Advances in Bioresearch
Adv. Biores., Vol4 (3) September 2013: 39-49
©2013 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr/abr.htm
CODEN: ABRDC3

ORIGINAL ARTICLE

Excluder Strategies in Response to Pb Toxicity in *Matricaria chamomilla*

Fatemeh Zarinkamar, Zohreh Saderi, Saiedeh Soleimanpour
Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
E-mail: zarinkamar@modares.ac.ir

ABSTRACT
Lead is among the most contaminant metals in ecosystem and has different effects on anatomical and morphological characters of different plant species. Matricaria chamomilla is an important medicinal plant which contains 120 chemical compositions. In this research, the effects of lead on production of apigenin as a significant medicinal substance were studied. Furthermore, structural changes of M. chamomilla were studied in different stages of development by electron and optic microscopes. So the plants in first stage of development, were transplanted to hydroponic culture with five-treatment design of 0, 60, 120, 180, 240 µM Pb. The plants under treatment were harvested in first stage of development, as well as in shooting and flowering stages for measurement of Pb absorption. According to this study, morphological changes were not visually observed during the treatment process in different stages; nevertheless, reduction in total biomass was detected in different stages compared to control plants. The results of SEM study of leaf indicated structural changes in stomata and epicuticular waxes in different stages. Also in anatomical studies we noticed several changes in cross section of leaf, stem and root. The obtained results indicated that in different stages, Pb accumulation in roots and shoots has increased under Pb stress during various stage of development. Moreover, Pb stress has no effect on apigenin content. Therefore, M. chamomilla is an excluder species which is tolerant to Pb stress.
Keywords: *Matricaria chamomilla L.; Lead; Anatomy; Apigenin; Phytoremediation.*
Abbreviation: *BCF: Bio-concentration factor, TF: Translocation factor, Ep: Epiderme, Cu: Cuticle, Ph: Phloem, Xy: Xylem, Co: Cortex, Pth: Pith, V: Vascular, S: Space*

Received 27/05/2013; Accepted19/07/2013

INTRODUCTION

Recently, increase in heavy metal content of the environment has become one of the serious ecological problems, which threatens ecosystems and human health [1, 2]. Meanwhile Pb is the most prominent heavy metal contaminant in the environment. Pb pollution of the environment has resulted from mining and smelting activities, Pb-containing paints, gasoline and municipal sewage sludge enriched in Pb [3]. Such a pollution induces negative effects on plant anatomical, morphological and physiological characters such as a significant reduction in biomass production, growth inhibition, induction of leaf chlorosis, reduction in photosynthesis, repression on seed germination, inhibition and activation of enzymatic activities, and a decrease or increase in secondary metabolites content[4, 5]. Besides, entry of heavy metals such as Pb into food chain has caused various diseases and disorders in living organisms which is of significant concern.

Because of the mentioned adverse effects, techniques have been developed in order to remediate contaminated soils, including solidification, soil washing and permeable barriers. These technologies are however expensive and cause further disturbance to the environment. Phytoremediation is a technique with low cost, while being safe to human health and the environment, which makes use of green plants to clean-up contaminated hazardous waste sites [6]. It is well known that plants have different resistances and very different mechanisms for tolerance to heavy metals pollution [7].

Based on tolerance mechanisms, plant species have been divided into two types: (I) *Accumulators*: the plants which actively take up heavy metals and translocation them into above-ground tissues. Bio-concentration factor (BCF): the ratio of metal concentration in plant to soil, and translocation factor (TF): the ratio of metal concentration in shoots to roots, are greater than 1.0 in this type; (II) *Excluders*: the plants which avoid excessive transport of metal ions from the roots to the shoots, and their shoot BCF and TF are lower than 1.0, but their root BCF is greater than 1.0 [8, 9, 10].

Chamomile (*Matricaria chamomilla*), one of the oldest pharmaceutical plants, is tolerant to heavy metals such as cadmium and accumulates high amounts of Cd preferentially in the roots and also in anthodia [11]. The species can be put into facultative metallophytes categories or metal excluders [12].
Hence, the criterion for defining Pb hyper accumulation has considered 1000 μg g^{-1} in shoot dry weight to have no toxic symptoms on treatment plants [13]. We studied response of *M. chamomilla* to Pb toxicity in different stages of development in order to determine the growth stage at which the highest content of Pb can be restored from environment. Moreover, we studied anatomical changes of *M. chamomilla* in Pb pollution. Besides, we investigate in this research the changes of apigenin concentration, one of the secondary metabolites in Pb stress. Apigenin is flavones which is an important medicinal substance.

