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Research Article

BIOACCUMULATION OF TOXIC HEAVY METALS IN THE FRESHWATER BIVALVES, *PARREYSIA CYLINDRICA* AND *LAMELLIDENS MARGINALIS* UNDER EXPERIMENTAL STRESS

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ABSTRACT
The aim of present study was to find the most appropriate sentinel bivalve species for metal pollution biomonitoring programme in the freshwater ecosystem. The freshwater bivalves namely, *Parreysia cylindrica* and *Lamellidens marginalis* were separately exposed to chronic concentration of heavy metal salts. The bivalves, *Parreysia cylindrica* were exposed to chronic $LC_{50/10}$ dose of copper suphate (0.0295ppm) and mercury chloride (0.0195ppm). The bivalves, *Lamellidens marginalis* were exposed to chronic LC $_{50/10}$ dose copper suphate (0.0350 ppm) and mercury chloride(0.0245ppm) up to 21 days in laboratory. Bioaccumulation level in whole body tissues of *Parreysia cylindrica* and *Lamellidens marginalis* were estimated after 7, 14 and 21 days. It was found that the freshwater bivalves, *Parreysia cylindrica* had highest bioaccumulation and concentration of copper (182.62µg) and *Lamellidens marginalis* showed highest concentration of mercury (5.87µg). Therefore, *Parreysia cylindrica* is proposed as sentinel organism for the biomonitoring of copper and *Lamellidens marginalis* for mercury in freshwater ecosystem.

Keywords: *Bioaccumulation,Mercury,Copper, Parreysia Cylindrica, Lamellidens Marginalis*

INTRODUCTION
Rapid industrial development, as well as the use of metals in production processes has led to the increased discharges of heavy metals into the environment (Koli *et al.,* 1977). The presence of toxic metals poses environmental problems due to their non-degradable and persistent nature (Sarabject and Dinesh, 2007). Heavy metals occur in aquatic systems from natural sources and anthropogenic activities. The pollution of aquatic environment by heavy metals affects aquatic biota posse's considerable environmental risks and concerns (Amisah *et al.,* 2009).
Compared with other types of aquatic pollution, heavy metal pollutants less visible but its effects on the ecosystem and humans are intensive and very extensive due to their toxicity and their ability to accumulate in the biota (Shanmugam *et al.,* 2007; Edem *et al.,* 2008).
Metal uptake pattern of aquatic macroinvertebrates is comparatively less known. Due to their often broad distribution and great abundances in both terrestrial and aquatic environments, their limited mobility and relative great accumulation potency for contaminants, molluscs - both snails (Gastropoda) and mussels (Bivalvia) are suitable indicators of toxic matters in aquatic habitats and for that reason are commonly studied around the world from the ecotoxicological point of view (Elder and Collins 1991). The heavy metals enter into the body of animals including man through the non vegetarian and vegetarian diet, drinking water and air and accumulate in the tissues, usually react with proteins and interfer the physiological activities and thus increase the risk of life in various ways. They are difficult to remove from the body.
The discharge of mine tailings and fly ash (the major source of solid Cu pollution), fertiliser production and algaecide and molluscicide runoff (Felts and Heath, 1984). Cu is classified as a heavy metal and has many physical properties that make it useful for various industrial applications. Its high electrical and thermal conductivity as well as its resistance to corrosion makes it an important element in the use of combustion sources (i.e. municipal incinerators and combustion of coal, gasoline, diesel and lubricating oils), tires and brakes of vehicles (WHO 1998; Rice *et al.,* 2002). However, once it enters aquatic

environments, it is only slightly soluble in freshwater, saline waters or mildly acidic solutions, but carbonate, which can be found in copious amounts in freshwater, can more readily dissolve Cu (WHO 1998). The biotic ligand model (BLM) is a good model that estimates dissolved metal toxicity, including Cu, based on natural occurring ions in the environment (Cruz & Delos 2010).

The BLM was first derived to look at the effects of metal toxicity to fish gills, but has recently been extended to other aquatic organisms, such as algae and crustaceans (Cruz & Delos 2010, Vijver *et al.*, 2004).

