



Marine Organisms in Toxicological Approach for the Assessment of Environmental Risk Associated With Cd, Cu, Pb and Zn

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Abstract:

Three marine organisms, *Mugil cephalus*, *Penaeus monodon* and *Perna viridis* were exposed to cadmium, copper, lead and zinc in 24-hrs static renewal acute toxicity test in the present study. Subsequently safe concentration and the ambient concentrations in the Ennore were estimated. The calculated 96-hour LC₅₀ values showed the sensitivity of mullet juveniles to metals are in the order of Cu>Cd>Pb>Zn. Juveniles of *P. viridis* were highly susceptible to copper; 96 h LC₅₀ value was very low and was tolerant to zinc. The order of sensitivity was Cu>Cd>Pb>Zn. Post larval stages of *P. monodon* exposed to heavy metals in acute toxicity test revealed vulnerability towards lead and tolerant to zinc. The order of sensitivity was Pb>Cu>Cd>Zn. Ambient concentrations in Ennore creek showed that the values were above the safe concentrations derived and the coastal standards. The heavy metal concentrations would have posed a great threat for the sustainability of juveniles in the Ennore estuary. Hence, there is an urgent need to implement stringent rules to prevent pollution of creek, so that the juveniles may grow, reproduce, and thrive in their waters contributing a sustainable ecosystem for the future.

Keywords: *Mugil cephalus*, *Penaeus monodon*, *Perna viridis*, Ennore creek, Heavy metal pollution

1.0 Introduction:

Impact of anthropogenic changes may have adverse effect on intertidal benthic communities, including their accumulation and subsequent biomagnification through the food chain (Seralathan *et al.*, 2008). Many metals have a wide range of uses, but these have come at a significant environmental price, have serious negative environmental consequences, yet our dependence on them continues to result in large inputs into our environment. Heavy metals are an important category of pollutants and as such have major detrimental impact on human and environmental health (Ahiamadjie *et al.*, 2011).

To balance the ecosystem structure and functions several directives are being adopted over time to protect estuaries and coasts. Water quality is a crucial facet for the survival and well-being of the living resources, especially in the coastal and estuarine areas. Assessing the toxicity of contaminants on aquatic life has been a long-standing practice (Smithwick *et al.*, 2005). The environmental quality standards rely on the concentrations of contaminants as quality objectives for comparing the sites. The ecological integrity is judged using water or sediment in toxicity tests (Tueros *et al.*, 2009). The United

States Environmental Protection Agency (USEPA) recommends the use of bioassays, biological and habitat data in addition to chemical data for water quality assessments (USEPA, 2002a & b). The toxicity tests measure the responses to the possible acute effects of contaminants. Test species should be sensitive enough to respond to different levels of contaminants and must be available for use from field collection throughout the year. Species dominance can also reflect pollution conditions since the tolerant species usually form the dominant population and develop higher individual abundance under the conditions of organic pollution. Measuring contaminants such as trace metals in bioindicators therefore provides a means of investigating spatial and temporal trends in ambient contaminant concentrations (Lurling and Scheffer, 2007, Rajkumar *et al.*, 2011b).

The risk assessment tools in toxicology promote the sustainability of ecosystems and identify early symptoms of exposure to prevent the series of environmental deprivation. Protection can be accomplished if effects are both quantifiable and to generate preventive guidelines. The guidelines should be protective and flexible to ensure the generation of new data would provide maximum protection to as many of the species in the

ecosystem. The challenge still remains for ecotoxicologists is what effects on the ecosystem are acceptable or unacceptable sensitive endpoints on the species level. Thus, developments in risk assessment should focus on the translation from laboratory species to field communities (OSPAR, 2002). Hence, in the present study the acute toxicity test was performed with *Mugil cephalus*, *Penaeus monodon* and *Perna viridis* in relation to cadmium, copper, lead and zinc and to predict the safe concentration with respect to the measured concentration in the Ennore creek for assessing the risk of heavy metals.

