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ACUTE TOXICITY OF CHROMIUM AND LEAD TO THE FRESHWATER LEBISTESRETICULATUS(PETER)

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Abstract:

Present study was carried out to delineate toxic level of metal to *Lebistesreticulatus* (guppy) (Poeciliidae). Freshwater fish *L. reticulatus* were subjected to fish bioassay test in the laboratory conditions using chromium (Cr) and lead (Pb) metals. Mortality was assessed at interval of 24 hour (h), 48h, 72h and 96h. Acute toxicity and median lethal concentrations (LC50) were determined by Sprague and Finney method. The 95% confidence interval was calculated as per the literature, Litchfield-Wilcoxon. Slope and regression (R2) were also calculated to confirm the authenticity of the results. LC50 decreased with increased mean exposure times for both metals. Results indicated that the Cr is more toxic than Pb. Pb was found to be least toxic for *L. reticulatus*. Relationships between 96 h LC50 and physico-chemistry of water showed negative correlation with pH and dissolve oxygen.

KEY WORDS:

Bioassay, Acute toxicity, LC50, heavy metal, 95% confidence interval, guppy.

INTRODUCTION

Pollution by heavy metals has become a serious environmental and public health hazard because the concentrations released into the environment from industrial processes often exceed permissible levels. Due to their bioaccumulative and non-biodegradable properties, heavy metals constitute a core group of aquatic pollutants (Vutukuru et al., 2007). Their high toxicity even in low concentrations can produce cumulative deleterious effects in a wide variety of fish and other aquatic organisms. Therefore, most of the heavy metals are toxic or carcinogenic in nature, posing threats to the human health and the environment (Farombiet al., 2007). In ecotoxicology, heavy metals have gained significant consideration because of their severe toxicity and amassing tendency in the aquatic biota (Javed, 2004). Various fish species have been employed to assess the health status of aquatic ecosystems to monitor metallic ion pollution that could be biologically magnified in the food chain and hence exhibiting devastating effects on the aquatic organisms (Raufet al., 2009; Farkaset al., 2002). Fish may accumulate large amounts of heavy metals from contaminated water (Olaifaet al., 2004) and primary consumer.

Chromium is found commonly in surface waters (Faraget al., 2006) in microquantities (Zhang et al., 1994). However, it is considered the most detrimental pollutant to the aquatic organisms, especially the fish (Al-AkelandShamsi, 1996). Sodium dichromate is extensively used to produce chrome pigments and chrome salts in leather tanning industry, as wood preservative, anti-corrosives and for caustic dying. The trivalent and hexavalent Cr are considered biologically important. Hexavalent Cr can cross the cell barrier quite easily and within the cell it reduces to trivalent form that attaches with DNA and other macromolecules and ultimately causing mutagenic and toxic effects within the cells (Goyer, 1986).

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Lead is a potentially toxic chemical that may be directly ingested by man or indirectly through aquatic animals like fish and shellfish. The effects of lead on man include mental retardation, learning dysfunction, and loss of coordination(Goodman and Gilman, 1992). Though the effect of lead and chromium toxicity is well elucidated in fish (Olaifa et al 2003; Hamda and Muhammad, 2011; Velma et al, 2009)

This work is therefore aimed at assessing the toxic stress of lead and chromium on fish using a static bioassay technique (Reish and Oshida, 1987). As bioassay technique has been the cornerstone of programmes

The fish *Lebistes reticulatus* is a small, larvicidal and toxic tolerant and there is paucity of information of metal toxicity on this species. This fish is an important food chain between primary consumer and tertiary consumer. Any disturbances in *L. reticulatus* directly affect the tertiary consumer and food chain. Therefore this species is selected for fulfilling the existing lacunae.

MATERIALS AND METHOD

L. reticulatus is a standard test organism recommended by EEC (1992) and OECD (1982) guidelines for toxicity evaluation. Some of the important contributions to the toxicity studies are reported by Yap (2008), Satyanarayanan et al. (2005), Castro et al. (2004), Clearwater et al. (2002), Widiannarko et al. (2000), Kumare et al. (1995).

The test fish *L. reticulatus* were procured from a "Public Health Centre and Malaria Prevention Department, Nagpur". Only healthy specimens 2-3 cm in length and 2-3.5 gm of weight were chosen for experimental work. The fish were acclimatized for one week in an aerated and dechlorinated tap water (dilution water) at $\pm 28^{\circ}\text{C}$. Physico-chemical characteristics of dilution water used in bioassay test are shown in Table 1.

Fish bioassay was carried out following Sprague (1969) method. For performing bioassay experiments, 20 randomly selected *L. reticulatus* were placed in 2-L beakers for each metal concentration, with replications done for each treatment and for untreated controls. Test was carried out at ambient temperature. The acute toxicity tests utilized a static method without aeration or feeding test solutions were renewed daily.

