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BIOACCUMULATION OF METAL IN FRESHWATER PELECYPOD MOLLUSCS UNDER EXPERIMENTAL CONDITION

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ABSTRACT
The objective of the 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, Corbicula striatella, Parreysia corrugata, Lamellidens corrianus, Lamellidens marginalis* and *Indonaia caeruleus* were separately exposed to chronic concentration of arsenic (0.1719 ppm), cadmium (0.1411 ppm), copper (0.033 ppm), lead (1.50ppm), mercury (0.0443 ppm) and zinc (1.8589 ppm) up to 30 days in laboratory. It was found that the freshwater bivalve, *Lamellidens corrianus* showed the highest concentration of arsenic (37.9μg) and lead (1235.4μg), *Lamellidens marginalis* showed highest concentration of copper (826.7μg) and mercury (5.87μg), while *Parreysia cylindrica* had highest concentration of cadmium (182.62μg) and *Corbicula striatella* showed highest concentration of zinc (4139.2μg) per gm dry tissues. The bioconcentration factor (BCF) was high for arsenic and lead in *Lamellidens corrianus,* copper and mercury in *Lamellidens marginalis,* cadmium in *Parreysia cylindrica* and zinc in *Corbicula striatella*. Therefore *Lamellidens corrianus* is proposed as sentinel organism for the biomonitoring of arsenic and lead, *Lamellidens marginalis* for copper and mercury, *Parreysia cylindrica* for cadmium and *Corbicula striatella* for zinc in freshwater ecosystem.

INTRODUCTION

The presence of toxic metals poses environmental problems due to their non-degradable and persistent nature (Sarabject and Dinesh, 2007). The ingestion of metal contaminants affect not only the productivity and reproductive capabilities of organisms, but ultimately affect the health of man that depends on these organisms as a major source of protein and eventually poses greater health risk.

Therefore, there is growing need to detect and assess the level of pollutants, particularly low concentration of increasingly complex mixtures of pollutants, such as metals in aquatic ecosystems. There is increasing concern that measurement of total pollution levels does not lead to a reliable estimate of water quality. Increased recognition is given to the use of biological monitors in biomonitoring programme that can give a direct and integrated measure of aquatic health (Abdullah, 2008). Many aquatic organisms have the ability to accumulate and biomagnify metals (Davies *et al.,* 2006), which leads to concentrations several orders of magnitude higher than those of the surrounding water (Casas *et al.,* 2008). Usually, the level of pollutant accumulated in such organism's tissues is used for assessing the level of pollution in its habitat (Abdallah and Moustafa, 2002).These organisms are potentially useful in revealing the presence of known and unknown pollutants, on a sabtial and temporal scale during chronic exposure. The use of biomonitors can therefore; complement the interpretation of physical and chemical measurements in the field studies (Salanki *et al.,* 2003).

Bivalves have instead been used by several authors as bioindicators of aquatic pollution (Kljakovic-Gaspic *et al.,* 2007). Molluscs are capable of achieving tissue concentrations of metals that are 100 to 1000 times higher than those in water concentrations (Hartwig, 1995). A bivalve's potential to accumulate metals from a medium into its tissue can be estimated using bioconcentration factor (BCF). By comparing BCF, one can compare the potential of different bivalve's to uptake metals from water. In case of bivalves, physiological processes of the organisms, as well as, abiotic factors such as the physical and chemical properties of the environment and the chemical nature of the heavy metal influence on the metal accumulation of bivalve (Van Roon, 1999 and Shulkin and Presley, 2003)

It is well established that organisms vary widely in their sensitivity to different pollutants, and that no single species or monitoring system is sensitive or suitable for the detection of all possible toxic pollutants (Forbes and Forbes, 1994). The knowledge of concentration of metal in native species is very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species.

In order to use the bivalve as bioindicators in pollution-monitoring programmes, there is a need to develop a bioaccumulation database using various bivalve species which might be used in finding the most appropriate sentinel bivalve species for metal pollution monitoring programme in the freshwater ecosystem**.**

Therefore in the present study different native species of fresh water bivalves, *Parreysia cylindrica, Corbicula striatella, Parreysia corrugata, Lamellidens corrianus, Lamellidens* *marginalis* and *Indonaia caeruleus* were selected to establish a local environmental monitoring network using bivalves as bioindicator species to assess trends of As, Cu, Hg, Pb and Zn in freshwater ecosystem.

