



Profile of Metal Accumulation in Aquatic Macrophytes

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ABSTRACT

Industrial development coupled with population growth has resulted in the over exploitation of natural resources. Life support systems viz; water, air and soil are thus getting exposed to an array of pollutants especially heavy metals released by anthropogenic activities. Tolerant species of aquatic plants are able to survive and withstand the pollution stress serves as pollution indicators and as tool for phytoremediation of heavy metals is an environment clean up strategy in which green plants are employed to remove toxic contaminants and operates on the principles of biogeochemical cycling.

The aquatic plants viz; *Salvinia molesta* and *Pistia stratiotes* were used for its toxicity and profile of metal accumulation (Cadmium –Cd) from synthetic media. The test plants were cultured in a modified Hoagland solution supplemented with cadmium nitrate Cd (NO₃)₂. The present study focuses on Cd toxicity on morphology, biochemical parameters and bioaccumulation potential of *Salvinia* and *Pistia*. The laboratory experiments were conducted for the assay of morphological index parameters (MIP), biochemical parameters, and profile of cadmium accumulation in test plants at various concentrations viz, 0.1, 0.5, 1.0, 1.5 & 2.0 ppm at 4 days regular intervals for 12 days exposure. The test plants show visible symptoms, like withering of roots, chlorosis, necrosis and in particular, at higher concentrations (2.0 ppm) lower leaves gets decayed. However, the lower concentrations i.e. 0.1 ppm shows normal growth. The estimation of biochemical parameters viz total chlorophyll, protein & carbohydrates of test plants showed significant increased at lower concentrations i.e. 0.1 ppm of Cd. The biochemical constituents decreased with increase in exposure concentrations i.e. 0.5 to 2.0 ppm. The toxic effect Cd was directly proportional to its concentrations and exposure durations. The profile of metal accumulation by both test plants was maximum at 4 days exposure irrespective concentrations and gradually decreases at subsequent exposure concentrations and duration.

Key Words: Biochemical parameters, Cadmium, Toxicity, Accumulation, Aquatic plants

INTRODUCTION

Heavy metal pollution is a major environmental problem facing the modern world (1, 2). The global heavy metal pollution is increasing in the environment due to increases of human activities. However, it is gaining importance day by day due to its obvious impact on human health through the food chain (3). As a result of rapid growth in the industrial sectors, India is now encountering several environmental problems, especially contamination of heavy metals in water. The danger of heavy metals is aggravated by their almost indefinite persistence in the environment because they cannot be destroyed biologically but are only transformed from oxidative state or organic complex to another. In addition, they are highly toxic for both aquatic flora and fauna. The heavy metal, cadmium, is selected as toxicant for the present

study because they are used in several industries in India and they are highly toxic to animals, humans and plants. Biological treatment of waste water through aquatic plants have a great potential for its purification which are effectively accumulates heavy metals (4). Aquatic macrophytes accumulates considerable amount of toxic metals and make the environment free from the pollutants. Thus, play significant role in cleaning up of environment and make the environment free from many pollutants. Many aquatic plants have been successfully utilized for removing toxic metals from aquatic environment (5). Similarly algae were also used to remove heavy metals from aquatic systems as they have capacity to accumulate dissolved metals (6, 7). The metal tolerance of plants may be attributed to different enzymes, stress proteins and phytochelatins (8). The accumulation of metals at higher concentration causes retardation of growth,

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ISSN: 2231-2196 (Print)

ISSN: 0975-5241 (Online)

Received: 21.06.2018

Revised: 12.07.2018

Accepted: 28.07.2018

biochemical activities and also generation of –SH groups containing enzymes (9).

In the present investigation *Salvinia molesta* Mitchell and *Pistia stratiotes* L, a common aquatic floating macrophytes are used to study the effect of different concentrations of cadmium on morphology, biochemical constituents and accumulation of Cd from the experimental pond under laboratory conditions.

MATERIALS AND METHODS

Salvinia molesta and *Pistia stratiotes*, free floating aquatic plants from unpolluted water bodies is maintained in cement pots (1 m diameter) under natural conditions at a temperature 28-30° C. About 20 g of young healthy *Salvinia* and *Pistia* were acclimatized for two weeks in Arnon and Hoagland nutrient solution maintaining pH between 7.1-7.4. The concentrations of Cd in the polluted water are in the range of 0.1, 0.5, 1.0, 1.5 and 2.0 mg/l and tap water as a control. Morphological Index parameters (MIP) viz, root length, leaf length and breadth were observed for

12 days at interval of 4 days. Photographs of *Salvinia* and *Pistia* which were taken by using Canon's Power Shot G₂ digital camera were treated with different concentrations of copper. For the further study the plants were harvested at the end of 4, 8 and 12 days exposure and are thoroughly washed with distilled water and used for the estimation of total chlorophyll, protein and carbohydrate and also for morphological observations. Plants harvested after 48 hrs were dried at 80° C for 2 days for metal extraction.

