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Heavy Metal Concentration from Biologically Important Edible Species of Bivalves (*Perna viridis and Modiolus metcalfei*) from Vellar Estuary, South East Coast of India

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Abstract

Commonly mollusks are filter feeding in habits so while feeding from the mud the unwanted chemicals and metals are accumulate in the organs like gills, foot and mantle. These edible bivalves can better be used for biomonitoring in case of the food safety at regularly used by the local fisherman community. So it is very important necessity to study about edible mollusks and with the aim of the present study is carried. Assessment of heavy metal such as Cd, Cr, Cu, Pb and Zn accumulation of seven different body parts viz, Foot, Mantle, Gonad, Adductor muscle, Byssal thread, Gills and shell from two different edible bivalve species of *Perna viridis* and *Modiolus metcalfei* were carried briefly. Among all the metals, Zn was higher and Cd was lower concentration were observed from two different bivalve and their values varied with respect to different body parts. The accumulation of these five heavy metals ranged as Cd (0.022-0.091 µg/g), Cr (0.147-0.447 µg/g) Cu (0.126-0.356 µg/g), Pb (0.145-1.57 µg/g) and Zn (0.964–8.607 µg/g) for *P.viridis* and *M. metcalfei* ranged as Cd (0.013-0.095 µg/g), Cr (0.092-0.495 µg/g) Cu (0.063-0.367 µg/g), Pb (0.528-1.263 µg/g) and Zn (2.172-11.113 µg/g). Cluster analysis (Bray-Curtis Similarity) was used for making the similarity percentage between different body parts of edible bivalves and comparison also done with sediment metal concentration. In general, all heavy metals are less than the permissible level according to WHO/EPA except Cr and Pb from both bivalves.

Keywords: Heavy metals; Estuary; Sediment; *Perna viridis; Modiolus metcalfei*

Introduction

Heavy metals are persistent pollutants in aquatic ecosystems. The trace metal occurs in all compartments of the marine environment and has a tendency to accumulate in organisms from different tropic levels of marine food webs. The accumulation of trace metals in aquatic organisms can pose a long-term burden on biogeochemical cycling in the ecosphere. Bioaccumulation becomes an environmental problem when chemicals accumulated are toxic. Toxicity may occur along the food chain when the contaminated species or a substance is consumed [1]. An unaltered chemical like copper element can be eliminated rapidly residues will not accumulate and tissue less likely damaged [2]. Once trace metals enter the food chain, they may accumulate to dangerous levels and be harmful to human health [3]. Marine molluscs such as oysters, mussels, cockle and clams have been widely employed as biomonitors for heavy metals pollution due to their ability to accumulate metals without harming themselves. The reliable use of marine molluscs as indicator organisms for metal contamination requires an understanding of how environmental parameters effect metal accumulation. Particularly bivalve molluscs have been considered as a potential biomonitor for metallic contamination in marine ecosystems [4]. They are sedentary and sessile filter-feeders, having a wide geographical distribution. Because of their abundance in coastal water and their ability to accumulate several classes of pollutants, they have been chosen as a suitable organism for mussel watch monitoring programs [5]. Marine organisms are characterized by a greater spatial ability to accumulate some metals when compared with bottom sediments [6]. Filter feeding bivalves capable to accumulate heavy metals in their tissues and numerous studies were attributed. Mussels can accumulate Cd in their tissues at levels up to 100,000 times higher than the level observed in the water in which they live [7].

The wide use of shellfish reflects not only the high capacity of these organisms to bioaccumulation of organic and inorganic contaminants and their widespread distribution, but also their importance, because shellfish represent an important source of protein for coastal communities. It has been predictable, for instance, that over 90% of human health exposure to several contaminants occurs through diet primarily seafood and meat [8,9]. The use of bivalves as biomonitors of heavy metal pollution has been widely reported. This is due to their characteristics from ecological and biological points of view which are advantageous for biomonitoring. *Perna viridis* and *Modiolus metcalfei* are usually found in the tropical intertidal area including mangrove trees and estuarine mudflats. Although their abundance and distribution are usually found in Vellar estuary, Southeast coast of India, Tamil Nadu, it's prospective as a biomonitor has not been reported. Hence the present study was estimate the heavy metal concentrations such as Cd, Cr, Cu, Pb and Zn in different tissue part of Green mussel (*Perna viridis*) and Horse mussel (*Modiolus metcalfei*) collected from Vellar Estuary.

