

## Phytotoxicity of cadmium on the physiological dynamics of *Salvinia natans* L. grown in macrophyte ponds

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**Abstract:** The objective of this experiment is to study the potential ability of *Salvinia natans* L. to use in phytoassay for the cadmium toxicity in ecotoxicological studies. It is a simple and cost competitive method. The sensitivity of *S. natans* in terms of biochemical changes and enzyme activities to Cd<sup>2+</sup> was remarkably noticeable. The catalase and protease activity was significantly decreased in the presence of cadmium, with increase of peroxidase and superoxide dismutase activity. The level of protein, carbohydrate and chlorophyll decreased and proline content increased in plants. The maximum permissible concentration of cadmium in different general water quality standards was evaluated and found that cadmium standard is not stringent enough.

**Key words:** Cadmium, Bioremediation, *S. natans*, Bioassay.

### Introduction

Several heavy metals such as Fe, Co, Cu, Mn, Mo and Ni are essential to plant metabolism and are often added to mineral fertilizers. In higher concentration, these heavy metals cause severe damage to plants (Mohan *et al.*, 1998). This has been extensively studied and reviewed by Fernandes and Henriques, 1991 and Markert, 1993. Knowledge of the mechanism of Itai-Itai disease (Friberg *et al.*, 1976) helped to understand more details about the bioaccumulation of heavy metals in plants and aquatic organisms. As the suitability of the aquatic plants as the wastewater purifiers, the rapid growth rate and hardiness of *Salvinia natans* shows its ability as a good potential candidate for wastewater purification.

The toxic effects of Cd was approached by the estimation of the biochemical changes and determination of the concentration causing 50% decrease in chlorophyll content (EC<sub>50</sub>) in response to Cd toxicity. The objective of this study is to develop a bio-assay using *Salvinia natans* L. and to compare the results with the existing water quality standards.

### Materials and Methods

Young plants of *Salvinia natans* L. were collected from uncontaminated freshwater ponds around Mangalagangothri campus, and the plants were grown in green house at 30±2°C for 3 to 5 days. Mature plants were used in the test system. Thirty (30) plastic tubs (20 litre capacity) were filled each with 10 litre of tap water. From that 15 tubs were selected for the Cd treatment to *Salvinia* plants at 5 different concentrations (0, 0.5, 1, 5 and 10 µMdm<sup>-3</sup>) so as to maintain triplicates of each concentration. Remaining 15 tubs were also added with the same concentration of metals to maintain triplicate at each concentration to check the loss of the metal from experimental system due to evaporation and adsorption on the walls of the container. A known weight of *Salvinia natans* L. plants were introduced into each plastic treatment

tubs and placed in open sunlight. They were allowed to grow in day and night condition for 8 days. Analysis was made with the treated plants at 4 days intervals. For protein estimation, phenolics compounds were removed by the methods of Sadasivam and Manikam (1992). After that protein estimation was carried out by Lowry *et al.* (1951) method using folin ciocalteau reagent. Carbohydrate, proline, chlorophyll pigments, catalase and peroxidase enzyme activities were determined according to Sadasivam and Manikam (1992). Protease activity was measured by the method of Greenberg (1958). Proteins were extracted from fresh tissue (0.2g) with phosphate buffer (pH 6.8, 4°C), centrifuged (17,000 g for 15 minutes at 4°C) and the supernatant was used to assay super oxide dismutase (SOD) according to Giannopolitis and Reis (1997). Heavy metals absorbed by plants were analysed at different intervals (1, 4 and 8 days) using atomic absorption spectrophotometer.

### Results and Discussion

Cadmium was found to be highly toxic to *Salvinia natans* L. because the plants exhibited chlorosis on day 4 at 1.0 µMdm<sup>-3</sup> of Cd concentration. The plants were affected to a greater extent above 1.0 µMdm<sup>-3</sup> of Cd. The uptake of metal increased gradually with increase in concentration and duration of exposure (Fig. 1). The effects of the metals on changes in protein and carbohydrate content after 4 and 8 days of contact time are shown in Fig. 2 and 3, while that of proline and chlorophyll levels are given in Figure 4 and 5. Protein content decreased slightly at the treatment above 1.0 µMdm<sup>-3</sup> Cd. However, the value remained almost the same as in control as well as at 0.5 µMdm<sup>-3</sup> treatment of Cd. The bivalent cadmium absorbed by the plants forms stable complexes with -COOH group of protein molecules, present in the plant cell, thereby preventing its degradation by protease enzymes (Sen *et al.*, 1994). The carbohydrate content in the plant body decreased, with the time of exposure and concentration of the toxicant. This may be attributed to the formation of complexes of carbohydrate

