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TOXIC EFFECTS AND BIOACCUMULATION OF CADMIUM, COPPER, LEAD AND ZINC IN POST LARVAL STAGES OF PENAEUS MONODON

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ABSTRACT

The acute toxicity and bioaccumulation of cadmium, copper, lead and zinc to *Penaeus monodon (*Tiger prawn) was evaluated in static tests. Each test lasted up to 4 d and LC_{50} values were also calculated. The toxicity of each metal increased with exposure time for cadmium, copper, lead and zinc. The 96 hours LC_{50} values for cadmium, copper, lead and zinc were 1.72, 0.66, 0.41, 2.36 mg/l. Lead was toxic metal to *P.monodon* than zinc followed by cadmium and copper tested. The order of toxicity was Pb>Cu>Cd>Zn. The bioaccumulation study showed that the zinc was accumulated in a higher concentration followed by copper, lead and cadmium.

INTRODUCTION

Estuaries are highly sensitive zones subjected to the heavy industrialization and overpopulation (Venkatachalapathy *et al.*, 2010). 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 because of their pervasiveness and persistence nature (Singh and Chandel, 2006). 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). A number of early life stage toxicity test protocols have been developed and effectively applied to characterize contaminants using Sea Urchin and Bay Scallop (Krassoi *et al.*, 1996). The role of developmental stage, size and salinity are very crucial in heavy metal toxicity to estuarine and marine organisms (Grosell *et al.*, 2007). Heavy metals may affect organisms by accumulating in their bodies or by transferring to the next trophic level of the food chain (Shah and Altindag, 2005). Accumulated heavy metals in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level (Yildirim *et al.*, 2009). Aquatic organisms exposed to a higher concentration of trace metals in water may take up substantial quantities of these metals. However, when metal contaminated aquatic organisms are transferred to clean water, metal depuration occurs (Kord *et al*., 2010). Species of prawn can be used to monitor the trace element pollution in the aquatic environment because they are omnivorous benthic animals that maintain their body in direct contact with the water and substrate of their environment and they tend to accumulate metals in their tissues (Barrento *et al.*, 2009). Studies have examined the accumulation of contaminants in tissues of a number of prawn species, and under different concentrations and times of exposure in the field and the laboratory (Yilmaz and Yilmaz, 2007). Elements that are passively adsorbed into the exoskeleton of a crustacean will contribute to the total body concentration in the crustacean which can accumulate trace elements in hepatopancreatic and edible tissues (Uluturhan and Kucuksezgin, 2007). Pollutants are inevitably permanently immobilized in sediments due to bioturbation and re-suspension constituting a potential danger and significant risk to estuarine fauna, particularly crustaceans. Prawns are amongst the most abundant and ecologically important species. These species play a major role in energy transfer in salt marsh ecosystems (Leight *et al.*, 2005). 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). Hence in the

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present investigation the post larval stages of *Penaeus monodon* were exposed to cadmium, copper, lead and zinc in the acute toxicity test to study the bioaccumulation and toxic effects of each metal.

MATERIALS AND METHODS

Toxicity test

Collected post larval stages of *Penaeus monodon* 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. Post larvae of *Penaeus monodon* were fed with mixed feed for *P.monodon* (Japan) throughout acclimatization period. (USEPA, 1996). Prior to toxicity tests and stock solution preparations, all the glassware's 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 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 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). These solutions were serially diluted to get the experimental concentration for the toxicity test.

