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Bioaccumulation and Distribution of Heavy Metals in Different Soft Body Tissues of The Freshwater Bivalve, Parreysia corrugata

KEYWORDS: Parreysia corrugata, bioaccumulation, digestive glands, bioindicator

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ABSTRACT *The freshwater bivalve, Parreysia corrugata were exposed to chronic concentration (LC50/10 96 h) of arsenic (0.1719 ppm), cadmium (0.1284 ppm), copper (0.033 ppm), lead (1.50 ppm) and zinc (1.8589 ppm) separately for 20 days. After 10 and 20 days of exposure animals were dissected and their mantle, gills, digestive glands and whole soft body tissues were removed and dried in oven at 80oC. The heavy metal concentrations were determined from dry powder of each tissue by using AAS. The obtained data revealed a significant increase in metal concentration in all soft body tissues of experimental bivalves with increase in exposure period as compared to the bivalves maintained as control. It was observed that different concentration of heavy metals found in different soft body tissues, this indicates different metal regulation and binding sites in these organs. It was also observed that digestive glands accumulated higher concentration of As (52.81 µg/g), Cd (71.18 µg/g), Cu (80.28 µg/g), Pb (286.08 µg/g) and Zn (1532µg/g) of dry tissue weight as compared to other studied tissues.*
Therefore, digestive glands of Parreysia corrugata is being proposed as bioindicator organ for monitoring of As, Cd, Cu, Pb and Zn metal pollution in freshwater ecosystem.

Introduction
The development of industry and agriculture promotes the rapid increase of environmental heavy metal pollution. The contamination of water, soils sediments and biota by heavy metals is of major concern because of their toxicity, persistence and bioaccumulative nature (Ikem et al., 2003). The most important metals from the point of view of water pollution are As, Cd, Cu, Pb, Hg, Ni and Zn (Li et al., 2002). Some of these metals (e.g. Cr, Cu, and Zn) are essential trace metals to living organisms, but become toxic at higher concentration. Heavy metal accumulated in benthic organisms might be further biomagnified in food webs. Hence consummation of such kind of animals may form a significant pathway in the human being and creating public health problems (Medeiros et al., 2012). Human health effects are highly depends on ingested doses of heavy metal. Even at very low level, metal ions causes serious health effects. It is therefore imperative to monitor them for public safety and other ecological concern.

To reveal very low undetectable concentration of heavy metals for early warning, the idea has been proposed to use a biomonitoring technique, which includes study of xenobiotic body load (bioaccumulation) in aquatic biota and this method is more efficient and sensitive (Cajaraville et al., 2000) as compared to conventional methods. The bioaccumulation studies led to adoption of the bioindicator concept. Bioaccumulative indicators such as bivalves are frequently regarded as biomonitors because they are sedentary, filter feeder, wide spread and have a long life span, greater ability to accumulate metal and provides a time integrated indication of environmental contamination, hence fulfilling the criteria as good bioindicators (Huang et al., 2007). Metal accumulation in living organism leads to concentration several orders of magnitude higher than dose of surrounding water (Casas, 2008). Bioaccumulation of toxicants is described as one of the many possible tool used in biomonitoring (Chapman, 1997). Usually the level of pollutant accumulated in tissues of such organisms is used for assessing the level of pollution in its habitat (Abdallah and Moustafa, 2002). According to the Abdullah (2008) metal studies in aquatic biota give an idea that metals in aquatic organisms could be more reliable water quality indicator than chemical analysis. Tissue metal concentration and mollusc in particular are sensitive biomonitors of anthropogenic metal inputs (Hendozko et al., 2010).