The fresh test plant samples of 1g is macerated in 100 ml of 80% (v/v) chilled acetone by using pestle and mortar. The centrifuged and supernatant was used for the estimation of total chlorophyll by standard method (10) using 652 nm against the solvent (80% acetone as a blank). The protein was estimated by Lowry's method (11) using Bovine Serum Albumin (BSA) as a standard, using 660 nm and carbohydrates by phenol sulphuric acid method (12) using glucose as standard at 490 nm. Morphological characters were identified with the help of photographs, using Canon's Power Shot G₂ -digital camera.

The estimation of metal Cd in the test plant was carried out by using standard method (13). The dried and powdered 1 g plant material was digested by using mixed acid digestion method in Gerhardt digestion unit. The digested samples were diluted with double distilled water and filtered through Whatman filter paper No-44. The estimation of Cd was done by AAS (GBC 932 Plus Australia) with air acetylene oxidizing flame and metal hollow cathode lamp at 217.00 nm wavelength. Working standards (SISCO-Chem-Bombay Lab) were used for the calibration of instrument.

STATISTICAL ANALYSIS

Data are presented as mean values \pm SE from two independent experiments with three replicates each. Data were subjected to Two - way ANOVA to know significance between concentrations and between exposure duration for the accumulation of heavy metal (Cd). Further, Dunet's test is also applied for multiple comparisons between control and other concentrations. Two – way ANOVA test is also extended to know the significance between concentration and duration for biochemical parameters.

RESULTS

Toxicity effect of cadmium on morphology. The test plants showed luxuriant growth, shows increase in the laminal length and breadth at low concentration (0.1 ppm) in both test plants. In *Salvinia* at 0.1 ppm of Cd was found to promote laminal length by 2.166 ± 0.169 , 2.200 ± 0.169 and 2.400 ± 0.094 and breadth by 2.066 ± 0.118 , 2.333 ± 0.118 and 2.366 ± 0.144 at 4, 8 and 12 days exposure duration. Similarly root length by 5.066 ± 0.383 , 5.330 ± 0.0356 and 5.533 ± 0.381 in *Pistia* at the same concentration (0.1 ppm) shows increase in laminal length by 1.660 ± 0.027 , 1.666 ± 0.027 and 1.738 ± 0.027 cm and breadth by 1.533 ± 0.027 , 1.666 ± 0.047 and 1.666 ± 0.027 at 4, 8 and 12 days exposure durations respectively. Similarly, the root length by 7.000 ± 0.072 , 7.130 ± 0.032 and 7.330 ± 0.027 at the same exposure duration.

However, in *Pistia* at 2.0 ppm Cd severely inhibit laminal length by 1.200 ± 0.047 , 1.000 ± 0.000 and 0.700 ± 0.000 and breadth 0.833 ± 0.027 , 0.813 ± 0.072 and 0.600 ± 0.072 at 4, 8 and 12 days exposure duration. Similarly root length inhibition by 4.000 ± 0.355 , 3.160 ± 0.027 and 2.900 ± 0.355 at the same exposure duration.

Salvinia also shows at 2.0 ppm concentration severe inhibition of laminal length by 1.300 ± 0.047 , 1.133 ± 0.072 and 1.116 ± 0.027 and laminal breadth by 1.600 ± 0.094 , 1.4 ± 0.216 and 1.106 ± 0.027 at 4, 8 12 days exposure duration. Similarly root length inhibition by 2.666 ± 0.196 , 1.866 ± 0.881 and 1.166 ± 0.259 cm at 4, 8 and 12 days exposure duration.(Table. 1 & 2).

Toxicity effect of Cadmium on biochemical parameters

The total chlorophyll content was very sensitive to heavy metal (Cd) toxicity. The results found that Cd at 0.1 ppm found to augment chlorophyll synthesis and was directly proportional to concentration and exposure duration in both the test plants. In *Salvinia* the chlorophyll content was increased by 3.65% (0.602 mg/g), 4.06 % (0.615 mg/g) and 4.56% (0.645 mg/g) respectively at 4, 8 and 12 days compared

to control pond. Similarly in *Pistia* the chlorophyll content was increased by 0.79% (0.382 mg/g), 1.04 % (0.385 mg/g), 1.30% (0.389 mg/g) respectively at 4, 8 and 12 days compared to control pond.

However, the higher concentration of Cd found to inhibit the chlorophyll synthesis in both the test plants. The inhibition at 2.0 ppm Cd by 20.05% (0.303mg/g), 31.49% (0.261 mg/g) and 39.58 % (0.232 mg/g) significant at $P > 0.95\%$ in *Pistia* and the inhibition at 2.0 ppm Cd by 24.09% (0.441 mg/g), 29.61% (0.416 mg/g) and 34.52% (0.402 mg/g) in *Salvinia*.

