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Effect of Lead on *Marsilea minuta* Linn. Under Hydroponic Conditions

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Authors' contributions

This work was carried out in collaboration between both the authors. Author JS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MSS managed the literature searches, analyses of the study, performed the spectroscopy analysis. Author JS managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Soil heavy metal contamination is becoming a matter of great global concern. The extent of Lead (Pb) concentration which causes toxicity in lower vascular plants, pteridophytes, remains unclear. So the present study was carried out to investigate the extent of Pb accumulation with its impact on physiological and biochemical basis of heavy metal tolerance in *Marsilea minuta* Linn. Lead salt in lower concentrations (0 µM, 0.1 µM, 0.3 µM, 0.5 µM and 1.0 µM) was allowed to absorb by the plants for 12 days in hydroponic culture and a significant deterioration of the plant biomass was recorded. However, roots absorbed more metals than the leaves. Changes in leaf area, biomass allocation, chlorophyll content in leaf, total carbohydrate and protein content were monitored. Higher concentrations upto 1.0 µM reduced biomass and chlorophyll contents. A noticeable decrease in total protein content was noticed on early days of treatment followed by a sharp increase in 0.3 µM, 0.5 µM and 1.0 µM treatments. Total carbohydrate content were decreased, as compared to control condition, in 1.0 µM and increased in 0.5 µM treatment.

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1. INTRODUCTION

Heavy metals being a toxic substance cause injury to the plants by modifying the metabolic process depending upon their oxidation state. It causes toxicity to plant cells in many ways includes inactivation of enzymes, functional groups blockage, replacement of certain metal ions from bio-molecules, conformational changes and the membrane disruption, culminating in photosynthesis inhibition, respiration, and disruption in enzymatic activities [1,2].

Lead (Pb^{2+}) is the most common heavy metal contaminant in the environment [3] and is not included in essential elements for plants. But the plants absorb lead when it is present in their environment. Lead pollution is resulted from mining and smelting activities, Pb^{2+} containing paints, gasoline and explosives as well as from disposal of municipal sewage sludge enriched in Pb [4] and emission by motor vehicles & industrial plants [5]. On entering in water it intrudes the food chain & adversely affects the flora & fauna. Significant reduction in plant height, root-shoot ratio, dry weight, nodule per plant, and chlorophyll content are the major consequences of enhanced level of lead in soil [6,7]. Especially in the rural areas, the soil is polluted by automotive exhausts and in field contaminated with fertilizers containing heavy metal ingredients [8].

The objective of the current work was to determine the critical Pb^{2+} activity associated with the growth of *Marsilea minuta*. Despite the worldwide importance of Pb^{2+} contamination, it remains unclear that, to what extent of Pb^{2+} concentration will cause toxicity in lower vascular plants, pteridophytes. Fern species by nature are over producer of a number of secondary metabolites and happens to be more efficient in antioxidation pathways against heavy metals [9]. It would be worth to describe the relationship between Pb^{2+} stress and aquatic pteridophytes because there is less report on the Marsilea species for metal phytotoxicity.

Dilute nutrient solutions were used to replicate conditions found in the soil solution as closely as possible. Growth of both the roots and shoots were related to Pb^{2+} activity as calculated from measured Pb^{2+} concentrations. Thus, the present investigation aims to elucidate the physiological mechanism for stress tolerance at lower doses.

2. MATERIALS AND METHODS

Marsilea minuta Linn., an aquatic fern of the family Marsileaceae was selected for the present study. The plants were collected from water pond and paddy fields in Nattika, Thrissur District, Kerala, India. The genus *Marsilea* is virtually a cosmopolitan in distribution. Plants were grown under natural light in hydroponics. Healthy plants with undamaged roots were collected and planted in tray filled with Hoagland's solution and were grown for 7 days and transferred for treatments.

The modified Hoagland's solution [10,11] was prepared and used for the present study. The pH of the final solution was adjusted to 6.8 using 0.1N HCl or NaOH. Trays were filled with 1000 ml of Hoagland nutrient medium for the control experiment and different concentrations of $PbCl_2$ along with nutrient solution for experiments. The present investigation includes the following sets:

1. Control plants growing in Hoagland's medium.
2. Plants grown in 0 µM, 0.1 µM, 0.3 µM, 0.5 µM and 1.0µM $PbCl_2$ solution along with modified Hoagland's solution.