Materials and Methods

Study area

The vellar estuary lies between (Lat 110º 30` N; Long. 79º 46`E), the southeast coast of India, which originates from the Shervarayan

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hills of Salam district in Tamilnadu, India. After traversing distance of about 480 Km, it forms an extensive estuarine system at parangipettai, before it joins with the Bay of Bengal. It has fringed with variety of mangrove plants, mud flat shrubs which serve as significant breeding ground for number of marine and brackish water organisms. It is one of the fertile estuaries in Tamil Nadu. Basically estuaries serve as both pollution sources for the coastal sea and marginal filter to the polluted runoff from the river drainage basin. Particularly Vellar estuary highly influenced by human inhabitation, mechanized boat withstand on estuary, agriculture and aquaculture activities, which could possibly introduce consideration level of contaminants into the water body as well as mussels. Due to this reason accumulation of metals such as Cd, Cr, Cu, Pb and Zn were estimated in sediment and different body parts of the *P. viridis* and *M. metcalfei* were as Foot, Mantle, Gonad, Adductor Mussel, Shell, Byssal Thread and Gills.

Sample collection and preparation

Sediment samples were collected using a pre cleaned and acid washed PVC corer and immediately kept in pre cleaned and acid washed polythene bags, which were sealed and kept in ice box until further analysis in the laboratory. Sediment samples were washed with metal free double distilled water. The sediment samples were dried in an over at 60ºC for 5-6 hours. Dried sediment samples were ground in a glass mortar and reduced into fine particles.

A total of 60 green mussels, *P. virids* and 100 of yellow banded horse mussel, *M. metcalfei* were collected randomly by hand picking during low tide from Vellar estuary. The mussels were identified based sufficient taxonomic tools [10]. Collected mussels were cleaned to remove adhering on mussels with the fresh water and soft tissues were removed from the shells with a plastic knife. Different body parts of the each species were dissected and pooled together with respective body parts for mass quantification to analyze the heavy metal concentrations. Further, the different body parts dried at 60ºC and ground well to obtain fine powder before analysis.

Determination of metal concentration

To estimate the metal content (Cd, Cr, Cu, Pb and Zn) samples were digested (1 g) with Conc. HNO_3 and Conc. $HClO_4$ (4:1). The samples were subjected to complete dryness by placing on a hot plate. The ash consequently obtained was made up to 20 ml solution using ultrapure water from Milli-Q® water system with 20% nitric acid. The mixture was filtered using Whatman filter paper (11 µm) and then the metal concentrations were determined using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Software- WinLab 32; Perkin Elmer, Optima 2IOODV). The different metals were analyzed by ICP-OES using followed wavelength such as Cd (228.802), Cu (324.752), Pb (220.353), Zn (213.857) and Cr (267.716).

Statistical analysis

All analysis was performed in triplicate and mean, SD were done through SPSS® (v16) software and Bray Curtis similarity index was carried out by Primer 6 (v6).

Results

In the present study, Cd, Cr, Cu, Pb and Zn concentrations were determined in sediment and dry weight of different body parts (Foot, Mantle, Gonad, Adductor Mussel, Shell, Byssal Thread and Gills) of two edible bivalves (*M. metcalfei* and *P. viridis*). The metal concentration and the corresponding mean standard deviations all the samples from both bivalves are shown in Tables 1 and 2. Concentration of the heavy metals significantly varied in the different body parts of samples collected from the two bivalves. In *M. metcalfei,* the concentration of Cd ranged from 0.013 ± 0.0011 (BT-Byssal Thread) to 0.095 ± 0.0012 (FT-Foot) µg/g, and Cr 0.092 ± 0.0011 (SL-Shell) to 0.495 ± 0.002 (BT-Byssal Thread) µg/g, and Cu 0.063 ± 0.0011 (BT-Byssal Thread) to 0.367 ± 0.0043 (FT-Foot) µg/g, and Pb 0.164 ± 0.002 (BT- Byssal Thread) to 1.263 ± 0.002 (GL-Gill) µg/g, and Zn 2.172 ± 0.0011 (BT-Byssal Thread) to 11.113 ± 0.023 (AR-Aductor) µg/g (Table 1). In *P. viridis*, the concentration of Cd ranged from 0.022 ± 0.0023 (GL-Gill) to 0.091 ± 0.0012 (FT-Foot) µg/g, and Cr 0.147 ± 0.0012 (FT-Foot) to 0.447 ± 0.012 (GD-Gonad) µg/g, and Cu 0.126 ± 0.071 (ML-Mantle) to 0.356 ± 0.002 (SL-Shell) µg/g, and Pb 0.145 ± 0.0012 (GL-Gill) to 1.57 ± 0.0012 (GD-Gonad) µg/g, and Zn 0.964 ± 0.002 (SL-Shell) to 8.607 ± 0.004 (FT-Foot) µg/g (Table 2). In *M. metcalfei*, accumulation of Zn was found to be higher followed by Pb, Cu, Cr and Cd and *P. viridis* also higher concentration present in Zn and followed by Pb, Cr, Cu and Cd. All metals were commonly found in all the body parts of edible bivalves, but accumulation rate different from body parts as well as mussels. Depending upon the overall average mean value of each metal, the metal accumulation was in the order of different body parts of *M. metcalfei* and *P.viridis* presented in

