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RESEARCH ARTICLE

CHLOROPHYLL RESPONSE OF DUCKWEED (LEMNA MINOR) IN HEAVY METAL (Cu AND Hg) POLLUTION

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ABSTRACT

Aim of present study is to assess the tolerance of chlorophyll change in duckweed plant (*Lemna minor*). Tolerance level and toxic effect of copper sulphate ($CuSO_4$) and mercuric chloride($HgCl_2$) in *Lemna minor* was investigated under laboratory conditions. For toxicity test plants of *L. minor* were exposed to different concentrations of $CuSO_4$ (5ppm, 10ppm and 20ppm) and $HgCl_2$ (1.0ppm, 3.0ppm and 5.0ppm) separately as well as in combination. The combination of metals ($CuSO_4$ + $HgCl_2$) were 2.5ppm Cu + 0.5ppm Hg, 5ppm Cu + 1.5ppm Hg and 10ppm Cu + 2.5ppm Hg. All experiments were conducted into two different seasons i.e. winter (December-January) and summer (May-June) and chlorophyll tolerance estimated at 2 days and 4 days post treatment with specific heavy metal/combination of metals respectively. There was decrease in the chlorophyll content with highest concentration being at 20ppm Cu and also when 5ppm Hg was added individually. Chlorophyll content was found decreased in all concentrations and was maximum at 20ppm Cu and 5ppm Hg concentration. Severity of symptoms was found toxicant concentration dependent. Possible mechanism of Cu and Hg toxicity to this bioindicator has been discussed in this paper. Tabulated data suggests the potential role played by *L. minor* in bioremediation of heavy metals within optimum concentrations

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INTRODUCTION

Heavy metal contamination in terrestrial and aquatic environment or ecosystem has been appeared as global environmental problem. Heavy metal pollution in aquatic ecosystem posed a serious threat to aquatic biodiversity and drinking water which turns caused severe health hazards in humans and animals (Shafi, Nuzhat, *et al.,* 2015). As excess level heavy metals are exposed into environment for example by industrial waste and fertilizers, cause serious concern in nature as they are non-degradable and accumulate at high levels (Divya Singh *et al*., 2012). At least 20 metals are classified as toxic with half of them emitted into environment in concentrations that pose great risk to human health (Akpor and Muchie, 2010). Heavy metals, released due to modern industrial activities, have significant impact on the land and water (Nriagu,1988, Verma and Suther, 2015). The indiscriminate disposal of agricultural and municipal waste reaches finally to lotic and lentic system, leading to water pollution, which may contain large quantities of organic and inorganic materials and also varying amounts of heavy metals. Heavy metals are classified in two main categories one is metals that are phytotoxic at both low as well as high concentrations like- Cd, Co, Hg and Cr and other in small quantities. Certain heavy metals are nutritionally essential for a healthy life but large amount of any of them may cause acute or chronic toxicity like Cu, Zn, Mn, Fe and Ni (Langille and Maclean, 2005, Singh Divya *et al.,* 2012). Copper is one of the oldest known metal and 25th most abundant element in the Earth's crust. Cu is introduced in aquatic ecosystem by natural process as well as by human interferences (Singh, P., 2015). The presence of Cu above trace levels in the environment is indicator pollution. On the other hand Cu is essential micronutrients for plants; Cu is structural and catalytic component of many proteins and enzymes involved in metabolic pathways (Teisseire and Vernet, 2000). However when concentration reaches beyond its optimum value, these essential metals become first inhibitor and then toxic. Cu is responsible for many alterations on plant cells (photosynthesis, respiration, pigment synthesis and enzyme activity) (Kanoun Boule, 2009). Each plant species has different resistance and tolerance level to different contaminants. Mercury is highly toxic heavy metal that is found naturally. Mercury poisoning also has become a problem of current interest as a result of environmental pollution on a global scale. Hg is strong phytotoxic as well as genotoxic metal. Hg has a wide variety of uses in industries, medicine, dentistry, batteries, paint and electrical equipments. Mercury pollution is currently a global environmental threat. Hg is anthropogenic in origin and liquid metal that has different salt forms, some of which are

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bioavailable, causing serious environmental problems world over, lacking adequate experimental data (Boening, 2000). Hg is non-essential, non-biodegeradable and toxic to life, especially when it is geochemically transformed into bio-available forms (Karuna Sagar *et al.,* 2006). Present work describes the usefulness of duckweed (*Lemna minor*) bioassay, an aquatic plant test system for monitoring the toxicity of heavy metals. The aquatic macrophytes of lemnaceae are most extensively used plants (Wang, 1992) and have been recommended as a standard test organism for testing phytotoxicity of waste waters, chemicals by OECD (2000); ISO (2001).