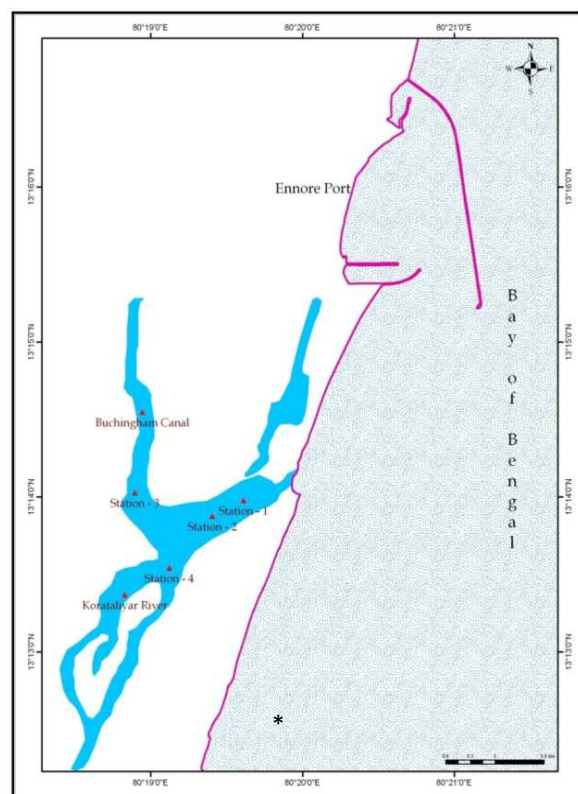
2.0 Materials and Methods:

Ennore is located on the northeast coast of Chennai. Ennore creek once encompassed with rich biodiversity and in due course of time has been totally wiped out by the petrochemical complex by pumping their effluents into the Ennore Creek. Consequently, the natural wealth is eroded to mere sewage channel and the biological productivity of the coast has come down (Jayaprakash *et al.*, 2005, Rajkumar *et al.*, 2011a). In order to assess the impact generated by the anthropogenic activities in the Ennore creek, water samples were collected at Station I- the bar mouth region (13°14'02.31" N, 80°19'49.47" E), Station II- creek (13°13'52.54" N, 80°19' 24.26" E), Station III- the Buckingham canal (north towards Pulicat lake) (13°14'02.72" N, 80°18' 54.18" E) and station IV- right down the railway bridge (13°13'30.39" N, 80°19' 02.30" E) (Fig 1). The dissolved metals in seawater was extracted in filtered water samples by chelating with 5 ml of 2 per cent Ammonium pyrrolidine-dithiocarbamate (APDC) followed by 15 ml of Methyl isobutyl ketone (MIBK) extraction in 500 ml separating funnel, according to the method described by El-Moselhy and Gabal (2004). Extracted metal concentrations were determined with a Varian Spectra AA 220FS Atomic Absorption Spectrophotometer (AAS) with an air/acetylene flame. Appropriate internal standards (Merck Chemicals, Germany) were used to calibrate the instrument. Analytical grade reagents were used to make up the relevant blanks.

Fingerlings of *M.cephalus* of mean 1.5 ± 0.4 cm in length and 0.13 ± 0.02 g in weight, juvenile specimens of *P. viridis* (1.6 ± 0.4 cm in length and 0.12 ± 0.01 g), and post-larval stages of *P. monodon* (PL-12) were collected from Ennore, Puducherry and Marakanam (Tamilnadu, India). Collected juveniles were immediately transported to the

laboratory in air filled plastic bags and acclimatized in glass aquaria with aerated natural filtered seawater for a period of 8 days at 28 PSU salinity, temperature of 28 ± 2 °C, dissolved oxygen of 5.6 mg/l and pH of 8.01. Captured wild organisms were quarantined immediately (Oxytetracycline). After a day of acclimatization, the juvenile *M.cephalus* was then fed with pellets of rice bran and oil cake, *P. viridis* was fed with mass culture of cyanobacteria (*Anabaena* sp.) and post larvae of *P. monodon* were fed with mixed feed for *P. monodon* (Japan) throughout acclimatization period. The dead animals and remaining detritus were removed by siphoning (USEPA, 1996).

