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Phytotoxicity of Cadmium and Lead in *Hydrilla verticillata* (l.f.) Royle

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Summary

Aquatic plants are well known in accumulating and in concentrating heavy metals. *Hydrilla verticillata* (lf.) Royle was subjected to different concentrations of Cd and Pb. Various physiological parameters i.e. fresh weight, total chlorophyll, carotenoid, NR activity, protein and bioaccumulation were studied. At lower metal concentrations, an increase in total chlorophyll, protein, and NR activity was noticed but at higher concentration these parameter were decreased. Treatment with 20.0 mg/l Pb and 2.5 mg/l & 5.0 mg/l Cd found to be more toxic. The accumulation of these metals (Pb and Cd) by *H. verticillata*, was more at lower concentration than higher concentration.

Key Words: Growth parameters, *H. verticillata*, Heavy metal, Bioaccumulation

Introduction

Contamination of the aquatic arena by various pollutants discharged through industrial applications such as heavy metals, polyaromatic hydrocarbons etc. have caused several level of entropy in the normal / natural functioning of the ecological system. Among these, heavy metals cause severe damage at various steps to the living systems.

Cadmimum (Cd) and Lead (Pb) were choosen for this investigation since they are common toxic metals found in waste water or polluted water. Cd, a non-essential toxic metal, enters into the aquatic area via industries like Nickel- cadmium batteries, electroplating, chemicals etc. Cadmium ranks the highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain [1]. Pb is one of the most abundant toxic metals in the earth crust. Elevated Pb in soils may compromise soil productivity and even a very low concentration can inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption with toxic symptoms of dark leaves, stunted foliage [2].

Aquatic macrophytes serve as convenient input for the assessement and monitoring of toxic heavy metals [3]. *Hydrilla verticillata* (l.f.) Royle is a submerged leafy aquatic plant, found to thrive well in waste water. It has been reported to scavenge Cadmium and Chromium [4]. After acknowledging the property of *H. verticillata*, the present task has been carried out to evaluate the tolerance ability of *H. verticillata* in response to Cd and Pb via analyzing different biochemical parameters.

Material and Methods

The submerged plants of *H. verticillata* (l.f.) Royle were obtained from pond of Plant Science Department, M.J.P Rohilkhand University, Bareilly (Uttar Pradesh, India). These plants were further acclimatized in 10% Hoagland's solution for six weeks during summer season. Selected healthy plants were treated with different concentrations of Cd (Cadmium Chloride, $CdCl_2$, 99% purified) i.e. 0.5mg/l, 1.0 mg/l, 2.5 mg/l, 5.0 mg/l and Pb as Lead nitrate $Pb(NO_3)_2$ i.e. 2.5mg/l, 5.0mg/l, 10.0mg/l, 20.0 mg/l separately during summer season. Plant's cultured in 10% Hoagland's solution without treatment served as control. Three replicates of each treatment were kept in 4.0 liters capacity tubs. Initially in each tub 5.0 gm of fresh biomass (wet weight) was added. The plants were harvested after 3days and 7days of exposure and the metal solutions were changed on every 2nd day. The harvested plants were properly washed with distilled water and used for estimation of plant growth (biomass), total chlorophyll, carotenoid, protein, In vivo NR activity and bioaccumulation of heavy metals.

Cholorophyll content was determined by extracting fresh leaves (100mg) with 80% chilled acetone and centrifuging at 10000 rpm as per method of Arnon [5]. Carotenoid was measured using the process of [6]. Protein was estimated by the method of Lowry [7]. Fresh leaves of plant was extracted in 3ml of 10% TCA and centrifuged at 10000 rpm for 10 min. 1 N NaOH was added in pellets after decanting supernatants which was then boiled for 15min. and then cooled. 5ml of Lowry solution was added to the 0.5 ml of final supernatant, and then incubated for 10min. at 30 ^{o}C. 0.5ml of Folin & Ciocalteu's phenol reagent was added and absorbance was read at 750nm after 45min using BSA as the standard. In vivo nitrate reductase activity was assayed following Srivastava [8]. Estimation of Cd and Pb was done after wet-digestion with Nitric acid: Perchloric acid, 3:1, v/v, mixture of the oven-dried plant material. The Cd and Pb contents were analysed by Perkin-Elmer (Analyst Model 300) atomic absorption spectrophotometer. Data was subjected to analysis of variance (ANOVA) and Microsoft excel for standard error.

Results and Discussion

The results relating to effect of different concentrations of Pb and Cd on biomass (Plant growth) yield of *H. verticillata* are depicted in Table no. 1. It was observed that at 2.5, 5.0, 10.0 mg/l Pb and 0.5, 1.0 mg/l Cd supported the plant growth where as at 20.0 mg/l Pb and especially Cd at 5.0 mg/l affected the plant growth severely, thereby reducing the biomass on 7d exposure as compared to control. Slight increase in biomass was observed in 10.0 mg/l Pb and 20.0 mg/l Pb at 7d exposure as compared to same treatment at 3d exposure where as significant decrease in biomass was noted in 5.0 mg/l Cd treatment from 3d to 7d exposure duration. Stunted growth, and chlorosis were observed as most common effect of Cd toxicity in plants [9]. The reduction in the growth in *H. verticillata* could also be due to the suppression of the elongation growth rate of cells, because of an irreversible inhibition exerted by Cd on the proton pump responsible for the process [10]. By estimating biomass yield, Cd was found to be more toxic than Pb.

