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Short communication

Effect of cadmium uptake on growth and physiology of water lettuce

Mayank Varun*, Clement O. Ogunkunle[1], C. Sarathambal[2], Manoj S. Paul, Bhumesh Kumar[3]
Department of Botany, St. John's College, Agra, Uttar Pradesh 282 002

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Cadmium (Cd) is a major pollutant metal that is extremely toxic to organisms. Cadmium contamination occurs through the use of phosphate fertilizers and sludge, and inputs from mining and smelting industries (McGrath *et al*. 2001). Daily consumption of Cd-contaminated foods poses a risk to human health (Shukla *et al*. 2007). In Japan, Cd-contaminated rice caused Itai-itai disease near the Jinzu River basin in the middle of the 20th century. Even in recent years; rice is the major source of Cd intake of people in Japan (Watanabe *et al*. 2000). Cadmium is not an essential nutrient and at high concentration inhibits plant growth (Aery and Rana 2003). It has also been reported that even at relatively low concentrations, it alters plant metabolism (Van Assche and Clijsters 1990). The ability of aquatic macrophytes to uptake nutrients directly from water bodies and to assimilate them into their body is the greatest benefit of phytoremediation (Galal and Shehata 2014). Several aquatic plants effective in heavy metal uptake have been identified world over (Khankhane *et al.* 2014).

Pistia stratiotes L. is an aquatic plant. It grows abundantly in stagnant ponds, lakes and water bodies. The toxic effect of Cd has amply been documented both in the laboratory and under natural conditions in aquatic plants. Thus there was great need to assess toxic potential of Cd in *P. stratiotes* under such conditions. This study presents the toxicity and bioaccumulation potential of Cd in *P. stratiotes*.

Uniform plant of *Pistia stratiotes* were collected from ponds and brought to laboratory. Plants were cultured in 3% Hoagland's nutrient solution (pH 5.7) in laboratory under controlled conditions, illuminated with a light intensity of 45 µmoles $m^2/s^1/$ at 12 h/12 h light and dark cycle, under the temperature of 27 + 2 ºC. Each jar was amended with cadmium in increasing concentrations of 0.5, 1, 2.5, 5, and, 10 mg/L. Jar without any Cd treatment was used as control. $CdCl_2.4H_2O$ was preferred due to its stability and high solubility. pH of the solutions was adjusted to 5.7. The experiment was set up in triplicate for each concentration and test duration. Plant samples from each container were separately harvested after 3, 6, 9, 12, and 15 days to analyze for biomass productivity and total chlorophyll content.

Chlorophyll content of each plant was estimated according to Arnon (1949). Fresh leaves (40 mg) were soaked in 10 ml of 80% acetone and kept in a bottle covered with black carbon paper to prevent the entry of light and kept in a refrigerator for 4-5 days. The bottle was sealed to prevent evaporation of acetone. Care was taken to ensure that leaves of same age were taken from each treated plant. The absorbance of pigment extract was measured at both 663 nm and 645 nm on spectrophotometer. For biomass study, plant samples were kept in an oven at 80 ºC for 48 hr till a constant weight is obtained. The dry weight of plants for each metal concentration and exposure time was expressed as percentage decrease of biomass relative to controls.

Roots and leaves were analyzed by dry ash method where the samples were ashed in a muffle furnace and 0.5 g cooled ash was dissolved in HNO_3 and boiled for 20 min on a hot plate. The filtrate in each case was analyzed for Cd content by atomic absorption spectrophotometer (Analyst100, Perkin Elmer, USA), using an air-acetylene flame. Statistical evaluation of the data has been made by Pearson's correlation coefficient at a significance level of $p < 0.05$ and $p < 0.01$ with SPSS 16.0 statistics software. Factorial analysis of variance (ANOVA) was employed to test variance among readings. To isolate which group (s) differed from the others Fisher's LSD test was employed as a multiple comparison procedure.

