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ASSESSMENT OF LEAD ACCUMULATION POTENTIAL AND ITS PHYTOTOXIC EFFECTS IN *EICHHORNIA CRASSIPES*

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ABSTRACT

Physico-chemical analysis and metal concentration were determined in effluent of two contaminated sites; Jajmau, Kanpur and Scooters India Limited (SIL), Lucknow. To assess the toxicity of Pb various parameters e.g. morphological, physiological, biochemical and genotoxicity were studied *in situ* as well as under laboratory conditions. Plants of *E. crassipes* were exposed to nutrient solution containing 0.01, 0.1, 2.5, 5.0 and 10.0 ppm of lead for 24, 48, 72 and 96 h. Leaf and root samples from treated culture were taken and analysed for bioconcentration of Pb. Accumulation of Pb more in roots (791 mg g^{-1} dw) in comparison to leaves (328.3 mg g^{-1} dw). The accumulation in root and leaf tissue was found to be both concentration and duration dependent. Phytotoxicity of Pb showed that chlorophyll a, b and total chlorophyll were stimulated from 0.01 to 0.1 ppm and inhibited at concentration ranging from 1.0 ppm to 10.0 ppm with the duration of exposure. Carotenoid content was induced from 0.01 2.5 ppm and decreased from 5.0 to 10.0 ppm. At lower concentration protein content and NR activity was induced, however, higher concentration had significant inhibitory effect on these two parameters. Root meristems were studied for mitotic index, which was induced from 0.01 to 0.1 ppm and decreased from 1.0 ppm to 10.0 ppm. It is concluded that lead inhibits cell division. Numerous micronuclei

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were also observed. The result indicated that water hyacinth is a good sensor to monitor low level of aquatic lead. Results showed high tolerance and accumulation potential of Pb by *E. crassipes*. The plant is found to be tolerant to the elevated lead concentration up to 0.1 ppm as there is no inhibition of chlorophyll and carotenoid. So this bioassay can be used for biomonitoring and control of heavy metal pollution in the aquatic environment.

INTRODUCTION

In recent years heavy metals have become ubiquitous in the environment due to rapid growth in industrialization and urbanization. Among the heavy metals, Pb is one of the 13 metal on the United States Environmental Agency's list of 129 priority pollutants (Keith and Teliard, 1979). Now-a-days much concern has been shown towards lead pollution of aquatic environment. The main source of Pb contamination of the aquatic systems are the industrial discharges from smelters, battery manufacturing units, run-off from contaminated land areas, atmospheric fallout and sewage effluents. Lead is known to induces the production of reactive oxygen species (ROS), which are potentially toxic and induced unspecific oxidation of proteins and membrane lipids and DNA injury (Schutzendubel and Poole, 2002). Excess level of Pb can also cause anemia, liver disease, paralysis, brain damage and death in human beings and other mammals. A recent study have shown that Pb effects 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 leydig cell steriodogenesis which result in reduction of testosterone and causes low sperm counts in animals as well as in human (Lin *et al*. 2003). Lead also affects the energy metabolism in human erythrocytes *in vitro* (Baranowska Bosiacka et al., 2003). Unlike, some of other metals, Pb is not required even in trace amount for the growth and development of plants as well as human beings.

In the context of above there is a pressing need to remove the Pb contamination in aquatic system. Aquatic macrophytes have been used during the last two decades for metal removal from contaminated water competing with other secondary treatments, being the principal mechanism for metal uptake adsorption through roots (Demirezen 2002). Among several aquatic plants, water hyacinth has become an important tool ub pollution treatment systems and used successfully to remove Pb (Wolverton and McDonald, 1979). It is also an excellent candidate for a plant standard reference material and genotoxicity testing because it has well developed root system (Beckert, 1978, Panda *et al*. 1988; Misra *et al*. 2006).

The present study has been carried out to evaluate the bioaccumulation, phytotoxicity, cytotoxicity and biomonitoring potential of lead using water hyacinth as a test model. In this study a comparative account of performance of the plant under both laboratory experiment and *in situ* condition was determined.