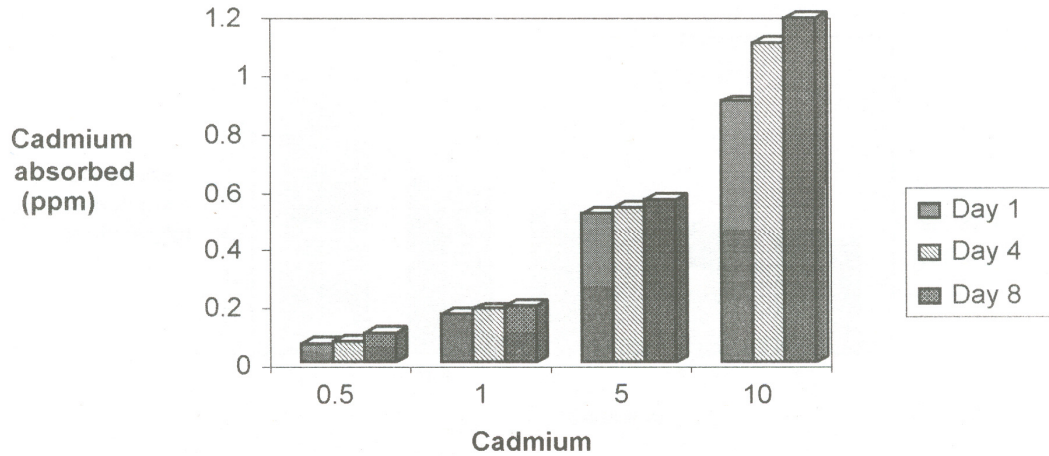


Fig. 1: Cadmium concentration in *Salvinia natans* plants.

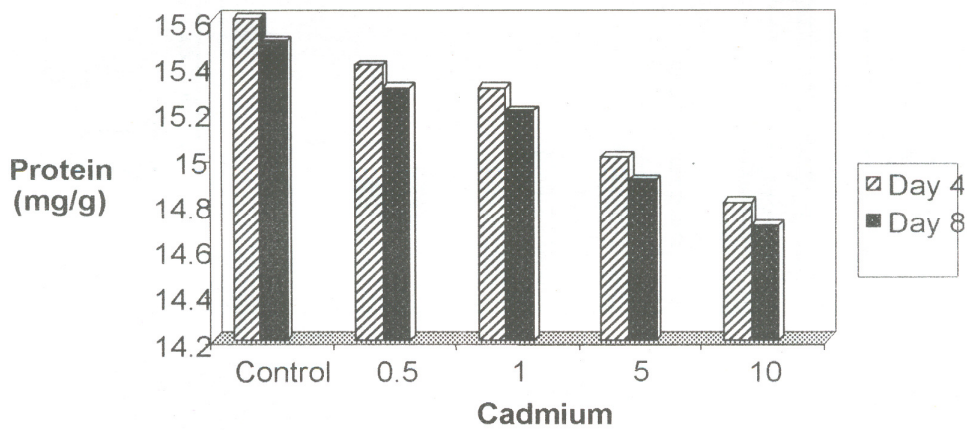


Fig. 2: Effects of cadmium on the protein content in the plant tissue.

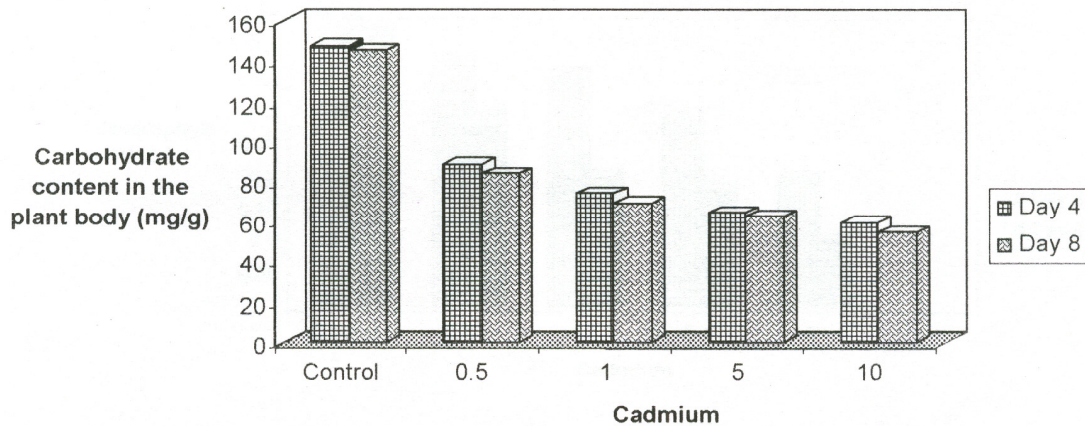


Fig. 3: Effect of cadmium on carbohydrate level in plant tissue.