2.1 Selection of Lead as heavy metal

In the present study, *Marsilea minuta* Linn., abundantly grown in lowland or water bodies was taken for consideration. The soil analysis of that area revealed that contaminants included the some heavy metals like copper (Cu), Zinc (Zn), lead (Pb) and iron (Fe) in lower concentration.

2.2 Preparation of Lead Chloride

One molar solution of lead chloride ($PbCl_2$) was prepared as the stock solution. Trial experiment was conducted by planting rooted propagules of *Marsilea minuta* plants in order to determine the optimum concentration in which the experimental plants survived and exhibited about 50 percent growth retardation. It was found that 0.2 µM was the optimum for the present study. Appropriate volumes of $PbCl_2$ solution were mixed with nutrient stock solution and final volume was made up to 1000 ml, so as to get the final volume of $PbCl_2$ as 0 µM, 0.1 µM, 0.3 µM, 0.5 µM and 1.0 µM in 1000 ml of nutrient solution. Plants were maintained to grow at field saturation capacity for 15 days at room temperature and light with changing solution once in three days in laboratory conditions.

Random sampling was followed by collecting a minimum of 5 plants from the trays of each treatment and control at an interval of 1, 3, 5, 7 and 9 days from the day of treatment up to 12th day. The collected plants were thoroughly washed in tap water, blotted to remove water adhered on it.

2.3 Physiological and Biochemical Studies

Growth of the treated as well as control plants was assessed in terms of tolerance index percentage [12] stomatal index percentage [13] and total biomass. Stomatal index percentage is calculated using the formula (Number of stomata per unit area /Number of stomata per unit area + Number of epidermal cells per unit area) X 100. Tolerance index percentage [12] is calculated using the formula TI% = (Observed value in solution with metal/ Observed value in solution without metal) X 100. For calculating Tolerance Index root length, runner length and leaf area were taken as parameters. The biochemical estimation of chlorophyll [14], total carbohydrate [15] and protein [16] were conducted. The fresh weight and dry weight of whole plant were also determined.

2.4 Statistical Analysis

The data were presented as mean±SE. The statistical analysis was performed by one-way ANOVA analysis, taking $P \leq 0.05$ as significant.

3. RESULTS

After 12 days of exposure to varying Pb concentrations there had been recorded hardly any visual or morphological toxic symptoms of the plants. The changes in development of rhizoids were the only noticeable morphological change in 0.3 µM, 0.5 µM and 1 µM lead chloride treated plants. Since the plants are less differentiated into rhizome and roots, in the present study both the organs were considered as shoot for metal accumulation.

The plants showed profuse development of new rhizoids from the nodal region of rhizome in control and 0.1 µM on the first day of growth itself. Lateral roots were developed on 7th day of growth in both these cases. It was hard to find out any morphological changes between control and 0.1 µM treatment.

In 0.3 µM, 0.5 µM and 1 µM treatments the root development was noticed on the 1st day of growth but further development was slow and started to cauterize on 7th day. In plant treated with 1 µM, root development was noticed from internodal region also. But such roots were very thin and short in appearance. It shared same anatomical features as that of rhizoids from the nodal region of rhizome. But it remains small sized upto 12 days of growth. As in the case of control plants, the Pb treated plants showed development of new leaves from the rhizome. But in 1µM treatment the circinate region was black in colour then in others.

The control and lead treated plants showed gradual but insignificant increase in the biomass content. A significant increase in the biomass was noticed in plants treated with 0.5 µM and 1.0 µM treatments during 7th and 12th days of growth. All other days showed no such significant increase in biomass (Table1).

