Meridian Vol. 5 (1), 32 - 38, 2016. ISSN 2278-750x

EFFECTS OF LEAD AND CHROMIUM ON GROWTH OF LEMNA MINOR L.

Dr. Asma. V.M.[1]

A study was conducted to know the effects of different concentration of lead and choromium on morphological, anatomical, physiological and biochemical features in *Lemna minor L.* The results of the experiment showed the possibility of *Lemna minor* in purifying the waste water. The favourable changes in chlorophyll content, protein content, pH and moisture content at 80ppm, 160ppm and 240ppm for lead showed that *Lemna minor* can with stand the heavy metal pollution such as lead up to these limits. But for chromium the results indicate that *Lemna minor* can withstand the heavy metal pollution up to a particular concentration such as 225ppm.

Keywords: *Lemna minor L,* Lead, Chromium, chlorophyll, protein, Phytoremediation.

Heavy metals include both essential and non-essential trace metals that may be toxic to living organisms, depending on their properties, availability and levels of concentration. Frequently, urban and industrial areas exhibit high concentrations of heavy metals. The persistence of some heavy metals in the environment can lead to transformations to more toxic compounds. In recent years, due to the high development of industrial activity, the levels of heavy metals in water system have substantially increased over time. Heavy metal pollution is of great ecological significance due to the toxicity and accumulation behavior of such metals in the ecological system (Hiremath, 2002).

Mercury, lead, cadmium and copper are the important metals that cause most concern. Lead is a serious cumulative body poison. Natural waters seldom contain more than 5mg/L, although much higher values have been reported. Lead in water supply may come from industrial, mine and smelter discharges or from the dissolution of old lead plumbing. It is the most serious toxicological danger to children according to the American Academy of Paediatrics. It is associated with damage to the central nervous system, mental retardation, anaemia and other blood disorders, birth defects and miscarriages. Chromium salts are used extensively in industrial processes and may enter a water supply through the discharge of wastes. Chromate compounds are frequently added to cooling water for corrosion control. Chromium may exit in water supplies in both hexavalent and the trivalent state although the trivalent form rarely occurs in potable water.

The aquatic plants such as *Lemna minor, Ipomea aquatic, Vallisneria spirals, Nymphia alba* etc have been reported to accumulate heavy metals industrial effluents (Dilipkumar et al., 2006). Phytoremediation as a clean up process is majestic since it is effective relatively inexpensive and environmental friendly was reported by Funetha and Guptha (2006). *Lemna minor L*. is a small aquatic

[1]*Associate Professor, Department of Botany, MES Asmabi College, P. Vemballur, Kodungallur, Thrissur, Kerala, India.*

Paper submitted October 2015. Revised and Accepted January 2016.

macrophyte widely distributed in quiescent fresh water and esturies ranging from tropical to temperate zones. It is the most common species of the family Lemnacea (also known as common duck weed) in the United States and many parts of the world. It is a morphologically simple, flowering plant consisting of only frond and root. The plant is colonial, multiplies either sexually or asexually and has a growth rate far exceeding those of other flowering plants. It was also found to be a good supplement to poultry feed. Its rapid growth rate and capacity of purifying waste water makes *Lemna minor* an efficient agent for water purification and waste water treatment (Mohan and Hosetti, 1999).

In the present work the effects of lead and chromium on the growth of *Lemna minor* was studied.

MATERIALS AND METHODS

The experimental study was carried out to understand the morphology, anatomy, physiological and biochemical changes in *Lemna minor* in different concentrations of heavy metals like lead and chromium. The first step of this experiment is to collect good and healthy looking *Lemna minor* plants from the ponds near the campus. The selected plants were kept in an artificial pond in the college campus. The plants were allowed to acclimatize in the laboratory. Range finding tests were conducted for each heavy metal as per standard toxicity procedures (APHA, 1995). In the range finding test (broad range and narrow range) a series of concentrations were examined. From the results of broad range a narrow range was selected.

