



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

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ABSTRACT

Industrial development coupled with population growth had 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. But tolerant species of aquatic plants are able to survive and withstand the pollution stress and serves as a pollution indicators and as tools for phyto remediation of heavy metals from the aquatic ecosystems. Phytoremediation is a biotechnological application based on "Green liver concept" and operates on biogeochemical cycling. The present study focuses on copper toxicity on morphology, biochemical parameters and bioaccumulation potential of *Pistia*. The laboratory experiments were conducted for the assay of morphological index parameter (MIP), biochemical parameters and accumulation profile of copper in the test plants at various concentrations viz, 2, 5, 10, 15 and 20 ppm, at 4 days regular intervals for 12 days exposure. The test plants show visible symptoms like withering of roots, chlorosis, necrosis and lower leaves gets decayed at higher concentrations (severe at 20 ppm), however, the test plant showed normal growth at lower concentration viz, 2 and 5 ppm. The estimation of biochemical parameters viz, total chlorophyll, protein and carbohydrates of test plants showed significant increase at lower concentrations (2 and 5 ppm) of Cu. The biochemical constituents decreased with increase in exposure concentrations (10, 15 and 20 ppm) and duration. The toxic effect of sewage was directly proportional to its concentrations and exposure duration. The accumulation profile of Cu by *Pistia* was maximum at 4 days exposure and gradually decreases at subsequent exposure duration.

Key Words: Copper, Accumulation, Toxicity symptoms, Biochemical parameters

INTRODUCTION

Industrialization and urbanization coupled with alarming rate of population growth have resulted in the large scale pollution of aquatic ecosystems by industrial and domestic waste water discharge. Natural erosion and anthropogenic activities are greatly responsible for water pollution particularly heavy metals like Zinc (Zn), Lead (Pb), Cadmium (Cd), Copper (Cu) etc. There is likelihood of phytotoxicity both micro and macrophytes and environmental risks (de-Fillipes and Pallghy, 1994; Wei, et al., 2003). Heavy metals persisting in sediments may be slowly released into the water and become available to the organisms. Some heavy metals viz, Zn, Cu, Iron (Fe), Manganese (Mn) etc. are represented as micronutrients (Reeves and Baker, 2000) and are only toxic when taken in excess quantities (Blaylock and Huang, 2000; Campenela, 2001), but nonessential ions like Pb, Cd and Ni

can inhibit various metabolic activities even in small quantities (Cerventes, *et al.*, 2001; Dinkar, *et al.*, 2001; Choudhary and Sharma, 2009).

The waste water emitting from source metals which could be toxic to flora and fauna. Biological treatment of waste water through aquatic macrophytes plants has great potential for its purification, which effectively accumulates the heavy metals (Brix and Schirup, 1989). Aquatic macrophytes accumulate considerable amount of toxic metals and make the environment free from the pollutants. Thus they play significant role in cleaning up of environment and make the environment free from toxic pollutants. So many aquatic plants have been successfully utilized for removing toxic metals from the aquatic environments (Satyakala and Kaiser Jamil 1992). The metal tolerance of plants may be attributed to different enzymes, stress proteins and Phytochelatins (Van-Asche and Clijsters 1990). Accumulation of metals at higher concentra-

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tion causes retardation of growth biochemical activities and also generation of –SH group containing enzymes (Weckx and Clijsters 1996).

In the present investigation *Pistia stratiotes*, a common aquatic macrophyte is used to study the effect of different concentrations of copper on morphology, biochemical constituents and accumulation profile of Cu from the experimental pond under laboratory conditions.

MATERIALS AND METHODS

Pistia stratiotes, a free floating aquatic plant 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 *Pistia* is acclimatized for two weeks in Arnon and Hoagland nutrient solution maintaining pH between 7.1-7.4. The concentrations of Cu in the polluted water are in the range of 02, 05, 10, 15 and 20 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 copper 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 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 (Arnon, 1949) using 652 nm against the solvent (80% acetone as a blank). The protein was estimated by Lowry's method (Lowry et al., 1951) using Bovine Serum Albumin (BSA) as a standard, using 660 nm and carbohydrates by phenol sulphuric acid method (Dubois et al., 1956) 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 Cu in the test plant 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 in Gerhardt digestion unit. The digested samples were diluted with double distilled water and filtered through Whatman filter paper No-44. The estimation of Cu 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 (SISCOPE-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 (Cu). 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