MATERIAL AND METHODS

Plant Cultivation and Treatment: Chamomile seeds were obtained from Isfahan Agricultural Research Centre. Seeds were first cultivated in the boxes under natural climatic conditions in October 2009 in the green house. Then, ninety-day-old *Matricaria chamomilla* L. plants were transplanted to ½ slightly modified Hoagland solution routinely with continual aeration used in our laboratory [14]. Homogeneous plants were selected cultivated in plastic boxes (9 plants per box) The whole experiment was performed in a growth chamber under controlled conditions: 16 h days length, the photon flux density was 210_μmol^{-2} s^{-1} PAR at leaf level, supplied by cool white fluorescent tubes (TLD 36W/77; Flora, Germany); 25/20 C° day/night temperature, and 60% relative humidity. Solutions were renewed every four days. After 8 days for adaptation of plants to new conditions, $PbNO_3$, $2H_2O$ was added to the medium to achieve concentrations of 60, 120, 180 and 240 μM Pb. Of course before the treatment, the morphological characters (dry weight of root and shoot, length of root, stem and leaf, leaf and rosette number) of plants were studied.
Harvesting the Samples in Different Stages of Development: Plants were harvested in three stages of development for anatomical analysis and Pb determination; the first harvesting was fulfilled in seventh day of treatment at which the plants form basal leaf rosettes only. The second harvesting was performed in fourteenth day in shooting stage. The third one was accomplished in flowering stage in day 21 after treatment. The production of biomass (harvested shoots and roots) was determined after 72 h of drying at 80°C for each of the three stages. Chamomile inflorescences were collected at the stage when about 2/3 tubular flowers were in full flower. For apigenin analysis, they were afterwards dried in the air at room temperature.
Determination of Lead Contents: Total Pb contents in the roots and shoot were determined by flame atomic absorption spectrometry by means of Varian-ICP-OES (Varian Australia Ltd., Vista-pro) equipped with 15 l/min plasma flow, 1/9 ml/min pump rate, and 15s for sample uptake delay. Powder of samples (0.5 g) was used for acid digestion. In this research, wet oxidation was applied for determination of Pb content [15]. Finally, the two indices BCF and TF were measured to evaluate the capacity of plants to accumulate heavy metals.
Sample Preparation for Apigenin-7-glucoside Determination: 20 ml methanol was added to 0.5 g of chamomile inflorescent powder. The mixture was sonicated for 20 minute and cooled to room temperature (28 ± 2°C). The extract was subsequently filtered through What man filter paper no. 41 (E. Merck, Mumbai, India) pore size 0/45μm, AG.37.70 Gottingen. Germany) and the filtrate were used as the sample solution for assay.
For quantification of apigenin-7-glucoside, the method described in Britannia Pharmacopoeia (2004) was applied. HPLC system included Knuwer pump and ODS -80 Ts column (4.6×250mm). The mobile phase comprised of acetonitrile and deionizer water in gradient mode. The flow rate was 1.0 ml/min and the temperature of column was controlled at 25 C°.
Preparation Necessary for Microscopic Study: Stem and root sections of approximately 5mm length were cut out from 2 cm above and 2 cm below the stem–root intersection. Leaf sections of 5 mm length were cut out from the middle segment of the most juvenile leaf from the base of the plant.
Light Microscopy (LM): Samples were stored in 30% alcohol before being sectioned. The sections were stained with Carmen and methyl green. We used normal transmitting light for the microscopic analysis (Olympus BH2). Digital photographs were taken with an Olympus camera DP 12. The anatomical descriptions were yielded from measurement software.
The statistical analyses were made using SAS 9.1 for Windows. Values reported here are means of three replicates. Data were tested at significant levels of $P < 0.05$. Analysis of variance was used for comparing the group means between control and treated groups by Duncan examination. Also, charts were drawn by Excel software.

RESULTS

Morphological observation: In hydroponics experiment, *M. chamomilla* grew well at different Pb concentrations, and no morphological symptoms such as chlorosis and necrosis were observed. However, the total biomass reduced in different stages of development which is a result of decreased number of leaves, fresh and dry weight, and length of root and shoot (Tables 1, 2, 3; Fig. 1).

Anatomical studies of leaf surface by SEM indicated that epicuticular waxes, size of guard cells and stomata ostiole were influenced by Pb concentrations. The epicuticular wax deposition in control leaf was less than that in Pb-treated leaves and it increased with enhancement of Pb concentration (Table 4; Fig. 3). The length of guard cells was also affected by Pb in both surfaces which increased with raise in Pb concentration especially and the tallest of guard cells were shown in 180 µM treatment in rosette stage (Figs. 2 and 3). Size of stomata ostiole was also reduced by Pb concentrations. Maximum level of decrease in stomata ostiole was observed in the treatment Pb 180 (pertaining to rosette stage) such that the stomata ostiole was completely closed. Stomata deformity was also observed in rosette stage.

Anatomical studies of leaf and stem: The statistical results of measurement in the transversal section of the leaf and stem revealed a reduction in leaf surface and stem diameter. The decrease observed in stem diameter and leaf thickness was due to the reduction of xylem and phloem tissue. It should however be noted that a considerable increase was also observed in cortical parenchyma, though it did not compensate the mentioned decrease. In addition, in transversal section of stem the veins experience a disorder caused by Pb treatment. Never the less, such disorder dose not influences the number and shape of secretary ducts (Tables 5- 9; Figs. 4 and 5).

Anatomical studies of root: Different effects on root anatomy were observed in the three stages of development. In the shooting and flowering stage, root diameter increased especially in xylem tissue, while it decreased in rosette (Tables 10 and 11; Fig. 5).

However in the three studied stages, Pb concentration was observed to cause thickness of cell walls of parenchyma. Pb concentrations oblige parenchyma cells to rapidly begin the secondary growth.

Pb concentrations in plant tissues: Analysis of Pb concentrations in plant organs showed that Pb concentrations in root were significantly higher than those in shoot in different stages of development, such that in rosette stage the ratio of Pb concentration in root to shoot in 180 µM treatment was eight-fold. In the three studied stages, the highest Pb concentration was observed in 180 µM treatment (Tables 13- 15).

According to results, TF in all development stages was measured to be less than 1and root BCF was greater than 1, except in 60 µM treatment (Tables 13- 15).

Apigenin evaluation: Analysis of apigenin percentage of *M. camomilla* by HPLC revealed that, apigenin content of shoot in pre-flowering stage (shooting) in comparison with apigenin content of flowers in flowering stage was 40-fold. However Pb stress had no significant effect on extracted apigenin contents in shooting and flowering stages (Table 16).

