Response of *Lemna minor L.*(Duckweed) to the heavy metal copper

PREETY SINGH

P.G. Department of Zoology
B.S.N.V.P.G.College,
Lucknow-226001, (U.P.), India

Abstract

Present study aims to assess the tolerance and toxic effect of copper on common duckweed aquatic plant *Lemna minor*. Copper tolerance in *Lemna minor* was investigated under hydroponics conditions between pH of 4.5 to 6.7 and natural photoperiod . For toxicity test *lemna minor* L. were exposed to different concentration of $CuSO_4$ (0.5, 1.0, 2.0, 4.0 mg/l and control) for measurement of frond numbers, general appearance and chlorophyll content. $CuSO_4$ exposure caused chlorosis and necrosis in all test groups. Frond dissociation was noticed at 1.0 mg/l, chlorophyll content was found decreased in all concentration and was maximum at 4.0 mg/l concentration. Severity of symptoms was found toxicant concentration dependent.Possible mechanisms of copper toxicity to these valuable bioindicators have been discussed. The purpose of this study was to investigate the tolerance to copper in duckweed as a first step to determine the use of this aquatic species to remove heavy metal from polluted river water.

Key words*: Lemna minor*, Heavy metal toxicity, Copper, Chlorophyll

Introduction

Lemna minor L. (duckweed) are aquatic plants, which often form dense floating mats in ponds and rivers (Driever et.al., 2005). The plants are fast growing and adapt easily to various aquatic conditions and can tolerate a wide pH range (4.5-8.3) (Cayuela et. al.,2007). The small size, simple structure and rapid growth make duckweed very suitable for toxicity test (OECD,2002). Some studies indicate that duckweed plants are sensitive to toxicity (Khellaf and Zerdaoui,2010). Other studies however, report that duckweed plants are tolerant to environment toxicity (Wang, 1990). To assess the tolerance of the species *lemna minor* to heavy metals plants were exposed to different concentration of copper. Toxic effect of pollutant on duckweed is generally evaluated by phytotoxicity tests based on growth inhibition (Geoffroy *et.al.,*2004).

Copper a " Gray listed " heavy metal, despite working as essential metal for several biological processes, also become toxic at higher concentration. Copper is introduced in aquatic ecosystem by natural process as well as by human interferences. Copper were chosen as the metals for this study for a number of reasons. Their presence above trace levels in the environment is an indicator of industrial pollution. On the other hand, they are essential micronutrients for plants; Copper is a structural and catalytic component of many proteins and enzymes involved in metabolic pathways (Teisseire and Vernet,2000). However, when the concentration reaches a threshold value, these essential metals becomes first inhibitory and afterwards toxic. Copper is responsible for many alterations of plant cells (respiration, photosynthesis, pigment synthesis and enzyme activity (Kanoun Boule *et.al*.,2009). Each plant species has different resistance and tolerance level to different contaminants (Kamal *et.al*.,2004).

The present study investigates copper toxicity to *Lemna minor* to determine tolerance of this aquatic species to copper. The goel was to assess the possibility to use *Lemna minor* for phytoremediation of copper contamination in water and investigate the tolerance to Cu in duckweed to determine the use of this aquatic species to remove heavy metal from polluted river water.

Materials and Methods:

Plant material and culture medium: *Lemna minor* L. A common duckweed plant (Angiosperm; family *lemnaceae)* is widely distributed and found floating in local rivers and ponds with flat fronds and fine roots. The plants were collected from Gomti river and cultured in Hoagland's medium at pH 6.1 (Hoagland's,1950). Healthy plants were used in the tests after one week of culturing (EPA,1975). A continuous aeration system provides oxygen for the *lemna* fronds and prevent root fungal diseases (Kamal *et.al.,*2004). Plants were cultured at 26±2^{0}C and natural photoperiods.

Toxicity test: The test protocol were derived from the standard draft guidelines 221 (OECD,2002). Duckweed growth was measured after four days of exposure to different concentration of copper (0.5.1.0,2.0,4.0 mg/l and control). The metal used for this study were supplied as $CuSO_4.5H_2O$. Nine to twelve lemna fronds were gently placed in 250 ml glass beaker containing 100 ml of metal solution diluted in distilled water. The dose response tests were performed in conditions similar to those of the plant cultures. Preliminary assays defined the variation of concentrations for each metal. The nominal concentrations selected were based on the response of the fronds in the presence of the metal ions, the upper limit of the concentration ranges were defined when the necrosis was observed. Chlorophyll content was determined by Arnon (1949). All data presented in the table are mean of three triplicate ± S.E. value.

