INTERNATIONAL JOURNAL OF ENVIRONMENT
Volume-3, Issue-2, Mar-May 2014 ISSN 2091-2854
Received:5 March *Revised:25 March* *Accepted:13 May*

BIOMONITORING OF LEAD (PB) TOXICITY THROUGH AQUATIC MACROPHYTE *EICHHORNIA CRASSIPES*

Kiran Gupta
Plant Genetic Unit, Department of Botany,
University of Lucknow, Lucknow-206007, INDIA
Corresponding author: sunrays79@gmail.com

Abstract

A study was performed for biomonitoring the toxicity of lead (Pb) in water hyacinth *(Eichhornia crassipes).* To assess phytotoxic response of the plant against Pb chlorophyll content, protein content and NR activity has been observed while genotoxiocity was analyzed by study of mitotic index and micronuclei (MNCs) as genotoxic end point. For this purpose, Plants of *Eichhornia crassipes* were exposed to various concentrations of Pb (0.0, 1, 2, 4 and 8 ppm) for 2 and 7 days durations. Pb induced phytotoxicity was evident by reduction in chlorophyll content, protein content and nitrate reductase activity (NR activity). All aforesaid parameters decreased in dose-duration dependent manner. Pb treatment also led to dose-duration dependent inhibition of mitotic index (MI) and induction of micronuclei in root meristematic cells of *E. crassipes*. Decline in MI reflects cytotoxicity that directly affects root growth and elongation. Pb may cause cell death, which may appear as decline in MI. Micronucleus induction involving the mitotic spindle and consequent production of laggard chromosomes during anaphase and loss of a complete chromosome. The present investigations revealed that *E. crassipes* exposed to Pb experienced phyto-genotoxicity, therefore it can be utilized as biomonitoring tool for toxicity of Pb assessment.

Key words: *Eichhornia crassipes*, Pb, Biomonitoring, phyto-genotoxicity

Introduction

Among the heavy metal, Pb is one of the 13 metal on the United States Environment agency's list of 129 priority pollutants (Pratap et al., 2006). The main source of lead pollution is the industrial discharges from smelters, battery manufacturing units, run off from contaminated land areas, atmospheric fall out and sewage effluent. Lead is known to induce Reactive oxygen Species (ROS), which are potentially toxic and induce unspecific oxidation of proteins, membrane lipids and DNA injury (Schutzendubel and poole, 2002). Excessive levels of Pb can also cause anemia, liver diseases, paralysis, brain damage and death in human being and mammals. Pb also affects the glutathione disulphide activity, thus altering

lipid peroxidation in liver and brain (Aykin-Burns et al., 2003). The metal has been found to influence directly leyding cells steriodogenesis which results in reduction of testosterone and causes low sperm counts in animals as well as in human beings (Lin et al., 2003). Lead also affects the energy metabolism in human erythrocytes *in vitro* (Baranowska-Bosiacka et al., 2003). Unlike other heavy metals, Pb is not required even in trace amount for the growth and development of plants as well as human beings.

Recently, many plants including water hyacinth have become important in pollution treatment systems and used successfully to remove Cr. It will also be used well for genotoxicity testing due to well-developed profuse root system for monitoring and phytoremediation of low level of cadmium in water (Mishra et al., 2007). The objective of present study was to assess bioconcentration, cytotoxicity, phytotoxicity and bioremediation potential of aquatic Cr by water hyacinth bioassay.

