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Determination of acute toxicity levels of mercury to the fish *Tilapia mossambica* (Peters)

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Abstract. Toxicity of inorganic mercury to the freshwater fish *Tilapia mossambica* was demonstrated by static bioassays. Statistical analysis was used for calculating 48 hr median tolerance limit, 95% confidence limits and the slope function (*S*). No mortality was observed within 48 hr in mercuric chloride concentration of 0.7 mgl^{-1} although the swimming activity of the fish decreased and the rate of opercular movement increased significantly. Concentrations of 0.9 mgl^{-1} and higher proved lethal. Symptoms of mercury poisoning and safe concentrations of mercury have been discussed.

Keywords. Toxicity; mercury; safe concentrations; *Tilapia mossambica*.

1. Introduction

The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to industrial pollution. High toxicity of mercury compounds has been known since a long time (Bidstrup 1964), but their hazardous nature as pollutants of aquatic environment became a matter of concern only after a large number of deaths occurred from poisoning in areas adjoining the Minamata Bay, Japan (Ui 1971; Kurland *et al* 1960). The use of mercury in the manufacture of many products such as chlor-alkali or in electroplating processes as also in the paper manufacturing industry, enlarges the interest regarding the influence of this metal on the aquatic organisms. In developing countries many freshwater and estuarine ecosystems receive mercury released during the operation of small-scale industrial units located in coastal towns.

Tilapia mossambica, a freshwater fish, was selected for the study of toxicity of mercury, because of its easy availability and also because this species responds well to aquarium conditions and can readily be kept under a wide range of environmental conditions.

2. Material and methods

Specimens of *T. mossambica*, collected from the Goa State Fisheries Farm, were brought to the laboratory and kept in several glass aquarium tanks (40×20×30 cm) containing aerated freshwater. The fish were obtained during January to March 1980 and acclimated to laboratory conditions for 2 weeks before the experiments began. Dissolved oxygen content of the water in the tank was always greater than

4.8 ml per litre and the temperature variations were within ±1°C. The fish were fed daily during the acclimation period on dry pellets made from a mixture of prawns and wheat flour. This food was readily taken by the fish and was nutritious to maintain them in apparently healthy condition.

Bioassays were conducted in static waters by adopting the procedure of Doudoroff *et al* (1951). The fish were not fed during the bioassays and for two days before the start of the experiment. The size of the fish varied from 4.7-6.2 cm (average 5.5 cm) and their weight ranged from 2.2-3.5 g (average 2.8 g). Stock solution of the metal (mercuric chloride) was prepared and diluted to obtain the desired degrees of concentration.

Exploratory tests were run by maintaining higher concentrations of toxicity in the beginning. Progressively lower concentrations were tested to locate a critical concentration range.

Six test animals were placed in each of the test containers, with a water-fish ratio of 2 l of water per 3 g of fish. Slow aeration was provided to maintain adequate dissolved oxygen concentration. Temperature during the test period was 29°C ± 1°C.

Seven concentrations, *viz.* 0.7, 0.9, 1.1, 1.3, 1.5, 1.7 and 1.9 mg Hg^{2+} l^{-1} and two controls were used. The constituents were identical in all the test tanks except for the absence of the toxic metal in the control tanks. The test animals were transferred from the acclimation tank to the test environment within 20 min after the preparation of the experimental solutions. The fish were handled carefully to avoid any injury during their transfer. Fish mortality was observed at intervals of 24 and 48 hrs. The experiments were duplicated for various test concentrations and satisfactory reproducibility in the results was noted.

The reactions of the toxicant on the behaviour of the test fish, *i.e.* loss of equilibrium and movement of the operculum, were observed during the test period. The fish was considered dead when there was no respiratory or other movements, and no response was found to gentle prodding.

The 48 hr LC_{50}, its 95% confidence limits, and the slope function were determined following the method of Litchfield and Wilcoxon (1949).

3. Results and discussion

The test animals, within a short period of their introduction in the experimental tanks, exhibited signs of distress. The gulping of air by hanging on to the surface with the hind part of the body turned downwards was very evident even when sufficient amount of dissolved oxygen was available in water. This was, however, not at all seen in the control tanks. Visible signs of poisoning were manifested by periodic bursts of erratic swimming, rapid opercular movements, surfacing and gulping of air. Disorders of the central nervous system were observed when the fish, in the lethal concentrations, lost their sense of equilibrium and turned with their belly upward making jerky movements. Finally, they sank to the bottom before death occurred. A whitish substance was seen at the bottom of the test vessels which was not seen in the control tanks.