MATERIALS AND METHODS

For *in situ* study, effluents were collected from the industrial complex at Jajmau, Kanpur and SIL, Lucknow. The plants of *E. crassipes* were also collected from these contaminated sites for estimation of Pb accumulation. Different morphological characters e.g. number of plantlets, petiole length with and without leaf, root length, number of roots lets and diameter of petiole were determined. For the assessment of genotoxicity micronuclei and mitotic index end points were scored (Panda *et al*. 1988).

For laboratory experiments, healthy plants of *Eichhornia crassipes* were collected from unpolluted pond in and around Lucknow and placed in cemented tank containing tap water in the campus of Lucknow University. Phytoplanktons and soil particles adhering at the roots were removed from the plants under running tap water. After acclimatization plants were cultured in 5% Hoagland's solution for 15 days (Hoogland and Arnon, 1950). In each set 5 plants of uniform size having a good number of healthy roots were placed in plastic tubs containing 5 liter of experimental solution at 0.01, 0.1, 1.0, 2.5, 5.0 and 10.0 mg ml^{-1} concentration of Pb for each exposure. The stock Pb solution for these experiments was prepared from lead acetate ($Pb(CH_3COO)_2.3H_2O$).

Treated and untreated dried plant samples (500 mg) were digested in concentrated HNO_3: $HClO_4$ (v/v, 3:1) at 80°C temperature till a clear solution was obtained. Lead estimation was done by using Atomic Absorption Spectrophotometer model **********

Chlorophyll and carotenoid were estimated in 80% chilled acetone extracts (Arnon, 1949), using Milton Roy 1201 Spectronic Spectrophotometer. Protein was estimated by the method of Lowry *et al*. (1951) using egg albumin as reference. *In vivo* nitrate reductase activity was measured following the method of Srivastava (1974) with some modification.

Table 1
Physico-chemical characteristics of effluents

Parameters	Values	
	Jajmau, Kanpur	Scooters India Ltd., Lucknow
Colour	Light grey	Greyish green
Odour	Unpleasant	Unpleasant
Temperature (°C)	29.0°C ±1.24	34.0°C ±1.89
pH	7.8±0.62	7.9±0.63
DO	0.27±0.07	0.21±0.10
BOD	7.6±2.01	5.3±1.62
COD	129±2.48	107±2.13
Pb	0.826±0.012	1.713±0.013
TS	3892±9.74	3754±8.04
TDS	3420±6.25	3315±7.59
TSS	472±3.79	439±2.34

Values are given in mg l^{-1} except pH and temperature. Mean ±S.E. (n=3)

Table 2

Morphological variations, Pb concent and cytological variationin *E. crassipes* growing in water bodies at Jajmau, SIL and laboratory

Parameters	Values		
	Control	Jajmau	Scooters India Ltd. (Lucknow) (Kanpur)
Number of plantlets	7.00±0.89	6.00±1.06	7.00±0.73
Petiole length without leaf	18.00±2.30	14.80±1.31	8.56±0.92
Petiole length with leaf	25.40±2.20	21.50±1.76	14.31±1.39
Root length	20.00±3.80	25.40±1.74	22.33±1.34
Number of rootlets	54.00±6.30	85.00±4.93	65.00±8.53
Diameter of petiole	4.80±0.99	6.61±0.68	8.03±0.66
Pb in leaf	-	48.3±4.25	76.8±3.45
Pb in root	-	675±7.13	1302±11.20
Mitotic index	7.51±0.14	4.32±0.12	5.02±0.11
Micronuclei cells	1.42±0.14	6.83±0.13	5.26±0.10

Metal accumulation are given in mg g^{-1} dw and length given in centimetre. Mean ±S.E. (n=3)

For cytological studies roots were excised at intervals of 24, 48, 72 and 96 hr and fixed in Carnoy's fluid (Darlington and Lacour, 1976). *E. crassipes* with its many small chromosomes (2n=32) was found unsuitable for analysis of chromosome aberrations or for sister chromatid exchange, instead the cytological end points mitotic index and the frequency of interphase cells with micronuclei (MNC) were scored following the standardized procedure for water hyacinth (Panda *et al*. 1988).

The experiments were conducted in 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).

