INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES Volume 3, No 1, 2012

Research article **ISSN 0976 – 4402**

Acute toxicity of cadmium, copper, lead and zinc to tiger shrimp *Penaeus monodon* postlarvae

Raj Kumar J.S.I
School of Biodiversity and Environmental Monitoring Lab, Department of Advanced Zoology and Biotechnology, Loyola College, Chennai-600034, Tamilnadu, India
drjsirajkumar@gmail.com
doi:10.6088/ijes.2012030131029

ABSTRACT

The acute toxicity of cadmium, copper, lead and zinc to *Penaeus monodon (*Tiger prawn) was evaluated in static renewal tests. Each test lasted up to 4 days and 96 hours LC_{50} values were calculated. The toxicity of each metal increased with exposure time for cadmium, copper, lead and zinc respectively. The 96 hours LC_{50} values based on the measured concentration for cadmium, copper, lead, and zinc were 1.72, 0.66, 0.41, 2.36 mg/l. Lead was toxic to *P.monodon* than zinc followed by cadmium and copper tested. The order of toxicity was Pb>Cu>Cd>Zn. Our results found that postlarvae are well protected by both the current CCME Canadian water quality guidelines for the protection of aquatic life, as LC_{50} values were at least 25 times higher than the guideline concentrations.

Keywords: Copper, cadmium, lead, zinc, tiger shrimp, *Penaeus monodon*, Postlarvae.

1. Introduction

In industrialized countries, environmental problems are less related to acute toxicity of environmental pollutants than to sublethal, synergistic and long-term effects which are difficult to detect and whose consequences for ecosystems are far from being understood (Watts and Pascoe, 2000). Urbanization poses a significant risk to estuarine fauna, particularly crustaceans. Prawns are amongst the most abundant and ecologically important species (Leight *et al.,* 2005). These species play a major role in energy transfer in salt marsh ecosystems (Kneib, 1997). Historically, it is also an economically important species supporting major fisheries and has been used extensively in toxicity testing because they are widely distributed, abundant, sensitive to environmental contaminants and relatively easy to hold and culture in the laboratory (Akpu *et al.,* 2010).

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 (Duquesne *et al*., 2004). The overall toxicity of heavy metals is commonly assessed using laboratory bioassays where organisms are exposed to contaminants (Chapman and Wang, 2001). Invertebrates are routinely used as candidate organisms in such bioassays, and early life stages of invertebrates are often the most sensitive to contaminants (Rand *et al.,* 1995). Environmental stress from pollutants seems to be an important determining factor signaled by the occurrence of increase in diseases (Lacoste *et al.,* 2001). Determination of Lethal Concentration (LC) for toxicants is an essential prerequisite in all toxicological investigations and several investigations have been carried out on heavy metal toxicity in prawns (Bat *et al.,* 2001). Toxicity of heavy metal to aquatic organisms has been intensely studied (Khallaf *et al.*, 2003; Filho *et al.*, 2004; Kalpaxis *et al*., 2004; Shaw and Handy, 2006). The presence of heavy metals in the environment has increased in some areas to levels, which threaten the health of aquatic and terrestrial organisms including man (Honda *et al*., 2008). A major challenge, therefore, is to predict the effects of contaminants on aquatic organisms and to establish toxicity criteria for acceptable levels of chemical contamination (Bat *et al.,* 2001). A reason for interest in heavy metals and behavior in aquatic communities is that heavy metals may have different behavioral effects at concentrations much less, 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 sublethal behavioural effects (Klaschka, 2008). Hence, in the present study the acute toxicity was conducted to study the impact of cadmium, copper, lead and zinc on the postlarval stages of tiger prawn.

2. Materials and methods

Postlarval stages of *Penaeus monodon* (PL-12) was collected from the local hatcheries in and around Marakanam (Tamil nadu, 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. Postlarvae 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).

Stock solutions of cadmium, copper, lead and zinc were freshly prepared by dissolving the proper metal salts ($CdCl_2 .2.5H_2O$ 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), $Pb (NO_3)_2$ for Pb (Lead (II) nitrate, CAS-10099-74-8, molecular weight-331.23) and $ZnSO_4.7H_2O$ for Zn (Zinc sulfate, CAS-7446-20-0, molecular weight-287.54) 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 for range finding test (preliminary tests) (USEPA, 2002a). Range finding tests were conducted to establish suitable concentration ranges for conducting definitive test for acute test. 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 20 animals in a 5 L glass trough. Test chambers were loosely covered to reduce evaporation, to minimize the entry of dust into solutions, and to prevent loss of test animals. 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). Water quality parameters were analyzed following (Grasshoff *et al.,* 1999). 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 and (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 LC_{50} values at the termination of each test and upper and lower 95 per cent confidence levels were also calculated.

