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# Effect of lead on plant growth, lead accumulation and biochemical changes of *Pistia stratiotes* L.

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**Key words:** Lead, Phytoremediation, *Pistia stratiotes*

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**Abstract**

Aquatic macrophytes are well known accumulators for heavy metals in contaminated water bodies. The objective of this study was to evaluate the effect of lead nitrate on the growth, accumulation and biochemical changes of *Pistia stratiotes* L. Plants were cultured in Hoagland's medium which was exposed to different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/l) of lead nitrate solutions. The toxicity symptoms of Pb on *P. stratiotes* showed chlorosis on leaves followed by significant decrease in the relative growth, biomass productivity, chlorophyll content, protein and carbohydrate content with increased concentrations. But with the increase in Pb concentration (20, 40, 60, 80, 100 ppm) an increased proline content (0.516±0.01, 0.739±0.01, 0.956±0.02, 1.434±0.03, 1.844±0.05 µg/g fresh weight) was observed in *Pistia stratiotes* irrespective of the period of treatment. Increased concentrations of Pb (100 mg/l) in the growth medium enhanced the bio-concentration factor of *P. stratiotes* up to an optimum value of 1239.56, while the relative growth of plants significantly decreased (0.79 g). These responses indicate the potential of *Pistia stratiotes* L. not only as a lead scavenger but also a suitable tool for phytoremediation in the aquatic environment.

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## Introduction

Since the industrial revolution, mankind has been introducing various toxic pollutants into the environment at an alarming rate (Sharma and Dubey, 2005, Leblebici, 2010). These pollutants are a cocktail of various organic compounds and heavy metals (HMs) which can drastically affect human and animal health as well as ecosystems (Lone, 2008). Heavy metals including such lead (Pb) are of major concern because of their persistence in the environment (Melegy, 2010). Lead is a toxic pollutant mainly introduced into the environment through smelting processes (Peralta *et al.*, 2009). Although Pb has no known functions in biological systems, it can impact the central nervous system especially in children leading to reduced growth of the brain (Butcher, 2009). It is generally ranked the number one heavy metal pollutant and number two of all hazardous substances by the Agency for Toxic Substances and Disease Registry (ATSDR, 2007; Gallando *et al.*, 2002). Cognizant of their effects on human and animal health and ecosystems, it is imperative to reduce the concentrations of HMs such as Pb from contaminated soil and water sources. Numerous engineering-based technologies have been used to remediate metal contaminated soils and aquatic systems but they are very expensive. Phytoremediation is becoming a more cost effective alternative (Chaney and Malik, 1997) because of the productive interdisciplinary cooperation of plant biochemists, molecular biologists, soil chemists, agronomists, environmental engineers, and federal and state regulators. The metals targeted for phytoremediation include Pb, Cd, Cr, As, and various radionuclides. The harvested plant tissue, rich in accumulated contaminant is easily processed by drying, ashing or composting (Raskin *et al.*, 1997).

Phytoextraction involves the recurrent cropping of plants in polluted soil/water, until the metal concentration is reduced to regulatory levels (Chaney *et al.*, 2007). The ability of the plants to account for the decrease in soil/water metal concentrations is a function of the extent of metal uptake and biomass production (Mc Comb *et al.*, 2012). Therefore, a suitable phytoextractive species must be able to tolerate the toxic metal and produce high biomass yield.

This study was conducted to 1) evaluate the growth and Pb accumulation ability of *Pistia stratiotes* exposed to various levels of Pb (supplied as lead nitrate) at different growth periods, 2) determine whether *P. stratiotes* plants can tolerate and translocate high concentrations of lead, and 3) to assess different biochemical changes in *P. stratiotes*. This study explored the potential Pb scavenging activity of *P. stratiotes*.

## Materials and methods

### *Culture of plants*

*Pistia stratiotes* L. were collected from natural ponds and cultured in 100% Hoagland's nutrient solution (pH 5.6) in laboratory under controlled conditions (illuminated with a light intensity of 45 µmoles $m^{-2}s^{-1}$, at 12hr/12hr light and dark cycle, under the temperature of 25±2°C).

### *Composition of 100% modified Hoagland's nutrient stock solution*

The 100% nutrient solution consisted of 5.0 mmol $L^{-1}$ $KNO_3$, 1.0 mmol $L^{-1}$ $NH_4H_2PO_4$, 4.0 mmol $L^{-1}$ $Ca(NO_3)_2$, 2.0 mmol $L^{-1}$ $MgSO_4$, 10.0 µmol $L^{-1}$ $MnSO_4$, 0.7 µmol $L^{-1}$ $ZnSO_4$, 0.3 µmol $L^{-1}$ $CuSO_4$, 50.0 µmol $L^{-1}$ $H_3BO_3$, 0.1 µmol $L^{-1}$ $Na_2MoO_4$ and 100.0 µmol $L^{-1}$ Iron(II) ethylene diamine tetra acetic acid (FeEDTA). All the nutrient solutions of different strengths were prepared in distilled water by appropriate dilution of the stock solution and their pH was maintained at 5.6 using $HNO_3$ and NaOH.