Figure1: Plant in rosette stage; A, control; B, 60µM; C, 120µM; D, 180µM; E, 240µM

Table 1. Influence of Pb on morphological characters of *M.chamomilla* in rosette stage.

Pb concentration (µM)	dry weight of root (g)	dry weight of (g) shoot	root length (mm)	leaf length (mm)	Rosette numbers	leaves numbers
0	0.173 a	0.979 a	1.3 a	4.06 a	4 a	5.67 a
60	0.152 b	0.684 c	0.93 b	2.63 ab	2.67 ab	4 ab
120	0.123 c	0.852 b	0.77 c	3.63 a	3.67 a	5.33 a
180	0.059 e	0.257 e	0.47 d	1.23 c	1.33 c	2.33 b
240	.0.091d	0.539 d	0.57 d	2.3 ab	2.33 ab	3.67 ab

Different letters in the same columnindicate significant differences ($P<0.05$) among the treatments and control.

Table 2. Influence of Pb on morphological characters of *M.chamomilla* in shooting stage.

Pb concentration (µM)	dry weight of root (g)	dry weight of shoot (g)	root length (mm)	shoot length (mm)	leaf length (mm)	Rosette numbers	leaves numbers
0	0.43 a	.3.02 a	3.1 a	5.5 d	4.57 a	4.33 a	6.67 a
60	0.37b	2.62 b	2.57 b	7.8 c	3.66 b	3 b	4.33 b
120	0./35 b	2.28 bc	2.33 b	9.3 b	2.82 c	2 c	2.69 c
180	0.29 c	2.1 c	1.27 c	11.1 a	2.1 d	1.33 d	2 c
240	.0.36 b	2.59 b	2.5 b	8.2 c	3.6 b	3 b	4 b

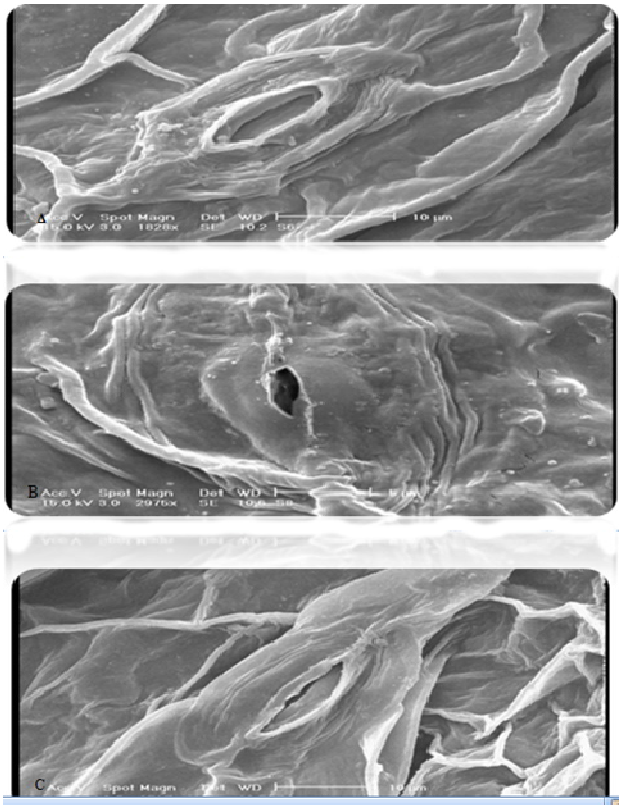

Figure 2: Adaxial stomata in 180 µM Pb; A, control; B, rosette stage; C, flowering stage

