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ORIGINAL ARTICLE

Predicting NOEC and Safe Concentration for *Mugil cephalus* and *Perna viridis* to Mercury

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ABSTRACT
No-effect concentration as well as safe concentration of mercury was evaluated by exposing Perna viridis and Mugil cephalus to 24-hour static renewal bioassay for 96-hours. The 96-hour LC_{50} value was 75 and 129 µg/l Hg for Mugil cephalus and Perna viridis. Juvenile mullets were more sensitive than green mussel. The safe concentration calculated were 0.75 and 1.29 µg/l Hg. The no-effect concentration calculated for Perna viridis was 51.05 µg/l and 27.14 µg/l for Mugil cephalus calculated in the present study.
Keywords: *acute toxicity, green mussel, mercury, mullet, no-effect concentration*

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INTRODUCTION

Environmental problems pertaining to aquatic pollution are related to acute toxicity of pollutants categorized as heavy metals than to and long-term effects rather difficult to detect and consequences for ecosystems are far from being understood and are unrecoverable [1]. Economically important species like fish and molluscs are supporting major fisheries and has been used extensively in laboratory toxicity testing bioassays since they are widely distributed in the wild, sensitive to contaminants and relatively easy to hold and culture in the laboratory are and designated as biological indicators [2]. Measuring biological response of marine organisms to heavy metals particularly at their sensitive early life stages, toxicity testing is a relatively simple laboratory bioassay [3]. The degree on the effect of toxicity of heavy metals is universally assessed through laboratory bioassays practices where test organisms are exposed to contaminants and acute test data are obtained for many native species, owing to simpler test methods [4]. Lethal Concentration (LC) determination for a given toxicant is a vital requirement in all toxicological investigations to derive for safe concentration for the survival of test organisms [5]. Toxicity tests in a legal context, to implement regulations for certain proof of harm to threatened biological resource. Perhaps for these reasons, environmental risk assessment focuses on a simple and straightforward end-point, lethality or survival (LC_{50}). This measurement forms a baseline data for toxicity and other referable safety concentrations [6].

Heavy metals are omnipresent in the environment and have increased in some areas especially in estuarine sediments, acting as a sink for pollutants threatening aquatic organisms specifically juveniles that thrive in estuarine conditions [7]. To predict the effects of contaminants exerted on aquatic organisms and to establish toxicity criteria for acceptable levels of heavy metal contamination has been a major challenge in ecotoxicological research. Fish and molluscs are an integral component of the aquatic ecosystems. In addition to being a source of protein, they play an important role in energy flow, nutrient cycling and maintaining community balances in aquatic ecosystem. Thus, utility of sentinel marine organisms for assessing environmental conditions in aquatic ecosystem has gained prominence. In recent years, fish and molluscs acted as potential biomonitors for environmental pollution [8]. Heavy metals may have different physical and biological effects at concentrations much lower for a longer period of time, than at which they have lethal effects, suggesting that regulatory pollution limits based upon standard toxicological studies may be too high to prevent damage to aquatic communities through the sub lethal behavioural effects [9]. Assessing the toxicity of contaminants on aquatic life has been a long-standing practice.

One of the largest difficulties in ecological risk assessment is to determine a "safe" concentration of chemicals that protects "most species" in nature [10]. Hence, in the present study the acute toxicity test was conducted to determine the impact of mercury on the survival rate of juveniles *Mugil cephalus* (Flathead mullet) and *Perna viridis* (Green mussel) to predict safe concentration and no-effect concentration (NOEC) for mercury.