Mercury is one of the most hazardous environmental pollutants. Mercury tends to concentrate in various organisms including fish due to reduced biodegradation of its derivatives. Consequently, gastropod snails are widely used as biomarkers for assessing heavy metal contamination level of aquatic environment and the health state of aquatic ecosystems.

Considering all these things, therefore, in the present study different native species of fresh water bivalves, *Parreysia cylindrica* and *Lamellidens marginalis* were selected to establish a local environmental monitoring network using bivalves as bioindicator species to assess trends of Cu and Hg in freshwater ecosystem.

MATERIALS AND METHODS

The freshwater bivalves, *Parreysia cylindrica* and *Lamellidens marginalis* were collected from various dams of Jalgaon district in Maharashtra state, India. After collection animals were brought to laboratory and were acclimatized in aquarium containing dechlorinated tap water for 4 days. During acclimatization and experiment, the animals were fed with freshwater algae and water of aquarium was changed after every 24 h. After acclimatization, the active, medium, uniform sized and healthy bivalves of each species were selected by measuring their shell length and width.

The freshwater bivalves, *Parreysia cylindrica* was divided into three groups. The bivalves group of A was maintained as control. The group B was exposed to chronic concentration ($LC_{50/10}$) of $CuSo_4$ (0.0295 ppm) and group C was exposed to chronic concentration ($LC_{50/10}$) of $HgCl_2$ (0.0195ppm) up to 21 days. The freshwater bivalves, *Lamellidens marginalis* was divided into three groups. The bivalves group of A was maintained as control. The group B was exposed to chronic concentration ($LC_{50/10}$) of $CuSo_4$ (0.0350ppm) and group C was exposed to chronic concentration ($LC_{50/10}$) of $HgCl_2$ (0.0245 ppm) up to 21 days in laboratory.

Previously calculated LC_{50} values for 96 h exposure were used in deciding the dose for experimentation. Ten animals from each of experimental and control group were dissected after 7 days, 14 days and 21 days of exposure period and the whole body mass of bivalves from all groups were collected after every seven days and were dried in oven at 70^{o}- 80^{oC} till constant weight was obtained. The 500 mg sample was taken for digestion. The tissue was digested in 10 ml of acid mixture ($HCL:HNO_3$ in (3:1) ratio) on hot plate till dryness. The digested mixtures were kept in water bath for 6-7 hours until the samples were cooled. Cool digested samples were filtered (Whatman grade 541). The total volume was diluted to 50 ml by double glass distilled water in volumetric flask.

The sample were analysed on the instrument atomic absorption sepctrophotometer (Chemito). Dry weight of each animal was used to calculate the metal concentration per unit body weight (μg/g). The concentration of Cu and Hg accumulation in the tissue of each exposure period was recorded and the results are given in the tables.

RESULTS AND DISCUSSION

Along the experiments, the patterns of accumulation of metals in two freshwater species of bivalves, after exposure to chronic concentration of Cu and Hg separately for 7, 14 and 21 day are summarized in Table A and B. The data revealed a significant increase in levels of all metal concentrations and bioaccumulation in the whole soft body tissues of experimental bivalves with increase in exposure period as compared to the bivalve maintained as control. It was observed that different species of bivalves showed different uptake levels for different metals.

The biaccumulation data from table A, indicates that the amount of Cu in whole body tissues of freshwater bivalve, *Parreysia cylindrica* on exposure to $CuSo_4$ (0.0295ppm) increased with increase in exposure period as compared to control.

The Cu contents are expressed in µgm/kg dry weight. The control group of animals showed minute quantity of Cu as compared to the experimental groups. The control group of animal showed 16.65, 14.34 and 13.28 µgm/Kg copper accumulation after 7,14 and 21 days. Cu in whole body tissue accumulation for 7 days was 109.24 µgm/Kg. The concentration in the tissues was raised after 14 days which was 112.36µgm/Kg. While after 21 days the rate of accumulation was 126.79 µgm/Kg. There was minute change in the accumulation in control animals.

The amount of Hg in tissues on exposure to $HgCl_2$ (0.0195ppm) increased with increase in exposure period as compared to control. The mercury contents are expressed in µgm/Kg dry weight. The control group of animals showed minute quantity of mercury as compared to the experimental groups. The control group of animal showed 25.79, 25.50 and 21.89 µgm/Kg mercury in whole body tissue after 7,14 and 21 days respectively.