Materials and Methods

For experiment, young plants of *E. crassipes* were collected from an unpolluted water body from Botany Department of Lucknow University and acclimatized in hydroponic tubs under natural condition at Lucknow University, Lucknow. From this young plants were selected for experimental purposes. Cultures were placed in a growth chamber (light: dark cycle 14:10 hr, temperature 28±2°C, 115 mol $m^{-2}s^{-1}$ illumination provided through day fluorescent tube light). Various concentration (0.0, 1.0, 2.0, 4.0 and 8.0 ppm) of Pb were prepared by adding the required aliquots of 1000 ppm stock solution of $PbNO_3$ to the 5% Hoagland. Plants of *E. crassipes* were transferred into 250 ml plastic beakers containing 200 ml Pb supplemented medium. Three sets, each of seven concentrations were placed separately in a growth chamber under conditions mentioned above. Plants placed in 5% Hoagland solution without chromium served as control. For the estimation of bio concentration experimental cultures were aerated 6 hr a day. One set of each concentration was harvested after 2 and 7 days of the treatment and washed three times with double distilled water. Oven dried (80^oC) plant tissues (leaves and roots) were digested in HNO_3:$HClO_4$ (3:1 v/v) and chromium concentration was estimated by a flame atomic absorption spectrophotometer (PerkinElmer 2380).The chlorophyll of fresh leaves was estimated by the method of Arnon (1949) using 80% acetone. Protein was estimated by the method of Lowry et al. (1951) using egg albumin as standard in roots or leaves. Nitrate reductase (NR) activity was assayed in leaves by the method of Srivastava (1974) and activity was expressed on fresh weight basis. Root meristems were fixed in Carnoy's fluid for mitotic index (Darlington and Lacour, 1976) and micronuclei end points (Panda et al., 1989).The experiments were conducted taking three replicates(n=3) for each parameter. The data was subjected to test the significance of variation among the each parameter through two way ANOVA (Gomez and Gomez, 1984).

Results

Total chlorophyll content was inhibited at all concentration and duration. Maximum inhibition in total chlorophyll (24.12%) content has been recorded at 8 ppm after 7 day time exposure (Fig-1). In comparison to unstressed plant protein content was inhibited in a concentration and duration dependent manner. Maximum inhibition in total chlorophyll

(35.65%) content has been recorded at 8 ppm after 7 day time exposure (Fig-2). Similarly, in *vivo* NR enzyme activity was decreased at all treatments with maximum inhibition of 39.34 at 8 ppm after 7 days exposure (Fig-3).

Lead inhibited mitotic index in dose duration dependent manner. Treated plant showed maximum inhibition of 2.65 mitotic index at 8 ppm after 7 day time duration (Fig-4) while micronuclei has been induced at dose - duration dependent manner. Result revealed maximum induction of 16.78 at 8 ppm after 7 days treatment (Fig-5,6a and 6b).