RESULT

Physico-chemical analysis of effluent samples were carried out (Table 1). The effluents were slightly alkaline (pH 7.8 and 7.9) having dissolved oxygen (DO) 0.27 and 0.21 mg l^{-1}, biochemical oxygen demand (BOD) 7.6 and 5.3 mg l^{-1}, chemical oxygen demand (COD) 129 and 107 mg l^{-1}, total solids 3892 and 3754 mg l^{-1}, total dissolved solids (TDS) 3420 and 3315 mg l^{-1}, total suspended solids (TSS) 472 and 439 mg l^{-1} and Pb content was 0.826 and 1.713 mg l^{-1} in Jajmau and SIL, respectively.

Different morphological parameters were measured in control, Jajmau and SIL. The number of plantlets in control and above mentioned sites were 7,6 and 7. The peticle length without and with leaf were 18.0, 14.8, 8.56 cm and 25.4, 21.5, 14.31 cm, respectively. The root length and number of rootlets were 20.0, 25.4, 22.33 and 54, 85, 65, respectively. Whereas the diameter of petiole was 4.8, 6.61 and 8.03 cm respectively. All the morphological parameters showed decrease in comparison to control except root length, number of root lets and diameter of petiole (Table 2). There are distinct morphological variation in *E. crassipes* collected from of Jajmau and SIL contaminated sites. Root length and number of rootlets were much greater in Jajmau and SIL plants in comparison to control. The diameter of petiole of these plants were also very much swollen which provide byoancy to the plants. Both morphological characters are showing ecological adaptations.

Table 3

Accumulation of Pb (μg g^{-1} dw) in leaves and roots of *E. crassipes* at different concentration and duration

Concentration (ppm)	Leaves				Root			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0.01	1.01±0.20	1.04±0.30	1.06±0.27	1.07±0.23	1.33±0.13	1.36±0.11	1.40±0.16	1.43±0.18
0.1	2.10±0.19	2.19±0.19	2.31±0.15	2.42±0.25	5.1±2.03	9.3±1.13	12.9±1.87	17.03±2.08
1.0	26.0±12.50	30.66±16.16	35.66±17.45	41.33±18.23	70.3±21.42	89.3±16.16	91.3±28.78	94.6±22.40
2.5	57.66±14.98	71.3±18.65	75.66±16.16	82.0±18.64	156.3±37.43	174±26.17	194±17.91	97.33±38.67
5.0	118.3±34.81	140.3±37.38	158.6±33.73	170.6±38.67	319±31.12	340±41.04	369.3±46.23	77.66±47.29
10.0	245±48.49	219.3±61.07	306.66±42.47	328.3±64.61	643.3±37.34	681.6±49.78	721.3±62.17	791±77.13

Mean ±SE (n=3). ANOVA (P<0.01) for bioaccumulation in leaves. F (expo.) = 3.02704** F (conc.) = 110.515** For root F (expo.) = 4.54767**, F (conc.) = 537.685**

Various physiological and biochemical parameters measured in Jajmau and SIL for e.g. chlorophyll 1.02 and 1.06 mg g^{-1} fw, chlorophyll b 0.18 and 0.19 mg g^{-1} fw, total chlorophyll 1.20 and 1.26 mg g^{-1} fw, carotenoid 0.66 and 0.64 mg g^{-1} fw, protein content 76.13 and 79.34 mg g^{-1} fw and NR activity 39.19 and 49.05 m mol $NO_2 g^{-1}$ protein h^{-1} respectively.

Lead accumulation by leaves and roots

Accumulation of Pb by leaves and roots of *E. crassipes* was high in SIL than Jajmau. Under laboratory conditions, the accumulation of Pb in leaves and roots was concentration and treatment duration dependent (Table 3). The result depicted in Table 3 show that by increasing the concentration of Pb in the medium, the metal accumulation increased significantly ($p<0.01$). At 0.01 ppm during 24 h treatment duration accumulated 1.01 and 1.33 mg g^{-1} dw Pb in leaves and roots respectively. While at 10 ppm during 96 h treatment duration highest amount of Pb i.e. 328.3 and 791 mg g^{-1} dw were accumulated in leaves and roots respectively ($p<0.01$). The roots accumulated higher amount of Pb as compare to leaves *in situ* as well as in laboratory conditions.

