ESTIMATION OF SAFE AND NO-EFFECT CONCENTRATION OF LEAD EXPOSED TO *THERAPON JARBUA* (FORSSKAL, 1775)

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Abstract-Toxicity test of acute nature was conducted to evaluate the safe concentration and prediction of no-effect concentration and 96 hour LC_{50} for *Therapon jarbua* with lead. The concentrations used for the toxicity test were 2, 4, 7, 14 and 26 mg/l. The recorded mortality and survival data estimated linearity (R^2=0.93) in terms of percentage with concentrations used in the experiment. The calculated 96 hour LC_{50} was 3.04 mg/l (2.28-4.83); this value applied for safe concentration arrived at a value of 30.4 µg/l. The Acute to chronic estimation software predicted the no-effect concentration as 183 µg/l (ALT), 324 µg/l (MPA) and 188 µg/l (LRA). The prediction methods were conducted to minimize the use of test organisms in chronic toxicity test. The concentration predicted as no-effect concentration would be prevailing in the ambient waters in the creek and need to be assessed for the conservation and protection of this species, which utilize the estuaries as breeding and nesting grounds.

Keywords- acute toxicity, lead, no-effect concentration, safe concentration, static renewal

Introduction

Heavy metal pollution and contamination has fascinated particular attention since they persist and degrade the estuarine ecosystems, disturbing the water quality rendering higher toxicity to aquatic flora and fauna and indirectly to humans [1]. Pollutants are finally diluted in the sea or deposited in the estuarine sediments from point and non-point sources of pollution [2]. Domestic and industrial runoff constitutes an important source of trace metals to aquatic ecosystems [3]. Owing to pollution of heavy metals, lead has become distinctive component of estuarine ecosystems. Lead is a prevalent trace pollutant of high toxic to aquatic animals. It is considered as being potentially more lethal than other metals. The major source of lead to the environment is constituted from metal plating and the manufacture of pigments, batteries, and plastics [4] and other sources including ore mines, metallurgical industries and sewage sludge's. Lead compounds are more toxic to marine organisms and data on the toxicity of lead to marine organisms are scarce. At a specific concentration these heavy metals become toxic [5]. Persistence of toxic pollutants in the aquatic ecosystems poses a potential threat to the flora and fauna, unless the concentration of pollutants discharged are restricted within certain limits [6]. Most reliable bioindicator of metal pollution in the aquatic ecosystem are fish, which takes up the readily available dissolved metals in the environment [7]. Effects of heavy metal concentrations are determined by specific toxicity tests which provide reliable evidence of responses to marine organisms [8].

Protection and restoration of the aquatic environment has gained increased importance during the last two decades. In industrialized countries, environmental problems are less related to acute toxicity of environmental pollutants than to sub lethal, synergistic and long-term effects which are difficult to detect and whose consequences for ecosystems are far from being understood [9]. Biological toxicity testing is a relatively simple laboratory bioassay that measures the biological response of marine organisms, particularly at their highly sensitive early life stages [10]. The aim of the present study was to investigate the relative toxicity of lead on mortality and survival approach of *Therapon jarbua* under the static renewal test to evaluate acute toxicity and to predict safe concentration, with no-effect concentration with acute to chronic estimation software.

Materials and Methods

The crescent perch, *Therapon jarbua* of mean 2.5 ±0.3cm in length were taken as test organisms. They were captured from Ennore creek (Northern Chennai, Tamil Nadu, India). Test organisms were immediately transported to the laboratory and were quarantined before acclimatization in FRP tanks (1000 litre)with aerated natural filtered seawater for a period of 7 days at 28‰ salinity, temperature of 27 ±2°C, dissolved oxygen of 5.4 mg/l and pH of 7.98. They were then fed with chopped clam meat and the remaining detritus was removed by siphoning [11]. Stock solutions of lead were freshly prepared by dissolving lead nitrate (Pb $(NO_3)_2$) in deionized water. Range finding tests were conducted to establish suitable concentration ranges for the definitive test. Five concentrations in a geometric

series including control were prepared for the test. The ranges of concentrations used were 2, 4, 7, 14 and 26 mg/l. Experiments were carried out in static renewal (acute toxicity) bioassays using five test concentrations and a control series for 4 days [12,13]. Dilution water for the experiment was collected from the unpolluted site and filtered through 0.45µm GFC Whatman filter paper. Each series of test chambers with 10 animals of *T.jarbua* in a 10 litre glass trough in triplicate. Test chambers were loosely covered to prevent loss of test fish. Fish was not fed during the treatment period. Temperature, pH, salinity, dissolved oxygen and test concentrations were measured to ensure the acceptability and validation of the tests, following standard methods [11]. Metal levels were measured by Atomic Absorption Spectrophotometer, Varian SpectraAA 220FS Model. The criterion for determining death was the absence of movement when the animals were gently stimulated. Dead animals were removed at each observation and survivors were counted. Maximum-allowable control mortality was 10% for a 96 hour period of testing. A computerized probit analysis was carried out according to the methods of Finney [14] for the calculations of LC_{50} values. While the safe concentration of the metals for 96 hour was determined by the methods of Miller and Miller [15]. Acute to chronic estimation (ACE) is a software model version 2.0 which runs on raw acute toxicity test data to predict the no-effect concentration (Chronic end point) (NEC) of the chronic test. This model runs on three methods, Accelerated life testing (ALT), Multifactor probit analysis (MPA), and Linear regression analysis (LRA) [16].

