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RESEARCH ARTICLE

TOXIC EFFECT OF CADMIUM ON AQUATIC MACROPHYTE, *SALVINIA MOLESTA* (MITCHELL)

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ABSTRACT

The present study focuses on cadmium toxicity on morphology and biochemical parameters of *Salvinia molesta* (Mitchell) to the cadmium stresses and its accumulation. 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. *Salvinia* 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 *Salvinia molesta* 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.

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INTRODUCTION

Natural soil erosion and anthropogenic activities are greatly responsible for water pollution. The water pollution leads to undesirable changes in the quality of physicochemical environment, biota and its status. There is likelihood of phytotoxicity and environmental risks. Heavy metal persisting in sediments, may be slowly released into the water and become available to organisms and are toxic when taken in excess amount (Blaylock and Haung- 2000). The non-essential ions like lead (Pb) and cadmium (Cd) can inhibit variety of metabolic processes even in small quantities (Begum and Harikrishna, 2010). However, the essential metals like zinc (Zn), copper (Cu), iron (Fe) are toxic at higher concentration (Campanella, 2001). The waste water emanating from the source contains metals could be toxic to flora and fauna. Biological treatment of waste water through aquatic macrophytes has a great potential for its purification which effectively accumulate heavy metals. Aquatic macrophytes accumulate considerable amount of toxic metals and make the environment free from the pollutants (Singh *et al.*, 2011). Thus, play a significant role in cleaning up of environment and make the environment free from the pollutants.

Similarly, algae were also used to remove heavy metals from the aquatic systems as they have capacity to accumulate dissolved metals (Sbihi *et al.*, 2012). The metal tolerance of plants may be attributed to different enzymes, stress proteins and phytochelatins. The accumulation of metals at high concentration causes retardation of growth, biochemical activities and also generation of -SH group containing enzymes (Weckx and Clijsters, 1996; Vestena *et al.*, 2011). The aim of this study was to determine Cd toxicity on morphological index parameters (MIP), content of total chlorophyll, protein and carbohydrates in submerged aquatic macrophyte, *Salvinia molesta*.

MATERIALS AND METHODS

Laboratory experiments were conducted for the study of biochemical responses and accumulation potential of *Salvinia molesta* (Mitchell) from the experimental ponds. The test plants were collected from Kelageri pond near Karnatak University Dharwad, India. The plant stock was maintained under laboratory conditions, experiments were carried out in triplicates. The young and healthy *Salvinia molesta* is selected and acclimatized for two weeks in Hoagland solution maintaining pH between 7.1 to 7.4 in the experimental ponds of 10 l capacity. About 50 gm plant material was introduced simultaneously into each experimental ponds containing 0.1,

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0.5, 1.0, 1.5 and 2.0 ppm concentrations of cadmium and tap water as 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. The plants were harvested at the end of 4, 8 and 12 days exposure and thoroughly washed with distilled water and used for the estimation of total chlorophyll, carbohydrate and protein content. Plants were harvested after 48 hours were dried at 80°C for two days for metal extraction. The fresh plant sample of 1 gm 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 (Arnon, 1949) using 652 nm against the solvent (80% acetone as a blank). The protein was estimated by the method of Lowry (1951) using bovine serum albumin (BSA) as a standard using 660 nm and carbohydrate by phenol sulphuric acid method (Dubois et al., 1956) using glucose as a standard at 490 nm. The estimation of cadmium in the plant material was carried out using standard method (Allen et al., 1974). The dried and powdered 1 gm plant material was digested by using mixed acid digestion method until the appearance of white fumes in (C. Gerhardt Gmb H and Co. Bornheimor Star Be 100, 53119 Bonn). The digested sample was diluted with double distilled water and filtered through Whatmann filter paper No 44 into 100 ml volumetric flask and volume was made to mark. The estimation of cadmium was done by atomic absorption spectrophotometer (GBC 932 plus, Australia) with air acetalene oxidizing flame and metal hollow cathode lamp at the wave length of 228.8 nm. The standard solution of heavy metal (1000 µg ml⁻¹) was obtained from Ms Sisco Research Laboratories Pvt. Ltd. Mumbai. Working standards were prepared by serial dilution of standard stock solution and used for calibration of instruments.

RESULTS AND DISCUSSION

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/ Hierarchical test also states that the concentrations are significantly toxic at 5% level but duration is not significant. MCA test is also represent maximum deviation at higher concentration compared to control (Table. 1). The plant showed normal growth at 0.1 ppm concentration. Similar observation was made by (Garg et al., 1994) in *Limnanthemum cristatum* 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 many researchers (Dogan et al., 2009; and Kopittke et al., 2007). Similar observations were made by (Yongpisanphop et al., 2005) in *Salvinia natanus* and (Wolf et al., 2012) in *Salvinia auriculata*.