***Corresponding author:** 30mayank@gmail.com
[1]Environmental Biology Unit, Department of Plant Biology, University of Ilorin, Nigeria 240 003
[2]ICAR - Indian Institute of Spices Research, Kozhikode, Kerala 673 012
[3]ICAR - Directorate of Weed Research, Jabalpur, Madhya Pradesh 482 004

Effect on biomass of weed

A significant reduction of biomass **(Figure 1)** was observed with the exposure time and increased metal concentration ($p<0.05$). The highest biomass (100%) was found in the control, while the lowest was found at 10 mg/L Cd (41.2%). The toxicity symptom in the form of chlorosis was observed in the leaves. The toxicity symptoms increased with increasing concentration and exposure time. Chlorosis started from the margin of leaves and extending towards the inner portion of the blades. By 15 day, leaves became brown and easily separated from other parts. However, no morphological changes were observed in the roots. Poskuta *et al.* (1987) who reported chlorosis because of a localized H_2O_2 production, oxidative stress and cell death in *Pisum sativum* exposed to Cd.

Effect on chlorophyll content

Total chlorophyll content of control slightly increased with increasing exposure time. The total chlorophyll contents in treated plants were significantly decreased (**Figure 2**) from that of control ($p<0.05$). Highest total chlorophyll content (2.8 mg/g) was observed in the control, and the lowest was observed at 10 mg/l Cd (0.78 mg/g) after 15 days. Comparatively, at the highest concentration of both metals, the total chlorophyll content of plants treated with Cd was significantly lower than that of Pb treated plants ($p<0.05$). Cadmium is an effective inhibitor of photosynthesis (Vassilev *et al.* 2005). The presence of Cd in the growth medium decreases the growth of soybean and chickpea plants (Hasan *et al.* 2007).

Cadmium uptake

A steady increase in Cd uptake and accumulation was observed for all treatments at all testing days (**Table 1**). The metal contents significantly increased when the exposure time and metal concentration were increased ($p<0.05$). By 15 days, the Cd contents in the roots and leaves increased to the maximum levels of 21, 118.4, 688.4, 912, and 1308 and 4.56, 19.3, 157.1, 208.1, and 543.3 µg/g dry wt at Cd concentrations at 0.5, 1, 2.5, 5, and 10 mg/L, respectively. The accumulation of Cd was more in roots as compared to the shoots. Translocation of metal from root to leaves also increased with increasing metal concentration and exposure period. The highest Cd content (1851.3 µg/g dry wt.) was found in plants exposed to 10 mg/L Cd. Another toxicity symptom observed as the result of Cd exposure was decreased in biomass of *P. stratiotes* when the exposure time and metal concentration were increased. Cd was found to inhibit growth in many aquatic plants such as *Lemna* (Mohan and Hosetti 1997), *Eichhornia* (Zhu *et al.* 1999). Accumulation of particular type of metal is a selective

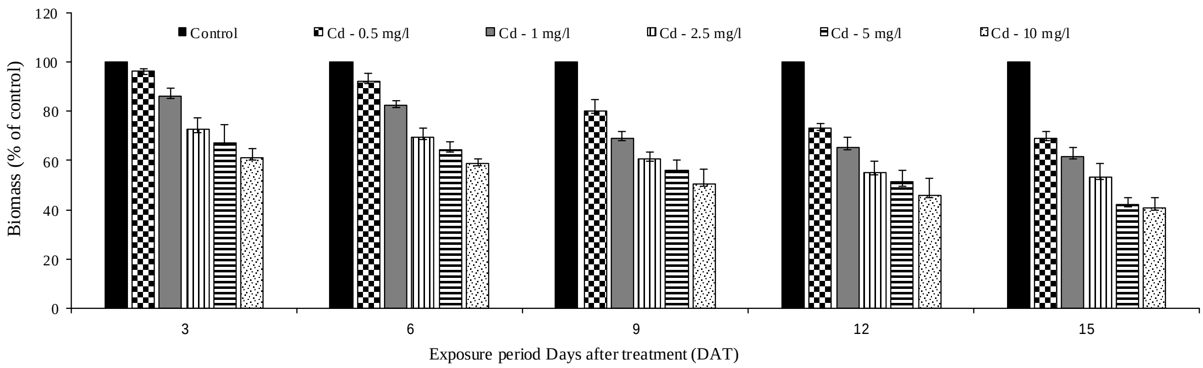

Fig. 1. Effects of Cd on biomass productivity (% control) of *P. stratiotes* at different concentrations and exposure period

Table 1. Cadmium uptake and accumulation (µg/g) in *P. stratiotes* (roots/leaves) at different days

