RESEARCH ARTICLE

ISSN: **0976-3031**

Available at http://www.recentscientific.com

International Journal of Recent Scientific Research

Vol. 2, Issue, 5, pp. 132 -135, May, 2011

LONG TERM EFFECTS OF CADMIUM, COPPER, LEAD AND ZINC EXPOSURE ON THE GROWTH OF JUVENILE GREEN MUSSEL (PERNA VIRIDIS)

Rajkumar, J. S. I[1*], John Milton, M. C[1] and Arockia Rita, J.J[2]

[1]Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034, Tamil Nadu, India.

[2]Department of Zoology, Quaid E Millath College (Govt), Chennai – 600 002, Tamil Nadu, India.

ABSTRACT

In this study *Perna viridis* was exposed to cadmium, copper, lead and zinc under long term toxicity test to investigate the changes in growth in terms of gain in length, weight and condition factor of the test organism. From the data obtained it is clear that the lower concentrations of cadmium, copper, lead and zinc under long term exposure significantly reduced the growth of Juvenile *P.viridis*. *P.viridis* is more sensitive to lead and cadmium compared to copper and zinc, making them suitable for environmental monitoring.

Key words: Growth, condition factor, cadmium, copper, lead, zinc and *Perna viridis*

INTRODUCTION

Aquatic organisms when exposed to heavy metals tend to accumulate in their body, essential and non-essential metals can produce toxic effects in fish by disturbing their growth, physiology, biochemistry, reproduction and mortality (Yilmaz, 2005). However, as sub-individual responses, biomarkers may, in some cases, lead to inconclusive predictions of ecological effects (Dang and Wang, 2009). Mussels 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. A variety of biomarkers has been used in this mussel to monitor the level of environment pollution (Nicholson and Lam, 2005).

Most of the toxicity studies on *P. viridis* have focused on the process of bioaccumulation in this animal and the organs that helps in bio-accumulation of metals. Environmental conditions can significantly influence the physiology of bivalves, and therefore, modify the growth potential (Guan and Wang, 2006). Physiological studies are useful for predicting 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 physiological changes in growth caused by exposure to pollutants may hence cause serious risks to the success of animal populations and disrupt aquatic communities (Gravato and Guilhermino, 2009). Hence in the present investigation the important biomonitoring agent, *Perna viridis* will be exposed to cadmium, copper, lead and zinc under long term toxicity test to study the changes in growth in terms of gain in length, weight and condition factor of the test organism under static renewal of five different concentrations with control.

***Corresponding author:** rajloyola83@gmail.com

MATERIALS AND METHODS

Juvenile specimens of *Perna viridis* (1.5 ±0.3cm in length and 0.22 ±0.02g) were collected from Puducherry old harbour (11°54'25.26" N, 79°47' 49.26" E) and 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 29 PSU salinity, temperature of 29 ±2 °C, dissolved oxygen of 5.9 mg/l and pH of 8. Captured wild organisms were quarantined immediately (Oxytetracycline). After a day of acclimatization, *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. 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 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. Commencement of the introduction of test organisms to the chronic toxicity test all the test organisms were subjected to physical measurements in terms of length and weight. Correspondingly at the end of the test the survived organisms underwent physical measurements. The calculated values were compared with control values. Condition factor (K) of the experimental animal was calculated by Williams (2000),

$$K = \frac{100\ W}{L^3}$$

The Total Length (TL) of the test organism was measured from the tip of the anterior or part of the mouth to the caudal fin (fish) using ruler calibrated in centimeters. Test organisms were measured to the nearest centimeter. Weight was measured after blot drying with a piece of clean hand towel. Weighing was done with a tabletop digital weighing balance (Metller), to the nearest gram.

RESULTS

The well being of the *P.viridis* was judged by the condition factor represented by per cent. In all the cadmium concentrations the gain in weight was significantly (P<0.001) reduced. Gain in length was affected significantly (P<0.001) exposed in 41, 66 and 105 µg/l. Significant change in length was also found significant in 16 (P<0.05) and 26 µg/l (P<0.01). The condition of the *P.viridis* exposed to cadmium significantly (P<0.01) affected in 105 µg/l (Table 1). High exceptional variation in the reduction of growth was observed in 23 and 34 µg/l. No significant (P<0.05) difference in the gain of length in 23, 34 and 51 µg/l was observed. Healthiness of the *P.viridis* decreased

Table 1. Gain in weight, length and condition factor of *P. viridis* exposed to cadmium in short-term chronic toxicity test

Concentration (µg/l)	Gain in Mean weight (%)	Gain in Mean length (%)	Condition Factor (%)
0	2.92 ±0.19	3.85 ±0.01	0.60 ±0.02
16	0.14 ±0.91***	0.97 ±0.03*	0.63 ±0.04
26	-3.26 ±0.01***	0.50 ±0.70**	0.52 ±0.02
41	-3.46 ±0.28***	2.44 ±0.67***	0.57 ±0.06
66	-2.88 ±0.41***	0.45 ±0.63***	0.49 ±0.01
105	-1.71 ±0.05***	0.00 ±0.01***	0.42 ±0.01**

***values are significant at P<0.001, ** values are significant at P<0.01, * values are significant at P<0.05. One way ANOVA Dunnetts multiple comparison test (α<0.05)); Values are the mean and standard deviation; the concentration used column (mg/l) contains '0' indicating control in the test conducted in triplicate.