3. Results and discussion

During the toxicity test, temperature were 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 postlarvae exposed to cadmium, copper, lead and zinc to acute toxicity test showed that *P.monodon* were sensitive to lead and tolerant to zinc. The order of toxicity was Pb>Cu>Cd>Zn (Table 1). The postlarvae exposed to higher heavy metal concentrations showed piercing character with rostrum to the glass trough, rapid moulting, and the moulted larvae were sensitive to metal concentration and cannibalistic behaviour was observed. The mortality of the *P.monodon* was observed by the red colouration of the whole body. The 96-hour LC_{50} values were compared to CCME (2006) in Table 2. Note that, although the CCME values in Table 2 are calculated for a hardness of 20 mg/L CaCO3 equivalents (the lowest recommended extrapolation), this hardness level (20mg/L) is still lower than the laboratory water used in this experiment (hardness, 1550 to 1786 ±11.3 mg/l).

Table 1: Results of 96 hour LC_{50} (mg/l) in range finding test (preliminary test) for conducting definitive acute toxicity test (static renewal)

Metal	Concentration used (mg/l)	96 hour LC_{50} (mg/l)	95% LCL-UCL
Cd	0.01, 0.1, 1, 10, 100	1.32	0.73 – 2.40
Cu	0.01, 0.1, 1, 10, 100	0.95	0.53 – 1.71
Pb	0.01, 0.1, 1, 10, 100	0.39	0.22 – 0.72
Zn	0.01, 0.1, 1, 10, 100	2.74	1.69 – 4.63

*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%)

Copper appears to be toxic to juvenile tiger prawn. Elfing and Tedegren (2002) reported high toxicity of copper at low salinity. This difference may be because of the ability of the low saline water to maintain the metals in solution or suspension (Cheng, 1988). The 96 hour LC_{50} for juvenile *P.monodon* obtained in the present study was 0.66 mg/l. Eisler (1971) reported 96 hour LC_{50} for juvenile grass shrimp of 0.42 mg/l. Nimmo *et al.* (1977) reported similar 96 hour LC_{50} of 0.76 mg/l for the same species. Eisler (1985) concluded in his synoptic review of cadmium hazards to fish and invertebrates that crustaceans were sensitive marine group in short-term tests. In the toxicity review of the effects of temperature and salinity on heavy metals to marine and estuarine invertebrates, McLusky *et al.* (1986) reported that the order of toxicity of metals was generally: Cd > Cu > Zn > Pb . In the present study *P.monodon* showed toxicity in the order of Pb>Cu>Cd>Zn. 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, which is true with the present study.

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.

Table 2: Lethal Concentration (LC_{50} (mg/l)) of *M. cephalus, P. viridis* and *P. monodon* exposed to cadmium, copper, lead and zinc in acute toxicity test

Metal	96 hour LC_{50} (95% LCL-UCL mg/l)	96 hour LC_{50} (µg/l)	CCME (µg/l)
Cd	1.72 (0.83 – 1.59)	1720	0.017
Cu	0.66 (0.49 – 0.91)	660	2
Pb	0.41 (0.29 – 0.53)	410	1
Zn	2.36 (1.85 – 2.96)	2360	30

*LCL-UCL indicates the lower confidence level and upper confidence level (95%); Values are mean and 95% lower and upper confidence levels in parenthesis each n=2

White shrimp juveniles were sensitive to cadmium than zinc, cadmium toxicity was 44 times greater than zinc. Pink shrimp, *P. duorarum* exposed to cadmium showed an LC_{50} of 0.31 mg/l, which is lower than the present study of 1.72 mg/l for *P.monodon* in 96 hour. Two species of grass shrimp *Palaemonetes pugio* and *P. vulgaris* showed an LC_{50} of 1.30 and 0.76 mg/l (USEPA, 2001). *P.monodon* exposed to cadmium exhibited 1.05 mg/l 96 hour LC_{50} (Chongprasith *et al.,* 1999a). Tiger prawn, *P.monodon* exposed to copper had 0.58 mg/l as the 96 hour LC_{50}. Tiger prawn, *P.monodon* exhibited 96 hour LC_{50} for lead as 0.29 mg/l (Wong and Tan, 1999). Chongprasith *et al.* (1999b) reported the following LC_{50} values of 96 hour for Tiger prawn *P.monodon* as 2.5mg/l for zinc 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. In this context this toxicity values derived is of great practical utility to provide biological criteria to establish quality standards that protect resources of the coastal environment, especially in the shrimp farms of the Tamilnadu coast of India.