The experiments were conducted with the following parameters:

a. Effect of Copper toxicity on morphology:

The test plant showed luxuriant growth and slight increase in the laminal length and breadth at 5 ppm concentration. The 5 ppm Cu found to promote length by 6.900cm (± 0.047) in comparison to control, 5.33cm (± 0.027) and laminal length 2.63 cm (± 0.047) and breadth, 2.33 cm (± 0.027) when compared to control (laminal length, 1.766 cm ± 0.027 and breadth 1.56cm ± 0.027) respectively during 12 days exposure. However, at 20 ppm Cu, severely inhibit the root length by 1.63 cm (± 0.034) in comparison to control 5.33 cm (± 0.027) and laminal length, 0.80 cm (± 0.047) and breadth, 0.63 cm (± 0.108), when compared to control (laminal length, 1.76 cm ± 0.027 and breadth 1.566 cm ± 0.027) respectively during 12 days exposure (Table 1). MCA test also represented maximum deviation at higher concentration compared to control.

b. Effect of Copper toxicity on biochemical parameters:

The chlorophyll content was very sensitive to copper toxicity. The results found that Cu at 5 ppm found to augment chlorophyll synthesis and was directly proportional to concentration and exposure duration. The chlorophyll content increased by 3.81% (0.381 mg/g), 4.10% (0.406mg/g) and 4.79% (0.415mg/g) respectively at 4, 8 and 12 days exposure compared to control pond. However, the higher concentration of copper found to inhibit the chlorophyll synthesis. The inhibition at 20 ppm Cu by 13.35% (0.318mg/g), 27.7% (0.284mg/g) and 46.71% (0.211mg/g) (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 were significant at p > 0.01 level but duration is not significant (Fig. 1).

The increase in the carbohydrate content of *Pistia* at 5 ppm Cu by 23.06% (32.0 mg/g), 35.71% (38.0 mg/g) and 36.66% (41.0 mg/g) respectively during 4, 8 and 12 days exposure duration. The severity of inhibition of carbohydrate synthesis was noticed at 20.0 ppm by 46.15% (14.0 mg/g), 57.14% (12.0 mg/g) and 68.75% (10.0 mg/g) respectively at 4, 8 and

12 days exposure in comparison to control (fig.).

The protein synthesis at 5 ppm Cu was promotive irrespective exposure duration. However, the protein content decreased at subsequent higher concentration and inhibition was directly proportional to the duration of exposure. The 5 ppm Cu promoted protein synthesis by 8.06% (6.7 mg/g), 18.75% (7.6 mg/g) and 19.11 (8.1 mg/g) respectively at 4, 8 and 12 days exposure duration. The reduction in protein content was observed with progressive increase in Cu concentration. The inhibition of protein content increase viz, 11.36% (3.9 mg/g), 32.60% (3.1 mg/g) and 43.75% (2.7 mg/g) was respectively at 4, 8 and 12 days exposure in comparison to respective control (Fig. 1)

Application of two-way ANOVA, it is found that the biochemical responses of test plant species with respect to concentrations were significant at $p < 0.01$ level. However, exposure duration was not statistically significant (Table 2).

c. Profile of Metal Accumulation

The accumulation data (Fig 2) revealed that 'Cu' accumulation in *Pistia* was directly proportional to its concentration and exposure duration. The *Pistia* grown in experimental pond containing 5 ppm accumulate 2812.04 $\mu\text{g/g}$, 3062.0 $\mu\text{g/g}$ and 3208 $\mu\text{g/g}$ and accumulation at higher concentration (20 ppm) was 8425.0 $\mu\text{g/g}$, 8750.0 $\mu\text{g/g}$ and 8770.0 $\mu\text{g/g}$ during 4, 8 and 12 days exposure respectively. Two-way ANOVA showed that both concentration and exposure duration were 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 plant. From the statistical analysis it is clear that concentration treatments are significantly differ with control (Table 3).

