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Assessment of Mercury Toxicity by the Changes in Oxygen Consumption and Ion Levels in the Freshwater Snail, *Pila globosa*, and the Mussel, *Lamellidens marginalis*

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Mercury is a highly toxic element to aquatic fauna. It is a group IIB metal, the behavior of which is significantly different from the other two members of the same triad zinc and cadmium. The total production of mercury greatly increased during this century with the increase in industrialization (National Academy of Sciences 1978). It has wide spread use in insecticides, fungicides, bactericides and pharmaceuticals. It is mainly used now in the manufacture of electrical equipment and in the electrolytic production of chlorine and caustic soda (Moore and Ramamoorthy 1984). The concentration of mercury in aquatic ecosystems, particularly freshwater ones, has been significantly increasing with the discharge of the effluents of these industries, leading to hazardous consequences. It can readily accumulate in tissues of aquatic fauna and has a high affinity for thiol groups, resulting in increased biotransport, distribution and toxicity (Jensen and Jernelov 1969). There are many studies on mercury toxicity in freshwater fishes (Mc Kim et al. 1976, Spehar et al. 1982), but very few on freshwater molluscs (Wright 1978) though they serve as bio-indicators of metal pollution. A few reports on marine gastropods and bivalves indicated the importance of these animals in metal toxicity studies (Zaroogian 1980). Hence, in the present study, the level of tolerance of the freshwater gastropod Pila globosa (Swainson) and of a freshwater bivalve Lamellidens marginalis (Lamarck) to mercury at lethal and sublethal levels was determined and compared with the rate of whole animal oxygen consumption and the level of sodium, potassium and calcium ions in the hepatopancreas and the foot of these animals. As the period of exposure is one of the important factors in toxicity studies, the level of tolerance was determined

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at 120 hours of exposure and the other parameters were analyzed at 1, 3 and 5 days in lethal and at 1, 7 and 15 days in sublethal concentrations.

MATERIALS AND METHODS

Freshwater snails and mussels weighing 15 ± 1g were collected from local ponds and wells and were maintained in the laboratory in 3'x2'x1' glass tanks, ten in each. Water from the local wells was used for their maintenance. It had pH 7.6 ± 0.2, total hardness 100 ± 5mg/l Ca CO_3, temperature 28 ± 1°C and oxygen content of 5.79 ± 0.4mg/l. Mussels and snails were fed with freshwater plankton and hydrilla, respectively, ad libitum. Water was changed once a day. The animals were adapted to laboratory conditions for ten days. Later, groups of thirty each were exposed to different concentrations of mercury, prepared from pure mercuric chloride, ranging from 2 mg/l to 8 mg/l, and the mortality rate was observed at 120 hrs of exposure. LC_{50} values for 120 hrs were determined from percent and probit mortality verses log concentration curves (Finney 1971) and were subsequently verified by Dregstedt and Behren's method (Carpenter 1975). Animals in freshwater with 1 ml of hydrochloric acid per liter, to nullify the chloride effect, served as controls. After the determination of LC_{50}s, groups of ten snails were exposed to lethal (120 hr LC_{50}), 4.5 mg/l, and sublethal (one fifth of 120 hr LC_{50}), 0.9 mg/l, concentrations and mussels to 3.0 mg/l and 0.6 mg/l. At the end of each exposure period, 1, 3 and 5 days in lethal and 1, 7 and 15 days in sublethal concentrations, the rate of whole animal oxygen consumption was measured using the Winkler iodometric method as described by Welsh and Smith (1953) in the animals survived, 9, 7 and 5 at the respective periods of lethal and 10, 10 and 9 of sublethal concentrations. Shells were then removed from the animals and the hepatopancreas and foot isolated. They were subjected to wet digestion in 1 : 1 (V/V) concentrated perchloric acid and nitric acid and evaporated to 100°C (Dall 1967). The residues were dissolved in distilled water and the levels of sodium, potassium and calcium were determined with a flame photometer (Elico Pvt. Ltd., Model CL-22A). The data were analyzed statistically, using Wilcoxon's t-test.