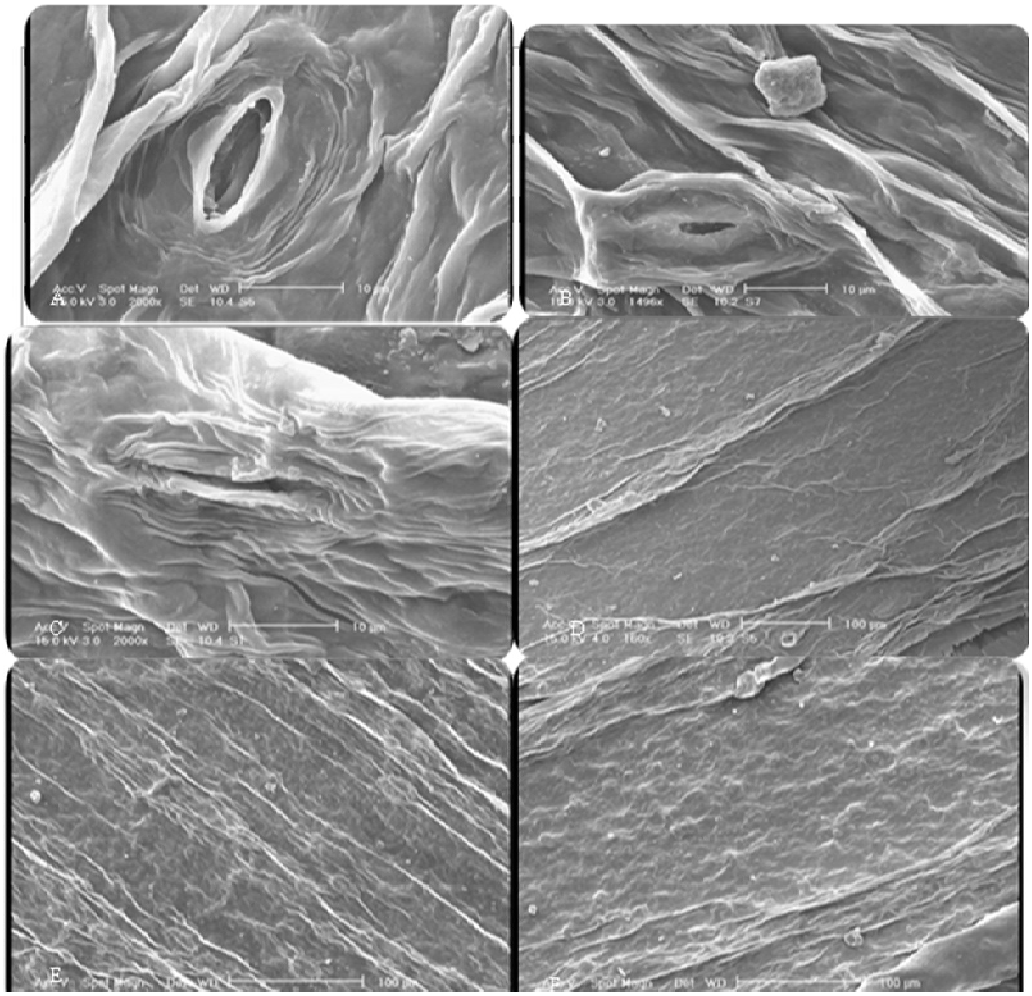

Figure 3: Abaxial stomata and epicuticular wax in superficial view in 180 µM Pb; A, D, control; B, E, rosette stage; C, F, flowering stage.

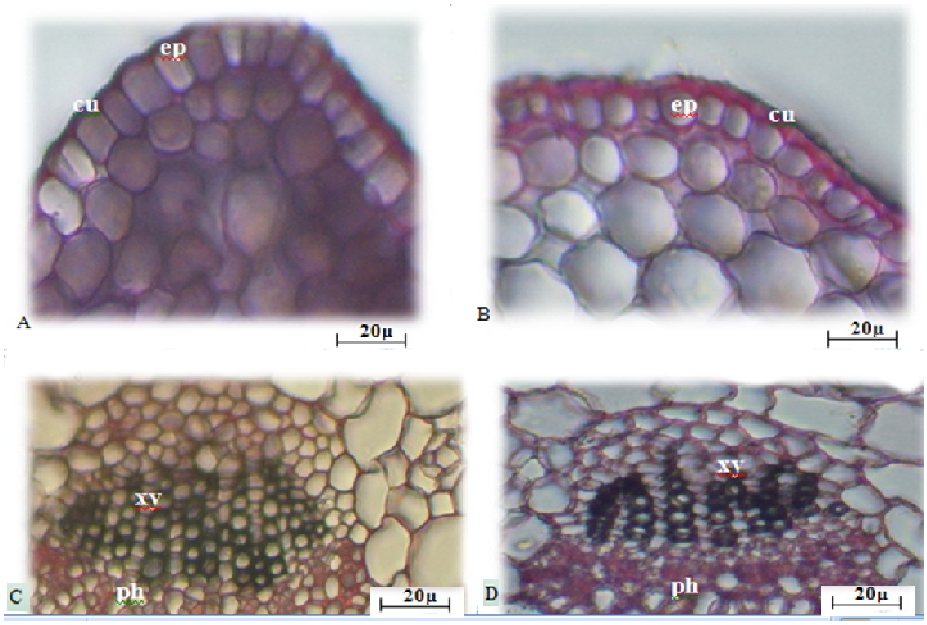

Figure 4, A-B: cross section of abaxial epidermis and midrib in flowering stage; A-C control; B-D, 180 µM Pb; Ep, Epiderme; cu, cuticle. Ph, Phloem; xy, xylem.

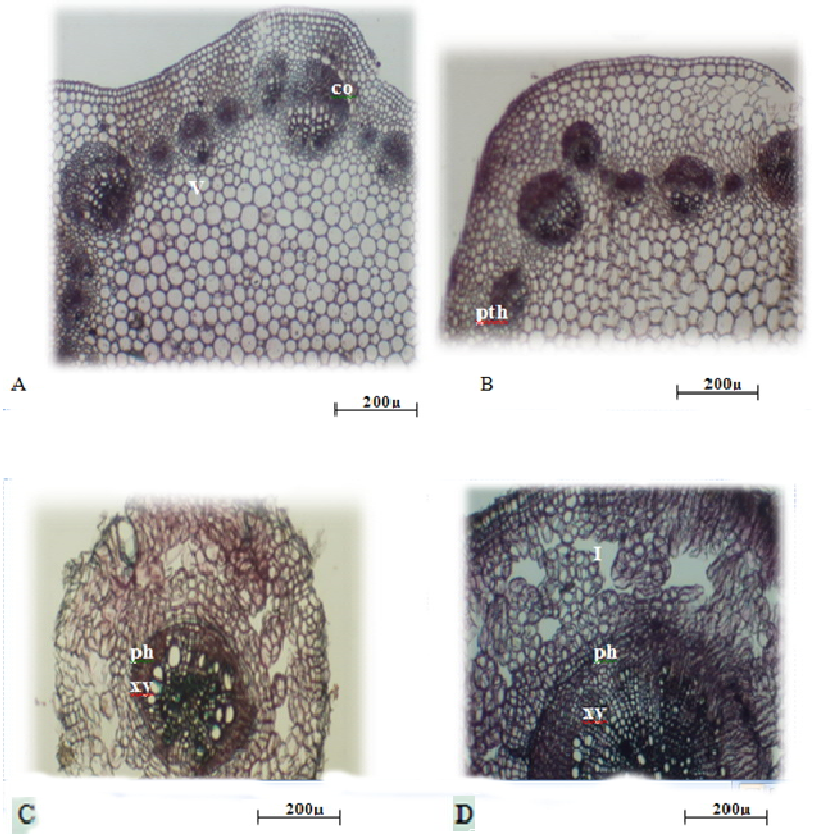

Figure 5: Cross section of stem and root; A-B, stem in shooting stage; A, control; B, 180 µM; C-D, root in rosette stage; C, control; D, 180 µM; co, cortex; pth, pith; V, vein. Ph, phloem; xy, xylem; I, intercellular space.

