AN INVESTIGATION ON HEAVY METAL CONTAMINATION IN EDIBLE MUSSELS DISTRIBUTED IN KOCHI BACKWATERS

A DISSERTATION SUBMITTED TO ST. TERESA'S COLLEGE (AUTONOMOUS), ERNAKULAM IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF

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CERTIFICATE

This is to certify that the dissertation entitled '**An investigation of heavy metal contamination in edible mussels distributed in kochi backwaters'** submitted to St. Teresa's College (Autonomous), Ernakulam, in partial fulfilment of the requirement of award of degree of Master of Science in Zoology is an authentic work carried out by **Ms. PRABITHA P P** (SM20ZOO007) in the academic year 2020 – 2022 under the guidance and supervision of Dr. Moncey Vincent, (External Guide) Assistant professor, Sacred Heart College (Autonomous),Thevara and Mrs. Indu Vasudevan, (Internal Guide), Assistant Professor, Department of Zoology, St. Teresa's College(Autonomous),Ernakulam.

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<u>CERTIFICATE</u>

This is to certify that this dissertation titled, "**An investigation of heavy metal contamination in edible mussels distributed in Kochi backwaters**" is an authentic record of work carried out by **Ms. Prabitha P P** of St. Teresa's College(Autonomous) Ernakulam, Kerala, under my guidance and supervision in the Department of Zoology , Sacred Heart College (Autonomous), Thevara during the period from 4th April to 30 th April 2022 in the partial fulfillment of requirements for the Degree of Master of Science in Zoology. I further certify that she has completed all the assigned work to my complete satisfaction.

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DECLARATION

I hereby declare that the dissertation entitled **''An investigation of heavy metal contamination in edible mussels distributed in Kochi backwaters"** submitted to St. Teresa's College (Autonomous), Ernakulam in partial fulfilment of the requirements, for the award of the Degree of Master of Science in Zoology is a record of original research work done by me under the supervision and guidance of Dr. Moncey Vincent, Assistant Professor, Sacred Heart College Thevara, during the period from 4th April to 30th April 2022 and Ms. Indu Vasudevan, (Internal Guide), Assistant Professor, Department of Zoology, St. Teresa's College, Ernakulam, to the best of my knowledge and belief, this project contains no material previously published or written by another person, except where due reference is made.

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LIST OF ABBREVIATIONS

1.	%	Percentage
2.	As	Arsenic
3.	Cd	Cadmium
4.	Fe	Iron
5.	*M.strigata*	*Mytella strigata*
6.	Mn	Manganese
7.	Pb	Lead
8.	PPM	Parts Per Million
9.	SD	Standard deviation
10.	*V.cyprinoides*	*Villorita cyprinoides*
11.	WHO/FAO	World Health Organisation/Food and agriculture Organisation of the United Nation
12	XRF spectroscopy	X-ray Fluoresce spectroscopy
13.	Zn	Zinc
14.	$\mu g/g$	Microgram per gram

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ABSTRACT

Bivalves are known for their ability to bioaccumulate the contaminants and hence they can be used as a good bioindicators of heavy metals contamination in coastal areas. This ability is due to their sessile life style, resistance to high accumulation of chemicals and ease of sampling. Kochi is a coastal area in the south west of Kerala. Bivalves of Kerala state include different species of clams, mussels and oysters. Kochi backwaters is a part of 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 sample bivalves 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 that the concentration of six heavy metals (As, Pb, Mn, Zn, Cd and Fe) were found in both *M.strigata* and *V.cyprinoides*. The concentration of heavy metal is more prominent in *M.strigata* than *V.cyprinoides*. From 2019 onwards the presence of *M.strigata* has been reported in various parts of Kerala. But during the studyperiod, its population seems to be declining, this suggest that heavy metal concentration has relationwith its population decline. 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. The bioaccumulation capability depends on the levels of contaminates in the environment and also depend on species. Therefore, *V.cyprinoides* and *M.strigata* can be used as biomonitoring organisms for aquatic pollution control in inland waters of kochi backwaters .The heavy metal concentration are above the safe limits which indicates that bivalves from the Kochi backwaters are not safe and suitable for consumption. Prolonged exposure may result in health issues in humans.

INTRODUCTION

Life on earth is threatened both by environmental pollution and overpopulation. Natural phenomena contributing to pollution have always existed, but anthropogenic activities are increasing contamination of air, soil and water. Aquatic habitats are especially polluted by metals. The main threats to life from toxic metals are associated with exposure to Pb, Cd, Hg and As, which accumulate in organisms. Other than pollution, the problem currently faced by nation is overpopulation. Overpopulation means more food is required but the amount of arable land is declining due to human requirements. One way of overcoming these consequences of overpopulation is to exploit seas and oceans. Thus, the consumption of seafood has increased in recent years, especially in coastal regions. Marine mussels are an excellent candidate for aquaculture. However, mussels accumulate a wide range of metals in their soft tissue. Thus, 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).

Kerala's coastal zone is unique with the presence of a large number of perennial or temporary backwaters (Kayals), endowed with rich biological and genetic diversity (Thomson, 2001). 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 due to pollution and toxicity problems 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. Reclamations over the past several decades have resulted in considerable shrinkage (40%) of the Cochin estuary (Gopalan *et al.*, 1983).

