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Physiological and biochemical study of *Hydrilla verticillata* (L.f.) Royle under cadmium heavy metal stress

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Abstract

Hydrilla verticillata, is a widespread submerged plant in Bhanjabihar is selected and inoculated in different concentration of cadmium (Cd) (1, 2, 3, 4, and 5ppm) as well as taken a control and studied on the physiological and biochemical parameters like total chlorophyll, protein, carbohydrate, amino acid and studies enzymatic activities like catalase and peroxidase under continuous light were studied in the summer season. The cadmium stress caused considerable inhibition of growth and synthesis of biomolecules with proportional to the concentration of metal. The cadmium toxicity decreased in biomolecules compounds like chlorophyll, protein, carbohydrate, amino acid and catalase and Peroxidase enzyme activity at lower concentration i.e., at 1ppm as well as higher concentrations like 2, 3, 4, and 5ppm. But at higher concentration of cadmium ie., 5ppm was found to be more toxic in the present study. There was a loss of total chlorophyll pigment shown in H. verticillata plant in cadmium stress. The protein content was decreased significantly ($P \leq 0.05$) up to 2ppm, then slightly increased in 3ppm was observed. Also, amino acid content was decreased in all concentration of cadmium stress when compared to control. Sugar content also decreased in all concentration of cadmium solution but less toxic effect found in 1ppm as the sugar content was nearby to the control. Same way the activity of catalase enzyme showed significantly ($P \leq 0.05$) decreased at the lowest concentration. With the increase of cadmium concentration, the decrease of catalase activity was found. Peroxidase enzyme also increased significantly ($P \leq 0.001$) at 1ppm, however, the highest percentage of increase in peroxidase enzyme activity was found at 5ppm. The study suggests that the plant H. verticillata as of a phytoremediation of cadmium and used as a bioindicator to access the metal toxicity in the aquatic system.

Keywords: Bhanjabihar, *Hydrilla verticillata* (L.f.) Royle, Cadmium, biomolecules.

Introduction

Environmental pollution is at present a global concerned which has been attracting the researchers, planners, and administration to find out the way to check the pollution. India being an industrialized country in the world which has been progressing towards development. This craze for development resulted in the unlimited exploitation of various natural resources. As a result of which it disturbs the delicate ecological balance between living and nonliving components of the Biosphere, which not only threatens the survival of humans itself but also other living organisms. The problems of pollution have gone to such an extent that a new discipline called Ecotoxicology came to limelight. Green plants are the primary producers and any damage may lead to disturbance in the entire food chain and this is called phytotoxicity which damage to the plant caused by an external agent and that is the toxic metal which is rarely found in the soil under natural condition but more commonly found in the chemical industries, fertilizer plants, mining waste disposal, pesticides and metal contaminated sludge's.

These are the important sources of metal dispersion in terrestrial and aquatic environment[1]. In the recent year, a number of reviews have been published on metal phytotoxicity[2-5].

The metals (trace elements) concentration above the tolerance limit often may cause some damage to the living system[3]. These elements are necessary for the normal metabolic functions of the plants, but relatively at high concentration, these metals are toxic and may severely interfere with physiological and biochemical functions of the plant and animals[6-7]. They also cause irreversible injury to the plants and cell membranes. Trace metals or elements like cadmium, lead, zinc, chromium known as toxic metal and among all metal cadmium was recognized many years ago to be a highly toxic element but it was not until recently that concern began to be expressed over the possible effects on human health of long-term exposure to low concentration of this element. It (Cadmium) ranks the highest in terms of damage to plant growth and human health. Cadmium has many anthropogenic uses; its uptake and accumulation in plants pose a serious health threat to humans via food chain[8]. The presence of excessive amounts of Cadmium in soil and water commonly evoke many stress symptoms in plants, such as reduction of growth, especially root growth, enzymatic activity, disturbances in mineral nutrition and carbohydrate metabolism[9], and may thus cause fiercely reduction of biomass production.

Among the different aquatic macrophyte *H. verticillata* is found to grow luxuriantly in various polluted water bodies. It is a

submerged macrophyte and an aquatic weed belonging to family Hydrocharitaceae. It has been reported by many researcher that Hydrilla plants scavenge cadmium[10]. Cadmium poses a serious threat to flora and fauna as well as to the environment.

Materials and methods

Test chemical: The test chemical used in the present study analytical grade, obtained from Merck laboratories, Mumbai. In the form of Cadmium Sulphate [Cadmium $SO_4.8H_2O$ (M.W. 769.51)]. Different concentration of the Cadmium sulfate was prepared by using distilled water as the solvent. The concentrations of the test chemical were used 1-5ppm.