Stock solutions of cadmium, copper, lead and zinc were freshly prepared by dissolving the proper metal salts ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ for Cd (Cadmium chloride hemi (pentahydrate), CAS-7790-78-5, molecular weight-228.36), CuCl_2 for Cu (Copper (II) chloride, CAS-7447-39-4, molecular weight-170.48), $\text{Pb}(\text{NO}_3)_2$ for Pb (Lead (II) nitrate, CAS-10099-74-8, molecular weight-331.23) and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ for Zn (Zinc sulfate, CAS-7446-20-0, molecular weight-287.54) in deionized (double distilled) water with glass standard flasks. Fresh stock solutions were prepared daily. These solutions were serially diluted to get the experimental concentration for the toxicity test.



Map was designed from ARCGIS 9.0; not to scale

Fig 1 Ennore creek

The experimental method includes static renewal (24-hour renewal) test by following the method of USEPA (2002a). Five concentrations in a geometric series including control were prepared for the test for 4 days in acute toxicity test; toxicant and seawater were replaced on daily basis (USEPA, 2002b). 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 of test chambers consisted of duplicates with 10 animals in a 5 L glass trough. Test chambers were loosely covered to reduce evaporation and to minimize the entry of dust into solutions and to prevent loss of test animals. All the experiments were conducted at salinity of 28 PSU, temperature of 28 ±2 °C, dissolved oxygen of 5.6 mg/l and pH of 8.01 with gentle aeration.

Test animals were not fed during acute test, temperature, pH, salinity, dissolved oxygen and test concentrations were measured to ensure the acceptability and validation of the tests, following standard methods (USEPA, 1996). Daily observations were recorded for survival and mortality. 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 per cent for a 96-hour period of testing (USEPA, 2002b). A computerized probit analysis program (USEPA probit analysis program version 1.5)

(Probit Program version 1.5) was carried out for the calculations of LC₅₀. Percentage of mortality was calculated and corrected using Abbott's formula (Abbott, 1925). One-way ANOVA method was performed with graphpad prism software v 5.00 and the correlation with draftsman plot and the dendrogram were prepared with primer v 6.00 (Clarke and Gorley, 2006). The safe concentration of the heavy metals (Cd, Cu, Pb and Zn) for 96 hour was determined by the methods of Miller and Miller (1986) (1/100th) of the 96 hour LC₅₀ value.

3.0 Results and Discussion:

Concentrations of cadmium, copper, lead and zinc in the surface waters of the Ennore creek varied significantly ($P < 0.001$) with respect to stations. The concentrations of heavy metals were above the coastal standards. Measured concentrations for cadmium, copper, lead and zinc was high in stations 3 and 4 when compared with stations 1 and 2, the correlation was significant at $P < 0.001$ ($\alpha = 0.05$). One-way ANOVA performed between the metal concentrations and the coastal standards showed a high significance indicating that all measured concentrations in all the seasons and stations ($P < 0.0001, 0.0001, 0.001$ and 0.005) were relatively high in Ennore creek (Table 1). The chief source of cadmium, copper, lead and zinc was found in stations 3 and 4 which were distributed throughout the creek correlating with a high significance in all the stations.

Table 1. Dissolved heavy metals (mg/l) in the surface waters of Ennore creek at different stations

Heavy metal	Station 1	Station 2	Station 3	Station 4	Coastal standards ^a
Cd	12.23 ±0.13	13.11 ±0.19	17.20 ±0.07	24.18 ±0.15	10
Cu	65.12 ±2.56	118.22 ±0.34	131.24 ±2.70	142.45 ±3.55	20
Pb	78.45 ±2.98	198.56 ±1.04	122.35 ±1.97	226.56 ±1.63	100
Zn	198.33 ±2.93	223.87 ±1.47	256.65 ±2.27	372.19 ±3.57	100

*Values were significant at $P < 0.05$ (one way ANOVA), $P < 0.001$ ($n = 4$), highly significant, with coastal standards were analyzed for station 1, 2, 3 and 4 respectively during all seasons; ($P = 0.0001, 0.0001, 0.001$ and 0.005); a, National Environmental Board report, 1994; Values are mean and standard deviation of $n = 2$