Two way ANOVA represents biochemical toxicity to the test plants, concentrations were significant at $P > 0.01$ level but duration is not significant (Table. 3 & 4).

The increase in carbohydrate content of *Salvinia* at 0.1 ppm Cd by 3.44% (30.0 mg/g), 12.88% (36.0mg/g) and 13.88% (43.0mg/g) respectively. Similarly, in *Pistia* the carbohydrate content increases marginally at 0.1 ppm concentration of Cd exposure by 8.82% (37.0 mg/g), 11.42% (39.0 mg/g) and 13.15% (43.0 mg/g) respectively at 4, 8 and 12 days exposure. However, the severity of inhibition is more pronounced in *Pistia* at 2.0 ppm of Cd by 47.05% (18.0 mg/g), 62.85% (13.0 mg/g) and 74.35 % (10.0 mg/g) respectively at 4, 8 and 12 days exposure (fig.). The 2.0 ppm of Cd found to inhibit carbohydrate synthesis by 27.58% (21.0 mg/g), 43.75% (18.0 mg/g) and 65.78% (13.0 mg/g) respectively at 4, 8 and 12 days exposure in comparison to control (Fig. 1 & 2).

The protein synthesis at 0.1 ppm of Cd was promotive irrespective of exposure duration in both test plants. However, the protein content decreased at subsequent concentration and inhibition was directly proportional to the exposure duration. The 0.1 ppm of Cd promoted the protein synthesis by 2.38% (4.3 mg/g), 4.65 % (4.5 mg/g) and 6.81 % (4.7 mg/g) respectively at 4, 8 and 12 days exposure duration. Similarly for *Pistia* at 0.1 ppm shows promotive by 2.22% (4.5 mg/g), 8.33% (5.2 mg/g) and 12.24% (5.5 mg/g) respectively at 4, 8 and 12 days exposure duration (Fig. 1 & 2).

The reduction in content was observed with progressive in Cd concentration in both the test plants. The inhibition of protein content increase viz, 35.7% (2.7 mg/g), 44.18% (2.4 mg/g) and 59.09% (1.8 mg/g) was noticed in *Salvinia* at 4, 8 and 12 days exposure. Similarly in *Pistia* also at 2.0 ppm inhibition by 3.4 mg/g (24.44 %), 2.6 mg/g (45.83%) and 2.0 mg/g (59.18%) respectively at 4, 8 and 12 days exposure duration (Fig. 1 & 2).

Application of two-way ANOVA, it is found that the biochemical responses of test plants species with respect to their concentrations were significant at $P > 0.01$ level. However, exposure durations are not statistically significant (Table. 3 & 4).

Profile of metal accumulation

Fig.3 shows the concentration of Cd accumulation in *Salvinia* and *Pistia* and was directly proportional to its concentration and exposure duration. The *Salvinia* grown in experimental pond containing 0.1 ppm found to accumulate 112.050 µg/g, 130.75 µg/g and 133.75 µg/g. Similarly *Pistia* also shows metal accumulation at the same concentration by 112.50 µg/g, 130.75 µg/g and 133.75 µg/g at 4, 8 and 12 days exposure duration (Fig. 3).

However, at higher concentration (2.0 ppm) accumulation in *Pistia* by 1060.50 µg/g, 1104.50 µg/g and 1125.00 µg/g and rate of accumulation in *Salvinia* also by 1270.0 µg/g, 1375.25 µg/g and 1381.00 µg/g during 4, 8 and 12 days exposure duration respectively (fig. 3).

Two way ANOVA showed that both concentration and exposure duration were significant at $P < 0.01$ level in both test plants and further Dunet's test was applied for the multiple comparison between control and different concentration treatments of test plant. From the statistical analysis it is clear that concentrations treatments are significantly differ with control (Table. 5).

DISCUSSION

Toxicity effect of Cd on morphology

1. Morphological toxicity: Morphometric assay is one of the quantitative tools for the assessment of toxicants measured by using Morphological Index Parameter (MIP). The rate of inhibition in the root and leaf (fronds) is directly proportional to the concentration of cadmium in both the test plants. Two way ANOVA test states the concentrations are significantly toxic at 5% level but duration is not significant. MCA test also represent maximum deviation is at higher concentration compared to control (Table 1). Both the test plants showed normal growth at their respective lower concentrations (i.e. 0.1 ppm). Similar observations were made by (14) in *Limnantherum cristatum* at 1 ppm concentration of Pb, Zn & Cr. The higher concentration of Cd (0.5 to 2.0 ppm) exhibited toxicity symptoms like chlorosis and leaf fall were observed, then brownish was occurred being marked in old leaves, respectively at higher concentration 2.0 ppm in both the test plants. Our results of toxicity symptoms of Cd at higher concentrations observed were similar to (15) and (16) and also in *Salvinia natans* (2). Sobero et. al (17) confirmed root elongation of Cd in some members of lemnaceae was found at different concentrations of Cd. The heavy metal induces morphological abnormalities in algae also (18).

