Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Environmental Sciences

ISSN 1819-3412
DOI: 10.3923/rjes.2018.160.165

Research Article
Influence of Cadmium Toxicity and its Accumulation in *Lemna polyrhiza* L.

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Abstract

Background and Objective: Aquatic plant species are widely used in water purification systems since long and many of them are used as scavengers of heavy metal ions from aquatic metal contaminated environments which significantly influence their growth and activity. The objective of the study was to evaluate the effect of various concentrations of cadmium (Cd) metal ion on biochemical (total chlorophyll, total carbohydrates, total proteins), stress parameters, catalase and guaiacol peroxidase and cadmium metal accumulation capability of *Lemna polyrhiza* L. after exposing the plant to select experimental concentrations and periods. **Materials and Methods:** In this study, *Lemna polyrhiza* L. were experimentally cultured in Hoagland's medium supplemented with various Cd ion concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 ppm along with one control for 2, 4 and 6 days of exposure period. The test plants were separately harvested after 2, 4 and 6 days and chlorophyll, protein and carbohydrates were estimated. Biochemical parameters namely chlorophyll, total carbohydrate and total protein as well as stress parameters such as proline and antioxidative enzymes were carried out using standard methods. Data were analyzed using SPSS. **Results:** The toxicity symptoms of Cd ions morphologically showed chlorosis on the leaves of the test plants after 6 days. The results revealed that increased metal ion concentration as well as the exposure period gradually reduced chlorophylls, carbohydrate and protein contents, while proline and antioxidative enzymes gradually increased. **Conclusion:** Cd treated plants of *Lemna polyrrhiza* L. showed decreased level of chlorophyll, carbohydrates and protein and increased in proline and antioxidative enzymes i.e., catalase and guaiacol peroxidase. Also, the plant was identified as hyperaccumulator of cadmium ion.

Key words: *Lemna polyrhiza* L., metal contaminate, cadmium ion, hyperaccumulator, biochemical parameter, stress parameters

Citation: Krupa Unadkat and Punita Parikh, 2018. Influence of cadmium toxicity and its accumulation in *Lemna polyrhiza* L. Res. J. Environ. Sci., 12: 160-165.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pollution of environment by toxic metals arises as a result of various industrial activities and has turned these metal ions into major health issues[1]. Although several adverse effects of the toxic metals have been known for a long time, exposure to heavy metals continues and is even increasing in some parts of the world, especially in developing countries. Heavy metal pollution is also a multi-element problem in many areas as they are easily taken up by plants and then enter the food chain, resulting in a serious health issue for humans. The increasing levels of heavy metals in the environment, their entry into the food chain and the overall health effects are of major concern to researchers in the field of environmental biology. There is a significant contamination of fresh water resources as well as artificial eutrophication of lakes and reservoirs and an accelerating accumulation of toxic metals in human food chain[2].

This has assumed serious proportion in the twentieth century and water, one of the most precious and vital components of the biosphere, is being constantly polluted. Effluents from electroplating plants constitute one of the important sources of heavy metal pollution in surface waters. Moreover, increased generation of these effluents containing trace metals poses not only a stress but also a major threat to the biological pool of the ecosystem[3].

Heavy metals severely inhibit root growth[4-12]. Bioavailability of heavy metal in soil, uptake of these heavy metal at phytotoxic level lead to growth retardation, affects palisade and spongy parenchyma cells in leaves[13,14], collated deposition in the vascular bundles and change in vacuoles with electron dense material along the walls of xylem and phloem vessel[5]. Cadmium is toxic metal, which has contaminated soil over a very long period, initially as a contaminant arising from smelting and mining of other metals[6]. Cadmium tolerance mechanisms may differ depending on the species because of Cd banding with certain plants products, the amount of Cadmium retained in active site can be small, then Cd toxicity is alleviated[7]. Cadmium decreases the chlorophyll content[8]. Cadmium drastically changed the lipid composition of membranes, the content of palmitic acid increased and the contents of linoleic and linolenic acids decreased in all classes of lipids[9].

Considering the above facts the present study was carried out with an objective to investigate the influence of Cd ion accumulation in *Lemna polyrhiza* L. and to characterize its interfering effects on biochemical changes.

MATERIALS AND METHODS

Study area: Harni Pond is situated in Harni area of Vadodara district. Harni pond is geographically located at latitude 22°20'18.83'' and 73°13'03.84" longitude. It has spread in large area with varieties of vegetation found in and surrounding area of pond.