MATERIALS AND METHODS

The freshwater bivalves, *Parreysia cylindrica, Corbicula striatella, Parreysia corrugata, Lamellidens corrianus, Lamellidens marginalis* and *Indonaia caeruleus* were collected from various dams of Maharashtra state, India. After collection animals were brought to laboratory and were 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.

Experimental design

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

1st group was maintained as control

2nd group was exposed to chronic concentration 0.1719 ppm ($LC_{50}/_{10}$) of As upto 30 days

3rd group was exposed to chronic concentration 0.1411 ppm ($LC_{50}/_{10}$) of Cd upto 30 days

4th group was exposed to chronic concentration 0.033 ppm ($LC_{50}/_{10}$) of Cu upto 30 days

5th group was exposed to chronic concentration 0.0443 ppm ($LC_{50}/_{10}$) of Hg upto 30 days

6th group was exposed to chronic concentration 1.50 ppm ($LC_{50}/_{10}$) of Pb upto 30 days

7th group was exposed to chronic concentration 1.8589ppm ($LC_{50}/_{10}$) of Zn upto 30 days

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 10 days, 20 days and 30 days of exposure period and the whole body mass of each animal was dried in oven at 70°-80°C. After oven drying dry, weight of the whole body was measured.

Analysis of metals

500 mg dry powder of whole soft body tissue of control and experimental bivalves was digested in 10 mL mixture of Nitric acid: Perchloric acid in (5:1) ratio. After half hour stirring the samples were left overnight and on next day samples were digested on hot plate till the clear white fumes appeared. 10 mL volume of solution was maintained by adding acidic mixture of Nitric acid and Perchloric acid drop by drop. After allowing the flask to cool, double distilled water was added to bring the volume to 50 mL by using volumetric flask and then solution was filtered through Whatman filter paper number 41. From each tissue and background water sample, respective metal was analyzed by using Atomic Absorption Spectrophotometer (AAS).

Dry weight of each animal was used to calculate the metal concentration per unit body weight (μg/g). The bioconcentration factor (BCF) of the metals in the tissues of the bivalves was calculated by dividing the concentration in the water in which the animals were exposed. Results were expressed as mean ± standard deviation (SD). Difference among the mean values of bioaccumulated metals of control and treated bivalves were analyzed by Student's t-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Along the experiments, physico-chemical parameters of water were determined are summarized in Table 1. The patterns of accumulation of metals in six freshwater species of bivalves, after exposure to chronic concentration of As, Cd, Cu, Hg, Pb and Zn separately for 10, 20 and 30 day are summarized in Table 2 and Figs. 1 to 6. Bioconcentration factors (BCFs) of six metals in different bivalve species have been calculated and are summarized in Table 3. The data revealed a significant increase in levels of all metal concentrations and bioconcentration factor (BCF) 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. Value of metals per gm dry tissues as shown in Table 2 and Figs. 1 to 6 shows that the freshwater bivalve, *Lamellidens corrianus* accumulated the highest concentration of arsenic (37.9μg/g) and lead (1235.4μg/g), *Lamellidens marginalis* accumulated highest concentration of copper (826.7μg/g) and mercury (5.87μg/g), while *Parreysia cylindrica* accumulated highest concentration of cadmium (182.62μg/g) and *Corbicula striatella* accumulated highest concentration of zinc (4139.2μg/g) among the studied bivalve species. 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.

In this study, BCF mollusc-water (BCF m-w) refers to the concentration of a particular metal in the tissue of bivalves per concentration of that metal in water. Tolerant species of bivalves tend to restrict water tissue transfer, and thus have less accumulation in tissue. Table 3 shows the higher value of bioconcentration factor (BCF m-w) for As (323.38) and Pb (962.00) in *Lamellidens corrianus,* for Cd (1761.04) in *Parreysia cylindrica,* for Cu (30171.53) and Hg (151.68) in *Lamellidens marginalis* and for Zn (2726.21) in *Corbicula striatella*. Respectively high bioaccumulated values show that these bivalve species are best bioindicators for monitoring these metals as pollutant in water. On the other hand other species exhibited low bioconcentration factor (BCF m-w). Such low value indicated limited ability/potential of these metals to be absorbed from the water to bioacumulate in bivalve tissue. The bivalves with low bioconcentration factor for the accumulation of metal are not good for monitoring of above mentioned metal pollutants.