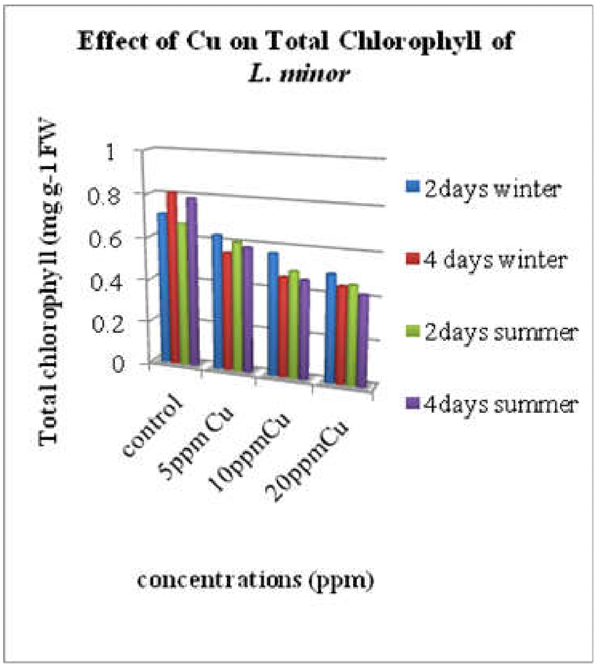
MATERIALS AND METHODS

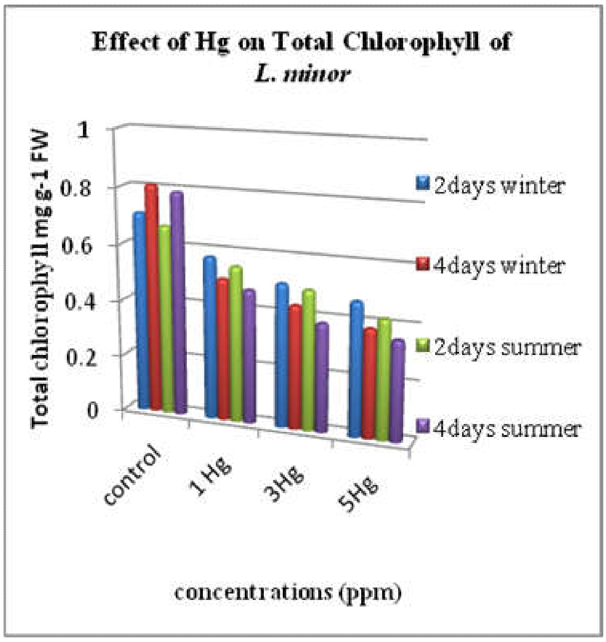
Duckweed plants were obtained from Nakatia River situated at Bareilly (U.P.). The plants were washed very well and rinsed in distilled water. The plants were exposed to different concentrations of Cu2+ and Hg2+ ions i.e. 5ppm, 10ppm and 20ppm of Cu and 1.0ppm, 3.0ppm and 5.0ppm of Hg separately as well as in combination (2.5ppm Cu+0.5 ppm Hg, 5ppmCu+1.5ppmHg10ppmCu+2.5ppmHg). Initially both Cu and Hg concentrations were used as same concentration (as in single) in binary combination (for sake of uniformity) but same concentration of both metals were highly toxic and *L. minor* could not survive beyond 4days. Therefore, the levels of Cu and Hg were reduced to half of single dose. The experiments were conducted in plastic tubs carrying 5g of biomass with triplicates for each treatment. Comparison of metal exposed plants was made with untreated (control) plants. The data pertaining to chlorophyll was obtained after 2days and 4days in two different seasons i.e. winter (December-January) and summer (June-July). All the chemicals used were of analytical grade reagent (Merck, India).

Determination of chlorophyll: Chlorophyll content was estimated according to Hiscox and Israelstam (1979). In a tube containing 10ml DMSO (Dimethyl Sulphoxide), 50 mg of finely chopped fresh leaves were added. The tubes were kept in the oven at 65^{0}C for 3 hrs. 1ml aliquot was mixed with 2ml DMSO and was vortexed. Absorbance was determined by photometric estimation at 645nm, 663nm with pure DMSO as blank.

RESULTS AND DISCUSSION

Treatment of aquatic plant *L. minor* with different concentrations of Cu and Hg showed variable growth rate during two different seasons i.e. winter and summer. Higher concentration of Cu (10ppm) and lower concentration of Hg (1ppm) showed morphological symptoms such as chlorosis, yellow coloration, less plant growth, decrease photosynthetic activity etc. These symptoms were more prominent during summer season as compared to winter season. The effect of different concentrations of Cu, Hg and combined metal (Cu+Hg) on total chlorophyll of *L. minor* is described in Table1 and Figure 1, 2, 3. In case of Cu, Hg and binary combination gradual decrease in total chlorophyll was observed at 2 and 4 days exposure in winter. Same pattern was following during summer season. Total chlorophyll declined progressively with increasing concentrations of metals in comparison with controls. The effect of Hg was more toxic than Cu. The results showed that combined metal concentrations also decreased the chlorophyll content during summer and winter.