MATERIALS AND METHODS

Juvenile specimens of *Mugil cephalus* (Flathead mullet) of 1.5 ±0.5 cm and *Perna viridis* (Asian green mussel) 1.5 ±0.5 cm in size were collected from Ennore (Tamilnadu, India) and *Perna viridis* from Pondicherry (old harbour, Pondicherry, India). Collected alive juveniles were immediately transported to the laboratory in oxygen filled plastic bags and acclimatized in different 1000 L FRP tanks with aerated natural filtered seawater for a period of 8 days at 28 ±2 PSU salinity, temperature of 27 ±2 °C, dissolved oxygen of 5.6 ± 0.3 mg/l and pH of 8.01 ± 0.03. The test organisms collected from wild were quarantined with oxytetracycline (OTC). Juvenile specimens of *Mugil cephalus* were fed with rice bran and oil cake and juvenile specimens of *Perna viridis* were live fed with *Chlorella Sp*. throughout acclimatization period. The dead animals were removed immediately and remaining detritus were removed by siphoning [11]. Stock solutions of mercury were freshly prepared by dissolving mercury (II) chloride ($HgCl_2$) in deionized (double distilled) water with glass standard flasks. Fresh stock solutions were prepared daily and were serially diluted to arrive at the experimental concentration. The experimental method includes static renewal (24-hour, 4 day) test by following the method for range finding test (preliminary tests) [15]. Range finding tests were conducted to establish suitable concentration ranges for conducting definitive test for acute toxicity test. Five concentrations (100, 150, 225, 338 and 500 µg/l) in a geometric series including control, toxicant and seawater were replaced on daily basis [12].

Dilution water for the experiment was collected from the unpolluted site (Neelangarai, India) and filtered through 0.45µm filter paper (HA-Millipore) using Millipore vacuum pump. Test organisms were added to test chambers within 30 minutes of addition of the test material to dilution water. Each series consisted of triplicate test chambers with 10 animals in a 10 L glass trough and test chambers were loosely covered to prevent loss of test animals. Temperature, pH, salinity, dissolved oxygen, total hardness and test concentrations was measured to ensure the acceptability and validation of the tests [13, 14]. Test animals were not fed during acute test. Daily observations were recorded for survival and mortality. The criterion for determining death was the absence of movement when the animals were gently stimulated and dead animals were removed. Maximum-allowable control mortality was 10 per cent for a 96 hour period of testing and [13]. A computerized probit analysis program (USEPA probit analysis program version 1.5) was carried-out for the calculations of LC_{50} values (24, 48, 72 and 96-hour) and upper and lower 95 per cent confidence levels were also calculated. The safe concentration (It is the $1/100^{th}$ of the 96-hour LC_{50} value) for mercury from 96-hour was determined following Miller and Miller [15]. No-effect concentration (NOEC) was calculated using survival data from acute toxicity test in DEBtox/DEBdeg v2.01 (2004) software (Hazard Model; Normal Kinetics).

RESULTS AND DISCUSSION

In the toxicity test, temperature were maintained at 28 °C ±0.3, salinity was maintained at 28 ±1.2 PSU, pH was 7.78 ± 0.02, and dissolved oxygen was maintained with 4.9 ±0.5 mg/l. The total hardness varied from 1550 to 1786 ±11.3 mg/l. The measured mercury concentration in the test chambers ranged from 83 to 95 per cent. The 96-hour LC_{50} value was 75 and 129 µg/l Hg for *Mugil cephalus* and *Perna viridis*. Green mussel seems to be much tolerant than mullet juveniles. Safe concentration calculated were 0.75 and 1.29 µg/l Hg for *Mugil cephalus* and *Perna viridis*. The no-effect concentration (NOEC) calculated for *Perna viridis* was 51.05 µg/l and 27.14 µg/l for *Mugil cephalus* (Figure 1 and 2). The fraction of survivors in terms of concentration and time profile for the toxicity test was calculated and represented in Figure 3, 4, 5 and 6. Clear evidence suggests that, juveniles of mullet are sensitive and juvenile green mussels were tolerant to mercury. The number of survivors decreased with respect to concentration and time profile for both *Mugil cephalus* and *Perna viridis*. The 24, 48, 72 and 96-hour LC_{50} values of Mercury in *Mugil cephalus* and *Perna viridis* are represented in Figure 7. At relatively low concentrations found at metabolically active sites mercury is highly toxic. Characteristics including neurotoxicity, nephrotoxicity, and gastrointestinal