Table no. 1: Effect of different concentrations of Cd and Pb on biomass (fresh wt.) in gm of *H. verticillata* at different exposure periods

Duration of Exposure	Control	Lead (Pb) in ppm				Cadmium (Cd) in ppm			
		2.5	5.0	10.0	20.0	0.5	1.0	2.5	5.0
3d	22.04 ± 0.029	22.22 ± 0.136	22.72 ± 0.096	22.68 ± 0.329	21.21 ± 0.086	22.07 ± 0.163	21.91 ± 0.200	21.32 ± 0.156	20.41 ± 0.132
7d	23.78 ± 0.162	24.61 ± 0.083	24.37 ± 0.360	23.63 ± 0.121	22.78 ± 0.129	23.54 ± 0.118	21.23 ± 0.257	20.98 ± 0.225	19.32 ± 0.372

Values are means of ± SE (n = 3)

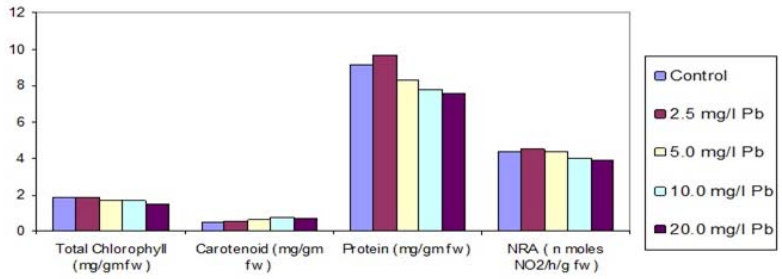

Figure 1: Effect of different concentrations of Pb on Total Chlorophyll, Carotenoid, Protein and NR activity of *H.verticillata* after 3d exposure period. Values are means of ± SE (n = 3)

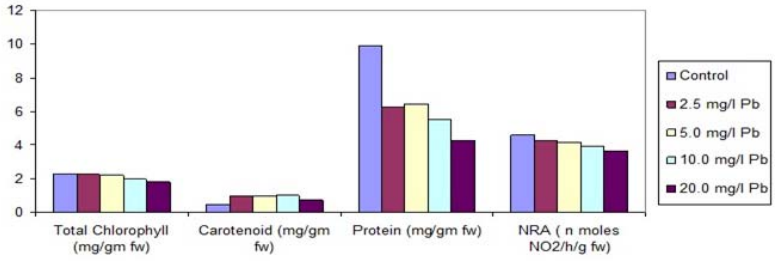

Figure 2: Effect of different concentrations of Pb on Total Chlorophyll, Carotenoid, Protein and NR activity of *H.verticillata* after 7d exposure period. Values are means of ± SE (n = 3)

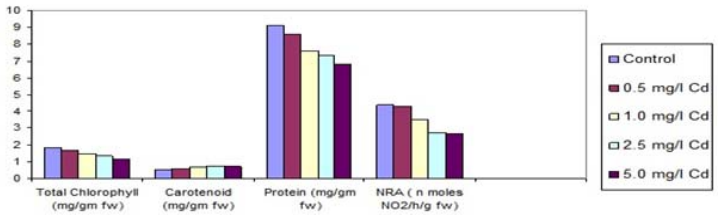

Figure 3: Effect of different concentrations of Cd on Total Chlorophyll, Carotenoid, Protein and NR activity of *H.verticillata* after 3d exposure period. Values are means of ± SE (n = 3)

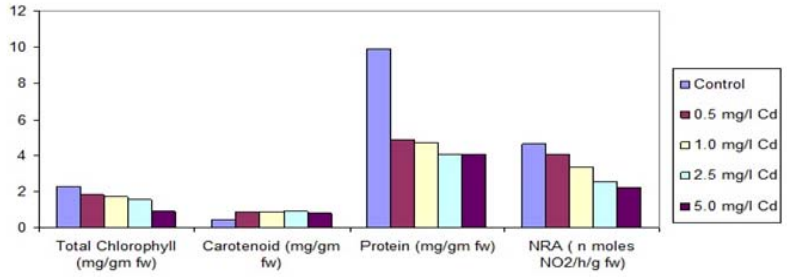

Figure 4: Effect of different concentrations of Cd on Total Chlorophyll, Carotenoid, Protein and NR activity of *H.verticillata* after 7d exposure period. Values are means of ± SE (n = 3)

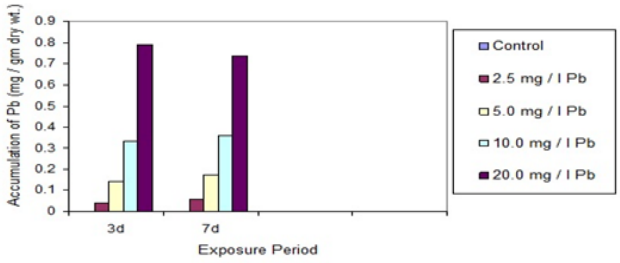

Figure 5: Accumulation of Pb (mg/gm dry wt.) by *H.verticillata* after 3d and 7d exposure period. Values are means of ± SE (n = 3)