Chlorophyll and carotenoid

Variation in chlorophyll a, b, total chlorophyll and carotenoid content of *E. crassipes* at various concentration and treatment duration was found signifi-

Table 4
Effect of different Pb concentrations Pb on chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (mg g^{-1} fw) in *E. crassipes* at various durations

Concentration	Chlorophyll a				Chlorophyll b			
(ppm)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	1.26± 0.03	1.31± 0.04	1.34± 0.05	1.35± 0.03	0.38± 0.06	0.41± 0.04	0.42± 0.02	0.44± 0.04
0.01	1.34± 0.03	1.37± 0.03	1.28± 0.05	1.16± 0.06	0.45± 0.06	0.44± 0.03	0.40± 0.03	0.36± 0.06
0.1	1.39± 0.04	1.41± 0.04	1.25± 0.03	1.13± 0.03	0.47± 0.03	0.49± 0.06	0.36± 0.06	0.30± 0.03
1.0	1.19± 0.04	1.18± 0.07	1.09± 0.05	1.05± 0.06	0.35± 0.06	0.32± 0.03	0.26± 0.06	0.19± 0.06
2.5	1.18± 0.04	1.06± 0.07	0.96± 0.19	0.86± 0.07	0.31± 0.06	0.28± 0.03	0.19± 0.03	0.15± 0.04
5.0	1.13± 0.03	0.95± 0.04	0.88± 0.05	0.71± 0.05	0.28± 0.03	0.18± 0.03	0.16± 0.02	0.14± 0.04
10.0	1.03± 0.02	0.86± 0.02	0.75± 0.08	0.62± 0.03	0.19± 0.04	0.16± 0.03	0.12± 0.03	0.10± 0.03
Concentration	Total chlorophyll				Carotenoid			
(ppm)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	1.66± 0.02	1.71± 0.02	1.76± 0.07	1.80± 0.07	0.35± 0.07	0.36± 0.07	0.37± 0.03	0.40± 0.03
0.01	1.77± 0.07	1.82± 0.06	1.68± 0.01	1.52± 0.07	0.42± 0.07	0.43± 0.06	0.46± 0.08	0.51± 0.09
0.1	1.85± 0.06	1.90± 0.07	1.62± 0.07	1.45± 0.02	0.45± 0.06	0.47± 0.09	0.53± 0.12	0.57± 0.09
1.0	1.54± 0.08	1.50± 0.10	1.35± 0.11	1.24± 0.11	0.51± 0.06	0.54± 0.06	0.60± 0.06	0.66± 0.08
2.5	1.49± 0.10	1.35± 0.11	1.15± 0.10	1.01± 0.12	0.55± 0.07	0.61± 0.06	0.64± 0.06	0.72± 0.07
5.0	1.42± 0.06	1.14± 0.07	1.04± 0.07	0.85± 0.01	0.61± 0.04	0.57± 0.09	0.55± 0.07	0.50± 0.07
10.0	1.22± 0.07	1.03± 0.09	0.88± 0.12	0.73± 0.06	0.65± 0.08	0.59± 0.06	0.56± 0.04	0.46± 0.07

Table 5
Effect of different concentration and duration of Pb on *in vivo* nitrate reductase activity (mmol NO_2 g^{-1} protein h^{-1}) and protein activity in *E. crassipes*

Concentration	Protein				Nitrate reductase activity			
(ppm)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	91.39±0.62	92.49±0.52	91.58±0.40	91.81±0.36	81.22±5.21	82.17±5.13	84.31±4.89	85.08±5.46
0.01	91.79±0.22	92.95±1.12	92.91±0.76	93.10±0.92	86.16±5.37	76.03±5.61	68.27±4.07	61.28±4.68
0.1	92.06±0.53	93.29±1.31	93.06±0.78	93.39±1.34	78.32±4.86	73.26±4.92	64.05±3.17	56.15±4.77
1.0	87.89±0.80	87.19±0.91	83.98±0.38	78.65±1.41	75.04±4.09	62.14±3.78	53.21±4.51	50.26±3.64
2.5	83.18±0.71	81.30±0.72	78.29±2.02	75.89±0.76	65.13±4.36	54.06±2.77	46.13±3.28	38.07±2.38
5.0	76.13±0.43	72.03±0.91	69.81±1.43	66.51±1.36	49.17±3.79	45.23±3.63	36.24±3.73	27.38±1.82
10.0	72.65±0.76	68.69±0.35	65.09±0.81	55.94±2.01	43.21±3.26	37.36±2.26	29.02±2.31	22.31±0.81