toxicity with ulceration and hemorrhage are exhibited with mercury toxicity [16]. Verlecar et al. [16] reported an LC_{50} of 450 µg/l for green lipid mussel (8 to 10cm). Verlecar et al. [17] reported an LC_{50} of 25 µg/l for typical mysid species, *Mesopodopsis zeylanica*, which is lesser than the calculated value for *M. cephalus* in the present study. Green mussels (65 to 85 mm shell length) showed an LC_{50} of 155 µg/l of mercury [18]. In general, acute toxicity (96 hour LC_{50}) ranges from 33-400 µg/l for freshwater fish, with seawater fish being less sensitive [19]. Estuaries are primary sensitive zones for heavy metal pollution subjected to heavy industrialization and overpopulation. Heavy metal contamination of the environment, which has been recognized as a serious pollution problem, is capable of exerting considerable biological effects even at low levels due to pervasiveness and persistence nature to lower stages of marine organisms. In order to study the response generated in the stressed ecosystem, environmental toxicology plays a crucial role [20]. To balance the ecosystem structure and functions several directives are being adopted over time to protect estuaries and coasts from pollution. The environmental quality standards rely on the concentrations of contaminants as quality objectives for comparing the state of vulnerable sites [21]. The ecological integrity is judged using water or sediment in toxicity tests. In other cases, concentrations of the contaminants are used to assess the ecological status of a location. The toxicity tests measure the integrated responses to the possible acute effects of contaminants, on these processes [22]. Biological toxicity testing is a relatively simple laboratory bioassay that measures the biological response of marine organisms. Invertebrates are routinely used as candidate organisms in such bioassays, and early life stages of invertebrates are often the most sensitive to contaminants. A number of early life stage toxicity test protocols have been developed and effectively applied to characterize contaminants using Sea Urchin and Bay Scallop [23]. The role of developmental stage, size and salinity are very crucial in heavy metal toxicity to estuarine and marine organisms [24].

Hazard model, Normal kinetics			ASD	Correlation coefficients		
Blank mortality rate	1.848e-009	d^{-1}	0.000			
No-effect concentration	27.14	$\mu g\ l^{-1}$	21.221	0.000		
Killing rate	0.005247	$1\ \mu g^{-1} d^{-1}$	0.001	0.000	0.603	
Elimination rate	2.098	d^{-1}	0.898	-0.000	0.208	-0.509
Deviance	5.623					

Fig 1. The NOEC values calculate for *M. Cephalus* for mercury in acute toxicity test, with survival data using DEBtox software

Hazard model, Fast kinetics			ASD	Correlation coefficients	
Blank mortality rate	5.938e-011	d^{-1}	0.000		
No-effect concentration	51.05	$\mu g\ l^{-1}$	21.944	0.000	
Killing rate	0.00232	$1\ \mu g^{-1} d^{-1}$	0.000	0.000	0.770
Deviance	16.54				

Figure 2. The NOEC values calculate for *M. Cephalus* for mercury in acute toxicity test, with survival data using DEBtox software

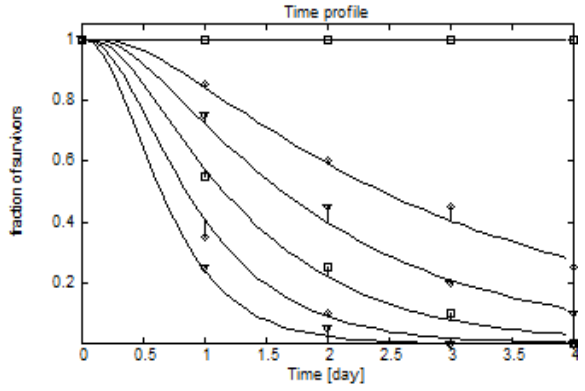

Figure 3. Graph representing the fraction of survivors with time 4 days (96-hours) for *M. Cephalus* for mercury in acute toxicity test, with survival data using DEBtox software

