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# Accumulation and Depuration of Mercury in a Catfish *Heteropneustes fossilis* (Pisces: *Heteropneustidae*) Exposed to Sublethal Doses of the Element

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## Abstract

The accumulation and depuration of mercury in exposed *Heteropneustes fossilis* to two sublethal levels of the element has been studied as a function of time. Exposure produced a time- and dose-dependent increase in the concentrations of mercury in all tested tissues. Animals exposed to both concentrations accumulated significant amounts of mercury, levels in the tissues decreasing in the following order: liver > gill > muscle > intestine. On transfer of the fish to uncontaminated water after 9 days of exposure, the mercury concentrations in tissues gradually declined. All tissues of catfish exposed to 0.01 or 0.03 ppm mercury recorded complete depuration of accumulated mercury over 37-123 days; individuals exposed to 0.03 ppm mercury took twice the time for excretion of the element as those exposed to 0.01 ppm.

## Introduction

Mercury is an extremely toxic heavy metal widely used in chlor-alkali plants, electroplating, paints, plastics and the paper and pulp industries. Effluents from such plants contribute to the entry of mercury into aquatic environments. Mercury pollution causes a serious and complex environmental problem resulting in the rapid depletion of resources and deleterious effects on living organisms. Most aquatic organisms have the capability for concentrating metals by feeding and metabolic processes, which can lead to the accumulation of high concentrations of trace elements in their tissues. Metals exhibit significant biological half-lives in many species (Simkiss and Mason 1984).

Published reports exist on the toxic effects of metals on behavioral and respiratory responses (James 1990), survival and biochemical changes (James et al. 1991), hematology (Banerjee and Verma 1987) and growth (Rodgers and Beamish 1982) in fish. Studies related to the bioaccumulation and elimination of metals in fish tissues are important from the viewpoint of human health. Several authors have studied heavy metal accumulation or elimination in fish (e.g., Menezes and Qasim 1984; Cuvin and Furness 1988; Giles 1988; Barak and Mason 1990). There is paucity of information on whether the depuration of metals is dose-dependent and on the rate of metal depuration. The present paper reports on the accumulation and depuration of mercury in the catfish *Heteropneustes fossilis* exposed to mercury.

## Materials and Methods

Catfish, *H. fossilis*, were collected from Korampallam tank near Tuticorin (latitude 8°46; longitude 75°5), Tamilnadu, India. The fish were acclimated to laboratory conditions for a month, during which they were regularly fed *ad libitum* with minced pieces of goat liver. Well-acclimated and active animals (15.5±1.2 g) were chosen for the experiment. Fish were not fed one day prior to the experiment or throughout the bioassay period. The water used was clear and unchlorinated. The dissolved oxygen content, temperature, pH and salinity of the medium during the experiment averaged 4.7 $mg \cdot l^{-1}$, 31±1°C, 7.5 and 0.2‰, respectively. Fish were exposed to different concentrations of mercury (Sprague 1973) and the 96-hour $LC_{50}$ value was determined following the method of Litchfield and Wilcoxon (1949).

After determining the 96-hour $LC_{50}$ of mercury (0.099 ppm), two sublethal concentrations (0.01 and 0.03 ppm) were selected as dosage levels for the experiment. Prior to the commencement of the experiment, three fish were sacrificed to establish their background mercury concentrations. Ten fish were exposed to the chosen sublethal levels of the element for 9 days in a plastic trough containing 15 l of the test medium. Triplicate samples were maintained for each concentration. Three fish were removed from each test exposure and liver, muscle, gill and intestine tissues were analyzed after 1, 3, 6 and 9 days of exposure. After 9 days of exposure, all

test individuals were transferred to uncontaminated water and maintained for a further 21 days to investigate the depuration of mercury from the tissues. Three fish were sacrificed on 6, 11, 16 and 21 days post-exposure, and these were analyzed for mercury. Regression analysis was carried out based on a least squares method, following Zar (1974).

Total mercury in the tissues was estimated by taking 0.5-1 g wet tissue and digesting it with a 1:2 mixture of concentrated $HNO_3$: $H_2SO_4$ for 4 hours at 60°C in a 250 ml conical flask. Immediately prior to analysis, 5 ml of 20% stannous chloride solution was added (Sadiq and Zaidi 1983). Samples were analyzed by cold vapor atomic absorption spectrophotometry. The instrument was calibrated using $HgCl_2$ as a standard.

