Journal of
Environmental & Analytical Toxicology

Ranjan and Babu, J Environ Anal Toxicol 2016, 6:4
DOI: 10.4172/2161-0525.1000385

Research Article Open Access

Heavy Metal Risk Assessment in Bhavanapadu Creek Using Three Potamidid Snails - *Telescopium telescopium, Cerithidea obtusa* and *Cerithidea cingulata*

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Abstract

The study area experiences the sea water influx during high tide and fresh water during rainfall, flooding season etc. The Creek area is beset mostly with dwarf mangroves on either side, aquaculture ponds, agriculture fields, saltpans etc. The Creek life is influenced by these activities either directly or indirectly throughout the year. It is an almost unexplored area and is represented with diverse fauna and flora. In observation of that, the current investigation was undertaken to study the status of bioaccumulation of heavy metals, specifically Cu, Cd, Zn, Pb, Ni and Fe in soft tissues namely, in foot, digestive caecum, total body and operculum and shells of three different species of molluscs namely *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* using the Flame Atomic Absorption Spectrophotometer. All the outcomes were expressed in µg/gram of dry weight and the end result reveals reflective amounts of heavy metals, except Cd, Pb and Ni. The statistical analysis of all *in vitro* studies of heavy metal concentration within and in between the matching organs of all tested species with oneway ANOVA showed significant difference except one. It can be concluded that, although molluscs provide information on the bioavailability of contaminants in ecosystems, it is hardly possible to derive any predictions of biological effects of these pollutants at the given level of exposure; such predictions are the main perspectives of biological effect monitoring.

Keywords: Bioaccumulation; *Cerithidea cingulata*; *Cerithidea obtusa*; Heavy metals; *Telescopium telescopium*

Introduction

Human activities can increase metal concentrations to higher than background levels. The mining and processing ores, domestic waste water effluents, storm water runoff and industrial wastes and discharges are certain main anthropogenic sources of heavy metal pollution [1]. Heavy metal pollution in aquatic ecosystem has been recognized as a serious environmental problem. In many cases, heavy metals occur in natural water bodies at levels below their toxic thresholds, however, due to their non degradable nature, such low concentrations may still pose risk of damage via uptake and subsequent bioaccumulation by organisms, which cannot be effectively metabolized and these absorbed metals are extracted. Several scientific observations have shown that heavy metals are bio concentrated or bioaccumulated in one or several compartments across food webs [2,3]. Besides, the contamination of resources with trace elements may have devastating effect on the natural ecosystem functioning, as well as cause a decrease of biodiversity and extinction of sensitive taxa [4]. Metal bioaccumulation can be of importance from the public health point of view, especially when humans consume the accumulators. Secondly, this phenomenon is now being exploited in the assessment of environmental quality, in addition to chemical surveys of water and sediment [5].

More and more attention has been drawn due to the wide occurrence of metal pollution in the aquatic system. Monitoring and prevention of heavy metal pollution is one of the hot topics in researches [6]. As shown in a review by Zhou et al. [6] many of the bioindicator papers were about metal pollution, wherein plants, invertebrates, fish and mammals were the dominant used bioindicator species. Each bioindicator shows the special merits for the biomonitoring of metal pollution in aquatic ecosystem when compared to the others.

Scrutinizing the literature on trial potamidid Snails, there is paucity of information on bioindicator studies with respect to heavy metal pollution of the Bhavanapadu creek study area. Hence the current investigation was undertaken to study the status of bioaccumulation of heavy metals, specifically Cu, Cd, Zn, Pb, Ni and Fe in soft tissues namely, in foot, digestive caecum, total body and operculum and shells of three different species of molluscs namely *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata*.

Materials and Methods

Chemicals

Chemicals and reagents used for the study were purchased from Merck. All additional chemicals used were analytical grade. Altogether the experiments were performed at room temperature unless otherwise stated.

Collection of test samples

To avoid differences in metal content because of size or reproductive stage, only the commercial sized, forty individuals each for three different species of molluscs namely *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* were collected according to Saavedra et al. [7]. The gastropods were dissected and pooled into different tissues such as foot, digestive caecum, total body, operculum and shell. The shells and all different categories of tissues were dried at 60°C to constant dry weights. The dried tissue was reduced into fine powder in a pestle and mortar and was stored in dessicator for further analysis. The shell of individual species of molluscs was also finely ground. The resulting powder was collected, using a plastic sieve with 0.2 mm opening size and was stored in desiccator for further analysis.