Methods: The plant was collected from the local pond of Bhanjabihar near Berhampur University and was cultured in different concentrations of Cadmium sulfate i.e. 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm. The healthy plant were selected with similar biomass (5gm fresh weight) and released into each metal treatment containing 250ml of treatment solution and plants also culture in pond water taken as control without the ant treatment which are before that the plants were acclimatized in 10% Hogland's solution for 5 weeks during the summer season. The containers were exposed to continuous light intensity. The source of light was two number of Philips fluorescent white cool tube lights. The plants were harvested after 3 days and properly washed with distilled water and used for the experiment of different physiological and biochemical frameworks like total Chlorophyll[11], Protein[12], Amino acids[13], Sugar[14], Catalaseas[15] and Peroxidase enzyme[15] were measured following the standard procedure.

The innoculated plants were harvested after 3 days of exposure and the metal solution were changed every alternate day. The harvested plants were properly washed with distilled water and used for analysis of total chlorophyll, protein, amino acid, carbohydrate and enzymatic activities like catalase and peroxidase. Total Chlorophyll was determined by extracting fresh leaves (100mg) with 80% chilled acetone and centrifuging at 10000rpm as per the method of Arnon[10]. Protein was quantified by the method of Lowry[11]. Fresh leaves of plant was extracted in 3ml of 10% TCA and centrifuged at 10000rpm for 10 min. and then 1 N NaOH was added to the supernatant which was then boiled for 15 min. and then cooled and added 5ml of Lowry solution to it then incubated for 10 min at 30^0C and 0.5 ml of Folin-Ciocalteu's phenol reagent added and the absorbance were measured at 750nm after 45min. using BSA as a standard. The Amino acid content was measured by the method[13]. 100mg of fresh leaves extracted with 70% ethanol and centrifuged at 8000rpm. then to 1 ml of final supernatant added 3ml of 0.3% Ninhydrin reagent and boiled in a water bath at 75^0C for 20 minutes, after cooling the sample the absorbance were read at 570nm. using L_Leiucine as a standard. The sugar content was measured by the method[14]. 100mg shoot material was ground with 70% ethanol (v/v) and centrifuged at 8000rpm for 15minutes and the supernatant were collected in a test tube. The final supernatant left for few minutes to evaporate the remained alcohol portion. The reduced amount was made up to 10ml by adding distilled water and to 1ml of supernatant, 5ml of Anthrone reagent (20mg anthrone in 10ml concentrated H_2SO_4) was added and was boiled in a water bath at 75^oC for 10 minutes, then cooled at room temperature and the absorbance were read at 620nm. The Catalase enzyme was assayed by the modified method[15]. About 200mg of shoot material of *Hydrilla verticillata,* extracted with 5ml of 0.1N phosphate buffer and the homogenate was centrifuged for 30min at 4^0C at about 15000rpm in a cooling centrifuge then to 1ml enzyme extract, 1ml of 0.005M H_2O_2 and 3ml 0.1M phosphate buffer (pH 7.0) was added and were incubated at room temperature for 1min, then the reaction was stopped by adding 10ml of 1.0M Sulphuric acid. This solution was titrated with 0.01N $KMNO_4$ (50ml). A blank was prepared by an acidified solution of reaction mixture without sample extract. Catalase activity was expressed at micromole hydrogen peroxide utilized gm per fresh wt. per min. Peroxidase extraction was similar to that of catalase. The Peroxidase enzyme was assayed by the modified method[15]. About 200mg of shoot material of *H. verticillata,* were extracted with 5ml of 0.1N phosphate buffer and centrifuged for 30min at 4^0C at about 15000rpm in a cooling centrifuge. To 1ml of enzyme extract, 3ml of 0.1M phosphate buffer, 1ml of pyrogyllol, and 1ml of 0.005m Hydrogen Peroxide were added. The reaction was stopped by adding 1ml sulphuric acid and after 5min incubation at 25 degree centigrade and the amount of purpurogallin was estimated by measuring the absorbance at 420nm in Peroxidase activity was expressed in absorbance (A) units.

Results and discussion

The present study was carried out the phytotoxic effects of cadmium on physiological and biochemical frame works of *H. verticillata*. The results are presented as table and figures and statistical analysis have been made wherever necessary. The data were summarized as mean value ± SD (n=3) in the tables.

