RESEARCH ARTICLE

ISSN: **0976-3031**

*Available at http://www.recentscientific.com*

***International Journal of Recent Scientific Research***

***Vol. 2, Issue, 5, pp. 139 -142, May, 2011***

# Analysis of inhibition concentration (ic) of cadmium, copper, lead and zinc in mugil cephalus, perna viridis and penaeus monodon under short term chronic toxicity tests

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## ABSTRACT

The present study was conducted to estimate the inhibition concentration ($IC_{25}$) causing 25 per cent reduction in growth of *Mugil cephalus*, *Perna viridis* and *Penaeus monodon* induced by cadmium, copper, lead and zinc in short term chronic toxicity test (30 days). The $IC_{25}$ for *M.cephalus* was high in all the metal exposure except cadmium. *P.monodon* had the lowest $IC_{25}$ of copper and cadmium and *P.viridis* showed lowest $IC_{25}$ for lead and zinc. Low concentrations of heavy metals; cadmium, copper, lead and zinc are good enough to bring 25 percent inhibition in growth for *P.monodon* and *P.viridis*. In the other hand, *M.cephalus* required higher concentrations to bring a considerable effect in growth. Signifying that mullet juveniles are tolerant to heavy metals when compared to *P.monodon* and *P.viridis*. Essential and non-essential metals can produce toxic effects in fish by disturbing their growth and mortality. The difference caused may be due to various ecological and physiological events of the toxicological experiments and duration of the study.



**Key words:** Inhibition concentration, cadmium, copper, lead, zinc, growth.

## 1. INTRODUCTION

Most commonly used stressor end points are variables related to growth performance. The body size correlates with many ecological as well as life history traits and may thus influence the abundance of species as well as population structure and dynamics (Sibly and Hone, 2002). Growth rate has been frequently used as a measure of performance of the individual and is believed to be a more appropriate measure of toxicological effects (Schamphelaere and Janssen, 2004). When toxicity tests are viewed within a 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 ($IC_{25}$ representing the inhibition concentration causing 25 per cent reduction in growth). This measurement represents a baseline for toxicity towards physiological responses (Ankley *et al.,* 2010). Inhibition concentration ($IC_p$), a point estimate interpolated from contaminant concentrations, at which effects start, is far more useful and realistic than the No-Effect-Concentration (NEC). The estimated effect level should be biologically significant; that is, it should protect a high proportion of all species and be predictive of a contaminant concentration that produces adverse effect in the receiving water (Grothe *et al.,* 1996). Estuarine regions are important areas for the reproduction and growth of many aquatic organisms and crustacean species (Blaber *et al.*, 2000). Many species utilize estuaries as nurseries for feeding and growth during their planktonic phase (Joyeux *et al.*, 2004). There is a growing concern for chemicals and metals that are suspected of disrupting reproduction in aquatic organisms (Figueroa *et al.,* 2007). These are tied to observations in humans and wildlife over the last 40 years of worrying trends of adverse effects (Berkun, 2005). Aquatic organisms are also widely used biological indicators of health in metal pollution (O'Connor, 2002). Molluscs show drastic changes in immune competence upon exposure to different categories of pollutants (Gagnaire *et al.*, 2004). *Perna viridis* has been used in toxicity studies as a bioindicator. Environmental conditions can significantly influence the physiology of marine organisms, and therefore, modify the growth potential (Guan and Wang, 2006). Behaviour studies are useful for studying effects of environmental pollutants because it can provide a bioassay to determine an ecological death that may occur after much lower exposures to the toxicant (Baker and Montgomery, 2001). Altered behaviours caused by exposure to pollutants may hence cause serious risks to the success of animal

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populations and disrupt aquatic communities (Gravato and Guilhermino, 2009). The present study aims at the estimation of inhibition concentration ($IC_{25}$) causing 25 per cent reduction in growth of *Mugil cephalus*, *Perna viridis* and *Penaeus monodon* induced by cadmium, copper, lead and zinc in short term chronic toxicity test (30 days).