RESULTS AND DISCUSSION

The percent and probit mortalities of P. globosa and L. marginalis increased with the increase in mercury concentration (Figure 1A & B). The 120 hr LC_{50}s for snails obtained from percent and probit mortality curves and Dregstedt and Behren's method are 4.467, 4.467

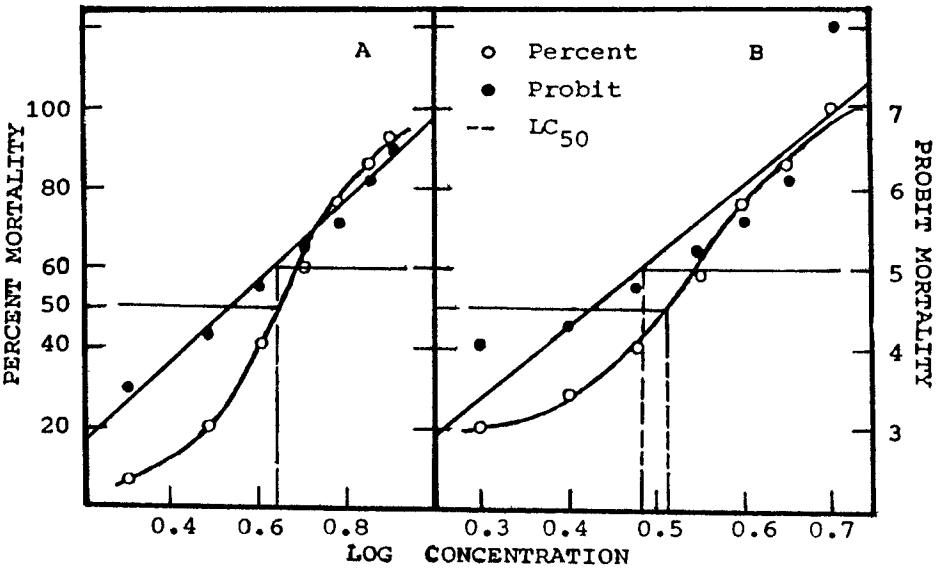

Figure 1. 120 hr percent and probit mortality of P. globosa (A) and L. marginalis (B) as a function of log concentration of mercury.

and 4.477 mg/l, respectively, and for mussels 3.311, 3.020 and 2.754 mg/l. These values reflect high tolerance of both the animals to mercury, as is also evident from studies in freshwater invertebrates where the 96 hr LC_{50} of this metal varies from 0.01 to 10 mg/l (Moore and Ramamoorthy 1984). This variation depends on the species, developmental stage, adaptive ability, amount of accumulation and environmental factors like temperature, pH, water hardness, size, etc. (Gardner et al. 1978). In the present study, the high tolerance capacity of snails and mussels to mercury could be due to their sedentary nature and/or adaptive ability, or because these animals may close up and not react to their environment. Further, the snails tolerated a higher mercury concentration in water than did mussels.

A few symptoms of mercury poisoning such as secretion of mucus sheet near the opening of shell valves, swelling of the foot and loss of control in closing the shell valves were noticed in the snails and mussels exposed to lethal concentrations. These symptoms were insignificant in the beginning and not noticed on prolonged exposure to sublethal concentrations.

The rate of oxygen consumption of snails and mussels decreased at the respective exposure periods studied in both lethal and sublethal concentrations of mercury. However, the percent suppression was more at lethal concentrations and increased over time ($1 < 3 < 5$ days)

without significant ($P > 0.05$) differences in between, whereas at sublethal concentrations it decreased over time (1 > 7 > 15 days) with significant ($P < 0.05$) differences. Further, the suppression was more in mussels exposed to lethal concentrations, but no significant differences were obtained between these two groups of animals at the sublethal concentration (Figure 2). Respiratory changes are good indicators of stress (Jones 1947); in gastropods and bivalves the action of gill cilia facilitates respiratory gaseous exchange. Hence, any alteration in gill epithelium would cause respiratory changes. The steep suppression in the rate of oxygen consumption of both species of animals from 1 to 5 days at the lethal concentration of mercury was probably due to the reduced efficiency of gills, besides the irreversible interaction of metal ions with cellular metabolic enzymes (Singh and Singh 1979). Less suppression of oxygen consumption in snails than in mussels indicates their high resistance to mercury. The amphibious nature of these animals could be one of the reasons for it. Although there was a high decrease in the rate of oxygen consumption of both the animals at 1 day of exposure at sublethal concentrations, a gradual recovery on further exposure indicates the efforts taken by these animals for their survival in a less toxic environment. Fortification of gill epithelium, high metal-storing capacity and synthesis of metal-binding proteins are some of the possible reasons for their metabolic recovery (Engle and Fowler 1979). Though mussels seem to be more sensitive to high concentrations of mercury than the snails, the adaptive ability of both the animals is almost the same in low concentrations.