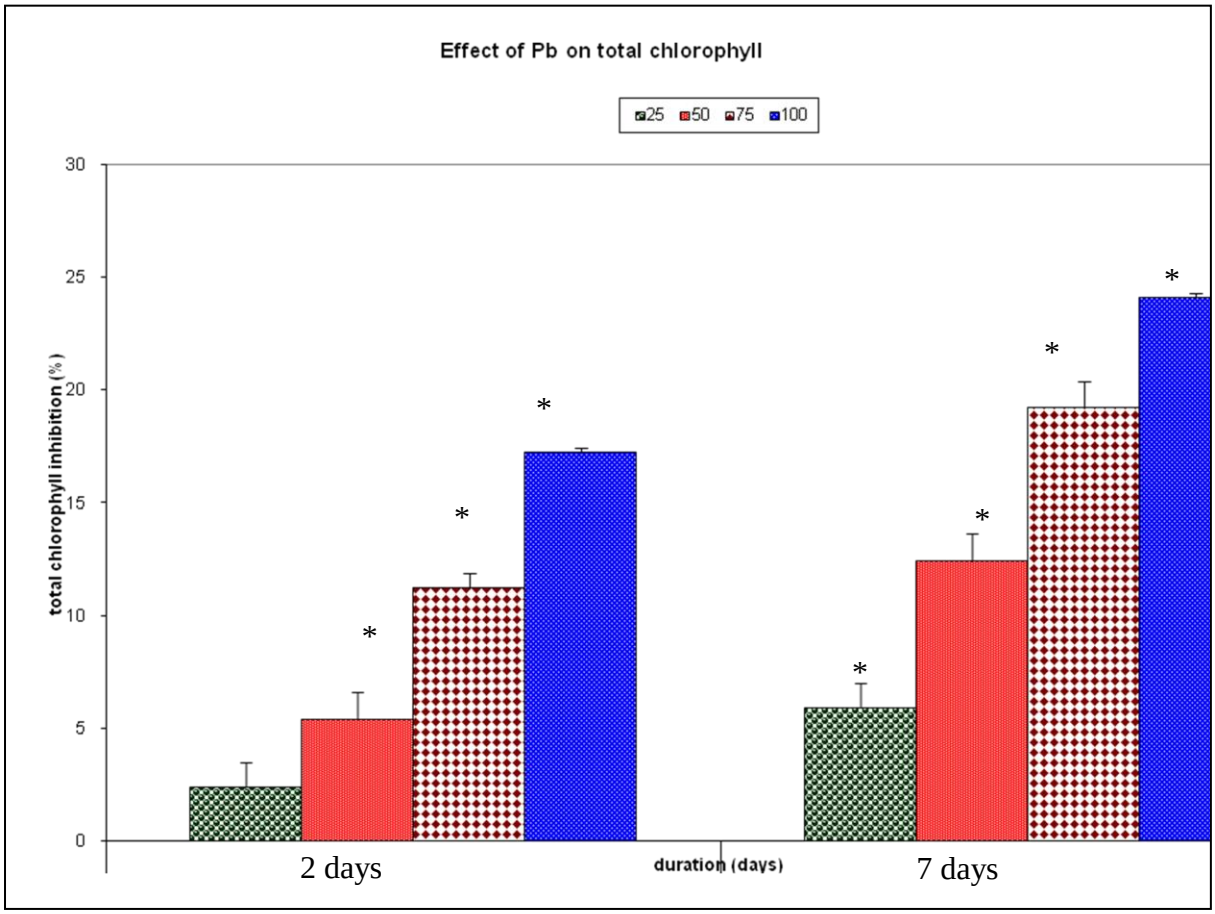

Fig:1 Effect of Pb on Total chlorophyll content inhibition in *E. crassipes* . All values are mean of triplicates ±S.D.* = Significance (p<0.01) compare to control

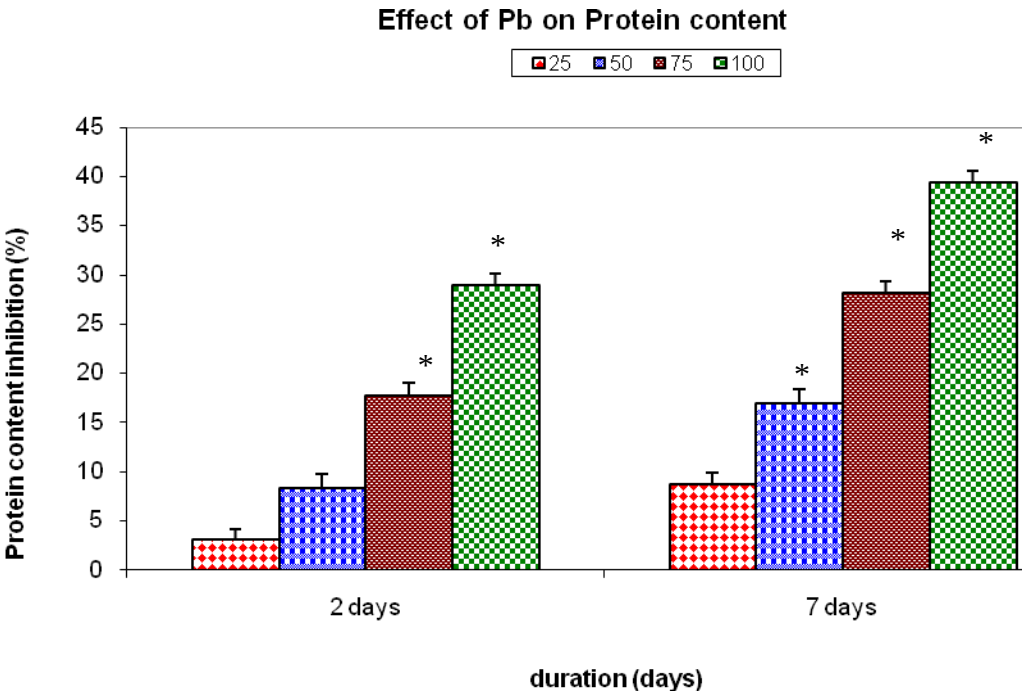

Fig: 2 Effect of Pb on Protein content inhibition in *E. crassipes* . All values are mean of triplicates ±S.D.* = Significance (p<0.01) compare to control