From our study, it is observed that the photosynthetic pigment content was found to be more in 1ppm than other concentrations of Cadmium but not more than control. 5ppm was found to be more toxic to the plant (Table-1, Figure-1). The reduction in chlorophyll content in hydrilla plants exposed to Cadmium stress is may be due to inhibition of δ-aminolevulinic acid dehydratase and monovinyl protochlorophyllide reductase[5] which are associated with chlorophyll biosynthesis. Cadmium stress also causes impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls. In our result decrease in chlorophyll content corroborating with the finding of other workers who were worked on the interaction of cadmium and iron and effect on Phaseolus vulgaris and found a decrease in chlorophyll content[16]. The result has shown that submerged plant species are more capable of accumulating metals than the floating and emergent plants[17]. Also, various abiotic stresses decrease the chlorophyll content of plants[18]. In our study, *H.*

verticillata showed the capability of accumulating significant amounts of Cadmium. This process seems to be concentration duration dependent. Impact of metal toxicity is gradually manifested on chlorophyll even at a very low ambient concentration of toxic metals. The plants of *H. verticillata* showed the decrease in chlorophyll content in all the concentrations of the cadmium treatment which was found to be the most susceptible parameter. This might be due to an interaction of Cadmium with–SH groups of various enzymes involved in chlorophyll synthesis[19].

Table-1: Effects of various cadmium concentrations on Total Chlorophyll contents of *H. verticillata* at 3 days exposure. Values are mean of replicates ± SD (n=3).

Concentrations of Cadmium SO_4 in ppm	Total Chlorophyll content in mg/g fresh weight
0 (Control)	2.44 ± 0.03
1	2.29 ± 0.02
2	2.23 ± 0.01
3	2.13 ± 0.05
4	2.03 ± 0.03
5	1.82 ± 0.005

Protein contents also found to declining at the lowest concentration of Cadmium i.e. 1ppm and 2ppm. ($P \leq 0.05$). But the protein contents were increased at 3ppm as compared to 2ppm, but not more than control. The protein contents again decreased with the increase in Cadmium concentration. But there is an overall decline of protein contents was found (Table-2, Figure-2). The similar trend was observed by Kumar et al. while working on *Elusine corocana* seedling[20]. Heavy metal stress inhibits synthesis of some proteins and promotes others[21]. The decrease in protein content in *H. verticillata* may be caused by enhanced protein degradation process as a result of increased protease activity which is found to increase under stress condition[22]. It is also likely that Cadmium may have induced lipid peroxidation in *H. verticillata* and fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content[23]. The similar trend was observed by Garg et al., in *H. verticillata*[24]. Neelu et al. also supported this view that total soluble protein decreases with increasing concentration of Cadmium[25].

Table-2: Effect of various Cadmium concentrations on Protein contents of *H. verticillita* at 3 days exposure. Values are mean of replicates ± SD (n=3).

Concentrations of Cadmium SO_4 in ppm	Protein content in mg/g fresh weight
0(Control)	8.66 ± 0.05
1	7.06* ± 0.15
2	5.96* ± 0.11
3	6.76 ± 0.05
4	4.53 ± 0.05
5	3.73 ± 0.05