The zinc concentrations were high followed by lead, copper and cadmium in all the stations. Ennore creek carries high load of heavy metals (Kannan *et al.*, 2007, Rajkumar and Samuel, 2012.). Buckingham canal and Korataliyar river are no

longer able to receive and assimilate effluents because they have fallen below minimum levels of flow. The treated effluents of the Madras Refinery Ltd, through the Buckingham canal and the Madras Fertilizers Ltd, through the Red Hills

surplus channel, reach the Ennore backwater. Brigden *et al.* (2005) reported lead concentrations between 17 and 247 times higher, exceeding guidelines of WHO (2004) by 190 to 2400 times in India (New Delhi and Bangalore). Dissolved metals are considered to be the most mobile, thus reactive in bioavailable fractions of the aquatic system and are cause for concern (Wong *et al.*, 2007). Dissolved heavy metal concentration in station 1 decreased, but in station 4 a complex behaviour exists with high heavy metal concentration in the downstream direction (Dauby *et al.*, 1994). This increase in dissolved heavy metals in Ennore creek is linked to the high industrialization of the surrounding areas, which produces large amounts of heavy metal-based effluents. In the present study conducted in Ennore creek the heavy metal concentrations were higher than the permissible limits in all four stations indicating pollution of heavy metals in the aquatic environment.

During the toxicity test, temperature was maintained at 28 °C ±0.3, salinity was maintained at 28 ±1.2 PSU, pH was 7.78, and dissolved oxygen was maintained with 4.9 mg/l. The total hardness

varied from 1550 to 1786 ±11.3 mg/l. The recovery of the concentrations in the test chamber ranged from 96.4 to 118 per cent of the nominal concentrations. The higher rates of mortality were observed with *M.cephalus* when exposed to copper than lead and zinc in the acute toxicity test. The animal experienced dark colouration, gasping of air, rapid movements of operculum and eyeballs, losing their stability, falling of scales, the juveniles remained tilted in the highest copper concentration. The juveniles of *P.viridis* exposed to heavy metals showed distinct behaviour in the test. The byssal thread formation was observed in the control, but not in treated test chambers. The post larvae of *P.monodon* exposed to heavy metal concentrations showed piercing character with rostrum to the glass trough, rapid moulting, and the moulted larvae were sensitive to metal concentration. The mortality of the *P.monodon* was observed by the red colouration of the whole body. The 96-h LC₅₀ values of copper, zinc and lead to *M.cephalus*, *P.viridis* and *P.monodon* are shown in Table 2.

Table 2. Results of 96 hour LC₅₀ (mg/l) and Safe concentration (SFC) (µg/l) in acute toxicity test

Test species	Metal	Concentration used (mg/l)	96 hour LC ₅₀ (mg/l)	Safe Concentration (µg/l)
<i>M.cephalus</i>	Cd	0.01, 0.1, 1, 10, 100	5.09 (2.91 – 8.69)	50.9
	Cu	0.01, 0.1, 1, 10, 100	2.74 (1.69 – 4.63)	27.4
	Pb	0.02, 0.2, 2, 20, 200	7.13 (4.14 – 12.23)	71.3
	Zn	0.02, 0.2, 2, 20, 200	7.73 (4.31 – 13.86)	77.3
<i>P.viridis</i>	Cd	0.01, 0.1, 1, 10, 100	2.53 (1.38 – 4.62)	25.3
	Cu	0.01, 0.1, 1, 10, 100	0.50 (0.28 – 0.89)	5.0
	Pb	0.01, 0.1, 1, 10, 100	3.16 (1.88 – 5.32)	31.6
<i>P.monodon</i>	Zn	0.01, 0.1, 1, 10, 100	4.11 (2.39 – 6.73)	41.1
	Cd	0.01, 0.1, 1, 10, 100	1.32 (0.73 – 2.40)	13.2
	Cu	0.01, 0.1, 1, 10, 100	0.95 (0.53 – 1.71)	9.5
	Pb	0.01, 0.1, 1, 10, 100	0.39 (0.22 – 0.72)	3.9
	Zn	0.01, 0.1, 1, 10, 100	2.74 (1.69 – 4.63)	27.4