### *Experimental procedure*

About 0.5 g fresh matter of plant was placed in each experimental pot, which contained 1000 ml of a control (metal free) and 10, 20, 30 to 100 mg/l of Pb solution [Pb $(No_3)_2$]. The pH of the solutions was adjusted to 5.6. Plant samples from each container were separately harvested after 8 and 15 days to analyze for toxicity symptoms, biomass productivity, total chlorophyll content, protein content, carbohydrate and proline content, and metal content. The experiments were set up in triplicates for each concentration.

*Root length and Biomass productivity*

Three plants from each triplicate were randomly selected for recording the root length. First the plats were soaked uniformly with the help of blotting paper. Their roots were measured by using centimeter scale and recorded. Then it was put on weighing machine and measurement was taken simultaneously. First the initial weights of the plant were taken separately. Then the final weights of the plants were taken after phytoremediation. After recording the fresh weights of harvested plants, they were dried at 60 °C for three days and subsequently the dry weights were determined. The difference in between initial weight and final weight gives the increase in biomass of the plants. Growth inhibitory rate (GIR) % was calculated according to Wilkins (1978). The relative water content (RWC) was also calculated as described by Chen *et al.*, (2009).

RWC (%) = [(FW-DW)/ FW] ×100, where FW-fresh weight & DW-dry weight.

GIR (%) = [(average value in the control- average value in the treated treatment)/ average value in the control] × 100.

*Chlorophyll Content*

Chlorophyll content was determined according to Porra *et al.,* (1989) method. For measurement of chlorophyll content 0.5 g of each plant material were put into test tubes containing 10 ml of methanol and kept in dark for 24 hours. After this the supernatant solution from each test tube was taken for measurement of absorbance at 470 nm, 652 nm and 665 nm by the help of UV spectrophotometer.

Calculation (µg/ml) =Chl a=16.29 $A_{665}$ – 8.54 $A_{652}$
Chl b=30.66 $A_{652}$ – 13.58 $A_{665}$Chl (a+b)=22.12 $A_{652}$ + 2.71 $A_{665}$

$$\text{Total carotenoid} = \frac{1000A_{470} - 2.86\text{Chl a} - 129.2\text{Chl b}}{245}$$

*Protein estimation*

For protein estimation 0.5 g of leaf sample was taken and homogenized in 10% ice cold TCA by a pre-chilled mortar and pestle incubated overnight at 40° C. Then centrifuged at 10,000 rpm for 10 minutes, successively washed with 80% ethanol/chloroform, diethyl ether to remove phenolic compounds. Pellet was washed and suspended in a known volume of 0.1N NaOH. Then protein was estimated by standard method (Lowry *et al.*, 1951).

*Carbohydrate estimation*

Carbohydrates are estimated from plant extracts by Anthrone method (Hedge *et al.*, 1962). Carbohydrates are first hydrolysed into simple sugars using anthrone reagent. In hot acidic medium glucose is dehydrated to polysaccharides into simple sugars and estimating the resultant monosaccharide with absorption at 625 nm.

*Estimation of proline*

For proline estimation the plant materials (0.5 g) were grinded in 10 ml of 3% sulfo-salicylic acid before the homogenized mixture was centrifuged at 3000 rpm for 10 minutes. Then to the 2 ml of supernatant 2 ml of acid ninhydrin reagent and 2 ml glacial acetic acid were added. This mixture was boiled in water bath at 100° C. The reaction was terminated, by placing the tubes in ice bath. 4 ml of toluene was added to each of the test tube containing samples of different treatments. It was then followed to separate into phases by mixing vigorously using a cyclo-mixture. The chromophore containing upper toluene layer was collected carefully with the help of micro-pipette and the absorbance was measured at 520 nm. Proline content was estimated as per the method described by Bates *et al.*, (1973).

All the experiments were done in triplicates and the data were analyzed statistically and standard errors of mean (SEM) was calculated.

*Instrumentation and chemicals*

Analytical grade chemicals and reagents were used in all experiments. Distilled water was used to prepare all aqueous solutions. The pH of solutions was measured using a pH meter (Thermo Russell Model RL060P). A muffle furnace (Lenton Model EF 11/8) was used to ash the plant material. Lead was analyzed using atomic absorption spectrophotometer (AAS; GBC Model 932 AB Plus) at the wavelength of 283.3 nm using air-acetylene flame.

*Digestion and analysis of plant material*

After recording the fresh weights of harvested plants after washing, they were dried at 60 °C for three days and subsequently the dry weights were determined. Plant biomass was digested by dry ashing according to Hoeing *et al.*, (1998). Dried, powdered plant sample in a crucible was placed in a cold muffle furnace and the temperature was progressively elevated to 450 °C over two hours and held for four hours. After cooling, a drop of distilled water was added, and then 5.0 ml conc. $HNO_3$ was added to the ash. The sample was slowly heated on a sand bath for 30 minutes at 120-130 °C. To this, 5.0 ml of hydrogen peroxide was added with care in small amounts to avoid strong foaming. The heating was continued at that temperature until a clear solution was obtained. The solution was cooled and its volume made up to 50 ml by adding distilled water. The samples were analysed by AAS to determine their lead content.

