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Research Article

Growth rate and Chlorophyll Response of Duckweed *Lemna minor* in Heavy metal Pollution

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Abstract

To assess the tolerance of growth and chlorophyll change in duckweed *Lemna minor*, 15 healthy fronds were exposed to 0.1g and 0.2g concentration of four heavy metals (Cu,Zn,Cr,Ag) under a daily regime of 8 hrs. Control sample was without exposure for eight days of experimentation period under laboratory conditions. At the end of time period0.2gAg metal exposure shows abrupt change in frond colour with decrease in chlorophyll content and growth rate however control sample shows highest growth rate of 25 fronds with increase in chlorophyll concentration. On the basis of visible symptoms, the toxicity of metal exposure in decreasing order of damage was 0.2g Ag>0.1gAg>0.2gCu> 0.2gZn> 0.1gCu> 0.2Cr >0.1g Zn>0.1gCr> control sample. Tabulated data suggests the potential role played by *Lemna minor* in bioremediation of heavy metals within optimum concentration and its toxic effects beyond such concentration.

Keywords: *Lemna minor*, Heavy metals,Chlorophyll, Toxicity

Introduction

Heavy metal pollution in aquatic ecosystem posed a serious threat to aquatic biodiversity and drinking water which in turn caused severe health hazards in humans. The economic aspects and side effects of conventional treatment technologies in aquatic ecosystems paved the way to phytoremediation technology.In phytoremediation, plants are used to ameliorate the environment from various hazardous pollutants. It is cost-effective and eco-friendly technology for environmental clean-up(Qian *et al*., 1999). Macrophytes are preferred over other bio-agents due to low cost, frequent abundance in aquatic ecosystems and easy handling. The efficiency and selection of potent aquatic plants is done through different experiments. Many widespread aquatic floating weeds, such as duckweeds, water hyacinth are commonly utilised for this purpose (Miranda and Ilangovan, 1996; Zhu *et al*., 1999).Free floating macrophytes have been the species of choice, and are often used as the representative species of all other vascular plants because of their small size, structural simplicity and rapid growth characteristics that contribute to their sensitivity to chemicals. *Lemna minor* is one of the free floating forms of macrophytes with a size of 2-4mm across and it is widely distributed in fresh waters and estuaries ranging from tropical to temperate regions (APHA, 2005; Hillman, 1961;Dirilgen N., 1998; Wang, 1990). The plant size is small, but the fronds are sufficiently large to be easily counted with the unaided eye.This also facilitates non-destructive, repeated calculation of growth patterns particularly important for the present experimental study which was carried out to monitor changes in physiological effects, number of plants and chlorophyll content in*Lemna minor* in different heavy metal concentrations.

Materials and Methods

Duckweed *Lemna minor* were collected from Dal lake an urban aquatic ecosystem of Kashmir Himalaya in sealed plastic bags and were washed properly. They were rinsed with 0.01 M of NaOCl for 30s to prevent algal outgrowth (APHA, 2005). Growth response tests were conducted using 200ml of 9 glass petridishes in which 15 healthy fronds of *Lemna minor* were exposed. The range of concentrations used in the experiment were 0.1g and 0.2g of $CuSo_4$, $ZnSo_4$, $AgNo_3$, $K_2Cr_2O_7$to each petridish with one control sample as shown in table below to investigate growth, increase in frond number, physical appearance, colour of frond and chlorophyll content. In the present study, 8-day period was selected in which frond replication visibly projecting from the edge of the parent frond were counted as separate frond and were considered to be dead when the colour of the fronds changed from green to black or partial chlorosis.

Metal	Form	Conc.
Cu	$CuSo_4$	0.1g, 0.2g
Ag	$AgNo_3$	0.1g,0.2g
Zn	$ZnSo_4$	0.1,0.2g
Cr	$K_2Cr_2O_7$	0.1g,0.2g
Control	Lake water sample	

Estimation of chlorophyll

Chlorophyll content (Chl a, Chl b and Total Chlorophyll) of *Lemna minor*was determined Spectrophotometrically, in acetone extracts prepared from samples using standard methods (Strain *et al.*, 1971). Acetone extract of the macrophytes was prepared by macerating 0.2gfresh weight of plant leaves with 80% aqueous acetone using a mortarand pestle. The decanted suspension was centrifuged for 3 minutes at1320 rpm. After centrifugation, the upper green clear solution was decanted from the colourless residue and then made up to 2ml with80% acetone in 5 ml test tubes. The solution was then subjected to centrifugation at 10,000 rpm for 10 minutes. The absorbance of the solution was determined using a spectrophotometer (Systronics-116) at wavelengths of 665 and 649 nm respectively. The results were expressed as µg/ml.