*The concentration used for the range finding test includes control and was conducted in duplicate; LCL-UCL indicates the lower confidence level and upper confidence level (95%) in parenthesis

The mortality of all animals tested increased with increasing copper, zinc and lead concentrations in seawater. However, the survival of *M.cephalus*, *P.viridis* and *P.monodon* was 93 per cent in the controls; demonstrating that the holding facilities, water and handling techniques were acceptable for the toxicity test, as required in the standard EPA/COE protocol, where mean survival should be $\geq 90\%$. The fingerlings of *M.cephalus* were sensitive to copper and tolerant to zinc concentrations. The sensitivity of mullet juveniles to metals are in the order of $Cu > Cd > Pb > Zn$. Juveniles of *P.viridis* were highly susceptible to copper; 96 h LC_{50} value was very low and was tolerant to zinc. The order of sensitivity was $Cu > Cd > Pb > Zn$. Post larval stages of *P.monodon* exposed to heavy metals in acute toxicity test revealed vulnerability towards lead and tolerant to zinc. The order of sensitivity was $Pb > Cu > Cd > Zn$. *P.viridis* and *M.cephalus* were sensitive to copper, while *P.monodon* was sensitive to lead. The three organisms showed tolerance behaviour towards zinc in the entire acute toxicity tests (Table 2).

Mohapatra and Rengarajan (1997) reported that mullet, *Liza parisa* exposed to lead, copper and zinc in acute toxicity test revealed the 96 hour LC_{50} of 64.7, 21.8 and 13.7 mg/l. Copper was sensitive to *L.parisa* than zinc and lead ($Cu > Zn > Pb$). Eisler (1985) concluded in his synoptic review of cadmium hazards to fish and invertebrates that decapods crustaceans were sensitive marine group in short-term tests. Copper was found to be more toxic to *P.monodon* than *M.cephalus* in this study. Eisler and Raymond (1977) studied the acute toxicity of several heavy metals to estuarine macrofauna and found the rank order of toxicity to be: $Cd > Zn$. In the present study the same order of toxicity to metals was observed when compared with literature observations. Concentrations of cadmium and copper used in our experiments are regarded to be high for *M.cephalus* (Sarnowski, 2004). 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 (Akan *et al.*, 2008). 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 (Yin *et al.*, 1997). Vanegas *et al.* (1997) reported 96 hour LC_{50} for White Shrimp, *P.setiferus*, juveniles exposed to cadmium and zinc were 0.99 and 43.87 mg/l. White shrimp juveniles were sensitive to cadmium than to zinc, cadmium

toxicity was 44 times greater than zinc. In the present study *P.monodon* were sensitive to cadmium than zinc. Chongprasith *et al.* (1999) reported the following LC_{50} values of 96 hour for Tiger prawn *P.monodon*, Common clam, *D.faba*, (Yellow-eye mullet) and *L. vaigiensis* (Diamond scaled-mullet) 2.5, 3.61 and 21.7 mg/l for zinc toxicity test. Svobodova and Kolarova (2004) found *Tinca tinca* susceptibility to toxic effect of copper similar to rainbow trout *Oncorhynchus mykiss* and similar to *M.cephalus* in the present study.

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.

