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Research Article

An Investigation on the Heavy Metal Contamination in the Edible Mussels distributed in Kochi Backwaters

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Abstract

Bivalves are known for bioaccumulation of environmental contaminants and hence they can be used as good bioindicators of heavy metal contamination in coastal areas. This ability is due to their sessile lifestyle, resistance to high accumulation of chemicals and ease of sampling. Kochi is a coastal area in the south west of Kerala. Kochi backwaters are part of the Vembanad lake which support a large production of bivalves. The samples for the study were collected from five different locations which are part of Kochi backwaters. *Villorita cyprinoides* (black clam) and *Mytella strigata* (an invasive mussel) are the two species used for the study. The analysis of Heavy metals was done with the help of EDXRF spectroscopy and was carried out in BARC. The study revealed the presence of four heavy metals (As, Pb, Zn, Cd) in both *M. strigata* and *V. cyprinoides*. The concentration of heavy metals is more prominent in *M. strigata* than *V. cyprinoides*. The results of this study enabled to conclude that both *V. cyprinoides* and *M. strigata* possess the ability for bioaccumulation of heavy metals from the surrounding. It is found that the heavy metal concentrations are above the safe limits which indicate that bivalves from the Kochi backwaters are not safe and suitable for consumption. Prolonged exposure may result in health issues in humans.

Keywords: *Mytella strigata*; *Villorita cyprinoides*; Parts Per Million (ppm); Handheld x-ray spectrophotopmeter

Introduction

The consumption of seafood has increased in recent years, especially in coastal regions. Marine mussels are a high demanding species for human consumption and also are an important candidate for mariculture. However, it is known that mussels are filter feeders and this may cause an accumulation of environmental contaminants in their soft tissue (Besada et al., 2011). The determination of the concentrations of potentially toxic substances in mussels is essential because of their usage as seafood and the potential adverse effects of their consumption on human health. Moreover, as contamination by metal pollutants continues and is even increasing in some parts of the world, particularly in less developed countries, it is also important to determine the level of pollution in the marine environment, especially in regions where aquaculture is foreseen and where the local population consumes large amounts of mussels (Stankovic et al., 2012).

Cochin backwaters, with an area of 256 km^2 extending from Cochin to Alleppy having two permanent openings, one at Cochin, which forms the main entrance (450 m wide) to Arabian sea and another opening, further north at Azhikode. The booming city of Cochin and 60% of the chemical industries of Kerala located in the vicinity of the backwater discharge nearly 0.105 million m^3/d of effluents

(Anon, 1998). Therefore, Kochi backwaters is greatly affected with pollutants, particularly from heavy metals, pesticides, PCBs and organotin compounds. Cochin backwater has thus been drawn considerable research interest for the last few decades because of the social and economic importance.

A number of industries located on the banks of Periyar River empty huge quantities of waste water into the Cochin backwaters. Previous studies have shown that the effluents from the industries contain high proportion of various heavy-metals and pesticides. In this context, Charru mussels (*Mytella strigata*), a recognized invasive species, were collected from three different sampling points along the Kochi backwaters and black clams (*Villorita cyprinoids*) which form particularly good fisheries in Kerala, were collected from two sites. Both are studied in order to identify any relationship between metal (oid) concentrations in charru mussel tissues and the geological and environmental conditions of the coastal area via biochemical analysis.

Mussels and Heavy Metal Residues

Bivalves were known to feed on suspended particles in the water column which could be contaminated by various contaminants (including heavy metals) derived from either anthropogenic activities or natural emissions (Langston et al., 1998). Sediments are considered contamination reservoirs which can act as sources of metals which may be released to the overlying water column (Lin et al., 2013; Rao et al., 2021). The metal loads present in the sediments have adverse effects on biota when in a bio-available state (Garcia-Ordiales et al., 2019). These bioavailable metals are detected in various organisms which live and obtain nutrients from the affected ecosystems (Blanco-Rayon et al., 2019; Signa et al., 2019). Regarding these kinds of biota, filter-feeding bivalve molluscs obtain nourishment from the overlying water column and add dissolved metal (oid)s to their organism. One of the most relevant bivalve mollusks are mussels, as they have been identified as one of the best biological indicators of coastal pollution (Cevik et al., 2008; Besada et al., 2014; Cunha et al., 2017).