While the amount of accumulation was of Hg in presence of $HgCl_2$ (0.0195ppm) for 7 days was 95.34 µgm/Kg. The concentration in the tissues was raised after 14 days which was 102.19µgm/Kg. While after 21 days the rate of accumulation was 115.67 µgm/Kg.

The biaccumulation data from table B, indicates that the amount of Cu in whole body tissues of freshwater bivalve, *Lamellidens marginalis* on exposure to $CuSo_4$ (0.0350ppm) increased with increase in exposure period as compared to control.

The Cu contents are expressed in µgm/kg dry weight. The control group of animals showed minute quantity of Cu as compared to the experimental groups. The control group of animal showed 25.96, 24.64 and 22.44 µgm/Kg copper accumulation after 7, 14 and 21 days. Cu in whole body tissue accumulation for 7 days was 285.70 µgm/Kg. The concentration in the tissues was raised after 14 days which was 297.53µgm/Kg. While after 21 days the rate of accumulation was 310.87 µgm/Kg. There was minute change in the accumulation in control animals. The amount of Hg in tissues on exposure to $HgCl_2$ (0.0245ppm) increased with increase in exposure period as compared to control. The mercury contents are expressed in µgm/Kg dry weight. The control group of animals showed minute quantity of mercury as compared to the experimental groups. The control group of animal showed 95.60, 84.32 and 81.40 µgm/Kg mercury in whole body tissue after 7, 14 and 21 days respectively. While the amount of accumulation was of Hg in presence of $HgCl_2$ (0.0245ppm) for 7 days was 360.48 µgm/Kg. The concentration in the tissues was raised after 14 days which was 377.52µgm/Kg. While after 21 days the rate of accumulation was 398.18 µgm/Kg.

Table A: Cu and Hg Content (µgm/Kg Dry Weight) in Whole Body Tissues of *Parreysia Cylindrica* After Chronic Treatment of $CuSo_4$ and $HgCl_2$

Sr. No.	Name of Bivalves Species	Bivalves Exposed in Days and Tissue		Cu and Hg Content (µgm/kg Dry Weight)			
				Treatment			
				A Control	B 0.0295 ppm $CuSo_4$	A Control	C 0.0195 ppm $HgCl_2$
1	*Parreysia Cylindrica*	7	W.B.	16.65	109.24	25.89	95.34
		14	W.B.	14.34	112.36	25.50	102.19
		21	W.B.	13.28	126.79	21.89	115.67

Table B: Cu and Hg Content (μgm/Kg Dry Weight) in Whole Body Tissues of *Lamellidens Marginalis* After Chronic Treatment of $CuSo_4$ and $HgCl_2$

Sr. No.	Name of Bivalves Species	Bivalves Exposed in Days and Tissue		Cu and Hg content (μgm/kg dry weight)			
				Treatment			
				A Control	B 0.0350 ppm $CuSo_4$	A Control	C 0.0245 ppm $HgCl_2$
2	*Lamellidens Marginalis*	7	W.B.	25.96	285.70	95.60	360.48
		14	W.B.	24.64	297.53	84.32	377.52
		21	W.B.	22.44	310.87	81.40	398.18

- -Compared with respective A, W.B.- Whole Body

Based on these results, it shows that the magnitude of heavy metal accumulation in bivalve tissues depend on the type of heavy metal and the species of the bivalve. Concentration of metals observed in the control animal body indicates presence of these metals in natural ecosystem of experimental bivalves. A reduced metal level in control bivalves indicates slow and gradual depuration of metals by bivalves the high bioaccumulated values show that these bivalve species are best bioindicators for monitoring these metals as pollutant in water. The bivalves with low bioconcentration factor for the accumulation of metal are not good for monitoring of above the observed differences in tissue metal concentration in bivalve species might be due to variation in body size and growth.

The accumulation of metal in different species is the function of their respective membrane permeability and enzyme system. The ratio between bioaccumulation and exposure concentration with periods of exposure has been shown by various investigators.

