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Effect of Cadmium Toxicity on Aquatic Macrophyte *Pistia Stratiotes* (L.)

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Abstract

The present study focuses on cadmium toxicity on morphology and selected biochemical parameters of *Pistia*. The laboratory experiments were conducted for the assessment of morphological index parameters (MIP), biochemical parameters and accumulation of cadmium in plants at its various concentrations (0.1, 0.5, 1.0, 1.5 and 2.0 ppm) at the regular interval for 12 days exposure. *Pistia* showed visible symptoms like withering of roots, chlorosis and necrosis at higher concentration (2.0 ppm), however, the plant showed normal growth at lower concentration (0.1 ppm). The estimation of biochemical parameters (Total chlorophyll, Protein and Carbohydrate) of test plants showed a significant increase at lower concentration (0.1 ppm) of cadmium. The biochemical parameters decrease with increase in exposure concentration and duration. The toxic effect of cadmium is directly proportional to its concentration and duration. The accumulation of cadmium by *Pistia stratiotes* was maximum at 4 days exposure duration and marginal at subsequent concentrations and exposure duration. With respect to biochemical parameters the concentrations are significant. However, metal accumulation is significant at concentrations and exposure duration.

Keywords: Accumulation; Biochemical parameters; Cadmium; Toxicity

Introduction

Heavy metal contamination in the water bodies is increasing at an alarming rate due to industrial and anthropogenic activities [1]. Heavy metals cannot be degraded and therefore, are persistent. Efforts are being made to develop technologies that use biological materials effectively in removing heavy metals from the environment. Plants possess the capacity to accumulate heavy metals. Higher plant species possess physiological traits that enable them to tolerance and hyper accumulate metals [2,3]. The metal tolerance of plants may be attributed to different enzymes, stress proteins and phytochelatins [4]. The accumulation of metals at higher concentrations causes retardation of growth, biochemical activities and also generation of -SH group containing enzymes [5].

The aim of this study was to determine Cd toxicity on morphological index parameters (MIP), contents of total chlorophyll, protein and carbohydrates in submerged aquatic macrophyte, *Pistia stratiotes*.

Materials and Methods

Pistia stratiotes, a free floating aquatic plant from unpolluted water bodies and maintained in cement pots (1 m diameter) under natural conditions at a temperature 28-30°C. About 20 g of young healthy *Pistia* is acclimatized for two weeks in Arnon and Hoagland nutrient solution [6] maintaining pH between 7.1-7.4. The concentrations of Cd in the polluted waters 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 *Pistia* treated with different concentrations of cadmium were taken by using Canon's Power Shot G2 digital camera. 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 plant sample of one g is macerated in 100 ml of 80% (v/v) chilled acetone by using pestle and mortar. The extract was centrifuged and supernatant was used for the estimation of total chlorophyll by standard method [7] using 652 nm against the solvent (80% acetone as a blank). The protein was estimated by Lowry's method [8] using Bovine Serum Albumin (BSA) as a standard using 660 nm and carbohydrates by phenol sulphuric acid method [9] using glucose as standard at 490 nm. Morphological characteristics with the help of photographs using Canons Power Shot G_2-digital camera.

The estimation of metal (Cd) in the test plant was carried out by using standard method [10]. 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 ± 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.

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Results and Discussions

Morphological toxicity

Morph metric assay is one of the quantitative tools for the assessment of toxicants was measured by using Morphological Index Parameter (MIP). The rate of inhibition of growth in the root and leaf is directly proportional to the concentration of Cd. Two-way ANOVA test states that the concentrations are significantly toxic at 5% level but duration is not significant. MCA test is also represent maximum deviation is at higher concentration compared to control (Table 1). The plant showed normal growth. Similar observation was made by Garg et al. [11] in *Limnanthemum cristastum* at 1 ppm concentration of Pb, Zn and 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 more marked in old leaves especially at 2.0 ppm Cd concentration such adverse effect of Pb on the growth has been reported by Dogan and Saygideger [12] and Kopittke et al. [13]. Similar observations were made by Yongpisanphop et al. [14] in *Salvinia natanus* and Wolff et al. [15] in Sal*vinia auriculata.*

