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Observations on the Experimental Assessment of Optimal Exposure Time for Mercury Detoxification by an Integrated Aquatic Macrophyte Base System

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Due to awareness of heavy metals for ecological toxic contamination, great thrust was given to the study of heavy metal accumulations by aquatic macrophytes for of metal recovery and removal from the aquatic environment (Wolverton, 1982; Reddy and Smith, 1987; Brix and Schierpup, 1989; Cooper and Findlater, 1990). *Eichhornia crassipes* and *Phragmites australis* have gained considerable attention in the last few years culminating in full scale macrophyte base system development for removal of heavy metals from aquatic bodies (Brix, 1991). Shrivastava and Rao (1997) proposed integrated aquatic macrophyte base system (IAMB) in which combination of weed groups constituting different types of weeds viz. floating, emergent and submerged types were used. This IAMB system recorded better results when compared to using individual weed plants. In the present study an assessment of integrated aquatic macrophyte system was made for overall removal of mercury within a laboratory scale. This study aims to assess the optimal exposure time for mercury detoxification by IAMB system. Which is currently being attempted in the tropical countries.

MATERIALS AND METHODS

Integrated aquatic macrophyte base system was designed and assembled in the laboratory. IAMB system constituting two floating weeds *Eichhornia, Salvinia* in the surface water layers, *Chara* and *Hydrilla* in column layer and *Vallisneria* in the bottom layers. The fish *Oreochromis mossambicus* was selected as test animal.

Mercuric chloride was used as toxicant. Fish LC-100 values and other toxicological data were obtained and computed in the earlier experiment. Fish lethal concentration 1.00 ppm of $HgCl_2$ was selected as toxicant concentration for the present study. Five experimental groups, constituting five selected weeds were subjected to established fish lethal concentration of mercury for a period of 24 hr, 48hr, 72hr, 96hr and 120hr to facilitate mercury absorption. The fish were then introduced. The details of experimental groups are as per details given under :

1. Control : Equal weight of five weeds (20 gms each for 10 litres) + water + fish
2. Lethal concentration - LC-100 concentration of $HgCl_2$ in experimental group water + fish.

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3. Experimental group 1 - Equal weight of live weeds in LC100 concentration of $HgCl_2$ (exposed for 24 hr) + fish
4. Experimental group 2 -Equal weight of five weeds in LC100 concentration of $HgCl_2$ (exposed for 48 hr) + fish.
5. Experimental group 3 - Equal weight of five weeds in LC100 concentration of $HgCl_2$ (exposed for 72 hr) + fish.
6. Experimental group 4 - Equal weight of five weeds in LC100 concentration of $HgCl_2$ (exposed for 96 hr) + Fish.
7. Experimental group 5 - Equal weight of five weeds in LC100 concentration of $HgCl_2$ (exposed for 120 hr) + Fish.

The mercury detoxification evaluation by IAMB system were based on fish bioassay (relative fish mortality, opercular movement, locomotory activity), and Hg incoporated value in weeds followed by reduction in mercury concentration in experimental water. Fish mortality was observed at an interval of 12hr upto 96hr of experimentation. Locomotory activity of fish in different groups was measured in aquaria with the base marked in one cm. squares (Sage 1965). Mercury concentrations in experimental weed and water was determined by Atomic Absorption Spectrophotometer Perkin Elmer 3100 (Shrivastava and Rao 1997).

RESULTS AND DISCUSSION

Over the years, a wide range of treatment technologies were suggested and used in an effort to restore and maintain the chemical, physical and biological integrity of water bodies (Tare et al., 1988). These technologies however could not result in a significant improvement, because they are too cost-intensive to be applied for sustaining on a mass scale. The aquatic weed plants absorb and incorporate the dissolved toxicant material in their own body tissues so rapidly and effectively that they are now considered for use in treatment of heavy metal contamination removal from effluents. In this scenario, this investigation on relative bioabsorbancy is a viable approach of heavy metal recovery from waste waters contaminated with toxic metals (Reddy and Smith, 1987; Oke and Juwarkar, 1996). Wolverton et al. (1983) suggested the use of vascular aquatic plants for inorganic and organic pollution removal. The aquatic plants absorb and accumulate calcium, nickel, mercury, silver, cobalt and others which become 4000-20,000 times more concentrated in plants than in the medium through the process of biomagnification (Moore, 1990).

Dhanekar et al. (1984) and Shrivastava and Rao (1989) reported highest capability of mercury removal by *Eichhornia* in laboratory conditions. Kaiser (1993) observed that *Eichhornia* is an adequate weed having a great capacity of removing heavy metals through roots. Oke and Juwarkar (1996) reveals that nearly 60% of heavy metals are retained in the root bed and 40% gets accumulated in plant biomass. Shrivastava and Rao (1997) suggested that the heavy metal load in natural condition is concentrated more in the hypolimnic layers where the floating weeds have less access. Hence the proposed integrated aquatic macrophyte base system (IAMB) in which two floating weeds *Eichhornia* and *Salvinia* in surface layer, *Chara* and *Hydrilla* in column layer and *Vallisneria* in the bottom layer provides efficient mechanism of metal absorption through their roots and record better results when compared to using individual weed plants.