The accumulation of several metals is due to the low capacity of these mollusks for discriminating among metals, which are similar in some characteristics such as ionic radius (Mitra *et al.*, 2000; Pragatheeswaran, 1987; Sayer *et al.*, 1989; Barber and Sharma, 1998; Senthiloathan *et al.*,1998; Jeffree *et al.*, 1993; Metcalfe Smith,1994). Heavy or toxic metals are metals with a density at least five times that of water. They are stable elements (meaning they cannot be metabolished by the body) and bio-accumulative are (passed up the food chain to humans). These include: mercury, lead, nickel, arsenic, cadmium, aluminium, platinum and copper. Heavy metals besides micronutrients have no fuction in the body and can be highly toxic. Studies confirm that heavy metals can directly influence behaviour of living organism including man.

Two obvious methods exist for expressing the heavy metal component of living organisms. Absolute may be assessed by considering the organisms, metal contens i.e. body burden and the metal component may be expressed as a fuction of the weight of individual organism.

According to the Gundacker (1999), a zebra mussel accumulates high amounts of potentially toxic metals and was widely used as a bio-monitoring organism. Avelar *et al.*, (2000) reported that Oyster and mussels can accumulate Cd in their tissues at levels up to 100,000 times higher than the levels observed in the water in which they live. Passow *et al.*, (1961) reported that lead can induce synthesis of specific proteins which selectively bind them. Inhibition of enzyme activities by heavy metals is either due to the direct binding with enzyme protein or due to damage of cell organelles or by toxic effect produced. The specific amoebocytes and or digestive vesicles within the cell may engulf metals outside the cell membrance (i.e. in the human digestive tract), then move back into the tissue carying their particulate burden (Owne *et al.*, 1966).

The pond snail (*Lymnaea stagnalis* L.), which is one of the most common snails of freshwater habitats in central Europe, have a good indicator potential, since more information about the features of heavy metal

accumulation, toxic pollution tolerance and impact of metals on the physiology of the genus *Lymnaea* are known (Królak 1998; Bogatov and Bogatova, 2009). The accumulation of metals in invertebrates is also dependent on functional feeding group and scrapers that feed on periphyton, such as snails, accumulated the largest concentrations of metals (Farag *et al.*, 1998).
The finding of this study showed that the concentration bioaccumulation value for Cu was highest in the *Parreysia cylindrica*. Hg was highest in *Lamellidens marginalis*. Therefore, these results indicate that *Parreysia cylindrica* is sentinel organism for the biomonitoring of Cu and *Lamellidens marginalis* for Hg in fresh water ecosystem

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REFERENCES
Amisah S, Adjei-Boateng D, Obirikorang KA and Quagrainie KK (2009). Effects of clam size on heavy metal accumulation in whole soft tissues of *Galatea paradoxa* (Born, 1778) from the Volta estuary. Ghana. *International Journal of Fisheries and Aquaculture* **1**(2) 14 -21.
Avelar WEP, Mantelatto FLM, Tomazelli AC, Silva DML, Shuhama T and Lopes JLC (2000). The marine mussel Perna perna (Mollusca, Bivalvia, Mytilidae) as an indicator of contamination by heavy metals in the Ubatuba Bay, Sao Paulo, Brazil. *Water, Air & Soil Pollution* **118** 65-72.
Barber D and Sharma MS (1998). Experimentally induced bioaccumulation and elimination of cadmium in freshwater fishes. *Pollution Research* **17** 99-104.
Bogatov VV and Bogatova LV (2009). Heavy metal accumulation by freshwater hydrobionts in a mining area in the south of the Russian far east. *Russian Journal of Ecology* **40** 187–193.
Cruz LA and Delos C (2010). *An Introduction to the Biotic Ligand Model,* (US Environmental Protection Agency, Washington D.C, USA).
Duffus JH (1980). *Environmental Toxicology*. (Edward Arnold (publishers) Ltd, London, Great Britain) 164.
Edem CA, Akpan B and Dosunmu MI (2008). A comparative assessment of heavy metals and hydrocarbon accumulation in Sphyrena afra, *Oreochromis niloticus* and lops lacerta from Anantigha Beach market in Calabar- Nigeria. *African Journal of Environmental Pollution and Health* **6** 61-64.
Elder JF and Collins JJ (1991). Freshwater molluscs as indicators of bioavailability and toxicity of metals in surfacewater systems. *Reviews of Environmental Contamination and Toxicology* **122** 37–79.
Felts PA and Heath AG (1984). Interactions of temperature and sublethal environmental copper exposure on the energy metabolism of bluegill, *Lepomis macrochirus* Rafinesque. *Journal of Fish Biology* **25** 445-453.
Farag AM, Woodward DF, Goldstein JN, Brumbaugh W and Meyer JS (1998). Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d`Alene Riverbasin, Idaho. *Archives of Environmental Contamination and Toxicology* **34** 119–127.
Gundacker C (1999). Tissue specific heavy metals (Cd, Pb, Cu and Zn) deposition in Natural pollution of Zebra mussel Dreissena polymorpha Pallas. *Chemosphere* **38** 3339-3356.
Jeffree RA, Markich SJ and Brown PL (1993). Comparative accumulation of alkaline earth metals by two freshwater mussel species from the Nepean River, Australia: consistencies and a resolved the paradox. *Australian Journal of Marine & Freshwater Research* **44** 609-34.
Królak E (1998). Concentration of heavy metals in the snails *Lymnaea* (*Radix*) *peregra* (O.F. Mull) and *Lymnaea stagnalis* (L) occurring in rivers near Siedlce town. *Polskie Archiwum Hydrobiologii* 45 553–563.
Koli AK, Williams WR, McClary EB, Wright EL and Burrell TM (1977). Mercury levels in freshwater fish of the state of South Carolina. *Bulletin of Environmental Contamination and Toxicology* **17**(1) 82-89.