The observed differences in tissue metal concentration in bivalve species might be due to variation in body size, growth,

Table 1: Physico-chemical parameters of water

S. No.	Sample	Parameters pH	Temperature(°C)	Conductivity ($Ohm^{-1}cm^{2}mol^{-1}$)	Chlorides (mg/L)	Salinity (mg/L)	Total alkalinity (mg/L)	Total hardness (mg/L)
1	Tap water	8.22 ± 0.62	25 ± 1.36	1.14 ± 0.40	78.95 ± 1.52	144.87 ± 3.81	378 ± 10.23	381.2 ± 8.25
2	Tap water + $ZnSO_4$	8.35 ± 0.52	25 ± 1.36	1.08 ± 0.51	79.52 ± 1.01	145.91 ± 5.24	400 ± 9.54	383.2 ± 6.15
3	Tap water + $CuSO_4$	8.46 ± 0.37	25 ± 1.36	1.09 ± 0.61	79.94 ± 1.15	146.70 ± 2.35	406.5 ± 8.57	383.2 ± 6.99
4	Tap water + $PbNO_3$	8.33 ± 0.86	25 ± 1.36	1.12 ± 0.35	78.10 ± 1.01	143.31 ± 3.26	375 ± 7.65	384 ± 7.90
5	Tap water + $CdCl_2$	8.34 ± 0.75	25 ± 1.36	1.12 ± 0.78	79.52 ± 1.15	145.91 ± 4.96	403 ± 9.10	382 ± 8.16
6	Tap water + $HgCl_2$	8.54 ± 0.95	25 ± 1.36	1.11 ± 0.52	81.36 ± 1.68	149.29 ± 2.12	411.5 ± 7.12	384 ± 4.50
7	Tap water + $AsHNa_2O_4$	8.46 ± 0.80	25 ± 1.36	1.11 ± 0.36	80.37 ± 1.59	147.47 ± 3.26	388 ± 7.12	386.6 ± 7.79

fitness, reproductive condition, genotype of the animal difference in metabolic rate and weight. Variability in metal body concentrations between closely related species are mainly caused by interspecific differences in the biokinetics of uptake, elimination and different physiological rates such as pumping, filtration and respiration. Both physiological/biochemical responses and metal geochemistry might be responsible for the differences in metal bioaccumulation as observed in different species. Differences in metal efflux rates are also important in determining interspecific differences in accumulated metal concentrations among the bivalves. Interaction of metals in body tissues seems to vary from species to species. At the same time the responses of the organism is specific for different element and substance.

The interspecific difference in the metal concentrations was evidence that different organisms display a range of capacities varying from low accumulation of certain elements to very high accumulation (Paez-Osuna *et al.,* 2000). Wang *et al.* (2002) reported that the interactions between metal geochemistry and animal physiology determine the differences in the bioavailability among metals. The inter-specific difference of metal assimilation efficiencies might be related to the species-specific digestive physiology and absorption rate of a metal across gut epithelium (Jung and Byeong, 2005). Abdullah *et al.* (2007) and Christopher *et al.* (2010) reported that the element concentrations in molluscs differ between different species due to species-specific ability/ capacity to regulate or

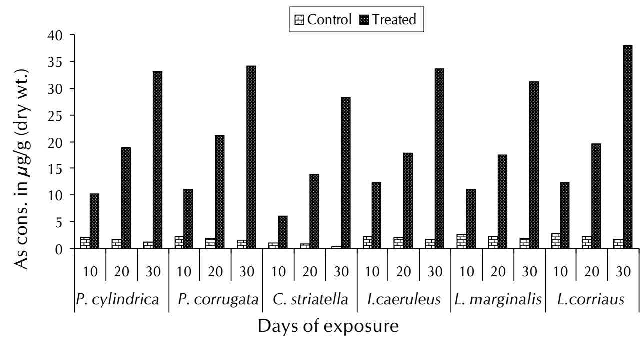

Figure 1: Arsenic concentration in exposed bivalves

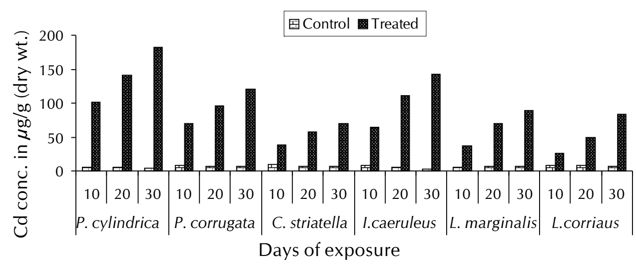

Figure 2: Cadmium concentration in exposed bivalves

accumulate trace metals.