Biochemical toxicity

Chlorophyll content is a parameter that is sensitive to heavy metal toxicity [16]. Cadmium at 0.1 mg/lt found to augment chlorophyll synthesis and directly proportional to the duration, the content increased by 0.79 %, 1.04% and 1.30 % at 4, 8 and 12 days exposure compared to control pond. Similar observation has been reported by Singh et al. [2] in *Hydrilla verticillata* and Dhir and Srivastava [17] in *Salvinia natanus* at 10 mg/lt of Cu, Fe, Zn, Co, Cr. The stimulations of chlorophyll synthesis may be due to phytochelatins (PCs) which plays role in detoxification [18]. However, the higher concentration of Cd found to inhibit the chlorophyll synthesis. The inhibition at 2.0 mg/lt Cd by 20.05 %, 31.49 % and 39.58 % (significant at P>0.95) at 4, 8 and 12 days exposure respectively compared to control. Two-way ANOVA represents biochemical toxicity to the test plant, concentrations are significant at P>0.01 level but duration is not significant. Two-way ANOVA represents, cadmium toxicity is at P>0.01 level significant towards but duration is not significant (Figure 1A).

Higher metal concentration, Pb 20 mg/lt and 5.0 mg/lt Cd showed decrease in total chlorophyll [2]. The decline in chlorophyll content in plants exposed to Cd 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 [19].

Carbohydrate content of *Pistia* at 0.1 ppm increased to the extent of 8.82 %, 11.42 % and 13.15 % respectively at 4, 8 and 12 days exposure compared to control. It is believed that Cd and Pb are inducers for phytochelatin synthesis and definite role of detoxification of Cd and Pb and hence increase in the carbohydrate content at lower (0.1 ppm) concentration [20]. However, the high concentration of Cd found to inhibit carbohydrate. The inhibition at 2 mg/lt of Cd by 47.05 %,

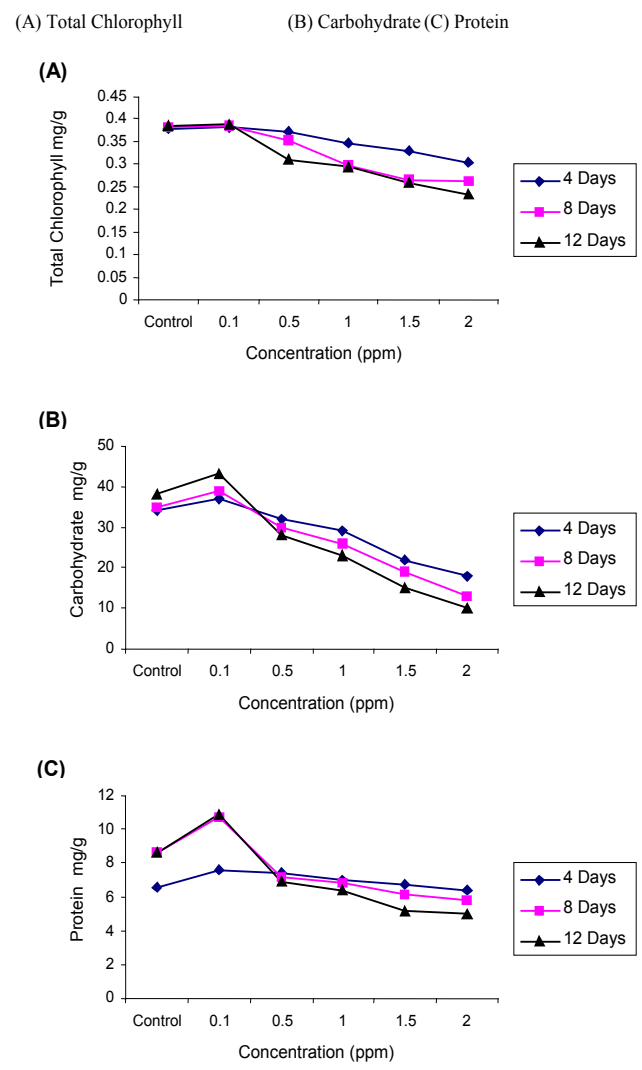

Figure 1: Biochemical effects of Cadmium on *Pistia*. (A) Total Chlorophyll (B) Carbohydrate (C) Protein.