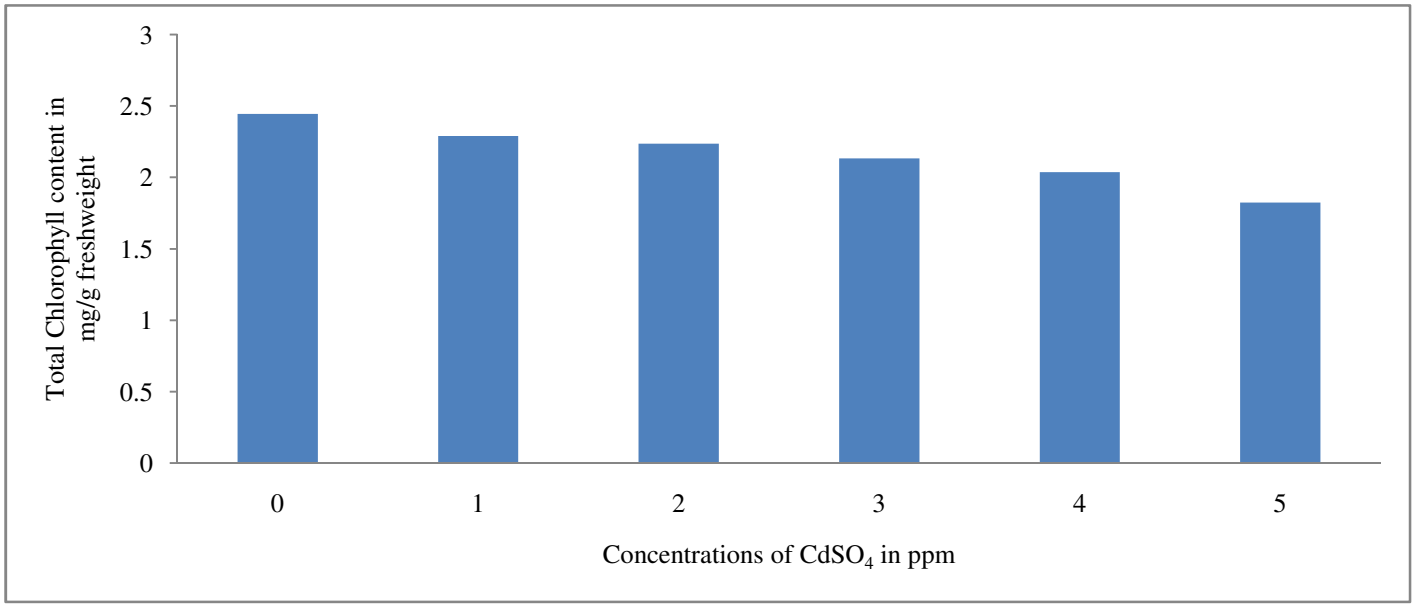

Figure-1: Effects of various Cadmium concentrations on Total Chlorophyll contents of *Hydrilla verticillata* at 3 days exposure. Values are mean of replicates (n=3).
Significant level:*p≤0.05 or **p≤0.01 or ***p≤0.001 or ns = non-significant.

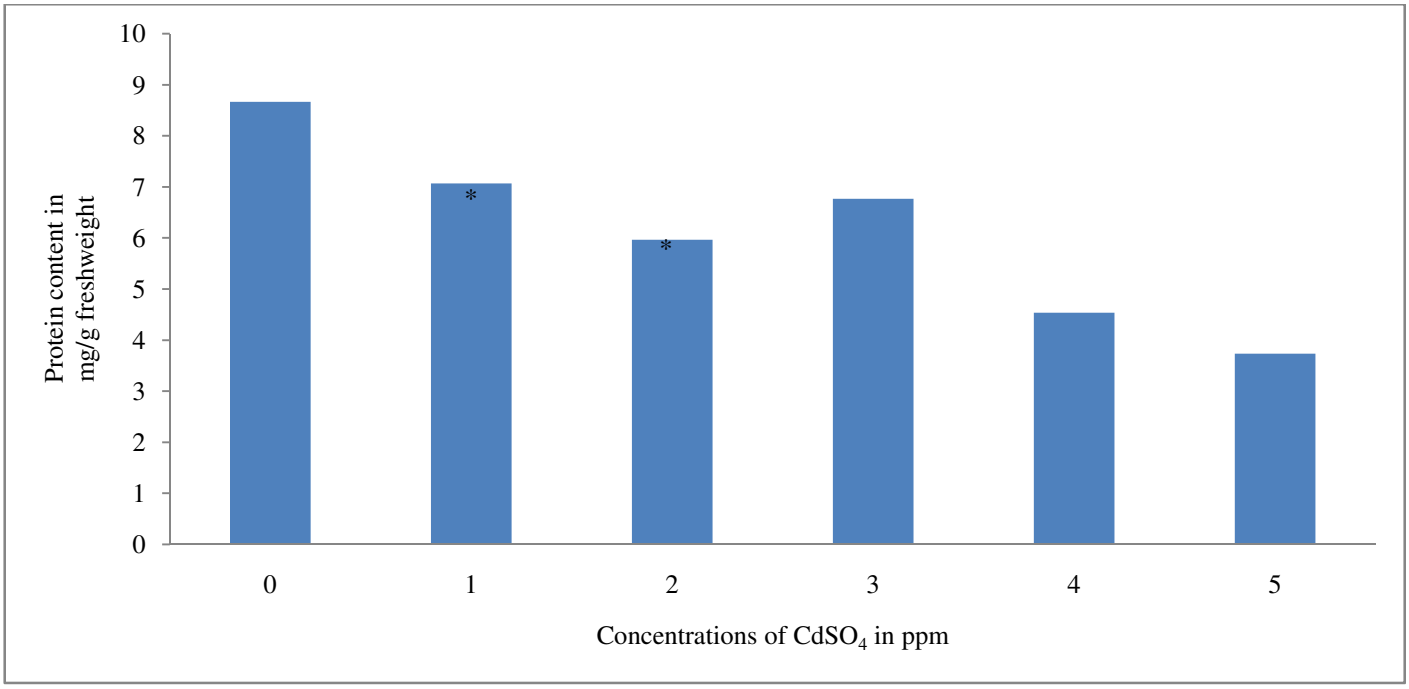

Figure-2: Effects of various Cadmium concentrations on Protein contents of *Hydrilla verticillata* at 3 days exposure. Significant level: *p≤0.05 or **p≤0.01 or ***p≤0.001 or ns = non-significant.

Different investigations of Cadmium on Hydrilla plant do not show any linear relationship between metal concentration and Amino acid content. In this study, it was observed that the lower concentration of Cadmium i.e. up to 2ppm the Amino acid shows a decline than control value, at 3ppm Amino acid content was elevated but not more than control.