Lemna minor L, were cultured in glass troughs. All experiments were started with plants from stock culture less than 10 days old. The duration of range finding tests for lead and chromium was 10 days. The range finding experiment for lead and chromium was conducted in 5 different concentrations such as 100ppm, 200ppm, 300ppm, 400ppm, and 500ppm. 12 troughs were taken for the range finding test. Two were taken as control and other 5 treatments in duplicate. At the time of inoculation, the length of roots and fronds of healthy plants were measured. 25 plants were put in to each trough. The growth was noted every day. Frond number, frond length, root length and the multiplication of plants were also observed along with the anatomy of the plants.

Experiment using lead and chromium as heavy metals

Narrow range observed for lead was 80ppm, 160ppm, 240ppm and 320ppm. The experiment was conducted for these concentrations. 10 glass troughs were taken for the experiment. Four of them were considered as treatments in duplicates. They contained lead nitrate solution, distilled water and nutrient solution. The duration of this experiment was 20 days. Narrow range observed for chromium was 75ppm, 150ppm, 225ppm, and 300ppm. The experiment contained 2 controls and 4 treatments in duplicates. Control contained distilled water and nutrient solution. Others contained potassium chromate solution, nutrient solution and distilled water. The duration of this experiment was 20 days

For the above two experiments morphology and anatomy of the plants, growth of plants and the pH were observed day by day. The physiological and biochemical changes of plants were observed at time interval of 5 days. Moisture content of the samples were noted by oven dry method and the pH was also determined. Chlorophyll content was estimated according to APHA (1998), total protein content was determined by using the biuret method (Dorsey et al., 1978). Anatomy of the plants was observed under the microscope and significant changes were photographed.

RESULT AND DISCUSSION

The different experimental results showed the capacity of *Lemna minor* to withstand the heavy metal pollution up to a particular limit. The experiments proved that *Lemna minor* constantly exposed to heavy metal, tolerate metal induced stress and act as a bioremediative agent by changing morphological, anatomical, physiological and biochemical features.

Effects of lead on the growth of *Lemna minor*

Morphological analysis of *Lemna minor* during the range finding test (Broad range) for lead showed the effect of lead on the growth rate of *Lemna minor.* Based on frond numbers under control 100ppm, 200ppm, 300ppm, 400ppm and 500ppm concentrations growth was not affected up to 300ppm. *Lemna minor* showed a reduction in the growth rate under 400ppm and 500ppm because of the formation of fewer colonies (Table 1). When compared the area covered by the fronds under control, 100ppm, 200ppm, 300ppm there was no significant difference at 400ppm and 500ppm concentrations of lead and the area covered by the frond become smaller as a result of decreased width and length. The fronds were also white and thinner (Table 2). Root growth was also inhibited.

During the tests under narrow range growth of *Lemna minor* in all of the concentrations of lead such as 80ppm, 160ppm, 240ppm and 320ppm showed noticeable lag phase often extending up to 4 days and this resulted in synchronous division and growth. The frond area in 80ppm, 160ppm and 240ppm concentration there is no difference showed by *Lemna minor*. But in 320ppm concentration 50% plants showed a marked reduction in frond area.

The fronds were greener and thicker in control. As the concentration of lead increases there is a marked decrease in green colour and thickness. The photograph showed the difference in the morphological features of *Lemna minor* at different concentrations. Anatomically the plants had small air spaces in controls but in 80ppm, 160ppm, 240ppm and 300ppm the plants showed abnormality in that they had more and larger air spaces.

The number of stomata on *Lemna minor* under the 4 concentrations of lead appeared to be equal to that on the controls. The chlorophyll content was also different under different treatments (Fig. 1). The initial chlorophyll content in all plants is 0.19mg/g. During the time interval chlorophyll content in each concentration indicates that the amount of chlorophyll decreases as the concentration of metal increases. The initial amount of protein present in all plants is 24mg/g. During the time interval amount of protein increased in 80ppm, 160ppm and 240ppm than the control. Plants growing in 320ppm showed reduction in protein content than the control (Fig. 2).

Percentage of moisture content in *Lemna minor* decreases with increasing concentration of lead. In 80ppm there is an increase in percentage of moisture content than the control. But the concentrations such as 160ppm, 240ppm and 360ppm showed decrease in percentage of moisture (Table 3). pH showed variation from 8.01-6.09. The control showed an increase in pH range during the time intervals. But the other concentrations such as 80ppm, 160ppm, 240ppm and 320ppm showed slight decrease in pH range (Table 4).