Table 2. Gain in weight, length and condition factor of *P.viridis* exposed to copper in short-term chronic toxicity test

Concentration (µg/l)	Gain in Mean weight (%)	Gain in Mean length (%)	Condition Factor (%)
0	3.08 ±0.67	3.85 ±0.01	0.58 ±0.01
10	1.87 ±0.97	1.00 ±0.02	0.56 ±0.01
15	-1.04 ±1.37	0.50 ±0.70	0.50 ±0.02**
23	-1.15 ±0.92*	0.59 ±0.69*	0.48 ±0.01***
34	1.29 ±0.14*	0.08 ±0.11*	0.43 ±0.02***
51	-2.91 ±0.44	0.00 ±0.01*	0.38 ±0.02***

***values are significant at P<0.001, ** values are significant at P<0.01, * values are significant at P<0.05. One way ANOVA Dunnetts multiple comparison test (α<0.05)); Values are the mean and standard deviation; the concentration used column (mg/l) contains '0' indicating control in the test conducted in triplicate.

Table 3. Gain in weight, length and condition factor of *P.viridis* exposed to lead in short-term chronic toxicity test

Concentration (µg/l)	Gain in Mean weight (%)	Gain in Mean length (%)	Condition Factor (%)
0	2.19 ±0.19	3.51 ±0.48	0.53 ±0.01
10	0.98 ±0.89	3.67 ±0.22	0.51 ±0.02
15	-1.98 ±0.76	0.81 ±0.25	0.46 ±0.01**
23	-3.01 ±0.32*	0.59 ±0.69	0.42 ±0.02***
34	-3.27 ±0.81*	0.08 ±0.11*	0.40 ±0.01***
51	-6.81 ±0.51*	-0.32 ±0.46*	0.38 ±0.01***

***values are significant at P<0.001, ** values are significant at P<0.01, * values are significant at P<0.05. One way ANOVA Dunnetts multiple comparison test (α<0.05)); Values are the mean and standard deviation; the concentration used column (mg/l) contains '0' indicating control in the test conducted in triplicate.

Table 4. Gain in weight, length and condition factor of *P.viridis* exposed to zinc in short-term chronic toxicity test

Concentration (µg/l)	Gain in Mean weight (%)	Gain in Mean length (%)	Condition Factor (%)
0	4.70 ±0.60	6.22 ±0.39	0.74 ±0.01
8	2.91 ±0.77	3.66 ±1.94	0.70 ±0.04
16	2.02 ±0.48*	1.75 ±1.07	0.73 ±0.06
32	-1.44 ±0.20**	0.59 ±0.69*	0.68 ±0.10
64	-1.52±1.04***	0.08 ±0.11**	0.60 ±0.07
128	-4.39 0.99***	-0.32 ±0.46**	0.55 0.01*

***values are significant at P<0.001, ** values are significant at P<0.01, * values are significant at P<0.05. One way ANOVA Dunnetts multiple comparison test (α<0.05)); Values are the mean and standard deviation; the concentration used column (mg/l) contains '0' indicating control in the test conducted in triplicate.

significantly (P<0.001) in 23, 34 and 51 µg/l copper concentrations (Table 2). Significant (P<0.01) difference was also observed in 15 µg/l. *P.viridis* exposed to lead in the short-term chronic toxicity test revealed significant (P<0.05) reduced in growth as 23, 34 and 51 µg/l. Gain in mean length was observed to have changes significantly (P<0.05) in 23 and 34 µg/l lead concentrations. The well being of the *P.viridis* in the lead exposed test showed extreme change in the condition factor highly significant (P<0.001) exposed in 23, 34 and 51 µg/l (Table 3). Significant (P<0.01) changes were also found in 15 µg/l. *P.viridis* exposed to 64 and 128 µg/l for 30 days showed significant (P<0.001) reduction in weight were observed in zinc concentrations. Significantly reduced weight was also observed in 16 (P<0.05) and 32 µg/l (P<0.01). Gain in mean length of the *P.viridis* exposed to 64 and 128 µg/l was significant at P<0.01. The condition factor of *P.viridis* had no significant changes except in 128 µg/l (P<0.05) (Table 4). Although many concentrations did not influence the weight and length of the *P.viridis* varied with the tolerance ability.