DISCUSSION

a. Effect of Copper toxicity on morphology: Morphometric assay, is one of the quantitative tool for the assessment of toxicants, was measured by using Morphological Index Parameters (MIP). The rate of inhibition of growth in the root and leaf was directly proportional to the concentrations of copper. The test plant show luxuriant growth and slight increase in the laminal length and breadth at 5 ppm concentration. However, at higher concentration (20 ppm) severely inhibit the root length, laminal length and breadth. Similar observations were made by Garg *et al.*(1994) in *Limnathemum cristatum* at 1 ppm concentration of Pb, Zn and Cr. Our results of toxicity symptoms of copper at higher concentrations observed were similar to Dagon and Saygideger (2011) and Kopitte *et al.*, (2007) and also of Yongpisamshap *et al.*, (2005) in *Salvinia natanas*. Two – way ANOVA states that the concentrations were significantly toxic at 5% level

but duration was not significant. MCA test also represented maximum deviation at higher concentration compared to control (Table. 1).

b. Effect of Copper toxicity on biochemical parameters:

Copper is essential trace element required by all plants. The accumulation of copper in plants lead to biochemical changes. Total chlorophyll content, a parameter, was a sensitive to heavy metal toxicity (Gupta and Chandra, 1996). Similar observations had been reported by Dhir and Srivastava (2013) in *Salvinia natanas* at 10 ppm of Cu, Fe, Zn, Co and Cr. The stimulation of chlorophyll synthesis may be due to phytochelatins (PCs) which plays role in detoxification (Rolli, *et al.* , 2010) however, the higher concentration of Cu found to inhibit the chlorophyll synthesis. The inhibition at 20 ppm Cu by 13.35% (0.318mg/gm), 27.7% (0.284mg/gm) and 46.71% (0.211mg/gm) (significant at $p > 0.95$) at 4, 8 and 12 days exposure respectively compared to control. Similar observations was made by Singh *et al* 2011 in *Hydrilla verticillata* at higher concentration of Pb at 20 ppm and Cd at 0.05 ppm. This is due to decline in chlorophyll content in plants exposed to Cu due to: 1) inhibition of important enzymes associated with chlorophyll synthesis, 2) peroxidation of chloroplast membranes resulting from heavy metals induced oxidative stress, 3) formation of metal substituted chlorophyll (Patsikka, *et al.*, 2002). Two-way ANOVA represents biochemical toxicity to the test plant, concentrations were significant at $p > 0.01$ level but duration is not significant. Two way ANOVA represents toxicity was at $p > 0.01$ level significant towards but duration was not significant.

Our investigation revealed that lower concentration of copper (5 ppm) promotes the carbohydrate synthesis. The carbohydrate content was increased at lower concentration of Cu, was due to detoxification free radicals by quenching / utilization by enzymatic superoxide dismutase (SOD), peroxidase (POD), catalases or glutathione reductase (Wang *et al.* 1997). Similarly Choudhary and Ramachandra (2005) observed stimulatory effect of carbohydrate in *Nostoc muscorum* at lower concentration (1.5 ppm) of Cu like other heavy metals. But the severity of inhibition of carbohydrate was noticed (Fig. 1). The heavy metals damaged the photosynthetic apparatus, in particular light harvesting complex II (Krupa, 1988) and photosynthesis I and II (Siedlecka and Krupa, 1996) and Hasan *et al.*, (2009).

The proteins play an important role in energy metabolism. In the test plant, the lower concentration of copper enhances the protein synthesis and is directly proportion to exposure duration. However, the protein content was decreased at subsequent higher concentration and inhibition was directly proportional to the exposure duration. Many studies show that protein content of many aquatic macrophyte was increased by accumulation of Pb at lower toxicity concentration. The stimulation of protein synthesis at lower concentration of

Cu (5 ppm) may be attributed to the synthesis of stress proteins. The phytochelatin (PC) and phytochelatin synthetase bind and regulate the Cu and sequesters the toxicity in the plants and thus showed metal tolerance (Mohan and Hosetti, 1997; Steffens, 1997). The reduction in protein content was observed with progressive increase in Cu concentration. The inhibition of protein content increase viz, 11.36% (3.9 mg/g), 32.60% (3.1 mg/g) and 43.75% (2.7 mg/g) was respectively at 4, 8 and 12 days exposure in comparison to respective control (fig. 1)

