Impact of sublethal levels of mercury on glycogen and selected respiratory enzymes in *Heteropneustes fossilis* and role of water hyacinth in reduction of Hg toxicity

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Mercury adversely affects various physiological and biochemical functions of fish (Snarski and Olson 1982; Muthukrishnan *et al.* 1986). Water hyacinth, *Eichhornia crassipes*, has been reported to have the capability of absorbing and removing some heavy metals from polluted water bodies (Wolverton and McDonald 1975; Chigbo *et al.* 1982). However, studies on utilization of aquatic plants for the reduction of mercury toxicity to fish are lacking. This study reports the sublethal effects of mercury on glycogen and selected respiratory enzymes in *Heteropneustes fossilis* and the role of water hyacinth in reducing the metal toxicity.

*Heteropneustes fossilis* (300) were collected from Korampallam pond near Tuticorin (Lat. 8º46'; Long. 75º5'), Tamil Nadu, and acclimated to the laboratory conditions and feeding schedule. Well-acclimated and active (126 fish) *H. fossilis* (weight 12.5 ± 1.0 g; length 14.5 ± 1.3 cm) were chosen from acclimation tank and were starved for 24 hr prior to the experiment. Static renewable bioassay method was adopted (Sprague 1973) and the 96 hr LC₅₀ was determined separately as 0.099 ppm following Litchfield and Wilcoxon (1949). Healthy individuals were divided into 7 groups. Triplicates were maintained for each group. Group 1 served as control. Individuals in groups 2, 3 and 4 were exposed to sublethal concentrations 0.01, 0.02 and 0.03 ppm of mercury and groups 5, 6 and 7 were exposed to the above concentration along with healthy water hyacinth, *E. crassipes*, of uniform sizes and number. Mercuric chloride (HgCl₂) was used for getting required concentration of mercury. The experiment was conducted in plastic trough containing 10 litres of test medium.

Test individuals were fed on minced pieces of goat liver *ad libitum*. The medium was changed daily (Sprague 1971) to maintain constant toxic concentration by adding appropriate amount of mercury stock solution. Salinity, dissolved oxygen content, temperature and pH of test medium averaged 0.13‰, 5.07 ml O₂/litre, 30°C and 7.35 respectively. The experiment was conducted for 15 days. Feeding was discontinued 1 day before the experiment. After exposing for 15 days the control and experimental fishes were sacrificed and tissue muscle, liver and gill were isolated and kept at 0°C for the estimation of glycogen and dehydrogenases. The glycogen content was estimated by the method of Kemp and Kits (1954). Succinate dehydrogenase (SDH) and glyceraldehyde
dehydrogenase (GDH) activities were estimated as per Kun and Abood (1949). The chlorophyll content of *E. crassipes* was estimated as per Amon (1949).

Of the 3 tissues analysed, liver was the major site of stored glycogen followed by muscle and gill (Table 1). Compared to control, the glycogen content in liver, muscle and gill was reduced by 82, 73 and 72% respectively in fish exposed to mercury alone at a concentration of 0.03 ppm whereas it was 60, 58 and 57% respectively in animals exposed to mercury + plant at 0.03 ppm. This indicated maximum utilization of glycogen in fish exposed to mercury alone. SDH activity showed a decline in all tested tissues both in fish exposed to metal alone and metal + plant combination. The activity of GDH, on the other hand, increased in all the tissues with the increase in mercury concentration. This increase in liver, muscle and gill at 0.03 ppm was 112, 95 and 75% respectively in fish exposed to mercury alone and 96, 65 and 58 respectively in fish exposed to metal + plant, as compared to controls.

The increase in the sublethal concentrations of mercury caused a significant reduction in chlorophyll content of *E. crassipes*. It was 0.34 mg/g wet tissue when reared in metal free water but it decreased to 0.08 mg/g wet tissue in plants exposed to 0.03 ppm of mercury (Table 2). The green colour of leaf gradually changed to brown during the experimental period due to mercury toxicity.