Metals are drawing much attention on account of their crucial effects on different life forms. Metals of biological concern can be divided in to 3 groups-ight metals (transported as mobile cations in aquatic media), transition metals (essential in low concentration but may be toxic in high concentration) and metalloids (toxic at low concentration, not require for metabolic activity). Transition metals and metalloids are collectively known as heavy metals; e.g., Cu, Zn, Hg, Pb, Mn, Co, Cd, Cr etc. They are generally water soluble, non-degradable and vigorous oxidizing agents. The essential metals like copper, zinc, iron and cobalt have important biochemical functions in living organisms at the levels, which allow the enzymes systems to function without interference. Heavy metals, commonly defined as - elements having a specific density of more than 5g/cm^3 (Hawkes, 1997), are potentially harmful to most organisms at some level of exposure and absorption. Heavy metal in effluents has emerged as one of the environmental contaminats in these days because of their inherent toxicity, vast sources, persistence, and non-degradability (Breierova et al., 2002). The concentrations of trace metals are generally higher in the organism than in water. However, due to excess amount of pollutants in the water if the concentration levels of these trace elements increase beyond the level required by the organism they act in an either actually or chronically toxic manner (Gulfaraz et al., 2001). Heavy metals like chromium, copper, zinc, nickel, lead etc, are some of the major components of industrial waste, which along with other products from industrial operations are discharged into the aquatic environment. These substances are toxic to aquatic life (Dutton et al., 1988; Bowlby et al., 1988).

The increased accumulation of heavy metal levels in the aquatic environment is disastrous to aquatic organisms and humans alike (Naji et al., 2010). Heavy metals are discharged into the aquatic environment from mining, smelting, agriculture, petrochemical industry, printing, aquaculture, electronic industry and municipal waste etc. and can be bioaccumulated by the organism and biomagnified through food chain (Ciji and Nandan, 2014). Cd, Hg, and Pb are non-essential metals for molluscs which means that these elements can cause harmful effects on bivalves even at low concentrations. These elements are considered the most hazardous metals with regard to the coastal ecosystem (OSPAR Commission, 2017).

Elements bioaccumulated in organisms could be transferred and biomagnified throughout the trophic chain, which in turn poses a significant environmental risk (Turritto et al., 2018). Thus, an accurate environmental assessment must include not only chemical analysis indifferent environmental compartments, but also established biological techniques with the aim of determining the relationship between metal concentrations and their effect on marine ecosystems (Bellas et al., 2014; Riani et al., 2018) Seafood is associated with many beneficial effects on human health. However, the overall level of contaminants in biota has increased over the last two centuries and seafood is one of the sources of oral exposition to contaminants (Chiesa et al., 2018). Bivalves are rich in omega-3 that can diminish cholesterol level and help in conditions causing coronary diseases, stroke and pre-mature birth (Daviglu et al., 2002; Patterson 2002). They are also rich in protein, calcium, phosphorus, fluorine, and iodine, polyunsaturated and unsaturated fats, and insoluble vitamins which have hypocholesterolic impact against atherosclerosis or cardiovascular diseases (Ismail, 2005; Ikem and Egiebor, 2005). Despite these benefits, shellfishes could bring about negative effects to health (Tan and Ransangan, 2015). There has been rising evidence of heavy metal intoxication that leads to health risk (Jin et al., 2011) such as weak immune system, mental retardation, organ damages as reviewed by previous studies (Kamaruzzaman et al.,2011; Alluri et al.,2007; Ismail and Rosniza, 1997; Gorell et al.,1997). Therefore, this work aimed to evaluate cadmium, lead, mercury and zinc presence in mussels and clams, from the Kochi backwaters.

Before the year 2018 the major species of edible mollusc collected from Kochi backwaters belong to the genus *Villorita* and *Paphia*. However, recently an exotic species is found to occur abundantly in several areas of the Kochi backwaters. This mussel species is known by the name 'Charru Mussel' belongs to the genus *Mytella*. It was observed that, in several regions of Kochi backwaters, this exotic and invasive mussel *viz. Mytella strigata* show considerable population reduction in the last 4 months (Pers. Observation). People are known to consume this species. The decline in population in the last few months is very significant that many places where this species were abundant are now a days contain only their dead remains (Varghese, 2022).