Biochemical toxicity Chlorophyll content is a parameter that is sensitive to heavy metal toxicity. Cadmium at 0.1 mg g⁻¹ found to augment chlorophyll synthesis and directly proportional to the duration, the content increased by 3.61% (0.601 mg g⁻¹), 4.06% (0.61mg g⁻¹) and 4.56% (0.465 mg g⁻¹) at 4, 8 and 12 days exposure compare to control pond. Similar observation has been reported by Singh et al., (2011) in *Hydrilla verticillata* and also in *Salvinia natanus* observed by Dhir and Srivastava (2013) at 10 mg l⁻¹ of Cu, Fe, Zn, Co, Cr. The stimulation of chlorophyll synthesis may be due to phytochelatin (PCs) which plays role in detoxification.

Table 1. Cadmium toxicity on morphology of *Salvinia molesta*

Concentration (ppm)	Exposure Duration (in days)								
	4			8					
	Root length			Leaf size					
	Length	Breadth	Length	Breadth	Length	Breadth			
Control	4.40 ± 0.31	4.53 ± 0.21	4.76 ± 0.23	1.76 ± 0.02	2.00 ± 0.04	1.76 ± 0.02	2.00 ± 0.04	2.03 ± 0.04	1.90 ± 0.04
0.1	5.06 ± 0.38	5.33 ± 0.035	5.53 ± 0.38	2.16 ± 0.17	2.06 ± 0.11	2.20 ± 0.16	2.33 ± 0.11	2.40 ± 0.09	2.36 ± 0.14
0.5	4.36 ± 0.17	4.16 ± 0.14	4.06 ± 0.13	1.93 ± 0.15	1.96 ± 0.07	1.86 ± 0.13	1.90 ± 0.08	1.73 ± 0.11	1.80 ± 0.04
1.0	4.06 ± 0.13	3.86 ± 0.15	3.73 ± 0.09	1.73 ± 0.10	1.66 ± 0.05	1.66 ± 0.05	1.50 ± 0.04	1.53 ± 0.02	1.36 ± 0.02
1.5	4.00 ± 0.04	3.30 ± 0.20	2.33 ± 0.27	1.66 ± 0.05	1.63 ± 0.15	1.60 ± 0.12	1.43 ± 0.15	1.53 ± 0.02	1.26 ± 0.07
2.0	2.66 ± 0.19	1.86 ± 0.38	1.16 ± 0.25	1.30 ± 0.04	1.60 ± 0.09	1.13 ± 0.07	1.4 ± 0.21	1.11 ± 0.02	1.10 ± 0.0

Values are expressed in cms Mean values ± standard error

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 parameter.

synthesis the inhibition at 2.0 mg l⁻¹ by 24.09% (0.441 mg g⁻¹), 29.64% (0.416 mg g⁻¹) and 34.52% (0.402 mg g⁻¹) (significant at P>0.95) at 4, 8 and 12 days exposure respectively compare to control. Higher metal concentration of Pb 20 mg l⁻¹ and 0.5 mg l⁻¹ showed decrease in total chlorophyll (Singh et al., 2011). The decline in chlorophyll content in plants exposed to Cd due to: 1) inhibition of important enzymes associated with chlorophyll biosynthesis 2) impairment in the supply of Mg⁺² and Fe⁺² required for the synthesis of chlorophylls 3) Zn⁺² deficiency resulting in inhibition of enzymes such as carbonic anhydrase ((Hasan et al., 2009). Two-way ANOVA represents biochemical toxicity to the test plants, concentrations are

significant at $P > 0.01$ level but duration is not significant. Two-way ANOVA represents, Cd toxicity is at $P > 0.01$ level significant towards but duration is not significant (Fig. 1A).