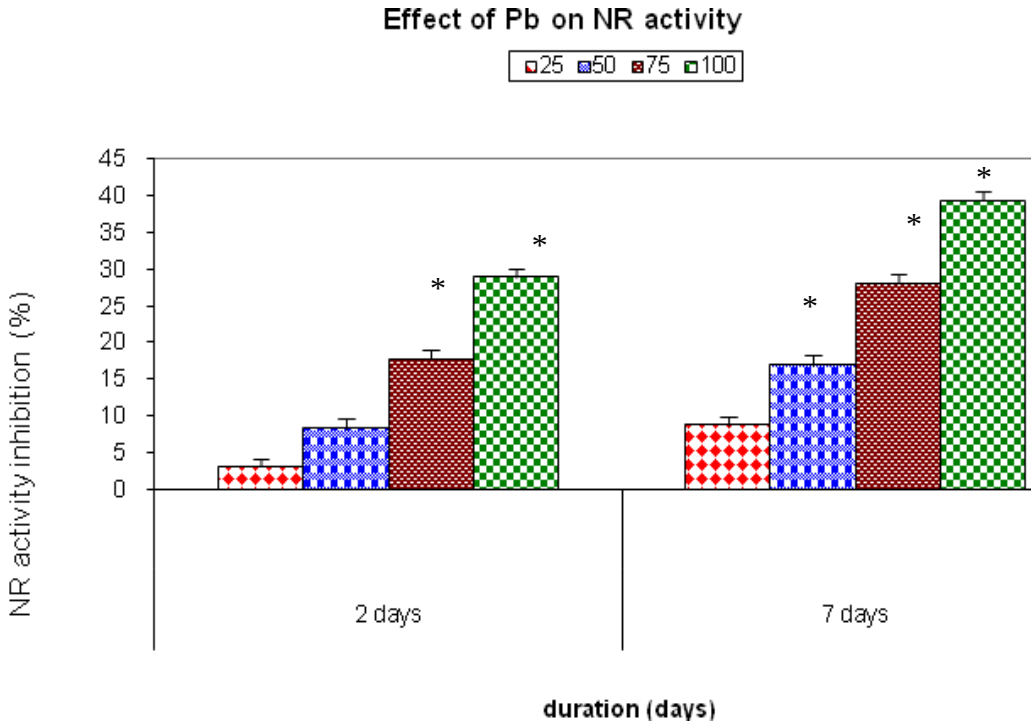

Fig: 3 Effect of Pb on NR activity inhibition in *E. crassipes*. All values are mean of triplicates ±S.D.* = Significance (p<0.01) compare to control