During the experiments, no mortality of fish was observed in the control tanks. This shows that there was no other injurious substance or condition causing the mortality of the fish except for the toxic metal introduced in the test tanks. The pH values in the experimental tanks varied from 7.5 to 7.9 during the test period. The percentage mortality of the fish in 24 and 48 hr is given in table 1.

Table 1. Dose mortality response of *T. mossambica* to mercury.

Concentration of mercury (ppm)	% Mortality after	
	24 hr	48 hr
Control	0	0
0.7	0	0
0.9	0	33.3
1.1	33.3	66.6
1.3	33.3	83.3
1.5	33.3	100
1.7	50.0	100
1.9	100	100

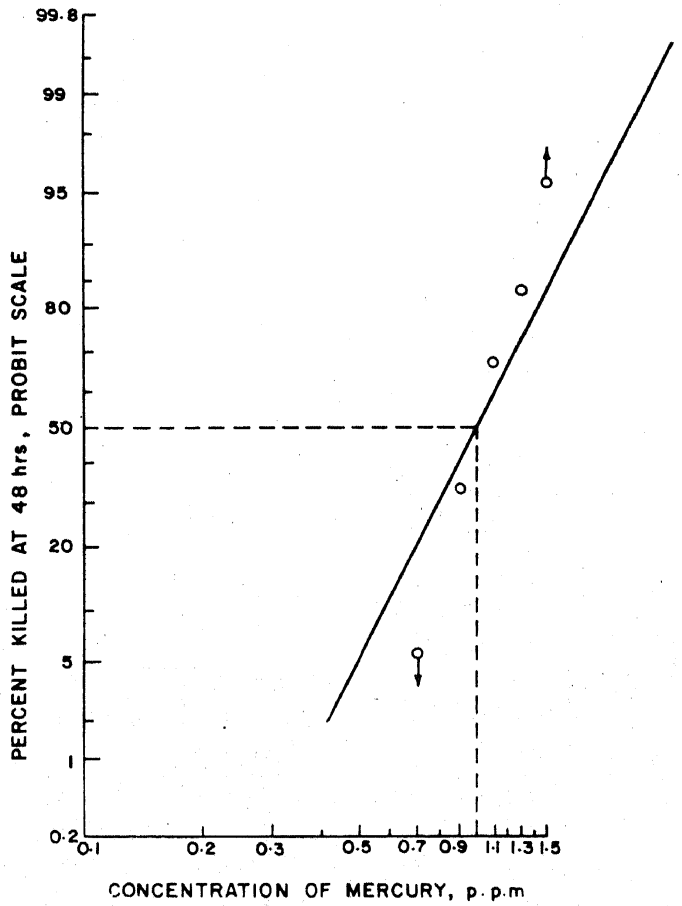

Figure 1. Dose-mortality response of mercury to *T. mossambica*.

Table 2. Toxicity of mercury to *T. mossambica* as indicated by acute toxicity tests.

Concentration of mercury (ppm)	Dead/Tested	Observed mortality (%)	Expected mortality (%)	Difference between observed and expected	Chi² contribution (from Nomograph 1)
0.7	0 / 6	0	19	19	0.230
0.9	2 / 6	33.3	40	6.7	0.017
1.1	4 / 6	66.6	60	6.6	0.018
1.3	5 / 6	83.3	73	10.3	0.050
1.5	6 / 6	100	86	14.0	0.150

As can be seen from table 1, at 0.7 ppm of mercury, no mortality was observed within 48 hrs of the test period. However, concentrations of 0.9 and above were observed to be toxic. The 48 hr LC_{50} value of mercury to *T. mossambica* was 1 ppm (figure 1). The 95% confidence limits were 1.261 (upper limit) and 0.792 (lower limit). The value of the slope *S* was 1.5075 (table 2).

Only limited data are available on the relative toxicity of mercury compounds to the aquatic organisms which could be compared with the present study. Jackim *et al* (1970) and Klaunig *et al* (1975) reported relatively high acute 96-hr LC_{50} of mercury (as mercuric chloride) to adult mummichog, *Fundulus heteroclitus* of 230 and 2010 µg/l respectively. Both the studies were performed under similar laboratory conditions, utilising the fish collected during the spring and summer months. Portmann (1972) obtained a 48 hr LC_{50} value of 3.3 ppm mercury for the fish, *Pleuronectes flesus* and between 3.3-10 ppm for the European brown shrimp, *Crangon crangon* and the European cockle, *Cardium edule*. The 24 hr LC_{50} for the rainbow trout fingerlings was 0.9 ppm mercury (Wobeser 1975a). The above experiments were carried out at 15 and 10°C respectively. Macleod and Pessah (1973) obtained 96 hr TLm values of 0.4, 0.28 and 0.22 ppm mercury, at 5, 10 and 20°C respectively for the rainbow trout fingerlings.