2. Toxicity effect of cadmium on biochemical parameters: A number of heavy metals required by plants as micronutrients and they act as co-factors of enzymes

as a part of prosthetic groups and involved in a wide variety of metabolic pathway, but higher concentration of heavy metals are toxic and induces physiological and genetical changes in plants (19, 20).

In the present investigation the lower concentration of Cd promotes the synthesis of chlorophyll in *Salvinia molesta* & *Pistia stratiotes*. The enhancement of chlorophyll content from 0.602 mg/g to 0.642 mg/g in *Salvinia* & 0.379 mg/g to 0.384 mg/g in *Pistia* from 4 to 12 days exposure. The percent enhancement of chlorophyll is 4.56% in *Salvinia* & 1.56% in *Pistia* when compared to control (respective) during 12 days exposure. The stimulatory of Cd at lower concentration (0.01 to 0.4 mg/l) was noticed in *Ceratophyllum demersum* (21). The phytochelatins (PCs) play an important role in cellular metal ion homeostasis and metal detoxification (22) and hence lower concentration of Cd shows stimulatory effect.

The Cd treatment at higher concentration decreases the chlorophyll content due to accelerated degradation of chlorophyll. In the present investigation, the inhibition varies from 0.441 mg/g to 402 mg/g in *Salvinia* and 0.303 mg/g to 0.232 mg/g in *Pistia*. The inhibition is 34.52% in *Salvinia* & 34.58% *Pistia* at 12 days exposure compared to their respective control. The Cd found to inhibit general metabolic activities in many species of aquatic plants viz, *Eichornia* species (23) and in *Salvinia natans* (24). Inhibition activity of Cd is due to inhibition of haemobiosynthesis and chlorophyll formation by integrating with functional -SH group of enzyme involved in the biosynthesis of chlorophyll (25). Similar observation was made by (26) in *Hydrilla verticillata* at higher concentration of Pb at 20 ppm and Cd at 0.05 ppm. The decline in chlorophyll content in plants exposed to 2.0 ppm of Cd is due to i) inhibition of important enzymes associated with chlorophyll biosynthesis ii) peroxidation of chloroplast membranes resulting from heavy metal induced oxidative stress and iii) formation of metal substituted chlorophyll (27).

Carbohydrates acts as osmoregulators which maintains water balance in plants (28). Lower concentration (0.1 ppm) of Cd increases the carbohydrate content from 30 mg/g to 36 mg/g in *Salvinia* and 37 mg/g to 43 mg/g in *Pistia* from 4 to 12 days exposure duration. The percent enhancement of carbohydrate at 12 days exposure is 13.88% in *Salvinia* and 13.55% in *Pistia* compared to respective control. However, higher concentration of Cd inhibits the synthesis of carbohydrate and vary from 21 mg/g to 3 mg/g in *Salvinia* and 18 mg/g to 10 mg/g in *Pistia* from 4 to 12 days exposure duration. The rate of carbohydrate at 2.0 ppm of Cd is 67.75% in *Salvinia* and 74.35% in *Pistia* during 12 days exposure compared to respective control. The reduction in carbohydrate content can be attributed to the reduced rates of photochemical activities. (18) and also succinic dehydrogenase (SDH) fall in cells indicate oxygen stress and energy crisis

and mitochondria disturbances (29).

The lower concentration of Cd (0.1 ppm) enhance the rate of protein synthesis. The protein content vary from 4.32 mg/g to 4.7 mg/g in *Salvinia* and 4.6 mg/g to 4.7 mg/g in *Pistia* from 4 and 12 days exposure duration. The percent enhancement is 6.81% in *Salvinia* and 12.24% in *Pistia* during 12 days exposure when compared to their respective control. The stimulation of protein synthesis at lower concentration of Cd may be attributed to the synthesis of stress proteins (30). The phytochelatins (PCs) are produced by Glutathione reductase (GR) and Phytochelatin Synthetase. These proteins bind and regulate the Cd and sequester the Cd toxicity and thus, plants shows metal tolerance (31).

However, the higher concentration of Cd inhibit protein metabolism in the plants. The protein content declines from 2.7mg/g to 1.8 mg/g in *Salvinia* and 3.4 mg/g to 2.0 mg/g in *Pistia* from 4 to 12 days exposure period. The percent inhibition is 59.09% in *Salvinia* and 59.18% in *Pistia* during 12 days exposure compared to respective at control. The Cd shows slight inhibitory effect 0.5 mg/l and severe inhibition of algal growth at higher concentration in some algae (32, 18). The DNA and RNA were inhibited, rather due to blocking of -SH group or to the inactivation of RNA and DNA polymerase activity (18, 33).