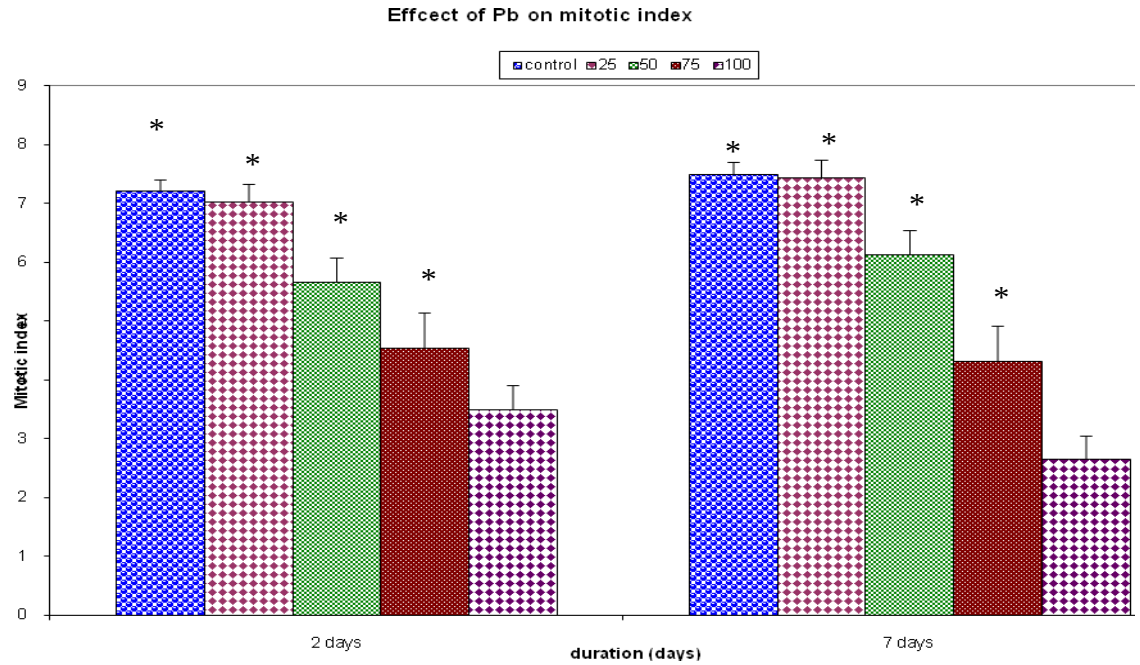

Fig:4 Effect of Pb on Mitotic index in *E. crassipes*. All values are mean of triplicates ±S.D.* = Significance (p<0.01) compare to control

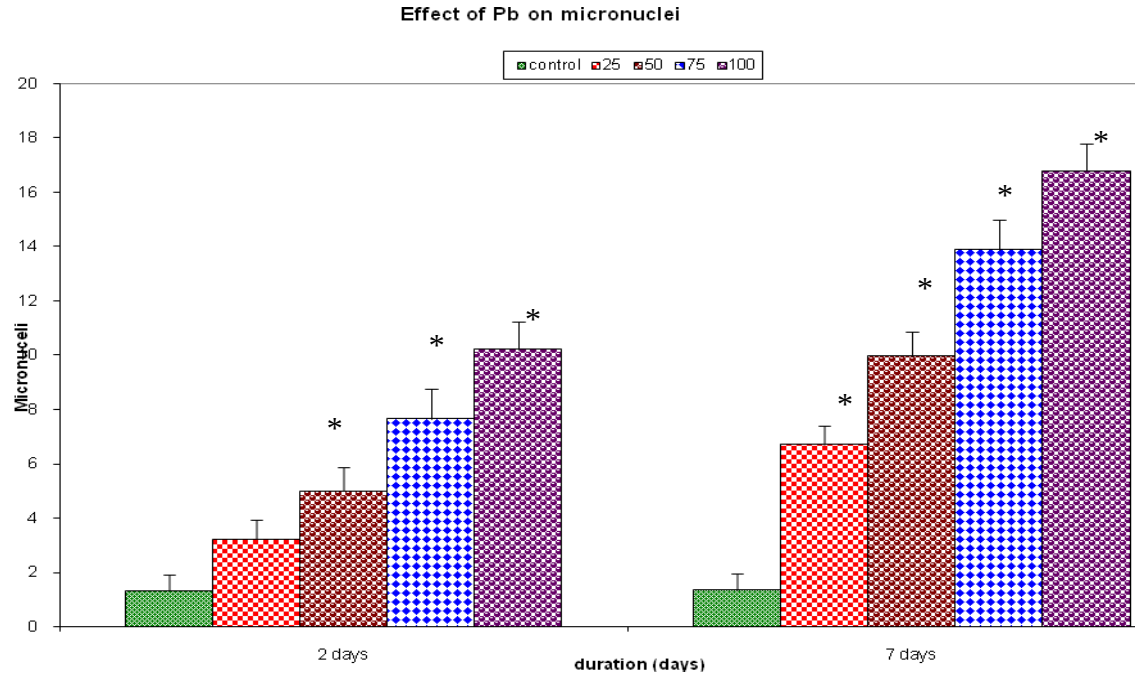

Fig:5 Effect of Pb on Micronuceli in *E. crassipes. All values are mean of triplicates ±S.D.* = Significance (p<0.01) compare to control

.