Chlorophyll 'a' (µg/ml)=11.63 (A665)-2.39 (A649)
Chlorophyll 'b' (µg/ml)=20.11 (A649)-5.18 (A665)
Total Chlorophyll (µg/ml)=6.45 (A665) +17.72 (A649).

Results

The experimental design of the present study allowed us to compare the sensitivity of *Lemna minor* towards heavy metal stress. The change in frond colour, frond number and chlorophyll response could be used as a rapidly determined phytoremediation tool and toxicity studies. Table: 1 depicted variation in frond number in different petridishes after exposure of 15 healthy fronds of *Lemna minor* to different range of metal concentrations for 8 days experimentation period.$AgNo_3$ was inhibitory for both selected concentrations while as $CuSo_4$ and $ZnSo_4$ showed moderate effect on fronds. Fronds showed contrasted differences in their colour with increase in time and metal concentration. Control sample containing lake water showed highest replication of fronds (35) with proper colouration compared to Cr exposure where number was reduced to 20 and lowest in Ag where only 8 live fronds were present. Fig.:1 demonstrates the effect of various metal concentrations on the colour response of fronds at different times (1^{st}-8^{th} day). Chlorosis (a progression of green to yellow colour on the frond) and frond disconnection (detachment of frond from colonies) were toxicity signs observed at the start of exposing *Lemna* frond to the 0.2 mg/l Cu metal concentration. These signs progressed to necrosis (death of frond) at the end of the treatment with both concentrations of Ag. The metals Zn and Cr caused visible damage to duckweed at concentration 0.1and 0.2 mg/l, respectively and changed its colour at the 8^{th} day

Table: 1 Variation in number of fronds during eight days period.

	Initial no.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
Cu (0.1g)	15	15	18	18	21	23	25	25	28
Cu (0.2g)	15	15	15	16	18	18	19	20	20
Ag (0.1g)	15	15	15	16	10	10	12	12	15
Ag (0.2g)	15	15	15	15	14	12	10	8	8
Zn (0.1g)	15	15	18	22	24	24	25	28	32
Zn (0.2g)	15	15	16	18	18	19	22	22	25
Cr (0.1g)	15	15	18	20	20	23	24	28	33
Cr (0.2g)	15	15	16	18	20	20	24	27	30
Control	15	15	19	22	25	28	30	32	35

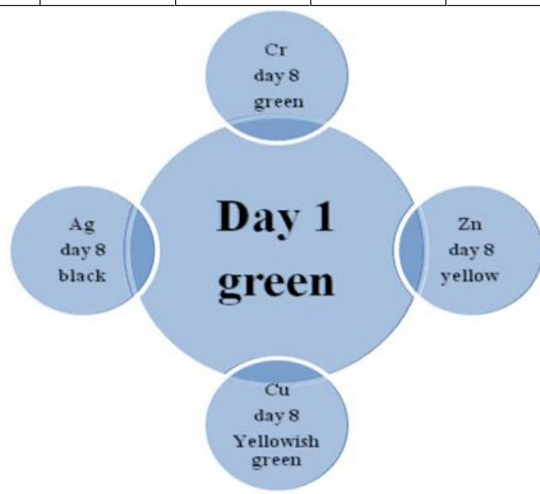

Fig. 1 Change in colour of fronds

Data on the pigment content of *Lemna minor* revealed significant differences for Chlorophyll-a, Chlorophyll-b and Total Chlorophyll in different groups exposed to metal concentrations. The levels of Chlorophyll-a, the primary photosynthetic pigment in the present study varied from 2.5µg/ml to 5.0 µg/ml. The values were highest in control sample(5.0µg/ml) and lowest in (2.5µg/ml).The Chlorophyll-b content recorded its highest value in control sample where the data observed was (2.8 µg/ml). The lowest Chlorophyll-b values were obtained in 0.2 mg Ag having a value of (1.0µg/ml).The Total Chlorophyll content also recorded its highest value in control sample(8.2 µg/ml) decreasing to a lowest of 3.8 µg/ml in 0.2 mg of Ag (Table: 2).In general fronds present in control petridish containing lake water sample were characterized by high pigment content while as lower pigment levels were obtained in petridishes containing different concentration of metals.