Profile of metal accumulation

Heavy metal pollution of water is a major environmental concern, is increasing at alarming rate due to anthropogenic activities and is drawing attention and gaining paramanual importance due to its obvious impact on health through the food chain (1, 34). In the present investigation aquatic macrophytes viz, *Salvinia* and *Pistia* are used in accumulation. The plants exposed to concentration of cadmium, i.e 0.1 ppm found to accumulate maximum in *Salvinia* (133.75 µg/g) followed by *Pistia* (128.50 µg/g) during 12 days exposure duration. Similarly at higher concentration i.e 2.0 ppm of Cd during 12 days exposure duration shows 1381 µg/g in *Salvinia* and 1125 µg/g in *Pistia*.

Generally in our experiments it was found that the rate of accumulation is maximum at 4 days exposure irrespective of concentrations and exposure duration, however, at subsequent concentrations and exposure durations it is marginal. Similar observations were also made by (35) in *Nasturtium officinale* and *Mentha aquatic* to the exposure concentrations of 0.1 and 0.5 ppm of Cd. Similar observation was made by (36) in the accumulation of Nickel in *Hydrilla verticillata* and Cd & Pb in *Salvinia cuculata* (24). The increase in the accumulation might be due to increased number of binding sites for the complexation of heavy metal ions, leading to the increased absorption, however, slow accumulation may be attributed to binding ions to the plants and establishment of equilibrium status between adsorbate and adsorbent (37, 38).

CONCLUSION

It is concluded from the findings that morphological, biochemical responses and profile of metal accumulation by *Salvinia* and *Pistia* were directly proportional to concentration of metal and maximum metal uptake was recorded at 4 days exposure and later it was marginal at subsequent concentrations and exposure durations *Pistia stratiotes* is found to be suitable candidate for toxicity evaluation. *Salvinia molesta* is the tolerant species and can be used for the remediation of heavy metals from aquatic ecosystem and environmental monitoring.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, B.L.D.E.A's Degree College, Jamkhandi (India), Research and Development centre, Bharthiar University, Coimbatore. Dept. of Botany, Karnataka University Dharwad for providing necessary facilities to carry out research work. Further, the author acknowledges the immense help received from the scholars whose articles are cited and included in references of this manuscript. The author is also grateful to authors / editors, publishers of all those articles, journals and books from where the literature for this article has been received and discussed

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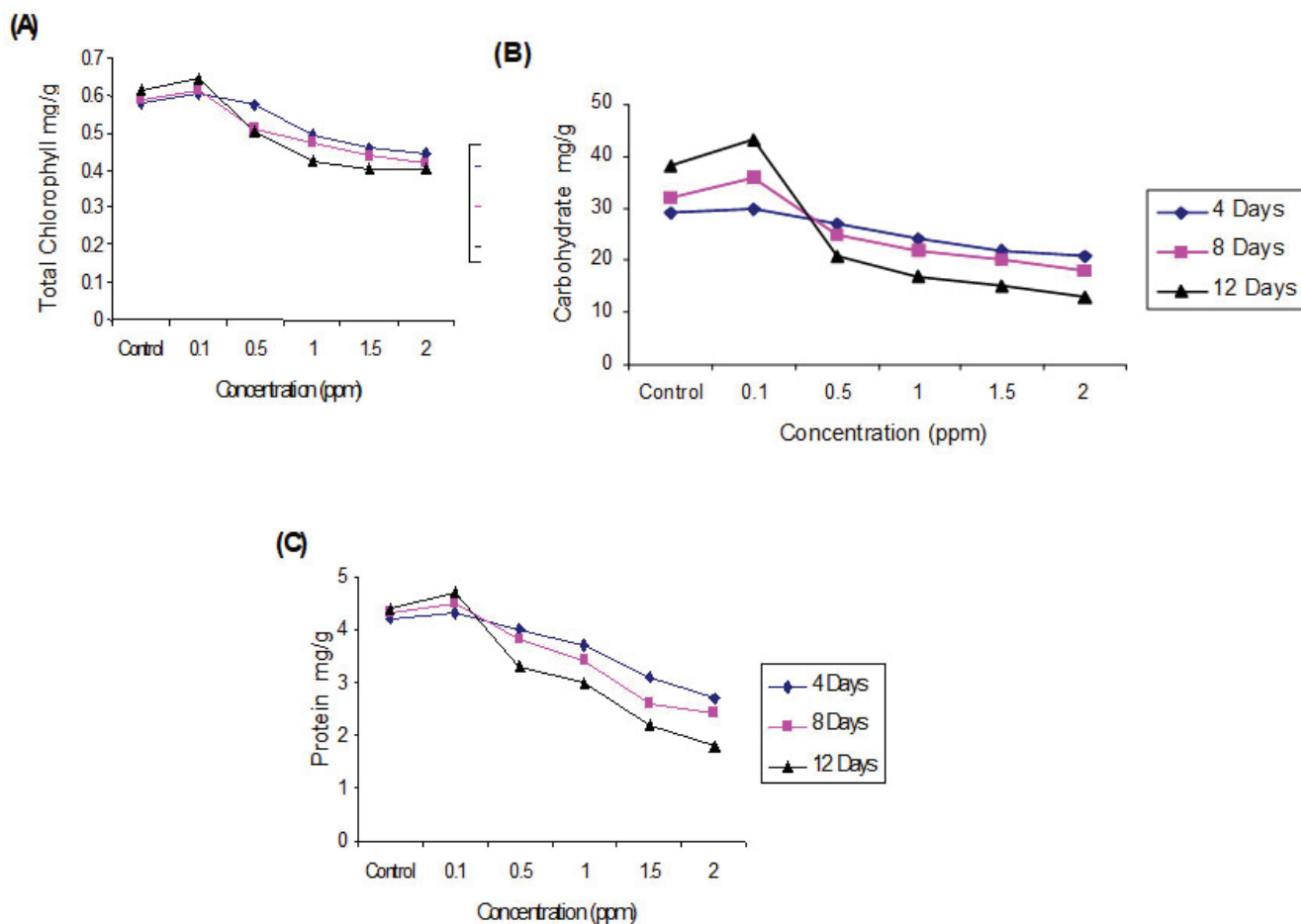