Pillai *et al.* (1986) estimated the metal load in clams, *Vellorita cyprinoides*, *Meretrix costa*, *Crassostrea madrasensis*, *Perna viridis* and *Perna indica* and found significant variations in the concentrations of iron, zinc, copper, lead, cadmium, nickel and cobalt. Amongst the bivalves, clams showed high concentration levels of all metals especially, iron and zinc. Jasmine *et al.* (1987) found that small oysters contained significantly higher mercury content than larger ones.

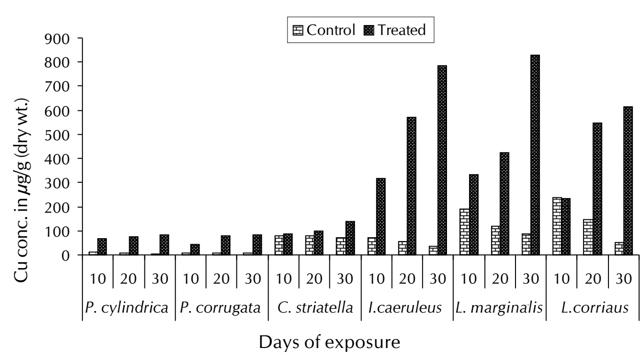

Figure 3: Copper concentration in exposed bivalves

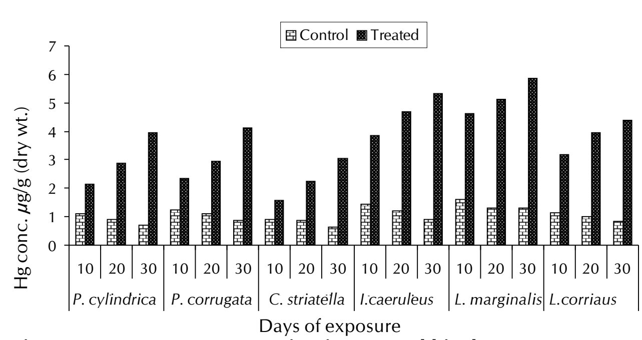

Figure 4: Mercury concentration in exposed bivalves

Table 2: Heavy metal concentrations in the whole soft body tissues of different freshwater bivalves after exposure to chronic concentrations of different heavy metals