*Relative growth and bio-concentration factor*

Relative growth (Lu *et al.*, 2004) of the plants during the experiment and the bio- concentration factor (BCF) were calculated as follows:

Relative growth = Final fresh weight / Initial fresh weight.

$$BCF = \frac{\text{Concentration of meral in dried plant tissue (mg/g)}}{\text{Intitial concentration of meral in dried plant tissue (mg/g)}}$$

Bio-concentration factor is a useful parameter to evaluate the potential of plants for accumulating metals (Lu *et al.*, 2004; Mun *et al.*, 2008).

The data in this study were analysed statistically using Microsoft office excel 2007, and all values are presented as Mean ±SEM (standard error mean).

## Result and discussion

*Effect of lead on growth, biomass and relative water content*

Growth inhibition is a common response to heavy metal stress and is also one of the most important agricultural indices of heavy metal tolerance (Malar *et al.*, 2014). Lead is not generally considered to be an essential element for plant growth.

**Table 1.** Effect of various concentrations of Pb on growth parameters of *Pistia stratiotes* L.

| Concentration of Pb (in ppm) | Inhibition of growth rate (%) |
|---|---|
| 0 | 00 |
| 10 | 6.11 |
| 20 | 16.53 |
| 30 | 20.65 |
| 40 | 22.46 |
| 50 | 26.00 |
| 60 | 34.99 |
| 70 | 38.43 |
| 80 | 42.63 |
| 90 | 47.32 |
| 100 | 56.11 |

All the values are mean of three replicates.

The effect of lead on plant growth seems to be different with regards to plant species, cultivars, organs and metabolic processes (Sharma and Dubey, 2005). *Pistia stratiotes* grown in different concentrations of Pb $(No_3)_2$ exhibited inhibition of both plant and root growth (Fig.1). After 15 days of lead treatment, the reduction of plant growth was 47% and 56% respectively at 90 ppm and 100 ppm concentration, when compared to the control (Table 1).

Plants do not show any visible toxicity symptoms up to 30 mg/l Pb treatment. However, Pb treatment at 100 mg/l concentration showed toxicity symptoms like chlorosis and drying at edges in plants (Fig. 3). Similar response to lead treatment was previously noticed in various plants (Piechalak *et al.*, 2002; Brunet *et al.*, 2008). Abubakar *et al.*, (2014) reported that, the growth and multiplication of the plant *P. stratiotes* is greater in control

than in other treatments of Pb $(No_3)_2$. Decreased plant growth might be associated with the inhibition of mitotic index noticed with Pb and Cd heavy metal treatment (Vecchia *et al.*, 2005).

**Table 2.** Effects of various concentrations of Pb on plant biomass and relative water content (RWC) of *Pistia stratiotes* L.

| Concentration of Pb (in ppm) | Fresh weight (in g) | Dry weight (in g) | Relative water content (in %) |
|---|---|---|---|
| 0 | 10.46 | 0.591 | 94.34 |
| 10 | 9.82 | 0.575 | 94.14 |
| 20 | 8.73 | 0.540 | 93.81 |
| 30 | 8.30 | 0.523 | 93.69 |
| 40 | 8.11 | 0.463 | 94.29 |
| 50 | 7.74 | 0.449 | 94.19 |
| 60 | 6.80 | 0.406 | 94.02 |
| 70 | 6.44 | 0.378 | 94.13 |
| 80 | 6.00 | 0.351 | 94.15 |
| 90 | 5.51 | 0.335 | 93.92 |
| 100 | 4.59 | 0.310 | 93.24 |

Relative water content (RWC) in *Pistia stratiotes* was decreased slightly when compared to the control (Table 2). RWC change has been suggested as an indicator of phytotoxicity after heavy metal stress (Zn and Cr) in Indian mustard and Chinese brake fern (Su *et al.*, 2005). It is most likely that lead treatment induced stomatal closure, triggered over the course of the experiment due to the atmospheric carbon fixing activities that were compromised as a consequence (Brunet *et al.*, 2008).

**Table 3.** Variation of mean bio concentration factor of *P.stratiotes* L. for Pb under different initial Pb concentrations.

| Initial Pb 2+ concentration in the medium (mg/l) | Mean bio concentration factor |
|---|---|
| 10 | 1011.23 |
| 20 | 1057.18 |
| 30 | 1071.26 |
| 40 | 1088.06 |
| 50 | 1097.85 |
| 60 | 1106.31 |
| 70 | 1153.08 |
| 80 | 1186.39 |
| 90 | 1205.73 |
| 100 | 1239.56 |

*Effect of lead on relative growth and Bio-concentration factor of P. stratiotes*

The relative growth of *P. stratiotes* exposed to Pb at each concentration decreased significantly with respect to the control (Fig.2). At high Pb concentrations, *Pistia* growth was reduced after 15$^{th}$ days. In a similar study Thayaparan *et al.*, (2013) reported that the relative growth of *Azolla pinnata* exposed to lead was significantly reduced when metal concentrations were increased.