## 2. MATERIALS AND METHODS

Fingerlings of *Mugil cephalus* of mean 1.5 ±0.4cm in length and 0.13 ±0.02g in weight, juvenile specimens of *Perna viridis* (1.6 ±0.4cm in length and 0.12 ±0.01g) and post-larval stages of *Penaeus monodon* (PL-12) were collected and immediately transported to the laboratory in air-filled plastic bags and acclimatized in glass aquaria. Fish fingerlings were acclimatized in 200 L Fiberglass Reinforced Plastics (FRP) tanks 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 fry specimens of *M.cephalus* were then fed with pellets of rice bran and oil cake, *P.viridis* was fed with mass culture of cyanobacteria (*Anabaena* sp.) Samples of cyanobacteria were isolation was done using serial dilution and streaking plate method (Rippka, 1988). Samples were diluted with sterilized ASN-III medium up to 10-25 dilution. Dilution tubes were incubated under constant light at room temperature of 28 ±3°C. Stock cultures were maintained at room temperature under diffused light. Post larvae of *Penaeus monodon* were fed with mixed feed for *P.monodon* (Japan) throughout acclimatization period. The dead animals were removed immediately. The remaining detritus were removed by siphoning (USEPA, 1996). Prior to toxicity tests and stock solution preparations, all the glasswares were washed in 10 per cent nitric acid and rinsed with deionized water. Stock solutions of cadmium, copper, lead and zinc were freshly prepared by

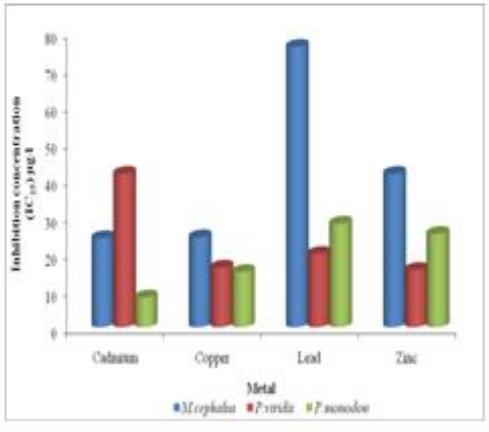


**Figure 1. Inhibition concentration (IC) causing 25 per cent reduction in growth of *M.cephalus, P.viridis* and *P.monodon***

in short-term chronic toxicity test induced by cadmium, copper, lead and zinc dissolving the proper metal salts ($CdCl_2$ .$2.5H_2O$ for Cd, $CuCl_2$ for Cu, Pb $(NO_3)_2$ for Pb and $ZnSO_4.7H_2O$ for Zn in deionized (double distilled) water with glass standard flasks. Stock solutions were acidified by the addition of 0.1 ml of concentrated nitric acid per litre of stock solution (Chapman, 1978). Fresh stock solutions were prepared daily. These solutions were serially diluted to get the experimental concentration for the toxicity test. 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 30 days for short-term chronic toxicity test (USEPA, 2002b). Toxicant and seawater were replaced on daily basis. 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 fed regularly three times a day. Inhibition concentration was calculated according to USEPA (2000b).

## 3. RESULTS

Inhibition concentration ($IC_{25}$) causing 25 per cent reduction in growth of *Mugil cephalus*, *Perna viridis* and *Penaeus monodon* induced by cadmium, copper, lead and zinc in short term chronic toxicity test (30 days) are given in Table 1. The $IC_{25}$ for *M.cephalus* was high in all the metal exposure except cadmium; *P.monodon* had the lowest $IC_{25}$ of copper and cadmium short-term chronic toxicity test (Figure 1). *P.viridis* had the lowest $IC_{25}$ for lead and zinc (Figure 1). In other context, the lower concentrations of cadmium, copper, lead and zinc are well enough to bring about the inhibition in growth for *P.monodon* and *P.viridis*. *M.cephalus* required higher concentrations to bring a considerable effect in growth. It also signifies that mullet juveniles are tolerant to heavy metals when compared to *P.monodon* and *P.viridis* (Figure 1).

**Table 1. Inhibition concentration (IC) causing 25 per cent reduction in growth of cadmium, copper, lead and zinc to *M.cephalus, P.viridis* and *P.monodon* in short-term chronic toxicity test**

| Metal | Test species | $ICp_{25}$ (µg/l) | 95% LCL-UCL |
|---|---|---|---|
| Cadmium | *M.cephalus* | 24.15 | 20.91 – 29.69 |
| | *P.viridis* | 41.61 | 31.69 – 54.63 |
| | *P.monodon* | 8.04 | 4.14 – 12.23 |
| Copper | *M.cephalus* | 24.39 | 15.31 – 33.86 |
| | *P.viridis* | 16.15 | 11.38 – 24.62 |
| | *P.monodon* | 14.95 | 10.28 – 20.89 |
| Lead | *M.cephalus* | 76.09 | 61.88 – 85.32 |
| | *P.viridis* | 19.86 | 12.39 – 22.73 |
| | *P.monodon* | 28.09 | 20.73 – 33.40 |
| Zinc | *M.cephalus* | 41.61 | 32.53 – 51.71 |
| | *P.viridis* | 15.52 | 10.22 – 19.72 |
| | *P.monodon* | 25.28 | 1.69 – 4.63 |