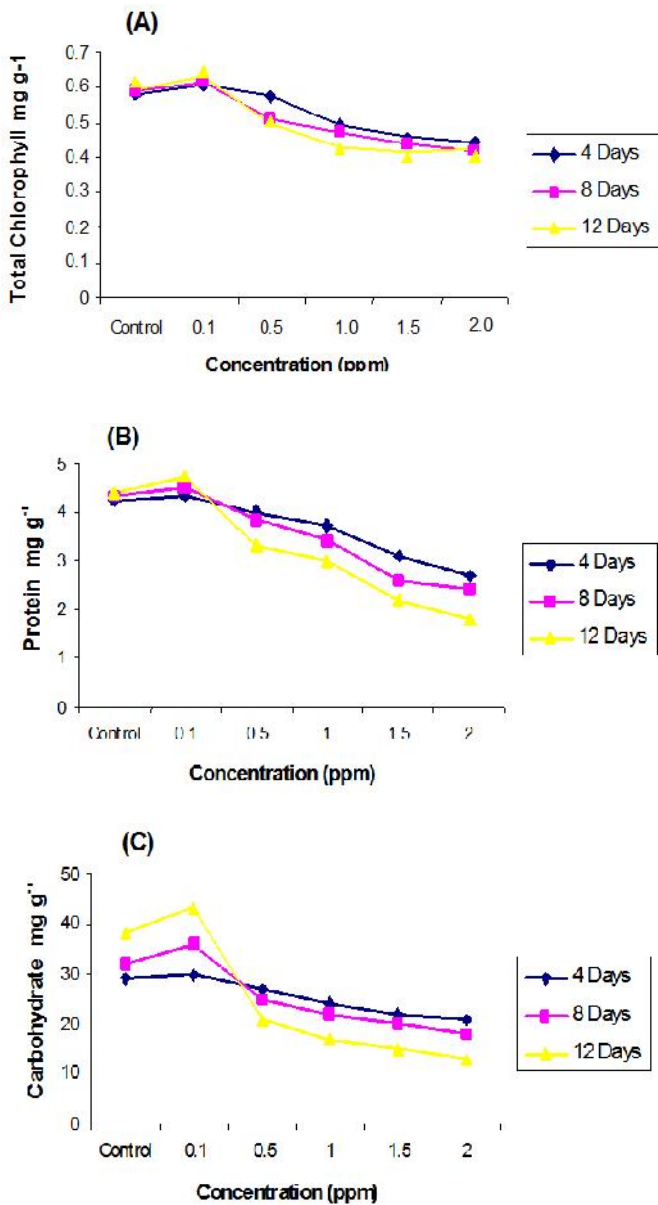


Fig.1. Biochemical effect of Cadmium on *Salvinia molesta*
A) Total chlorophyll B) Carbohydrate C) Protein

Carbohydrate content of *Salvinia* at 0.1 ppm increase to extent of 3.44% (30 mg g⁻¹), 12.88% (36 mg g⁻¹) and 13.88% (43.1 mg g⁻¹) respectively at 4, 8 and 12 days exposure respectively compared to control. It is believed that Cd and Pb are inducers for phytochelatin synthesis and have definite role of detoxification of Cd and Pb and hence in increase in the carbohydrate content at lower (0.1 ppm) concentration (Bhattacharya and Choudhary, 1995). However, the high concentration (2.0 ppm) of Cd found to inhibit carbohydrate. The severity of inhibition of carbohydrate synthesis was noticed at 2.0 ppm by 27.58% (21 mg g⁻¹), 43.75% (18.0 mg g⁻¹) and 65.78% (13 mg g⁻¹) (significant at $P > 0.05$) respectively at 4, 8 and 12 days exposure compared to control. Our results corroborate with the findings of Ahmed *et al.*, (2006) noticed the increase in the soluble sugars at lower concentrations of

salt stress and decrease at higher concentrations in *Pisum sativum*. The decreased carbohydrate in *Salvinia* is due to photosynthetic inhibition or stimulation of respiration rate. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulose triphosphate carboxylase observed in *Tilia argentea* and *Quercus cerris* (Johj *et al.*, 2008). 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 (Fig. 1B).

Many studies shows that the protein content of many aquatic macrophytes was increased by accumulation of Pb at lower toxicity concentration (Mohan and Hosetti, 1997). The protein content increase marginally at 0.1 ppm at 2.38% (4.3 mg g⁻¹), 4.65% (4.5 mg g⁻¹) and 6.81% (4.7 mg g⁻¹) 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 phytochelatin (PC) and phytochelatin synthetase bind and regulate the Cd and sequester the toxicity in the plants and thus, shows metal tolerance (Steffens, 1997). The reduction in protein content was observed with progressive increase in Cd concentration (fig. 1C). Protein content in *Salvinia* decreased by 37.7% (2.7 mg g⁻¹), 44.18% (2.4 mg g⁻¹) and 59.09% (1.8 mg g⁻¹) respectively at 4, 8 and 12 days exposure at 2.0 ppm concentration compared to control ($P > 0.01$) (Fig.1C). The Cd induce 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 (Romero, 2007) and (Garg *et al.*, 1994). The decrease in protein content of macrophyte may be due to the above reasons.

Metal Accumulation: Fig. 2 shows the concentration of Cd accumulated in *Salvinia*. 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.

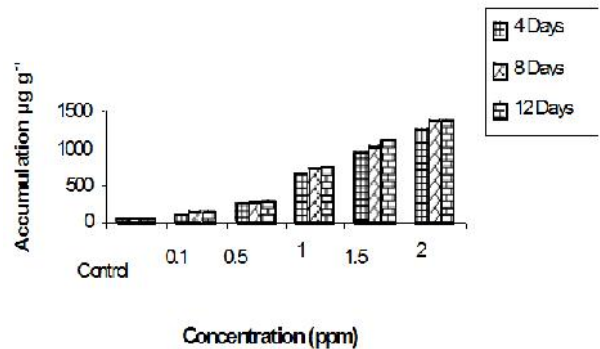


Fig. 2. Accumulation profile of Cadmium by *Salvinia molesta*

The *Salvinia* 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.*, 1990) 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 (Rai and Kumar, 1999; and Sbihi *et al.*, 2012). 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. The present investigation showed that *Salvinia molesta* found to accumulate significant concentrations of Cd from the sewage and proves to be a suitable plant species for phytoremediation of Cd from the sewage.

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