## Results

Fig. 1 shows the accumulation of mercury in *H. fossilis*. Increases in the exposure period and sublethal concentrations of mercury elicited significant time- and dose-dependent increases in accumulation of the metal in all tested tissues. Catfish exposed to 0.03 ppm attained concentrations of 11.7, 7.1, 4.4 and 3.2 $\mu g \cdot g^{-1}$Hg wet tissue in liver, gill, muscle and intestine, respectively. Similar trends were obtained in test animals exposed to 0.01 ppm of mercury; however, the amount of mercury accumulated in the tissues at this exposure level was significantly lower than that in fish exposed to 0.03 ppm. Regression equations obtained for mercury accumulation in liver, gill, muscle and intestine for *H. fossilis* exposed to 0.03 ppm are shown in Table 1. Mercury accumulation in the tissues decreased in the following order: liver > gill > muscle > intestine (Fig. 1). Catfish exposed to 0.01 ppm also showed the same ranking of tissues. The correlation coefficient 'r' was calculated in the two test groups and it was found to be positive and significant ($P<0.05$; see Table 1).

On transfer of exposed catfish to uncontaminated water after 9 days of exposure, mercury concentrations decreased in all tested tissues. Mercury levels after 21 days of depuration had decreased to 56.4, 48.4, 43.4 and 28.5% of the post-exposure concentrations in gill, muscle, intestine and liver, respectively in *H. fossilis* exposed to 0.01 ppm. Mercury levels returned to pre-exposure concentrations in

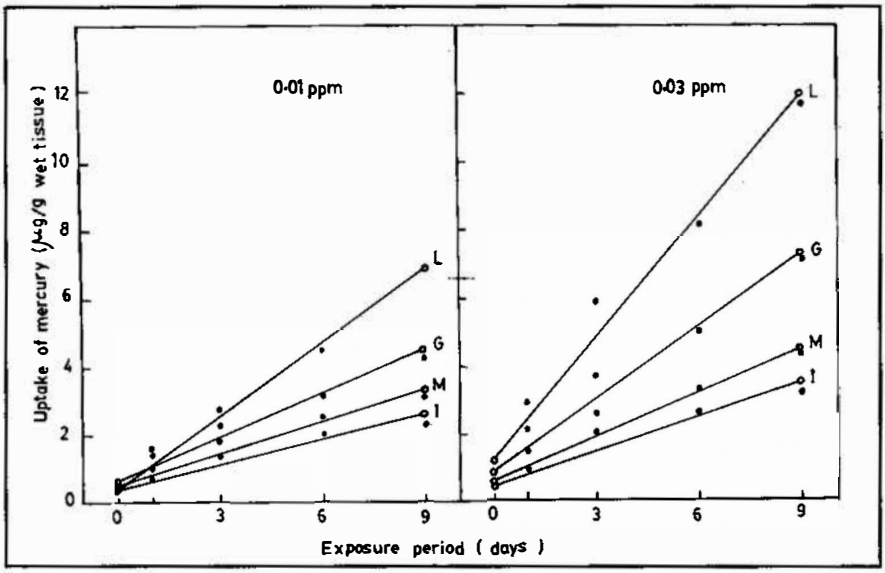


Fig. 1. Uptake of mercury (µg·g$^{-1}$ wet tissue) in *Heteropneustes fossilis* exposed to 0.01 and 0.03 ppm mercury as a function of exposure period. L=liver, G=gill, M=muscle and I=intestine.