*Pistia stratiotes* in the aquatic system is an efficient cosmopolitan, metal hyper-accumulator because of its extensive root system that

favours the selective metal uptake (Irfan, 2015). Bio concentration factors (BCF) for *P. stratiotes* grown in different concentrations of Pb are given in Table 3.

The potential of a plant for phytoremediation process is often judged by its bio-concentration factor. The BCF values over 1000 are considered as evidence of a useful plant for phytoremediation (Zayed *et al.*, 1998).

**Table 4.** Effect of various concentrations of Pb on the pigmentation in *Pistia stratiotes* L.

| Conc. of Pb (in ppm) | Chlorophyll 'a' (mg/g fresh weight) | | Chlolorophyll 'b' (mg/g fresh weight) | | Total Chlorophyll 'a+b' (mg/g fresh weight) | | Carotenoids (mg/g fresh weight) | |
|---|---|---|---|---|---|---|---|---|
| | 8th day | 15th day | 8th day | 15th day | 8th day | 15th day | 8th day | 15th day |
| 0 | 0.315±0.03 | 0.277±0.03 | 0.354±0.02 | 0.314±0.06 | 0.398±0.03 | 0.379±0.09 | 0.253±0.02 | 0.242±0.01 |
| 10 | 0.301±0.01 | 0.265±0.02 | 0.317±0.01 | 0.289±0.09 | 0.360±0.03 | 0.354±0.05 | 0.226±0.02 | 0.238±0.02 |
| 20 | 0.299±0.02 | 0.260±0.01 | 0.300±0.03 | 0.287±0.11 | 0.355±0.07 | 0.350±0.07 | 0.217±0.06 | 0.226±0.01 |
| 30 | 0.293±0.01 | 0.211±0.01 | 0.291±0.01 | 0.227±0.01 | 0.319±0.01 | 0.279±0.02 | 0.208±0.01 | 0.203±0.09 |
| 40 | 0.270±0.03 | 0.195±0.03 | 0.286±0.05 | 0.213±0.03 | 0.311±0.01 | 0.260±0.02 | 0.198±0.03 | 0.184±0.03 |
| 50 | 0.268±0.03 | 0.176±0.09 | 0.275±0.02 | 0.181±0.04 | 0.289±0.01 | 0.225±0.01 | 0.173±0.01 | 0.167±0.01 |
| 60 | 0.251±0.02 | 0.168±0.02 | 0.270±0.09 | 0.172±0.06 | 0.279±0.02 | 0.212±0.01 | 0.168±0.01 | 0.155±0.02 |
| 70 | 0.233±0.02 | 0.162±0.01 | 0.261±0.01 | 0.151±0.03 | 0.271±0.01 | 0.194±0.01 | 0.152±0.04 | 0.148±0.03 |
| 80 | 0.215±0.09 | 0150±0.03 | 0.256±0.01 | 0.149±0.03 | 0.264±0.03 | 0.187±0.03 | 0.144±0.03 | 0.146±0.03 |
| 90 | 0.209±0.01 | 0.131±0.04 | 0.247±0.07 | 0.124±0.02 | 0.255±0.02 | 0.159±0.02 | 0.125±0.03 | 0.119±0.01 |
| 100 | 0.187±0.01 | 0.110±0.03 | 0.226±0.09 | 0.122±0.01 | 0.237±0.01 | 0.148±0.01 | 0.111±0.01 | 0.108±0.01 |

Values of 3 replicate ±SEM.

The present experiment revealed that BCF of *P. stratiotes* for Pb increased significantly with increasing Pb concentration in the growth medium, and the highest BCF was observed at 100 mg/l. *P. stratiotes* is a potential candidate for removal of Pb from waterways polluted with effluents containing Pb. Rijal *et al.*, (2016) reported that *P. stratiotes* and *Lemna flava* were very potential as agents of phytoremediation because it can accumulate Pb and Cd in large amounts with a short period of time.