Metcalfe Smith JL (1994). The influence of species and sex on metal residues in freshwater mussels (Family Unionidae) from the St. Lawrence River, with implications for biomonitoring programs. *Environmental Toxicology and Chemistry* **13**(9) 1433-1443.

Mitra A, Mitra S, Hazra S and Chaudhari A (2000). Heavy metal concentration in India. Coastal Fishes. *Research Journal of Chemistry and Environment* **4** 35.

Owen G (1966). In: *Physiology of Mollusca II*, (edition K.M. Wilbur and C.M. Younge). (Academic Press, New York, USA) 53-88.

Passow H, Rothetein A and Clarkson TW (1961). The general pharmacology of the heavy metals. *Pharmacological Review* **13** 185-224.

Pragatheeswaran V (1987). Effect of Zinc, Copper and mercury on *Ambassis Commersoni* (Cuvier). Ph.D. Thesis, Annamalai University, India.

Rice KC, Conko KM and Hornberger GH (2002): Anthropogenic sources of arsenic and copper to sediments in a suburban lake, Northern Virginia. *Environmental Science and Technology* **36** 4962-4967.

Sayer MD, Reader JP and Morries R (1989). The effect of cadmium concentration on the toxicity of Copper, Lead, Zinc to Yolk Sac of brown trout, *Salmo trout*. L. In soft and acid water. *Journal of Fishes Biology* **35** 323-32.

Senthiloathan S, Balasubramanian T and Venugopal VK (1998). Metal concentration in mussel *Perna vridis* (Bivalvia) and Oyster *Crassostrea madrasensis* (Bivalvia) from some parts of southeast coast of India. *Indian Journal of Marine Sciences* **27** 206-10.

Senthiloathan S and Balasubramanian T (1998). Heavy metal concentration in the oyster Crassostrea *madrasensis* (Bivalve) From the Uppanar. Vellar and Kaduviar estuaries of Southeast coast of India. *Indian Journal of Marine Sciences* **27** 211-16.

Shanmugam A, Palpandi C and Kesavan K (2007). Bioaccumulation of some trace metals (Mg, Fe, Zn, Cu) from Bowl *Cymbium melo* (Solander, 1786). *Research Journal of Environmental Sciences* **1**(4) 191-195.

Sarabject SA and Dinesh G (2007). Biosorption: A solution to pollution. *International Microbiology* 3.17-324.

Vijver MG, Van Gestel CAM, Lanno RP, Can Straalen NM and Peijnenburg WJGM (2004). Internal metal sequestration and its ecotoxicological relevance: A review. *Environmental Science & Technology* **38** 4705-4712.

WHO (1998). Geneva. Environmental Health Criteria 200: Copper.