4. Conclusions

Of the metals, tested postlarvae were the most sensitive to lead, followed by copper, cadmium, and then zinc. The relatively high tolerance to zinc is not surprising given that zinc is essential elements, which likely can be regulated. A surprising finding is that copper, which is essential, was more toxic than cadmium, which is not essential. Generally, cadmium is more toxic to organisms than copper. *P.monodon* is at least in part due to their ability to maintain internal calcium balance even with continued cadmium exposure. *P.monodon* seem to be very interesting object in toxicological studies because it has a reputation of being a resistant that can survive in the environment. Based on the CCME, we would expect cadmium, followed by copper, then lead, to be the most toxic; however, in this study, lead was found to be the most toxic, followed by copper, then cadmium. The LC_{50} values for each metal are all orders of magnitude above the CCME guidelines. Thus, *P.monodon* are well protected by the current CCME guidelines. 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.

5. References

1. Akpu. I.A.F., Allison. M.E., Sikoki. F.D., and Maria C.R.S., (2010), Survival and gill morphology of different life stages of Tilapia guineensis exposed to the drilling fluid, Ciencia Rural, 40(3), pp 611-616.

2. Bat. L., Bilgin. S., Gundogdu. A., Akbulut. M., and Culha M., (2001), Individual and combined effects of copper and lead on the marine shrimp Palaemon adspersus Rathke, 1837 (Decapoda: Palaemonidae), Turkish journal of marine sciences, 7, pp 103-117.

3. CCME. (2006), Canadian Council of Ministers of the Environment (CCME) (updated in 2006), Canadian water quality guidelines for the protection of aquatic life. Excerpt from publication No. 1299; ISBN 1- 896997-34-1

4. Chapman A., (1978), Toxicities of cadmium, copper, and zinc to four juvenile stages of Chinook salmon and steelhead, Transactions of the American fisheries society, 107, pp 841-847.

5. Chapman. P.M., and Wang F., (2001), Assessing sediment contamination in estuaries, Environmental Toxicology and Chemistry, 20, pp 3-22.

6. Cheng T.C., (1988), In vivo effects of heavy metals on cellular defense mechanisms of Crassostrea virginica, Total and differential cell counts, Journal of invertebrate pathology, 51, pp 207-214.

7. Chongprasith. P., Utoomprurkporn. W., and Rattikhansukha C., (1999a), Asean marine water quality criteria for cadmium. Marine environment division, water quality management bureau, pollution control department, pp 1-20. Retrieved from, http://www.marinepcd.org/document/marine/cadmium.pdf

8. Chongprasith. P., Utoomprurkporn. W., and Rattikhansukha C., (1999b), Asean marine water quality criteria for zinc. Marine environment division, water quality management bureau, pollution control department, pp 1-14. Retrieved from, http://www.marinepcd.org/document/marine/zinc.pdf.

9. Duquesne. S., Liess. M., and Bird D.J., (2004), Sub-lethal effects of metal exposure: Physiological and behavioural responses of the estuarine bivalve Macoma balthia, Marine environmental research, 58, pp 245-250.

10. Eisler R., (1971), Cadmium poisoning in Fundulus heteroclitus (Pisces: Cyprinodontidae) and other marine organisms, Journal of the fisheries board of Canada, 28, pp 1225-1234.

11. Eisler R., (1985), Cadmium hazards to fish, wildlife, and invertebrates: A synoptic review, U.S fish and wildlife service biological report, 85(1.2), pp 46.

12. Eisler. R., and Raymond J.H., 1977. Acute Toxicities of Cd $^{2+}$, Cr $^{+6}$, Hg $^{2+}$, Ni $^{2+}$ and Zn $^{2+}$ to Estuarine Macrofauna, Achieves of environmental contamination and toxicology, 6, pp 315-323.

13. Elfing. T. and Tedengren M., (2002), Effects of copper on the metabolism of three species of tropical oysters, Saccostrea cucculata, Crassostrea lugubris and C. belcheri, Aquaculture, 204, pp 157-166.

14. Filho. O.E.C., Lopes. R.M., and Paumgartten F.J.R., (2004), Comparative study on the susceptibility of freshwater species to copper-based pesticides, Chemosphere, 56, pp 369-374.

