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ACCUMULATION OF SOME HEAVY METALS Cd, Cu, Pb AND Zn IN MUGIL CEPHALUS, PERNA VIRIDIS AND PENAEUS MONODON

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ABSTRACT

In the present study *Mugil cephalus, Perna viridis* and *Penaeus monodon* was exposed to cadmium, copper, lead and zinc under long term chronic toxicity test to investigate the bioaccumulation pattern of cadmium, copper, lead and zinc to the three marine test organisms. Treated test organisms under long term chronic toxicity test showed that the concentration of heavy metals in the tissues were highly significant ($P<0.001$) when compared with control. The order of concentration in the tissues of *M. cephalus* was Zn>Cu>Pb>Cd, *P.viridis* accumulated in the order of Cd<Pb<Cu<Zn and Penaeus monodon accumulated heavy metals in the order of Cu>Zn>Pb>Cd. Knowledge on accumulation and distribution of metals in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals. More studies should be conducted in the future to determine the potentials of *Mugil cephalus, Perna viridis* and *Penaeus monodon* as biomonitoring agents in heavy metal pollution.

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INTRODUCTION

Aquatic pollution started long back but intensified during the last few decades, and now the situation has become alarming, especially in India (Girija *et al.,* 2007). Environmental pollution in urbanized countries have deteriorated the quality of the water (Zhao *et al.,* 2011). Contamination of aquatic ecosystems with heavy metals has been receiving increased worldwide concern (Tsangaris *et al.*, 2007). Copper enters the aquatic environment chiefly through leaching from paints on the hulls of boats and ships (Singh and Turner, 2009). Due to persistence in the environment and tendency to accumulate in the biota, copper pose a potential hazard to environmental and human health. It is the most poisonous heavy metal when present in excess (De Boeck *et al.,* 2006). Zinc is the fourth most widely used metal in the world. Its major uses include galvanized steel for alloy production, and as an ingredient in rubber and paints (USEPA, 1991). Cadmium is toxic to fish even at low concentrations (Jarup and Akesson, 2009). Due to its long biological half-life and strong ability to accumulate in animal tissues, residual cadmium forms a serious threat to the performance and survival of aquatic biota (Seebaugh *et al.*, 2005). Sources of lead in marine environments include natural sources from rock weathering, riverbank and coastal erosion, and anthropogenic sources from urban and industrial emissions (Kelly *et al.,* 2009). The impacts vary relatively minor to major disruptions due to bioaccumulation and biomagnifications processes (Altun *et al.,* 2008). The ecological integrity is judged using toxicity tests (Tueros *et al.*, 2009). The toxicity tests measure the integrated responses to the possible acute or chronic effects of contaminants, on these processes (Watts and Pascoe, 2000). Test species should be sensitive enough to respond to low levels of contaminants and must be available for use from field collection throughout the year. Chronic toxicity tests data are generally more reliable, providing responses related to a complete or part of life cycle of the test-species (Nascimento *et al.*, 2000). Aquatic organisms exposed to a higher concentration of trace metals in water may take up substantial quantities of these metals (Kord *et al.*, 2010). Accumulation of heavy metals in tissues mainly depends upon concentration of metals in water and exposure period; although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in metal accumulation (Blackmore and Wang, 2003). Marine organisms are characterized by a greater spatial ability to accumulate some metals when compared with bottom sediments (Kaladharan *et al.,* 2005). Mussels have been considered as a potential biomonitor for metallic contamination in marine ecosystems (Jung and Zauke, 2008), Fish are the good bioindicator for monitoring metal pollution (Tyrrell *et al.*, 2005). Species of prawn can be used to monitor the trace element pollution in the aquatic environment because

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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). Hence in the present study the *Mugil cephalus*, *Perna viridis* and *Penaeus monodon* was exposed to cadmium, copper, lead and zinc under long term chronic toxicity test for 30 days to estimate the bioconcentration factors of cadmium, copper, lead and zinc.