Table 1. Effect of lead on biomass (grams) in Marsilea

Concentration of Pb in µM	Days of sampling					
	0	1	2	5	7	12
Control	1.8±0.2	1.8±0.2	1.9±0.2	2.0±0.27	2.2±0.22	4.6±0.20
0.1	1.8±0.2	1.8±0.2	1.9±0.2	2.0±0.20	2.1±0.09	4.6±0.20
0.3	1.8±0.2	1.8±0.2	2.0±0.2	2.2±0.12	2.5±0.12	4.7±0.11
0.5	1.8±0.2	1.8±0.2	2.1±0.2	2.4±0.27	2.7±0.3	5.8±0.03
1	1.8±0.2	1.8±0.2	2.±0.2	2.4±0.21	2.9±0.27	6.2±0.12

Increase of biomass from day 7 to day 12 is significantly at $P \leq 0.05$

3.1 Tolerance Index

Tolerance index percentage with respect to root length showed decrease in plants treated with lead. It decreased regularly upto 12 days of growth. Tolerance index percentage showed a gradual decrease among the treatments and was lowest in highest lead concentration of treatment (Table 3).

In plants treated with lead the tolerance index percentage with respect to rhizome length also showed a significant reduction. Tolerance index percentages among the trea ments were equally lower among treatments of 0.1 µM to 1.0 µM (Table 3).

Tolerance index percentage with respect to leaf area showed a significant decrease in treated plants. In 0.1 µM and 0.3 µM treatments tolerance index was higher on 12th day of treatment after a sharp decrease on 7th day of treatment. But in other treatments it was decreasing day by day (Table 3). In 0.5 µM the increase in tolerance index on 12th day after a decrease on 7th day was not significant (P<0.1).

3.2 Stomatal Index percentage

The leaves of Marsilea are dorsi-ventral. Abaxial and adaxial epidermises are uniseriate and comprise of irregularly shaped epidermal cells. The outer cell walls of the epidermal cells are well cuticularised with cuticular ridges. carbohydrate content.

Leaves are with significantly larger sized guard-cell. The stoma is of diacytic type. The epidermal cell showed change in shape in 0.5 µM and 1.0 µM $PbCl_2$ treatments. In control 0.1 µM and 0.3 µM treatments the epidermal cell was irregular in shape but in treatments 0.5µM and 1.0µM the cells are rectangular in shape.

Stomatal index showed a gradual increase in control and treatments (Fig. 1). In treatments on increasing the concentration of lead the present study noticed a gradual increase in stomatal index along with the increase in lead concentration. In control and 0.1 µM treated plants stomatal index was almost similar and the other treatments 0.3 µM, 0.5 µM and 1.0 µM showed similar pattern of stomatal index.

3.3 Chlorophyll

Chlorophyll content was increased throughout the experimental period. More than four times increase in total chlorophyll content was observed on 12th day (Table 2) in control and treatments. In control and 0.1µM treated plants a sudden increase in the chlorophyll content was observed on 2nd day of growth. Thereafter the increase was gradual. In these plants Chlorophyll b was observed in higher concentration. In treatments such as 0.3 µM, 0.5 µM and 1.0 µM an increase in chlorophyll content was noticed on 2nd day of growth and thereafter the chlorophyll content showed no further increase or decrease (Table 2).

In control and treatments such as 0.1 µM and 0.3 µM Chla/b ratio was significantly decreasing during 12 days of growth. But in treatments 0.3 µM, 1.0 µM the ratio remained almost equal. The difference (decrease/increase) during 1st to 12th day of growth was insignificant (P<0.1).

3.4 Total Carbohydrate

In control and lead treated plants the total carbohydrate content was almost similar upto 2nd day of growth. But on 5th day 0.5 µM, and 1.0 µM Pb treated plants showed a deviation from others. In 1.0 µM Pb treated plants there was a sharp decrease in total carbohydrate content than in others on 5th day whereas in 0.5 µM Pb treated pants showed a sharp increase in total carbohydrate content. On 7th day of growth 0.5 µM Pb treated plants showed a decrease in total carbohydrate content but it was still higher than the control, 0.1 µM, and 0.3 µM lead chloride treatments. In 1.0 µM Pb treated plants, on 7th day of growth, total carbohydrate content showed a sharp increase but it was still lower than the control, 0.1 µM, and 0.3 µM treated plants. On the 12th day of growth all the samples showed almost similar amount of total carbohydrate content (Fig. 2).