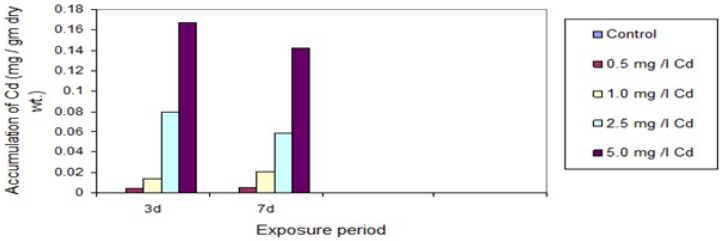

Figure 6: Accumulation of Cd (mg/gm dry wt.) by *H.verticillata* after 3d and 7d exposure period. Values are means of ± SE (n = 3)

Pb at 2.5 mg/l and Cd at 0.5 mg/l showed significant increase in total chlorophyll while at higher concentration (20.0 mg/l Pb and 5.0 mg/l Cd) they showed decrease in total chlorophyll during both exposure periods (figure 1,2,3&4) . After 3d there was marginal increase in total chlorophyll at 2.5, & 5.0 mg/l of Pb and 0.5, &1.0 mg/l of Cd. As the metal concentration increased, total chlorophyll content decreased at 3d and 7d exposure period as compared to control. Higher metal toxicity leads to chlorosis & stunted growth due to which total chlorophyll content observed to be decreased. Various abiotic stresses decrease the chlorophyll content in plants [11]. The decline in chlorophyll content in plants exposed to Cd^{2+} and Pb^{2+} stress is believed to be due to (a) inhibition of important enzymes associated with chlorophyll biosynthesis ;(b) impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls ;(c) Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase [12].

Carotenoid parameter increased with increase in concentration at 3d of exposure with maximum increase in 10.0mg/l Pb & 20.0 mg/l Pb and 2.5mg/l Cd & 5.0 mg/l Cd of exposure as compared to control. It was observed that after 7d of exposure, there was decrease in carotenoid content at 2.5 and 5.0 mg/l Cd concentrations as compared to other treatments of Pb and Cd (as showed in figure 1,2,3&4). Carotenoid, a non-enzymatic antioxidant, is a part of photosynthetic pigment, playing an important role in protection of chlorophyll pigment under stress conditions. Recently, Sinha et al [13]. reported an increase in carotenoid content in submerged plant of *Najas indica* at lower concentration of Fe under repeated metal exposure after 3day.

Protein found to be increase in 2.5 mg/l Pb as compared to control at 3d of exposure period. Due to metal stress, protein decreased at higher concentration of Cd and Pb in both the duration as compared to control (as depicted in figure 1, 2, 3&4). Gupta et. al. [14] reported an increase in protein content with increase in copper concentration (upto 80 μM) in plant of *H. verticillata* upto 96h, however, protein content decreased at 8 μM onwards after 168h. Protein was found to be increase at lower metal concentrations while reverse pattern was observed at higher metal concentrations in 3d of exposure where as in 7d of exposure period, lower metal concentration of Pb and Cd both resulted in decreased protein as compared to control. Nitrate reductase activity found to be slightly increased at 2.5 mg/l Pb & 5.0 mg/l Pb and 0.5 mg/l Cd at 3d exposure whereas

higher metal concentration of Pb and Cd after 7d found to be more toxic which resulted, decrease in NR activity as compared to control.

Submerged plants possess significant potential to bioconcentrate metals due to their greater surface area as compared to non-submerged plants [15, 16, 17]. The rate of accumulation of Cd and Pb was higher at lower concentrations. For lower concentrations i.e. 2.5 mg/l, 5.0mg/l, 10.0 mg/l Pb and 0.5 mg/l, 1.0 mg/l Cd respectively, the uptake was concentration and duration dependent. However, at higher concentrations i.e. 20.0 mg/l Pb and 5.0 mg/l Cd, the uptake of both metals was lower and after 7d of exposure, the absorption was almost stagnant due to the toxic effect caused to the plant (as depicted in figure 5 & 6). Rahmani and Sternberg [18] observed the complete die – off in *L. minor* at high doses of Pb. Some literature data show a higher Cd accumulation in shoots than in roots [19] as well, although other authors reported a higher content in roots than in shoots. This present study showed that Cd is more toxic than Pb.

Conclusion

Thus the exposure of *H. verticillata* to different concentrations of Cd and Pb shows an increase in biomass, total chlorophyll, carotenoid, protein, *in vivo* nitrate reductase activity at lower concentration as compared to control during both 3d and 7d exposure period whereas at higher metal concentration total chlorophyll, protein and NR activity were reduced more during 7d exposure period than 3d exposure duration. Higher concentrations of Cd effected the growth and development of *H. verticillata* more significantly than higher concentration of Pb.

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