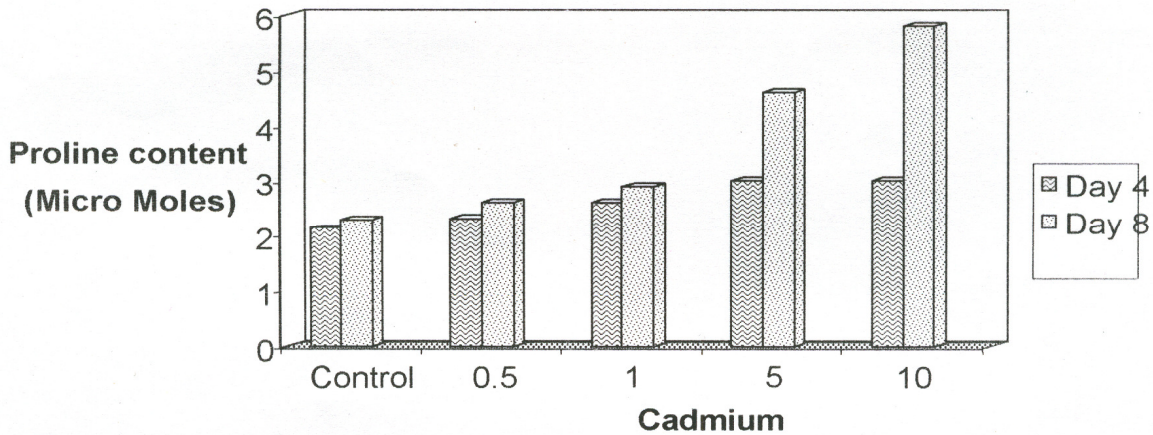
with Cd molecules which prevents the enzymatic degradation by changing the conformation of the carbohydrate. The concentration of proline increased with the increase in the concentration of Cd. Though, the molecular mechanism has not yet been established for the increased levels of proline under stress condition of cadmium toxicity, one of the hypothesis

refers to the breakdown of proteins into amino acids and conversion to proline for storage (Mohan and Hosetti, 1998). Decrease in the chlorophyll content of the plant with the increase in the concentration of metal with time was observed (Fig. 1 and 5). Chlorosis was the initial symptom of Cd toxicity in *Salvinia natans*L. It gave way to necrosis as the concentration

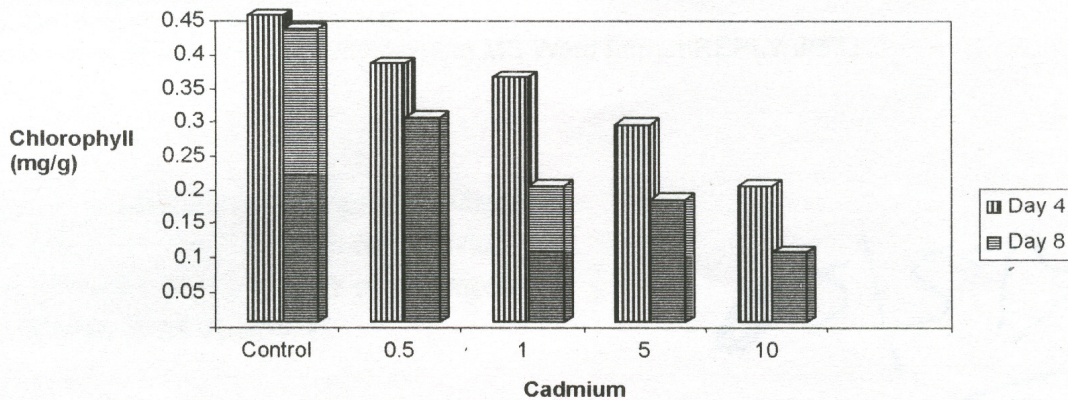
**Table – 1:** Toxic effects of cadmium on catalase, peroxidase, protease and superoxide dismutase (SOD) activity in *Salvinia natans* L.

Cd Conc. ↓ ( $\mu\text{Mdm}^{-3}$ )	Catalase		Peroxidase		Protease		SOD					
	Treatment											
	Day		Day		Day		Day					
	0	4	8	0	4	8	0	4	8			
Control	26.8±1.8	26.3±1.5	25.9±1.9	82.5±3.5	82.2±3.6	83.4±2.1	545±2.2	539±2.5	532±2.8	1.43±0.2	1.39±0.2	1.40±0.3
0.5	26.8±1.8	18.4±1.7	17.3±1.6	82.5±3.5	92.1±4.2	93.8±3.8	515±5.2	484±6.3	428±7.4	1.43±0.2	1.52±0.3	1.69±0.2
1.0	26.8±1.8	13.8±1.6	12.6±1.7	82.5±3.5	94.5±3.6	95.8±3.9	515±5.2	439±4.1	412±5.4	1.43±0.2	1.54±0.2	1.86±0.3
5.0	26.8±1.8	10.2±1.6	9.4±1.8	82.5±3.5	97.2±3.5	102±3.7	515±5.2	417±5.8	394±3.1	1.43±0.2	2.79±0.3	3.98±0.2
10.0	26.8±1.8	8.7±1.8	8.4±2.0	82.5±3.5	99.4±4.2	103±3.9	515±5.2	389±5.5	367±5.3	1.43±0.2	3.2±0.2	4.1±0.3