Table 1. Lethal Concentration (LC_1, LC_{50} and LC_{99} (mg/l)) of *P. monodon* exposed to cadmium, copper, lead and zinc in acute toxicity test

Test species *P. monodon*	96 hour LC50 mg/l (95%LCL-UCL)			
	Cd	Cu	Pb	Zn
	1.72	0.66	0.41	2.36
	(0.83 – 1.59)	(0.49 – 0.91)	(0.29 – 0.53)	(1.85 – 2.96)

*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

Table 2. Recovery of trace elements in certified reference material (DOLT-3) Dogfish liver certified reference material for trace metals

Element	Certified values (mg/kg)	Measured concentration (mg/kg)	Recovery (%)
Cd	19.4 ±0.6	20.03 ±0.11	103.26
Cu	31.2 ±1.0	31.7 ±0.63	101.45
Pb	0.319 ±0.045	0.311 ±0.05	97.19
Zn	86.6 ±2.4	85.62 ±3.22	98.87

*Measure concentration (mg/kg) is the mean and standard deviation of n=12

Table 3. Concentrations of cadmium, copper, lead and zinc in tissues of *P.monodon* exposed to acute toxicity

Metal	Units	Concentrations (mg/l)					
Cd	a	0	0.3	0.5	1	2	4
	b	0.60 ±0.07	6.38 ±0.32	10.50 ±0.14	20.83 ±0.32	33.08 ±1.17	56.15 ±0.64
Cu	a	0	0.1	0.3	0.5	1	2
	b	2.68 ±0.11	9.45 ±0.14	18.30 ±0.71	38.48 ±0.32	75.88 ±0.39	166.60 ±0.78
Pb	a	0	0.3	0.5	1	2	4
	b	1.18 ±0.35	5.83 ±0.32	12.43 ±0.35	27.10 ±0.71	50.60 ±0.64	99.63 ±0.25
Zn	a	0	1	2	4	8	16
	b	13.08 ±1.17	25.53 ±2.29	44.40 ±0.71	73.50 ±1.20	85.30 ±1.98	175.35 ±3.82

Values were significant at $P<0.05$, One way ANOVA: Dunnetts multiple comparison ($\alpha=0.05$), $P<0.05$ values compared with control were highly significant (***, <0.001). a, exposed concentration (mg/l), b, concentration in tissues (µg/g dry weight); Values are mean and standard deviation each n=2; The concentration column (mg/l) contains '0' indicating control in the test conducted in triplicate

The experimental method includes static renewal (24 hour renewal) test wherein the toxicant and seawater were replaced on daily basis (USEPA, 2002a & b). 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). Each toxicity test lasted 4 days. Daily observations were recorded for survival and mortality. 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). Water samples for the determination of metal concentrations from different experiment were collected and extracted according to the method described by Koirtyohann and Wen (1973) and updated by El-Moselhy and Gabal (2004). 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, upper and lower 95 per cent confidence levels were also calculated.

Bioaccumulation

The tissue samples of the survived test organisms were removed using a Teflon scalpel, the tissue was washed with distilled water and dried at 95 °C in hot air oven and grinded to a fine powder with pestle and mortar and the metal analysis was carried out by UNEP (1984). To ensure the accuracy and precision in the sample analysis, it was cross-examined with respect to certified reference material (DOLT-3, Dogfish liver certified reference material for trace metal, from national research council Canada) (DOLT-3, 1999). Metal analysis was carried out using Varian SpectraAA 220FS Atomic absorption spectrophotometer 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. The corresponding standards were prepared by adding increasing amounts of Cd, Pb, Cu and Zn to the matrix.

RESULTS

Metal toxicity

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 post larvae of *Penaeus 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, cannibalistic behaviour was also observed. The results of the toxicity test are given in Table 1. The mortality of the *P.monodon* was observed by the red colouration of the whole body. Post larval stages of *P.monodon* exposed to cadmium, copper, lead and zinc in acute toxicity test revealed vulnerability towards lead and tolerant to zinc. The order of sensitivity was Pb>Cu>Cd>Zn.

Bioaccumulation

The recovery of trace elements for the bioaccumulation study (CRM) (DOLT-3) Dogfish liver certified reference material for trace metals, cadmium recovery was found at 103.26 percent, copper, lead and zinc were recovered with 101.45, 97.19 and 98.87 percent respectively (Table 2) *P.monodon* exposed to heavy metals depicted high significance ($P<0.001$) with the concentration of heavy metals in tissues when compared with control (One way ANOVA: Dunnetts multiple comparison test ($\alpha=0.05$)) (Table 3). The concentration of heavy metals in the tissues of *P.monodon* follows in the order of Zn>Cu>Pb>Cd.