Flame atomic absorption spectrophotometer

About 0.5 gm of the test samples were digested in 10 ml of concentrated nitric acid (AnalaR Grade; 69%). They were placed in a

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Received June 21, 2016; **Accepted** July 11, 2016; **Published** July 19, 2016

Citation: Joseph TUR, Ramesh KB (2016) Heavy Metal Risk Assessment in Bhavanapadu Creek Using Three Potamidid Snails - *Telescopium telescopium, Cerithidea obtusa* and *Cerithidea cingulata*. J Environ Anal Toxicol 6: 385. doi:10.4172/2161-0525.1000385

hot block digester first at low temperature (40°C) for one hour and were then fully digested at high temperature (140°C) for at least three hours. The digested samples were then diluted to a volume of 40 ml with double distilled water. The sample was then filtered through Whatman No.1 filter paper (Dia: 110 mm), then they were analysed for Cu, Cd, Zn, Pb, Ni and Fe by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Varian 220 Spectra AA. The samples were analyzed in three replicates. The data was presented in µg/g of dry weight. Multi-level calibration standards were analyzed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/L stock solutions of each metal (Merck Titrisol). All the glassware and plastic materials used were acid-washed in 10% concentrations of acid in order to minimize external contamination [8].

Statistical analysis

Oneway analysis of variance (ANOVA) was used to know the significance of heavy metal concentration within and in between the matching organs of all tested species. 'P' value less than 0.003 was considered as significant difference in the analysis. All the statistical analysis was resolved using SPSS software.

Results and Discussion

Metal bioavailability to organisms depends on various factors including geochemical and biological processes. Determination of metal concentration in an organism provides information on the bioavailable fraction of metal. However, measurement of metal in tissues does not provide information regarding the process controlling metal intake. Previously in Asia, investigations on the measurement, distribution and fate of heavy metals in the marine environment have been reported for a number of countries including Thailand, Malaysia, Japan, Korea and China [9-14]. Bhavanapadu Creek (Long: 18° 33' 52" to 18° 32' 11" N; Lat: 84° 21' 26" E to 84° 18' 22" E) is located on the North East coast of Andhra Pradesh, adjoining the Bay of Bengal (Figure 1). The Bhavanapadu Creek mouth is an ecosystem harbouring rich and vulnerable species [15]. Luxuriant mangroves cover an approximately 2000 hectares of mangrove marshy land, mostly dominated by eight species of halophytes, two sea grasses [16] and nineteen molluscs [17]. The study area experiences the sea water influx during high tide and fresh water during rainfall, flooding season etc. The Creek area is beset mostly with dwarf mangroves on either side, aquaculture ponds, agriculture fields, saltpans etc. The Creek life is influenced by these activities either directly or indirectly throughout the year. It is an almost unexplored area and is represented with diverse fauna and flora including the molluscs, among which the abundantly existing three Potamidid snails-*Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* (Figure 2) were identified and selected for the cram.

It was observed that the magnitude of heavy metal accumulation in snails tissues depend upon type of heavy metal and the species of the snail. The observed differences in tissues metal concentrations between snail species might be due to variation in reproductive condition, genotype of the animal, difference in metabolic rate, body weight, trophic position, presence or absence of enzyme system that can degrade the pollutants [18]. Variability between closely related species was reflected by difference in the biokinetics of uptake, elimination and different physiological rates such as pumping, filtration and respiration. These qualities are specific for different species. Element concentrations in molluscs differ between different species due to species-specific ability/ capacity to regulate or accumulate trace metals [19] and might be related to the species-specific digestive physiology and absorption rate of a metal across gut epithelium [20]. Differences in metal efflux rates are also important in determining interspecific differences in accumulated metal concentrations among the snail's species. 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 [21]. Therefore, two species that live in a same place can differ in the types and concentrations of metals they accumulate [22]. The species which are more tolerant to respective metal accumulates more metal, while less tolerant species accumulate less metal or may not survive. It was also known that aquatic molluscs possess very diverse strategies in the handling and storage of accumulated metals [23].

