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Biochemical responses and accumulation of cadmium in *Spirodela polyrhiza*

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Abstract: *The present study focused on biochemical responses of Spirodela polyrhiza to cadmium stresses and its accumulation. The laboratory experiments were conducted for the assessment of biochemical responses and accumulation of cadmium in plants at its various concentrations (0.1, 0.5, 1.5 and 2.0 ppm) at the regular interval for twelve days exposure. Spirodela showed visible symptoms like withering of roots and chlorosis 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 changes 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 Spirodela polyrhiza was maximum at four days exposure duration and gradually decreases.*

Key words: *Cadmium, Accumulation, Toxicity, Biochemical parameters*
PDF of full length paper is available online

Introduction

The indiscriminate disposal of industrial, municipal and agricultural wastes into the aquatic ecosystem lead to water pollution, which contains large amount of organic and inorganic materials, in addition to the presence of variable amounts of heavy metals (Brar *et al.,* 2000). The pollution leads to undesirable changes in the quality of physicochemical environment, biota and its status. The use of sewage may limits its long term use for agricultural purpose. Natural soil erosion and anthropogenic activities are greatly responsible for water pollution, particularly, heavy metals *viz*, Zn, Cd, Pb. There is likelyhood of phytotoxicity and environmental risks. Heavy metals persisting in sediments, may be slowly released into the water and become available to organisms and are toxic when taken in excess amounts (Blaylock and Huang, 2000). The non essential ions like Pb and Cd can inhibit variety of metabolic processes even in small quantities (Cerventes *et al.,* 2001; Siddhu *et al*., 2008; Dinakar *et al.*, 2009; Chaudhary and Sharma, 2009). However, the essential metals like Zn, Cu, Fe are toxic at higher concentration (Campanella, 2001).

The waste water emanating from source contains metals which could be toxic to flora and fauna. Biological treatment of waste water through aquatic macrophytes has a great potential for its purification which are effectively accumulates heavy metals (Brix and Schierup, 1989). Aquatic macrophytes accumulate considerable amount of toxic metals and make the environment free from the pollutants. Thus, play a 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 environments (Satyakala and Kaiser Jamil, 1992). Similarly, algae were also used to remove heavy metals from aquatic systems as they have capacity to accumulate dissolved metals (Wilde and Benemann, 2003). The metal tolerance of plants may be attributed to different enzymes, stress proteins and phytochelatins (Van-Asche and Clijsters, 1990). 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).

In the present investigation, *Spirodela polyrhiza*, a common aquatic floating macrophyte, is used to study the effect of different concentration of cadmium on biochemical constituents and accumulation of Cd from the experimental pond under laboratory conditions.

Materials and Methods

Laboratory experiments were conducted for the study of biochemical responses and accumulation of Cd by *Spirodela polyrhiza* (L) Schleid from the experimental pond. The test plants were sampled from Srinagar pond near Karnatak University Dharwad, India. The plant stock was maintained under laboratory conditions, experiments were carried out in triplicates. The young and healthy *Spirodela polyrhiza* species were selected and acclimatized for two weeks in Hoagland solution in the experimental ponds of 10 l capacity. About 50 g plant material was introduced simultaneously into each of experimental ponds containing 0.1, 0.5, 1.0, 1.5 and 2.0 ppm concentrations of cadmium and tap water as control. The plants were harvested at the end of 4, 8 and 12 days exposure and thoroughly washed with distilled water and used for

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an estimation of total chlorophyll, carbohydrate and protein content and plants were dried in oven at 48°C to get a constant weight for metal extraction.

The fresh plant samples mention the amount were 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 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 Cd in the plant material was carried out by using standard method (Allen *et al*., 1974). The dried and powdered 1 g plant material was digested by using mixed acid digestion method until the appearance of white fumes in Gerhardt digestion unit (C. Gerhardt Gmb H and Co. Bornheimor Star Be 100, 53119 Bonn). The digested sample was diluted with double distilled water and filtered through Whatman filter paper No 44 into 100 ml volumetric flask and volume was made to mark. The estimation of Cd was done by atomic absorption spectrophotometer (GBC 932 plus, Australia) with air acetylene oxidizing flame and metal hollow cathode lamp at the wavelength 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 instrument.

Statistical analysis: The data was statistically analyzed by employing two way ANOVA test to know the significance between concentrations and between exposure durations for the biochemical and accumulation of heavy metal. Further, Dunet's test is applied for multiple comparison between control and concentrations.