Figure 1: Biochemical effects of Cadmium on *Salvinia molesta*

(A) Total Chlorophyll (B) Carbohydrate (C) Protein

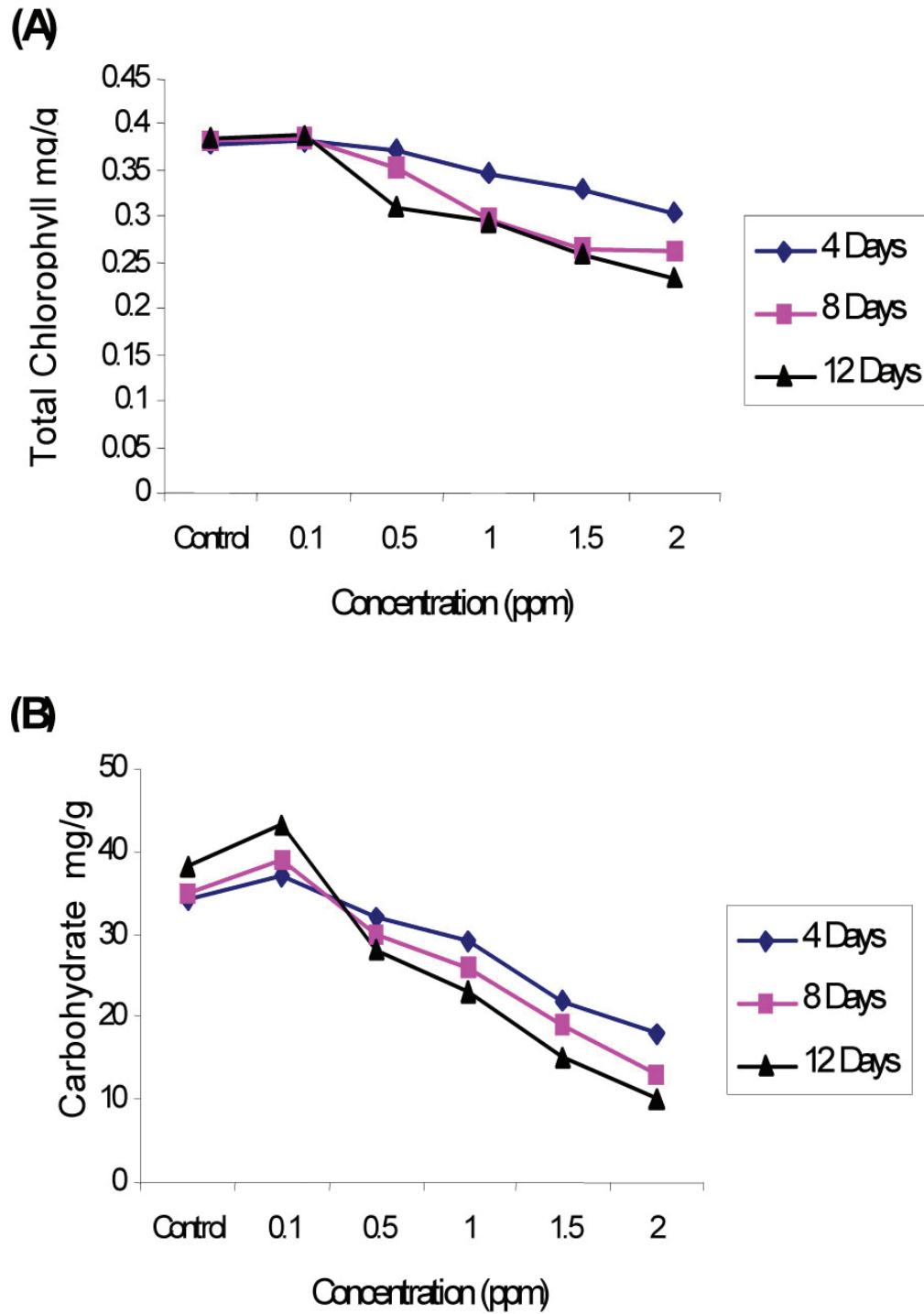


Figure 2: Biochemical effects of