Mean ±S.E. (n=3). ANOVA ($p<0.01$) F (expo.) = 21.7041**, F (conc.) = 56.2544**

Table 6
Effect of concentration and duration of Pb on mitotic index and micronuclei cell

Concentration	Mitotic index				Micronuclei cell			
(ppm)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	7.51±0.14	7.65±0.08	7.48±0.13	7.45±0.20	1.42±0.14	1.43±0.13	1.41±0.10	1.36±0.17
0.01	7.57±0.13	7.66±0.12	7.10±0.17	6.90±0.20	5.21±0.10	6.27±0.20	7.03±0.21	7.16±0.20
0.1	7.61±0.21	7.72±0.18	6.95±0.25	6.62±0.23	6.40±0.15	7.31±0.24	9.25±0.16	11.15±0.25
1.0	7.07±0.12	6.50±0.19	6.06±0.32	5.07±0.32	8.15±0.51	9.19±0.16	11.11±0.17	17.24±0.26
2.5	6.02±0.22.	5.21±0.19	4.84±0.19	3.89±0.15	10.10±0.33	12.31±0.23	13.87±0.17	19.24±0.24
5.0	5.26±0.16	4.61±0.18	3.71±0.19	2.75±0.24	11.41±0.20	14.28±0.22	16.31±0.23	-
10.0	4.07±0.26	3.49±0.17	1.18±0.22	-	13.31±0.18	16.71±0.18	21.12±0.14	-

Mean ±S.E. (n=3). ANOVA ($p<0.01$) F (expo.) = 21.7041**, F (conc.) = 56.2544**

cant ($p<0.01$). With increase in Pb concentration there was gradual decrease in chlorophyll content, except at low dose i.e. 0.01 and 0.1 ppm (Table 4). Chlorophyll a, b and total chlorophyll ranged from 1.26 to 1.35, 0.38 to 0.44, 1.66 to 1.80 mg g^{-1} fw, respectively during growth period in control plants. Maximum reduction was observed during 96 h exposure at 10 ppm Pb that were 0.62, 0.10 and 0.73 mg g-1 fw in chlorophyll a, b and total chlorophyll, respectively.

The carotenoid content ranged from 0.35 to 0.40 mg g^{-1} fw during growth period in control plants (Table 4). With increase in Pb concentration there was gradual increase in carotenoid content (Table 4). Maximum induction of carotenoid content was observed during 96 h exposure at 2.5 ppm that was 0.72 mg g^{-1} fw.

Protein and NR activity

In control plant, the protein content ranged from 91.39 to 91.81 mg g^{-1} fw during growth period (Table 5). During 24 h exposure the protein content ranged between 91.39 to 72.65 mg g^{-1} fw, while during 96 h exposure decrease in protein content ranged between 91.81 to 55.94 mg g^{-1} fw ($p<0.01$). Maximum reduction was observed during 96 h at 10 ppm i.e. 55.94 mg g^{-1} fw, however, it showed increase at 96 h at 0.1 ppm concentration i.e. 93.39 mg g^{-1} fw.

During growth period NR activity ranged from 81.22 to 85.08 mmol NO_2^- g^{-1} protein h^{-1} in control plant (Table 5). During 24 h exposure NR activity ranged between 81.22 to 43.21 mmol NO_2^{-1} g^{-1} protein h^{-1}, while during 96 h exposure ranged between 85.08 to 22.31 m mol NO_2^{-1} g^{-1} protein h^{-1}. At lower concentration NR activity was induced i.e. at 0.01 ppm. However, higher concentrations have significant ($p<0.01$) inhibitory effect on NR activity.

Micronuclei assay

In situ conditions as well as in experimental conditions the mitotic index decreased and micronuclei cell frequency increased with increase in Pb concentration (Table 2, 6). In experimental conditions, concentration of 10.0 ppm was toxic at 96 h exposure period ($p<0.05$).