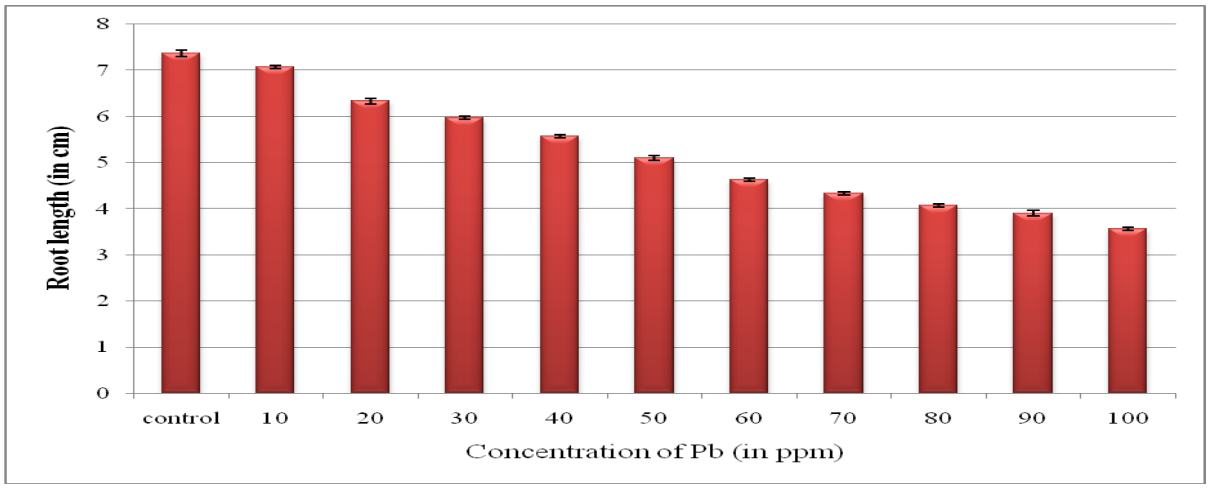


**Fig. 1.** Effect of various concentrations of Pb on root length of *Pistia stratiotes* (All values are mean ± SEM of three replicates).

*Effects of Lead on Photosynthetic pigments Chlorophyll a, b and total chlorophyll*

Photosynthetic pigments, chlorophyll-a, chlorophyll-b and total chlorophyll was evaluated after 8 and 15 days of lead nitrate exposure, and their observed values are depicted in Table 4.

As the concentration increased, progressive decrease was recorded in the chlorophyll-a, chlorophyll-b and total chlorophyll. When different concentration of lead was applied there was a significant decrease in chlorophyll content. The estimation of chl a, chl b, and total chlorophyll were found to be 0.187 mg $g^{-1}$,

0.226 mg $g^{-1}$, 0.237 mg $g^{-1}$ at100 mg $g^{-1}$ on 8th day, while it was reached a minimum value of 0.110 mg $g^{-1}$, 0.122 mg $g^{-1}$, and 0.148 mg $g^{-1}$ fresh weight on 15th day with 100 mg $g^{-1}$.

The lead was toxic for the growth and development of plants and at high levels could be a strong inhibitor of photosynthesis.

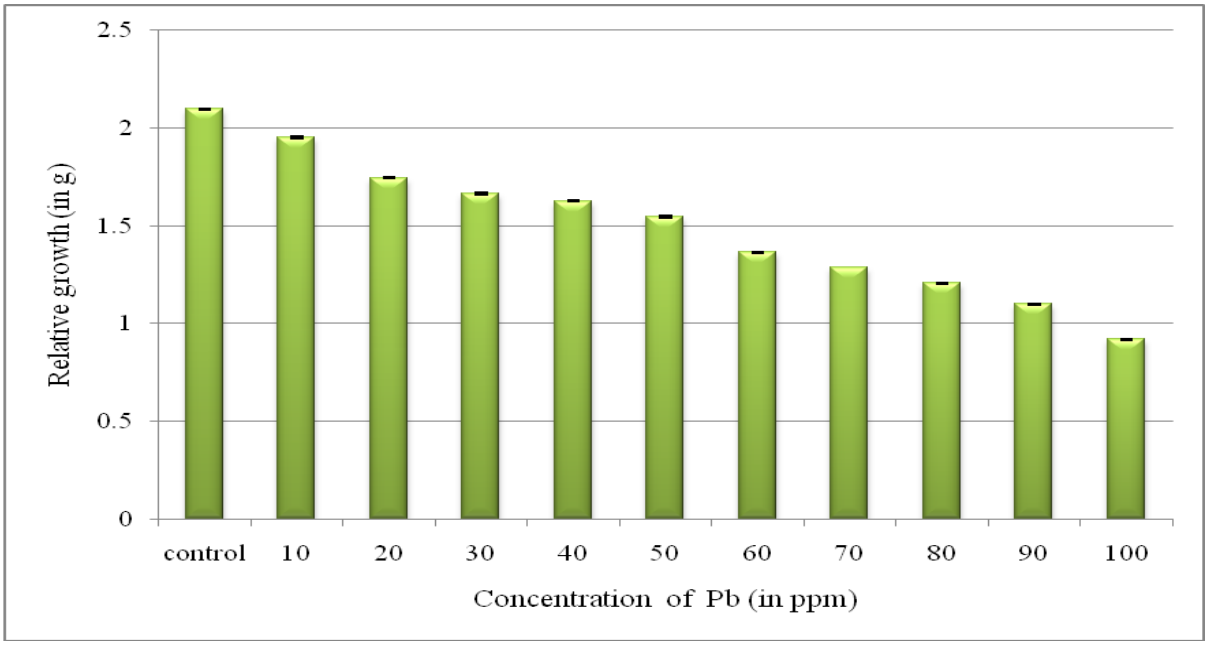


**Fig. 2.** Relative growth of *Pistia stratiotes* L. in various concentrations of Pb compared to the control after 15 days of culture in Hoagland's medium (Bars indicate mean ± SEM, where n= 3).