The proteins played an important role in energy metabolism. A decrease in protein content could be due to inactivation of protein synthesizing enzymes in the cell. The Cu induced oxidative stress by generating reactive oxygen species (ROS). These disrupted cellular homeostasis, thus, enhanced the production of ROS. These ROS reactions with proteins, lipids, nucleic acids causing membrane damage and enzyme content of macrophyte may be due to above reasons (Garg et al., 1994; Romero et al., 2007). (fig. 1)

Application of two-way ANOVA, it is found that the biochemical responses of test plant species with respect to concentrations were significant at $p < 0.01$ level. However, exposure duration was not statistically significant (Table 2).

c. Profile of Metal Accumulation

Heavy metal pollution of a water is a major environmental concern, is increasing at (Dushenkar et al. 1995) alarming rate due to anthropogenic activities and is drawing attention and gaining paramount importance due to its obvious impact on health through the food chain (Prasad 1997). The aquatic plants are able to accumulate heavy metals from sediment water. In the present investigation aquatic macrophyte viz, *Pistia stratiotes* is used in accumulation. The accumulation data (Fig 2) revealed that 'Cu' accumulation in *Pistia* was directly proportional to its concentration and exposure duration.

It was observed that the rate of accumulation is maximum at 4 days exposure irrespective of concentrations and exposure duration, however, at remaining durations it is marginal. Similar observations were made by Bendra et al., (1990) in *Cladophora glomerata* at the concentration of 0.1M solution of Cd. Initial increase in the accumulation might be due to the availability of increased number of binding sites for the complexation of heavy metal ions leading to the increased 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 (Rai and Kumar, 1999; Sibih, et al., 2012).

Two-way ANOVA showed that both concentration and exposure duration were 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 plant. From the statistical analysis it is clear that concentration treatments are significantly differ with control (Table 3)

CONCLUSION

It is concluded from the findings of the present investigation, it is concluded that morphological, biochemical responses and profile of Cu accumulation by *Pistia stratiotes* are directly proportional to concentration of the media and exposure duration. Regular harvest of the plants at the interval of 4 days help to cleanup aquatic environment.

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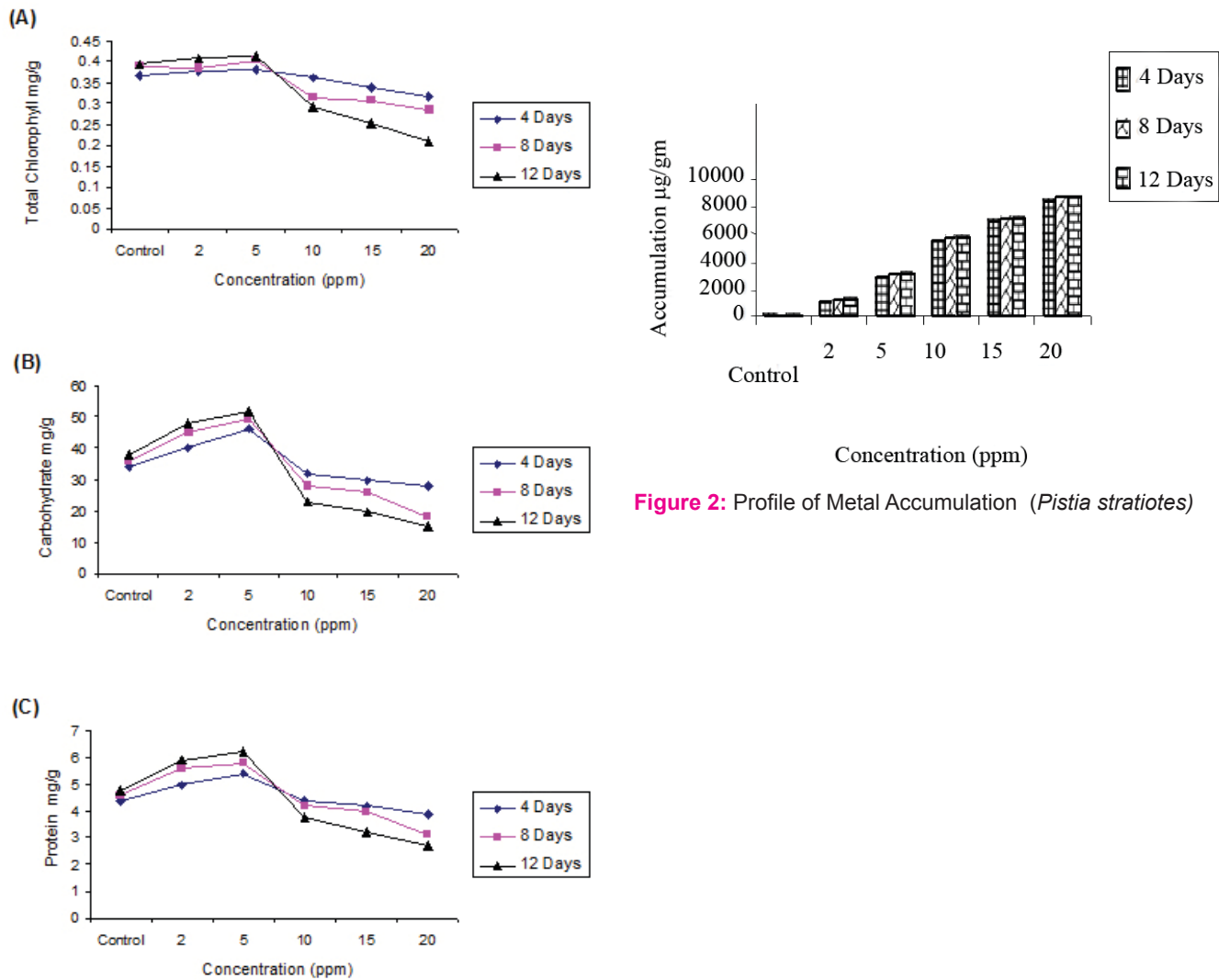