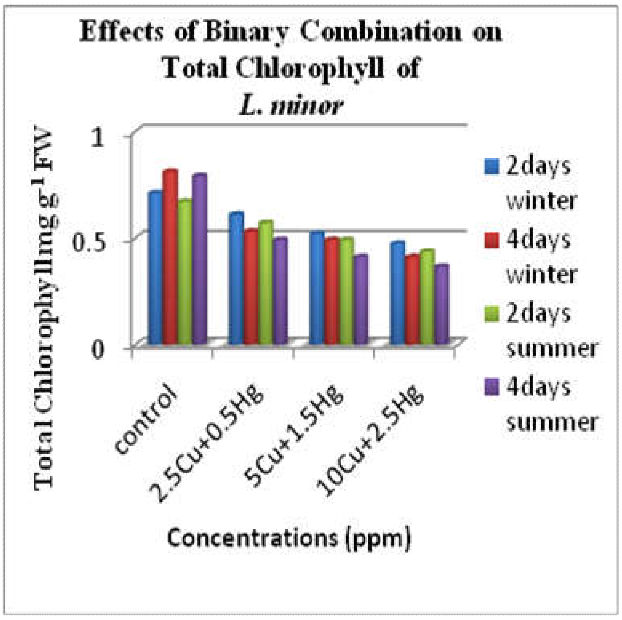


Table 1. Effect of different concentrations of Cu, Hg and binary combination (Cu+Hg) on total chlorophyll (mg g^{-1} FW) of *L. minor* at 2 and 4days post exposure to heavy metal during winter and summer season. Values are means of replicates ± SE (n=3)

Concentrations (ppm)	Total chlorophyll content (Winter) Mean± SE		Total chlorophyll content (Summer) Mean± SE	
	2 days	4 days	2 days	4 days
Control	0.710±0.035	0.810±0.035	0.670±0.010	0.790±0.040
5 Cu	0.630±0.034	0.553±0.029	0.606±0.018	0.586±0.043
10 Cu	0.570±0.030	0.466±0.014	0.496±0.003	0.460±0.015
20 Cu	0.500±0.015	0.450±0.021	0.460±0.006	0.420±0.005
1 Hg	0.576±0.012	0.503±0.008	0.550±0.025	0.473±0.033
3 Hg	0.506±0.046	0.436±0.021	0.493±0.014	0.383±0.017
5 Hg	0.470±0.046	0.383±0.017	0.420±0.020	0.353±0.008
2.5 Cu + 0.5 Hg	0.610±0.031	0.530±0.006	0.570±0.003	0.490±0.012
5 Cu + 1.5 Hg	0.520±0.017	0.490±0.024	0.490±0.013	0.410±0.032
10 Cu + 2.5 Hg	0.473±0.037	0.410±0.010	0.436±0.031	0.366±0.017

Many studies have demonstrated influence of heavy metals on chlorophyll content in plants such as-Ouzonidou, 1996; Ralph and Burchett, 1998; Xiong *et al.,*2006; Tanyolac *et al.,*2007; Singh *et al*.2007. In this study heavy metals (Cu, Hg) treatment also was found to reduce total chlorophyll content. Various abiotic stress decrease the total chlorophyll content in plants (Ahamad *et al*., 2007). Several reports show chlorophyll biosynthesis inhibition by metals in higher plants (Prasad and Prasad, 1987, Fikriye and Sevda, 2007). The mechanism of heavy metals on photosynthetic pigments may be owed to (John, R. *et al*.,2008; Fikriye and Sevda, 2007)- (a) inhibition of important enzymes, such as $^{\delta}$ -aminoevulinic acid dehydratase (ALA- dehydratase) and protochlorophyllide reductase (Van Assche and Clijsters,1990). (b) impairment in the supply of Mg^{2+} and Fe^{2+} required for synthesis of chlorophyll. (c) Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters, 1990). (d) the replacement of Mg^{2+}ions associated with the tetrapyroll ring of chlorophyll molecule. Our results of decrease in chlorophyll content corroborated with the findings of Balsberg Pahlsson, 1989; Vajpayee *et al*.,2000; Siedlecka and Krupa,1996; Ahamad *et al*., 2007 who also found decrease in chlorophyll content with heavy metal stress in many plants such as *Zea mays, Acer rubrum, L. minor* etc. Our results also indicate that high level of Hg^{2+} is strongly phytotoxic to cells and can decrease the chlorophyll content.

Conclusion

In conclusion our results indicate that exposure of *Lemna minor* to different concentrations of copper Cu and mercury Hg which were provided as $CuSO_4$ and $HgCl_2$ results is decrease in total chlorophyll content in cell. Hg was more toxic than Cu. Thus it can be concluded that duckweed is sensitive to the variations in the metal concentrations and is capable of high metal enrichment at test levels. Although growth was reduced at these concentrations, the aquatic species could be a good candidate for cleaning up waste water polluted with Cu and Hg at low level.

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