Materials and Methods

Study Area

The study area, Kochi backwaters, is a shallow brackish water which lies between 09° 40'-10° 12'N and 76°15'-76°25'E in the Vembanadu lake extending from Alleppey to Azhikode. Mussels were collected from five area which are part of Kochi backwaters *viz*., Thoppumpady, Cheppanam, Palluruthy, Chellanam and Kallencherry. Thoppumpady is a region in the city of Kochi. It connects the tourist destinations Mattancherry, Fort Kochi, Palluruthy and Willingdon Island. Cheppanam is a small village near Panagad in Ernakulam District. It is a calm and quiet place with some beautiful backwater view.

Palluruthy is part of the water bound west Kochi, lying westward to the Kochi mainland. Chellanam is on a narrow landform about 10 km in length. Chellanam harbour, one of the finest picturesque harbours in Kochi is located at south chellanam. Kallencherry is a place in Kumbalangi, an island village in the outskirts of Kochi city. Situated amidst backwaters, around 12 km (7.5 mi) from the city centre, Kumbalangi is a major tourist attraction and is famous for its Chinese fishing nets.

Sample Collection

Samples were collected with the help of local fishermen. Five individuals of *Mytella strigata* from two sites were collected of which four were from seaward side and one from riverine side. In addition to this, two samples of common edible clams (*Villorita cyprinoides*) were collected from two different sites, where clams were harvested for marketing. The collected samples brought to the laboratory and placed in the refrigerator for preservation.

Sample Analysis

The shells of the mussels were removed with the help of scalpels and the tissue were extracted. The flesh of each individual collection were seperately dried in oven at a temperature ranging 65-80^{0}C, for two days, so as to ensure all the moisture content is removed. The samples were then ground using mortar and pestle to fine powder. The powdered material then transferred to different sample bottle and each one labelled accordingly. The estimation of different metals was done using HHXRF spectroscopy (Hand held X -ray fluorescence spectroscopy) at Bhabha Atomic Research Centre, University of Mumbai.

Metal Analysis by Handheld x-ray spectrophotometer (HH-XRF)

In the XRF spectrometer, samples were excited by the X-rays emanating from an X-ray tube (Rh X-ray tube) having enough energy for ejecting electrons from various inner shells belonging to the atoms in the specimen. The vacancies in the inner shells of atoms are then occupied by electrons coming from the outer shells of the atoms causing the emission of characteristic X-rays; conventionally it can be referred to as X-ray fluorescence (XRF). In HHXRF, a silicon drift detector (SDD) was employed for the measurement of X-ray energies. The Silicon Drift Detector (SDD) contains graphene window that enables the detection of low Z elements (Al, S and P). These low Z elements usually cannot be detected by the conventional XRF system which uses a beryllium window. The powdered samples were placed in a cubic box and irradiated by Rhodium X-ray tube. Spectrum has been obtained for each sample in twenty seconds. The energy of the beams used for beam 1 is between 12 and 36 keV, whereas for beam 2, it varies between 0 to 12 KeV. The HHXRF experimental set-up is as shown in Fig. 1. The validity of the HHXRF set up was performed by analyzing Certified reference material (CRM) obtained from European Commision - Joint Research Centre, Institute for Reference Materials and Measurements - (ERMBB422 - Fish muscle) - was used for quantification of the elements and verifying the reliability of the data obtained by the present system.

Statistical analysis

Data in the present study are represented as mean + SD.

Results and Discussion

The tissue of mussels collected from five different regions of Kochi backwaters, were analysed for heavy metals such as Cadmium (Cd), Manganese (Mn) and Zinc (Zn), Lead (Pb), Arsenic (As), Iron (Fe) using XRF spectroscopy. In mussels, metal mean levels varied between species and also between collection sites. The results indicate that the highest levels of metals were observed in *Mytella strigata* that are collected from Palluruthy, Thoppumpady and Panangad (Cheppanam) than *Villorita cyprinoides* collected from Chellanam and Kallencherry. Among all the metals, Pb was recorded in one of the samples collected from Thoppumpady.