MATERIALS AND METHODS

Collected 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 immediately transported to the laboratory in air filled plastic bags and acclimatized in glass aquaria and fish fingerlings 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 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. Dilution water for the experiment was collected from the unpolluted site (Neelangarai, Tamilnadu, India) and filtered through 0.45µm filter paper (HA-Millipore) using Millipore vacuum pump. 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. 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 (USEPA, 2002b). The metal content of the tissue samples was based on dry weight. The organisms collected from the test chambers of the chronic test were sacrificed and was stored to -4°C and then transported to the laboratory and stored at -20°C in the deep freezer until analysis. During the course of analysis, 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) (Table 1). Nearest gram of the dried tissue powder was transferred to a Teflon crucible. To the tissue added 8-10 ml of concentrated acid (60 per cent nitric acid (HNO_3): 70 per cent perchloric acid ($HClO_4$)), such that the tissue was totally wet and with slight excess of acid and left it at room temperature for 12 h. The digested samples were heated slowly to 180°C on hot plate, till the sample volume was reduced to 2-3 ml. The resulting colourless solution was made up to 25 ml in standard glass flask and stored in 50 ml Polyethylene-Tereftalate (PET) bottles and was analysed for metals in Varian SpectraAA 220FS Atomic absorption spectrophotometer. Suitable internal chemical standards (Merck Chemicals, Germany) were used to calibrate the instrument. All the reagents used were analytical grade of high purity. The results were expressed as µg/g dry weight.

RESULTS

Treated heavy metals *M. cephalus* for short-term chronic toxicity test showed that the concentration of heavy metals in the tissues were highly significant (P<0.001) when compared with control (One way ANOVA: Dunnetts multiple comparison test (α=0.05)). Zinc concentrations in the tissues were higher than copper. The order of concentration in the tissues of *M. cephalus* was Zn>Cu>Pb>Cd (Table 2). Values were highly significant (P<0.001) when compared with control in the test conducted (One way ANOVA: Dunnetts multiple comparison test (α=0.05)). The order of decreasing concentration in the tissue of *P.viridis* was Cd<Pb<Cu<Zn (Table 3). Post larvae of *P.monodon* exposed to cadmium, copper, lead and zinc in the short-term chronic toxicity test revealed that the copper was concentrated more than zinc. Values were highly significant (P<0.001) when compared with control in the test conducted (One way ANOVA: Dunnetts multiple comparison test (α=0.05)). The order of concentration in the tissue was Cu>Zn>Pb>Cd (Table 4).

DISCUSSION

In the long term chronic toxicity test conducted for 30 days showed that the accumulation of heavy metals and the essential metals such as copper and zinc in the tissues of *M.cephalus* was high when compared with cadmium and lead which had significant (P<0.0001) increase with control. The

Table 1. 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 2. Concentrations of cadmium, copper, lead and zinc in the tissues of *M.cephalus* exposed under short-term chronic toxicity test

Metal	Units	Concentrations (mg/l)					
Cd	a	0	10	20	40	80	160
	b	0.43 ±0.04	7.05 ±0.14	9.13 ±0.32	12.15 ±0.78	15.50 ±0.07	26.95 ±0.35
Cu	a	0	10	20	40	80	160
	b	4.20 ±0.21	9.78 ±0.46	22.55 ±0.21	43.35 ±0.78	79.98 ±1.66	147.20 ±2.26
Pb	a	0	51	76	114	171	256
	b	0.43 ±0.35	4.68 ±0.11	8.35 ±0.78	11.98 ±0.39	21.60 ±0.78	35.03 ±1.59
Zn	a	0	29	46	74	118	188
	b	11.93 ±0.32	20.75 ±1.20	44.10 ±1.06	78.35 ±7.85	127.75 ±7.00	241.55 ±3.96

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 (***, $P<0.001$). a, exposed concentration (mg/l), b, concentration in tissues (µg/g dry wt.); Values are mean and standard deviation each n=2; The concentration column (mg/l) contains '0' indicating control in the test conducted in triplicate

Table 3. Concentrations of cadmium, copper, lead and zinc in the tissues of *P.viridis* exposed under short-term chronic toxicity test

Metal	Units	Concentrations (mg/l)					
Cd	a	0	16	26	41	66	105
	b	1.65 ±0.07	3.78 ±0.35	8.00 ±0.07	11.43 ±0.39	20.50 ±0.14	38.80 ±0.78
Cu	a	0	10	15	23	34	51
	b	1.90 ±0.07	3.80 ±0.01	7.95 ±0.56	12.40 ±0.21	25.30 ±0.35	52.90 ±4.59
Pb	a	0	10	15	23	34	51
	b	1.73 ±0.35	3.65 ±0.14	7.78 ±0.11	15.83 ±0.32	31.40 ±0.42	42.43 ±0.39
Zn	a	0	8	16	32	64	128
	b	11.93 ±0.32	13.25 ±0.78	21.88 ±0.39	40.03 ±0.81	70.75 ±1.20	113.75 ±3.25