Metals	Gonad	Mantle	Aductor	Shell	Byssal Thread	Gill	Foot	Sediment	WHO/EPA Standard
Cd	0.042 ± 0.0012	0.044 ± 0.002	0.015 ± 0.0023	0.014 ± 0.0011	0.013 ± 0.0011	0.023 ± 0.0011	0.095 ± 0.0012	0.135 ± 0.001	0.0-0.2
Cr	0.383 ± 0.001	0.097 ± 0.002	0.124 ± 0.002	0.092 ± 0.0011	0.495 ± 0.002	0.142 ± 0.0012	0.123 ± 0.0012	0.521 ± 0.0012	0.1-0.15
Cu	0.295 ± 0.001	0.324 ± 0.002	0.073 ± 0.002	0.125 ± 0.002	0.063 ± 0.0011	0.227 ± 0.0012	0.367 ± 0.0043	0.389 ± 0.0012	0-10
Pb	0.327 ± 0.0012	0.194 ± 0.0012	0.195 ± 0.002	0.175 ± 0.0023	0.164 ± 0.002	1.263 ± 0.002	0.528 ± 0.0012	2.057 ± 0.02	0-1.5
Zn	2.374 ± 0.002	5.795 ± 0.002	11.113 ± 0.023	3.626 ± 0.002	2.172 ± 0.0011	4.324 ± 0.0023	5.343 ± 0.016	9.486 ± 0.003	58-150

Table 1: Heavy metal concentration in different body parts from *M. metcalfei* (µg/g).

Metals	Gonad	Mantle	Aductor	Shell	Byssal Thread	Gill	Foot	Sediment	WHO/EPA Standard
Cd	0.057 ± 0.013	0.035 ± 0.015	0.025 ± 0.002	0.038 ± 0.0012	0.071 ± 0.002	0.022 ± 0.0023	0.091 ± 0.0012	0.135 ± 0.001	0.0-0.2
Cr	0.447 ± 0.012	0.234 ± 0.123	0.269 ± 0.0012	0.184 ± 0.002	0.334 ± 0.015	0.196 ± 0.002	0.147 ± 0.0012	0.521 ± 0.0012	0.1-0.15
Cu	0.314 ± 0.0012	0.126 ± 0.071	0.187 ± 0.0012	0.356 ± 0.002	0.281 ± 0.002	0.153 ± 0.0023	0.239 ± 0.0023	0.389 ± 0.0012	0-10
Pb	1.57 ± 0.0012	0.736 ± 0.002	0.362 ± 0.0012	0.355 ± 0.002	0.199 ± 0.0012	0.145 ± 0.0012	0.209 ± 0.0034	2.057 ± 0.02	0-1.5
Zn	2.57 ± 0.002	1.164 ± 0.0012	1.514 ± 0.0012	0.964 ± 0.002	2.246 ± 0.0012	4.445 ± 0.0023	8.607 ± 0.004	9.486 ± 0.003	58-150

Table 2: Heavy metal concentration in different body parts from *P. viridis* (µg/g).

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Heavy Metal	*M. metcalfei*	*P.viridis*
Cd	SM>FT>ML>GD>GL>AR>SL>BT	SM>FT>BT>GD>SL>ML>AR>GL
Cr	SM>BT>GD>GL>AR>FT>ML>SL	SM>GD>BT>AR>ML>GL>SL>FT
Cu	SM>FT>ML>GD>GL>SL>AR>BT	SM>SL>GD>BT>FT>AR>GL>ML
Pb	AM>GL>FT>GD>AR>ML>SL>BT	SM>GD>ML>AR>SL>FT>BT>GL
Zn	AR>SM>ML>FT>GL>SL>GD>BT	SM>FT>GL>GD>BT>AR>ML>SL

FT-Foot; ML-Mantle; GD-Gonad; GL-Gill; AR-Aductor; SL-Shell; BT-Byssal Thread; SM-Sediment

Table 3: Order of the metal concentration recorded in different body parts of edible mussels from Vellar Estuary.