Sample	***Study Area***	***PPM***					
		As	***Cd***	***Mn***	***Pb***	***Zn***	***Fe***
Mytellastrigata	Thoppumpady	3	23	130	4	88	1385
		ND	19	93	ND	90	4980
	Palluruthy	8	26	101	ND	113	3540
		6	33	85	ND	81	2441
	Panagad (Cheppanam)	3	34	200	ND	85	843
Mean±SD		4±3.0	27±6.4	121.8±46	0.8±1.78	91.4±12.5	2637.8±1668
Villorita cyprino-ides	Chellanam	ND	24	28	ND	136	182
	Kallencherry	5	34	50	ND	94	1504
Mean+SD		2.5±3.5	29±7.07	39±15.55	ND	115±29.69	843±934.79

Table 1: Heavy metal content in *Mytella strigata* and *Villorita cyprinoides* (given as PPM).

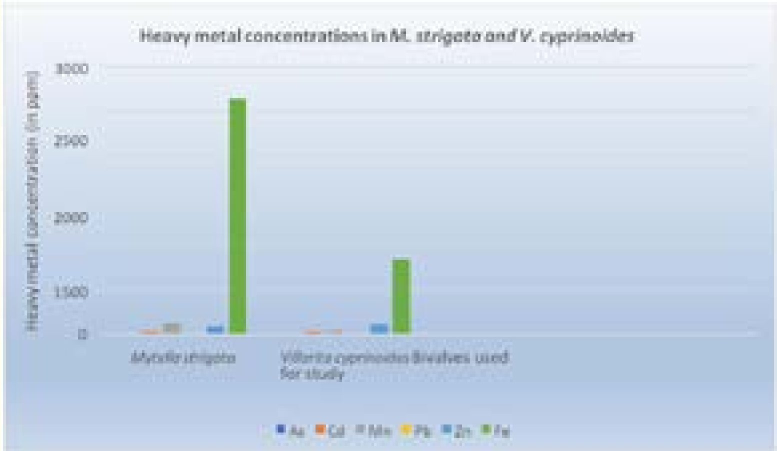
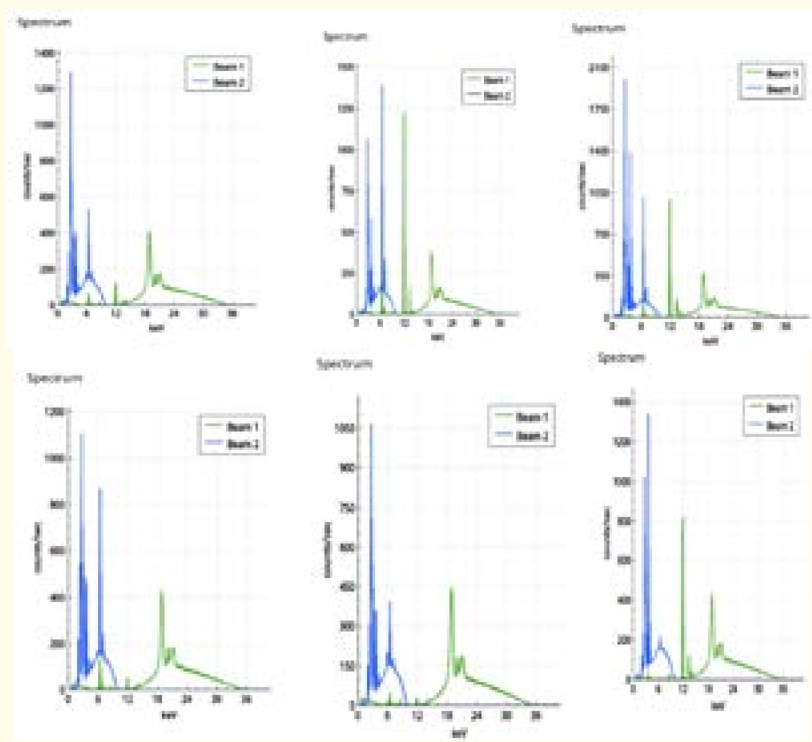


Figure 1: Graph showing the heavy metal concentration in *Mytella strigata* and *Villorita cyprinoides*.