Results and discussion :

Toxic effect of copper on general appearance,frond number and root structure were shown in Table-2. The metal copper caused visible damage to duckweed at concentration 0.5, 1, 2 and 4 mg/l respectively. Chlorosis (a progression of green to yellow colour on the frond) and frond disconnection (detachment of fronds from colonies) were toxicity signs observed at the start of exposing *lemna* fronds to the metal elements. These signs progressed to necrosis at the end of the treatment with Cu. Copper was a very toxic metal for *Lemna minor*. At low concentration of Cu, fronds were chlorotic and some fronds separated from the others. Necrosis was observed after 24 hour of exposure of plants to 0.5 mg/l of Cu. Some biochemical parameters showed inhibitory effect in *lemna minor* at high concentration of copper (4 mg/l). Toxic water inhibited the chlorophyll content (Table-1). The chlorophyll-a and chlorophyll-b and total chlorophyll was decreased by 20% at 2mg/l Cu concentration as compared to control. Copper when present in the nutrient solution at concentration ≤ 2mg/l was an essential element for the development of *lemna* fronds because of its important role in cellular metabolism. At a concentration higher than 4 mg/l, Cu caused the photosystem alteration by reducing electron transport. This effect was explained by a rapid development of chlorosis. Chlorophyll is an important pigment of the photosynthetic activity for primary productivity. Many studies have demonstrated influence of heavy metals on chlorophyll content in higher plants (Prasad *et.al*, 2001 ; Xiong *et.al.,*2006; Zengin and Kirbag,2007). Heavy metal inhibit uptake and transportation of other metal elements such as Fe, Zn and Mn by antagonistic effects, and therefore plants lose the capacity of synthesis of pigments (Lin and Wu, 1994, Liu *et.al.,*2004). The results supported sensitivity of *lemna minor* to copper toxicity, when present in aquatic bodies as reported by other workers (Cayuela *et.al*.,2007; Singh and Singh,2006). Some macrophytes also used as test plants for indicator of aquatic pollutants

(Wang,1986; Verma *et.al.,*1999). Our finding suggest that duckweed plant very sensitive towards toxicity of copper. Heavy metal toxicity in water suppressed biomass production and metabolic activities.

The presence of toxic chemicals and heavy metals in aquatic bodies due to discharge of industrial effluents, accumulates in the aquatic plants and cause toxicity effects (Rai and Raizada,1989; Huebert and Shay,1993; Sental,1994).Ultimately transfer into food web, when consumed by various living organism (Sharma *et.al*.,2001; Sahu *et.al*.,2007).

Conclusion:

In this work, the tolerance of Lemna minor to copper and the potential accumulation of this metal has been investigated. The metal was tolerated by lemna at 0.5 mg/l. This result revealed high tolerance of this aquatic plant to the heavy metal copper.

Table-1: Effect of Copper on Chlorophyll contents of *Lemna minor*

Conc. Of $CuSO_4$ (mg/l)	Chlorophyll-a (µg/g fresh wt.)	Chlorophyll-b (µg/g fresh wt.)	Total Chlorophyll (µg/g fresh wt.)
Control	0.07	0.330	0.4
0.5	0.039	0.313	0.352
1.0	0.055	0.307	0.362
2.0	0.301	0.068	0.369
4.0	0.069	0.056	0.125

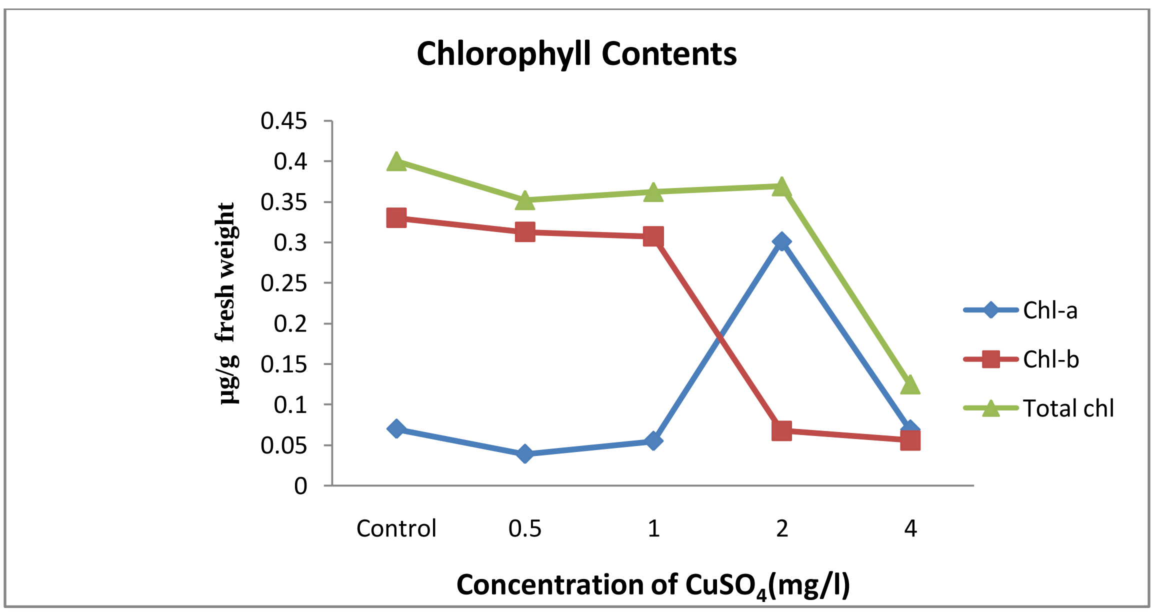

Table-2: Effect of Copper on General appearence of *Lemna minor*

Conc. Of $CuSO_4$ (mg/l)	**Appearence**	**Frond number (Total)**	**Root structure**
Control	No chlorosis, good healthy plants	41	Normal white colour roots of normal length
0.5	Mild chlorosis & necrosis in few number of fronds	35	Roots comparatively longer than control
1.0	Moderate chlorosis with starting of dislocation of fronds	30	Roots longer than control
2.0	Moderate chlorosis & necrosis with moderate dissociation of fronds	33	Roots shorter than control along with few black pattern at tip
4.0	Severe chlorosis and necrosis in about 80% plants, fronds were found highly dissociates and scattered	24	Roots shorter than control,black patches more pronounced in root tips upto 70%,breakage of root tips.