The in progress exploration reveals that, in *Cerithidea cingulata* towering amount of copper was found in digestive caecum 143.0 ± 4.1 and in foot 124.0 ± 4.9 and moderate amounts were found in total body and in operculum with 94.7 ± 5.1 and 59.1 ± 4.0. Very less amount 5.3 ± 1.5 was observed in shell. In *Telescopium telescopium* modest amounts of copper was found in operculum, foot, digestive

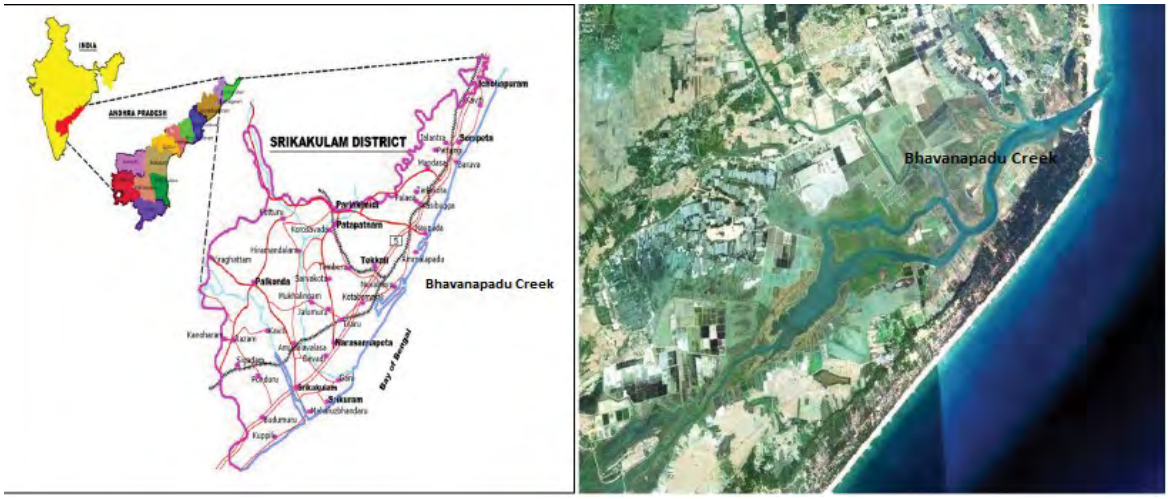

Figure 1: Study area and satellite map.

Telescopium telescopium *Cerithidea cingulata*

Cerithidea obtusa

Figure 2: Test molluscs.

caecum and total body; 50.7 ± 1.9, 95.1 ± 4.8, 88.2 ± 4.2 and 68.9 ± 2.5 respectively. Diminutive amount 9.9 ± 0.95 was experiential in shell. In *Cerithidea obtusa* high amount of copper was found in foot and total body with 116.2 ± 7.2 and 94.7 ± 4.1. Modest amount was found in digestive caecum and in operculum with 74.3 ± 1.4 and 42.3 ± 2.7 and less amount 3.47 ± 0.43 of copper was found in shell.

Elevated amount of cadmium was observed in shell of *Telescopium telescopium* with 3.12 ± 0.96 and 2.78 ± 0.44, 4.3 ± 0.98 and 1.79 ± 0.45 were observed in operculum, foot and in total body correspondingly. In *Cerithidea cingulata* high amount of cadmium was practical in operculum by means of 2.46 ± 0.16, where as in total body, foot and in shell were experienced with 1.4 3± 0.07, 1.22 ± 0.21 and 0.01 ± 0.007 in that order. Traces of cadmium was observed in *Cerithidea obtusa*, operculum, foot, shell and total body by means of 0.03 ± 0.007, 0.02 ± 0.003. 0.36 ± 0.07 and 0.001 ± 0.0002 respectively. Captivatingly no cadmium was observed in digestive systems of all three tested samples.

