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Acute toxicity of chromium and mercury to *Lates calcarifer* under laboratory conditions

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Abstract
Heavy metals are naturally occurring, highly toxic environmental pollutants. The objective of the present study, *Lates calcarifer* was exposed to an acute concentration of individual and mixed metals *viz.*, chromium (45 ppm), mercury (0.8 ppm) and chromium plus mercury (26 ppm) for a period of 96 hours, the acute toxicity levels were derived from LC_{50} concentration of the heavy metals. The survival time of *L. calcarifer* was reduced in chromium plus mercury concentration compared with mercury and chromium concentration respectively. Physiological responses like rapid opercular movement and frequent gulping for air due to respiratory rate impairment, darkening of the body, sudden and quick movement, rolling movement was observed during the initial stages of exposure after which it became occasional. All these observations can be considered to monitor the quality of aquatic ecosystems and severity of pollution.

Keywords: Chromium, Mercury, *Lates calcarifer*, Lethal Concentration, Toxicity test.

1. Introduction
Due to complications and the immensity of worldwide water pollution, there is a need to develop rapid and sensitive screening methods as an aid in water monitoring for the presence of toxicants; besides water qualities, necessity for studying aquatic organisms as indicators of pollution was felt long back and quite a large number of organisms have been identified for this purpose [1]. A toxicity test using aquatic organisms plays an important role in the development of proposals for environmental management and protection, especially for the aquaculture environment [2].

Heavy metal contamination severely interferes with ecological balances of an ecosystem and anthropogenic inputs like waste disposal directly adds to the burden of environmental degradation [3]. The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate the harmful chemicals in the aquatic ecosystem and consequently, to humans who depend on aquatic products as sources of food. Since heavy metals can accumulate in the tissues of aquatic organisms, these tissue concentrations of heavy metals can be of public health concern to both organisms and humans [4].

Lethal Concentration of 50% (LC_{50}) tests can measure the susceptibility and survival potential of animals to particular toxic substances such as heavy metals. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in animals [5]. Heavy metals such as mercury and chromium are toxic to aquatic animals at very low concentrations and are never beneficial to living beings [6, 7].

The acute toxicity test is used to determine the concentration of a test material or the level of an agent that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled conditions [8]. All toxicants are capable of severally interfering with the biological systems that producing damage to the structure and function of a particular organism and ultimately to its survival. Acute toxicity test constitutes only one of the many tools available to the aquatic toxicologists, but they are the basic means of provoking a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material [9].

Toxicity is a characteristic feature of an individual organism's response to a chemical at a particular concentration or dosage for a specific period. Comparative toxicity of mercury and chromium compounds was assay in the fish *Clarias batrachus* (Linn) by [8].

Majority of the studies concerning the effects of heavy metals on fish has been confined to the acute toxicity test with the death of fish as an end point. Hence, in the present study, the chromium, mercury and chromium plus mercury on Seabass *L. calcarifer* is very scanty, have an attempt was made to determine the survival of fish to evaluate the acute and sublethal toxic levels.

2. Materials and Methods

Specimens of *Lates calcarifer* were collected from Rajiv Gandhi Centre for Aquaculture (RGCA), Thirumullaivasal, Sirkali, Tamil Nadu, India. The collected specimens were acclimatized to laboratory conditions at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai for 15 days. Fish were fed *ad libitum* with dry fish and the fed on flour pellets, twice a day. Fish are ranging from 7- 8 cm in length and weighing 8-10 g were selected for experimental purpose. The quality of the water was determined according to APHA [10] and was as follows: Dissolved oxygen 6.2 ± 0.01 mg/l; pH 8.6 ± 0.1; Water Temperature 25.2 ± 2.0 °C; Salinity 28 ± 0.07 ppt; Total hardness 8.2 ± 2.0 mg/l; Calcium 5.0 ± 0.1 mg/l; Magnesium 3.0 ± 2.0 and Total alkalinity 16.0 ± 06 mg/l.

For preliminary studies, chromium, mercury and chromium plus mercury were carried out to find the median lethal concentration for 96 hrs. For this appropriate amount of chromium trioxide, mercury chloride and chromium trioxide plus mercury chloride were dissolved in fresh seawater every time to prepare a stock solution of 1000 ppm for each toxicant. For sub-lethal studies, 80 L of water were taken in each 100 L three glass tanks. For the first, second and third tanks 1/10 of 96 hrs LC_{50} concentrations of chromium (45 ppm), mercury (0.8 ppm) and chromium plus mercury (26 ppm) was added into each fish tanks respectively, while the 4th tank served as control. Then, 10 fishes were introduced into the each tank and the experiment was maintained for a period of 96 hours. No mortality was observed throughout the experimental period. After this period, the fish were sacrificed and percentage of mortality in static bioassay was converted into probit mortality following the method of Doudoroff [11].