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 (***, $P<0.001$). a, exposed concentration (mg/l), b, concentration in tissues (µg/g dry wt.); Values are mean and standard deviation each n=2; The concentration column (mg/l) contains '0' indicating control in the test conducted in triplicate

Table 4. Concentrations of cadmium, copper, lead and zinc in the tissues of *P.monodon* exposed under short-term chronic toxicity test

Metal	Units	Concentrations (mg/l)					
Cd	a	0	10	15	20	25	30
	b	1.28 ±0.35	2.83 ±0.35	5.90 ±0.34	12.43 ±0.39	22.60 ±0.28	35.83 ±0.32
Cu	a	0	8	13	21	34	54
	b	4.55 ±0.14	10.08 ±0.21	22.55 ±0.67	43.35 ±0.78	79.98 ±1.66	153.33 ±3.22
Pb	a	0	2	4	8	16	32
	b	2.98 ±0.39	4.03 ±0.11	7.78 ±0.11	15.83 ±0.32	31.40 ±0.42	44.65 ±0.35
Zn	a	0	15	23	34	51	76
	b	16.60 ±0.78	16.10 ±0.64	27.75 ±0.71	40.03 ±0.81	70.75 ±1.20	108.60 ±2.83

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 (***, $P<0.001$). a, exposed concentration (mg/l), b, concentration in tissues (µg/g dry wt.); Values are mean and standard deviation each n=2; The concentration column (mg/l) contains '0' indicating control in the test conducted in triplicate

deficiency of zinc can provoke serious consequences, as decrease of growth and sexual immaturity (Ansari *et al.*, 2004). The excess of some metals can cause harmful effects in fish such as alterations in oxygen consumption and damages in the gills (Zagatto and Bertoletti, 2006). Copper exposures leads to increased ammonium production (Kunwar *et al.*, 2009). This may be the reason for the reduced swimming performance of copper exposed fish, and could be caused by an increase in the catabolism of proteins (Waser *et al.*, 2009). It is well known that heavy metals accumulated in substantially high levels can be very toxic for fish, especially for young and eggs which are very sensitive to pollution (Kalay and Erdem, 1995).

Usero *et al.* (2004) reported in *Liza aurata*, *Anguilla anguilla* and *Solea vulgaris* the priorty of metal concentrated in the tissues were generally Cu = Zn>Cd. Metal levels in *Diplodus annularis*, *Scorpaena porcus* and *S.scrofa* in the order Zn>Cu>Cd (Chaffai *et al.*,1995). Besides concentrations (Zn >Cu >Cd), Chen *et al.* (2004) reported metal concentrations in *Liza macrolepis*, with descending order similar to results of the present study (Zn >Cu > Cd). In the present study *M.cephalus* accumulated heavy metals in the tissues of an order of Zn>Cu>Pb>Cd, which were comparable with all the above studies. Only levels of zinc and copper were comparable to those reported elsewhere (Tyrrell *et al.*, 2005). Biney and Ameyibor (1992) reported that the accumulation of copper, lead, zinc and cadmium in pink shrimp (*Penaeus notialis*) were lower than the present study. High concentrations of copper and zinc in both fish and shrimp were similar to findings previously recorded for crustacean (Hossain and Khan, 2001) and fish (Tyrrell *et al.*, 2005). Cadmium and zinc concentrations found in the muscle tissue of *P.monodon* were similar to those found in the shrimps reported by Eisler (1981) and in Pacific shrimps by Harding and Goyette (1989). Copper concentrations were similar to those recorded for brown and rock shrimps from the USA (Texas) continental shelf (Horowitz and Presley, 1977), but intermediate between levels of Australian shrimps *P. merguiensis* and *P. monodon* (Darmono and Denton, 1990) and North-east Pacific shrimps *Pandalopsis dispar* and *Pandalus borealis* (Harding and Goyette, 1989).