In *Telescopium telescopium* soaring amount of zinc was experimental in total body by 113.0 ± 5.7 and little amount was observed in shell by way of 5.91 ± 1 and restrained amount was observed in digestive caecum, foot and in operculum with 65.0 ± 5.1, 56.9 ± 2.9 and 43.9 ± 4.0 in the same way. In *Cerithidea cingulata* elevated amount of zinc was observed in total body and in foot through 113.0 ± 5.74 and 93.0 ± 3.4 respectively. Whereas, in digestive system and in operculum 77.5 ± 6.5 and 38.9 ± 3.3 were noticed likewise. Traces of zinc specifically 3.33 ± 0.3 were pragmatic in shell. In *Cerithidea obtusa* high amount of zinc was observed in total body with 77.3 ± 4.5 and moderate amount in foot and digestive system with 49.1 ± 4.6 and 35.6 ± 1.9. Stumpy amount of zinc was observed in operculum and in shell by way of 14.3 ± 2.8 and 6.36 ± 0.84 in that order.

Lofty amount of lead was observed in shell of *Telescopium telescopium* through 54.2 ± 4.5. Traces of lead were observed in operculum and in total body by 9.45 ± 2.19 and 10.0 ± 2.8. In *Cerithidea cingulata,* shell restrained high amount of lead 38.6 ± 0.54 and operculum with 16.3 ± 2.9, whereas stumpy amount was in total body with 0.94 ± 0.28. In *Cerithidea obtusa,* 0.89 ± 0.13 and 21.36 ±4.8 of lead was observed in total body and in shell respectively. Foot and digestive caecum of *T. telescopium, C. cingulata* and along with the above, operculum of *C. obtusa* were not distinguished with lead.

In *Telescopium telescopium* gigantic and a reduced amount of nickel was observed in shells by means of 10.4 ± 1.6 and operculum with 0.22 ± 0.09, where as in total body 4.77 ± 0.9 of nickel was practical. In *Cerithidea cingulata* 2.89 ± 0.29, 0.34 ± 0.12 and 1.79 ± 0.67 of nickel was observed in operculum, shell and in total body respectively. No nickel was observed in foot and digestive caecum of *Telescopium telescopium* and in *Cerithidea cingulata*. In *Cerithidea obtusa,* operculum and shell were identified with 0.24 ± 0.1 and 1.22 ± 0.33 nickel correspondingly. Whereas in the foot, digestive caecum and total body nickel was not found.

In *Telescopium telescopium*, *Cerithidea cingulata* and in *Cerithidea obtusa* high amount of iron was noticed in total body with 1782.0 ± 5.6, 1543.0 ± 4.5 and 1458.0 ± 8.2 in that order. In *Telescopium telescopium*, operculum had 921.0 ± 5.3 and digestive caecum with 851.4 ± 2.1 of iron. Fewer amounts were observed in foot and shell 166.5 ± 4.0 and 248.2

± 11.0 respectively. Operculum and digestive caecum of *Cerithidea obtusa* had 844.0 ± 4.1 and 406.0 ± 7.3 of iron. Diminutive amount 173.3± 4.2 was identified in shell. Iron content of *Cerithidea cingulata* operculum, digestive caecum, foot and shell was 638.0 ± 12.5, 406.0 ± 3.9, 255.0 ± 6.0 and 189.5 ± 5.9 respectively. All the above mentioned results were expressed in µg/gram of dry weight. The statistical analysis of all *in vitro* (n=3) studies of heavy metal concentration within and in between the matching organs of all tested species with oneway ANOVA showed significant difference except one. All the above fallouts were put on show in Table 1 and oneway ANOVA between heavy metals in different parts of three tested species were also displayed in addition (Tables 2-6).

In a number of previous investigations on the metal pollution of coastal sites and estuaries, various number of the indigenous biota have been engaged to evaluate the bioavailability levels of metals in the marine environment. It is known that molluscs accumulate organic and metallic pollutants at concentrations several orders of magnitude above those observed in the environment [24]. The accumulation of heavy metals has been reported in different molluscan species by different authors. Irato et al. [25] evaluated the accumulation of heavy metals in three species of bivalve molluscs *M. galloprovincialis*, *Scapharca inaequivalvis* and *T. philippinarum.* Shanmugam et al. [26] estimated the bioaccumulation of some trace metals in a marine neogastropod *Cymbium melo*. Similarly from bivalves in *P. viridis* [27] and in 15 species of benthic invertebrates [28]. Kone et al. [29] studied the comparative survey of the levels of contamination of heavy metals in gastropod *Tympanotonus fuscatus.* Gabr and Gab-Alla [30] studied the effect of transplantation of heavy metals in two species namely *Ruditapes decussates* and *Venerupis pullastra*. Kesavan et al. [31] in *Telescopium telescopium* and Asha et al. [32] in *Marcia opima* and *Donax cuneatus.*