The Amino acid content again decreased as the higher concentration of Cadmium (Table-3, Figure-3). The similar trend has been observed by Kumar while working on *Elusine corocana* seedlings[26]. Garg et al., also reported that low concentration of Cadmium in *H. verticillata* causes the decrease in cysteine content which is then increased an as high concentration of Cadmium was given[24]. But again decreased at higher concentration. This was also reported before it[27].

An excess cellular concentration of Cadmium either inhibits the utilization of amino acids or causes degradation of amino acids thus causes the decrease in amino acid content[28].

The sugar contents in *Hydrilla* plant decreased with the increase of Cadmium concentrations. So, there is a linear relationship between sugar content and Cadmium concentration (Table-4, Figure-4). The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive center of ribulose bisphosphate carboxylase[28] thus causes the decrease in the sugar content in *Hydrilla* plant. In response to Cadmium, there was a steady decrease in the sugar content in *H. verticillata.* The total carbohydrates got inhibited if Cadmium concentration is more than 5mg/kg soil[29].

Table-3: Effects of various Cadmium concentrations on Amino acid content of *Hydrilla verticillita* at 3 days exposure. Values are mean of replicates ± SD (n=3).

Concentrations of Cadmium SO_4 in ppm	Amino acid content in mg/g fresh weight
0 (Control)	11.26 ± 0.05
1	9.73 ± 0.05
2	8.4 ± 0.1
3	10.23 ± 0.15
4	6.36 ± 0.05
5	4.76 ± 0.15

Table-4: Effect of various Cadmium concentrations on the Sugar content of *H. verticillata* at 3 days exposure. Values are mean of replicates ± SD (n=3).

Concentrations of Cadmium SO_4 in ppm	Sugar content in mg/g fresh weight
0 (Control)	3.76 ± 0.02
1	3.26 ± 0.003
2	2.53 ± 0.02
3	2.13 ± 0.003
4	1.7 ± 0.02
5	1.26 ± 0.003