Table 3. Influence of Pb on morphological characters of *M.chamomilla* in flowering stage.

Pb concentration (µM)	dry weight of root (g)	dry weight of shoot (g)	dry weight of flower (g)	root length (mm)	stem length (mm)	leaf length (mm)	Rosette numbers	leaves numbers
0	0.7 a	4.82 a	0.024 d	4 a	9.02 c	3.59 a	4.67 a	5.32 a
60	0.49 b	3.8 b	0.034 c	0.03 b	13.2 b	2.87 b	3 b	3.33 b
120	0.39 c	2.83 c	0.056 b	2 c	17.32a	1.82 c	2 c	1.67 c
180	0.32 c	2.1 d	0.067 a	1.92 c	18.73a	1.71 c	1.33 c	1.33 c
240	0.48 b	3.66 b	0.039 c	2.87 b	14.2 b	2.54 b	3 b	3 b

Table 4. Influence of Pb on stomata of *M.chamomilla* in different stage of development.

Stage of development	Pb concentration (µM)	adaxial stomata closure (mµ)	abaxial stomata closure (mµ)	adaxial guard cell length (mµ)	abaxial guard cell length (mµ)
rosette	0	4.16 a	4.12 a	18.42 d	19.7 c
	180	2.96 b	1.06 c	33.77 a	29.97 a
	240	3.3 b	2.06 b	23c	27.1 a
flowering	180	2.06 d	0 d	28.8 b	23.27 b
	240	2.56 c	0 d	26.23 b	21.5 b

Table 5. Influence of Pb on leaf structure of M.chamomilla in rosette stage.

Pb concentration (Mμ)	Adaxial epidermis (mμ)	Adaxial epidermis (mμ)	Adaxial cuticular (mμ)	Adaxial epidermis (mμ)	midrib length (mμ)	xylem length (mμ)	Phloem length (mμ)	Glandula duct (mμ)	Leaf area (μm²)
0	10.45 a	12.32 a	1.2 d	1.74 d	91.44 a	34.56 a	12.74 a	28.44 a	383.54 a
60	9.99b	11.52 b	1.4 c	2.08 c	79.9 c	28.8 c	11.72 bc	27.86 a	374.12 b
120	9.95 b	10.11 c	1.84 b	2.58 b	85.55 b	31.74 b	12.12 b	27.15 a	377.17 b
180	9.02 d	8.61 e	2.2 a	3.04 a	70.79 e	23.62 d	10 d	25.34 a	353.06 d
240	9.49 c	9.33 d	2.03 ab	2.73 b	75.01 d	26.69 c	11.35 c	26.7 a	360.56 c

Table 6. Influence of Pb on leaf structure of *M.chamomilla* in shooting stage.

Pb concentration (Mμ)	Adaxial epidermis (mμ)	Adaxial epidermis (mμ)	Adaxial cuticular (mμ)	Adaxial epidermis (mμ)	midrib length (mμ)	xylem length (mμ)	Phloem length (mμ)	Glandular duct (mμ)	Leaf area (μm²)
0	11.28 a	11.72 a	1.45 d	1.81 d	96.91 a	35.56 a	14.24 a	29.47 a	400.35 a
60	11 ab	11.4 a	1.93 c	2.46 c	86.55 b	32.13 b	12.96 b	28.92 a	394.21 a
120	10.32 c	10.71 b	2.52 ab	2.79 ab	78.9 c	30.79 b	11.88 c	28.14 a	373.4 b
180	9.52 d	10.15 c	2.68 a	3.04 a	73.94 d	25.41 c	10.52 d	25.87 a	359.57 c
240	10.7 bc	11.03 b	2.2 bc	2.73 bc	84.14 b	30.14 b	12.58 b	28.03 a	378.92 b

Table 7. Influence of Pb on leaf structure of *M.chamomilla* in flowering stage.

Pb concentration (Mμ)	Adaxial epidermis (mμ)	Adaxial epidermis (mμ)	Adaxial cuticular (mμ)	Adaxial epidermis (mμ)	midrib length (mμ)	xylem length (mμ)	Phloem length (mμ)	Glandular duct (mμ)	Leaf area (μm²)
0	11.61 a	10.69 a	1.71 c	1.84 c	98.92 a	39.56 a	15.36 a	30.24 a	407.02 a
60	11.21 a	11.58 a	2.29 b	2.7 b	89.8 b	36.79 b	13.6 b	29.55 a	398.88 a
120	10.62 b	10.87 b	2.56 ab	2.92 ab	79.7 c	31.8 c	13.01 c	28.67 a	378.07 b
180	10.09 c	10.48 c	2.84 a	3.1 a	77.34 c	29.6 c	12.29 d	26.447 a	365.24 c
240	10.67 b	11.08 b	2.32 b	2.76 b	87.48 b	36.24 b	13.68 b	28.89 a	382.59 b

Table 8. Influence of Pb on stem structure of *M.chamomilla* in shooting stage.