Effects of chromium on the growth of *Lemna minor*

Morphological analysis of *Lemna minor* during the range finding test (Broad range) for chromium showed the effect of chromium on the growth rate of *Lemna minor.* Based on the frond numbers under control 100ppm, 200ppm, 300ppm, 400ppm and 500ppm concentrations growth was not affected up to 200ppm concentrations. *Lemna minor* showed a stagnant growth in 300ppm, 400ppm, 500ppm because of the absence of colony formation (Table 5).

When comparing the frond area under control 100ppm, 200ppm and 300ppm no significant differences were observed for *Lemna minor* at any of the three concentrations. In 400ppm and 500ppm concentrations of chromium, the fronds were also yellow and thick. During the tests under narrow range growth of *Lemna minor* in all of the concentrations such as 75ppm, 150ppm, 225ppm and 300ppm showed an increase in growth rate up to 12 days. After that the plants showed no increase in growth rate under 225ppm and 300ppm concentrations. Whereas in control, 75ppm and 150ppm the plants showed a slight increase in growth rate up to 20 days. In 300ppm concentration, *Lemna minor* showed a marked reduction in area covered by the fronds. The fronds were yellow due to chlorosis (Table 6).

Anatomically the plants showed abnormalities. The number of stomata per fronds decreased in concentrations such as 225ppm and 300ppm than the control. The guard cells were lengthened. The cells adjacent to the guard cells were black coloured and showed more air space between them (Fig 3a). Some of the plants present in 300ppm concentration showed disintegration of fronds (Fig. 3b). There were almost no roots in this concentration and the mother fronds were bleached after 16 days.

Chlorophyll content was found to be different in *Lemna minor* under different treatments. The initial chlorophyll content in all plants is 0.19mg/g. In 75ppm concentration the plants showed a slight reduction in chlorophyll content than control. But as the concentrations increases the plants showed a marked decrease in chlorophyll content during the time intervals (Fig. 4). Protein content also found to be varied in *Lemna minor* under chromium treatments. The initial amount protein present in all plants is 24mg/g. The amount of protein increases at lower concentrations. So it was found that the protein content at lower concentrations were more than the control and at higher concentration it was less than the control (Fig. 5).

pH showed variation from 8.01-5.89. The control showed an increase in pH range from 8.01-8.28 during the time intervals. But the other concentrations such as 75ppm, 150ppm, 225ppm and 300ppm showed a decrease in pH range (Table 7). Dry weights of fronds were affected by chromium treatment. *Lemna minor* showed 6%-8% decrease in dry weights as the concentration increased (Table 8).

Aquatic plants have also been used frequently to remove suspended solids, nutrients, heavy metals, toxic organics and bacteria from acid mine agricultural landfill and urban storm water runoff. In addition considerable research has been focussed on determining the usefulness of macrophytes as biomonitors of polluted environment and as bioremediative agents in waste water treatment (Lytle et al., 1998; Mohan and Hosetti, 1999; Fritioff and Greger, 2003; Kamal et al., 2004).The immobile nature of macrophytes make them particularly effective bioindicator of metal pollution as they represent real levels present at the site. Data on phytotoxicity studies are considered in the development of water quality criteria to protect aquatic life, the toxicity evaluation of municipal and industrial effluents (APHA-1998). The result of the present study also supports this view.

For *Lemna* the acceptable pH range is 5 to 9 although better growth possible in pH range of 6.5 to 7.5 (Leng et al., 1995). In *Lemna minor* there was a 26% decline in the dry weight per frond while in *Spirodela* there was 27% decrease at 0.75ppm SO2 (Loats et al., 1981). These observation suggest that the duckweeds may recover the fumigations (Fankhauser et al., 1976) and the need for an indepth analysis the rate of the photosynthetically assimilated carbon. Present study also suggested these peculiarities. Chatterjee and Nag (1991) have shown how *Lemna minor* can effectly be utilised for the removal of cadmium, mercury and copper. *Lemna* species has a potential and represents a very alternative among other aquatic plants for the recycling and treatment of anaerobic effluents from digested pigwaste was studied by Hernadez et al. (1997). The present study also proved that *Lemna minor* can be used as a potential bioremediative agent in the purification of waste water containing heavy metals like lead and chromium.