Conclusion

Snails have been successfully used as bioaccumulation indicators or monitors in the past and will also play a prominent role in this area of environmental surveillance in the future. It is noted that particular organ may be more effective tool than the whole soft tissue to monitor given metal in the mangrove mudflat. Nevertheless, it has to be considered that such studies can only offer rather limited insights into the ecological and ecotoxicological relevance of the actual pollutant exposure in the environment. Although they provide information on the bioavailability of contaminants in ecosystems, it is hardly possible to derive any predictions of biological effects of these pollutants at the given level of exposure; such predictions are the main perspectives of biological effect monitoring.

Species	Different organs	Copper (Cu)	Cadmium (Cd)	Zinc (Zn)	Lead (Pb)	Nickel (Ni)	Iron (Fe)
T. telescopium	Operculum	50.7 ± 1.9	2.78 ± 0.44	43.9 ± 4.0	9.45 ± 2.19	0.22 ± 0.09	921.0 ± 5.3
	Foot	95.1 ± 4.8	4.3 ± 0.98	56.9 ± 2.9	-	-	166.5 ± 4.0
	Digestive caecum	88.2 ± 4.2	-	65.0 ± 5.1	-	-	851.4 ± 2.1
	Shell	9.9 ± 0.95	3.12 ± 0.96	5.91 ± 1.0	54.2 ± 4.5	10.4 ± 1.6	248.2 ± 11.0
	Total body	68.9 ± 2.5	1.79 ± 0.45	113.0 ± 5.7	10.0 ± 2.8	4.77 ± 0.9	1782.0 ± 5.6
C. obtusa	Operculum	42.3 ± 2.7	0.03 ± 0.007	14.3 ± 2.8	-	0.24 ± 0.1	844.0 ± 4.1
	Foot	116.2 ± 7.2	0.02 ± 0.003	49.1 ± 4.6	-	-	252.3 ± 10.4
	Digestive caecum	74.3 ± 1.4	-	35.6 ± 1.9	-	-	406.0 ± 7.3
	Shell	3.47 ± 0.43	0.36 ± 0.07	6.36 ± 0.84	21.36 ± 4.8	1.22 ± 0.33	173.3 ± 4.2
	Total body	94.7 ± 4.1	0.001 ± 0.0002	77.3 ± 4.5	0.89 ± 0.13	-	1458.0 ± 8.2
C. cingulata	Operculum	59.1 ± 4.0	2.46 ± 0.16	38.9 ± 3.3	16.3 ± 2.9	2.89 ± 0.29	638.0 ± 12.5
	Foot	124.0 ± 4.9	1.22 ± 0.21	93.0 ± 3.4	-	-	255.0 ± 6.0
	Digestive caecum	143.0 ± 4.1	-	77.5 ± 6.5	-	-	406.0 ± 3.9
	Shell	5.3 ± 1.5	0.01 ± 0.007	3.33 ± 0.3	38.6 ± 0.54	0.34 ± 0.12	189.5 ± 5.9
	Total body	94.7 ± 5.1	1.43 ± 0.07	113.0 ± 5.74	0.94 ± 0.28	1.79 ± 0.67	1543.0 ± 4.5

Each value represents the mean ± SD of three replicates

Table 1: Heavy metal concentrations (µg/gram of dry weight) in unlike soft tissues and shells of tested species.

	Operculum	Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	423.36	2	211.68	22.909	0.002
	Within Groups	55.44	6	9.24		
	Total	478.8	8			
Cd	Between Groups	13.57	2	6.785	89.452	0
	Within Groups	0.455	6	0.076		
	Total	14.025	8			
Zn	Between Groups	1506.32	2	753.16	65.115	0
	Within Groups	69.4	6	11.567		
	Total	1575.72	8			
Pb	Between Groups	403.461	2	201.73	46.225	0
	Within Groups	26.184	6	4.364		
	Total	429.645	8			
Ni	Between Groups	14.106	2	7.053	201.677	0
	Within Groups	0.21	6	0.035		
	Total	14.315	8			
Fe	Between Groups	128454	2	64227	958.85	0
	Within Groups	401.9	6	66.983		
	Total	128855.9	8			

P<0.003 was considered as significant difference

Table 2: Heavy metal concentration in operculum of three tested species through ANOVA.