Pb concentration (Mμ)	epidermis (mμ)	cuticle (mμ)	cortex (mμ)	Diameter of vascular system(mμ)	xylem length (mμ)	Phloem length (mμ)	Pith of stem (mμ)	Diameter of stem (mμ)
0	7.52 a	0.68 a	41.34 c	70.91 a	30.22 a	10.52 a	495.49 a	600.35 a
60	7.17b	0.69 a	44.08 b	65.44 b	27.44 b	10.01 b	485.46 b	583.54 b
120	6.22 c	0.72 a	54.48 a	47.25 d	23.61 c	9.16 c	476.93 b	576.07 b
180	6.16 c	0.73 a	55.86 a	44.55 d	22.08 c	8.91 c	468.38 c	567.57 c
240	6.97 b	0.7 a	45.03 b	60.26 c	26.12 b	9.98 b	481.69 b	578.92 b

Table 9. Influence of Pb on stem structure of *M.chamomilla* in flowering stage.

Pb concentration (Mμ)	epidermis (mμ)	cuticle (mμ)	cortex (mμ)	Diameter of vascular system(mμ)	xylem length (mμ)	Phloem length (mμ)	Pith of stem (mμ)	Diameter of stem (mμ)
0	7.56 a	0.68 a	43.12 c	71.91 a	31.22 a	11.72 a	496.82 a	601.85 a
60	7.23 a	0.69 a	45.64 b	66.01 b	28.56 b	11.21 b	491.37 a	585.14 b
120	6.23 b	0.72 a	55.68 a	49.58 c	24.71 c	10.59 c	479.22 b	578.74 b
180	6.12 c	0.73 a	56.79 a	47.22 c	23.09 c	10.02 d	469.32 c	568.52 c
240	7.01 b	0.7 a	46.36 b	63.18 b	27.13 b	11.18 b	482.16 b	579.65 b

Table 10. Influence of Pb on root structure of *M chamomilla* in rosette stage.

Pb concentration (µM)	Diameter of central vascular (mµ)	xylam length (mµ)	phloem length (mµ)	Diameter of root (mµ)
0	259.31 c	170.47 c	53.69 a	624.77 c
60	263.2 b	179.43 b	52.85 b	630.1 b
120	263.9 b	184.61 b	50.38 b	632.33 b
180	268.89 a	194.63 a	45.45 c	641.96 a
240	267.64 a	189.81a	47.26c	638.36 a

Table 11. Influence of Pb on root structure of *M chamomilla* in shooting stage.

Pb concentration (µM)	Diameter of central vascular (mµ)	xylam length (mµ)	phloem length (mµ)	Diameter of root (mµ)
0	270.31 a	188.54 a	54.54 c	630.11 c
60	267.05 b	183.36 b	55.36 b	638.57 b
120	264.81 bc	180.1 bc	56.55 b	642.36 ab
180	264.59 c	177.36 c	57.71 a	644.73 a
240	266.61 bc	183.84 b	55.74 b	638.76 b

Table 12. Influence of Pb on root structure of *M chamomilla* in flowering stage.

Pb concentration (µM)	Diameter of central vascular (mµ)	xylam length (mµ)	phloem length (mµ)	Diameter of root (mµ)
0	270.43 c	189.4 c	56.05 c	635.84 c
60	274.76 ab	193.64 ab	58.51 ab	650 a
120	273.31 b	192.67 b	57.57 b	645.36 b
180	272.88 b	192.55 b	57.63 b	644.57 b
240	276.6 a	195.37 a	59.44 a	651.83 a

Table 13, Lead concentrations in root and shoot of *M.chamomilla* in rosette stage.

Pb concentration (µM)	shoot (ppm)	root (ppm)	root BCF	TF
0	22.71 e	56.25 e	-	-
60	121.82 c	208.32 d	0.72	0.58
120	92.34 d	794.13 c	1.37	0.12
180	256.47 a	2159.22 a	2.48	0.12
240	172.02 b	1572.74b	1.21	0.12

Table 14, Lead concentrations in root and shoot of *M.chamomilla* in shooting stage.

Pb concentration (µM)	shoot (ppm)	root (ppm)	root BCF	TF
0	15.01 d	50.98 c	-	-
60	38.42 c	412.23 b	1.42	0.09
120	54.38 b	410.71 b	0.71	0.12
180	60.2 a	517.73 a	0.6	0.12
240	41.19 c	407.85 b	0.35	0.1

Table 15, Lead concentrations in different parts of *M.chamomilla* in flowering stage.

Pb concentration (µM)	shoot (ppm)	root (ppm)	flower (ppm)	root BCF	TF
0	15.01 d	50.98 c	10.2 d	-	-
60	38.42 c	412.23 b	79.53 c	0.65	0.42
120	54.38 b	410.71 b	218.17 a	0.18	0.88
180	60.2 a	517.73 a	109/12 b	0.8	0.12
240	41.19 c	407.85 b	71.89 c	013	0.5

Table 16, Influence of Pb on apigenin contents in *M.chamomilla* in shooting and flowering stages.

