Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

# Effect of lead and cadmium on aquatic plant *Hydrilla verticillata*

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**Publication Info**

*Paper received:*
16 January 2012

*Revised received:*
01 June 2012

*Accepted:*
15 July 2012

**Abstract**

Absorption of different concentrations of Lead (Pb) and Cadmium (Cd) by aquatic plant *Hydrilla vertcillata* was measured during winter season for two different durations i.e. 3 days and 7 days. Effect of Pb and Cd was evaluated by analyzing various parameters such as biomass, total chlorophyll, carotenoid, protein, nitrate reductase activity, SOD (super oxide dismutase) and heavy metal uptake. Increase in biomass, total chlorophyll, protein and nitrate reductase activity was noticed at lower concentration of both metals whereas at higher metal concentrations of Pb and Cd, decrease in these parameters was observed i.e. it was concentration and duration dependent. Increase in carotenoid and SOD levels at high concentration of Pb and Cd indicated its ability of stress tolerance. Accumulation of Pb by test plant was found to be more than Cd at low concentration. Higher concentration of Cd and Pb caused toxicity which resulted in reduced plant growth and physiological activities.

**Key words**

Biochemical parameters, *H. verticillata*, Heavy metals, Toxicity

## Introduction

Heavy metals have become ubiquitous in the environment due to rapid growth in industrialization and urbanization. Occurrence of toxic heavy metals in lakes, reservoir and river water affects the lives of local people that depend upon these water sources for their daily requirements (Rai *et al.*, 2002). Mainly reducible fractions of heavy metals and metalloids constitute potential risk to living being, especially because of their solubility, in aquatic environment (Boughriet *et al.*, 2007). The most important challenge is how to improve the efficiency of phytoremediation by increasing the accumulation of metals in plants, or by improving key plant biological traits that should enhance metal uptake (Wu and Tang, 2009; Sarma 2011). Lead and cadmium are common toxic metals found in waste water or polluted water. Effluents emanating from industrial sources and urban runoff contain high level of toxic metals like Pb, Cd, Cr, Ni that are discharged into nearby water bodies. Both Pb and Cd are toxic, non-essential metals of no known function in biological systems. Increased levels of Pb and Cd in soil may affect soil productivity and even low concentration of these metals can inhibit vital metabolic processes in plant (Sharma, 2005; Main and Sune, 2001).

Many aquatic macrophytes like *Eichhornia crassipes, Azolla pinnata, Lemna* sp., *Hydrilla verticillata* possess the ability to remove heavy metals from aquatic environment and are being used in waste water renovation systems (Kadlec *et al.*, 2000; Rai *et al.*, 2008). These plants absorb metallic ions and deposit them in different parts of the plant body depending upon their affinity towards particular metal. In view of above, the present study deals with Pb and Cd accumulating potential of *Hydrilla verticillata* during winter season and also reports related phytotoxicity.

## Materials and Methods

**Experimental set-up :** The submerged leafy, aquatic plants

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of *H.verticillata* (l.f.) Royle were collected from the pond of Plant Science Department, M.J.P.Rohilkhand University, Bareilly. These plants were further acclimatized in tap water during winter season ( mid-November – December ) for six weeks. Selected healthy plants were treated with different concentrations of Pb (2.5, 5.0, 10.0, 20.0 ppm ) and Cd (0.5, 1.0, 2.5, 5.0 ppm) separately during winter season (December–January). Initially in each tub, 20 g of healthy plants was added. Three replicates of each treatment were kept in 4 l capacity tubs. Plant's in tap water without treatment served as control. The plants were harvested after 3 and 7 days of treatment. Metal solutions were changed on every 2nd day. The harvested plants were washed with distilled water and used for estimating various parameters.

**Estimation of heavy metals in plant tissue:** Accumulation of Pb and Cd in treated plants were estimated after wet-digestion of the oven-dried plant material with nitric acid : perchloric acid (3:1, v/v) mixture (APHA, 2005). The metal contents were analyzed by Perkin-Elmer (Analyst Model 300) atomic absorption spectrophotometer.

**Determination of biochemical parameters :** Total chlorophyll and carotenoid content in fresh leaves (100 mg) of control and treated plants was determined following the method of Arnon (1949) and Duxbury and Yentsch (1956) respectively. Protein content was measured by the method of Lowry *et al.* (1951). *In vivo* nitrate reductase activity was estimated following the procedure of Srivastava (1974).

SOD activity was assayed by the method of Giannopolitis and Reis (1977) using enzyme extract. Reaction mixture (3ml) contained 50 mM potassium phosphate buffer (pH 7.8), 1.3 µM riboflavin, 0.1mM EDTA, 13 mM methionine, 63 µM NBT, 0.05 M sodium carbonate (pH 10.2) and enzyme extract (100µl). The photoreduction of NBT was measured at 560 nm. One unit of SOD activity is defined as the amount of enzyme, which is required to cause 50% inhibition in the reduction of NBT.