Biomonitoring agents can be assessed by analyzing heavy metals in the whole tissue or certain parts or tissues of the organisms. One of the methods to increase the validity of metal data in order to truly reflect bioavailability and contamination by metals is the use of different soft tissues in the biomonitors (Yap et al., 2006a, 2006b). Knowledge on the accumulation and distribution of the metals in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals by different parts of the molluscs. It could reduce the inaccuracies of determining the heavy metal levels by using the total soft tissues (Yap et al., 2006b). It was observed that different tissues of any organisms accumulate metals at different concentration and at different rates and the biological half-lives of metals at each type of soft tissues also differ from one another (Yap et al., 2007b). The identified organ that accumulates higher metal concentrations when compared with other organs can be a potential biomonitoring organ to more effectively monitor a particular metal contaminant (Yap et al., 2006a; Edward et al., 2009). Therefore detail studies on bioaccumulation of heavy metals by different parts of the different mollusc species are essential. Numerous biotic and abiotic factors may affect the bioaccumulation of heavy metals in mollusk tissues (Chong and Wang, 2001; Mubiana et al., 2006).

Different studies on metal levels in different soft tissues of molluscs were reported in the literature. Yap et al. (2003) studied accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel Perna viridis (Linnaeus). Yap et al. (2005) studied tissue distribution of Cd, Cu, Pb and Zn in the green-lipped mussel, Perna viridis. Yap et al. (2006b) studied the different soft tissues of Perna viridis as biomonitors of bioavailability and contamination by heavy metals. Yap et al. (2008) studied the comparison of heavy metal concentrations in the different parts of Telescopium telescopium.

The objective of the present investigation was to assess trends of heavy metal (As, Cd, Cu, Pb and Zn) accumulation in different soft body tissues like mantle, gills, digestive glands and whole soft body tissues as well as to find the most

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

appropriate bioindicator organs/tissues to monitor the heavy metal pollution in freshwater ecosystem. The freshwater bivalve, Parreysia corrugata are selected for present study, because these are fairly cosmopolitan, abundant, and there have never been any published reports on the background of metal accumulation in different soft body tissues in this species.

Materials and Methods

Freshwater bivalve, Parreysia corrugata were collected from Girna dam 39.6 km away from Chalisgaon city of Jalgaon district of Maharashtra state, India. After collection animals were brought to laboratory and were cleaned and acclimatized in aquarium containing dechlorinated tap water for 10 days. During acclimatization and experiment, the animals were fed with freshwater algae and water of aquarium was changed after every 24 h. Physico-chemical parameters of tap water and metal added water were analyzed (APHA, 1998).

After acclimatization, the active, medium, uniform sized and healthy bivalves were selected by measuring their shell length, width and divided into six groups as below.

1st group was maintained as control

2nd group was exposed to chronic concentration 0.1719 ppm ($LC_{50/10}$) of As up to 20 days

3rd group was exposed to chronic concentration 0.1284 ppm ($LC_{50/10}$) of Cd up to 20 days

4th group was exposed to chronic concentration 0.033 ppm ($LC_{50/10}$) of Cu up to 20 days

5th group was exposed to chronic concentration 1.50 ppm ($LC_{50/10}$) of Pb up to 20 days

6th group was exposed to chronic concentration 1.8589 ppm ($LC_{50/10}$) of Zn up to 20 days

For each metal LC_{50} values for 96 h were determined by Probit analysis method (Finney, 1971). The average values of LC_{50} for each metal were calculated and average concentration was diluted ten times to expose to the experimental bivalve for each metal separately. Ten animals from each of experimental and control groups were dissected after 10 and 20 days of exposure period and their soft body tissues like mantle, gills, digestive glands and whole soft body tissues of each animal were dried in oven at 80^0C till constant weight was obtained. After oven drying, dry tissues were weighed and blended into powder. From each tissues powder, respective metal was analyzed by using Atomic Absorption Spectrophotometer (AAS) (Thermo Scientific, U.K. make, Solaar S series model).

Each observation was confirmed by taking at least three replicates. Results are expressed as mean (±) standard deviation (SD). Difference among the mean values of control and experimental animal groups were analyzed by Student's t test (Bailey, 1995). Differences were considered statistically significant at, *P<0.05, **P<0.01, ***P<0.001, NS-Non significant.