SUMMARY AND CONCLUSION

Recently public awareness about metals (cadmium, lead, nickel and chromium) contamination in environment has increased. The disposal of sewage/industrial effluents and city wastes into water bodies is becoming major problem. Such materials contain various toxic metals and pollute the water. Their accumulation or high concentration in water causes toxicity and adversely affect the plants and animals.

The macrophytes act as potential purifier of the polluted water from the various hazardous chemicals. The majority of the people are not yet fully aware from this great biological potential in this regard. Therefore the present study was conducted to know the effects of different concentration of lead and choromium on morphological, anatomical, physiological and biochemical features in *Lemna minor.* The plant was selected as test organism because they can be grown in sterile clonal cultures in defined media and being small, they are easy to manipulate. Their relatively simple structure and aquatic habitat add to their suitability as experimental organisms.

The results of the experiment showed the possibility of *Lemna minor* in purifying the waste water was also significant in the result. The favourable changes in chlorophyll content, protein content, pH and moisture content at 80ppm, 160ppm and 240ppm for lead showed that *Lemna minor* can withstand the heavymetal pollution such as lead up to these limits. But for chromium the results indicate that *Lemna minor* can withstand the heavy metal pollution up to a particular concentration such as 225ppm. The morphological and anatomical characteristics also support this view. At lower concentrations of lead and chromium, the plant showed an enhancement in growth. Lead concentration beyond 240ppm and chromium concentration beyond 225ppm proved to be toxic and inhibited all the growth parameters. Decreasing trends in the growth parameters were found with increasing metal concentration. Maximum decrease over control was found at 320ppm for lead and 300ppm for chromium.

From the study it was confirmed that common duckweed is an easy, economically affordable and eco-friendly material for phytoremediation purposes.

Table 1: Effects of lead on the growth of Lemna minor-Number of plants

	Control	100ppm	200ppm	300ppm	400ppm	500ppm
2nd day	-	-	-	2	4	15
4th day	-	-	-	1	20	10
6th day	-	-	2	2	-	-
8th day	-	-	1	3	-	-
10th day	-	2	4	5	0	0

Table 2: Number of plants showing chlorosis in different conc. of lead

	Control	100ppm	200ppm	300ppm	400ppm	500ppm
2nd day	29	34	30	31	27	26
4rd day	35	37	40	33	27	25
6th day	33	31	29	26	25	25
8th day	28	29	27	26	25	25
10th day	30	29	29	28	25	25

Table 3. Variation in percentage of moisture content in different conc. of lead

	Control	80ppm	160ppm	240ppm	320ppm
Intial	96%	96%	96%	96%	96%
Final	96%	98%	95%	94%	90%

Table 4. Variation in pH in different conc. of lead

	Control	80ppm	160ppm	240ppm	360ppm
1st day	8.01	8.02	7.74	7.2	6.64
3rd day	8.15.	8.01	7.66	7.01	6.39
5th day	8.18	7.98	7.6	6.91	6.3
7th day	8.18	7.21	7.13	6.89	6.25
9th day	8.21	7.11	6.98	6.86	6.22
11th day	8.23	7.08	6.95	6.82	6.19
13th day	8.25	7.08	6.92	6.75	6.15
15th day	8.26	7.05	6.89	6.74	6.14
17th day	8.28	7.02	6.85	6.71	6.11
19th day	8.28	7.01	6.81	6.71	6.09

Table 5. Effects of chromium on the growth of Lemna minor-Number of plants

	Control	100ppm	200ppm	300ppm	400ppm	500ppm
2nd day	31	25	25	25	25	25
4th day	33	35	31	25	25	25
6th day	31	30	29	27	25	25
8th day	27	28	26	27	25	25
10th day	29	27	27	26	25	25

Table 6. Number of plants showing chlorosis in different conc. of chromium

	Control	100ppm	200ppm	300ppm	400ppm	500ppm
2nd day	-	-	-	-	15	20
4th day	-	-	7	10	3	1
6th day	-	-	1	1	3	2
8th day	-	-	2	-	-	-
10th day	-	-	-	1	-	-