Pb concentration (µM)	Apigenin contents in shooting stage (mg/ml)	Apigenin contents in flowering stage (mg/ml)
0	0.008 b	0.36 a
60	0.007 b	0.32 a
120	0.008 b	0.34 a
180	0.008 b	0.35 a
240	0.007 b	0.3 a

DISCUSSION

Chlorosis of leaves is one of the most prevalent morphological changes and one of the first observable symptoms of heavy metal toxicity which is a result of decreased rate of chlorophyll biosynthesis and content. In our study, the treatment plants showed no morphological changes. In this study we report that, up to 180 µM concentration, lead acts as repressor of the growth of root and aerial tissue. A decrease in cell division increased the thickness of cell wall, and disorder in activity of hormones like auxin in chamomile roots exposed to Pb stress has been shown to be the cause of restricted root growth [16, 17, 18, 12]. On the other hand, reduction of shoot growth in the presence of lead is due to reduction of photosynthesis. In comparison with the control samples, it was observed that the smaller biomass in treatment plants is accompanied by the restriction of growth, increased rate of development and accelerated entry into the generative phase. The short life cycle of treatment plants is possibly the strategy for large reproductive effort and maintaining the tolerance.

Plant cuticles, which are the main barriers between the interior and the outer environment of the leaf, can hence serve as diagnostic marker of exposure to air pollution; furthermore alteration of epicuticular wax is one of the most important pollution symptoms [19]. In our study, Pb stress caused formation of contiguous layer on leaf area which could prevent the loss of water and dehydration of cells, similar to

findings of Douchiche [20]. Decrease in ostiole diameter cause a decrease in transpiration and stomata conduction [21, 22, 23]. Indeed, deformation of stomata and decrease of its diameter in Pb exposure caused to reduce photosynthesis [24] On the other hand, reduction of leaf surface in plant treatment decreases transpiration and gas exchange and reduction of leaf midrib causes to distribution of water and nutrient transfer and reduced photosynthesis [23].

According to our results, root as the first organ of plant in different stages of development makes use of different mechanisms in the presence of heavy metals. In the early vegetative growth, high uptake of Pb by root was accompanied by increase in diameter of xylem and phloem and also root cortex; however after entry of treatment plants into the generative phase, the mention parameters decreased. On the other hand, continuation of treatment period until flowering stage caused an increase in diameter of vascular system and root cortex in 240 µM treatment, which might have developed mechanisms to compensate for growth induction. Moreover, decrease in the intercellular spaces of cortical cells in root and increased of thickness in their cell wall in high concentrations of Pb especially in 240 µM treatment were observed, which might be due to Pb accumulation in cortical cell wall.

Production and accumulation of secondary metabolites have been recognized as a defensive mechanism in stress exposure. However, in this study Pb stress caused no effect on apigenin concentrations [25, 12, 26]. This result might be due to biosynthesis pathway of apigenin. Flavone biosynthesis is possibly feedback-inhibited by apigenin because a 50% increase in production of apigenin would reduce naringenin accumulation by 48% [27].

In the present research, Pb contents were higher in the roots than in the shoots, it may be due to either: I) the detoxification processes started soon after the initial accumulation of Cd in root tissue, thereby minimizing the amount of residual Cd for uptake by the shoots; or II) the means for detoxification were stronger in the aerial parts, especially in the stem [28]. Also immobility of Pb and existence of the root endodermis (stele) restricts Pb transport by means of its storage in cell walls and vacuoles, or by binding to metallo-thioneins or phytochelatin [29].

Pb concentrations were higher in early vegetative growth stage of *M. chamomilla* because of a relatively high nutrient uptake compared to growth rate and were caused by increase of control on transport accompanied by maturity [30, 31].

CONCLUSION

Different responses to Pb toxicity in root, leaf and stem of *M. chamomilla* were observed in different stages, but no change in apigenin content was seen in this condition.

Pb content in shoot tissue of chamomile showed that, the species could not be introduced as a hyper accumulator species. But due to accumulation of high amounts of Pb in its roots especially in rosette stage and considering the calculated values of BCF and TF as well as observing no toxic morphological symptoms, *M. chamomilla* can therefore be considered a Pb excluder. The yielded results indicate that *M. chamomilla* may potentially be useful for restoring Pb contaminated sites especially in early vegetative growth stage.

REFERENCES

1. Lasat, M. (2002) Phytoextraction of toxic metals: A review of biological mechanism. *Environ. Qual* 31: 109-120.
2. Kachout, S. S., Leclerc, J.C., Mansoura, A. B., Rejeb, M. N. and Ouerghi, Z. (2009) Effects of Heavy Metals on Growth and Bioaccumulation of the Annual Halophytes *Atriplex Hortensis* and *A. Rosea*. *J. Applied Sciences Research* 7: 746-756.
3. Sharma, P., Dubey, R. (2005). Lead toxicity in plant. Plant physiology. 17: 32-52.
4. Trillini, B., Ricci, A., Pintore, G., Chessa, M. (2006). Induction of hypericin in *Hypericum perforatum* in response to choromium. *Fitoterapia*.77: 164-170.
5. Murch, S., Haq, K., Rupasingh, H., Saxena, P. (2003). Nickel contamination affects growth and secondary metabolite composition *Hypericum perforatum* L. *Environmental and Experimental Botany*. 49. 251-257.
6. Chaney RL, Malik M, Li YM, Brown SL, Angle JS Baker AJM (1997) Phytoremediation of soil metals. *Opinions Biotechnol*. 8: 279-284.
7. Baker A, McGrath S, Reeves R, Smith J. (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of Metal-Polluted Soils. In: Terry N, Banuelos G, editors. *Phytoremediation of Contaminated Soil and Water*. Boca Raton: Lewis Publishers; pp. 85–108.
8. Baker, A. (1981) Accumulator and excluders-strategies in the response of plants to heavy metals. *J. Plant Nutr* 3:643–654.
9. Zayed, A., S. Gowthaman and N. Terry, (1998) Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *J. Environ. Qual*., 27:715–721.
10. Zhang, S., Chen, M., Li, T., Xu, X., Deng L. (2010) A newly found cadmium accumulator—Malva sinensis Cavan. *Journal of Hazardous Materials.* 173 705–709.