*LCL-UCL indicates the lower confidence level and upper confidence level (95%

## 4. DISCUSSION

Physiological effects of lead exposure have been studied for the green mussel, *P.viridis*. Tan and Lim (1984) reported that the first visible symptom of lead toxicity to *P.viridis* was an increase in mucous secretion resulting in foaming and frothing of the water. Lead inactivates amylase activity in the digestive glands of *P. viridis*. The concentration of lead that caused a 50 per cent inhibition reduced mussel growth since its digestive capacity was affected was $IC_{50}$ equivalent to 5.7 µg/l (Tan *et al.*, 1996), inhibition reduced mussel growth since its digestive capacity was affected. Waiwood and Beamish (1978) showed that 4, 23, and 168 µg/l of copper can produce 20 per cent reduction in growth rate. The Inhibition Concentration ($IC_p$) in terms of growth for cadmium exposed to *P.monodon* was sensitive; it was also sensitive to copper. *P.viridis* had the lowest $IC_{25}$ concentrations for lead and zinc. Marr *et al.* (1996) reported that fish exposed to 4.6 µg/l Cu were 23.5 per cent smaller than control, and the extrapolated $IC_{20}$ from the data is 4.2 µg/l Cu. In the short-term chronic toxicity test the *M.cephalus* exposed to cadmium, copper, lead and zinc showed 25 per cent effect in 24.15 µg/l Cd, 24.39 µg/l Cu, 76.09 µg/l Pb, and 41.61 µg/l Zn. These values were higher than the cited literature values. $IC_{01}$, $IC_{10}$, $IC_{20}$ and $IC_{50}$ reported by Hansen *et al.* (2002) to rainbow trout (1.1, 10.8, 21.6 and 54 µg/l Cu). The lack of mortality or growth retardation in fish chronically exposed to dietary cadmium (500 mg/kg) for 30 days was reported by Chowdhury *et al*. (2004). Chronic exposures to copper affect the growth of juvenile fish (DeBoeck *et al*., 1997). Waiwood and Beamish (1978) observed a 20 per cent reduction in growth rate in rainbow trout exposed to 23 µg/l Cu over a 30-day test. Similarly, Seim *et al.* (1984) observed that fish exposed to 31 µg/l Cu were approximately 20 per cent smaller than controls.

The growth inhibition in the group receiving the highest heavy metal concentration observed in our experiment could be due to the influence of heavy metals on food intake and assimilation. It was shown that cadmium decreased food intake and assimilation and led to the decrease of growth rate in marine organisms. Growth inhibition could also be an effect of cadmium on fish activity and food gaining ability (Abowei, 2009). Cadmium exerted a high effect on the growth rate of *Argopecten ventricosus* juveniles reported by Figueroa *et al*. (2007). Wo *et al*. (1999) observed a 50 per cent decrease in the growth rate of the gastropod *Nassarius festivus* exposed to 0.22 mg/l of cadmium for 40 days. Likewise, Stromgren (1982) detected a decrease in the growth of *M.edulis* at level of exposure below 100 µg/l of cadmium, whereas Pesch and Stewart (1980) found that cadmium at 1310 µg/l induced a 50 per cent decrease in the size of *A.irradians* (Atlantic scallop). The present study agrees that exposed cadmium concentrations inhibit growth with Szebedinszky *et al*. (2001). Heavy metal that interferes with this activity is likely to reduce the fitness of organisms and could involve ecological death (Scott and Sloman, 2004). Inhibition effects on growth due to cadmium exposure were also reported by Baird *et al.* (1990). It is well known that the defense and repair mechanisms depend on energy requiring processes such as active transport and synthetic activity (Smolders *et al.*, 2005). Therefore, confronting stress is likely to be energetically costly for the stressed organisms (De Coen and Janssen, 2003).

## 5. CONCLUSION

The estimate the inhibition concentration ($IC_{25}$) causing 25 per cent reduction in growth of *Mugil cephalus*, *Perna viridis* and *Penaeus monodon* induced by cadmium, copper, lead and zinc in short term chronic toxicity test (30 days). The $IC_{25}$ for *M.cephalus* was high in all the metal exposure except cadmium. Low concentrations of heavy metals; cadmium, copper, lead and zinc are good enough to bring 25 per cent inhibition in growth for *P.monodon* and *P.viridis*. In the other hand, *M.cephalus* required higher concentrations to bring a considerable effect in growth. Signifying that mullet juveniles are tolerant to heavy metals when compared to *P.monodon* and *P.viridis*. In environmental studies, biomarkers have the advantage of detecting early adverse effects resulting from stress exposure, well before they become visible at the population level. In addition, a suite of biomarkers, as measurable endpoints at molecular, cellular and physiological levels, may be necessary for a successful perception on the health status of populations, and once selected for a particular case, they can be adopted to different ecological scenarios.

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