Cd (mg/l)	Cadmium content in *P. stratiotes* (µg/g dry wt.)									
	3 DAT		6 DAT		9 DAT		12 DAT		15 DAT	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
0.5	1.68±0.6	0.63±0.2	6.12±1.7	1.68±0.7	8.26±2.3	2.18±0.8	19.2±2.1	2.52±0.5	21±1.9	4.56±1.4
1	41.7±2.1	7.74±1.4	85.3±8.3	9.24±1.8	106.6±8.2	12.3±1.7	116.3±11.7	17.5±16.1	118.4±11.7	19.3±2.6
2.5	265.6±6.2	104.2±7.6	490.2±17.2	114.8±27.1	612.7±18.7	117.1±17.2	655.2±2.2	128.1±21.1	688.4±28.2	157.1±21.2
5	352±12.3	138.1±13.2	649.6±38.2	152.2±17.2	812.1±10.3	153.7±26.1	868.3±21.6	169.8±9.7	912.2±11.5	208.1±19.1
10	613.7±9.3	178.4±18.2	664.5±14.8	232.4±28.2	830.6±9.5	237.4±11.8	1224±43.2	248.9±27.2	1308±41.7	543.3±26.3
Control	-	-	-	-	-	-	-	-	-	-

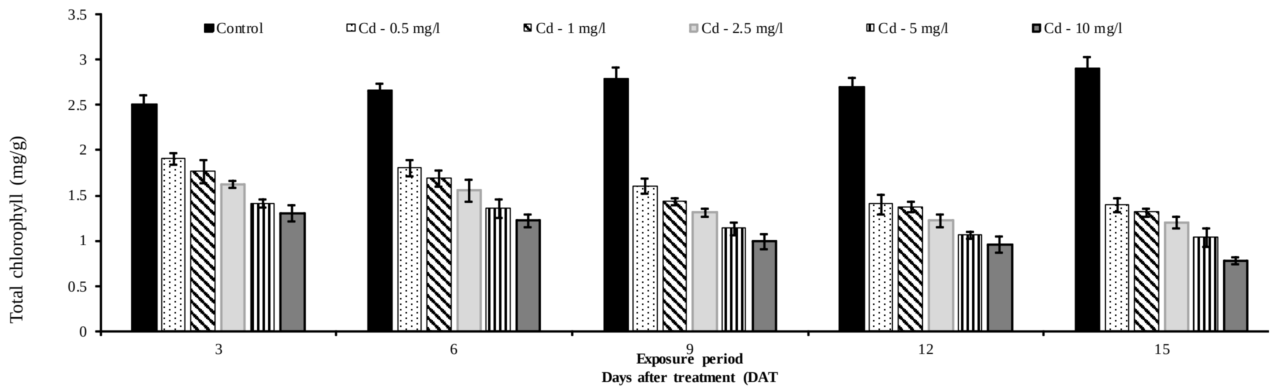

Fig. 2. Total chlorophyll content of *P. stratiotes* (mg/g) at different concentrations and exposure period

process that may vary from plant to plant. Metal concentrations were reported to be higher in the roots in most studies.

It was concluded that *P. stratiotes* was found to tolerate Cd concentrations up to 10 mg/kg, which confirms the ability of this plant to establish and grow well in Cd-contaminated water and accumulate substantial amount of Cd. Therefore, *P. stratiotes* is a promising plant for Cd accumulation and it can be employed for phytoremediation.

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SUMMARY

This study was aimed to determine the cadmium uptake and accumulation potential of *Pistia stratiotes* for phytoremediation of cadmium contaminated water bodies. Plants were grown in Hogland's medium spiked with 0, 0.5, 1, 2.5, 5 and 10 mg/L Cd, individually. Plant samples (roots and leaves) were analyzed for Cd content at 3, 6, 9, 12, and 15 days after treatment. A steady increase in Cd accumulation with increasing metal concentration and exposure period was observed for all treatments. The toxicity symptoms of Cd showed chlorosis on leaves. A significant reduction in the relative growth, biomass productivity and total chlorophyll content with the exposure time and concentration was observed. Accumulation of cadmium was more in roots (1308 µg/g) as compared to shoots (543.3 µg/g). Statistically significant difference ($p \leq 0.001$) in mean metal content in root and shoot at successive days of study was recorded.

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