The loss of chlorophyll content could be due to peroxidation of chloroplast membranes or replacement of magnesium in chlorophyll molecules by Pb ions (Matlock *et al.*, 2002). The effect of lead on *Pistia stratiotes* is detrimental and there was an inverse relation between concentration of lead and chlorophyll content.

*Carotenoids*

The accessory photosynthetic pigments carotenoid was also estimated on 8th and 15th day after lead exposure and the data are presented in Table 4.

Though it is non-enzymatic antioxidants its content adversely affected by the higher concentration. Further there was a gradual decrease in carotenoid content as the concentration of the solution increases. Similar observation was made in *A. microphylla* when exposed to the heavy metal Pb (Mishra *et al.*, 2014).

Under stress conditions carotenoid pigments are less affected than chlorophyll. Since carotenoids are less affected it also act as an antioxidant metabolite, it protects chlorophyll and photosynthetic membrane from oxidative damage, therefore decline in carotenoids could have serious consequences on chlorophyll as well as thylakoid membrane which may lead to reduction in photosynthetic capability of *Pistia stratiotes* (Chris *et al.*, 2006; Dai *et al.*, 2006).

*Effect of Pb on protein content*

Being essential macromolecules of living cells protein plays a paramount role in metabolic pathway. Analysis of protein content was done on 8th and 15th day of Pb exposure in *Pistia stratiotes* (Fig.4).

The concentration of protein was found maximum in control whereas a considerable decrease in protein content was noticed in the treated plants with the increase in Pb concentration. Further, there was a gradual decrease in protein content of the treated plant species recorded with the increase in the days of retention period.

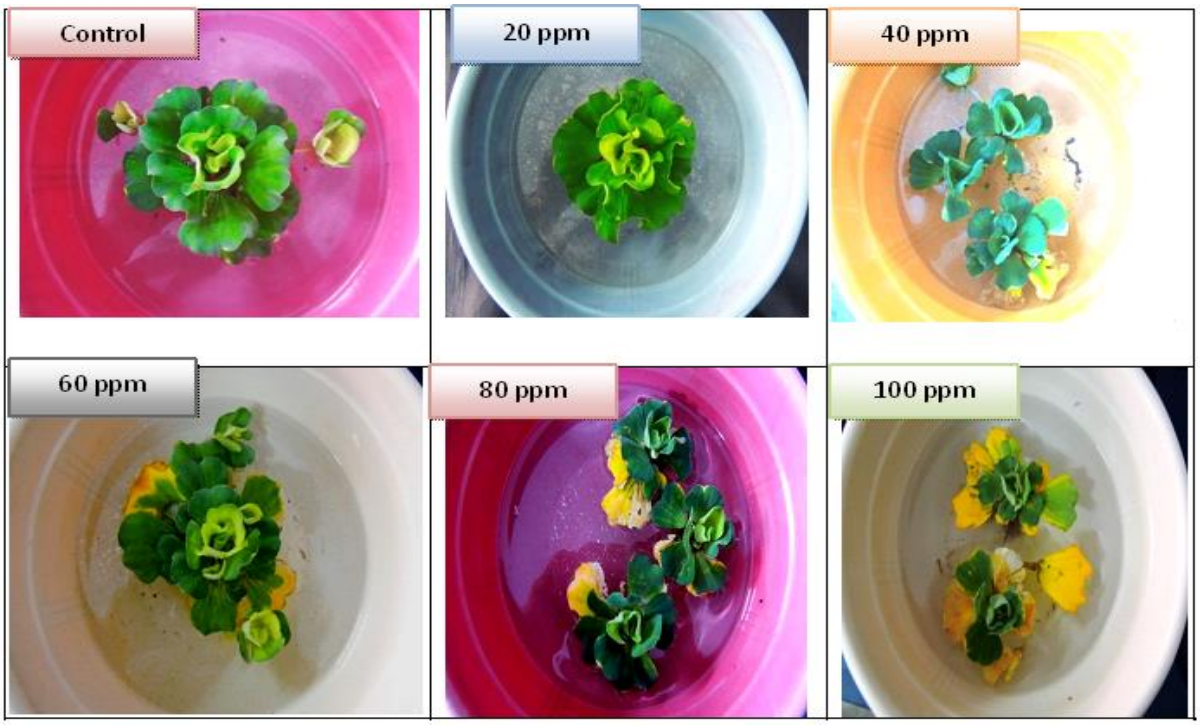


**Fig. 3.** Effect of various concentrations of Pb on morphological features of *Pistia stratiotes* L.

The findings of the present investigation corroborated with the findings of Costa and Spitz (1997) who reported a decrease in soluble protein content under heavy metal stress in *Lupinus albus*. Mohan and Hosetti (1997) found more pronounced decrease in the protein content with Cd as compared to Pb treatment in *Lemna minor*. Similarly John *et al.* (2008) reported the declined trend in soluble protein in *Lemna polyrrhiza* under different concentrations of Pb and Cd. Pillai *et al.* (2016) observed reduced protein content at higher concentrations of Pb treatment in *P. stratiotes*.