Table 7. Variation in pH in different conc. of chromium

	Control	75ppm	150ppm	225ppm	300ppm
1st day	8.01	7.98	7.63	7.52	6.73
3rd day	8.15	7.51	7.22	6.98	6.7
5th day	8.18	7.33	7.2	6.21	6.15
7th day	8.19	7.3	7.2	6.18	6.11
9th day	8.21	7.15	7.19	6.15	6.09
11th day	8.23	7.09	7.02	6.08	6.05
13th day	8.25	7.07	7.01	6.03	6.02
15th day	8.26	7.02	6.95	6.01	5.99
17th day	8.28	6.97	6.85	6.01	5.98
19th day	8.28	6.95	6.81	5.95	5.89

Table 8. Variation in percentage of moisture content in different conc. of chromium

	Control	75ppm	150ppm	225ppm	300ppm
Intial	96%	96%	96%	96%	96%
Final	96%	94%	91%	90%	87%

Fig. 1: Chlorophyll content under different treatments of lead

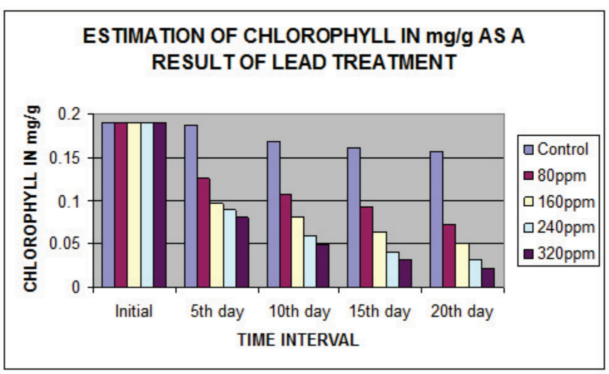


Fig. 2: Protein content under different treatments of lead

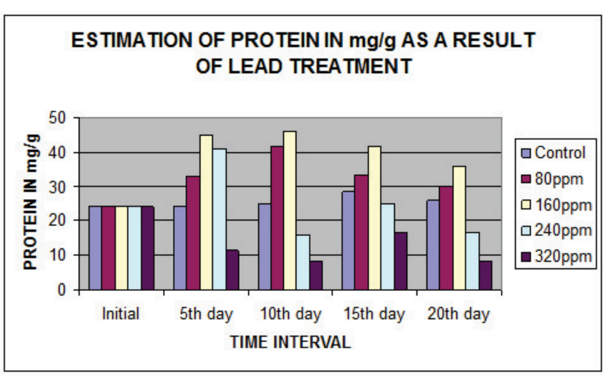
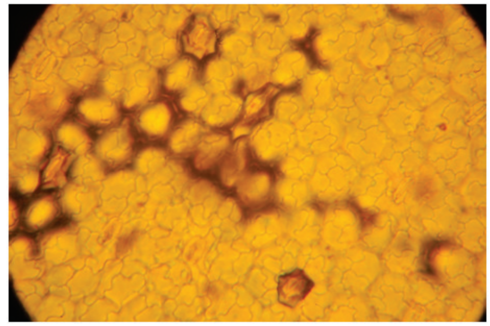
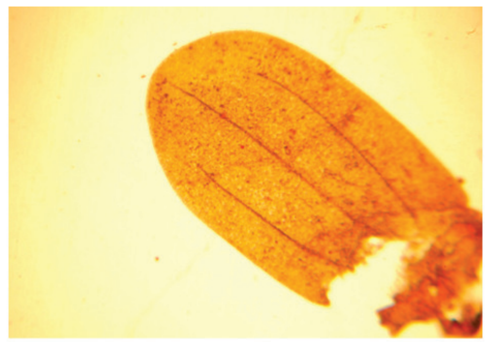