**Statistical analysis :** Data were summarized as mean ± SD. Treatments were compared by two factor analysis of variance (ANOVA) and the significance of mean difference within and between the treatments was done by Duncan multiple range test (DMRT). A two-sided $p<0.05$ was considered statistically significant.

## Results and Discussion

In the present study, plants of *H. verticillata* treated with different concentrations of Pb and Cd accumulated metals in a concentration-duration manner except at higher concentrations of Pb and Cd on 7th day. The plants accumulated significantly ($p<0.001$) higher amount of Pb as compared to Cd (Fig.1a). *H.verticillata* has been reported to accumulate high amount of metals like Cd, Cr and As within their tissue (Rai *et al.*, 1995; Garg *et al.*, 1997; Gupta *et al.*, 1996 and Srivastava *et al.*, 2009) from wastewater.

In response to metal accumulation, a significant ($p<0.01$ or $p<0.001$) increase in biomass was observed upto 10 ppm Pb and 0.5 ppm Cd treatment after 3 days and upto 2.5 ppm Pb treatment after 7 days of exposure as compared to control plants. However, a significant decrease ($p<0.05$ or $p<0.001$) in biomass was observed at 5.0 ppm (1.32%) to 20.0 ppm Pb (6.64%) and 0.5 (0.64%) to 5 ppm (9.34%) Cd treatment as compared to control after 7 days of exposure during winter season (Fig. 1b) Increase in biomass at lower doses of Pb and Cd may be due stimulatory effect. Mishra *et al.* (2007) reported stimulatory effect of Cd on dry weight of *E. crassipes* Decrease in biomass production in response to heavy metal accumulation has been earlier reported in several aquatic plants like *H.verticillata, B. monnieri, C. demersum, Salvinia* and *E. crassipes* (Singh *et al.*, 2011; Shukla *et al*., 2007; Singh *et al*., 2008).

As compared to untreated plants, Pb and Cd treated plants of *H. verticillata* showed increase in total chlorophyll content upto 5.0 ppm Pb and 0.5 ppm Cd treatment after 3 days of exposure. However, at higher concentrations a significant ($p<0.01$ or $p<0.001$) decrease in total chlorophyll content was observed after 3 and 7days of exposure as compared to control during winter season (Fig.1c). Inhibition of chlorophyll content in response to $Cd^{2+}$ and $Pb^{2+}$ stress has been reported in aquatic plants due to following reasons: inhibition of important enzymes associated with chlorophyll biosynthesis; impairment in the supply of $Mg^{2+}$ and $Fe^{2+}$ required for the synthesis of chlorophylls or $Zn^{2+}$ deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Vajpayee *et al.,* 2000; Mishra *et al.*, 2006).

In the present study, a dose and time dependent increase in carotenoid content was observed in Pb and Cd treated plants of *H.verticillata* at highest Pb and Cd concentration where carotenoid level decrease significantly ($p<0.001$ or $p<0.01$) on 7th day (Fig.1d).. Maximum carotenoid content was found at 10 ppm Pb and 2.5 ppm Cd after 7 days of exposure. This increase in carotenoid level is a defence strategy adopted by plants to nullify the toxic effect of free radicals generated during metal stress. Kenneth *et al.* (2000) reported that many plants have quite large difference in the absolute level of carotenoid and synthesize zeaxanthin in response to stress condition.

Fig. 2a shows the effect of Pb and Cd on the protein content of *H. verticillata*. As compared to control, protein content (5.75%) increased upto 2.5 ppm Pb treatment after 3 days and decreased further at all concentrations and durations. On 7th day, a maximum decrease of 23.56% in protein content was observed at 20.0 ppm Pb treatment as