In the present study an assessment of IAMB system was made for removal of mercury through fish Bioassay test. The fish *Oreochromis mossambicus* was subjected to its lethal concentration of $HgCl_2$ (1 ppm) recorded 100% fish mortality at 96 hr. of experimentation. The same concentration was used in all experimental set up (Table 1). Experimental groups 1 and 2 yielded low mortality rate 40% and 20% respectively in IAMB which were exposed for 24 hr and 48 hr of experimentation. IAMB system which were exposed for 72 hr., 96 hr. and 120 hr. (Experimental group 3,4 and 5) have shown no mortality at 96 hr. of experimentation. The present study indicates 100% fish mortality recovery in 72, 96 and 120 hr by experimentation 60% and 80% mortality recovery was recorded in 24 and 48 hr of experimentation respectively. Recovery in mortality rates established the Hg. removal capacity of IAMB system. All the data show that mercury removal efficiency increases with increasing exposure time.

Table 1. Mercury concentration, fish mortality response and mercury toxicity recovery in different experimental groups at 96 hr.

Experimental group	Average Mercury concentration		% Fish mortality	% Hg Toxicity recovery
	Weed mg/kg	Water mg/l		
1. Control group	0.20	Nil	Nil	Nil
2. Lc100 group	Nil	1.00	100%	Nil
3. Experimental Group 1	0.42	0.84	40%	16%
4. Experimental Group 2	0.54	0.44	20%	56%
5. Experimental Group 3	0.68	0.24	Nil	76%
6. Experimental Group 4	0.80	0.19	Nil	81%
7. Experimental Group 5	0.86	0.18	Nil	82%

The fish stress study in the form of opercular movements and locomotor-y activity exposed to mercury toxicity and their recovery efficiency in the experimental group of IAMB system was established. The opercular movements of experimental fish showed maximum hyperactivity in the lethal concentration at 36 hours after which the activity gradually decreased leading to death in mercuric chloride exposed fish as shown in figure 1. In control group fish initial hyperactivity was observed upto 12 hours and then attained basal level of opercular movements indicating initial handling effect and acclimatization in experimentation. The experimental groups 3,4 and 5 which were exposed for 72, 96 and 120 hr. respectively have shown similar trend of opercular movements as found in control group. These results show recovery of mercury chloride toxicity stress on fish.

As can be seen from fig.2 locomotor-y activity of fish was observed higher in control groups. Lowest values of locomotory activity were reported in mercuric chloride exposed fish. In the experimental groups 1 and 2 comparatively low values of locomotary activity was observed. Experimental group 3,4 and 5 which were exposed for 72, 96 and 120 hr. show similar pattern as found in the control. group. These observations also support that increasing exposure time to IAMB system resulted in less toxicity stress. Increased opercular movements and decreased locomotary activity were reported in the present study in the fish exposed to mercuric chloride (Fig. 1 and 2). It is probable that hyperactivity of opercular movements is correlated with decreased locomotory activity and compensate for the loss of efficiency in oxygen uptake by decreasing the physiological oxygen demand and increasing the amount of oxygen passing over the brachial tissues per unit time (Menezes and Qasim, 1983 and Dhanekar et al., 1984).

To deduce optimal time exposure for maximum mercury removal performance of IAMB system in the laboratory scale accumulation studies were made for estimating Hg incorporation in the weeds and in the experimental water, which show variable incorporation levels in each weed. Weeds in the control group also show mercury incorporation level 0.20 ppm (Table 1 & 2) which may be due to background concentration levels. The results indicate maximum mercury accumulation in *Eichhornia.* Next in order were *Vallisneria, Salvinia, Hydrilla,* and *Chara* (Table 2). Eventhough individually *Eichhornia* removes maximum quantity of mercury from the medium and in *Vallisneria, Salvinia, Hydrilla,* and *Chara* comparatively it capacity to remove lesser rates yet it was quite significant in terms of total percentage removed at, middle and bottom zones.

The fish lethal concentration of mercury was observed to decrease in different IAMB experimental group due to variable time of IAMB exposure (Table 1). The maximum recovery 82% was observed in 120 hr. exposed group 5. Next in order were 81%, 76%, 56% and 16% recovery in IAMB system exposed for 96, 72, 48 and 24 hr. at 96 hr. of experimentation (Experimental group nos. 4,3,2,1). The present results indicate that maximum quantity of mercury was accumulated by weeds of IAMB system with greatest rapidity upto 72 hr. of exposure, eventhough 56% recovery take place in first 48 hr of exposure. After the critical period of 72 hr. the rate of toxicant absorption suddenly reduces and becomes negligible. This may be due to the fact that the system might have achieved its maximum incorporation level and become saturated. It is therefore suggested that moderate required plant growth after 72 hr period can be harvested regularly, each time allowing the regrowth of a new crop, so that the IAMB water purification system becomes a continuous process. Thus a permanent treatment plant can be maintained in an Industrial area on a continuous basis. Studies with regard to aquatic macrophyte combination to be used in treatment pond and the period of macrophyte replacement should be seriously