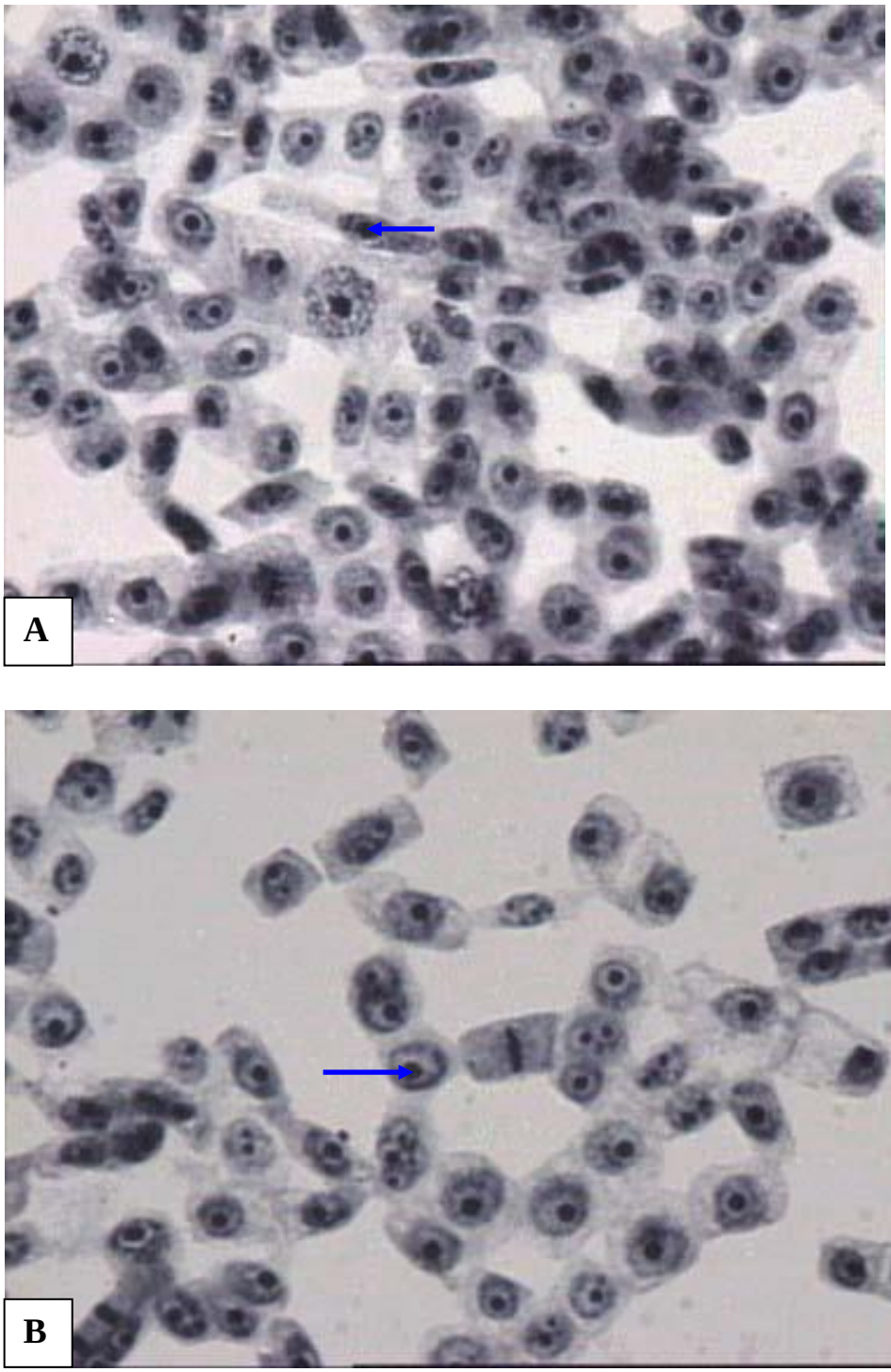

Fig: 6 An arrow showing micronuclei in (A) and (B)

Discussion

The chlorophyll content decreased with increase in concentration and duration of lead treatment. Lead is well known from numerous studies to interfere with & inhibit photosynthesis and transpiration rate with supply of metal (Seregin & Ivanov, 2001). Heavy metal accumulation in vascular plant is known to produce significant physiological and biochemical responses (Jana, 1988). A decrease in chlorophyll content may either be due to inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions (Chandra et