	Foot	Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	1339.193	2	669.597	20.001	0.002
	Within Groups	200.865	6	33.478		
	Total	1540.059	8			
Cd	Between Groups	29.245	2	14.622	43.257	0
	Within Groups	2.028	6	0.338		
	Total	31.273	8			
Zn	Between Groups	3288.999	2	1644.5	120.588	0
	Within Groups	81.824	6	13.637		
	Total	3370.823	8			
Pb	Between Groups	0	2	0	.	.
	Within Groups	0	6	0		
	Total	0	8			
Ni	Between Groups	0	2	0	.	.
	Within Groups	0	6	0		
	Total	0	8			
Fe	Between Groups	15218.347	2	7609.173	142.284	0
	Within Groups	320.873	6	53.479		
	Total	15539.22	8			

P<0.003 was considered as significant difference.

Table 3: Heavy metal concentration in foot of three tested species through ANOVA.

	Digestive caecum	Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	7915.94	2	3957.97	322.31	0
	Within Groups	73.68	6	12.28		
	Total	7989.62	8			
Cd	Between Groups	0	2	0	.	.
	Within Groups	0	6	0		
	Total	0	8			
Zn	Between Groups	2776.22	2	1388.11	57.147	0
	Within Groups	145.74	6	24.29		
	Total	2921.96	8			
Pb	Between Groups	0	2	0	.	.
	Within Groups	0	6	0		
	Total	0	8			
Ni	Between Groups	0	2	0	.	.
	Within Groups	0	6	0		
	Total	0	8			

Fe	Between Groups	396821.71	2	198410.85	8.08E+03	0
	Within Groups	147.287	6	24.548		
	Total	396969	8			

P<0.003 was considered as significant difference.

Table 4: Heavy metal concentration in digestive caecum of three tested species through ANOVA.

	Shell	Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	65.671	2	32.836	29.364	0.001
	Within Groups	6.709	6	1.118		
	Total	72.381	8			
Cd	Between Groups	17.412	2	8.706	27.825	0.001
	Within Groups	1.877	6	0.313		
	Total	19.29	8			
Zn	Between Groups	16.04	2	8.02	13.208	.006*
	Within Groups	3.643	6	0.607		
	Total	19.683	8			
Pb	Between Groups	1619.121	2	809.56	56.546	0
	Within Groups	85.901	6	14.317		
	Total	1705.021	8			
Ni	Between Groups	187.608	2	93.804	99.335	0
	Within Groups	5.666	6	0.944		
	Total	193.274	8			
Fe	Between Groups	9321.583	2	4660.791	80.466	0
	Within Groups	347.533	6	57.922		
	Total	9669.116	8			

*denotes insignificance; P<0.003 was considered as significant difference.

Table 5: Heavy metal concentration in shells of three tested species through ANOVA.

	Total body	Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	1331.28	2	665.64	40.828	0
	Within Groups	97.82	6	16.303		
	Total	1429.1	8			
Cd	Between Groups	5.372	2	2.686	38.354	0
	Within Groups	0.42	6	0.07		
	Total	5.792	8			
Zn	Between Groups	2548.98	2	1274.49	44.589	0
	Within Groups	171.5	6	28.583		
	Total	2720.48	8			
Pb	Between Groups	165.078	2	82.539	30.172	0.001
	Within Groups	16.414	6	2.736		
	Total	181.492	8			
Ni	Between Groups	34.898	2	17.449	38.41	0
	Within Groups	2.726	6	0.454		
	Total	37.624	8			
Fe	Between Groups	169311.74	2	84655.868	2.15E+03	0
	Within Groups	236.607	6	39.434		
	Total	169548.34	8			

P<0.003 was considered as significant difference.

Table 6: Heavy metal concentration in total body of three tested species through ANOVA.

Acknowledgements

The authors are grateful to the Head, Department of Marine Living Resources, Andhra University for the facilities.

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