Figures 3 to 5 show a decrease in sodium, potassium and calcium levels in hepatopancreas and an increase in sodium levels in the foot of snails and mussels exposed to lethal and sublethal concentrations. Further, except in mussels at 3 and 5 days at lethal concentration, potassium levels also increased in the foot of these animals. Calcium levels in the foot of snails decreased, but was not measured in mussels. All these changes were significant ($P < 0.001$) from controls except at 15 days of exposure to sublethal concentrations. In lethal concentrations the percent decrease in these ions increased over time (1 < 3 < 5 days), whereas the percent increase decreased over time (1 > 3 > 5 days) without any significant ($P > 0.05$) differences in between. Hence, there was a gradual net loss of these ions from 1 to 5 days. But, in sublethal concentrations the decrease and/or increase was in the order 1 > 7 > 15 days with significant ($P < 0.05$) differences in between. Further, the decrease in all these ions was greater in mussels than in snails, but

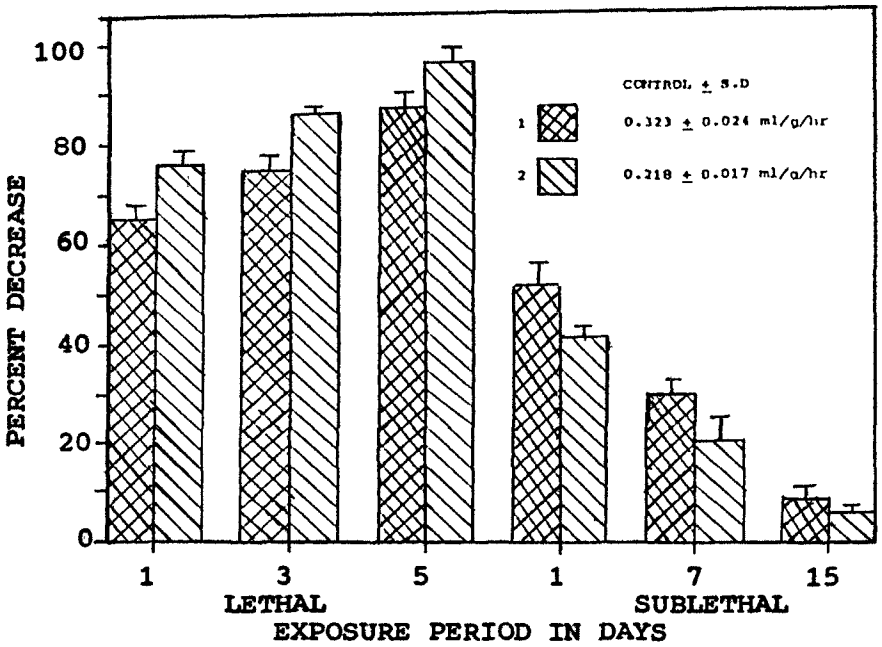

Figure 2. Percent decrease in the rate of oxygen consumption of P. globosa (1) and L. marginalis (2). Vertical bars represent standard deviation.

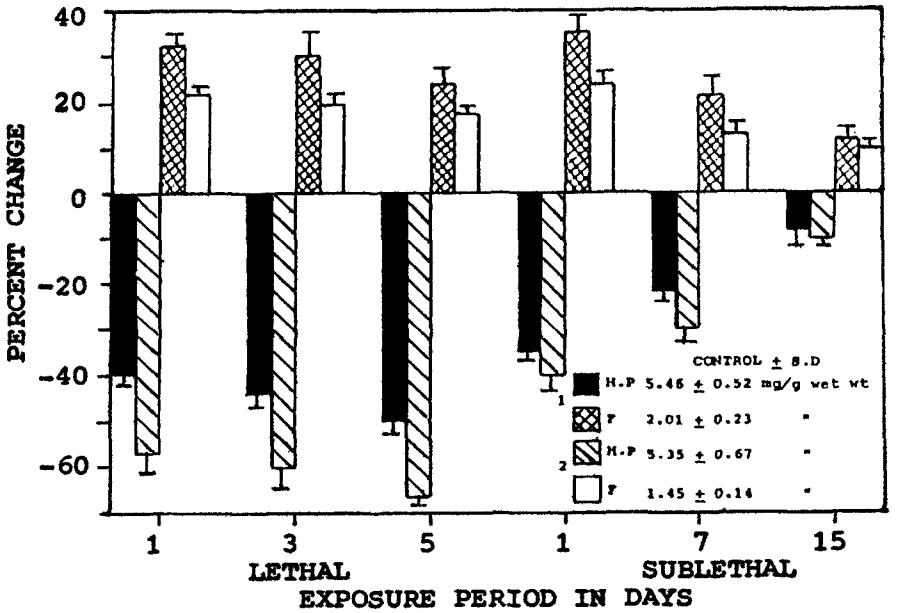

Figure 3. Percent change over control in sodium level in hepatopancreas (H.P) and foot (F) of P. globosa (1) and L. marginalis (2). Vertical bars represent standard deviation.