Results and Discussion

The physico-chemical parameters of tap water and metal added water was analyzed during experiment and are summarized in table no. 1. Table no. 2 shows the levels of accumulated heavy metal concentration (µg/g of dry tissue weight) in different soft body tissues of experimental freshwater bivalve, Parreysia corrugata after chronic exposure to As, Cd, Cu, Pb and Zn for 10 and 20 days. Obtained results revealed that concentration of heavy metals was gradually increased in all soft body tissues of experimental bivalves as the exposure period was increased and also bivalve's ability to limit the bioaccumulation of different metal varied from organ to organ. Higher accumulation of heavy metals was observed in the digestive glands of experimental bivalves as compared to other studied tissues. The order of heavy metal accumulation observed in different soft body tissues of experimental bivalve was, digestive glands > whole soft body >gills > mantle.

The obtained results indicate that the amount of heavy metal accumulation depends on the kind of bivalve tissue and chemical nature of heavy metal. Concentration of metals observed in the control animal's body tissues indicates presence of these metals in natural ecosystem. A reduced metal levels in control bivalves point out slow and gradual depuration of metals by bivalves.

The organ wise pattern of heavy metal accumulation observed during the present investigation was due to the fact that, the physiologic function of each tissue is independent (Bebianno and Serafim, 2003). This directly influences the distribution of metals in the different soft body parts (Yap et al., 2007a). Due to the affinity of certain metals towards the muscles of organisms, different levels of accumulations were observed in different body parts. The difference in the affinities of the metals to the binding sites of the metallothioneins in the different soft tissues (Viarengo et al., 1985) could affect the different metal levels found in the bivalves. The binding of toxic metal to metallothionein could fix the metals within the different tissues, which could result in slow turnover times of the heavy metals (Roesijadi, 1980). The formation of a metal-thiolate complex with cysteine residues located inside the lysosomes caused the slower depuration of the metals found in different tissues (Yap et al., 2003). Different rates of accumulation and excretion of metals in different tissues can also result in different concentrations found in each of the tissues of molluscs (Yap et al., 2003). The function or the location of a specific organ in the bivalves could also be associated with the metal accumulation in different tissues.

In the present study the digestive glands of the experimental bivalve, accumulated the highest concentrations of As, Cd, Cu, Pb and Zn as compared to other studied soft body tissues. The obtained results are in agreement with results reported previously by several other investigators (Bustamante et al., 2004; Zorita et al., 2007; Machreki-Ajmi et al., 2008). The digestive glands of the molluscs play an important role in heavy metal metabolism and contribute to their metal detoxification (Saha et al., 2006). This could explain the high metal concentrations in these organs. Elevated metal concentrations in digestive glands probably are associated to high amounts of metallothioneins in digestive glands (Amiard et al., 2006). The intracellular digestion and absorption of the food takes place in the digestive cells of the digestive gland. This may be attributed to the high concentration of metals observed in the digestive gland (Krishnakumar et al., 1990).

Different concentrations of heavy metals in different soft tissues of molluscs have been reported in the literature (Yap et al., 2003; Szefer et al., 2006; Yap et al., 2006a). The inherently high levels of metallothionein in the digestive gland of Littorina littorea explain the role of this tissue as a major site for cadmium storage (Bebianno and Langston, 1992). Bargagli et al. (1996) reported that the Antarctic scallop Adamussium colbecki accumulated as much as 100 µg g^{-1} dry weight of Cd in their digestive glands. Jantataeme et al. (1996) observed increase in the accumulation of lead in the digestive gland of snail F. m. martensi after long term exposure period.

Gagnon et al. (2006) reported higher concentrations of bioaccumulated Cu and Zn in the digestive glands of caged mussel, Elliptio complanata after exposure to metals in a primary treated municipal wastewater plume. Connan et al. (2007) noted from investigation that, the organs with the highest concentration of ^{210}Po are the digestive glands, the gills and the mantle in oysters. Berandah et al. (2010) reported highest concentrations of Cd in the digestive glands of Chicoreus capucinus L. Waykar and Shinde (2011), Waykar et al. (2011)

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

and Waykar and Deshmukh (2012) reported that heavy metal accumulation was increased with increase in exposure period in freshwater bivalves after chronic exposure to As, Cd, Cu, Hg, Pb and Zn.