Results and Discussion

There was 100% survival at the initial exposure in all the concentrations, but the survival rate started declining with increase in concentration and time of exposure. The mortality of *T. jarbua* increased with increase in concentrations with difference in time (Figure 1). At 24 hour 85% of *T. jarbua* of 26 mg/l Pb and about 95% were dead at 72 hour. Lead produced 10%, 5% and 0% survival at 96 hour respectively at 26 mg/l. Lead recorded 95% and 80% survival 96 hour at 2 mg/l (Figure 1).

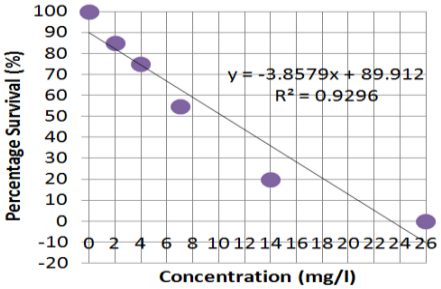

Figure 1. Survival percentage of T.jarbua to lead in acute toxicity test

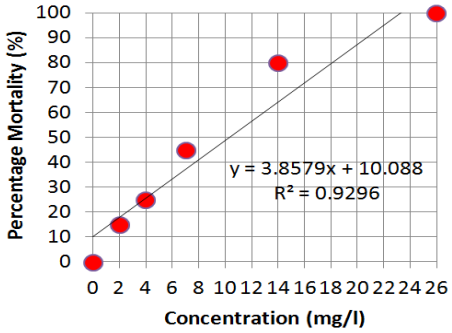

Figure 2. Mortality percentage of T.jarbua to lead in acute toxicity test

The study was conducted to determine the range of metal concentrations that elicit metal tolerance in *T. jarbua* and to find a significant impact on the mortality and survival of test fish. The 96 hour LC_{50} values was 3.04 mg/l. Significant differences ($P<0.05$) in the toxicities were observed in the different heavy metals used. *T. jarbua* produced 100% mortality at 26 mg/l Pb and 80% mortality at 14 mg/l Pb at 96 hour virtually 15% mortality was recorded at 2 mg/l Pb at 96 hour (Figure 2).

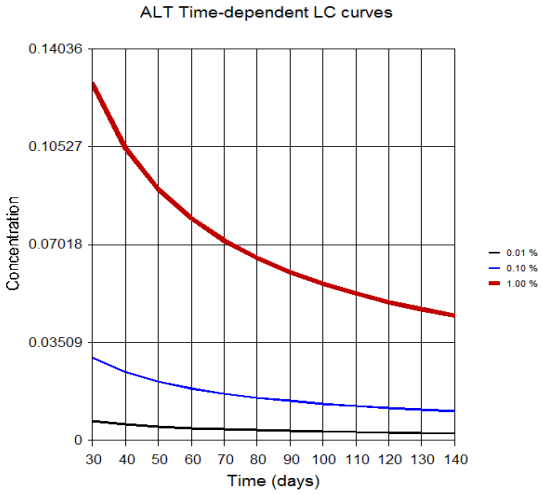

Figure 3. Prediction of no-effect concentration with ALT

The results clearly confirm that *T. jarbua* weakened progressively with time prior to mortality to all the lead concentrations. However, the rate of swimming behaviour in test organisms slowed down might be due to the amount of potency to lead. The safe concentration arrived at a value of 30.4 µg/l. The Acute to chronic estimation software predicted the no-effect concentration as 183 µg/l (ALT) (Figure 3), 324 µg/l (MPA) (Figure 4) and 188 µg/l (LRA) (Figure 5).

Tropical marine fishes are generally less sensitive to heavy metals [17], toxicity of lead depends on the water hardness, salinity and pH of the test media, lead is highly toxic in fresh water than in seawater, the toxicity decreases as salinity of the test media increases.