3.5 Total Protein

In control and 0.1 µM, a similar amount of protein content was noticed during 12 days of growth. Both these plants were showing to be maintaining the amount of protein during these days. In the case of Pb treatments such as 0.3 µM, 0.5 µM and 1.0 µM a sharp increase in protein content was noticed on 1st and 2nd day of growth and on the 5th day the protein content showed a sharp decrease in amount in all these plants. Thereafter on 7th day of growth the present study noticed a sharp increase in protein content. In 0.5 µM and 1.0 µM Pb treated plants the protein content was almost three times than on the 5th day. In 0.3 μM Pb treated plants only a small amount of increase was noticed. On 12th day in 0.3 μM Pb treated plants the protein content was almost doubled. Whereas in 0.5 μM and 1.0 μM Pb treated plants there was no significant difference in protein content (Fig. 3).

4. DISCUSSION

Sensitivity/tolerance of plants towards heavy metals varies from species to species and metal to metal [17]. In the present investigation on the effect of heavy metal toxicity, optimal concentrations are being selected based on trial experiment with various concentrations in which plants can be retained for 12 days. Since the plants are less differentiated into rhizomes and roots, in the present study the whole plant is used. Apart from this, the increase in biomass was significant through the due course in treatments such as 0.5 μM and 1.0 μM during 7th and 12th day of growth (Table 1). This may be due to increase in accumulation of Pb along with increasing concentration of treatments. Similar findings with the Chinese Brake fern showed 12–71% biomass increase compared to the control when the plants were treated with arsenic [18]. Significant reduction of biomass is an important effect of heavy metal stress [19] and it comes contradictory with the present finding.

In 0.3 μM, 0.5 μM and 1 μM treatments, the root development was noticed on the 1st day of growth but further development was slow and started to cauterize on 7th day (Table 3). In plant treated with 1μM, root development was noticed from inter nodal region also. But such roots were very thin and short in appearance. In control and 0.1μM the plants showed profuse development of new rhizoids from the nodal region of rhizome on the first day of growth itself. Lateral roots were developed on 7th day of growth in both these cases. But there was no lateral root development in 0.3 μM, 0.5 μM and 1μM treatments till 12th day. In Norway spruce, the primary toxic effect of Pb was a reduction in root growth [20]. It has been reported that Pb reduces root growth by restricting cell division [21] and cell elongation [22]. Reductions in root growth possibly results from the displacement of Ca from the cell wall by Pb [20].

As in the case of control plants the Pb treated plants also showed development of new leaves from the rhizome. But in 1 μM treatment the circinate region was black in colour then in others. A slight reduction in rhizoid growth was noticed in 1 μM treated Marsilea from 5th day of treatment. Other than a reduction in growth, the only symptom of Pb^{2+} toxicity in the shoot was a slight chlorosis of the younger leaves with 0.5 μM and 1.0 μM Pb in solution, possibly caused by Pb-induced Mg deficiency. These observations are similar to some reports for bean, in which Pb reduced root growth, the shoots showed no obvious toxicity symptoms other than a reduction in leaf surface area [23]. Chlorophyll content is often measured as a parameter in order to assess the impact of environmental stresses on plants, since the changes of pigments are linked with visual symptoms of growth disorders and photosynthetic productivity [24]. In *Marsilea* under lead stress, chlorophyll a/b ratio showed significant reduction compared to the control (Table 2). In wheat treated with heavy metals, total chlorophyll content was decreased to 50-70% and this reduction might be the result of inhibition of enzymes responsible for chlorophyll biosynthesis [25]. Hyper-accumulation of heavy metals also interferes with chlorophyll synthesis in plants [26].

Tolerance index values calculated on the basis of rhizoid length ratio of experimental and control shows a very small variation (Table 3) and rhizome length also exhibited only slight variation in the net tolerance index (Table 3). However, leaf area is found to be adversely affected by the treatment with Pb. The tolerance index values expressed on the leaf area basis is significantly reduced in the plants under lead treatment.