Fig. 3: Effects of chromium on the frond of Lemna minor



a. Internal structure of frond treated with chromium



b. Frond of *Lemna minor* treated with chromium

Fig. 5: Chlorophyll content under different treatments of chromium

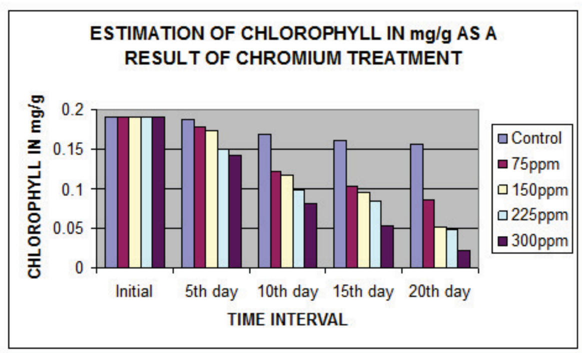
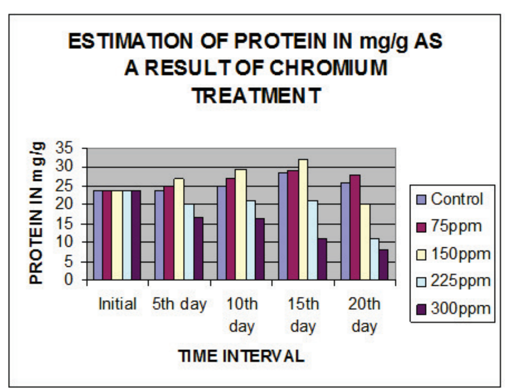


Fig. 6: Protein content under different treatments of chromium



REFERENCES

APHA- American Public Health Association, 1995.*Standard methods for the examination of water and waste water*. 19th edition, Andrew. D. Eaton ,Lenore .S .Clesceri & Arnold E. Greenberg.(Eds.).pp.8-40 – 8-43.

*APHA- American public Health Association,1998. *Standard methods for estimation water and waste water.* 20ed, Eaton, D.D .,Clesceri, L .S . and A. E.s Greenberg (Eds.),Washington D C.

*Chatterjee ,A. K. & Nag, 1991. *Intl. J. Toxicol. Occup. and Environ. Hlth* 1:166.

Dilipkumar Verma, Anoop singh & S. B. Agarwal. 2006. 'Pollution mitigation through plants'. *Environment and people* .pp .29.

Dorsey, T.E., Mc Donald ,P. & O.S. Roels. 1978. Measurement of phyto protein content by heated biuret folin assay. *Phycology*. 14(2):167-171.

Fankhauser, H ., Brunold, C. and K.H. Erismann. 1976. The influence of sublethal concentrations of sulphurdioxide on morphology ,growth and product yield of the duckweed *Lemna minor* L. *Oecologia* 23: 201-209.

Fritioff, A. & M. Gregar. 2003. Aquatic and terrestrial plant species with potential to remove heavymetal from storm water. *Int. J. phytorem*. 5: 211-224.

Funetha, H . & K .A .Guptha . 2006. Phytoremediation of heavy metals for sustainable development .*Agrobios News letter*. 4:32-35.

Hernandez, E .,Eugenia. J. Olgein, Sandra Trugillo & Jaqueline Vivano. 1997. Recycling and treatment of anaerobic effluents from pigwaste using Lemna sps temperate climate conditions. In:Glo. Enviri. Biotech. 293-304 .

Hiremath, K. G. 2002.Effect of heavy metal pollution on aquatic plants:a review .In: Hosetti, B. B(Eds.), Wetlands -Conservation and Management , Pointer publishers, Jaipur 302003 (Raj) India .pp.299-303.

Kamal,M., Ghaly ,A. E., Mahamoud, N . and R .Cote . 2004.Phytoremediation of heavymetals by aquatic plants. Environment International, 29:1029-1039.

Leng, R .A .,Stambolie, J .H. and R. Bell . 1995. “Duckweed a Potential high-protien feed resource for domestic animals and fish”. Live stock reaserach for rural Developmant 7(1).

Loats, K .V., Noble ,R. and B.L. Takemoto. 1981. Photosynthesis under low level SO2 and CO2 enchancement conditions in three duckweed species. In: Bot. Gaz (3) 305-310.

Lytle ,C. M .,Lytle, F. W . ,Young,N., Qion ,J. H .,Hansen, D., Zayed ,A. and N. Terry. 1998.Reduction of Cr(VI) to CR(iii) by wetland plants; potential for insitu heavy metal detoxification. Environ. Sci. Technol. 32:3087-3093.

Mohan, B .S .and B. B. Hosetti.1999. Aquatic plants for toxicity assessment, Environ. Res. 81:259-274.