DISCUSSION

Physico-chemical analysis of effluent revealed its alkaline nature having low DO level. Penfound and Earle (1948) reported that rapid growth of water hyacinth takes place when the dissolved oxygen level is 3.4 to 4.8 mg l^{-1}. Effluent containing high BOD and COD showing its toxic nature. Water hyacinth shows effective reduction in TS and BOD of sewage (Wolverton, 1975; 1979). It posses tremendous potential to reduce the level of Pb, BOD and to improve other physico-chemical characteristics of waste water.

All morphological variation accured in plants due to accumulation of Pb. Pb accumulation also caused cytotoxicity and disfunction of spindle which resulted in inhibition of mitotic index and induction of micronuclei (MNCs). The lack of toxicity symptoms or any significant effects of Pb on plant growth

appear to be the result of poor mobility as reported for other aquatic plants (Dabin *et al*. 1978; Peter *et al*. 1979). The ability of water hyacinth to bioconcentrate metals in its roots has been attributed to the occurrence of certain metal binding complexes.

The chlorophyll content decreased with increase in concentration and duration of Pb treatment. Metal accumulation led to changes in the metalloenzymes by displacement or replacement of metal ions, resulting the changes in photosynthetic activity. Metals are thought to reduce the chlorophyll biosynthesis by reacting with –SH group of d-aminolevulinic dehydrates. An increase in carotenoid content was observed in Pb treated plants of *E. crassipes*. Increased carotenoid concentration for the protection from free radical formation is a common response to xenobiotics (Ralph and Burchett, 1998; Kenneth *et al*. 2000). Lead is well known from numerous studies, to interfere with and inhibit photosynthesis and transpiration rates with increasing supply of the metal (Bazzaz *et al*. 1974; Seregin and Ivanov, 2001).

The results indicate significant increase in protein content by the lower concentration of Pb while it decreases at high concentrations. The decline in protein content under heavy metal stress in aquatic plants has been reported (Jana *et al*. 1982; Mazhoudi *et al*. 1997). The decrease in protein content in the 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.

Presence of Pb in the growth medium regulated NR activity (Gupta and Chandra, 1994). A pronounced stimulation and inhibition of *in vivo* NR activity by low and high Pb supply suggested that Pb acts at the level of synthesis. However, it has been suggested that NR activity depend upon active photosynthesis or production of photosynthates and required photosynthetically generated reductant (NADH) and energy (Raghuram and Sopory, 1995). Hence reduction in NR activity in Pb treated *E. crassipes* plants could be probably due to the inhibition of chlorophyll biosynthesis leads to lower photosynthetic rates concomitantly supply of lower level of photosynthates. A positive correlation between NR activity and protein content has been demonstrated in earlier studies (Rai *et al*. 1992).

The mitotic index reflects the frequency of cell division and it is regarded as an important parameter when determining the rate of root growth. The mitotic index decreased progressively with increased Pb concentration *in situ* as well as in experimental conditions. The mitotic index can be correlated with the rate of root growth, suggesting that the inhibition of growth resulted from the inhibition of the cell division. Lead toxicity in many plants were reported to be associated with the disturbance of mitosis, toxicity to nucleoli (Liu *et al*. 1994), induction of binuclear cells (Swieboda, 1976) and the inhibition of root elongation (Lane and Martin, 1980).

Heavy metal Pb is known to induce MNC in the root meristematic cells of water hyacinth through impairment of spindle function in mitosis (Panda *et al*. 1988). The frequencies of the cells with MNC in the root meristems of water hyacinth were concentration dependent. The micronuclei test has been

described as an indicator of the genetic damage induced by physical and chemical agents (Schmid, 1973). Micronuclei are the manifestation during interphase of acentric fragments (Read, 1959) and lagging chromosomes (Schmid, 1973). The presence of micronuclei cells (MNC) showed that lead induced chromosomal alterations in root tip cells of the water hyacinth. So, micronuclei cell frequency and mitotic index can be used as genotoxic end points in *E. crassipes*.

Thus it may be concluded from the results that *E. crassipes* is a good absorber and accumulator of Pb. It can be used safely at lower doses because showing stimulatory effect at 0.01 to 0.1 mg ml^{-1} studied physiological parameter. Therefore, water hyacinth can be used for biomonitoring and control of lead pollution in aquatic environment.

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