Figure 1: Effect of Copper toxicity on biochemical parameters (*Pistia stratiotes*)
 (A) Total Chlorophyll (B) Carbohydrate (C) Protein

Table 1: Effect of Copper toxicity on morphology (*Pistia stratiotes*)

Concentration (ppm)	Exposure Duration (in days)								
	4			8			12		
	Root length			Length		Breadth		Leaf size	
Control	5.266 ± 0.027	5.300 ± 0.124	5.333 ± 0.027	1.700 ± 0.047	1.500 ± 0.047	1.733 ± 0.054	1.500 ± 0.047	1.766 ± 0.027	1.566 ± 0.027
2.0	6.460 ± 0.165	6.566 ± 0.098	6.766 ± 0.098	1.900 ± 0.047	1.600 ± 0.047	1.960 ± 0.054	1.633 ± 0.054	2.000 ± 0.00	1.766 ± 0.027
5.0	7.166 ± 0.072	7.100 ± 0.027	6.900 ± 0.047	2.566 ± 0.054	2.433 ± 0.027	2.600 ± 0.047	2.466 ± 0.047	2.633 ± 0.047	2.333 ± 0.027
10.0	4.933 ± 0.072	4.800 ± 0.047	3.666 ± 0.072	1.466 ± 0.072	1.366 ± 0.072	1.400 ± 0.047	1.266 ± 0.054	1.300 ± 0.047	1.111 ± 0.054
15	3.366 ± 0.118	2.900 ± 0.047	2.700 ± 0.047	1.400 ± 0.047	1.300 ± 0.047	1.333 ± 0.054	1.233 ± 0.072	1.100 ± 0.047	0.900 ± 0.047
20	3.066 ± 0.072	2.766 ± 0.15	1.633 ± 0.0341	1.300 ± 0.047	1.233 ± 0.072	1.133 ± 0.072	0.900 ± 0.094	0.800 ± 0.047	0.63 ± 0.108

Values are expressed in cms
 Mean values ± Standard Error

Table 2: Two way ANOVA for biochemical effects of Copper on *Pistia stratiotes*

	Total chlorophyll	Carbohydrate	Protein
F-Value (between concentration)	7.329**	16.907**	13.090**
F- value (between duration)	1.074	0.363	0.120

** Significant at P < 0.01 level

Table 3: Two way ANOVA with Dunet's test for multiple comparison for accumulation of Copper by *Pistia stratiotes*

	Pistia
F-Value (between concentration)	4687.60
F- value (between duration)	16.59**
Dunet's Value	136.34
Control V/s 2 ppm	1078.66
Control V/s 5ppm	2860.33
Control V/s 10 ppm	5530.33
Control V/s 15 ppm	7016.33
Control V/s 20 ppm	8481.33

** Significant at P < 0.01 level