Sample collection: The study was conducted during monsoon season in the month of June-September, 2014. The total time required to complete the entire study was 4 months. The test plants *Lemna polyrhiza L.* was collected from the pond at Harni, Vadodara (Fig. 1). They were allowed to acclimatize for 15 days in lab condition. Plants were washed thoroughly under a running tap water and were grown and propagated for 4 weeks in quarter strength Hoagland's solution[10].

Preparation of cadmium aqueous solution: All experimental work was done using deionized water and all reagents were of analytical grade. The 1 N $CdCl_2$ stock solution served as a source of metal ion which was diluted further to obtain desired experimental concentrations.

Determination of LC_{50} value: Plants of same size were selected for the experiment. In the pilot scale experiment, the test plants were exposed to wide range of the metal ion concentrations i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppm for 10 days. In the subsequent experiments, it was revealed that the concentration mortality (LC_{50}) of cadmium chloride on exposed plants were 0.5 ppm during 120 h. Therefore, the trace element under study Cd as cadmium chloride was supplied at 0.1, 0.2, 0.3, 0.4 and 0.5 ppm for 2, 4 and 6 days. Nutrient solution devoid of trace element served as a control. Both the control and the treated solutions were maintained at pH 5.5 using dilute HCl or NaOH. Experimental plants (in triplicates) were placed in nutrient solution. Solutions were replenished every 3 days to prevent depletion of metals and nutrients.

Biochemical and oxidative stress parameters: After each experimental period, (2, 4 and 6 days after exposure to cadmium), harvested plants were washed in running tap water and rinsed with deionized water. Extraction and estimation of total chlorophyll was done using Arnon method[11], total soluble protein by Lowry method[12] and carbohydrates by Anthron method[15] whereas proline, catalase and peroxidase in test plants[16].

Fig. 1: Map of collection site

Statistical analysis: The data obtained were subjected to descriptive statistical analysis such as mean, standard deviation and t-test (at 0.05 level) using statistical package for Social Sciences (SPSS) software (version 10.0 for windows, SPSS 22.0).

RESULTS AND DISCUSSION

Total chlorophylls content: The results of effect of Cd metal ion concentration on total chlorophyll content of *Lemna polyrhiza* L. is represented in Fig. 2. The chlorophyll content showed gradual decline as the treatment, period and the metal ion concentration increased. The prolonged exposure of 6 days to high concentration of Cd i.e., 0.5 ppm significantly reduced chlorophyll than the control.

A significance difference was observed when chlorophyll content of control plant was compared with various treatment period ($p = 0.001 < 0.05$) followed by paired t-test. A significant difference were not obtained when similar results followed by chi-square test ($p = 0.85 > 0.05$). Present study results of decrease in chlorophyll content corroborated with the findings of Siedlecka and Krupa[17], who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery.

Total carbohydrate contents: Figure 3 shows the effect of Cd ion on carbohydrate content of *L. polyrhiza L.* that total

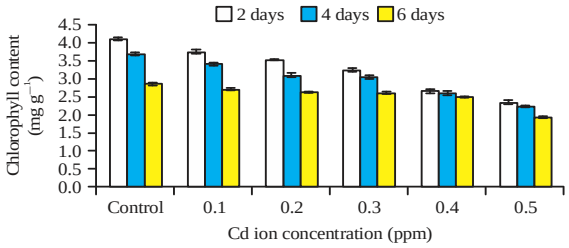

Fig. 2: Effect of Cd on total chlorophylls content of *L. polyrhiza* L.
Total chlorophylls content expressed as Mean±SD (n = 5)

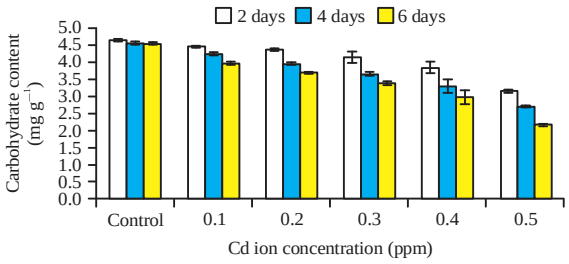

Fig. 3: Effect of Cd on total carbohydrates content of *L. polyrhiza* L.
Total carbohydrates content expressed as Mean±SD (n = 5)

carbohydrate content decrease as concentration of Cd ion increased. A negative correlation was recorded with the increased metal ion concentrations and the treatment period.