The plants treated with lead showed a significant increase in the stomatal indices (Fig. 1). Effect of heavy metals on plants like *Arabidopsis thaliana* has been shown to render stomatal conductance by osmoregulation of guard cell-water relations [27]. In *Marsilea* increased stomatal index may induce enhanced transpiration rate and/or interfere with bioaccumulation potential and/or phytovolatilization process of mercury in the leaves. Reduced stomatal index in the leaves of plants treated with cadmium has been reported in plants, which are not accumulators of this metal [28]. This characteristic shows the capacity of heavy metal accumulation by the plant. Histochemical studies showed that in the roots of plants treated with lead the stelar region is found to be filled with brownish spots, which are absent in control.

Table 2. Effect of lead on the chlorophyll a/b ratio and total chlorophyll content in Marsilea leaves

Concentration of Pb in µM	Days of sampling											
	0		1		2		5		7		12	
	Chl a/b	Total Chl	Chl a/b	Total Chl	Chl a/b	Total Chl	Chl a/b	Total Chl	Chl a/b	Total Chl	Chl a/b	Total Chl
Control	0.832	22.9	0.732	26.5	0.21	85.2	0.206	87.9	0.217	88.7	0.24	90.9
	± 0.1	± 1.8	± 0.11	±1.4	± 0.1	± 2.1	± 0.1	± 1.9	± 0.1	± 1.1	± 0.1	±1.2
0.1	0.832	22.9	0.732	26.5	0.21	85.2	0.21	88.2	0.217	88.7	0.24	90.9
	± 0.2	± 1.8	± 0.15	±1.0	± 0.1	± 2.3	± 0.1	± 2.4	± 0.1	± 1.1	± 0.1	± 1.4
0.3	0.832	22.9	0.738	46.4	0.786	43.4	0.465	35.6	0.506	39.0	0.524	45.06
	± 0.2	± 1.8	± 0.14	±1.2	± 0.1	± 1.9	± 0.1	± 3.1	± 0.1	± 1.8	± 0.1	± 2.2
0.5	0.832	22.9	0.868	49.7	0.889	50.31	0.911	50.12	0.907	50.22	0.9	50.42
	± 0.1	± 1.	± 0.90	±1.6	± 0.1	± 2.2	± 0.1	± 1.1	± 0.1	± 2.0	± 0.1	± 1.2
1	0.832	22.9	0.759	50.3	0.77	50.0	0.835	48.12	0.799	48.98	0.775	48.61
	± 0.23	± 1.8	± 0.01	±1.1	± 0.2	± 2.1	± 0.1	± 2.1	± 0.1	± 2.1	± 0.1	± 1.6

Difference is significantly at P ≤ 0.05 between control, 0.1, 0.3, 0.5 and 1.0µM from day 2

Table 3. Effect of lead on the tolerance index percentage with respect to root length, rhizome length and leaf area in Marsilea

Part of the plant	Concentration of Pb in µM	Days of sampling					
		0	1	2	5	7	12
Root length	Control	100	100	100	100	100	100
	0.1	93.8	93.3	95.2	98.5	78.1	85.5
	0.3	109.9	76.0	81.6	94.3	99.5	77.1
	0.5	110.6	69.7	85.3	82.8	91.9	72.7
	1	96.9	62.8	79.4	97.9	85.6	60.5
Rhizome length	Control	100	100	100	100	100	100
	0.1	96.4	132.0	91.6	73.5	74.3	71.1
	0.3	94.1	102.4	79.6	61.0	57.3	49.9
	0.5	95.3	120.0	76.7	61.0	64.9	49.2
	1	100.6	100.0	76.7	61.0	57.3	43.4
Leaf area	Control	100	100	100	100	100	100
	0.1	100.0	100.0	89.0	64.2	35.1	53.8
	0.3	100.0	100.0	89.0	62.6	31.2	41.2
	0.5	100.0	100.0	52.5	54.1	26.1	35.1
	1	100.0	100.0	52.5	54.1	26.1	26.1