3. Results

Results show that the median lethal concentration (LC_{50}) of the mortality rate of fish *L. calcarifer* exposed to chromium, mercury and chromium plus mercury concentrations are presented in **Table. 1 Fig. 1.** The experimental fishes were exposed to different concentration chromium (42 to 49 ppm), mercury (0.4 to 1.1 ppm) and chromium plus mercury (23 to 30 ppm) for different hours 24, 48, 72 and 96 hours respectively. Based on the different hours of exposure the LC_{50} concentration of chromium (45 ppm), mercury (0.8 ppm) and chromium plus mercury (26 ppm) at 96 hours duration was selected for further experimental studies.

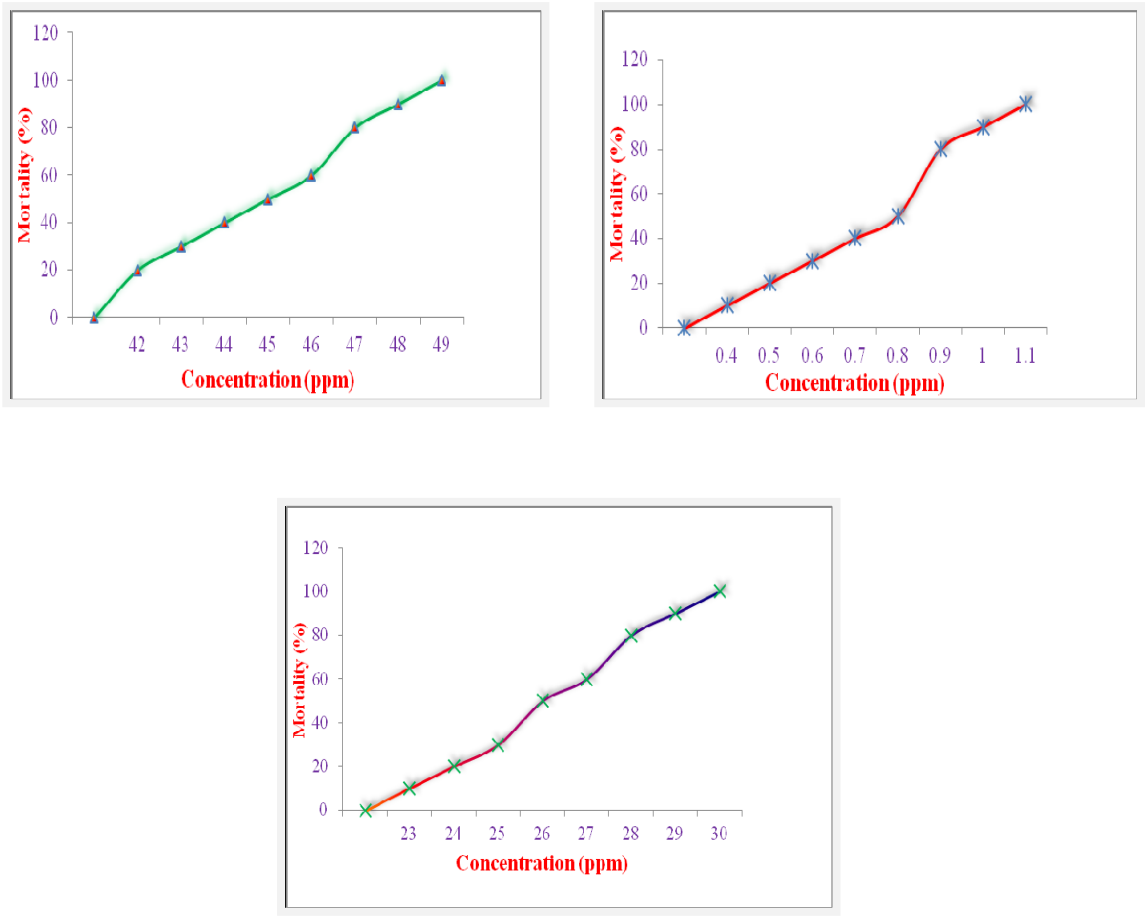

Fig 1: Morality of the fish, *L. calcarifer* exposed to different chromium, mercury and chromium plus mercury concentration at different duration of exposure (Narrow range trail)

Table 1: Morality rate (%) of the fish, *L. calcarifer* exposed to different chromium, mercury and chromium plus mercury concentration at different duration of exposure (Narrow range trails)

