peroxisomes are the main place for the production of CAT in cellular plants. The enzyme is able to catalyse dismutation reactions, without requiring any reductant (Anjum et al. 2016). The results are similar to the other research; for example, Abed et al. (2017) found at higher levels of Pb, the antioxidative response (CAT production) of Radish (*Raphanus sativus*) and cress (*Lepidium sativus*) were similar to that of the control treatment.

The stress of heavy metals significantly decreased *M. chamomilla* pigment contents including chla, chlb, chlt and carotenoid. Due to the production of reactive oxygen species by heavy metal stress, the integrity of cellular membrane, and the cellular organelles such as chloroplast (as the main site for the production of plant pigment contents), is damaged (Alyemeni et al. 2018; Huang et al. 2019). In another research by Rizvi and Khan (2019), it was indicated that the effects of cadmium were more toxic than chromium and nickel in corn. The heavy metal stress significantly decreased plant growth and enhanced the antioxidant activities of the plant including the production of proline (Sofy et al. 2020). The use and the increased production of proline under Pb stress in maize improved plant growth and alleviated the stress. Additionally, under the Pb stress the production of sugar also increased in corn plants.

Ahmad et al. (2016) found increased levels of Cd in mustard (*Brassica juncea* L.), significantly increased plant sugar and osmolyte contents including proline and glycine betaine. Although not investigated in the present research, it has been indicated that essentials oils of medicinal plants are not contaminated under heavy metals stress, showing the great potential of medicinal plants for the bioremediation of heavy metal polluted soils (Pandey et al. 2019). Leaf relative water increased under heavy metal stress, which can be due to the decreased growth of plant, suppressed activity of stomata, and reduced physiological activities of plant, which all collectively result in the less consumption and subsequent accumulation of water in plant.

Conclusion

The heavy metal tolerance of *M. chamomilla* was examined at different growth stages, in the greenhouse and in the field. The results indicated with increasing Cd and Pb concentrations, plant accumulated higher heavy metals, and the roots, and field conditions resulted in significantly higher accumulation of Cd and Pb. The most important results of the present research indicate it is possible to grow *M. chamomilla* in heavy metal polluted soils as: (1) the plant is able to accumulate high concentrations of Cd and Pb under the stress, and (2) the accumulation of Cd and Pb in *M. chamomilla* significantly affected plant biochemical properties including plant pigment contents (chlorophyll and carotenoid), proline, plant relative water, sugar, lipid peroxidation, and superoxide dismutase activity. The other important aspect of the research is to illustrate the role of plant growth stage affecting plant growth and physiology under stress as plant responses were significantly different during stemming, tillering and flowering. The results also indicated plant growth conditions (greenhouse and field) can also significantly affect plant response under the stress of Cd and Pb. The main conclusion is *M. chamomilla* can be used for the bioremediation of soils polluted with Cd and Pb affecting plant biochemical properties and hence plant nutritional, medicinal and pharmaceutical values. Such results are of economic significance because the enhanced production of such biochemical compounds can also affect the production of secondary metabolites in *M. chamomilla*. However, the important point, which determines the usability of *M. chamomilla* grown under heavy metals stress, is the level of accumulated Cd and Pb, as at levels higher than the permissible ones, the use of *M. chamomilla* may not be favorable for human health.

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