EFFECT OF COPPER AND MERCURY ON GROWTH PARAMETERS IN *LEMNA MINOR*

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Abstract : Theability of aquatic plants to accumulate heavy metals from water is well documented. In this study, duckweeds plants (*Lemna minor*) were exposed to different concentrations of Cu and Hg. Various growth parameters (fresh weight, dry weight and growth index) in different seasons(summer and winter) were studied. The effect of all concentrations on plant parameters was toxic. The plant growth was decreased as the concentrations of heavy metals were increased. The results suggest that the *L.minor* can be effectively used as phytoremediator for waste water polluted with more than one heavy metal at moderate concentrations.

Keywords : Cu and Hg, Heavy metal, *Lemna minor*, Plant-toxicity, Phytoremediation

INTRODUCTION

Heavy metal pollution is an important environmental problem due to wide spread use of metals for industrial and agricultural purposes in the world. Pollution of environmentby toxic metals arises as a result of various industrial activities and has turned these metals ions intomajor health issue (Waisberg *et al.*, 2003). Although several adverse effects of the toxic metal have been known for a long time exposure to heavy metals continues, and is even increasing in some parts of the world, is particular in less developed countries. Heavymetal pollution is also a multi element problem in many areas (An *et al*.,2004). Under these circumstances synergistic and antagonistic interaction may be important and predicted impact based on individual effects of each metal species is likely to be erroneous (Ting *et al*.,1991). In contrast with most organic material metals cannot be transformed by microorganisms and therefore accumulate in water, air, soil, bottom sediments and living organisms (Miretzky *et al*.,2004). There is therefore a clear need to understand the interactive effects produced by combinations of metal ions at different concentrations. The possible adverse effects of heavy metal pollution and their phytotoxic effects have been reported by several workers (Chiba and Takahashi,1977; Haele and Ormrod,1983; Lebloava *et al*.,1986).

Copper is one of the oldest known metals and is the 25^{th} most abundant element in the Earth's crust. Copper was identify as a plant nutrient in the 1930s (A.L. Somer,1931; C.B. Lipman and Mackinney, 1931).Cu contributes to several physiological processes in plants including photosynthesis, respiration,corbohydrate distribution, nitrogen and cell wall metabolism (Kabata-Pendias and Pendias,2001). The higher concentration of Cu may account for the suppressed root growth, leaf chlorosis, observed in plants (Baker and Walker, 1989).Mercury is a toxic heavy metal that is found naturally in the environment in various forms. Mercury poisoning has become a problem of current interest as a result of environment pollution on a global scale. Hg is a strong phytotoxic as well as genotoxic metal(Fridovich I,1986; Suszeynsky E.M. *et al.*, 1995). Toxic effect of Hg in plantsinclude growth reduction, decreased chlorophyll content and nitrate reductase activity (Vyas and Puranik, 1993). Decreased water uptake and antioxidant enzymes, biomass etc. (Gardea – Torresdey *et al*., 2005).Duckweed (family Lemnaceae) is a small, fragile, free floating aquatic plant that flourishes in quiescent shallow water bodies (Rahmani and Sternberg, 1999). Due to its special features, it is used as a test organismfor aquatic studies and for waste water treatment. In the present study, the effect of Cu and Hg on *Lemna minor* was examined by exposing the aquatic plants separately to each of the two metal species and then to combination of the two at various concentrations. Effects on biomass growth were studied.

MATERIAL AND METHOD

Duckweed plants obtained from Nakatia river situated in Bareilly (U.P.). The plants were washed very well and rinsed in distilled water three times for five minutes. Plants were exposed to individual trace elements $CuSO_4$ and $HgCl_2$ i.e. 5.0 mg/l , 10.0 mg/l , 20.0 mg/l and 1 mg/l , 3 mg/l, and 5 mg/l respectively, separately as well as in combination (2.5 mg/l Cu and 0.5 mg/l Hg; 5mg/l Cu and 1.5 mg/l Hg; and 10 mg/l Cu and 2.5 treatment . Initially in each tub 5.0 mg/l of biomass *(L. minor*) was added. Comparisons of metal exposed plants were made with untreated (control) plants. The data pertaining to plant growth (fresh weight, dry weight, biomass index) were obtained after three and five days after treatment. All experiments were conducted in summer season (June) and winter (December) in 2010.

Fresh Weight : colonies were transferred to pre weighted polystyre tubes with small (1 mm) holes in the rounded bottoms. The tubes were then centrifuged at 3000 rpm for 10 minutes at room temperature. Tubes, containing the now dried colonies are re weighed and the fresh weight is calculated by substracting the weight of empty tubes.

Dry weight: colonies were collected from each of test vessels and rinsed with distilled or deionised water. They were blotted to remove excess water and then dried at 60̊ C to a constant weight. Any root fragments also be included.

Biomass index : a plant growth index was calculated as follows

Growth index= Biomass (end of the test)/ Biomass (initially biomass).