Cadmium on *Pistia stratiotes*.

(A) Total Chlorophyll

(B) Carbohydrate (C) Protei

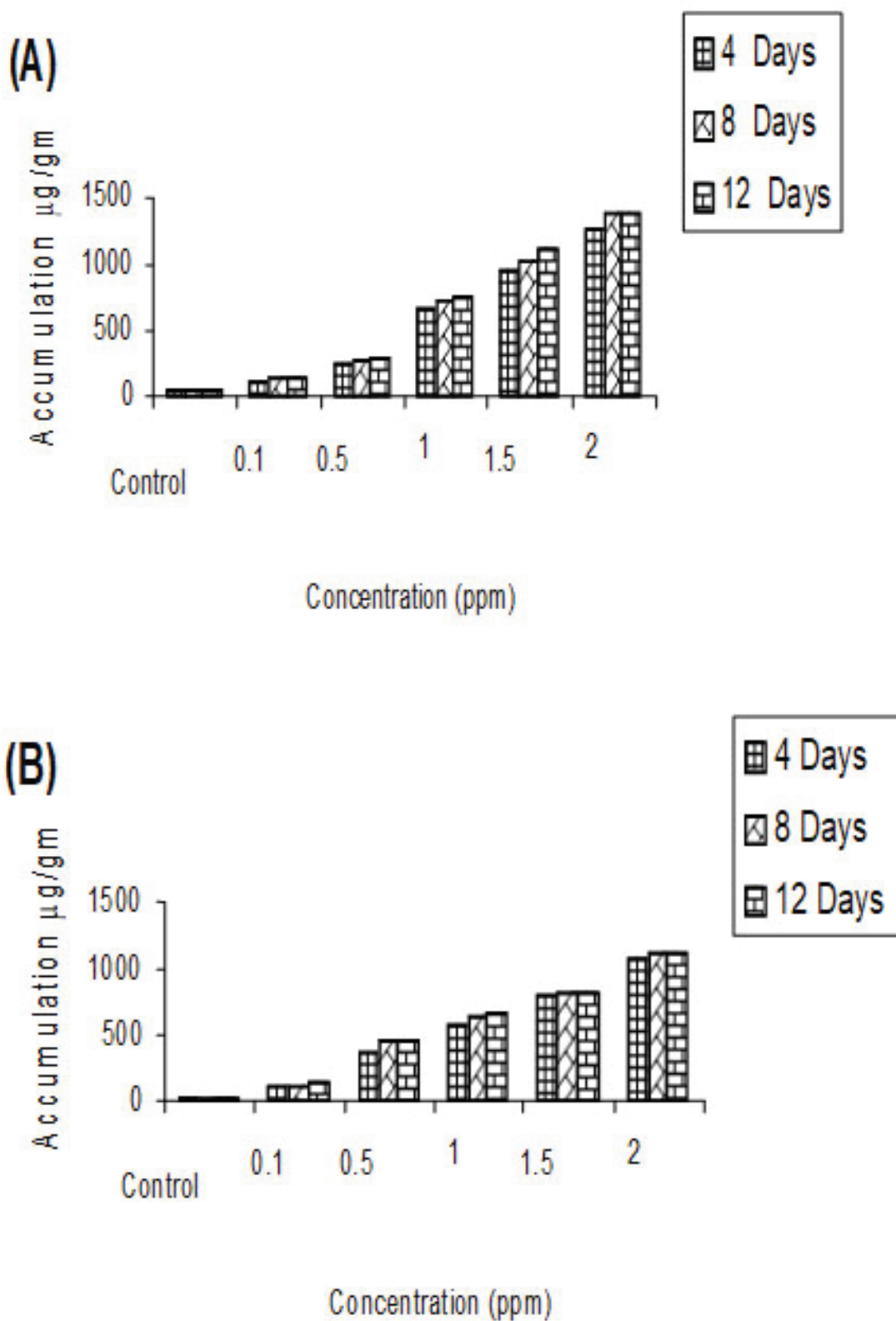


Figure 3: Accumulation profile of Cadmium by aquatic macrophytes.