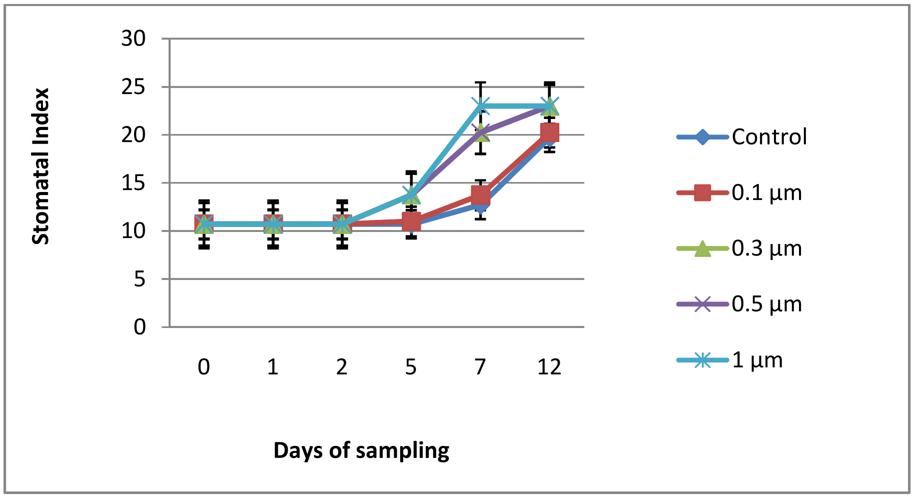

Fig. 1. Effect of lead on the stomatal index percentage (lower) in Marsilea

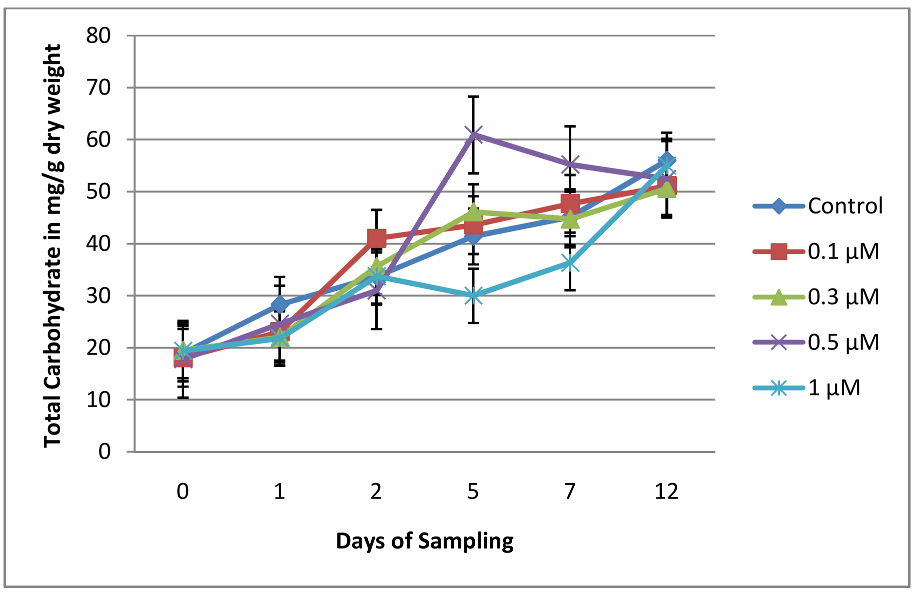

Fig. 2. Effect of on total carbohydrate content (mg g^{-1} dry weight) in Marsilea

Total carbohydrate content showed a significant decrease on 5^{th} and 7^{th} day of treatment in 1.0 μM treated plants. This may be due to adverse effects of heavy metal on photosynthesis. In the present study a sharp decrease in total chlorophyll is noticed during 5th day onwards. The plants exhibit a relationship between Pb application and reduction in photosynthesis [29] and the Pb accumulated inside the plant might be inhibiting chlorophyll synthesis [30]. The present study also showed a decrease in total chlorophyll content in 1.0 μM Pb^{2+} treated plants.

The most general symptoms of metal toxicity in plants are stunting and chlorosis [17]. Yield reductions could occur by excess metal directly or indirectly inhibiting either assimilates production in source leaves, translocation from source to sink, utilization in sink regions, or several of these. Various metal ions have adverse effects on respiration and photosynthesis in intact organisms, ranging from algae and lichens to vascular plants, as well as on isolated mitochondria and chloroplasts [31].