Table 1. Regression equations and correlation coefficients obtained for the accumulation and depuration of mercury in *H. fossilis* exposed to different concentrations of the element.

| Tissues and sublethal levels of mercury | Accumulation period | | Depuration period | |
|---|---|---|---|---|
| | Correlation coefficient (r value) | Regression (Y = a + bX) | Correlation coefficient (r value) | Regression (Y = a + bX) |
| Liver | | | | |
| A | 0.993 | Y=0.390 + 0.72 X | -0.995 | Y=7.048 - 0.097 X |
| B | 0.981 | Y=1.172 + 1.20 X | -0.996 | Y=11.16 - 0.098 X |
| Gill | | | | |
| A | 0.971 | Y=0.580 + 0.43 X | -0.997 | Y=4.135 - 0.115 X |
| B | 0.970 | Y=0.903 + 0.71 X | -0.993 | Y=7.18 - 0.094 X |
| Muscle | | | | |
| A | 0.954 | Y=0.512 + 0.31 X | -0.992 | Y=3.149 - 0.069 X |
| B | 0.952 | Y=0.614 + 0.43 X | -0.776 | Y=4.238 - 0.050 X |
| Instestine | | | | |
| A | 0.943 | Y=0.389 + 0.24 X | -0.976 | Y=2.259 - 0.046 X |
| B | 0.954 | Y=0.486 + 0.33 X | -0.967 | Y=3.213 - 0.036 X |

A = 0.01 ppm; B = 0.03 ppm.
r = All are significant at 5% level

fish exposed to 0.01 ppm of the element in 74 (liver), 37 (gill), 46 (muscle) and 54 (intestine) days. The respective times for catfish exposed to 0.03 ppm of mercury were 123, 75, 94 and 100 days, respectively (Fig. 2). This indicates that animals exposed to 0.03 ppm of mercury took almost double the time for complete depuration of the element as fish exposed to 0.01 ppm of mercury. Depuration rates of mercury in the different tissues decreased in the order of gill > muscle > intestine > liver. A negative correlation

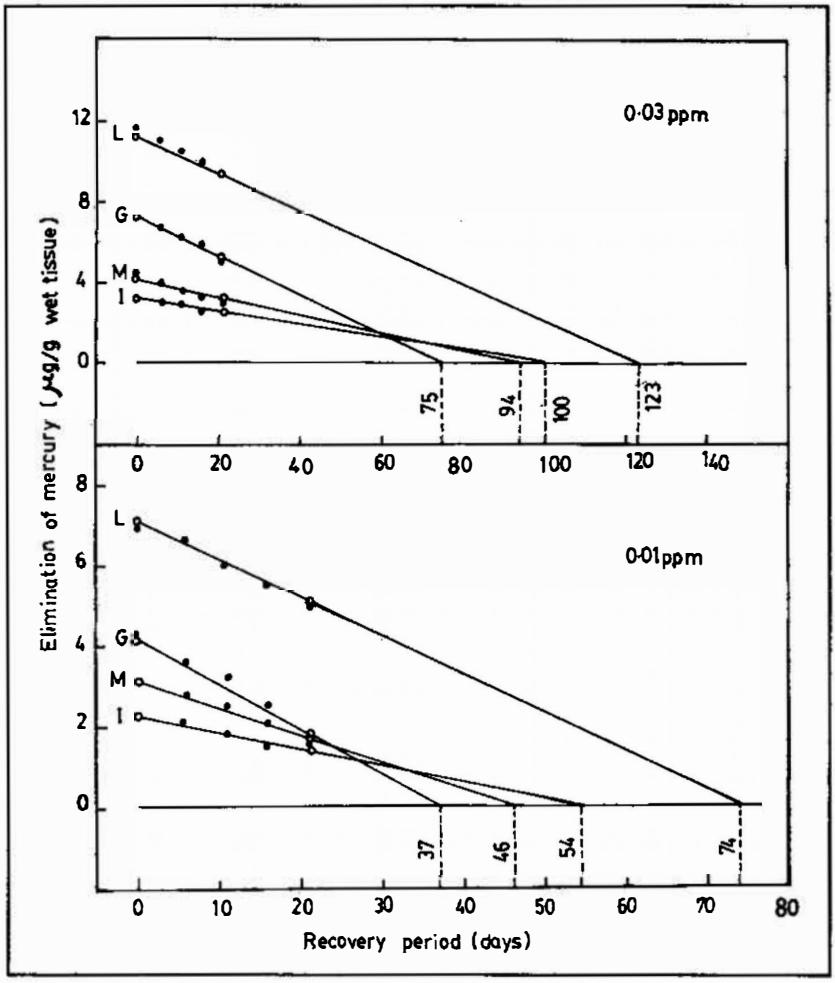


Fig. 2. Elimination of mercury ($\mu g \cdot g^{-1}$ wet tissue) in *Heteropneustes fossilis* exposed to two sublethal levels of mercury after transfer to uncontaminated water. L=liver, G=gill, M=muscle and I=intestine.

coefficient was obtained for the relationship between the depuration period and the amount of mercury lost, and this was statistically significant ($P<0.05$) for animals exposed to both concentrations.