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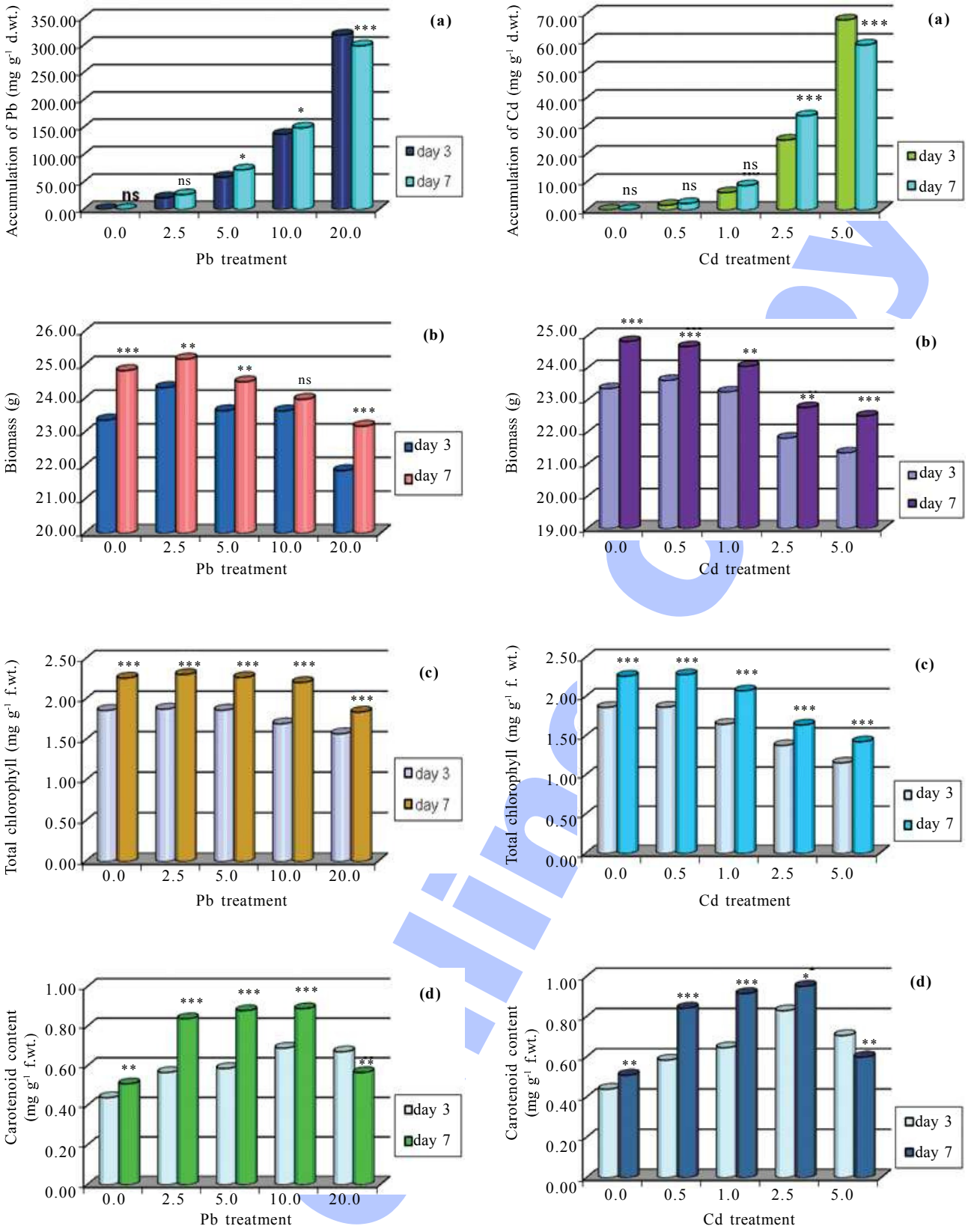


**Fig. 1 :** (a) Pb and Cd accumulation in *H.verticillata* and effect of Pb and Cd on (b) biomass (c) total chlorophyll (d) carotenoid content in *H.verticillata* after 3 and 7days of exposure during winter season. ns $p>0.05$ or $^{*}p<0.05$ or $^{**}p<0.01$ or $^{***}p<0.001$- day 3 vs. day 7