15. Grasshoff. K., Ehrhardt. M., and Kremling K., (1999), Methods of sea water analysis, 3rd edition, Verlag Chemie, Weinheim, Germany, pp 419-600.

16. Honda. R.T., De-Castilho. F.M., and Val A.L., (2008), Cadmium-induced disruption of environmental exploration and chemical communication in matrinxa, Brycon amazonicus. Aquatic Toxicolology, 89, pp 204-206.

17. Kalpaxis. D.L., Theos, C., Zaplanteri, M.A., Dinos, G.P., Catsiki, A.V., and Leotsinidis M., (2004), Biomonitoring of Gulf of Patras, N. Peloponnesus, Greece. Application of a biomarker suite including evaluation of translation efficiency in Mytilus galloprovincialis cells, Environmental research, 94, pp 211-220.

18. Khallaf. E.A., Galal. M., and Authman M., (2003), The biology of Oreochromis niloticus in a polluted canal, Ecotoxicology, 12, pp 405-416.

19. Klaschka U., (2008), The info-chemical effect-a new chapter in ecotoxicology, Environmental science and pollution research, 15, pp 452-462.

20. Kneib R.T., (1997), Early life stages of resident nekton in intertidal marshes, Estuaries, 20, pp 214-230.

21. Lacoste. A., Jalabert. F., Malham. S., Cueff, A., and Poulet S., (2001), Stress and stress-induced neuroendocrine changes increase the susceptibility of juvenile oysters (Crassostrea gigas) to Vibrio splendidus. Applied and environmental microbiology, 67, pp 2304-2309.

22. Leight. A.K., Scott. G.I., Fulton. M.H., and Daugomah J.W., (2005), Long term monitoring of grass shrimp Palaemontes sp. population metrics at sites with agricultrual runoff influences, Integrative and comparative Biology, 45, pp 143-150.

23. McLusky. D.S., Bryant. V., and Campbell R., (1986), The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates, Oceanography and marine biology: An annual review, 24, pp 481-520.

24. Nimmo. D.W.R., Lightner. D.V., and Bahner R.C., (1977), Effects of cadmium on the shrimps, Penaeus duorarum, Palaemonetes puqio and Palaemonetes vulqaris. In: Vernberg, F.J., A. Calabrese, F.P. Thurberg and W.B. Vernberg. (Eds.). Physiological responses of marine biota to pollutants, Academic Press Inc, New York, 131.

25. Rand. G.M., Wells. P.G., and McCarty L.S., (1995), Introduction to aquatic toxicology. In fundamentals of aquatic toxicology, Rand, G.M. (Eds.). Taylor and Francis, Washington, DC, 3-70. Retrieved from: http://www.marinepcd.org/document/marine/lead.pdf

26. Shaw. B.J., and Handy R.D., (2006), Dietary copper exposure and recovery in Nile tilapia, Oreochromis niloticus, Aquatic Toxicology, 76, pp 111-121.

27. USEPA. (1996), United States environmental protection agency. Ecological effects test guidelines OPPTS 850.1075 fish acute toxicity test, Freshwater and marine, prevention, Pesticides and toxic substances (7101), pp 96-118.

28. USEPA. (2000a), Revised baseline ecological risk assessment Hudson river PCBs reassessment. Phase 2 reports. Prepared by TAMS consultants, Menzie-Cura and associates for USEPA region 2. Washington, DC, USA.

29. USEPA. (2000b), United States environmental protection agency. Understanding and accounting for method variability in whole effluent toxicity applications under the national pollutant discharge elimination system. EPA 833- R-00-003.

30. USEPA. (2001), Update for ambient water quality criteria for cadmium, retrieved from: http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/pollutants/cadmium/index.cfm

31. Vanegas. C., Espina. S., Botello. A.V., and Villanueva S., (1997), Acute toxicity and synergism of white shrimp, Penaeus setiferus, Cadmium and Zinc in Juveniles, Bulletin of environmental contamination and toxicology, 58, pp 87-92.

32. Watts. M.M., and Pascoe D., (2000), A comparative study of Chironomus riparius Meigen and Chironomus tentans Fabricius (Diptera: Chironomidae) in aquatic toxicity tests, Achieves of environmental contamination and toxicology, 39, pp 299-306.

33. Wong. M.K., and Tan W.L., (1999), Asean marine water quality criteria for lead. Marine environment division, water quality management bureau, pollution control department, pp 1-13.