S. No.	Metal	Exposure period in days	Bivalve species *Parreysia cylindrica* Control	Treated	*Parreysia corrugata* Control	Treated	*Corbicula striatella* Control	Treated	*Indonia caeruleus* Control	Treated	*Lamellidens marginalis* Control	Treated	*Lamellidens corrianus* Control	Treated
1	As	10	2.15 ±0.79	10.24* ±1.42	2.27 ±0.48	11.07^{NS} ±1.07	1.01 ±0.43	6.04^{NS} ±1.74	2.31 ±0.58	11.02* ±1	2.62 ±0.27	12.28^{NS} ±1.3	2.84 ±0.87	12.37^{NS} ±1.73
		20	1.75 ±0.50	18.89^{NS} ±1.51	1.95 ±0.49	21.12^{NS} ±1.39	0.78 ±0.051	13.77^{NS} ±1.8	2.13 ±0.38	17.48* ±1.48	2.31 ±0.59	17.84* ±1.03	2.26 ±0.57	19.64* ±1.26
		30	1.21 ±0.4	33.12^{NS} ±1.61	1.48 ±0.48	34.05^{NS} ±1.88	0.42 ±0.014	28.23^{NS} ±1.79	1.70 ±0.67	31.23* ±1.37	1.93 ±0.26	33.6^{NS} ±1.97	1.74 ±0.71	37.9^{NS} ±2.3
2	Cd	10	5.46 ±1.13	101^{NS} ±1.63	8.51 ±1.25	69.39^{NS} ±2.1	9±1.64	38.04^{NS} ±1.65	7.65 ±1.31	37.02^{NS} ±2	5.73 ±0.78	64.87^{NS} ±2.37	8.09 ±1.41	26.06* ±2.17
		20	4.8 ±0.75	141.67* ±2.24	7.53 ±1.54	95.74^{NS} ±1.87	7.02 ±1.24	57.92^{NS} ±1.64	5.14 ±0.87	70.06^{NS} ±1.2	6.2 ±0.81	111.08^{NS} ±2.2	8.08 ±1.57	49.53* ±4.12
		30	4.05 ±1.34	182.62^{NS} ±1.91	6.55 ±1.04	121.01^{NS} ±1.74	6.61 ±1.43	69.9^{NS} ±2.16	2.52 ±0.48	88.71* ±1.24	6.7 ±0.49	142^{NS} ±64	7.08 ±0.48	83.28^{NS} ±2
3	Cu	10	11.75 ±1.40	65.84^{NS} ±3.33	9.05 ±0.47	42.12^{NS} ±1.96	81.21 ±1.72	89.06^{NS} ±2.06	70.5 ±2.87	318.5^{NS} ±3.4	190.7 ±2.94	334.4* ±3.72	236.6 ±3.81	232.2* ±3.1
		20	8.43 ±1.26	74.79^{NS} ±1.42	8.22 ±0.46	77.87^{NS} ±1.36	79.44 ±1.59	99.8^{NS} ±1.83	56.6 ±1.57	572.5* ±5.39	117.7 ±2.54	424.5^{NS} ±5.4	147.1 ±7.22	548.8^{NS} ±7
		30	5.18 ±1.29	84.19* ±1.81	7.37 ±0.49	83.97^{NS} ±1.42	72.28 ±1.92	137.04* ±1.89	33.9 ±1.15	783.23^{NS} ±7	89 ±1.75	826.7* ±4.09	50.5 ±1.87	613.4* ±5.23
4	Hg	10	1.11 ±0.28	2.15^{NS} ±0.68	1.25 ±0.28	2.35^{NS} ±0.80	0.91 ±0.15	1.59^{NS} ±0.77	1.43 ±0.47	3.84* ±0.68	1.61 ±0.26	4.61* ±0.89	1.13 ±0.2	3.18* ±0.83
		20	0.9 ±0.11	2.87^{NS} ±0.82	1.09 ±0.28	2.95^{NS} ±0.94	0.88 ±0.08	2.24^{NS} ±0.88	1.19 ±0.34	4.68* ±0.87	1.32 ±0.26	5.12^{NS} ±0.84	1±0.26	3.96* ±0.81
		30	0.71 ±0.14	3.96^{NS} ±0.77	0.88 ±0.08	4.12^{NS} ±1.09	0.62 ±0.12	3.06^{NS} ±0.98	0.9 ±0.21	5.34* ±0.87	1.3 ±0.28	5.87* ±0.82	0.85 ±0.11	4.38* ±0.46
5	Pb	10	54.97 ±1.69	195.27^{NS} ±2.32	79.58 ±2.22	168.75* ±19	116.6 ±2.66	327.54* ±2.16	46.2 ±1.85	169.4^{NS} ±3	275.4 ±3.54	256.7* ±2.64	33.28 ±2.13	288.1^{NS} ±3.31
		20	54.28 ±1.86	231.49^{NS} ±2.14	39.33 ±1.72	400.02^{NS} ±19	80.77 ±1.7	413.29* ±1.96	34.7 ±1.38	180.6* ±2.37	189.2 ±2.62	355.7* ±4.18	96.4 ±5.18	927.1^{NS} ±10
		30	53.53 ±2.27	267.01^{NS} ±1.91	12.5 ±1.1	602.01* ±131.79	62.15 ±2.07	729.6^{NS} ±2.43	30.12 ±1.34	235.6^{NS} ±5.3	117.6 ±1.29	656.1^{NS} ±3.3	196.4 ±4.38	1235.4* ±12
6	Zn	10	1518.6 ±11.39	1853.3* ±14.95	884 ±8.02	1256.5* ±17	817.7 ±11.44	1232.2* ±12.5	935.1 ±7.38	1326.5* ±11	1208.3 ±11.78	1111^{NS} ±10.2	925.4 ±9.76	1271.5* ±13
		20	1477.2 ±9.59	1903.4^{NS} ±11.13	734.1 ±7.06	2521* ±15.66	672.5 ±9.05	1632.2* ±13.2	694.9 ±6.39	1704.4^{NS} ±14	1108.2 ±8.05	1483.9* ±12	672.4 ±7.38	1569.3^{NS} ±13
		30	1095.4 ±170.22	3538.6* ±14.59	719 ±8.64	3182.5* ±15	447.4 ±10.63	4139.2* ±17.47	552.4 ±5.49	3032.3^{NS} ±14	977.8 ±7.38	2661.4^{NS} ±15	356.2 ±5.28	1639.6^{NS} ±12