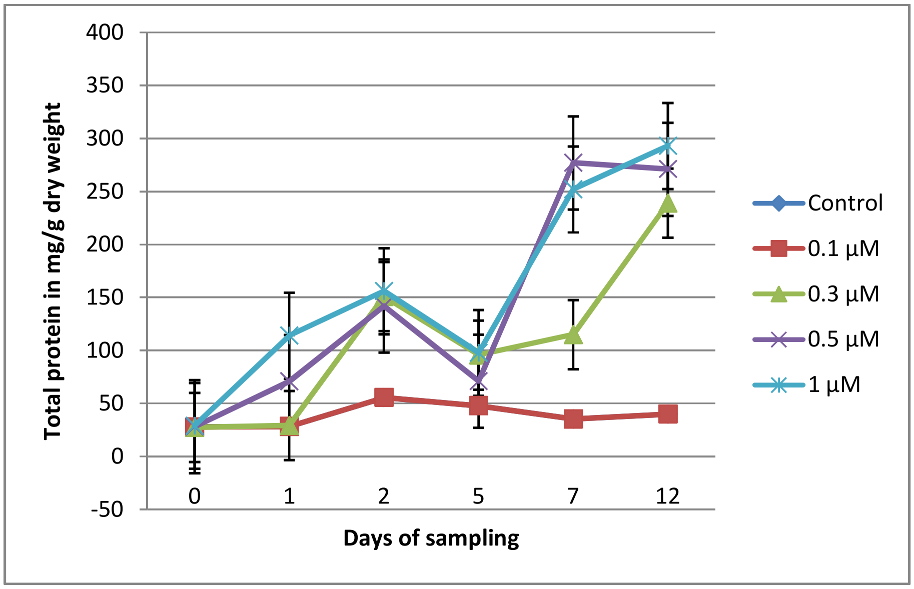

Fig. 3. Effect of lead on total protein content (mg g-1 dry weight) in Marsilea

High concentrations of heavy metal may decrease the protein pool of the plant due to acute oxidative stress of reactive oxygen species [32,33]. But in contradictory to this, in the present study, the total protein content of the plant treated with 0.3 µM, 0.5 µM and 1.0 µM showed a sharp decrease in protein content on 5th day of growth in lead followed by a significant increase (Fig. 3). This may be due to certain amino acids, like proline, which increase under lead stress and plays a major role in the tolerance of the plant to heavy metal. In *Cassia angustifolia* there was a sharp increase in amino acid content under lead stress [34].

5. CONCLUSION

Data obtained in this study revealed that it was hard to visualise morphological toxic symptoms of the plants. But the plant showed some features of lead toxicity, which includes increased stomatal index, decreased tolerance index, reduction of biomass and chlorophyll content, wavering in carbohydrate content and increase in protein content.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh RP, Tripathi RD, Sinha SK, Maheshwari R, Srivastava HS. Response of higher plants to lead contaminated environment. Chemo-sphere. 1997;34: 2467-2493.
2. Farooq MA, Ali S, Hameed A, Ishaque W, Mahmood K, Iqbal Z. Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. Ecotoxicology and Environmental Safety. 2013;96:242-249.
3. Watanabe MA. Phytoremediation on the brink of commercialization. Environmental Science and Technology. 1997;31:182-186.
4. Chaney RL, Ryan JA. Risk Based Standards for Arsenic Lead and Cadmium in Urban Soils.ed. Dechema, Frankfurt, Germany; 1994.
5. Friedland AJ. The movement of metals through soils and ecosystems. In: Shaw AJ, Editors. Heavy Metals Tolerance in Plants: Evolutionary Aspects. FL, Boca Raton, CRC Press; 1990.
6. Tomar M, Kaur I, Neelu, Bhatnagar AK. Effect of enhanced lead in soil on growth

and development of *Vigna radiata* (L.) Wilezek. Indian Journal of Plant Physiology, 2000;5(1):13-18.