Conc (ppm)	Exposure Duration (in days)								
	4	8	12	4		8		12	
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control	6.1 ± 0.04	6.16 ±0.07	6.3 ±0.12	1.4±0.04	1.33± 0.02	1.46 ±0.02	1.36 ±0.02	1.47 ±0.02	1.37 ± 0.04
0.1	7.0 ± 0.072	7.13 ±0.03	7.33 ±0.02	1.66 ± 0.02	1.533 ± 0.02	1.66 ±0.02	1.53 ±0.04	1.73 ±0.02	1.66 ± 0.02
0.5	6.36 ± 0.07	6.26 ±0.07	6.1 ±0.047	1.5 ± 0.047	1.333 ± 0.027	1.5 ±0.047	1.33 ±0.02	1.33 ±0.02	1.11 ± 0.02
1.0	4.76 ± 0.04	4.6 ± 0.08	4.29 ±0.04	1.4 ± 0.047	1.333 ± 0.027	1.4 ±0.047	1.33 ±0.02	1.3 ± 0.02	1.21 ± 0.02
1.5	4.53 ± 0.07	4.46 ±0.15	3.24 ±0.04	1.33 ± 0.02	1.066 ± 0.720	1.2 ± 0.00	1.06 ±0.07	1.0 ± 0.00	1.1 ± 0.027
2.0	4.0 ± 0.355	3.16 ±0.02	2.9 ± 0.35	1.2 ± 0.047	0.833 ± 0.027	1.0 ± 0.00	0.81 ±0.07	0.7 ± 0.00	0.6 ± 0.072

Values are expressed in cms
Mean-values ± standard Error

Table 1: Morphological response of *Pistia stratiotes* to Cadmium.

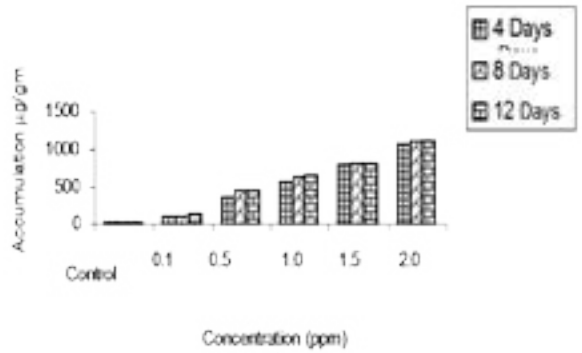

Figure 2: Accumulation profile of Cadmium by *Pistia*.

62.85% and 74.35% (significant at P > 0.05) at 4, 8 and 12 days exposure respectively compared to control. Two way statistical ANOVA represents biochemical toxicity to the test plant concentrations are significant at P > 0.01 level but duration is not at significant level (Figure 1B).

Many studies show that the protein content of many aquatic macrophytes was increased by accumulation of Pb at lower toxicity concentration [21]. The protein content increase marginally at 0.1 ppm at 2.22%, 8.33% and 12.24% respectively at 4, 8 and 12 days exposure compared to control. The stimulation of protein synthesis at lower concentration of Cd (0.1ppm) may be attributes to the synthesis of stress proteins. The phytochelatins (PC) and phytochelatin synthetase bind and regulate the Cd and sequester the toxicity in the plants and thus, shows metal tolerance [18,22]. The reduction in protein content was observed with progressive increase in Cd concentration. Protein content in *Pistia* decreased by 24.44 %, 45.83% and 59.81% respectively at 4, 8 and 12 days exposure at 2.0 ppm concentration compared to control (P>0.01) (Figure 1C). The Cd induces oxidative stress by generating reactive oxygen species (ROS). These disrupt cellular homeostasis, thus, enhances the production of ROS. These ROS reacts with proteins, lipids, nucleic acids causing membrane damage and enzyme inactivation [11,23]. The decrease in protein content of macrophyte may be due to the above reasons.

Metal Accumulation

Figure 2 shows the concentration of Cd accumulated in *Pistia*. There was increase in the accumulation may be due to availability of increased number of binding sites for the complexation of heavy metal ions leading to the increased absorption. In the test plant the accumulation of Cd is directly proportional to exposures and concentrations. The *Pistia* grown in experimental pond containing 0.1 ppm accumulate 112.50 µg/gm, 130.75 µg/gm and 133.75 µg/gm and accumulation at high concentration (2.0) was 1270.0 µg/gm, 1375.25 µg/gm and 1381.00 µg/gm during 4, 8 and 12 days exposures respectively. It was observed that the rate of accumulation is maximum at 4 days exposure irrespective of concentrations; however, at remaining durations it is marginal. Similar observations were made by Bendra et al. [24] in *Cladophora glomerata* at the concentration 0.1 M solution Cd Initial increase in the accumulation may be due to the availability of 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 almost all ions to the plants and establishment of equilibrium status between adsorbate and adsorbent [3,25].

Two way ANOVA represents concentration and exposure duration are significant at P < 0.01 and Dunet's test reveals the concentrations are significantly differ with control.

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