References:

Arnon D I. Copper enzymes in isolated chloroplasts polyphenol oxidase in beta vulgaris. Plant physiol,24:1-15,(1949).

Cayuela,M.L., Millner P, Slovin J and Roig A. (2007). Duckweed (Lemna gibba) growth inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composting.Chemosphere,67:36-43.

Driever SM, Nes EHV, Roijackers RMM (2005). Growth limitation of Lemna minor due to high plant density.Aquat. Bot.,81:245-251.

EPA,Approach for the evaluation of toxicity to aquatic vascular plants.pp.3.117-3.120,(1975).

Geoffroy L,Franckart C and Eullaffroy P (2004). Comparison of different physiological parameter responses in Lemna minor and scenedesmus obliquus to herbicide flumioxazin. Environ.Pollution,131,233-241.

Hoagland DR and Arnon DI: The water culture method for growing plants without soil.Calif.Agr.Exp.Station.Circ.347:32,(1950).

Huebert,DB and Shay M (1993). The response of Lemna trisulea L. to ccadmium. Environ.Pollut.,80: 247-353.

Kamal M, Ghaly AE, Mahmoud N,Cote R (2004). Phytoaccumulation of heavy metals by aquatic plants.Environ.Int.,29:1029-1039.

Kanoun-Boule, Vicentea JAF, Nabaisa C, Prasad MNV and Freitas F (2009). Ecophysiological tolerance of duckweed exposed to copper. Aquatic Toxicol.,91(1),1-9.

Khellaf N and Zerdaoui M (2010). Growth response of the duckweed *Lemna minor* to heavy metal pollution. Iran J. Environ. Health Sci. Eng., Vol.6,No.3,pp.161-166.

Lin,S and Wu L (1994). Effect of copper concentration of mineral nutrient uptake and copper accumulation in protein of copper tolerant and non tolerant Lotus purshianus. Ecotoxicol. Environ,Saf, 29:214-228.

Liu,J, Xiong,Z, Li T and Huang H (2004). Bioaccumulation and ecophysiological responses to copper stress in two population of Rumex dentatus L. from Cu contaminated and non contaminated sites. Environ.Exp.Bot.,52:43-51.

OECD (2002). Guidelines for the testing of chemicals. Lemna spp.Growth inhibition test, Draft guideline.221.

Prasad,M N V., Malec P, Waloszek A., Bajko M and Strzlka K (2001). Physiological responses of Lemna trisulea (duckweed) to cadmium and copper bioaccumulation. Plant Sci.,161:881-889.

Rai LC and Raizada M (1989). Effect of bimetallic combination of Ni, Cr and Pb on growth uptake of nitrate and ammonia, C-4 fixation and nitrogenise activity of Nostock muscorum. Ecotoxicol . Environ.Saf.,17:75-85.

Sahu RK, Katiyar S,Tiwari J and Kisku GC (2007). Assessment of drain water receiving effluent from tanneries and its impact on soil and plant with particular imphasis on bioaccumulation on heavy metals. J.Environ.Biol.,28:685-690.

Sharma K, Chaturvedi RK, Bhardwaj M and Sharma KP.(2001). Heavy metals in vegetables and cereals growing around Sanganer town Jaipur, Rajasthan (India).J. Ind. Bot.Soc.,80:103-108.

Telesseire H, Couderchet M and Vernet G (2000). Toxic responses and catalase activity of Lemna minor L. exposed to toipet, copper and their combination. Ecotox. Environ.Safe.,40, 194-200.

Verma Y, Hargan MC, Ruperelia SG and Kulkarni PK (1999). Toxicity testing of tannery effluents using duckweed (Lemna minor L.) bio assay.Pollut. Res.,18(4),497-500.

Wang W (1990). Literature review on duckweed toxicity testing. Environ.Research.52(1) 7-22.

Wang,W (1986). Toxicity tests of aquatic pollutant by using common duckweed. Environ.Pollutant.,11:1-14.

Wang,W (1990). Literature review on duckweed toxicity testing. Environ.Res.,52:7-22.

Xiong Z T, Liu C and Zeng B (2006). Phytotoxic effects of copper on nitrogen metabolism and plant growth in Brassica pekinensis Rupr. Ecotoxicol. Environ.Saf.,64:273-280.

Zengin,FK and Kirbag S (2007). Effect of copper on proline, protein and abscisic acid level of sunflower (*Helianthus annus* L.) seedlings.J. Environ.Biol.,28: 561-566.