The test duration was kept at 96 h. The mortality and morphological changes of fish and behavioural pattern were observed. The fish were not fed either during the experimental period in the test chamber or prior to experimentation for 48 h. The reason for stopping feeding 48 h before the experiment is to prevent/minimize the build-up of food and metabolic wastes and resulting oxygen demand.

Acute toxicity and median lethal concentration (LC50) for 24, 48, 72 and 96 h were determined by the methods of Sprague (1969) and Finney (1971). The 95% confidence interval was calculated as per the literature (Litchfield-Wilcoxon, 1949). Slope and regression (R2) were also calculated to confirm the authenticity of the results.

RESULT

Range finding test for Cr metal was performed at concentrations between 1 mg/L to 10 mg/L. Hundred per cent mortality occurred at 9 mg/L and at 4 mg/L no mortality was observed. Therefore detailed confirmatory test was performed between these concentrations. Cr toxicity to fish was comparatively lesser than Cd and Cu. LC50 values for 24, 48, 72 and 96 h exposure was 7.0 mg/L, 6.4 mg/L, 6.0 mg/L and 5.5 mg/L respectively. NOEC for 24 h exposures was around 4.5 mg/L and the same for 96 h exposure was 3.0 mg/L. The 95% confidence interval were calculated while slope function and regression were calculated statistically and shown in Fig. 1 and Table 2.

Range finding test for Pb metal was carried out for concentration between 5 mg/L to 25 mg/L. Hundred percent mortality was found at 22 mg/L and no mortality was observed at 15 mg/L. Therefore confirmatory test was carried out between these concentrations. LC50 values for 24, 48, 72, 96 h were observed as 20 mg/L, 19 mg/L, 18 mg/L and 17 mg/L respectively. NOEC values for 24, 48, 72 and 96 h were 15 mg/L, 14 mg/L, 13 mg/L and 12 mg/L respectively. The 95% confidence interval were calculated graphically while slope function and regression were calculated statistically is shown in Fig. 2 and Table 3.

DISCUSSION

Cr metal depicted moderate behavioural changes. At highest concentration bulging of eyes of fish with darkening of body colour was observed. Severe body bending, curling of tail fin, loss of balance as observed in Cd and Cu were not encountered in case of Cr. But a unique feature observed in case of Cr toxicity to fish was the shrinkage of gills giving it an appearance of a small single flap opening and closing.

The acute toxicity studies for 96 h in *Pimphalespromelans* when exposed to pure chromium metal solution was found to be 120 mg/L and 52 mg/L. (Adelman and Smith, 1976) indicating *L. reticulatus* is very sensitive to chromium toxicity.

Pb metal also exerted some behavioural changes in *L. reticulatus* along with blackening of the skin of the fish. Fish exhibited distress and showed increased respiratory rate, which was evident from quick opercular movements. Fish also gathered at the surface of water and gulped atmospheric oxygen for initial stages. But as the time elapsed they settled down with normal swimming. It clearly shows that impact of Pb during initial stages exerts more agitation but once the fish gets to adjust itself to the presence of Pb, the behavioural changes reduced considerably. At lower concentration, Pb metal did not exhibit any toxicity. At as high as 15 mg/L concentration, no toxicity was observed at 24 h exposure.

Muley et al. (2000) found significant alterations in the DNA and RNA contents in gills, liver and brain of the common carp, *Cyprinuscarpio* exposed to 96 h LC0 504 ppm and LC50 594 ppm concentrations of lead acetate. Lead acetate decreased DNA content in all tissues. Pb toxicity decreased RNA content in liver and brain and increased in gills is reported in literature.

Sehgal and Saxena (1987) found safe concentration for guppy to be 492 mg/L for male and 487 mg/L for female to Pb. The 96 h LC50 was found to be 1620 mg/L (Male) and 1630 mg/L (Female).

SAFE CONCENTRATIONS CALCULATION

Accordingly, in the evaluation of environmental damage resulting from pollutants or the establishment of water quality criteria to protect aquatic life, we always use 96-h LC50 values multiplied by a factor of 0.1-0.01 to arrive at a biologically safe concentration. In compliance with such an evaluation, biologically safe concentrations for *L. reticulatus* are 2.063 mg/L Cr and 9.27 mg/L Pb. Therefore in view of the need to protect most natural resources, a stricter criterion should be adopted.

STATISTICAL ANALYSIS:

Correlation coefficients between acute toxicity of Cr and Pb with water quality variables were determined in Table 4. The 96 h LC50 of Cr and Pb for *L. reticulatus* test media showed negative relationships with pH and dissolved oxygen (DO). pH is significant at 0.01 level while DO is significant at 0.05 level.

CONCLUSIONS

Cd metal is readily available to the aquatic organisms as they are more mobile. From the results obtained it can be inferred that the heavy metals Cr and Pb are toxic to the fish in general. Present findings showed that Cr is more toxic than Pb. Pb was toxic only at very high concentrations.

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