11. Kovacik, J., Tomko, J., Backor, M., Repcak, M. (2006). *Matricaria chamomilla* is not a hyperaccumulator,but tolerant to cadmium stress. Plant Growth Regul. 50. 239–247.
12. Grejtovský, A., Markušová, K., Eliašová, A. (2006). The response of chamomile (*Matricaria chamomilla* L.) plants to soil zinc supply. *Plant Soil Environ*. *52*. 1–7.
13. Baker, A., Brooks R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistery. *Biorecovery*. 1. 81-126.
14. Utmazian, M., Wieshammer, G., Vega, R. and Wenzel, W. (2007). Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. *Environmental Pollution*. 148. 155-165.
15. Gupta, P. (2000) Soil, plant, water and fertilizer analysis. *Agro bios*, New Delhi, India. 438.
16. Dauda, M. K., Variatha, M. K., Shafaqat, A., Najeeba, U., Jamilb, M., Hayat, Y., Dawooda, M., Khand, M. I., Zaffar, M., Cheemad, S. A., Tonga, X. H., Zhua, S. (2009). Cadmium-induced ultramorphological and physiological changes in leaves of two transgenic cotton cultivars and their wild relative. *J. Hazardous Materials*. 168. 614–625.
17. Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J., Jansen, M. A. K. (2007). Stress-induced morphogenic responses: growing out of trouble. *Plant Science*, 12. 98-105.
18. Almeida, A. F., Valle, A. A., Mielke, M. S., Gomes, F. P., Braz, J. (2007). Tolerance and prospection of phytoremediator woody species of Cd, Pb, Cu and Cr. *Plant Physiol*. 19. 83-98.
19. Verma, A., Singh, S. N. (2006). Biochemical and ultra structural changes in plant foliage exposed to auto-pollution. *Environmental Monitoring and Assessment*. 120. 585–602.
20. Douchiche, O., Rihouey, C., Schaumann, A.,·Driouich, A., Morvan, C. (2007). Cadmium-induced alterations of the structural features of pectins in Xax hypocotyls. *Planta*. 225. 1301–1312.
21. Pierzynski, G. M., Schnoo, J. L., Youngman, A., Licht, L., Erickson, L. E. (2002). Poplar Trees for Phytostabilization of Abandoned Zinc-Lead Smelter. *Pract. Periodical of Haz., Toxic, and Radioactive Waste Mgmt*. 6. 177-183.
22. Ying, R. R., Qiu, R. L.,Tang, Y. T., Hua, P. J., Qiu, H., Chen, H. R., Shi, T. H., Morel, J. L. (2010). Cadmium tolerance of carbon assimilation enzymes and chloroplast in Zn/Cd hyperaccumulator *Picris divaricata. J. PlantPhysiology*. 167. 81–87.
23. Kosobrukhov, A., Knyazeva, I., Mudrik, V. (2004). Plantago major plants responses to increase content of lead in soil: Growth and photosynthesis. *Plant Growth Regulation*. 42. 145–151.
24. Molas, J. (1997). Changes in morphological and anatomical structure of cabbage (Brassica oleracea L.) outer leaves and in ultrastructure of their chloroplasts caused by an in vitro excess of nickel. *Photosynthetica*. 34. 513-522.
25. Grejtovsky, A., Repcak, M., Gianits, L. (1998). The influence of soil cadmium eliminating sorbents on Chamomilla recutita. *Environ Sci Health*. 33. 307–316.
26. Dardanelli, M. S., Manyani, H., Barroso, S.G., Carvajal, M. A., Serrano, A. M., Espuny, M. R., Javier, F. (2009). Effect of the presence of the plant growth promoting rhizobacterium (PGPR) Chryseobacterium balustinum Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. *Plant Soil*. 328; 483-493.
27. Leonard, E., Yan, Y., Lim, K. H., and Koffas, M. A. G. (2005). Investigation of Two Distinct Flavone Synthases for Plant-Specific Flavone Biosynthesis in *Saccharomyces cerevisiae. Environmental Microbiology*. 71. 8241–8248.
28. Khudsar, T., Uzzafar, M., Soh, W. Y., Iqbal, M. (2000). Morphological and Anatomical Variations of *Cajanus cajan* (Linn.) Huth Raised in Cadmium-Rich Soil. Journal of *Plant Biology*. 43. 149-157.
29. Windhama, L., Weisb, J.S., Weisc, P. (2003) Uptake and distribution of metals in two dominant salt marsh macrophytes, Spartina alterniflora (cordgrass) and Phragmites Australia (common reed). Estuarine, *Coastal and Shelf Science*. 56: 63–72.
30. Seregin, V., Kozhevnikova, A. D. (2008). Roles of Root and Shoot Tissues in Transport and Accumulation of Cadmium, Lead, Nickel, and Strontium. *Physiology Rastenii*. 55:3–26.
31. Pulford, I. D., Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees. Review. *Environment International*. 29. 529– 540.

Citation of This Article
Fatemeh Zarinkamar, Zohreh Saderi, Saiedeh Soleimanpour. Excluder Strategies in Response to Pb Toxicity in *Matricaria chamomilla*. Adv. Biores., Vol4 (3) September 2013: 39-49.