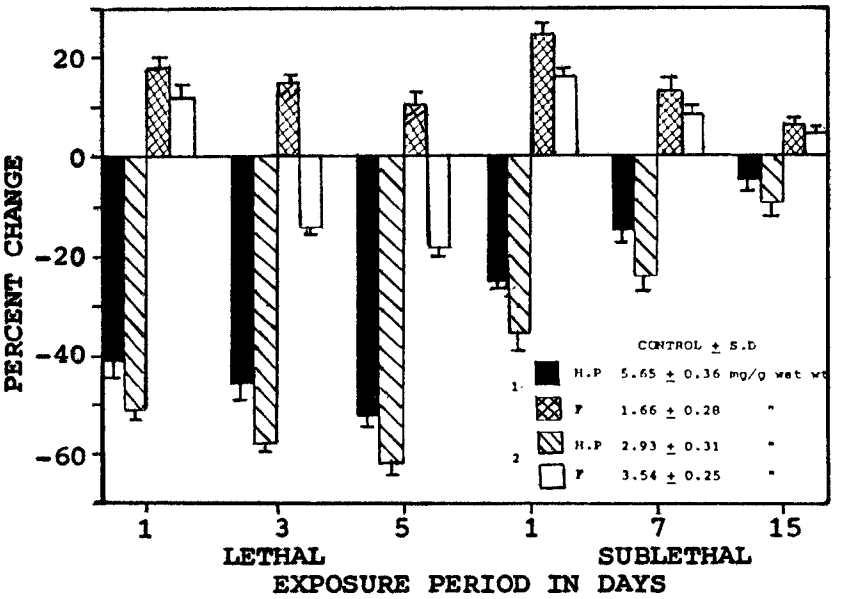

Figure 4. Percent change over control in potassium level in hepatopancreas (H.P) and foot (F) of P. globosa (1) and L. marginalis (2). Vertical bars represent standard deviation.

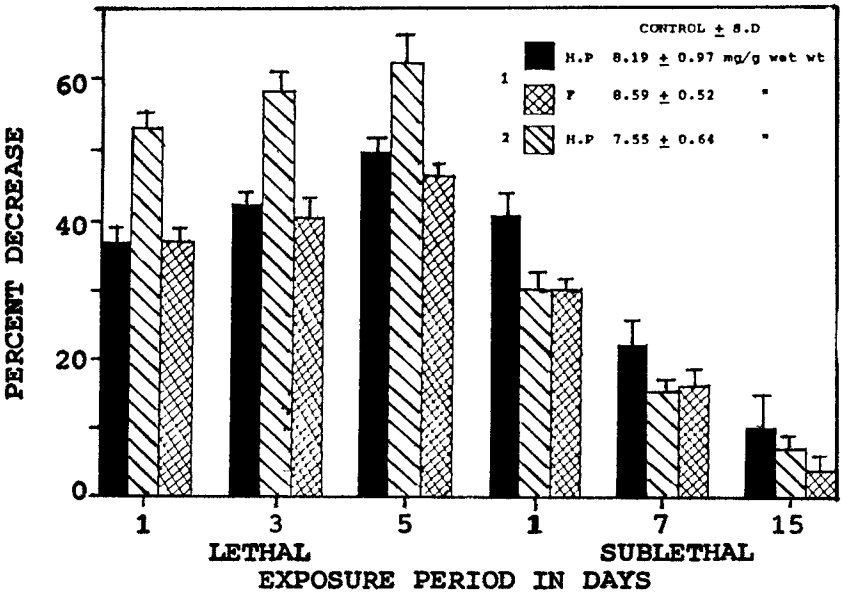

Figure 5. Percent decrease over control in calcium level in hepatopancreas (H.P) and foot (F) of P. globosa (1) and hepatopancreas (H.P) of L. marginalis (2). Vertical bars represent standard deviation.

the increase was more in snails.

For the maintanence of osmo-concentration of body fluids, sodium, potassium and calcium ions are essential and their role in this aspect has been well demonstrated in molluscs (Schoffeniels and Gilles 1972). The changes in the levels of these ions in the hepatopancreas and foot of the snails and mussels exposed to mercury could be correlated to the differential permeability properties of hepatic and muscle cell membranes to maintain electrolyte balance during toxic stress. However, the gradual net loss of these ions in lethal concentrations, along with drastic decrease in the rate of oxygen consumption, could cause an imbalance in osmo- and ionic-regulation of their body fluids. That this imbalance was more significant in mussels than in snails indicates once again their susceptability to high concentrations of mercury. In sublethal concentrations, the slow regain of these ions and the gradual recovery in the rate of oxygen consumption to normalcy indicate the attainment of homeostasis on prolonged exposure of these animals to chronic toxic stress. These compensatory adjustments were more or less similar in both animal groups.

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