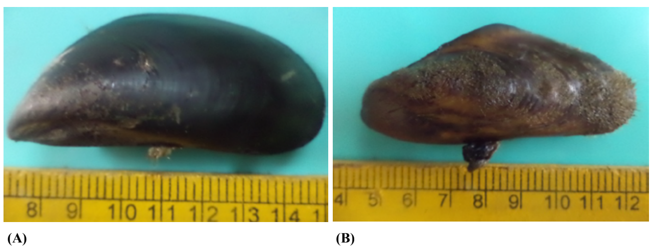

Figure 1: Study animal: A- *Perna viridis* (7.8cm length) and B- *Modiolus metcalfei* (7.6cm length).

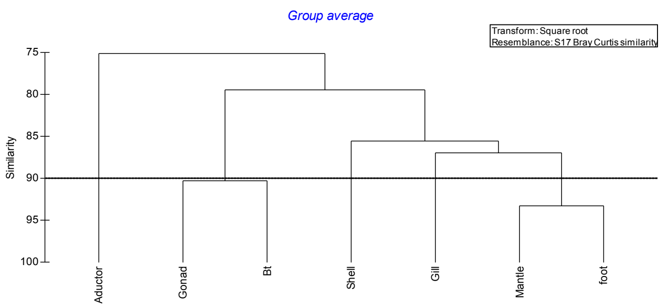

Figure 2: Bray Curtis similarity index versus different body parts of *M. metcalfei*. (Bt-Byssal Thread).

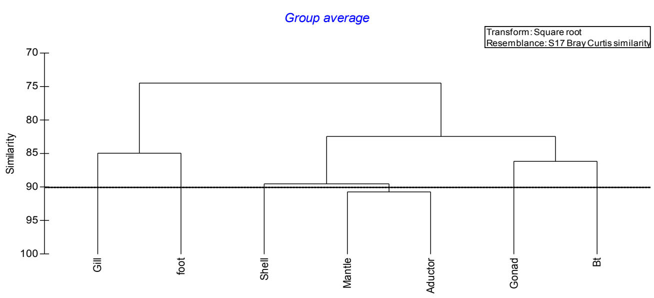

Figure 3: Bray Curtis similarity index versus different body parts of *P. viridis*. (Bt-Byssal Thread).

Table 3. All the metals are permissible level according to WHO/EPA except Cr and Pb from both bivalves. Sediment metal concentrations, comparatively higher than the both bivalves different body parts except Aductor (AT) of *M. metcalfei*. Moreover, there are the comprehensible relationships between concentrations of heavy metals in the mussels and sediment (Figure 1).

Dendrograms (Figures 2 and 3) are drawn to identifying the similarity percentage of heavy metal accumulation at different body parts using group linkage clustering technique (Bray Curtis Similarity coefficient of similarity). Mantle (MT) and Foot (FT) parts were at the highest level of (93.25%) of similarity at *M. metcalfei* followed by Gonad (GD) and Byssal Thread (BT) were successfully grouped by the next level of similarity (90.26%). In *P. viridis*, highest level of similarity present between (90.07%) Mantle (ML) and Aductor (AT).

Discussion

Sediments are one of the major sinks of trace metals in the aquatic environment and may be good indicators of long and medium term metal loads. Likewise, molluscan shell and tissue are also good indicator of metal pollution as they are sessile and sedentary and they reflect the heavy metal concentration of that particular area [11]. As expected, heavy metal concentrations in sediments greatly exceeded those in the surrounding water. In aquatic environments, heavy metals discharged from industrial or sewage effluents or from atmospheric deposition may be rapidly removed from the water column and transported to the bottom sediments [12]. The availability of metals in sediment provides an opportunity for aquatic animals to biomagnify these metals and later remobilized them through the food chain. In the present study, *P.viridis* and *M. metcalfei* from Vellar estuary was studied in different body part of the tissue for the level of accumulation of various metals apart from comparing the concentration with that of the sediment. Previously [13] reported that the level of heavy metal concentration from marine mollusc and sediment were reported. Cr in sediment was ranging between 7.10 µg/g and 31.44 µg/g and Cu concentration ranged from 11.28 µg/g to 37.24 µg/g and Zn concentration (36.14 to 56.14 µg/g) the sediment of Vellar estuary. Likewise copper in sediments collected from the Lukang culture area of central Taiwan and also evaluates the relationship by purble clams (*Hiatula diphos*) and venus clams (*Gomphina aguilatera*) [14].