Dumalagan and Gonzales (2010) reported that among the zinc was the most bioaccumulated in the soft tissues of mussels *P.viridis* followed by copper, than lead. In the present study the *P.viridis* exposed to cadmium, copper, lead and zinc in short-term chronic toxicity test revealed that at the end of the 30th day the metal concentrations were significantly (P<0.0001) higher than the green mussels exposed in control. Yap *et al.* (2003) reported high accumulation of zinc in soft mussel tissues of *P. viridis* and suggested that the byssus of mussels is a better biomonitoring organ for zinc contamination. The most abundant trace metals were zinc and copper followed in much lower concentrations by lead and cadmium in clam samples (Das *et al.*, 2009). For bivalves there appear to be trends in metal accumulation patterns of essential trace metals depending on the group, their metabolism and their ability to either excrete or store particular trace elements (Rainbow, 1993). Zinc concentrations of >2000 µg/g dry weight are regularly reported in soft tissues of different oyster species (Hayes *et al.*, 1998). The zinc tissue concentrations determined in the present study for the *P.viridis* were below 113.75 µg/g which is more typical less. Copper concentrations in *P.viridis* in the general ranged 3.8-52.9 µg/g can be compared with those for other bivalves. They are similar to concentrations reported for the European cockle *Cerastoderma edule* and mussel *Mytilus edulis* (Hummel *et al.*, 1997). They are also similar to the copper concentrations in the pearl oyster *Pinctada radiata* (Al-Sayed *et al.*, 1994), where the average range was 3.5 and 15.3 µg/g from the Arabian Gulf. The copper concentrations in the *P.viridis* were however lower than some other bivalves including *Macoma balthica* and mussels (Hummel *et al.*, 1997). Lead concentrations observed in *P. viridis* in the present study were 2-4 times lower than those reported by Krishnakumar *et al.*, (1998).

Elevated zinc concentration in invertebrates is associated with the presence of a sulphide-transporting protein and with zinc at its active site (Flores *et al.*, 2005), the bivalve gill, for instance, is widely known to lose bioaccumulated metals rapidly when placed in a metal-free environment (Ng and Wang, 2004). Although findings have shown body size to be a critical factor influencing metal concentration in marine bivalves (Chong and Wang, 2001), extremely high concentrations of zinc in green mussels during the whole period of accumulation indicate an important role of mucus in the depuration of metals.

Conclusion

Our study has shown that ability of *Mugil cephalus* to accumulate primarily zinc followed by copper, lead and cadmium, *Perna viridis* accumulated cadmium primarily followed by lead, copper and zinc. *Penaeus monodon* accumulated heavy metals in the order of Cu>Zn>Pb>Cd exposed under long term chronic toxicity test. High significance was observed in treated (P<0.001) when compared with control. This study shows that *Mugil cephalus*, *Perna viridis* and *Penaeus monodon to* be a better biomonitoring agents in heavy metal pollution of environmental monitoring.

REFERENCES

Al-Sayed, H.A., Mahasneh, A.M and Al-Saad, J. 1994. Variations in trace metal concentrations in seawater and pearl oyster *Pinctada radiata* from Bahrain (Arabian Gulf). *Mar. Poll. Bull.*, 28: 370-374.

Altun, O., Sacan, M.T and Erdem, A.K. 2008. Water quality and heavy metal monitoring in water and sediment samples of the Kucukcekmece Lagoon, Turkey (2002-2003). *Environ. Monit. Assess.*, 151(1-4): 345-62.

Ansari, T.M., Marr, I.L and Tariq, N. 2004. Heavy metals in marine pollution perspective-a mini review. *J. Appl. Sci.*, 4(1): 1-20.

Barrento, S., Marques, A. Teixeira, B. Carvalho, M.L. Vaz Pires, P and Nunes, M.L. 2009. Accumulation of elements (S, As, Br, Sr, Cd, Hg, Pb) in two populations of *Cancer pagurus*: Ecological implications to human consumption. *Food Chem. Toxicol.*, 47: 150-156.

Biney, C.A and Ameyibor, E. 1992. Trace metal concentrations in the pink shrimp, *Penaeus notialis* from the coast of Ghana. *Wat. Air soil Poll.*, 63: 273-279.

Blackmore, G and Wang, W.X. 2003. Inter-population differences in Cd, Cr, Se, and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. *Aqua. Toxicol.*, 62: 205-218.

Chaffai, H.A., Cosson, R.P. Triquet, C.A and El-Abed, A. 1995. Physico-chemical forms of storage of metals (Cd, Cu and Zn) and metallothionein-like proteins in gills and liver of marine fish from the Tunisian coast: Ecotoxicological consequences. *Comp. Biochem. Physiol.*, 111((2)C): 329-341.

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.

Chen, Y.C., Chen, C.Y. Huang, H.J. Chang, W.B and Chen, M. H. 2004. Comparison of the metal concentrations in muscle and liver tissues of fishes from Erren River, Southwestern Taiwan, after the restoration in 2000. *J. Food Drug Anal.*, 12: 358-366.

Chong, K and Wang, W.X. 2001. Comparative studies on the biokinetics of Cd, Cr, and Zn in the green mussel *Perna viridis* and the Manila clam *Ruditapes phyilippinorum*. *Environ. Pollut.*, 115: 107-121.