RESULT AND DISCUSSION

It was observed that 5 mg of Cu and 1 mg of Hg sharply decreased the growth of *Lemna minor*at the end of three days and five days in both seasons. The results pertaining to effect of different concentration of heavy metals on biomass yield of *Lemna minor* are depicted in tables.The concentrations of 20mg/l cu and 5 mg/l of Hg proved to be toxic affecting the plant growth severely. Fresh weight after five days decreased from 6.01 to 3.97 gm in summer and 6.44 gm to 4.07 gm in winter with 20 mg/l of Cu, in summer fresh weight after five days decreased from 6.01 gm to 3.83 gm and 6.44 gm to 3.92 gm in winter with 5 mg/l of Hg. Whereas higher concentration of mix (Cu and Hg) metals decreased fresh weight after five days from 6.01 gm to 3.92gm in summer and 6.44gm to 3.97gm in winter.It was observed that dry weight also effected severely. 20 mg/l of Cu decreased dry weight from .254 to .205 mg in summer and .262 to .208 in winter, 5 mg/l of Hg decreased dry weight from .254 to .183 in summer and .262 to .186gm in winter and 10 mg/l of Cu and 2.5 mg/l of Hg decreased dry weight .254 to .195 in summer and .262 gm to .199 in winters.Biomass index also decreased in all three manners in both seasons. According to N. Khellaf et al. (2009) Cu when present in the nutrient solution at concentrations less than or equal to 0.2 mg/l a concentration higher than 0.4 mg/l Cu caused the photosystem alteration by reduction electron transport. According to Teisseire and Vernet (2000), CuSO4 at 10 µM was inhibitory for *L. minor*, at this concentration activities of glutathione S- transferase and glutathione reductase were inhibited. Zayed *et al*. (1998) used *L. minor* for phytoaccumulation of copper in quarter strength Hoagland's solution at pH 6; the lowest Cu concentration causing > 10 % growth reduction was 5 mg/l. G. Quzounidou *et al*.(1992) and W. Maksymiec *et al.* (1995) also supported the results that in excess, the absorbed copper can be considered as a toxic element leading to growth inhibition.

It is generally accepted that heavy metal toxicity to plants is positively correlated to the concentration of metals in plant tissues; higher metal concentrations in the tissues usually induce stronger damage to the plants.Several studies have shown that most *Lemna* species retain less than 3% of their weight biomass after drying (Landesman 2000; Mkandawire, 2005). This means that the highest percentage of *Lemna* content is water just like many other aquatic emersed and even submersed macrophytes and algae (Mkandawire and Dudel, 2007). The results showed that for the growth of *L. minor* in winter season was very good. It was supported by Classen et al., 2000. According to Cheng *et al*. (2002) duckweed cold tolerance allows it to be used for year round wastewater treatment in areas where tropical macrophytes, such as water hyacinths can only grows in summer, Mkandawire *et al.* (2005) also showed that optimal temperature for *L. minor* is 18- 24° c.

Table 1. Effect of different concentrations of Cu on the fresh weight (gm) of *Lemna minor*

Cu (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	5.54	6.01	5.84	6.44
5	4.87	4.62	5.02	4.82
10	4.63	4.26	4.85	4.36
20	4.34	3.97	4.52	4.07

Table 2. Effect of different concentrations of Hg on the fresh weight (gm) of *Lemna minor*.

Hg(ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	5.54	6.01	5.84	6.44
1	4.42	4.28.	4.45	4.32
3	4.30	4.08	4.31	4.15
5	4.05	3.83	4.05	3.92

Table 3. Effect of different concentrations of Cu+Hg on the fresh weight (gm) of *Lemna minor*.

Cu + Hg (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	5.54	6.01	5.84	6.44
2.5+0.5	4.52	4.38	4.64	4.44
5+1.5	4.40	4.21	4.48	4.26
10+2.5	4.17	3.92	4.25	3.97

Table 4. Effect of different concentrations of Cu on the dry weight (gm) of *Lemna minor*.

Cu (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	.241	.254	.247	.262
5	.228	.224	.231	.226
10	.226	.214	.228	.219
20	.212	.205	.218	.208

Table 5. Effect of different concentrations of Hg on the dry weight (gm) of *Lemna minor*.

Hg (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	.241	.254	.247	.262
1	.218	.207	.222	.213
3	.205	.198	.214	.202
5	.197	.183	.200	.186

Table 6. Effect of different concentrations of Cu+Hg on the dry weight (gm) of *Lemna minor*.

Cu +Hg (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	.241	.254	.247	.262
2.5+.5	.226	.214	.228	.220
5+1.5	.217	.203	.221	.208
10+2.5	.207	.195	.213	.199

Table 7. Effect of different concentrations of Cu on the biomass index of *Lemna minor*.

Cu (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	1.108	1.202	1.168	1.288
5	.977	.925	1.005	.964
10	.927	.852	.957	.872
20	.872	.795	.904	.814

Table 8. Effect of different concentrations of Hg on the biomass index of *Lemna minor*.

Hg(ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS
Control	1.108	1.202	1.168	1 .288
1	.886	.856	.890	.898
3	.861	.817	.863	.830
5	.810	.767	.818	.784

Table 9. Effect of different concentrations of Cu+Hg on the biomass index of *Lemna minor*.

Cu +Hg (ppm)	SUMMER		WINTER	
	3 DAYS	5 DAYS	3 DAYS	5 DAYS

Control	1.108	1.202	1.168	1.288
2.5+0.5	.905	.877	.929	.889
5+1.5	.880	.842	.896	.853
10+2.5	.834	.784	.850	.794

The result that Hg is more toxic than any other metal was also supported by Servilla et al.(2005). They showed it on tomato growth. N.Dirilgen (2011)also showed that Hg is more toxic than Pb for *L.minor*. The effect of single metal is known to be influenced by the presence of other metals,resulting in inhibited or enhanced growth of one metal in the mixture (An et al.,2004). Several studies reported that the presence of one metal influenced the uptake of other metal (Peralta-Videa et al.,2002). Our studies show a higher effect of Hg than Cu, which confirms the results of An et al.(2004). The results of binary metal interactions was also supported by N. Dirilgen (2011). In this study it was observed that the metal effect efficiency of *L.minor* decreased in the presence of binary combinations of Cu and Hg with the increase in the toxicant concentrations as compared to cases where either metal was alone in the medium. This was classified as an antagonistic interaction.

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