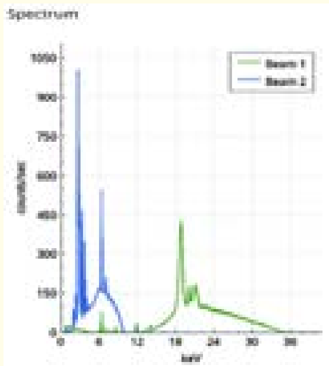


Figure 2: Spectrum of heavy metal concentration in the mussels (Beam 1: 12 to 36 KeV and Beam 2: 0-12 KeV).

Heavy metals are serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms (Veena et al., 1997). Due to bioaccumulative property, the bivalves have gained great importance as indicator organisms. Chemical hazards associated with seafood forms a major health risk of global concern. Elevated levels of toxic metals have already been reported in various seafood products especially in bivalves from different parts of the world including India (Kureishy, 1985, Lakshmanan, 1988, Krishnakumar et al., 1990, 1998, Prema et al., 2006, Sivaperumal et al., 2007). In the present study Cd, Pb, As, Fe, and Zn were analysed in the soft tissues of the mussels from the Kochi backwaters. It was observed that *Mytella strigata* (Charru mussel) and *Villorita cyprinoides* (Blackclam) show different uptake levels for different metals. Among same species also there is difference in heavy metal concentration. The invasive mussel, *Mytella strigata* accumulated the highest concentration of Fe, Pb, Mn, As and Zn with mean values of 2637.8μg/g, 0.8μg/g, 121.8μg/g, 4μg/g, 91.4μg/g respectively. While in *V cyprinoides*, the presence of Pb is not detected and the highest concentrate metal found was Fe (843μg/g) followed by Zn (115 μg/g). Based on these results, it shows that the magnitude of heavy metal accumulation in bivalve tissues depend on the type of heavy metal, exposure period and bivalve species. Considering all metals and samples, the mean values obtained decreased in the following order for *Mytella strigata*: Fe > Mn>Zn >Cd >As> Pb and for *Villorita cyprinoides* the order is as follow: Fe>Zn>Mn>cd>As. Based on a comparison of the permissible limits set by WHO for Zn (50.0ppm), Pb (2 ppm),As (4.0ppm), Cd (2ppm) and Mn (20ppm as per Turkish guidelines) all the mean values of analysed metals were higher than WHO and Turkish guidelines for heavy metal concentration with the exception of Pb whose mean concentration in M. strigata is lower than the permissible level. The high levels for As, Cd, Mn and Zn in mussels were probably related to discharge from urban areas and industries discharging into the Kochi backwaters.

Heavy metal concentration in M.strigata

Metal concentration in study area 1-THOPPUMPADY

The mean concentration of As in *M.strigata* is1.5ppm which is lower than WHO/FAO limits i.e., It doesn't exceed the permissible limit and may not cause health hazards while consumption of the mussel. In this area higher concentration of metals found were Fe (3182.5 ppm) and Mn (111.5 ppm). The permissible limit of metal concentration of Pb by WHO is 2ppm and its highest concentration is found in this sampling area and its was not found in any other samples which suggest that this area is contaminated with lead. In this study the concentration of Cd found to be 21ppm, which is ten times higher than the recommended level of Cd by WHO. So, it could be inferred that consumption of the *M. strigata* from this area could lead to health hazards in man.

Metal concentration in study area 2-PALLURUTHY

The mean concentration of as recorded is 7 ppm, which is higher than the permissible limit of metal by WHO. Among all the samples under study the highest amount of Fe reported is 2990.5 ppm and it was reported in this area. The concentration of Mn recorded in this area was 93 ppm, which also lies above the permissible limit by WHO. The heavy metals like Cd and Zn are also found to be present in large quantity in this area compared to other study area. The highest concentration of heavy metal was found in this area. The present work indicates that Palluruthy is highly polluted and metal accumulation follows the order: Fe>Zn>Mn>Cd>As

Metal concentration in study area 3-PANAGAD (chepanam)

The least amount of the sample was collected from this area. The concentration of metals found to be 85 ppm, 34 ppm, 3 ppm and 200 ppm for Zn, Cd, As and Mn respectively. The study suggest that the decreased amount of M. strigata in this area is may be due to the presence of higher levels of certain metals like Mn and Cd, which seems to be higher in this area compared to other area.