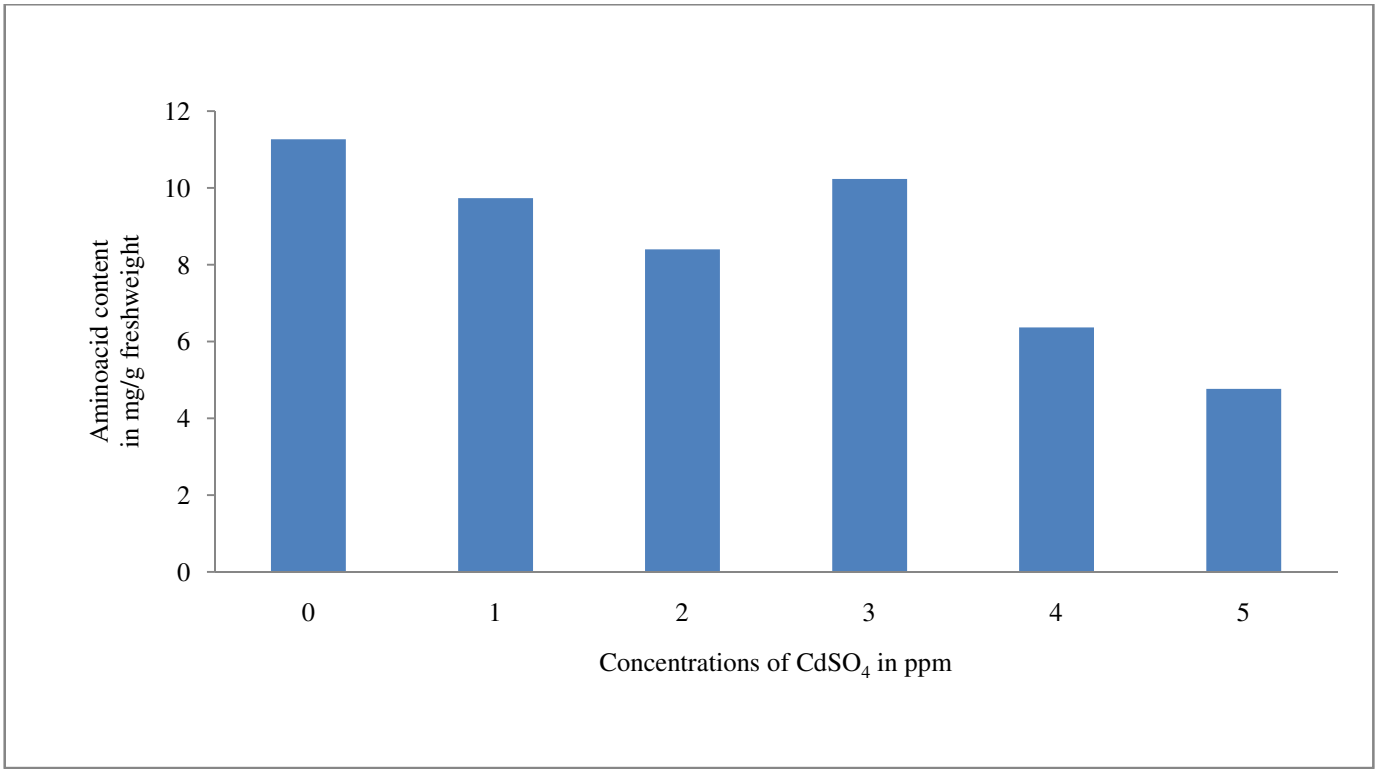

Figure-3: Effect of various Cadmium concentrations on Amino acid content of *H. verticillata* at 3 days exposure. Significant level:*p≤0.05 or **p≤0.01 or ***p≤0.001 or ns = non-significant.

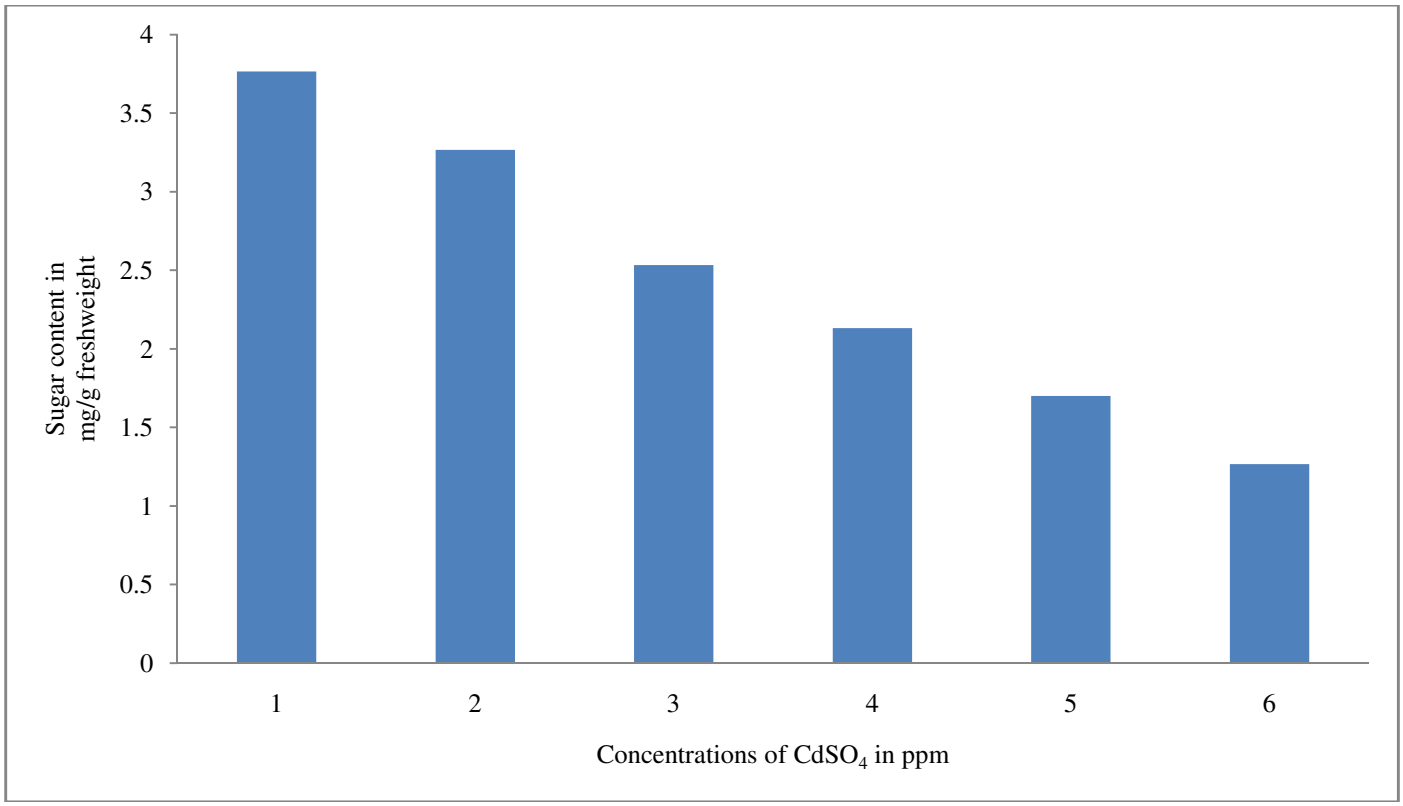

Figure-4: Effect of various Cadmium concentrations on the Sugar content of *Hydrilla verticillata* at 3 days exposure. Significant level: *p≤0.05 or **p≤0.01 or ***p≤0.001 or ns = non-significant.