7. Bekiaroglou P, Karatagli S. Effect of lead and zinc on *Mentha spicata*. Journal of Agronomy and Crop Science. 2002; 188(3):201-205.
8. Adriano DC. Trace elements in Terrestrial Environments: Biochemistry, Bioavailability and Risks of Metals. 2nd ed. Springer-Verlag, New York; 2010.
9. Hawa ZE, Jaafar HI, Mohd EK. Phenolics and flavonoid Compounds, phenylalanine ammonia lyase and antioxidant activity responses to elevated CO_2 in *Labisia pumila* (*Myrisinaceae*). Molecules. 2012; 17(6):6331-6347.
10. Epstein E. Mineral Nutrition of Plants: Principles and Perspectives. ed. John Wiley & Sons, New York; 1972.
11. Taiz L, Zeiger E. Plant Physiology. 3rd ed. Sinauer Associates, Sunderland, Massachussetts, .Inc. Publishers; 2002.
12. Turner AP. The responses of plants to heavy metals. In: Ross SM, Editors. Toxic Metals in Soil-Plant Systems. New York, John Wiley & Sons; 1994.
13. Meidner H, Mansfield LA. Physiology of Stomata. ed. McGraw-Hill, London; 1968.
14. Arnon DI. Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. Plant Physiology. 1949;24:1-15.
15. Hedge JE, Hofreiter BT. Methods in Carbohydrate Chemistry. In: Whistler RL, BeMiller JN, Editors. Carbohydrate chemistry. Academic Press, New York; 1962.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagent. Journal of Biological Chemistry. 1951;193:265-275.
17. Foy CD, Chaney RL, White MC. The physiology of metal toxicity in plants. Annual Review of Plant Physiology. 1978;29:511-566.
18. Xinde C, Lena QM, Cong T. Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). Environmental Pollution. 2004;128(3):317-325.
19. Kumar PBAN, Dushenkov V, Motto H, Raskin I. Phytoextraction: The use of plants to remove heavy metals from soils. Environmental Science and Technology. 1995;29(5):1232-1238.
20. Godbold DL, Kettner C. Lead influences root growth and mineral nutrition of *Picea abies* seedlings. Journal of Plant Physiology. 1991;139:95-99.
21. Eun SO, Youn HS, Lee Y. Lead disturbs microtubule organization in the root meristem of *Zea mays*. Physiologia Plantarum. 2000; 110:357-365.
22. Malkowski E, Kita A, Galas W, Karez W, Michael K. Lead distribution in corn seedlings (*Zea mays* L.) and its effect on growth and the concentration of potassium and calcium. Plant Growth Regulation. 2002;37:69-76.
23. Wozny A, Jerczynska E. The effect of lead on early stages of *Phaseolus vulgaris* L. growth in vitro conditions. Biologia Plantarum. 1991;33:33-39.
24. Parekh D, Puranik RM, Srivastava HS. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. Biochemie und Physiologie der Pflanzen. 1990;186:239-242.
25. Oncel I, Kele Y, Ustun AS. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environmental Pollution. 2000; 107:315-320.
26. Stiborova M, Doubravova M, Brezinova A, Friedrich A. Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley (*Hordeum vulgare* L.). Photosynthetica. 1986;20:418–425.
27. Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant Journal. 2002;32: 539-548.
28. Prasad MNV. Inhibition of maize leaf chlorophylls, carotenoids and gas-exchange functions by cadmium. Photosynthetica. 1995;31:635-640.
29. Bazzaz FA, Carlson RW, Rolfe GL. The inhibition of corn and soybean photosynthesis by lead. Physiologia Plantarum. 1975;34:326-329.
30. Sengar RS, Pandey M. Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments. Biologia Plantarum. 1996;38:459-462.
31. Puckett KJ. The effects of heavy metals on some aspects of lichen physiology. Canadian Journal of Botany. 1976;54: 2695-703.
32. Chatterjee C, Dube BK, Sinha P, Srivastava P. Detrimental effects of lead phytotoxicity on growth, yield, and

metabolism of rice. Communications in Soil Science and Plant Analysis. 2004;35(1–2): 255-265.

33. Piotrowska A, Bajguz A, Godlewska-Zylkiewicz B, Czerpak R, Kaminska M. Jasmonic acidas modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (*Lemnaceae*). Environmental and Experimental Botany. 2009;66(3):507-513.

34. Qureshi M, Abdin M, Qadir S, Iqbal M. Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Bioliologia Plantarum. 2007;51(1): 121-128.

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