Compare to previous study we observed less concentration of Cr, Cu and Zn in the sediment samples due to the continuous runoff water in the estuarine environment. But sediment metal concentrations, comparatively higher than the both bivalves different body parts except Aductor (AT) of *M. metcalfei*. Moreover, there are the comprehensible relationships between concentrations of heavy metals in the mussels and sediment. All the metals are permissible level according to WHO/EPA except Cr and Pb from sediment.

The use of molluscan shells as sentinels for metal pollution monitoring in marine waters has several advantages over that of the soft tissues. The shells are easy to store and handle and appear to be sensitive to environmental heavy metals over the long term. Since shell growth occurs incrementally they can provide a signal over a discrete time period, unlike the tissues which are strong accumulator of metals and integrate the chemical contamination signal over the life of the organism. Refinement of techniques for determining element using bivalves is important if global monitoring is to become a reality [15]. Another important observation in the present study, we estimated in different body parts of the edible bivalves such as Foot, Mantle, Gonad, Adductor Mussel, Shell, Byssal Thread and Gills. Previously same kinds of finding as bioaccumulation of some heavy metals (Cd, Fe, Zn and Cu) were done from fresh parts of two bivalves (*Pincta daradiata* and *Brachidonets pharaonsis)* from Akkuya Bay [16]. Similarly [17] reported that bioaccumulation of heavy metals such as magnesium, iorn, zinc and copper concentration in different body parts and shell of *C. melo* from Cuddalore coast. Previously [18] reported that, level of accumulation of heavy metals such as mercury, cadmium, lead, zinc, copper and arsenic in soft tissues of 15 species of benthic invertebrates from Zhejiang coastal waters, East china. The concentration of iron in the different soft tissues and byssus and also studied the potential role of the byssus as an excretion route for iorn in *P. viridis* [19]. In the present observation of heavy metals concentration also considerably varied in the different body parts of two edible bivalves. The concentration of Cd was minimum 0.013 ± 0.0011 µg/g (BT-Byssal Thread) and Cr had maximum (0.495 ± 0.002 µg/g) ranges observed from *M. metcalfei*. In case of Cu, Pb and Zn was minimum (Byssal Thread (BT)) ranges compare to other body parts.

Cu in the different soft tissues of the bivalve might be due to various mechanisms which included homoeostatic processes in the body in response to varying metabolic demands and entrapment of Cu under certain conditions by additional mucilage production/extrusion by animal [20]. Cadmium is widely distributed at low level in the environment and most foods have an inherently low level of Cd which has been shown to bind to the protein and accumulate significantly in higher level [21]. In present investigation, *P. viridis* metal concentration of Cd ranges minimum (0.022 ± 0.0023 µg/g) Gill part (GL) and maximum 0.091 ± 0.0012 µg/g recorded at Foot (FT) part [22]. Reported that Cd level is almost 10 times higher in shell fishes than in finfishes. This could also be due to fertilizer application in nearby area are transported to the estuaries by leaching and erosion as agriculture is also an important activity of the village folks besides fishing. According to Li [23], bivalves do not regulate Cd usually accumulate this element. Hence, green mussel might be able to regulate Cd in their body. Zinc is an essential trace element for all living organisms. Previously Vellar eastuarine gastropod Cd concentration was found fluctuating from 0.48 to 2.44 µg/g in the body tissues of *Nerita crepidularia* [13]. In the earlier, Cd concentration was found to be (6.88 ± 1.02) µg/g in *Nerita albicilla* from Taiwan coast [24], 1.78-2.87 µg/g in *Nerita albicilla* from marine environment, Hong Kong [25] and 2.83 µg/g in *N. lineata* from Peninsular, Malaysia [26]. Comparatively present study, Cd concentration were observed form both edible bivalves concentration was low. Similarly Zinc concentration of foot part in *M. metcalfei* (5.343 ± 0.016) and *P. viridis* (8.607 ± 0.004) was lower when comparing the results for 63.90-81.75 µg/g in Sunderban mangroves [27] in *Erita articulata*; 150-130 µg/g in Taiwan coastal waters [24] in *Nerita albicilla* and 31-680 µg/g in *S. Khatib*, Singapore [28] in *N. lineata*.

Conclusion

In the present study, the present status of heavy metal concentration from most edible bivalves was examined. Both edible bivalve may be used as an indicator species of heavy metal pollution and therefore can be used as sentinel organism to monitor pollution in the estuarine environment due to the following reasons. The tissues of different body parts were polluted by Zn and Cd compare to other heavy metals (Pb, Cr and Cu) and Cr and Pb beyond the limit of permissible level according to WHO/EPA from both bivalves as well as sediment also, it may be used as a biomonitor of certain heavy metals in Vellar estuary environment.

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