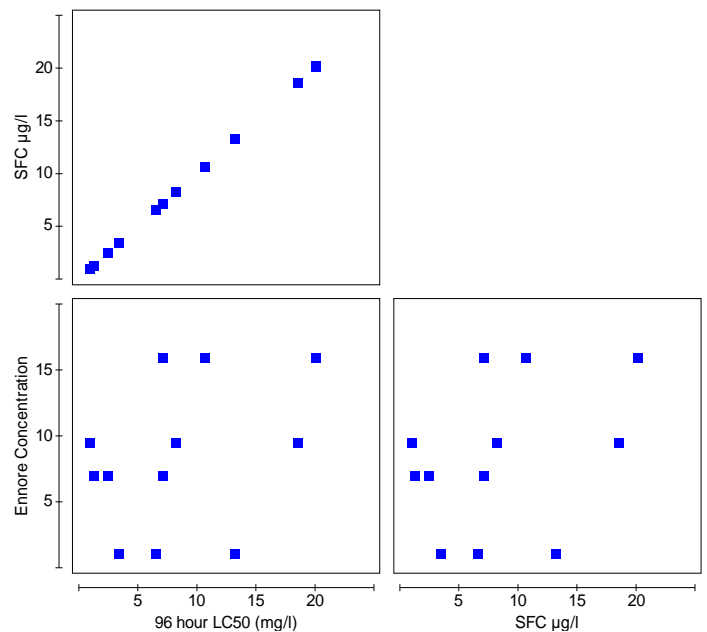


Fig 2. Correlation between the 96-hrs LC_{50} , Safe concentration (SFC) and the ambient concentration at Ennore through draftsman plot from Primer (Clarke and Gorley, 2006)

The present study with acute toxicity test generated LC_{50} values for predicting safe concentration to compare with the ambient concentration in the Ennore creek. Fig 2 shows that high correlation between the 96-hrs LC_{50} values and the predicted safe concentration. The data's clearly predict that the ambient concentrations were higher than the safe concentration and the coastal standards. The

Ambient concentrations at Ennore were significant with the 96-hrs LC₅₀ values and the safe concentration (Fig 3). The assessment of Ennore creek involving laboratory and field experiments revealed that the prevailing concentrations in the creek posed severe threat to the juvenile stages of marine organisms.

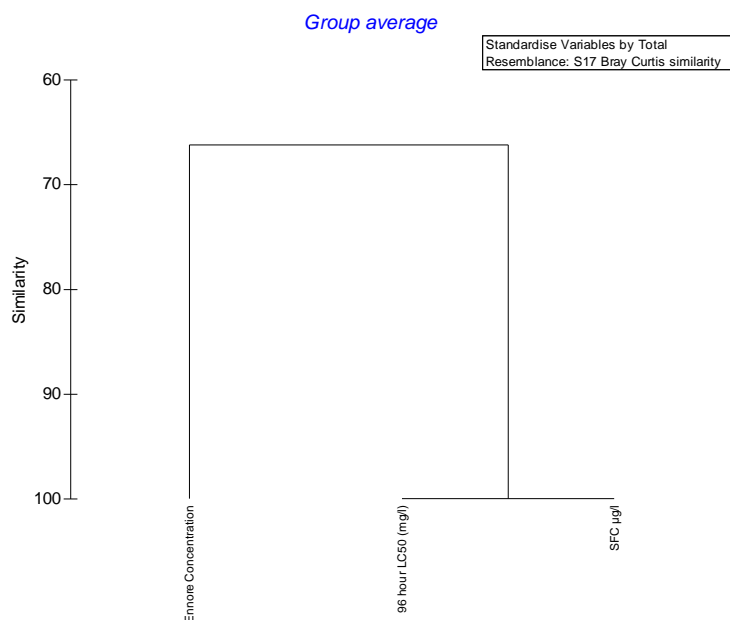


Fig 3. Similarity between the 96-hrs LC₅₀, Safe concentration (SFC) and the ambient concentration at Ennore through linkage diagram from Primer (Clarke and Gorley, 2006)

Hence there is an urgent need to implement stringent rules to prevent pollution of creek, so that the juveniles may grow and reproduce and thrive in their waters contributing a sustainable ecosystem for the future.

4.0 Conclusions:

The assessment of Ennore creek involving laboratory and field experiments revealed that the prevailing concentrations in the creek posed severe threat to the juvenile stages of marine organisms. The ambient concentrations were well above the coastal standards and derived trigger values. Hence there is an urgent need to implement stringent rules to prevent pollution of creek, so that the juveniles may grow and reproduce and thrive in their waters contributing a sustainable ecosystem for the future.

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