DISCUSSION

Elfing and Tedegren (2002) also 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). Copper appears to be toxic to juvenile tiger prawn. The 96 h 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. Cadmium, copper, zinc and lead is toxic to *P.monodon* than *M.cephalus*. The 96 hour LC_{50} for *M.cephalus* exposed to cadmium in this study was 4.29 mg/l. 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. 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. In the present study the same order of toxicity to metals was observed when compared with literature observations. Vanegas *et al.* (1997) reported 96 hour LC_{50} for White Shrimp, *Penaeus 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. 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. *P.monodon* seemed to be very interesting object of toxicological studies because it has a reputation of being a resistant that can survive in the environment. However, the knowledge of the effects of heavy metals on the *P.monodon* including lower stages of this test organisms are very limited, though there are papers describing their behaviour with heavy metals in adults. The impact of toxicants in nature may depend on other environmental and biological variables that act as stressors, 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).

Nugegoda and Rainbow (1987) reported that the decapod *Palaemon elegans* regulates the body concentration of zinc when exposed to a wide range of dissolved zinc concentrations, in good agreement with the present study. There have been several studies on concentrations of heavy metals in shrimp species, copper and zinc concentrations of essential elements in the muscles were notably higher than those of non-essential metals (Pourang *et al.,* 2004). Osuna and Mayen (1996) also reported the maximum concentration of zinc in *P.vannamei*. The high concentration of zinc appears to be the case common among crustaceans (Paul and Gupta, 1995). Zinc has a much lower toxicity to crustaceans than copper (Canli and Furness, 1993). In this study highest concentrations of zinc were found in *P.monodon.* Aquatic invertebrates show a large variability in heavy metal concentrations in the tissues among both metals and taxa (Rainbow, 2007). Metal bioavailability to marine invertebrates is influenced by a number of both physicochemical and extrinsic factors and biological or intrinsic factors (Barrento *et al.,* 2009). Cadmium has been shown to inhibit molting of the crab *N. granulate* (Moreno *et al.,* 2003) and to produce histopathological injury in the white shrimp *Litopenaeus vannamei* (Wu *et al.,* 2008).

Decapod crustaceans have been reported to have relatively higher concentrations of copper in their bodies because this metal is a component of the respiratory pigment haemocyanin (Uluturhan and Kucuksezgin, 2007). However, above a certain concentration of copper in the external medium, the regulation breaks down, and decapod crustaceans concentrate copper (Fordsmand and Depledge, 1997). Essential metals, such as copper and zinc, can be regulated and do not accumulate in decapods crustaceans until certain environmental threshold levels are reached (Rainbow, 2002). Crustacean cannot regulate the body concentration of non-essential metals, such as cadmium and lead (Rainbow and White, 1989). Hence in the present study the *P.monodon* have accumulated zinc and copper beyond the threshold limit in the ambient water, hence no regulation of cadmium and lead is mechanized in the marine organisms they do depurate it, unless and otherwise the concentration in the ambient water is not in steady state, when the concentrations are kept in a steady state, definitely the organism may concentrate the heavy metals in higher proportion (Yilmaz and Yilmaz, 2007).

Conclusions

Many experimental studies on the effects of pollutants, especially heavy metals on aquatic invertebrates have mainly been bioassays to determine acute toxic concentrations of pollutants. This type of bioassay is usually carried out in laboratory conditions, and the validity of defining safe levels. The results of bioassays are much more meaningful. Lead was toxic to the post larval stages of the *P.monodon* followed by copper, cadmium and zinc. Surprisingly copper an essential metal was toxic than the non-essential metal cadmium to the post larval stages of *P.monodon*. Zinc was primarily accumulated followed by copper, lead and zinc in the acute toxicity test. The assessment of acute lethal toxicity is the first step to determine the chronic effects on estuarine organisms, which will be evaluated in a future time.

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