Percentage (%) of morality at different hours of exposure														
Chromium					**Mercury**					**Chromium plus mercury**				
Concen-tration (ppm)	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**Concen-tration (ppm)**	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**Concen-tration (ppm)**	**24hrs**	**48hrs**	**72hrs**	**96hrs**
Control	0	0	0	0	Control	0	0	0	0	Control	0	0	0	0
42	0	0	10	20	0.4	0	0	10	10	23	0	0	0	10
43	0	10	20	30	0.5	0	0	10	20	24	0	0	10	20
44	10	20	30	40	0.6	0	10	20	30	25	0	10	20	30
45	**20**	**30**	**40**	**50**	0.7	10	20	30	40	**26**	**20**	**30**	**40**	**50**
46	10	40	50	60	**0.8**	**20**	**30**	**40**	**50**	27	20	20	40	60
47	30	50	60	80	0.9	40	50	60	80	28	30	40	50	80
48	50	70	80	90	1.0	50	60	70	90	29	40	60	80	90
49	60	80	90	100	1.1	60	70	90	100	30	50	70	90	100

4. Discussion

Bioassay is a necessity to determine the concentration of a toxicant, which could be allowed in waters without adverse effects on the living organisms [12]. In the present study, the acute mercury concentration was increased; the survival time of *L. calcarifer* was reduced. This work was supported by Ebrahimpour M [13] which he reports that the concentration of $HgCl_2$ increased, fish mortality also increased, indicating that there is a direct proportional relationship between mortality and concentration of $HgCl_2$. Hedayati [14] reported that the mortality of fish *Cyprinus carpio* exposed to mercury chloride might be due to respiratory epithelium damage by oxygen culmination during the formation of a mucus film over the gills of fish. In the present study also, the decrease survival of fish in mercury treatment may be due to respiratory epithelium damage by oxygen culmination.

McDonald [15] reported that the effects are to be used for predictive purposes (e.g., sublethal bioassays, field surveys), it will be necessary to understand which effects will cause death under a variety of conditions. McDonald [15] pointed out that at critically mercury levels, where mortality is 100% and death occurs within hours rather than days, a failure of O_2 delivery to the tissues is probably of primary importance. In the present study, the sudden death of fish in the mercury level of below 0.8 ppm may be due to the failure of oxygen delivery to the tissues concentration of $HgCl_2$ and respiration epithelium damage as suggested by the above authors.

In the present study the chromium level was increased, the survival time of fish. *L. calcarifer* was reduced. The above observation was supported by Azmat and Javed [16] who reports that the disruption of the gill epithelium, production of mucus on gills, inability to osmoregulate of the chromium of the blood have all been found to be associated with harmful chromium levels and a pronounced accumulation of mucus on the gills and a sloughing off of the gill epithelial tissue may severely impair bronchial O_2 diffusion. This combined with marked reduction in blood O_2 carrying capacity (Root effect) due to heavy metals, results in eventual cellular anoxia. In the present study also a similar mechanism may be operating in fish when it was exposed to chromium level below 45 ppm. Hence, the present study clearly explains when chromium levels are increased, the mortality rate of fish increased significantly.

In the present acute study, the chromium plus mercury level was increased; the survival time of *L. calcarifer* was reduced. Similar work was carried out by [14] in *Cyprinus carpio* exposed to combined metals, mercury chloride and lead chloride and zinc sulphate. Fish that are highly susceptible to the toxicity of one metal may be less or even not susceptible to the toxicity of another metal at the same level of that metal in the ecosystem. The gills are considered the main site of entry for the dissolved metals. Thus, they represent the target for the toxic action of metals [17]. However, the variety of cell-types of the gills (chloride cells, mucus cell, pillar cells and undifferentiated cells) makes it difficult to interpret the possible mechanisms of metal accumulation [18]. These responses resulted in decreased oxygen tension in the blood or might be connected to a disorder in osmoregulation. It may lead dead of the fish. In the present study also a similar mechanism may be operating in fish when it was exposed to chromium plus mercury level below 26 ppm supporting the views of the above authors.

In the present study, decreased survival rate of fish in the concentration of metals such as chromium, mercury and chromium plus mercury toxicity can cause all forms of physiological changes. Fish exposed to different doses of pollutants displayed marked behavioural changes. Those symptoms were hyper-activity and attempts to jump out due to skin irritation, restlessness, respiratory distress, loss of balance, gulping for air due to respiratory rate impairment, darkening of the body, sudden and quick movement, rolling movement, back stroke, excessive accumulation of mucus, all these ending in death. The heavy metals may be an important tool for assessment of the effects of pollutants in aquatic ecosystems. The two metals used in our experiment to demonstrate their potential for use in bioassays.

5. Conclusion

In conclusion, it is observed that both the metals are toxic and have a synergistic effect on all the parameters estimated, these findings proved that the metals were toxic to fish alone and in combination. The study also concludes that such metals from a sole threat to aquatic organism, which will affect the whole food chain and affect non target organisms.

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