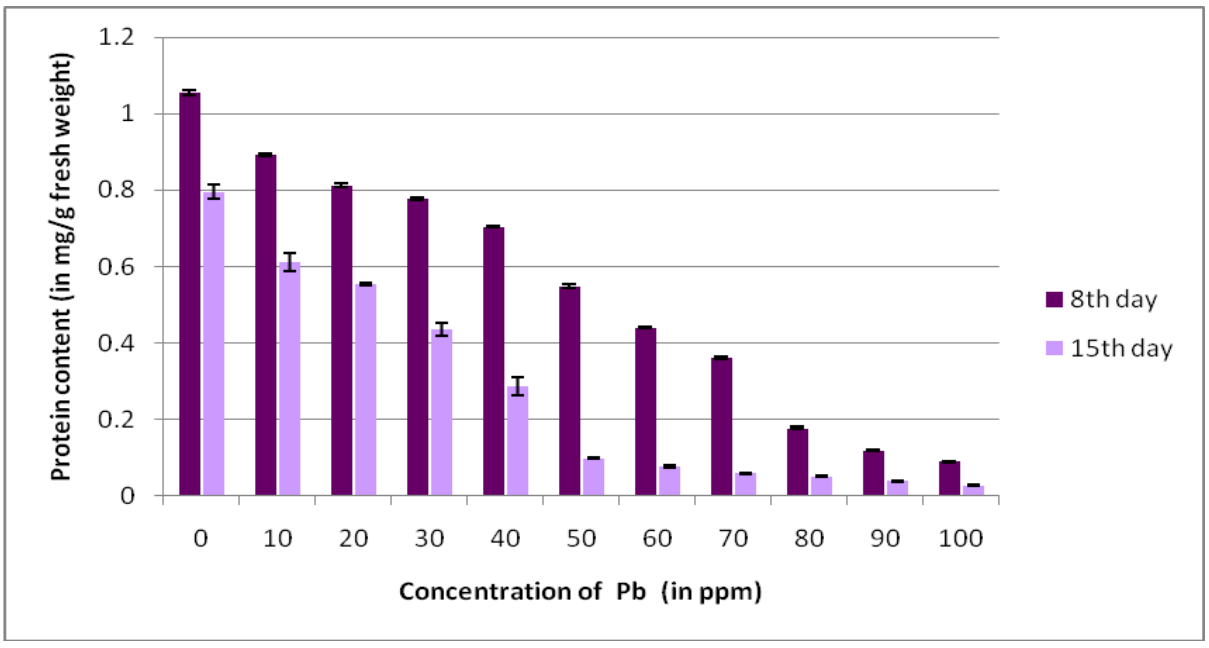


**Fig. 4.** Effect of various concentrations of Pb on protein content of *Pistia stratiotes* L. on 8th and 15th day of inoculation.

The decrease in protein content in plants may be caused by enhanced protein degradation process as a result of increased protease activity (Palma *et al.*, 2002) that is found to increase under stress conditions.

It is also likely that these heavy metals may have induced lipid peroxidation in aquatic plants and fragmentation of proteins due to toxic effects of reactive oxygen species leading to reduced protein content (Davies *et al.*, 1987).