Table: 2 Variation in pigment content

	1st day (µg/ml)			8th day (µg/ml)		
Metal	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.
Cu (0.1g)	3.2	1.9	5.0	4.4	1.6	6.28
Cu (0.2g)	3.2	1.9	5.0	3.4	1.5	5.4
Ag (0.1g)	3.2	1.9	5.0	2.9	1.4	4.9
Ag (0.2g)	3.2	1.9	5.0	2.5	1.0	3.8
Zn (0.1g)	3.2	1.9	5.0	4.7	2.3	7.4
Zn (0.2g)	3.2	1.9	5.0	3.7	1.8	5.9
Cr (0.1g)	3.2	1.9	5.0	4.4	2.6	7.8
Cr (0.2g)	3.2	1.9	5.0	3.6	2.0	6.0
Control	3.2	1.9	5.0	5.0	2.8	8.2

Discussion

In this work, it was aimed to compare the growth response of *Lemna minor*, a free floating macrophyte to heavy metals (Cu, Cr, Zn, Ag,) and control exposure. Metal phytotoxicity was assessed through the visible symptoms of change in frond colour, replication in fronds and determination of the chlorophyll content in 8 days experimental period. On the basis of these parameters, phytoremediation quality of hydrophyte was assessed along with toxicological stress. The toxicity of different metals to *Lemna minor* in decreasing order of damage was: 0.2g Ag>0.1gAg>0.2gCu> 0.2gZn> 0.1gCu> 0.2Cr >0.1g Zn>0.1gCr> control sample. Cu when present at concentration ≤ 0.1 mg/lis essential to replicate the fronds of *Lemna minor* and higher than 0.1 mg/l this effect was manifested by a rapid development of chlorosis supported by (Zayed*et al.*,1998; Teisseire and Vernet, 2000) in their experiment on *Lemna* fronds and find out activities of glutathione S-transferase and glutathione reductase were inhibited beyond optimum concentration. The present experiment showed that with as little as 0.2g of Cr exposure there was not substantial inhibition of growth, but with increase in time greater effect was observed may be attributed to the greater bioconcentration of metal (Vasseur, *et al*.,1993). Exposing *Lemna* to the Zn (2 or 3 days), the fronds colour changed from green to yellow and some fronds were separated from the colonies could be the result of reduction in photosynthesis and chlorophyll production(Vaillant *et al.*,2005).Similarly Ag exposure decrease *Lemna* frond number from 15 to 8 was supported by Wang (1990) that even lowest concentration of Ag can cause hindrance in their growth and replication.

In the present work, maximum pigment content of chl. a, chl. b and total chl. was observed in control sample characterized by optimum nutrients of lake water and without any added metal and were significantly lower in Ag exposed fronds maybe attributed to the positive role of metal exposure on decrease in chlorophyll concentration. At the exposure times of 7to 8 days, the difference between the control and lowest pigment concentration of the test substance was almost double. The present data revealed that *Lemna minor* is less sensitive to some metals like Cu, Cr and low concentrations of Zn and can be used to remove these toxicants from water bodies also supported by (Tripathi and Chandra, 1991) in their studies on sewage waters..

Conclusion

Growth of *Lemna minor* reduced metal concentration Cu, Cr and Zn in the medium to a certain level supports the importance of of these plants in phytoremediation technology. The metals were tolerated by *Lemna minor* at 0.1 and-0.2 mg/l respectively without visible toxicity signs on growth rate and chlorophyll concentration and beyond this level of metal exposure toxicity signs appear which progressed to necrosis (dead fronds).This suggested *Lemna minor* is good accumulator of metals at low concentration and can be used in bioremediation of metal polluted aquatic ecosystems. Though protocols need to be developed and further studies are required to understand the mechanisms involved in the uptake of pollutants so that maximum potential can be utilized for use in phytoremediation technology.

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