al., 2009). The decline in protein content under heavy metal stress in aquatic plants has been reported. A decrease in protein content in presence of heavy metal ions may be due to the breakdown of soluble protein or due to the increased activity of protease or other catabolic enzymes, which were activated and destroyed the protein molecules (Mishra et al., 2009). A positive correlation between NR activity and protein content has also been demonstrated in earlier studies (Vajpayee et al., 2000). The present findings are in agreement with earlier reports on Cr toxicity to chlorophyll, NR activity and protein content (Rai et al., 1992). Lead toxicity in many plants were reported to be associated with the disturbance of mitosis , toxicity to nucleoli , induction of bi nuclear cells and the inhibition of root elongation (Pratap et al., 2006). The mitotic index reflects the frequency of cell division and it regarded as an important parameter to determine the rate of root growth. The mitotic index can be correlated with the rate of root, suggesting that the inhibition of growth resulted from the inhibition of the cell division. Micronuclei cell frequency increased with increase in Pb concentration and duration. The presence of micronuclei indicates genetic damage induced by physical and chemical agents. MNC are induced through disturbance of spindle or through chromosome breakage (Gupta et al., 2012). Panda et al., (1989) suggested the use of MNC assay as a genotoxic end point. The present findings about mitotic index and micronuclei are in agreement with Pratap et al. (2006).

Thus it can be concluded from present result that *E. crassipes* is good biomonitoring tool for aquatic environmental pollution caused by Pb.

Acknowledgment

Authors are thankful to Head of Botany Department, Lucknow University for providing all required facility and Dr. Kumkum Mishra, Associate Professor of the same department for her valuable suggestions.

References

Arnon, D.I., 1949. Copper enzymes in violated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiology, 24, 1-15.

Aykin-Burns, N., Lacgeler, A., Kellog, G. and Ercal, N., 2003. Oxidative effect of Lead in young and adult fisher 344 Rats. Environment Contamination Toxicology. 44(3),417-420.

Baranowska-Bosiacka, I and Hlynczak, A.J., 2003. The effect of lead ion on energy metabolism of human Erythrocytes *in vitro* Comp. Biochemical Physiological C. Toxicology and Pharmacology. 134(3), 403-416.

Chandra, R., Bhargava, R.N., Yadav, S. Mohan, D., 2009. Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica compestries* L.) irrigated with distillery and tannery effluent. Journal of Hazardous Materials 162, 1514-1521.

Darlington, C.D. and Lacour, L.F., 1976. The handling of chromosomes. Allen and Unwin, London. p. 201.

Gomez, K.A. and Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. John-Willey and Sons, New York.

Gupta, K. Guamat, S. and Mishra, K., 2012. Studies on phytogenotoxic assessment of tannery effluent on *Allium cepa.* Journal of Environmental Biology 33, 557-563.

Jana, S., 1988. Senescence in submerged aquatic angiosperms: effect of Heavy metals . New phytology 99, 477-484.

Lin, M.Y., Leu, S.F., Yang, H.Y. and Huang, B.M., 2003. Inhibitory mechanism of lead on streoidogenesis in MA-10 Mouse leyding tumor cells. Archives of andrology 49(1), 29-38.

Lowry, O.H., Rasebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with folin-phenol reagent. Journal of Biology and chemistry 193, 265-275.

Mishra, K., Gupta, K. and Rai, U.N., 2009. Bio concentration and phytotoxicity of chromium in *Eichhornia crassipes.* Journal of Environmental Biology 30 (4), 521-526.

Mishra, K., Rai U.N. and Prakash, O., 2007. Bio concentration and phytotoxicity of Cd in *Eichhornia crassipes*. Environment Monitoring Assessment 130, 237-243.

Panda, K.K., Lenka, M. and Panda, B.B., 1989. *Allium* micronucleus (MNC) assay to assess bioavailability, bioconcentration and genotoxicity of mercury from solid waste deposits of a chloralkali plant, and antagonism of L. cysteine. Science Total Environment 79, 25-36.

Pratap, S., Gupta, K. and Mishra, K.K., 2006. Assessment of lead accumulation potential and its phytotoxic effects in *Eichhornia crassipes*. Ecology Environment and Conservation 12, 563-569.

Schutzendubel, A. and Poole, A., 2002. Plant response to abiotic stresses: Heavy metal induced oxidative stress and protection by michorrhization. Journal of Experimental Botany 53, 1351-1365.

Seregin, I.V. and Ivanov, V.B., 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. Russian Journal of plant Physiology 48,523-544.

Srivastava, H.S., 1974. In vivo activity of nitrate reductase in maize seedlings. Indian Journal of Biochemistry and Biophysics 11, 230-232.

Vajpayee, P., Tripathi, R.D., Rai, U.N., Ali, M.B. and Singh, S.N., 2000. Chromium accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content of *Nymphaea alba*. Chemosphere 41, 1075-1082.