Results and Discussion

Biochemical responses: The biochemical responses of *Spirodela polyrhiza* (Fig. 1 A) revealed that 0.1 ppm of Cd found to augment the chlorophyll synthesis and is directly proportional to the exposure duration. The chlorophyll of the experimental plant was increased by 7.27, 11.68 and 12.92% at 4, 8 and 12 days exposure at lower concentration of 0.1 ppm experimental pond as compared to control pond. Similar observations have been reported by Ornes and Sajwan, (1993) in *Ceratophyllum demersum* at the concentration of 0.01 to 0.4 μg l^{-1}. The synthesis of chlorophyll stimulation is associated with the formation of phytochelatins (PCs) which plays role in detoxification (Prasad, 2004). However, higher concentration (0.5, 1.0, 1.5 and 2.0 ppm) of Cd found to inhibit chlorophyll synthesis. The inhibition of chlorophyll at higher concentration (2.0 ppm) Cd by 12.68, 43.90 and 59.38% at 4, 8 and 12 days exposure respectively was observed. The presence of Cd decrease the content of chlorophyll and carotenoides and increase non photochemical quenching in *Brassica napus* (Larsen *et al*., 1998). Similarly the synthesis and level of chlorophyll decreased in other plant species under the influence of cadmium (Griffiths *et al*., 1995; Ferretti *et al*., 1993; Imai *et al*., 1996; Phetsombat *et al*., 2006; Pandey *et al*., 2007; Hasan *et al*., 2009).

Table - 1: Two way ANOVA for biochemical effects of cadmium on *Spriodela polyrhiza*

	Total chlorophyll	Carbohydrate	Protein
F-Value (between concentration)	15.114**	15.764**	5.919**
F- value (between duration)	0.298	0.327	0.478

** Significant at p<0.01 level

Table - 2: Two way ANOVA with Dunet's test for multiple comparison for accumulation of cadmium by *Spirodela polyrhiza*

	Spirodela polyrhiza
F-Value (between concentration)	441.39**
F-Value (between duration)	8.15**
Dunet's value	69.89
Control V/s 0.1 ppm	125.16
Control V/s 0.5 ppm	381.16
Control V/s 1.0 ppm	764.75
Control V/s 1.5 ppm	1104.25
Control V/s 2.0 ppm	1308.33

** Significant at p<0.01 level

The increase in the carbohydrate content of *Spirodela* at 0.1 ppm Cd by 14.28, 29.03 and 30.03% respectively during 4, 8 and 12 days exposure was determined. It is believed that Cd and Pb are inducers for PC synthesis and have definite role in detoxification of Cd and Pb and hence, at lower concentration (0.1ppm) carbohydrate content increases (Bhattacharya and Choudhary, 1995). The severity of inhibition of carbohydrate synthesis was noticed at 2.0 ppm by 32.14, 51.61 and 66.66% respectively at 4, 8 and 12 days exposure as compared to control. The heavy metals damages the photosynthetic appertus, in particular, the harvesting complex II (Krupa, 1988) and photosynthesis I and II (Siedlecka and Krupa, 1996; Hasan *et al.,* 2009).

The protein content in *Spirodela* increases marginally at 0.1ppm and is 15.15, 24.4 and 26.74% respectively at 4, 8 and 12 days exposure (Fig. 1 C). The stimulation of protein synthesis at lower concentration of Cd may be attributed to the synthesis of stress proteins. The phytochelatins (PC) and phytochelatin synthetase bind and regulate the Cd and sequester heavy metals in the plants and thus, show metal tolerance (Steffens, 1997).

Our investigation revealed that higher concentration of Cd inhibit protein metabolism in experimental plants. The protein content declines from 3.03, 32.55 and 41.86% respectively at 4, 8 and 12 days exposure as compared to control. A decrease in protein content could be due to inactivation of protein synthesizing enzymes in the cell.

