

Bioaccumulation and Biochemical Response of *Liza parsia* to Copper

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Abstract: *The test organism Liza parsia exposed to copper concentrations in static bioassay was evaluated to study toxicity, bioaccumulation rate and oxidative stress responses. The 96-hour LC₅₀ was found to be 1.25 mg/l (0.883-1.672) in static renewal bioassay. The bioaccumulation study revealed significant increase (P<0.05) in the accumulation of copper in L. parsia with increase in the ambient concentration. L. parsia accumulated 3.38, 16.45, 27.10, 48.85, 86.18 and 175.62 µg/g dry weight of copper in 0.5, 1, 2, 4 and 8 mg/l of exposed copper concentrations. Significant (P<0.05) decrease in concentration of protein and acetylcholinesterase activity, and significant (P<0.001) increases in lipid peroxidation content and catalase activity was recorded. Lipid peroxidation and catalase were found to be the promising biomarkers in heavy metal pollution with special reference to copper.*

Keywords: *Liza parsia*, copper, biomarker, bioaccumulation, static bioassay

1. Introduction

Heavy metals are natural components of the aquatic environment, but their levels have increased due to anthropogenic activities. Due to persistence in the environment and tendency to accumulate in the biota, copper pose a potential hazard to environmental and human health [1]. The ecological integrity is judged using water in toxicity tests. When toxicity tests are viewed under legal context as needed to implement regulations, they are also accepted based on the ease and expense of performing them, the acquisition of irrefutable proof of harm and financial implications of the lost or threatened resource. Perhaps for those reasons, environmental risk assessment focuses on a simple and straightforward end point, lethality or survival [2].

The measurement of cellular responses to chemical contaminants in sentinel organisms are used as bio-indicators from aquatic environment allowing early detection of biological effects as well as assessment of the extent of contamination of pollutants [3]. Oxidative stress is also of ecological significance, particularly in the aquatic environment, which provides a sink for many pollutants that are capable of causing oxidative stress. Changes in the activity of enzymes and other biomarkers are possible tools for aquatic toxicological research [4]. Accumulation of heavy metals in tissues mainly depends upon concentration of metals in water and exposure period. Fish are specific indicators of different environmental compartments in relation to their habitat

and food web position, and they exhibit different rates of bioaccumulation with respect to xenobiotics and heavy metals in general [5]. Hence, in the present study *Liza parsia* was exposed to copper in acute toxicity test under static renewal bioassay. Biochemical assay such as protein, lipid peroxidation, acetylcholinesterase and catalase activity were also analyzed with bioaccumulation data with support.

2. Materials and Methods

2.1 Toxicity tests

Juvenile *L. parsia* collected from Kovalam creek (12°14'16.99" N, 80°14' 52.94" E, Tamil Nadu, India) were transported to the laboratory in air-filled plastic bags. Test organisms were acclimatized in 1000L FRP tanks with aerated natural filtered seawater for a period of eight days with 28 ppt salinity, temperature of 29 ±2 °C, dissolved oxygen of 5.5 mg/l and pH of 7.9. *L. parsia* was fed with pellets of rice bran and oil cake. Stock solutions of copper were freshly prepared by dissolving the cupric chloride (CuCl₂) in double distilled water. These solutions were serially diluted to get the experimental concentration for the toxicity test. The experimental method includes static renewal test by following the method of USEPA [6]. Five concentrations in a geometric series including control were prepared for the test. Each series of test chambers consisted of triplicates with ten animals in a 10 L glass trough. Dead animals were removed at each observation and survivors were counted. Maximum-allowable control

mortality was ten per cent for a 96-hour period of testing [7].