Where, * $p < 0.05$ (Significant t-test), NS- Not significant

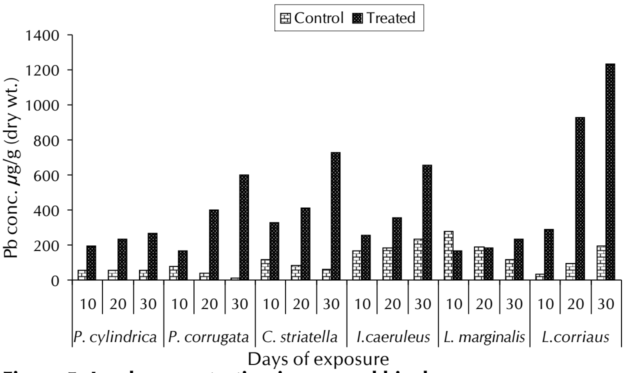

Figure 5: Lead concentration in exposed bivalves

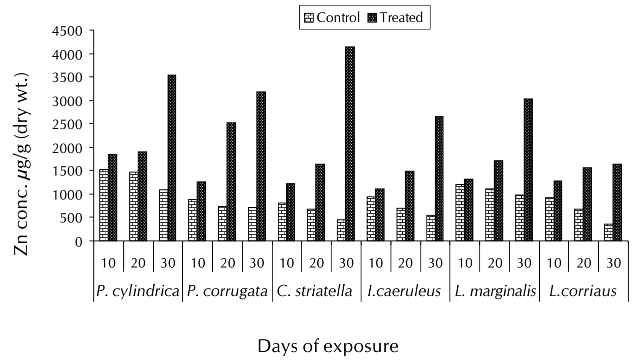

Figure 6: Zinc concentration in exposed bivalves

Table 3: Bioconcentration factor (BCF) of the whole soft body tissues of different species of freshwater bivalves after exposure to chronic concentration of different heavy metals

S. No.	Heavy metal	Metal in background water (mg/L)	Exposure period in days	Bioconcentration factor (BCF) of bivalve species *Parreysia cylindrica*	*Parreysia corrugata*	*Corbicula striatella*	*Indonia caeruleus*	*Lamellidens marginalis*	*Lamellidens corrianus*
1	As	0.1172	10	87.37	94.45	51.54	94.03	104.78	105.55
			20	161.18	180.20	117.49	149.15	152.22	167.58
			30	282.59	290.53	240.87	266.47	286.69	323.38
2	Cd	0.1037	10	973.96	669.14	366.83	356.99	625.55	251.30
			20	1366.15	923.24	558.53	675.60	1071.17	477.63
			30	1761.04	1166.92	674.06	855.45	1369.34	803.09
3	Cu	0.0274	10	2402.92	1537.23	3250.37	11624.09	12204.38	8474.45
			20	2729.56	2841.97	3642.34	20894.16	15492.7	20029.2
			30	3072.63	3064.60	5001.46	28585.04	30171.53	22386.86
4	Hg	0.0387	10	55.56	60.72	41.09	99.22	119.12	82.17
			20	74.16	76.23	57.89	120.93	132.21	102.33
			30	102.33	106.46	79.07	137.98	151.68	113.18
5	Pb	1.2842	10	152.06	131.40	255.054	131.91	199.89	224.34
			20	180.26	311.49	321.83	140.63	276.98	721.93
			30	207.92	468.78	568.14	183.46	510.90	962.00
6	Zn	1.5183	10	1220.64	827.57	811.57	873.67	731.74	837.45
			20	1253.64	1660.41	1075.02	1122.57	977.34	1033.59
			30	2330.63	2096.09	2726.21	1997.17	1752.88	1079.89

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.

The finding of this study showed that the concentration and BCF value for arsenic and lead was highest in the *Lamellidens corrianus*, for copper and mercury was highest in *Indonaia caeruleus*, and for cadmium was highest in *Parreysia cylindrica* while *Corbicula striatella* showed the highest concentration and BCF value of zinc. Therefore these results indicate that *Lamellidens corrianus* is sentinel organism for the biomonitoring of arsenic and lead, *Lamellidens marginalis* for copper and mercury, *Parreysia cylindrica* for cadmium and *Corbicula striatella* for zinc in fresh water ecosystem.

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