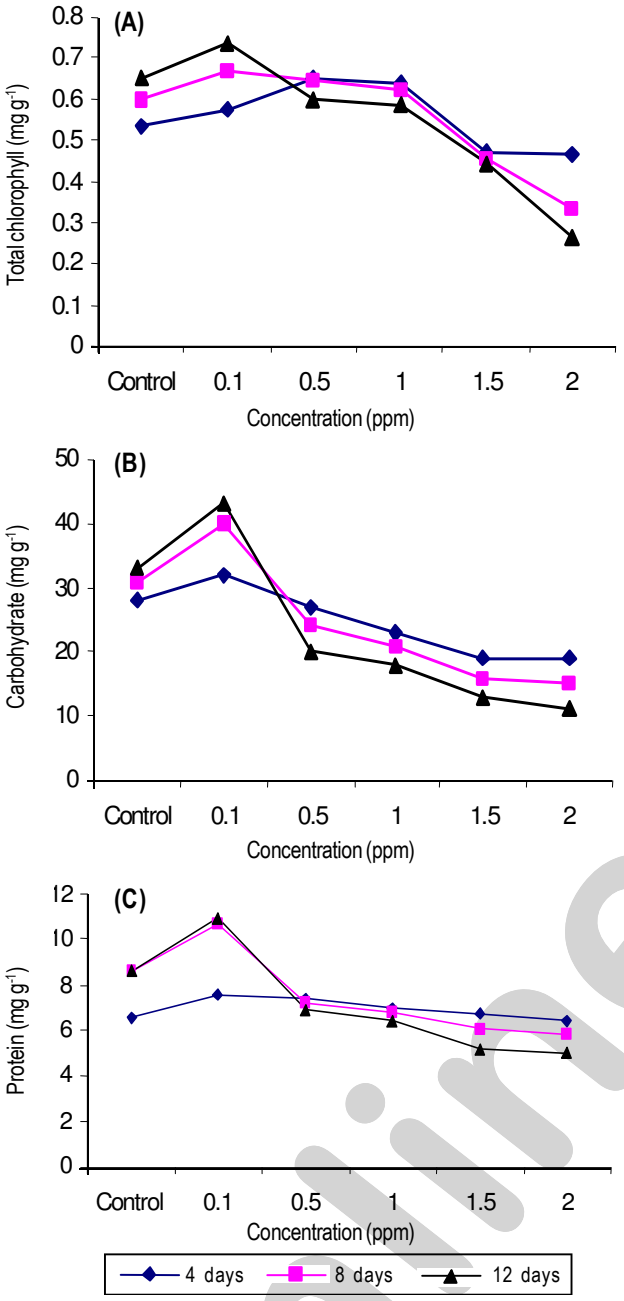

Fig. 1: Biochemical effects of cadmium on *Spirodela polyrhiza*: (A) Total Chlorophyll, (B) Carbohydrate and (C) Protein

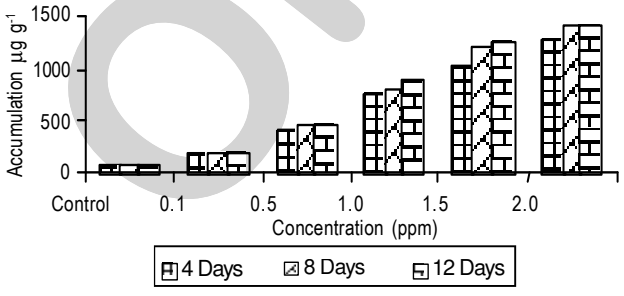

Fig. 2: Accumulation profile of cadmium by *Spirodela polyrhiza*

Two way ANOVA shows that the biochemical responses of the test plant species with respect to concentrations are significant at $p<0.01$, but exposure duration is not statistically significant (Table 1).

Accumulation of cadmium: The accumulation data (Table 2) reveals that Cd accumulation in *Spirodela* is directly proportional to its exposure concentration and duration. The *Spirodela* grown in experimental pond containing 0.1 ppm Cd found to accumulate 172.0, 198.25 and 205.0 µg g^{-1} and accumulation of Cd at 2.0 ppm concentration was 1305.75, 1398.0 and 1421.50 µg g^{-1} during 4, 8 and 12 days of exposure duration respectively. It is observed that the rate of accumulation is maximum at 4 days. There was dramatic uptake of zinc in *Cladophora glomerata* within first 10 minutes as reported by Bendra *et al.* (1990).

In the present investigation accumulation of Cd is linear and significantly correlated with Cd concentration in the effluent. Similar observation was made by Tripathi *et al.* (2003). The accumulation of zinc and cadmium using *Eichornea,* shows that the uptake was rapid in all the concentrations and absorption capacity gradually reduced by *Eichornea* with increase in the number of days (Sridevi *et al*., 2003) The 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 the binding of almost all ions to the plants and establishment of equilibrium between adsorbate and adsorbent (Rai and Kumar, 1999). Wang and Lewis (1997) reported that metal accumulation by aquatic macrophytes under controlled laboratory condition was dependent on metal concentration in the water. Two way ANOVA show that both concentration and exposure duration are significant at $p<0.01$ level in test plants and further Dunet's test was applied for the multiple comparison between control and different concentration treatments of test plants. From the statistical analysis it is clear that concentration treatments are significantly differ with control (Table 2).

It is concluded from the findings of the present investigation that biochemical responses and accumulation of cadmium by *Spirodela polyrhiza* are proportional to concentration and duration. Regular harvest of the plant at the interval of 4 days helps to cleanup aquatic environment.

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