The activity of catalase enzyme was studied in the *Hydrilla verticillata* plant and at the lowest concentration i.e. at 1ppm it was significantly (P≤0.05) decreased. With the increasing concentrations of Cadmium, the catalase activity was decreased at the highest percent that is at 5ppm. So this shows a linear relationship between them. (Table-5, Figure-5). The catalase activity decreased following exposure to elevated Cadmium concentrations[30]. The activity of several enzymes like RNAse also decreases due to the toxic action of Cadmium compounds. The Enzyme catalase showed a decreased trend in response to increasing concentrations of Cadmium in *Hydrilla verticillata* plant after 3 days of treatment. Like catalase, Peroxidase is another enzyme which is for scavenging of H_2O_2. This enzyme has received maximum attention in pollution studies.

Table-5: Effect of various Cadmium concentrations on Catalase Enzyme activity of *Hydrilla verticillata* at 3 days exposure. Values are mean of replicates ± SD (n=3).

Concentrations of Cadmium SO_4 in ppm	Catalase Enzyme activity in µ moles of H_2O_2 utilized/min/g fresh weight
0 (Control)	78.73 ± 0.59
1	76.92* ± 0.55
2	74.84 ± 0.51
3	74.45 ± 0.53
4	72.96 ± 0.32
5	71.95 ± 0.04

The activity of peroxidase enzyme was also studied in the *Hydrilla verticillata* plant and the lowest concentration i.e. at 1ppm it was significantly (P≤0.001) increased. With the increasing concentrations of Cadmium, the peroxidase activity was also increased. The highest percentage of increase in Peroxidase activity was found at 5ppm (Table-6, Figure-6).

In our present investigation, there was an induction of Peroxidase activity in *Hydrilla* plant in response to different concentrations of Cadmium after 3 days treatment There are few reports that the Peroxidase activity was induced by the induction of Cadmium heavy metal[31-32].

Table-6: Effect of various Cadmium concentrations on Peroxidase Enzyme activity of *Hydrilla verticillata* at 3 days exposure. Values are mean of replicates ± SD (n=3).

Concentrations of Cadmium SO_4 in ppm	Peroxidase Enzyme activity in Absorbance (A) units
0 (Control)	1.45 ± 0.015
1	1.52*** ± 0.01
2	1.90 ± 0.015
3	2.33 ± 0.03
4	2.63 ± 0.02
5	3.13 ± 0.02