## Discussion

The present study reveals that the uptake of mercury was greatest in liver of *H. fossilis*, followed by gill, muscle and intestinal tissues. High accumulation of mercury in the liver may be related to the storage, interconversion and detoxification functions of this organ. A higher uptake of mercury, cadmium and lead was reported in the liver than in the muscle of eels *Anguilla anguilla* and roach *Rutilus rutilus* (Barak and Mason 1990) and the same trend has been observed in rainbow trout *Salmo gairdneri* (*Oncorhynchus mykiss*)(Giles 1988). The gill also accumulates significant levels of mercury through direct contact with contaminated water and through its respiratory, osmotic and ionic regulatory functions. The high uptake of mercury in the gills of *Rangia cuneata* was considered by Dillion and Nelf (1978) to be due to their functional activity and position relative to the incoming ambient water. The absorption of metal ions by the mucous membrane of gills may also add to the concentrations accumulated (Smith et al. 1975). Muscle and intestine showed the lowest levels of mercury accumulation in *H. fossilis*, which support the findings of previous authors (Sangalang and Freeman 1979; Wilson et al. 1981). The mercury accumulation pattern in different tissues of *H. fossilis* indicates a degree of organ specificity, which may be related to the differences in physiological functions (Krishnakumar et al. 1990).

The present study also showed a time- and concentration-dependent linear accumulation of mercury (Fig. 1). Menezes and Qasim (1984) reported similar results for the accumulation of mercury in *Tilapia mossambica*. Similar observations were also made in *Gambusia affinis* (Paulose and Mahajan 1987; Newman and Doubet 1989), in freshwater clams (Smith et al. 1975) and in *Lymnaea accuminata* (Paulose 1987) exposed to mercuric compounds. The tissue burden of cadmium increased linearly with time in the liver, kidney, intestine and stomach of *O. mykiss* exposed to cadmium for 180 days (Giles 1988). Rodgers and Beamish (1982) reported whole body mercury concentrations of rainbow trout *O. mykiss* fed on

diets containing methylmercury to increase in a linear fashion with the exposure period. Cuvin and Furness (1988) found that the accumulation of mercury increased in a linear manner in minnows *Phoxinus phoxinus* on exposure to inorganic mercury for 24 days.

Fig. 2 shows the ability of different tissues of *H. fossilis* to eliminate mercury. The element was lost from gill tissues faster than from the other tissues, possibly through direct contact of the gills with the ambient medium. Holcombe et al. (1976) reported that gill tissues lost lead more rapidly than the liver of *Salvelinus fontinalis* exposed to the element. Mercury elimination rates in the tissues of *H. fossilis* in the present study decreased in the following order: gill > muscle > intestine > liver. Miettinen et al. (1972) reported that a considerable amount of mercury was retained in the visceral organs of *Tapes decussatus* and *M. galloprovincialis*. The greater retention of mercury in liver, intestine and muscle tissues compared to gills may be due to the reduced rate of internal flow of blood in such tissues, as reported by Dillion and Nelf (1978). Viarengo et al. (1985) suggested that the slow depuration of mercury from viscera and muscle of aquatic biota could be related to the chemical and physical affinity of metallothioneins in such tissues for the element.

In the present investigation, catfish exposed to 0.03 ppm of mercury took longer for complete depuration of the element, than fish exposed to 0.01 ppm (Fig. 2). Predicted times for the complete depuration of accumulated mercury in gill were 37 or 75 days and 74.5 or 123 days for liver tissues in fish exposed to 0.01 and 0.03 ppm, respectively, reflecting the faster loss of the element by gill tissues. Holcombe et al. (1976) found a similar pattern of depuration for lead in the tissues of trout, *O. mykiss*. Reichert et al. (1979) have shown that after 37 days of depuration, lead was completely eliminated in all tissues other than the kidney of *O. kisutch*.

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