A significant difference was observed when carbohydrate content of the treated plants were compared with control on

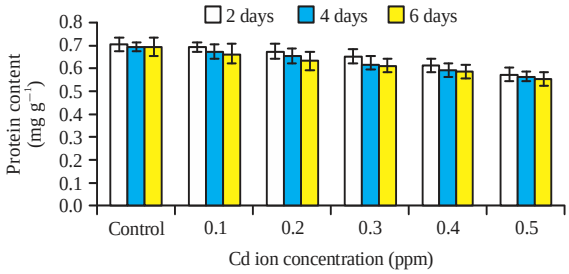

Fig. 4: Effect of Cd on total protein content of *L. polyrhiza* L.
Protein content expressed as Mean±SD (n = 5)

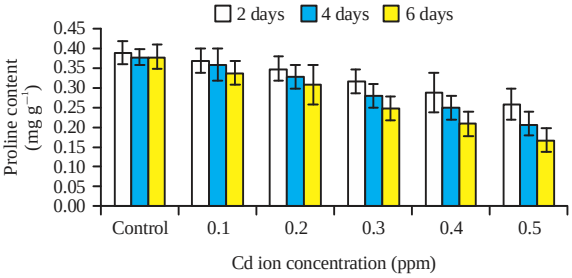

Fig. 5: Effect of Cd on proline content of *L. polyrhiza* L.
Proline content expressed as Mean±SD (n = 5)

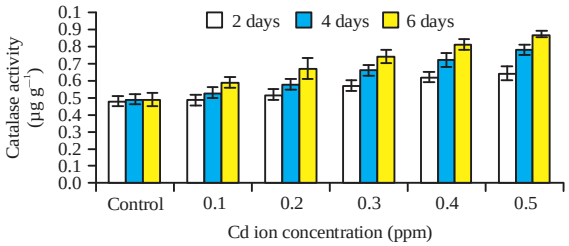

Fig. 6: Effect of Cd on catalase enzyme of *L. polyrhiza* L.
Catalase activity content expressed as Mean±SD (n = 5)

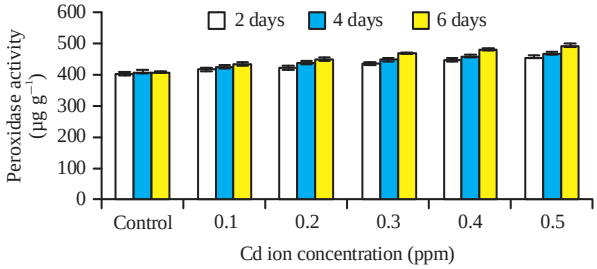

Fig. 7: Effect of Cd on peroxidase of *L. polyrhiza* L.
Peroxidase activity content expressed as Mean±SD (n = 5)

2nd day ($p = 0.017<0.05$). In addition to that, significant difference was found when carbohydrate content of 4th day ($p = 0.002<0.05$) and 6th day ($p = 0.006>0.05$) treated plants were compared with control followed by paired t-test. Additionally, in chi-square test, significant results ($p = 0.57>0.05$) was not find. The decrease in carbohydrate content of stressed levels probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Higher starch accumulation in damaged leaves of *Tilia argentea* and *Quercus cerris* may result both in the higher resistance of their photosynthetic apparatus[17] and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase[18,19].

Protein content: All the experimental concentrations shows decline in protein content as the period of treatment increased from 2-6 days and with increased Cd ion concentrations in all the treatment periods (Fig. 4). Protein showed 20% decline when the plants were treated with 0.5 ppm Cd ions following exposure of 6 days.

Significant difference was obtained when protein content of 5th, 6th and 7th day ($p = 0.00<0.05$) treatment plant when compared with control followed by paired t-test. Furthermore, in chi-square test, no significant results were obtained ($p = 1.0>0.05$). Present study of soluble protein content coincides with the findings of Singh and Sinha[20], who found decrease in soluble protein content in *Brassica juncea* when grown on various amendments of tannery waste containing heavy metals. Decrease in the protein content has also been found in aquatic plants when treated with metalliferous wastewater.

Proline content: The effect of Cd ion on proline content of the *L. polyrhiza L.* is represented in Fig. 5. A concentration dependent rise in the level of proline was observed during all experimental exposure period. But negative correlation was found between the period of exposure and level of proline content.