Darmono, D and Denton, G.R.W. 1990. Heavy metal concentrations in the banana prawn, *Penaeus merguiensis*, and leader prawn, *P. monodon*, in the Townsville region of Australia. *Bull. Environ. Contam. Toxicol.*, 44: 479-486.

Das, Y.K., Aksoy, A. Baskaya, R. Duyar, H.A. Guvenc, D and Boz, V. 2009. Heavy metal levels of some marine organisms collected in Samsun and Sinop coasts of Black Sea in Turkey. *J. Anim. Vet. Adv*., 8: 496-499.

De Boeck, G., Vlaeminck, A and Blust, R. 1997. Effects of sublethal copper exposure on copper accumulation, food consumption, growth, energy stores, and nucleic acid content in common carp. *Arch. Environ. Contam*., 33: 415-422.

DOLT-3. 1999. Dogfish liver certified reference material for trace metals, national research council of Canada, institute for national measurement standards, M-12, Montreal road, Ottawa, Ontario, Canada K1A 0R6.

Dumalagan, H.G.D and Gonzales, A.C. 2010. Trace metal content in mussels, *Perna viridis* L., obtained from selected seafood markets in a metropolitan city. *Bull. Environ. Contam. Toxicol*., 84: 492-496.

Eisler, R. 1981. Trace Metal Concentrations in Marine Organisms. Pergamon Press, New York, NY, USA. 687.

Flores, J. F., Fisher, C.R. Carney, S.L. Green, B.N. Freytag, J.K. Schaeffer, S.W and Royer, W.E. 2005. Sulfide binding is mediated by zinc ions discovered in the crystal structure of hydrothermal vent tubeworm haemoglobin. *Proc. Natl. Acad. Sci.*, 102: 2713-2718.

Girija, T.R., Mahanta, C and Chandramouli, V. 2007. Water quality assessment of an untreated effluent imparted urban stream: The Bharalu Tributary of the Brahmaputra River, India. *Environ. Monitor. Assess.*, 130: 221-236.

Harding, L and Goyette, D. 1989. Metals in northeast Pacific coastal sediments and fish, shrimp and prawn tissues. *Mar. Poll. Bull.*, 20:187-189.

Hayes, W.J., Anderson, I.J. Gaffor, M.Z and Hurtado, J. 1998. Trace metals in oysters and sediments of Botany Bay, Sydney. *Sci. Tot. Environ*., 212: 39-47.

Horowitz, A and Presley, B.J. 1977. Trace metal concentrations and partitioning in zooplankton, neuston and bentos from the south Texas outer continental shelf. *Arch. Environ. Contam. Toxicol.*, 5: 241-255.

Hossain, M.S and Khan, Y.S.A. 2001. Trace metals in penaeid shrimp and spiny lobster from the Bay of Bengal. *Sci. Asia*., 27: 165-168.

Hummel, H., Moddermam, R. Triquet, F.R.A. Duijn, N.V. Herssevoort, M. Desprez, M. Marchand, J. Sylvand, B. Amiard, J.C. Rybarczyk H and de Wolf, L. 1997. A comparative study on the relation between copper and condition in marine bivalves and the relation with copper in the sediment. *Aquat. Toxicol.*, 38: 165-181.

Jarup, L and Akesson, A. 2009. Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.*, 238: 201-208.

Jung, K and Zauke, G.P. 2008. Bioaccumulation of trace metals in the brown shrimp *Crangon crangon* (Linnaeus, 1758) from the German Wadden Sea. *Aquat. Toxicol*., 88: 243-249.

Kaladharan, P., Prema, D. Valsala, K.K. Leelabhai, K.S and Rajagopalan, M. 2005. Trends in heavy metal concentrations in sediment, fin fishes and shellfishes in inshore waters of Cochin, southwest coast of India. *J. Mar. Biol. Ass.*, 47(1): 1-7.

Kalay, M and Erdem, C. 1995. Accumulation of copper in liver, kidney, gill, muscle, brain and blood tissues of *Tilapia nilotica* (L.) and its effects on some blood parametrers. *Turk. J. Zool*., 19: 27-33.

Kelly, A.E., Reuer, M.K. Goodkin, N.F and Boyle, E.A. 2009. Lead concentrations and isotopes in corals and water near Bermuda, 1780-2000. *Eart. Planeta. Sci. Lett*., 283: 93-100.