The finding of this study indicate that the digestive glands of experimental freshwater bivalve, P. corrugata accumulated higher levels of arsenic, cadmium, copper, lead and zinc than mantle, gills and whole soft body tissues . Therefore these results indicate that, digestive glands of experimental bivalves are a potential biomonitor organ for monitoring of ambient levels of dissolved metals in the freshwater ecosystem.

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Table 1: Physico chemical parameters of tap water and metal added water.

Sr. No.	Sample	Parameters						
		pH	Temperature ^{0}C	Conductivity (µmho/cm)	Chlorides (mg/l)	Salinity (mg/l)	Total alkalinity (mg/l)	Total hardness (mg/l)
1	Tap water	7.30±0.62	25±1.36	215.3±5.13	57.13±1.52	103.15±3.81	109.4±5.23	197.2±5.25
2	As	7.71±0.80	25±1.36	228.3±5.36	60.14±1.59	108.58±3.26	126.14±4.12	238.1±7.79
3	Cd	7.51±0.75	25±1.36	229.7±4.78	58.07±1.15	104.85±4.96	127.1±2.10	214.2±8.16
4	Cu	7.49±0.37	25±1.36	234.1±6.61	64.13±1.15	115.78±2.35	122.7±5.57	224.8±6.99
5	Pb	7.42±0.86	25±1.36	230.1±5.35	61.24±1.01	110.57±3.26	134.2±4.65	231.5±7.90
6	Zn	7.38±0.52	25±1.36	221.2±7.51	59.24±1.01	106.96±5.24	118.3±3.54	218.3±6.15

Table 2: Metal concentrations in mantle, gills, digestive glands, and whole soft body tissue of freshwater bivalve, Parreysia corrugata after chronic exposure to different metals (values in µg/g of dry tissue weight)

Sr. No.	Treatment	Mantle		Gills		Digestive glands		Whole soft body	
		10 day	20 day	10 day	20 day	10 day	20 day	10 day	20 day
1	Control	1.15±1.12	0.87±1.35	3.15±1.11	2.95±0.78	6.12±2.38	4.21±1.57	3.71±1.12	2.57±1.35
2	As	20.47*±2.59	33.86*±2.02	25.78^{NS}±2.09	35.18**±2.66	30.45**±2.72	52.81^{NS}±2.41	28.45**±2.19	42.51^{NS}±3.58
3	Control	2.96±0.42	2.74±0.12	9.46±1.45	7.95±1.81	14.21±1.11	13.49±1.34	6.92±1.07	5.04±0.87
4	Cd	17.96^{NS}±1.73	36.60^{NS}±1.54	25.61^{NS}±3.81	54.51*±4.73	47.74**±3.24	71.18*±2.83	27.79***±2.67	60.16^{NS}±2.80
5	Control	8.83±1.05	8.09±1.12	13.03±1.37	11.72±1.26	19.12±1.49	18.10±1.37	14.71±2.60	13.97±2.49
6	Cu	21.98^{NS}±1.37	39.6**±2.88	29.39^{NS}±1.56	49.17*±1.30	46.23**±2.36	80.28*±2.19	35.19^{NS}±2.22	56.21*±3.01
7	Control	51.78±1.88	50.89±0.79	63.87±1.61	60.53±1.39	128.61±1.97	102.79±1.15	87.98±3.67	84.34±2.59
8	Pb	80.34^{NS}±1.53	145.94***±1.48	151.32^{NS}±2.15	216.59^{NS}±3.65	178.69*±3.28	286.08^{NS}±4.12	166.71*±4.39	238.51*±3.21
9	Control	675.1±15.23	657.6±13.65	805.1±11.42	761.9±10.24	961±13.13	928.7±14.58	808.3±11.57	761.5±12.28
10	Zn	809.8*±11.02	998.7^{NS}±11.45	1074.8^{NS}±14.19	1203.9*±13.12	1324*±11.62	1532^{NS}±10.58	1096.1*±13.97	1397.7**±13.28

(±) indicates standard deviation

*P< 0.05, **P< 0.01, ***P<0.001, ^{NS}- Non significant

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

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