There is significant difference between proline content of the treated and control plants and treatment periods (2, 4 and 6 days) ($p = 0.00<0.05$). While in chi-square test, no significant results were obtained ($p = 1.0>0.05$). Plants have been shown proline accumulation under environmental stress[5]. Proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to Cd^+ toxicity has been demonstrated by Dhir *et al.*[21]. Similar results of increasing proline content by Cd^+ was also reported by Dhir *et al.*[21] in sunflower.

Catalase and peroxidase activity: The results depicted that the activity of both catalase and peroxidase were significantly higher in treated plants in comparison with the control plants (Fig. 6 and 7). Greater activities of

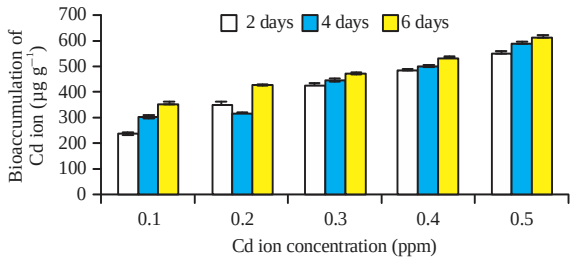

Fig. 8: Bioaccumulation of Cd ion of *L. polyrhiza* L.
Bioaccumulation of Cd ion expressed as Mean±SD (n = 5)

catalase and guaiacol peroxidase indicated that the treated plant were under oxidative stress.

In the present investigation, it was reported that activities of catalase and peroxidase enhanced linearly with increased metal ion concentration. Significant difference was found when catalase activity of the treated (exposed at 2, 4 and 6 days) were compared with control ($p = 0.00<0.05$) and peroxidase activity ($p = 0.00<0.05$) followed by paired t-test. Furthermore, in chi-square test, no significant results were obtained ($p = 1.00>0.05$) for catalase and peroxidase activity. Observed enzyme activity reductions are indicative of a limited protection against oxidative stress[22,23].

The intensity and direction of the antioxidative response to Cd appeared to be dependent on the plant species, tissue analyzed, metal identity[24], duration of metal exposure[12] and stage of plant development[24].

Bioaccumulation of Cd metal: Bioaccumulation of Cd ion in the test plants at different concentration is shown in Fig. 8. Exposure to 0.1-0.5 ppm increased the accumulation of 90% of Cd metal ion in all the experimental exposure periods.

There is significant difference between Cd metal uptake of the test plants when compared with control on 2, 4 and 6 days ($p = 0.0 3<0.05$) followed by paired t-test. Furthermore, no significant results were obtained in chi-square test ($p = 1.00>0.05$).

As phytoremediation technology is site-specific and not economically feasible everywhere. Hence, a stable and efficient phytoremediation system need to be established before its use in field trials. In spite of this limitation, the current study has created a new direction for the development of hyperaccumulating species through genetic engineering technology.

CONCLUSION

It was concluded that Cd treated plants showed toxicity of this metal ion when concentration and exposure period increased. Cd treated cells in addition to all these abnormalities also showed increase in antioxidative stress and proline increase. Hence, *L. polyrhiza* L. is valuable species for the study of metal hyperaccumulation mechanisms, which is of particular importance in the context of the potential use of this plant in phytoremediation. *Lemna polyrhiza* L. may be utilized as an eco friendly and economic plant for wastewater detoxification applicable to wide range of contaminated water system.

SIGNIFICANCE STATEMENT

This study discovers that *Lemna polyrhiza* L. can effectively be used as phytoremediator of cadmium ion that can be beneficial for reducing cadmium ion concentration in effluents of pharmaceutical and metal finishing industries. Additionally, this study will help the researchers to uncover the critical areas of heavy metal pollution which is perceptible to be more environmentally friendly, low tech alternative as well as more active and intrusive remedial methods that many researchers were not able to explore. Also, the physiological responses of plants to any type of stress including metal exposure are active research areas that can be even used for developing stress tolerance. Thus, a new theory on adopting molecular approaches for assessing metal tolerance in plants develops.

ACKNOWLEDGMENT

The authors acknowledged the support to Head of Department, Botany, The M.S. University of Vadodara, Vadodara, Gujarat for providing infrastructure to work, laboratory instruments and necessary facilities.

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