2.2 Biochemical estimation

For the analysis, 1g tissue was homogenized in chilled pestle and mortar with 5ml homogenization buffer (0.25M sucrose, 10 mMTris, 1 mMEDTA, and pH 7.4) and centrifuged at 5,000 rpm for 15 mts at 4°C. The resulting supernatant was the homogenate used for the estimation of various biochemical assays using Hitachi 700 UV-Spectrophotometer. Lipid peroxidation level was assayed by measuring Malondialdehyde (MDA). Hydroperoxides were determined by the thiobarbituric acid reaction as described by Ohkawa et al. [8]. The absorbance was read at 532 nm. Catalase (CAT) activity was measured at 240 nm by determining the decay of hydrogen peroxide (H₂O₂) levels followed by Beers and Seizer [9]. Acetylcholinesterase (AChE) activity was determined using Ellman's reagent, DTNB (5, 5'-dithio-bis (2-nitrobenzoic acid); 0.5mM) and acetylthiocholine iodide (ACTI) as substrate. The rate of change of absorbance at 412 nm was recorded over two minutes at 25°C [10]. The protein concentration of each of the sample extract was determined according to Lowry [11] using bovine serum albumin as the standard.

2.3 Bioaccumulation

The soft tissues of the test organisms were removed using a Teflon scalpel, rinsed with distilled water and was frozen at -80°C till analysis. During the analysis, the tissue was washed with distilled water and dried at 95 °C in hot air oven and grinded to a fine powder with pestle and mortar and the metal analysis was carried out by UNEP [12]. To ensure the accuracy and precision in the sample analysis, it

was cross-examined with respect to certified reference material (DOLT-3). Metals were analyzed in Varian Spectra AA 220FS atomic absorption spectrophotometer. Suitable internal chemical standards (Merck Chemicals, Germany) were used to calibrate the instrument. Probit analysis program was carried out for the calculation of LC₅₀ values 96-hour, and upper and lower 95 per cent confidence levels. PRIMER version 6 (6.1.7) was used to study the parameters through complete linkage using elucidan distance method.

3. Results and Discussion

The calculated LC₅₀ was 1.25 mg/l (0.883-1.672) for copper in the present study. Taylor et al. [13] reported that mullet, *Chelon labrosus* LC₅₀ value was 1.4 mg/l for copper, in continuous flow through system (CFTS) for four days. Mohapatra and Rengarajan [14] reported that mullet, *L. parsia* exposed to copper in acute toxicity test revealed a 96-hour LC₅₀ of 21.8 mg/l. Lydy and Wissing [15] reported LC₅₀ for fantail (*Etheostoma flabellare*) and johnny (*E. nigrum*) darters exposed to copper was 0.39 and 0.60 mg/l. Based on 96-hour LC₅₀, copper was found to be toxic to *L. parsia* in the present study [16]. Due to accidental industrial discharges of heavy metals into the aquatic environment, fish may have shorter or longer contact with such concentrations of heavy metals [17].

The total protein content significantly (P<0.05) decreased with increase in copper concentration. The amount of lipid peroxidation and the activity of catalase increased significantly (P<0.001). The activity of AChE was reduced in the present study (Table 1).

Table 1. Biochemical parameters of *L. parsia* exposed to copper in acute toxicity test

Concentrations (mg/l)	Protein (mg protein/g tissue)	LPO (nmol TBARS formed /mg protein)	AChE (nmol ACTI /min/mg/protein)	CAT (μmol of hydrogen peroxide consumed /min/mg/protein)
Control	11.73 ±0.71	12.73 ±0.71	16.31 ±0.28	390.85 ±8.58
0.5	12.09 ±0.20*	15.79 ±0.62*	15.05 ±0.78	399.49 ±8.26
1	9.89 ±0.47	16.95 ±1.07**	9.62 ±0.57***	416.78 ±9.69
2	10.73 ±0.71	20.89 ±0.58***	8.15 ±0.49***	426.83 ±11.37*
4	9.73 ±0.52	22.78 ±0.64***	6.95 ±1.13***	419.65 ±9.23
8	9.14 ±0.72	20.65 ±0.56***	4.95 ±0.64***	492.78 ±6.30***

Values are mean ±S.E; n=3; * P<0.05; ** P<0.01; *** P<0.001

The mortality percentage and biochemical data in the present study revealed a closer relationship with total protein, lipid peroxidation and catalase, which makes them

as a strong promising biomarker (Figure 1). Presence of low concentration of scavenging enzymes in the juveniles makes them susceptible to oxidative damage when attacked by Reactive oxygen species (ROS) [18]. *L. parsia*

exposed to copper concentrations experienced severe oxidative stress characterized by significant changes in the levels of biomarkers, which had also been observed in brain samples of the mullet [19]. Mullet (*Mugil sp.*) from contaminated Spanish areas revealed increased activities of antioxidant and detoxifying enzymes [20].