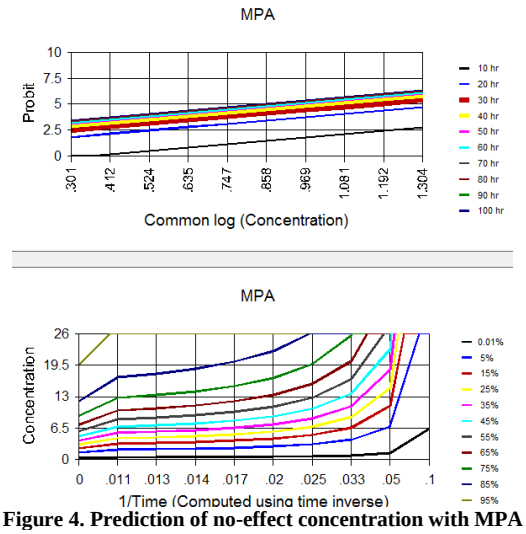

Figure 4. Prediction of no-effect concentration with MPA

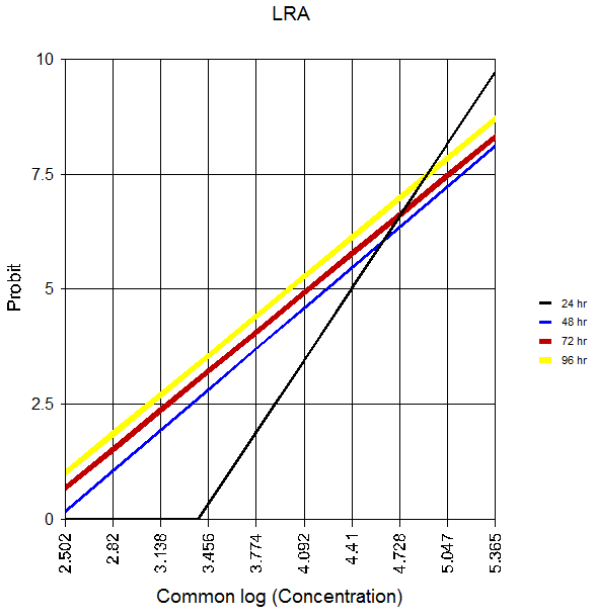

Figure 5. Prediction of no-effect concentration with LRA

The present study observed lethal concentrations in the toxicity of Pb on *T. jarbua* revealing that lead was not tolerated by the *T. jarbua* due to the fact that fish may become more sensitive to the presence of lead even in lower concentrations [18]. Heavy metals react with mucus on the surface of the gills causing precipitation and coagulation thus interfering with the normal exchange of gases and resulting in suffocation and death ultimately. The animal experienced dark colouration, gasping of air, rapid movements of operculum and eyeballs, losing their stability and falling of scales. Fish are relatively tolerant of lead as compared with other marine organisms [19]. Acute toxicity might be strongly influenced by environmental variables. Toxicity varies according to species, duration of exposure, concentration, and water quality characteristics such as salinity and pH which influence the bioavailability of lead. Freedman et al. [20] demonstrated that at low pH, toxicity effect is enhanced. The presence of other salts reduced the availability of lead. Hence, increase in salinity will reduce lead toxicity. Taylor et al. [21] reported that mullet, *Chelon labrosus* LC_{50} value was >4.5 mg/l for lead. Mohapatra and Rengarajan [22] reported that mullet, *Liza parisa* exposed to lead, in acute toxicity test revealed the 96 hour LC_{50} of 13.7 mg/l. Concentrations of lead used in our experiments are regarded to be high for *T. jarbua*. Such concentrations do not occur permanently in surface waters. However, 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 [23]. This may be dangerous for fish, especially for larvae that are considered to be more vulnerable to intoxication caused by heavy metals than embryos or older individuals [24]. Calabrese et al. [25] reported LC_0, LC_{50} and LC_{100} values for American Oyster, *Crassostrea virginica* exposed to lead were, 1.0, 3.80 and 6.0 mg/l. Square tail mullet, *Liza vaigeniensis* showed a LC_{50} of 98 mg/l for lead in 96 hour. Tiger prawn, *P.monodon* exhibited 96 hour LC_{50} for lead as 0.29 mg/l and green mussel, *P. viridis* exposed to lead in acute toxicity test showed the literature value of 4.46 mg/l [19]. Rajkumar et al. [26] reported an LC_{50} for lead as 0.29 mg/l for *Mugil cephalus* in static renewal toxicity test. The present study results were comparable to the authors related to the acute toxicity and sensitivity of test animals to the heavy metals in static renewal. However, the knowledge of the effects of heavy metals on the three marine organisms including lower stages of this test organisms are very limited, though there are papers describing their behaviour with heavy metals in adults.

The safe concentration was derived from the 96 h of LC_{50} and no-effect concentration derived from ACE value would provide shelter confining for the easy propagation of fish fingerlings in the natural ecosystem that is under threat. This study provides an in-depth strategy for mortality and possibility of reducing test organisms in the laboratory. Furthermore the safe concentration derived will be useful in screening potentially toxic substances in Integrated risk assessment for environmental and ecological issues is the most valuable and can give a balanced view. The study of environmental and human health issues can achieve more realistic results if human and ecological risk assessment were combined he marine environment. This contrasts sharply with human health risk assessment, which seeks to protect only a single species. In recent years, methodology of ecological risk assessment has been developed and applied frequently for addressing various circumstances where ecological impacts are suspected or have occurred due to environmental contamination.

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