Temperature has been the most important environmental factor controlling the rate of mercury uptake. The toxicity tolerance of fish decreases with an increase in temperature and fish become less tolerant to mercury in tropical conditions (Macleod and Pessah 1973; Boetius 1960). Active metabolic rate, although increases at higher temperatures, gets depressed by an exposure to mercuric chloride and higher temperatures thus augment the depressent effect indirectly (Macleod and Pessah 1973). These depressions are indicative of the damage done to the tissues of the vital systems.

In the present study, at mercury concentrations of 0.7 mg l^{-1} and higher, the rate of opercular movement was observed to be greater than in the controls. It was also noticed that the swimming activity of the fish exposed to 0.7 mg l^{-1} and higher concentrations decreased considerably within 20 min after the exposure. It is probable that such an increased opercular rate, associated with decreased swimming activity, compensates for the loss of efficiency in the oxygen uptake by decreasing the physiological oxygen demand and increasing the amount of oxygen passing over the branchial tissue per unit time. At concentrations of 0.9 mg l^{-1} and above, fish were no longer able to significantly increase the rate of opercular

movement, and therefore, no further compensation could be made for the losses occurring in the efficiency of oxygen uptake. The lethality of heavy metals (lead, copper, zinc, mercury) has been ascribed to coagulation of mucus (*i.e.* precipitation of insoluble metal-protein compounds) on the gill surfaces, damage done to the gill tissues and consequently to respiratory failure (Doudoroff and Katz 1953). Increased opercular rates have been observed in fish exposed to some metals (Brafield and Mathiessen 1976). The gills are known to accumulate significant amounts of heavy metals as compared to the other organs of the exposed fish. In conjunction with such accumulations, hypertrophy of the branchial epithelial cells has been reported in Hg, Cr, Cu, Cd and Zn toxicity (Wobeser 1975 a,b).

The symptoms of acute mercury poisoning in fish include rigidness of body, spreadout fins, slow movement of the fish and hanging on the surface with the hind part of the body turned downwards (Boetius 1960). These symptoms are followed by the loss of balance and finally sinking to the bottom before death occurs. All these symptoms were clearly visible in the test fish during the present study. One of the underlying problems with mercury pollution is the effect it provides to the nervous system by attacking the centres in the brain of human beings and other mammals (Suzuki 1969). Whether such a damage also occurs in the aquatic organisms has not been clearly demonstrated. However, similar effect seems to prevail in fish also, because some of the functions which are controlled by the nervous system such as the maintenance of equilibrium, have already been demonstrated to get disturbed with low concentrations of mercury in the fish (Lindahl and Schwanbom 1971a,b).

There is no simple formula to translate the various LC_{50} values into permissible concentrations considered safe for long periods of exposures. Although a chemical may not be directly toxic to a given fish, its cumulative action may eventually eliminate the species indirectly. In spite of these limitations in the estimation of long-term safe concentrations from the acute toxicity assays, some workers have developed certain formulae to translate the experimental data into numerical and usable values. Thus, the Ohio River Sanitation Commission (1955) has recommended an arbitrary factor of 1/10 to the 48 hr TLm values. Hart *et al* (1945) suggested the following formula for finding out the presumably harmless concentration for the toxicant, $C = (48 \text{ hr } TL_{50} \times 0.3)/S^2$ where $C =$ is presumably a harmless concentration, $S = 24 \text{ hr } TL_{50}/48 \text{ hr } TL_{50}$.

Many estimates made for the safe levels have been in the range of 0.1 and 0.4 toxic units. For some years, 0.1 of the 48 hr LC_{50} has been used in USA as an indicator of safe levels. Warner (1967) states that in Holland, Germany and Switzerland, application factors of 0.1 or 0.05 of the 20 day LC_{50}, which is equal to incipient LC_{50}, have been widely accepted. Recommendations for the maximum levels are 0.1 or 0.05 toxic units for the non-persistent pollutants, and 0.1-0.01 toxic units for the persistent chemicals and pesticides (Sprague 1971).

Based on the safe levels indicated above, the concentration of 0.01 to 0.04 ppm of mercury can be considered safe for *T. mossambica*.

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