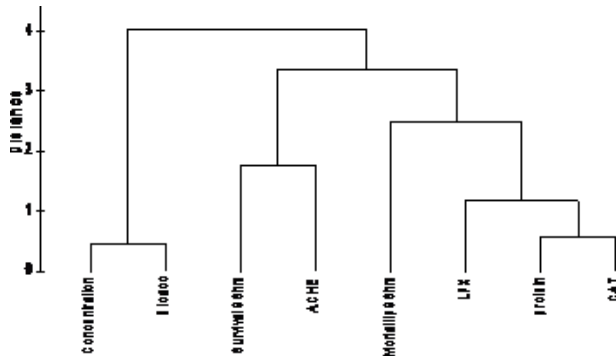


Figure 1. Complete linkage between the significance of accumulation and oxidative stress with mortality and survivalist of *L.parsia* exposed to copper in acute toxicity test in percentage

Enzymatic antioxidants have great potential to indicate the toxic effects of heavy metals, since they generate free radicals, which cause lipid peroxidation of cell membranes [21]. Increased levels of lipid peroxidation (LPO) have been observed in fish under experimental conditions, upon exposure to different xenobiotics [22]. There are evidences that heavy metals produce increased LPO levels in *L. parsia* [23]. Acute toxicity of xenobiotics has traditionally been attributed to inhibition of acetylcholinesterase (AChE) [24]. The level of antioxidant enzymes have been extensively used as an early warning indicator of aquatic pollution (Rajkumar, 2012).

Mullet species are more contaminated when compared with *Tilapia sp.*, which makes mullet species a good indicator of pollution [25]. The excess of some metals can cause harmful effects in fish such as alterations in oxygen consumption and damages in the gills. Copper exposures leads to increased ammonium production and results in the apoptosis process [26]. This may be the reason for the reduced swimming performance of copper exposed fish, and could be caused by an increase in the catabolism of proteins. It is well known that heavy metals accumulated in substantially high levels can be very toxic for fish, especially for juveniles which are very sensitive to pollution [27]. Heavy metal bioaccumulation in *L.parsia* by copper increased with increase in heavy metal concentration (Figure 2).

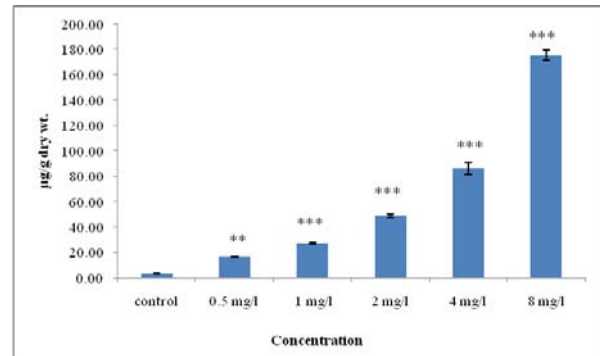


Figure 2. Rate of bioaccumulation of *L. parsia* exposed to copper in acute toxicity test in mean \pm S.E with significance ** $P < 0.01$ and *** $P < 0.001$

In the present study, copper values of the *L.parsia* were higher than to those reported in fish tissue from the lakes of Nasser, Manzalah and Ataturk [28] and lower than those reported from *L.abu* in Ataturk Dam lake [29] and *Abramis brama* from Balaton lake [30]. The copper concentrations in the tissue of six marine fishes (*Arius thalassinus*, *Plotosus anguillaris*, *Dasyatis zugei*, *Gastrophysus lunaris*, *Setipinna taty* and *Johnius carutta*) from the Klang estuary, were at 0.41 (range 0.04 - 0.98) $\mu\text{g/g}$ wet weight [31]. Studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals due to the reason that, most of the organisms inhabit estuaries [32]. Different biochemical biomarkers have been successfully implemented in fish in the recent decades. These biomarkers have been applied to the analysis of both pollution impacts in water courses and toxicity risk assessment of specific contaminants, both isolated and in combination.

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