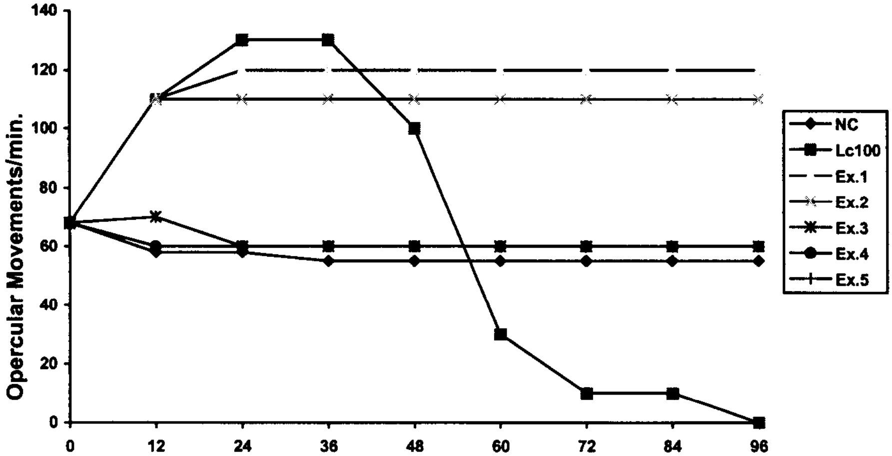

Figure 1. Opercular movements of *O.mossambicus* in different expermental groups

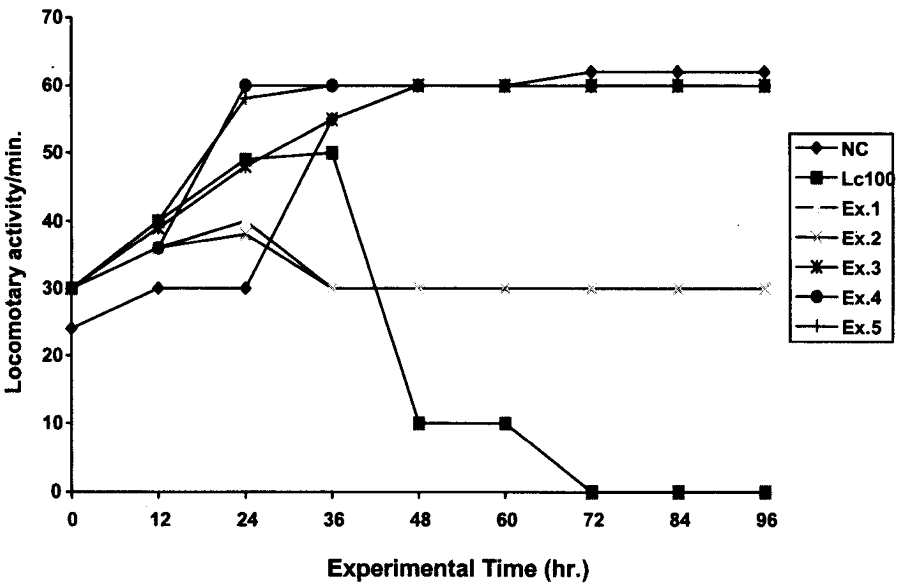

Figure 2. Locomotary activity of *O.mossambicus* in different expermental groups

undertaken for developing a more efficient natural and cheap integrated macrophyte base system, which will be most advantageous for toxicant removal.

This study demonstrated that IAMB system performed very well in treating waters contaminated with mercury. The IAMB system based biotechnologies are ideal for treating large quantities of industrial waste waters. Moreover, this system also has the potential use in management of ecological system and conservation of their biological diversity (Lucia et al., 1996). Since the weeds remove simultaneously a variety of toxic substances, such a system will be simple, economic and therefore much more viable than the traditional chemical treatment. The subproducts of the waste water treatment after incineration are of great commercial value as organic fertilizers, textile fiber, paper, tiles and other bioproducts.

Table 2 : Mercury concentration in different weeds of 1AMB system at 96 hrs. of experimentation.

Experimental Group	Average Mercury Concentration (mg/kg)				
	Eichhornia	Chara	Salvinia	Hydrilla	Vallisneria
1. Control group	0.35	0.15	0.15	0.15	0.20
2. Experimental Group 1	0.92	0.18	0.30	0.30	0.40
3. Experimental Group 2	1.40	0.20	0.40	0.40	0.32
4. Experimental Group 3	1.68	0.28	0.50	0.43	0.55
5. Experimental Group 4	1.90	0.32	0.64	0.48	0.65
6. Experimental Group 5	2.05	0.34	0.69	0.50	0.72

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