Kord, B., Mataji, A and Babaie, S. 2010. Pine (*Pinus eldarica* Medw.) needles as indicator for heavy metals pollution. *Int. J. Environ. Sci. Tech*., 7(1): 79-84.

Krishnakumar, P.K., Bhat, G.S. Vaidya, N.G and Pillai, V.K. 1998. Heavy metal distribution in the coastal waters of Karnataka, west coast of India. *Ind. J. Mar. Sci*., 27: 201-205.

Kunwar, P.S., Tudorache, C. Eyckmans, M. Blust, R and De, B.G. 2009. Influence of food ration, copper exposure and exercise on the energy metabolism of common carp (*Cyprinus carpio*). *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol*., 149: 113-119.

Nascimento, I.A., Smith, D.H. Gomes, M.G.S. Santos, G.V and Pereira, S.A. 2000. Ecotoxicological diagnosis of Aratu Bay, Bahia, Brazil: A new approach to validate a reactive short-term toxicity end-point by comparison with intertidal benthic activity. *Aquat. Ecosyst. Heal. Manag.*, 3(4): 449-458.

Ng, T.Y.T and Wang, W.X. 2004. Detoxification and effects of Ag, Cd, and Zn pre exposure on metal uptake kinetics in the clam *Ruditapes philippinaipum*. *Mar. Ecol. Prog. Ser*., 268: 161-172.

Rainbow, P.S. 1993. The Significance of Trace Metal Concentrations in Marine Invertebrates. In: Dallinger, R., and P.S. Rainbow (Eds.). Ecotoxicology of Metals in Invertebrates, USA. Chelsea, Lewis Publishers. 3-23.

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.

Seebaugh, D.R, Goto, D and Wallace, W.G. 2005. Bioenhancement of cadmium transfer along a multi level food chain. *Mar. Environ. Res*., 59(5): 473-491.

Singh, N and Turner, A. 2009. Trace metals in antifouling paint particles and their heterogeneous contamination of coastal sediments. *Mar. Poll. Bull*., 58: 559-564.

Tsangaris, C., Papathanasiou, E and Cotou, E. 2007. Assessment of the impact of heavy metal pollution from a ferro-nickel smelting plant using biomarkers. *Ecotoxicol. Environ. Saf.*, 66(2): 232-243.

Tueros, I., Borja, A. Larreta, J. Rodriguez, J.G. Valencia, V and Millan, E. 2009. Integrating long-term water and sediment pollution data, in assessing chemical status within the European Water Framework Directive. *Mar. Poll. Bull.*, 58: 1389-1400.

Tyrrell, L., Mchugh, B. Glynn, D. Twomey, M. Joyce, E. Costello, J and Mcgovern, E. 2005. Trace metal concentrations in various fish species landed at selected Irish Ports. *Mar. Envirn. Heal. Ser*., 20: 1-19.

UNEP. 1984. Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry reference methods for marine pollution studies. 11: 1.

USEPA. 1991. Pollutants of concern in Puget Sound. U.S. Environmental Protection Agency, Office of Puget Sound, Region 10, Seattle, Washington. United States, EPA 822-R-03-026.

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.

Usero, J., Izquierdo, C. Morillo, J and Gracia, I. 2004. Heavy metals in fish (*Soela vulgaris Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. *Environ. Int*., 29: 949-956.

Waser, W., Bausheva, O and Nikinmaa, M. 2009. The copper induced reduction of critical swimming speed in rainbow trout (*Oncorhynchus mykiss*) is not caused by changes in gill structure. *Aquat. Toxicol*., 94(1): 77-79.

Watts, M.M and Pascoe, D. 2000. A comparative study of *Chironomus riparius* Meigen and *Chironomus tentans* Fabricius (Diptera: Chironomidae) in aquatic toxicity tests. *Arch. Environ. Contam. Toxicol.*, 39: 299-306.

Yap, C.K., Ismail, A and Tan, S.G. 2003. Concentrations of Cd, Cu, Pb and Zn in different parts of byssus of the green-lipped mussel *Perna viridis* (Linnaeus). *Pakistan Journal of Biological Sciences.*, 6: 789-792.

Zagatto, P.A and Bertoletti, E. 2006. Ecotoxicologia Aquatica: Princıpiose Aplicacoes. Rima Editora, Sao Carlos. 464.

Zhao, X., Shen, Z.Y. Xiong, M and Qi, J. 2011. Key uncertainty sources analysis of water quality model using the first order error method. *Int. J. Environ. Sci. Tech.*, 8(1): 137-148.

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