Catalase = Enzyme units/hour/gram of tissue;      Protease = Protein hydrolysed/hour/gram of tissue  
Peroxidase = Enzyme units/hour/gram of tissue;      SOD = Enzyme units/hour/gram of tissue



**Fig. 4:** Effect of cadmium on praline content in plant tissue.



**Fig. 5:** Effect of cadmium on the chlorophyll content in the plant tissue.

of treatment was increased. Maximum of 0.425mg/g of fresh mass of total chlorophyll was recorded in the control and a minimum of 0.109mg/g of chlorophyll was observed in plants grown at Cd concentration of  $10\mu\text{Mdm}^{-3}$  after 8 days of treatment. The decrease in the chlorophyll content in the plant after treatment with different levels of Cd is either because of reduced synthesis or accelerated degradation of the chlorophyll pigment. It is understood that during photosynthesis protochlorophyllide reductase enzyme binds NADH and it contains sulphhydryl groups. In presence of toxicity, the  $\text{Cd}^{+2}$

interference with sulphhydryl groups of enzyme involved in biosynthesis of chlorophyll. The decrease in chlorophyll content in the *Salvinia natans* L. plant might be due to interference of  $\text{Cd}^{+2}$  at the sulphhydryl site of all enzymes involved in the chlorophyll biosynthesis (Muthuchelian *et al.*, 1988). The current trend of bioassay is to conduct acute lethal toxicity up to 4 days and sub-lethal chronic toxicity for 7 days or still longer (APHA, 1995; Michael *et al.*, 2005). For this study, the initial total chlorophyll content in the plants at different concentration on day 4 was measured in order to determine the

EC<sub>50</sub> value. Krupa *et al.* (1996) investigated the changes in the content of total chlorophyll in the first leaves of rye seedlings treated with cadmium and concluded that the determination of total chlorophyll is the reliable marker of cadmium toxicity in higher plants. A dose effect relationship was used to determine 50% effective concentration (EC<sub>50</sub>). The EC<sub>50</sub> is that concentration of a toxicant which produces 50% reduction of chlorophyll content in experimental plants as compared to control ones. The EC<sub>50</sub> of cadmium to *Salvinia natans* was 0.476mg/l (4.25µMdm<sup>-3</sup>) for first 4 days of exposure. The maximum permissible concentration (MPC) was calculated by considering 10% of EC<sub>50</sub> as a conventional safety factor for the hitherto studies. The calculated MPC in our experiment was 0.0476mg/l (0.425µMdm<sup>-3</sup>). The MPC value is compared with the existing general water quality standards such as WHO (World Health Organization) and general water quality standards of United States Environmental Protection Agency (USEPA). According to these standards, the permissible concentration of Cd in water is 0.05mg/l, but the MPC value obtained in present study was less than these standard value suggesting that the Cd standard is not stringent enough to protect aquatic plants such as *Salvinia natans*.

The toxic effects of Cd on the activities of protease, catalase, peroxidase and superoxide dismutase after 4 and 8 days of contact are shown in Table 1. In all the treatments, activities of protease and catalase decreased while the activities of peroxidase and SOD increased with increase of Cd concentration. The increased activity of the enzyme may be due to the senescence of the plants and indicated increased production of secondary metabolites like hydrogen peroxide, such excretory products might envisage the production of more peroxidase which breakdown H<sub>2</sub>O<sub>2</sub> into water and oxygen rendering it nontoxic. Abiotic stress are known to act as a catalyst in producing free radicle reaction resulting in oxidative stress in various plants where reactive oxygen species (ROS) such as superoxide radicle (O<sup>2-</sup>), hydroxyl radicle (OH<sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and alkoxy radicle (RO) are produced (Hernandez *et al.*, 2000). The toxic superoxide radicle has a half life of one second and is usually rapidly dismutated by SOD to H<sub>2</sub>O<sub>2</sub>, a product which is relatively stable and can be detoxified by peroxidase. Increased SOD activity is known to confer oxidative stress tolerance (Slooten *et al.*, 1995, Boominathan and Doran 2003). A decline in the activity of catalase and protease might be due to the formation of protein complexes with Cd, changing the conformation and solubility of the proteins. The results of the present study suggest that *Salvinia natans* is a promising indicator of aquatic toxicity.

Hence it is advocated that the bioassay using *Salvinia natans* should be further explored.

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