(A) *Salvinia* (B) *Pistia*

Table 1: Effect of Cadmium on morphology of *Salvinia molesta*

Concen- tration (ppm)	Exposure Duration (in days)								
	4	8	12	4	8	8	12		
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control	4.400 ± 0.309	4.533 ± 0.212	4.766 ± 0.232	1.766 ± 0.027	2.000 ± 0.047	1.766 ± 0.027	2.000 ± 0.047	2.030 ± 0.047	1.900 ± 0.047
0.1	5.066 ± 0.383	5.330 ± 0.035	5.533 ± 0.381	2.166 ± 0.178	2.066 ± 0.118	2.200 ± 0.169	2.333 ± 0.118	2.400 ± 0.094	2.366 ± 0.144
0.5	4.366 ± 0.178	4.166 ± 0.144	4.066 ± 0.136	1.933 ± 0.151	1.966 ± 0.072	1.866 ± 0.136	1.900 ± 0.081	1.732 ± 0.118	1.800 ± 0.047
1.0	4.066 ± 0.136	3.866 ± 0.151	3.733 ± 0.098	1.733 ± 0.108	1.666 ± 0.054	1.666 ± 0.054	1.500 ± 0.047	1.533 ± 0.022	1.366 ± 0.027
1.5	4.000 ± 0.047	3.300 ± 0.205	2.333 ± 0.276	1.666 ± 0.054	1.633 ± 0.151	1.600 ± 0.124	1.433 ± 0.151	1.533 ± 0.027	1.266 ± 0.072
2.0	2.666 ± 0.196	1.866 ± 0.381	1.166 ± 0.259	1.300 ± 0.047	1.600 ± 0.094	1.133 ± 0.072	1.4 ± 0.216	1.116 ± 0.027	1.106 ± 0.027

Values are expressed in cms
Mean values ± Standard Error

Table 2: Effect of Cadmium on morphology of *Pistia stratiotes*

Concen- tration (ppm)	Exposure Duration (in days)								
	4	8	12	4	8	12			
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control	6.100 ± 0.047	6.160 ± 0.072	6.300 ± 0.124	1.400 ± 0.047	1.330 ± 0.027	1.466 ± 0.027	1.366 ± 0.027	1.476 ± 0.027	1.376 ± 0.047
0.1	7.000 ± 0.072	7.130 ± 0.032	7.330 ± 0.027	1.660 ± 0.027	1.533 ± 0.027	1.666 ± 0.027	1.533 ± 0.047	1.738 ± 0.027	1.666 ± 0.027
0.5	6.360 ± 0.072	6.260 ± 0.072	6.100 ± 0.047	1.500 ± 0.047	1.333 ± 0.027	1.500 ± 0.047	1.333 ± 0.027	1.333 ± 0.027	1.111 ± 0.027
1.0	4.766 ± 0.047	4.600 ± 0.08	4.290 ± 0.047	1.400 ± 0.047	1.333 ± 0.027	1.400 ± 0.047	1.333 ± 0.027	1.300 ± 0.027	1.212 ± 0.02
1.5	4.533 ± 0.072	4.466 ± 0.151	3.240 ± 0.047	1.333 ± 0.027	1.066 ± 0.720	1.200 ± 0.00	1.060 ± 0.072	1.000 ± 0.00	1.100 ± 0.027
2.0	4.000 ± 0.355	3.160 ± 0.027	2.900 ± 0.355	1.200 ± 0.047	0.833 ± 0.027	1.000 ± 0.00	0.813 ± 0.072	0.700 ± 0.00	0.600 ± 0.072

Values are expressed in cms
Mean values ± Standard Error

Table 3: Two way ANOVA for biochemical effects of Cadmium on *Salvinia molesta*

	Total chlorophyll	Carbohydrate	Protein
F-Value (between concentration)	29.987**	8.057**	27.085**
F- value (between duration)	1.585	0.088	3.333

** Significant at P < 0.01 level

Table 4. Two way ANOVA for biochemical effects of Cadmium on *Pistia stratiotes*

	Total chlorophyll	Carbohydrate	Protein
F-Value (between concentration)	17.647**	32.381**	13.658**
F- value (between duration)	6.166	1.065	1.850

**Significant at P < 0.01 level

Table 5: Two way ANOVA with Dunet's test for multiple comparison for accumulation of Cadmium by aquatic macrophytes

	Salvinia	Pistia
F-Value (between concentration)	733.87**	1820.94**
F- value (between duration)	8.13**	13.27**
Dunet's Value	53.60	26.57
Control V/s 0.1 ppm	79.25	97.08
Control V/s 0.5 ppm	225.83	399.41
Control V/s 1.0 ppm	662.00	600.91
Control V/s 1.5 ppm	982.41	785.91
Control V/s 2.0 ppm	1295.66	1074.25

** Significant at P < 0.01 level