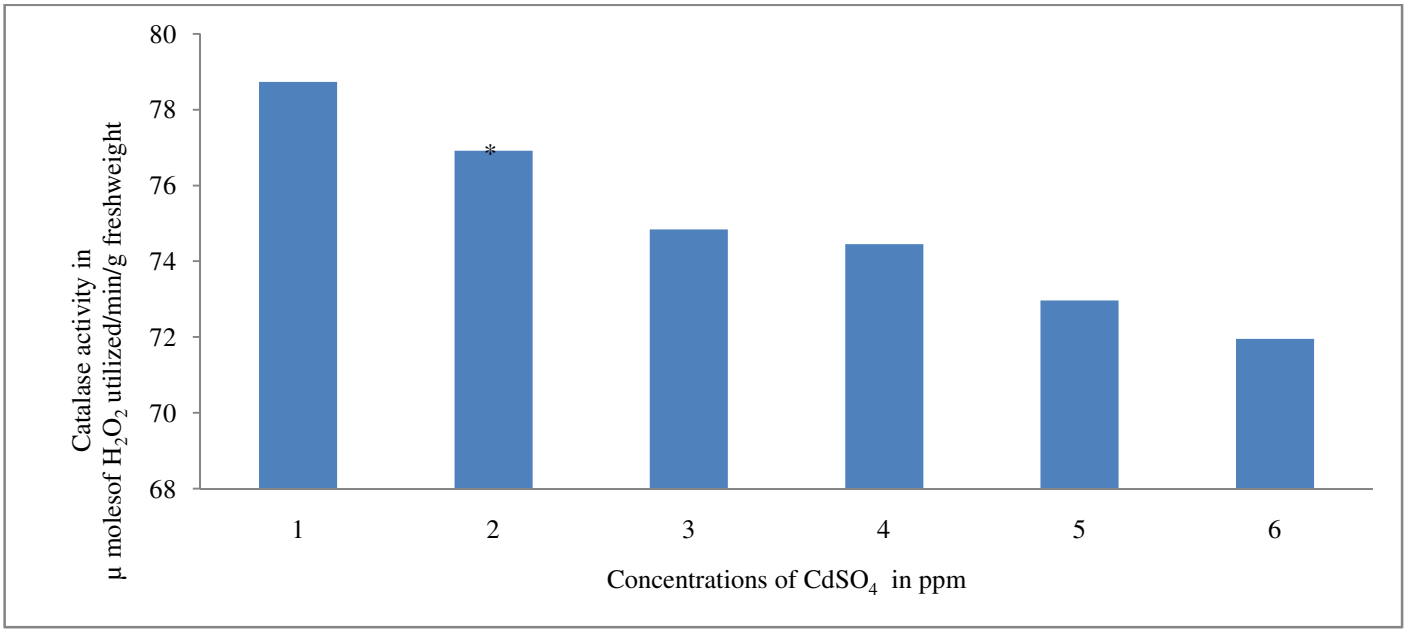

Figure-5: Effect of various Cadmium concentrations on Catalase Enzyme activity of *Hydrilla verticillata* at 3 days exposure. Significant level:*p≤0.05 or **p≤0.01 or ***p≤0.001 or ns = non-significant.

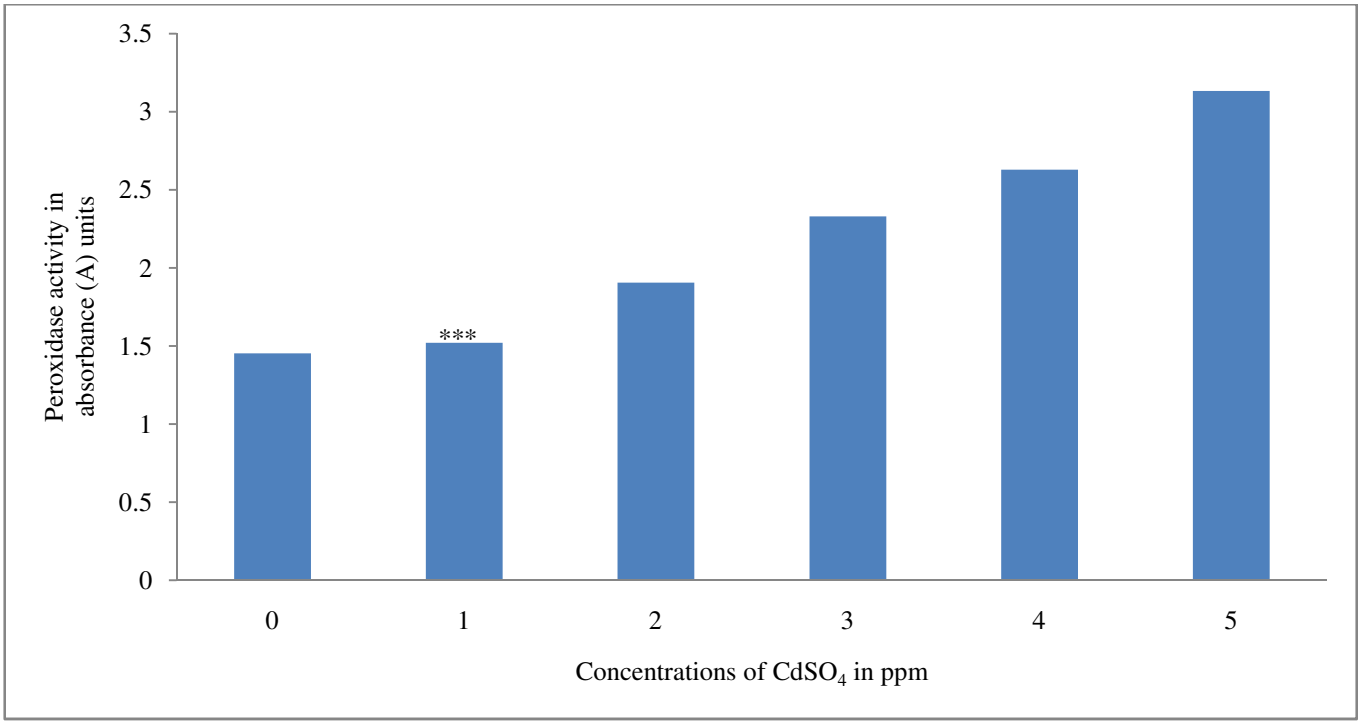

Figure-6: Effect of various Cadmium concentrations on Catalase Enzyme activity of *Hydrilla verticillata* at 3 day s exposure. Significant level: *p≤0.05 or **p≤0.01 or ***p≤0.001 or ns = non-significant.

Conclusion

In the present investigation, an effect of different concentrations of Cadmium showed there is a maximum decrease in the photosynthetic pigment and other biomolecules like protein, amino acid, carbohydrate catalase enzyme and maximum decreased in enzyme Peroxidase activity at higher concentrations. The study suggests that the plant *Hydrilla verticillata* may be taken as of a phytoremediator of Cadmium and can be used as an indicator plant to assess the metal toxicity in an aquatic system. But before that, the study also suggests needing further an intensive research work.

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