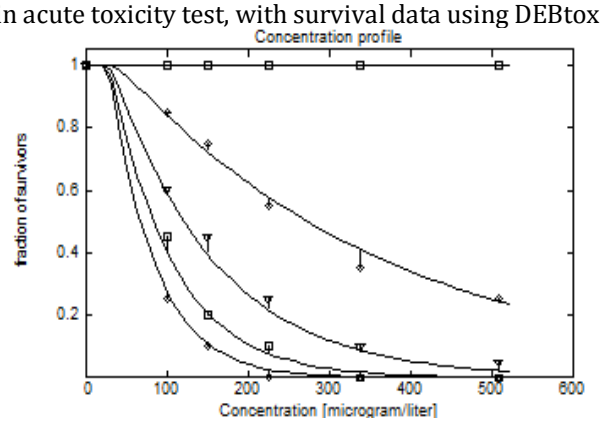

Figure 4. Graph representing the fraction of survivors with concentrations (100, 150, 225, 338 and 500 µg/l) for *M. Cephalus* for mercury in acute toxicity test, with survival data using DEBtox software

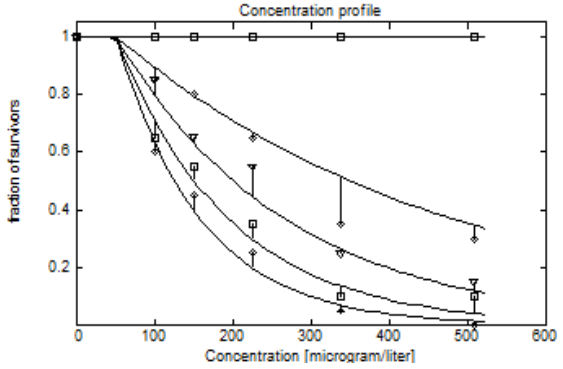

Figure 5. Graph representing the fraction of survivors with time 4 days (96-hours) for *P.viridis* for mercury in acute toxicity test, with survival data using DEBtox software

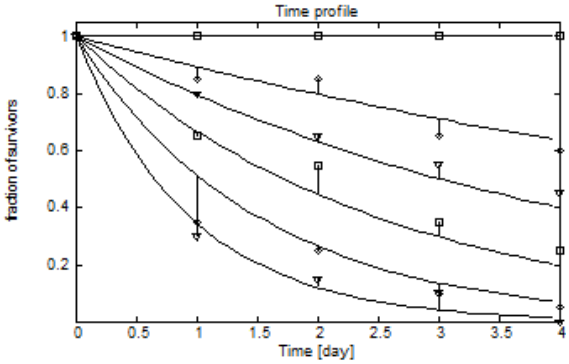

Figure 6. Graph representing the fraction of survivors with concentrations (100, 150, 225, 338 and 500 µg/l) for *P.viridis* for mercury in acute toxicity test, with survival data using DEBtox software

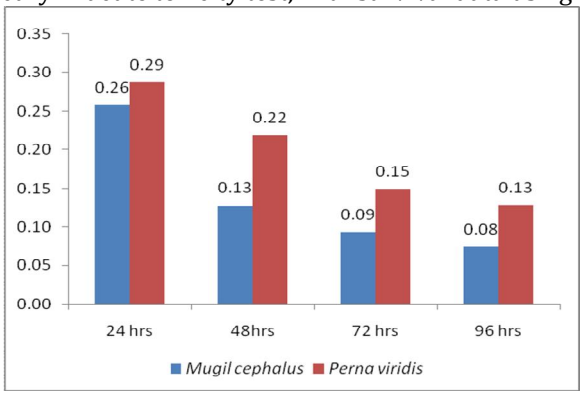

Figure 7. LC_{50} values of *M.cephalus* and *P.viridis* exposed to mercury in acute toxicity test

CONCLUSION

Safe concentration and no-effect concentration was predicted for mercury in the present study. The concentrations were higher for *P.viridis* and lower for *M.cephalus*. The levels may be used as criteria for mercury pollution. To reduce the use age of test organisms from the wild, biological and toxicological software's are widely used; one such incidence was used in the present study to predict NOEC. Pollution may result in a cascade of events, beginning with effects in individuals and extending through population, communities, ecosystems and landscapes. The biological indicators have helped substantially to establish ecotoxicological endpoints. The complexity of the large-scale effects on ecosystems results in a challenging research environment for environmental toxicologists and ecotoxicologists.

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