Heavy metal concentration in V. cyprinoides
CHELLANAM

Heavy metals like Zn (136 ppm), Fe (182), Mn (28 ppm) and Cd (24 ppm) were present in the sample collected from this area and are detected above the permissible heavy metal level by WHO. The presence of Arsenic was not detected in this sample. The increasing order of metal accumulation is as follows: Cd<Mn<Zn<Fe.

KALLENCHERRY

Among the *V.cyprinoides* samples the highest concentration of Fe (1504 ppm), Cd (34ppm) and Mn (50ppm) were detected in this area. Compared to the samples collected from Chellanam the concentration of Zn is low (44ppm) which almost lies near to the permissible metal concentration by WHO.

Comparison between metals- Pb, Zn, As, Mn and Fe

Pb concentrations in mussels (*M. strigata*) sampled during the study was 4ppm and is present only in one of the samples collected from Thoppumpady and was above the limits set by the WHO. The presence of lead during the summer could be related to an increase of urban populations during this period, anthropogenic sources, or shipping, where other metals are contained in Pb protective paints (FAO, 1983). This increase in the bioaccumulation of Pb in mussels during the summer depends not only on environmental concentrations but also on the chemistry of metals in seawater and the physiology of mussels (USFDA, 1993). Further, the values exceeding the limits are probably due to high lead concentrations in area sediments (Klassen et al., 1986, Rouane et al., 2015) Pb is a toxic, bioaccumulative heavy metal with no known biological function. Its absorption may constitute a serious risk to public health (Topcuoglu et al., 2003) The international Agency for Research on Cancer (IARC) classified inorganic Pb as being likely carcinogenic to humans. During the study, Zn concentrations were found to be higher than the recommended limits established by WHO (Table 1). Seafood is a major source of zinc and an important of human diets. Zinc is an essential trace element. However, in excess quantities, essential elements can also be poisonous and cause serious threats to human health (Dokmeci et al., 2009, EC 2006).

In the present study the total (inorganic and organic) As (Arsenic) concentration in *M. strigata* And *V.cyprinoides* (4ppm and 2.5ppm respectively) were found to be within the safe limits specified by the WHO. However, the individual concentration in the samples seems to be higher than the permissible limit. The fact that the arsenic content of mussels, especially in the summer periods, was higher than the limiting values may indicate anthropogenic (agricultural and industrial) activities in the region. Factories there allow uncontrolled discharges of pollutants into ground and surface water. The toxicity of organic arsenic compounds is relatively low, and they are eliminated faster from the organism (Topcuoglu et al., 2004). A wide range of arsenic compounds, including inorganic arsenic, has been reported in marine organisms. The percentage of inorganic As in seafood is 1-5%; while in bivalve mollusks, they are 1.9-6.5%, and mussels contain approximately 1-2% of inorganic As compounds; the great majority of seafood arsenic consists of complex organic arsenical compounds (Joksimovic et al., 2011). Mn levels recorded were very higher compared to the WHO/FAO limits. In the present study, the concentration of Mn in *M. strigata* (121.8ppm) and *V. cyprinoides* (39ppm) exceed the permissible limit, so it could be inferred that consumption of the mussels could lead to health hazards in man. The concentration of Fe in the study is the highest among all the metals. In *M. strigata* the concentration is 2637.8 ppm and 843 ppm in *Villorita cyprinoides*. The copper and iron form important components in oxygen carrying metallo-protein, hemocyanin and haemoglobin respectively. So mussels are a good source of iron. Mercury and Ni were not detected in any of the samples studied and does not pose a risk to public health.

Conclusion

The results of this study show that both *V. cyprinoides* and *M. strigata* possess the ability for bioaccumulation of heavy metals from the surrounding. It is found that the heavy metal concentrations are above the safe limits which indicate that bivalves from the Kochi backwaters are not safe and suitable for consumption. Prolonged exposure may result in health issues in humans.

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