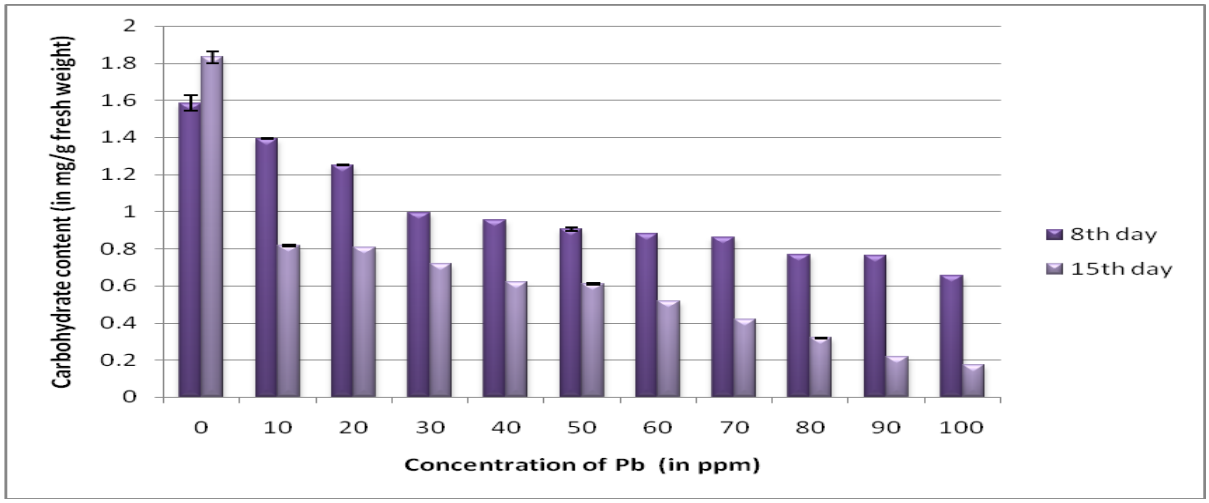


**Fig. 5.** Effect of various concentrations of Pb on carbohydrate content of *Pistia stratiotes* L.

*Effect of lead on carbohydrate content*

Experiment conducted in order to know the effect of lead on carbohydrate content revealed that soluble carbohydrate content in plants was decreased with increasing concentration of heavy metal Pb. The highest amount of carbohydrate i.e. 1.83 mg/g & 1.58 mg/g at control and lowest i.e. 0.65 mg/g and 0.18 mg/g fresh weight at 100 ppm of Pb were found in *P. stratiotes* both in 8$^{th}$ and 15$^{th}$ days experiment (Fig.5). The declined trend in total carbohydrate with respect to higher levels of Pb during the present investigation might be due to its role on the enzymatic reactions related to the cycles of carbohydrates catabolism.

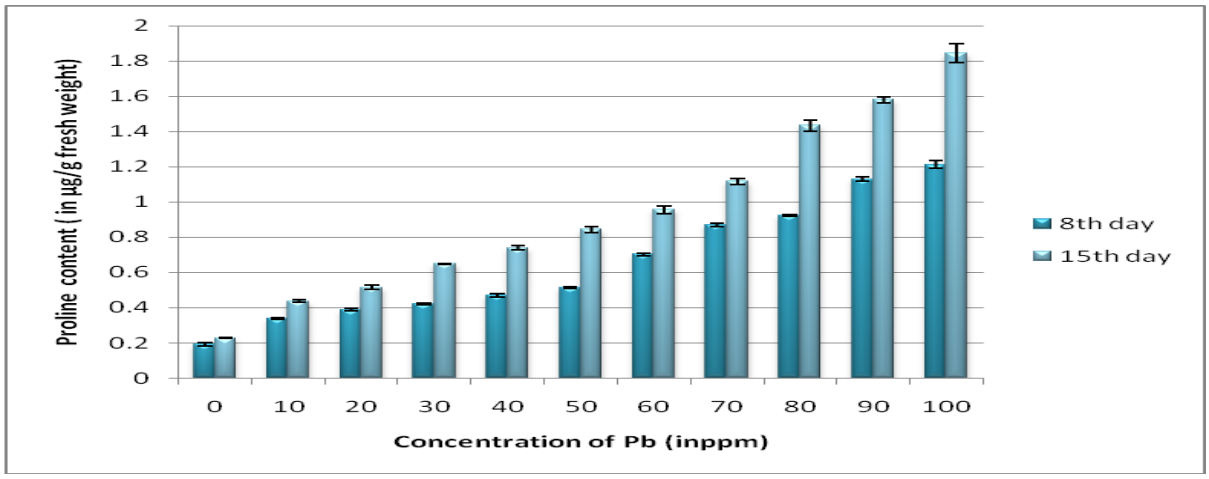


**Fig. 6.** Proline content of *Pistia stratiotes* L. in various concentrations of Pb solution.

The results of the present study is in agreement with the finding of Bharadwaj *et al.*, (2009) who reported the decreased carbohydrate with the increase in concentration of Pb and Cd in *Phaseolus vulgaris* L.

The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase (Stiborova *et al.*, 1987).

Similarly Rabie *et al.*, (1992) reported that Ni solutions gradually decreased all carbohydrate fractions in broad bean and corn plants.

*Effect of Pb on proline content*

With the increase in Pb concentration an increase in proline content was observed in *Pistia stratiotes* irrespective of the period of treatment (Fig.6). John *et al.* (2008) reported that proline content was increased with increase in Pb concentration in *Lemna polyrrhiza* L. The amount of proline found in *Vigna mungo* L. increased more under Pb stress than Ni (Sing *et al.*, 2012). Enhanced Proline concentration at increased stress indicated a possible role of this amino acid in osmoregulation. Korai (1989) reported that in matured leaves there is decreased protein degradation and increased concentration of free amino acids such as proline.

Proline also acts as a major reservoir of energy and nitrogen, which can be used in resuming the growth after the stress removal (Chandrashekhar and Sandhyarani, 1996). Proline is supposed to participate in the reconstruction of chlorophyll, activate the Krebs cycle and also in the energy source (Saxe, 1991).

## Conclusion

Based on the result, it can be concluded that, lead had inhibitory effect on growth and biochemical parameters of *P. stratiotes*. This study also showed that *P. stratiotes* has the capacity to uptake Pb, and the level of accumulation depends upon the concentration of the heavy metal in the culture solution. The BCF of the heavy metal increased along with the Pb level in the culture solution. Thus, *P. stratiotes* is a good accumulator for Pb and is a potential aquatic weed for removal of Pb from contaminated water. This floating hydrophyte can be used to decontaminate the water bodies polluted with heavy metals.

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