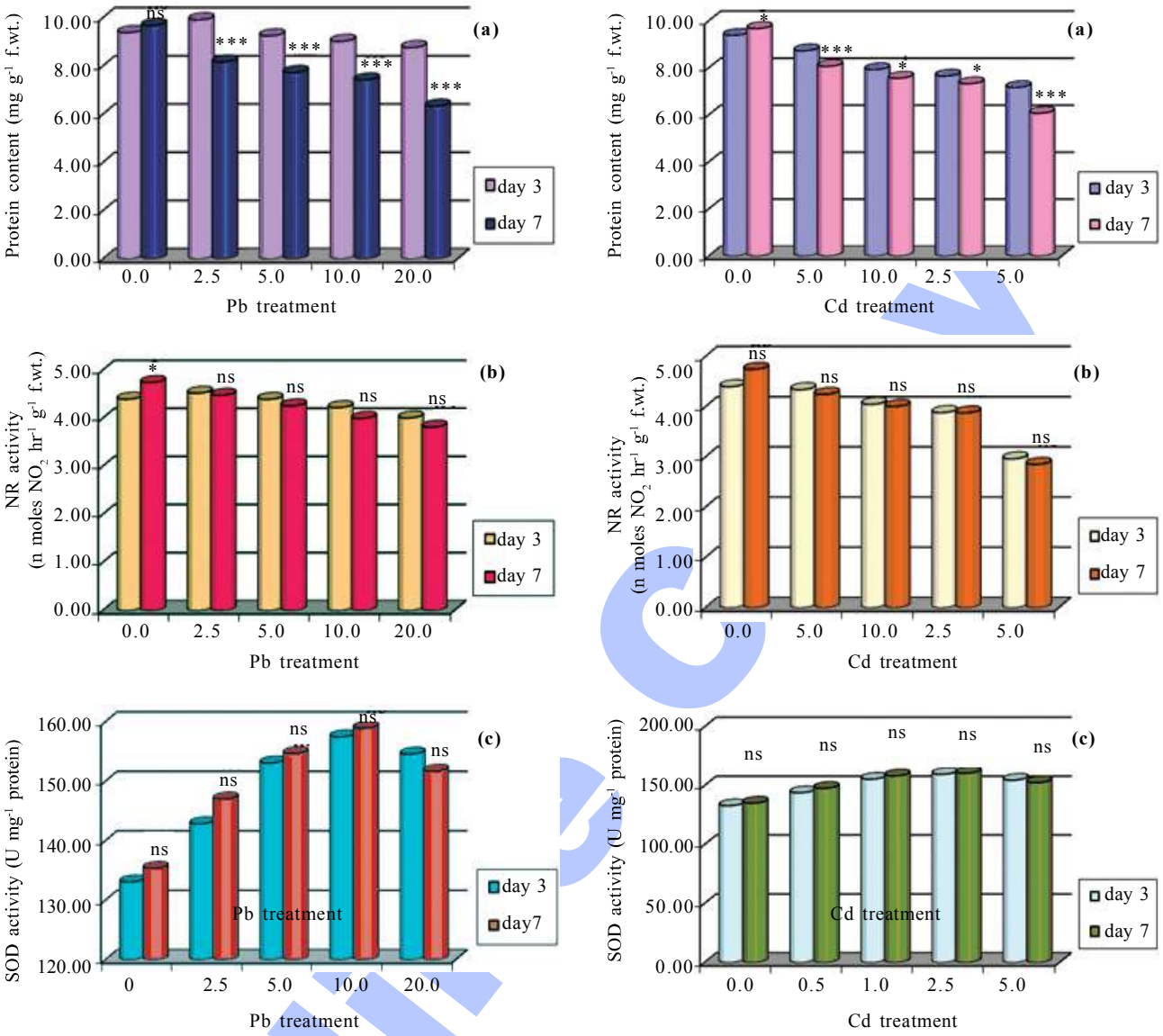


**Fig. 2 :** Effect of different concentrations of Pb and Cd on (a) Protein (b) *in-vivo* NR activity (c) SOD activity in *H.verticillata* after 3 and 7days of exposure during winter season. ns $p>0.05$ or $^{*}p<0.05$ or $^{**}p<0.01$ or $^{***}p<0.001$- day 3 vs. day 7

comparted to control. Whereas Cd treatment decreased protein content in a concentration-duration manner in *H. verticillata.*

The effect of Pb and Cd during winter season on *in vivo* NR activity of *H.verticillata* is shown in Fig. 2b. *In vivo* NR activity marginally increased by 2.95% at 2.5 ppm Pb treatment as compared to control after 3 days of exposure and decreased further at higher concentrations and duration. Mishra *et al.* (2007, 2008) reported inhibition of *in vivo* NR activity due to Cr and Cd accumulation in the *Eichhornia crassipes*.

In the present study, SOD activity in *H. verticillata* increased at all treatments of Pb and Cd as compared to control plants. As compared to control, maximum SOD activity of 18.33 and 20.05% was observed at 10 ppm Pb and 2.5 ppm Cd treated plants after 7 days of exposure (Fig. 2c). SOD is the key enzymatic antioxidant activated due to production of ROS under different stress condotions (Scandalios, 1993). Singh *et al.* (2006) and Mishra *et al.* (2006) reported induction of SOD activity due to production of ROS in response to Cd and Pb treatment in *B. monnieri*.

In conclusion, the study reveals that plants of *H.verticillata* treated with different concentrations of Pb and Cd accumulated significant amount of metal in their tissues and showed related phytotoxicity. Cd was found to

be more toxic than Pb as it hampered plant growth to a greater extent.

## Acknowledgments

We would like to thank the Head, Deptartment of Botany, Bareilly College, Bareilly for the support and assistance provided during research work. We highly acknowledge and thank the Director, Institute for Data Computing and Training (I.D.C.T.), Lucknow, for providing valuable assistance in statistical analysis.

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