DISCUSSION

The reduction of feed conversion rate in marine organisms at sublethal levels of heavy metals might be due the tissue burden of more heavy metals, which in turn could cause reduction food intake, increase in metabolic cost and poor food conversion efficiency (James *et al.*, 1992). 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).

Harkantra (1975) showed that the young ones exhibit greater growth rate. General condition of the marine organisms were investigated through condition factor (K), which has often been used as an indication of general fitness of the organism (Bolger and Connolly, 1989) as well as to investigate the effects of contaminants (Pyle *et al.*, 2005). Lett *et al.* (1976) attributed the growth reduction in copper exposed *Salmo gairdneri* partly to increased metabolic cost and reduced food consumption. Zinc acts on the reproductive output of *Gammarus pulex* thought energy allocation rather than by killing broodlings or eggs (Maltby and Naylor, 1990). Moore and Farrar (1996) also showed that growth rates and reproduction of *Hyalella azteca* decrease significantly with reduced food ratios. Movement is a highly ecologically relevant behavioural marker as well, since locomotion is required to find food, escape predation and obtain mates. Heavy metal that interferes with this activity is likely to reduce the fitness of organisms and could involve ecological death (Scott and Sloman, 2004). The present study agrees that exposed cadmium concentrations inhibit growth with a with Berntseen and Lundebye (2001). Dang and Wang (2009) reported that the cadmium exposed and control fish, had no significant difference in wet weight, Standard Length (SD), condition factor (K). Present study reveals that the weight, length and the condition factor of test organisms varied significantly (P<0.001 and P<0.01) with exposed concentrations.

Studies on non-salmonid species showed a similar relationship where a reduction in growth was observed at concentrations that were lethal, but no effect on growth was observed at sublethal exposures (Eaton, 1974). Reduced growth was observed in Atlantic salmon alevins exposed to 0.47 µg/l Cd (Rombough and Garside, 1982). The sensitivity of a species to growth effects caused by cadmium, copper, lead and zinc exposure may be influenced by the relative growth rate of the species. Multigenerational effects of cadmium on survival, growth, and reproduction have been observed in *Daphnia magna* (Guan and Wang, 2006). Effects on growth may reflect bioenergetics effects of cadmium, since reductions in scope for growth have been reported in *Gammarus pulex* (Stuhlbacher and Maltby, 1992), *Callinectes sapidus* (Guerin and Stickle, 1995) and *D. magna* (Baillieul *et al.*, 2005) following chronic exposure to cadmium. From these results, it is important to highlight that the concentrations of cadmium, copper, lead and zinc under long term exposure affects the growth of Juvenile *P.viridis.*

CONCLUSION

The study shows that the *P.viridis* is more sensitive to lead and cadmium compared to copper and zinc. Under certain conditions these metals produces a toxic effect on the green mussels. Our experiment also highlighted that cadmium, copper, lead and zinc concentrations under long term exposure affect the growth of Juvenile *P.viridis.* This data suggests that *P.viridis* could be used in growth monitoring programs for metal pollution. *P.viridis* is more sensitive making suitable for environmental monitoring.

REFERENCES

Baillieul, M., Smolders, R and Blust, R. 2005. The effect of environmental stress on absolute and mass-specific scope for growth in *Daphnia magna* Strauss. *Comp. Biochem. Physiol. Part C*. 140: 364-373.

Baker, C.F and Montgomery, J.C. 2001. Sensory deficit induced by cadmium in banded kokopu, *Galaxias fasciatus* juveniles. *Environ. Biol. Fish*., 62: 455-464.

Berntseen, M.H.G and Lundebye, A.K. 2001. Energetics in Atlantic salmon (*Salmo salar* L.) fed elevated dietary cadmium. *Comp. Biochem. Physiol*., 128C : 311-323.

Bolger, T and Connolly, P.L. 1989. The selection of suitable indexes for the measurement and analysis of fish condition. *J. Fish Biol*., 34: 171-182.

Chapman, A. 1978. Toxicities of Cadmium, Copper, and Zinc to Four Juvenile Stages of Chinook salmon and Steelhead. *Trans. Am. Fish. Soc*., 107: 841-847.

Dang, F and Wang, W.X. 2009. Assessment of tissue-specific accumulation and effects of cadmium in a marine fish fed contaminated commercially produced diet. *Aquat. Toxicol*., 95: 248-255.

Eaton, J.G. 1974. Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus*, Rafinesque). *Transact. Am. Fishery Soc.*, 4: 729-735.

Figueroa, S., Alma, S. Martinez, C. Botello, C. Alfonso, V and Nogueira, G.N. 2007. Effect of cadmium, chromium, lead and metal mixtures on survival and growth of juveniles of the scallop *Argopecten ventricosus* (Sowerby II, 1842). *J. Environ. Sci. Heal. Part A*. 42(10):1443-1447.