The significant decrease in glycogen reserves in tested tissues on exposure to mercury indicated that carbohydrate metabolism is altered. Perhaps the reserve glycogen was mobilized and broken down through glycogenolysis to meet the high energy requirement caused due to mercury stress. Mercury exposure may stimulate hormones that accelerate glycogen breakdown or inhibition of those associated with glycogen synthesis (Sahib et al. 1983). The decrease in glycogen content was relatively more in fish exposed to

### Table 1. Effect of sublethal concentrations of mercury alone and with water hyacinth on glycogen, succinate dehydrogenase and glyceraldehyde dehydrogenase in chosen tissues of *Heteropeustes fossilis* (each value is the mean + SD of 3 observations)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Mercury alone (ppm)</th>
<th>Mercury (ppm) + Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>9.13 ± 0.38</td>
<td>7.10 ± 0.53</td>
<td>5.81 ± 0.39</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.54 ± 0.17</td>
<td>3.19 ± 0.20</td>
<td>2.21 ± 0.24</td>
</tr>
<tr>
<td>Gill</td>
<td>2.23 ± 0.13</td>
<td>1.51 ± 0.21</td>
<td>1.01 ± 0.06</td>
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<tr>
<td>Liver</td>
<td>98.41 ± 1.49</td>
<td>67.05 ± 0.85</td>
<td>51.91 ± 0.54</td>
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<tr>
<td>Muscle</td>
<td>66.33 ± 0.19</td>
<td>49.74 ± 0.63</td>
<td>38.30 ± 0.69</td>
</tr>
<tr>
<td>Gill</td>
<td>54.10 ± 0.39</td>
<td>41.05 ± 0.19</td>
<td>35.72 ± 0.13</td>
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</table>

A. Glycogen

B. Succinate Dehydrogenase

C. Glyceraldehyde Dehydrogenase

* A. Values expressed as mg glucose/g of wet tissue; B and C, values expressed as µg reduced TTC/100 mg wet tissue/hr; *P < 0.01; **P < 0.05
Table 2. Effect of sublethal concentrations of mercury on chlorophyll content of *Eichhornia crassipes* (each value represents the mean of 3 observations)

<table>
<thead>
<tr>
<th>Mercury concentration (ppm)</th>
<th>Chlorophyll content (mg/g wet tissue)</th>
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<tbody>
<tr>
<td>0</td>
<td>$0.34 \pm 0.09$</td>
</tr>
<tr>
<td>0.01</td>
<td>$0.22 \pm 0.05$ NS</td>
</tr>
<tr>
<td>0.02</td>
<td>$0.16 \pm 0.06^*$</td>
</tr>
<tr>
<td>0.03</td>
<td>$0.08 \pm 0.01^{**}$</td>
</tr>
</tbody>
</table>

$^{*}P < 0.01$; $^{**}P < 0.05$; NS, Not significant

Mercury alone than those exposed to mercury + plant. Qayyam and Shaffi (1977) found reduction in stored tissue glycogen in *H. fossilis* exposed to mercuric nitrate. Mayes (1977) suggested that glycogen reserve may be utilized for countering the toxicant stress.

Suppression of SDH activity in tissues of fish exposed to mercury alone and mercury + plant indicated the impairment of oxidative metabolic cycle. However, elevation in GDH activity was an evidence of a shift in energy metabolism of fish from aerobiosis to anaerobiosis under mercury stress. It is likely that mitochondrial disruption (Vasllos et al. 1976) lead to a decrease in the activity of oxidative enzymes (Brierley 1977, Deung et al. 1978) and an increase in glycolytic enzymes (Swami et al. 1983). Elevation of GDH activity also indicated the activation of compensatory mechanism for adequate energy supply.

Liver, being the organ for interconversion and storage of foodstuffs and a centre for all detoxification mechanisms, demands more energy. Drastic shifts in SDH and GDH activities of liver in *H. fossilis* indicated a high energy demand for metabolic coordination and continuous detoxification mechanisms. Another vital organ, gill, experienced drastic changes in energy cycles as it was in direct contact with polluted water and performed respiratory and osmo-and ionic-regulatory functions. Similar observations were also made on *Tilapia mossambica* by Koundinya and Ramamurthy (1978) and on *Channa striatus* by Natarajan (1981).

Animals exposed to mercury alone showed more reduction in glycogen and suppression in SDH activity than those exposed to mercury + plant. It was hence, inferred that *E. crassipes* removed mercury from the medium and thereby indirectly reduced the toxicity on animals. Earlier studies also proved water hyacinth, *E. crassipes*, as an efficient absorber of mercury and lead (Wol- verton and McDonald 1975), and also arsenic and cadmium (Chigbo et al. 1982) from polluted water bodies. The decline in chlorophyll content and colour changes in leaves of *E. crassipes* in metal contaminated water indicated the absorption of mercury from the medium. This also indicated that mercury has adverse effects on water hyacinth at sublethal levels. Control plant, however, did not show any change in colour and chlorophyll content. Presence of water hyacinth, *E. crassipes*, in polluted water would thus help in reducing the toxicity of mercury on organisms.

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251


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