Gagnaire, B., Thomas H. Guyon and T. Renault. 2004. In vitro effects of cadmium and mercury on Pacific oyster, *Crassostrea gigas* (Thunberg), haemocytes. *Fish Shellf. Immunol*., 16: 501-512.

Gravato, C and Guilhermino, L. 2009. Effects of benzo(a)pyrene on seabass (*Dicentrarchus labrax* L.): Biomarkers, growth and behaviour. *Hum. Ecol. Risk. Assess*., 15: 121-137.

Guan, R and Wang, W.X. 2006.Multigenerational cadmium acclimation and biokinetics in *Daphnia magna*. *Environ. Poll*., 141: 343-352.

Guerin, J.L and Stickle, W.B. 1995. Effects of cadmium on survival, osmoregulatory ability and bioenergetics of juvenile blue crabs *Callinectes sapidus* at different salinities. *Mar. Environ. Res*., 40: 227-246.

Harkantra, S.N. 1975. Some observations on the clam beds of Kali Estuary, Karwar. *Mahasagar. Bull. Nat. Inst. Oceanogr.*, 8(1 and 2): 101-108.

James, R., Sampath, K. Pattu, V.J and Devakiamma, G. 1992. Utilization of *Eichhorina crassipes* for the reduction mercury toxicity on food transformation in *Heteropneustes fossilis. J. Aqua. Trop*., 7: 189-196.

Lett, P.F., Farmer, G.J and Beamish, F.W.H. 1976. Effect of copper on some aspects of the bioenergetics of rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can*., 33: 1335-1342.

Maltby, C and Naylor, C. 1990. Preliminary observations on the ecological relevance of the *Gammarus* 'scope for growth' assay: effect of zinc on reproduction. *Funct. Ecol*., 4: 393-397.

Moore, D.W and Farrar, J.D. 1996. Effect of growth on reproduction in the freshwater amphipod, *Hyalella azteca* (Saussure). *Hydrobiol*., 328: 127-134.

Nicholson, S and Lam, P.K.S. 2005. Pollution monitoring in Southeast Asia using biomarkers in the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia). *Environ. Int*. 31: 121-132.

O'Connor, T.P. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. *Mar. Environ. Res*., 53: 117-143.

Pesch, G and Stewart, E. 1980. Cadmium toxicity of three species of estuarine invertebrates. *Mar. Environ. Res*., 2: 145-156.

Pyle, G.G., Rajotte, J.W and Couture, P. 2005. Effects of industrial metals on wild fish populations along a metal contamination gradient. *Ecotoxicol. Environ. Saf*., 61: 287-312.

Rippka, R. 1988. Isolation and purification of cyanobacteria. In: Methods in Enzymology. 167: 1-7. (Eds.). Glazer. A.N., and L. Packer. Academic press, San Diego, California.

Rombough, P.J and Garside, E.T. 1982. Cadmium toxicity and accumulation in eggs and alevins of Atlantic salmon *Salmo salar*. *Canad. J. Zool*., 60: 2006-2014.

Scott, G.R and Sloman, K.A. 2004. The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. A review. *Aquat. Toxicol*., 68: 369-392.

Stromgren, L.M. 1982. Effects of heavy metals (Zn, Hg, Cu, Cd, Pb, Ni) on the length, growth of *Mytilus edulis*. *Mar. Biol*., 72: 69-72.

Stuhlbacher, A and Maltby, L. 1992. Cadmium resistance in *Gammarus pulex* (L.). *Arch. Environ. Contam. Toxicol*., 22: 319-324.

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). 96-118.

USEPA. 2002a. Consolidated Assessment and Listing Methodology: Toward a Compendium of Best Practices. US Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds. <http://www.epa.gov/owow/monitoring/ calm.html>.

USEPA. 2002b. Methods for measuring acute toxicity of effluent and receiving waters for fresh water and marine organisms, 5th Oct 2002. United States environmental protection agency, office of water (4303T), Washington DC, EPA 821-R02-012. 275.

Williams. J.E. 2000. The Coefficient of Condition of Fish. In: Schneider, James C. (ed.). Manual of fisheries survey methods II: With periodic updates. Michigan Department of Natural Resources, Fisheries Special Report 25. Ann Arbor.

Wo, K.T., Lam, P.K. and Wee, R.S. 1999. A comparison of growth biomarkers for assessing sublethal effects of cadmium on a marine gastropod, *Nassarius festivus*. *Mar. Poll. Bull*., 39: 165-173.

Yilmaz, A.B. 2005. Comparison of Heavy metal levels of Grey Mullet (*Mugil cephalus* L.) and Sea Bream (*Sparus aurata* L.) caught in Iskenderun Bay (Turkey). *Turk. J. Veterin. Ani. Sci*., 29: 257-262.

****