Cochin backwaters, one of the largest tropical lakes along the west coast of India, face serious environmental threats by intertidal land reclamation, pollution discharges, expansion of harbour development, dredging activities and urbanization (Gopalan *et al.*, 1983). A number of industries located on the banks of Periyar River empty huge quantities of wastewater 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 recognised 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

The molluscs, comprising of different groups like mussels, clams, chunks, cowrie, squids and cuttlefish form a sustenance fishery of India. Mussels and clams are excellent bioindicators because they have a wide geographic distribution range, are sedentary, can easily be sampled and analysed and in particular because they are able to accumulate both inorganic and organic contaminants in their tissues (Besada *et al.*, 2011).

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) Bivalves are known to filter between twenty and one hundred litres of surrounding waters a day. In doing so, they accumulate natural or anthropogenic contaminants. Upon consumption, they present these harmful contaminants to the consumers (Richards, 1988; Lees, 2000; Robertson, 2007).

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 bioavailablestate (Garcia-Ordiales *et al*., 2019b). 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 relevantbivalve molluscs are mussels, as they have been identified as one of the best biological indicators ofcoastal 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 into 3 groups- light 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 5 g/cm3 (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 most pressing problems 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).

Intertidal areas are the natural habitats of marine mussels and they are usually close to estuaries. Therefore, the chance of exposure to many contaminants from land -based activities through the riverine systems as well as sea-based sources is high, and one of its attributes is the possible usage of mussels as a biomonitoring agent for the estimation of pollutant metal concentrations in a sea environment. Among the environmental pollutants, trace metals represent natural constituents in tissues of marine organisms and their basal levels can be affected by marked seasonal fluctuations. Several environmental and biological factors mutually compete to determine such variations, (Fattorini *et al.*, 2008).

Prolonged exposure to a polluted ecosystem results in the bioaccumulation of several metals in the mussels (Kumar *et al.*, 2015; Martínez-Colon *et al.*, 2021). This bioaccumulation can cause a significant increase in the metal concentrations in the biota with respect to the metal loads occurring in the surrounding water (Casas et al., 2008; Besada *et al*., 2011; Nannoni *et al*., 2015).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 in different 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 nowadays contain only their dead remains (Varghese, 2022).

In this context we planned a study about the heavy metal content in the edible parts of mussels in order to evaluate whether accumulation of heavy metals have any role in their population decline.

Also, we use this work to analyse the amount of heavy metals in edible mussels of the Kochi backwaters. Because, heavy metals are already reported to be present in dangerous amounts in fishes living in the bottom part of the Kochi backwaters, we also suspect such a high amount of harmful material accumulation in the edible mussels distributed in the same area.

AIM AND OBJECTIVES

AIM

This present investigation was carried out to study about the heavy metal content in the edible parts of backwater mussels in order to evaluate whether accumulation of heavy metals have any role in their population decline and also to analyse the health implications associated with it by the consumption of these mussels by humans.

OBJECTIVES OF THE STUDY

- To determine the presence of heavy metal and assessment of carcinogenic agents in the selected sample.
- To determine the variation in the heavy metal concentration among the charru mussel collected from different sites.
- To study the difference of heavy metal content in common edible black clams and charru mussel.

This information may be useful to discern the level of impact of the possible metal sources along the coast in order to evaluate the environmental quality and ecological risks of this area with regards to the environmental guidelines.

RELEVANCE OF THE STUDY

- Bivalve molluscs specially the mussels are sessile and filter feeding organisms and they bioaccumulate compounds present in the environment and it provide a time integrated measure of pollution at a given site used in environment pollution monitoring.
- Mussels and clams are high in omega PUFAs and good source of essential amino acids and so it is consumed as a food source by many people so knowing the concentration of metal content in them is of great significance to study the effect of these heavy metal in human body.

REVIEW OF LITERATURE

Bryan (l976b) suggested that bivalves can be used to assess the quantity of heavy metals available in the aquatic environment. The capacity of bivalves to regulate trace metal content of the body by metabolic pathways is limited (Schulz-Baldes, 1974). The ability to accumulate trace metals and other toxicants by bivalves has led to the selection of this group as an important bio-indicator. Mhatre, 1991defined bioindicator as a plant or an animal which indicates the presence of a substance in its vicinity by showing some typical symptoms which can be due to the effects of other natural or anthropogenic stresses.

Commit *et al* (1989) concluded that Periyar has been performing a pivotal role in shaping the economic prospects of Kerala, as it helps in power generation, domestic water supply, irrigation, tourism, industrial production, collection of various inorganic resources and fisheries. However, as in the case of many other inland water bodies, river Periyar is gradually undergoing ecodegradation throughout its course of flow due to various anthropogenic stresses, which include indiscriminate deforestation, domestic- agricultural- industrial water pollution, excessive exploitation of resources, large scale sand mining, various interferences in the flow of water, etc.

According to Phillips and Rainbow (1994) and Cossa (1989) the accumulation of heavy metals is directly related to the bioavailability, which is closely related to the life cycle of the mussels, essentially their maturity and age. Additionally, many factors influencing accumulation are body size, internal cavity volume (Mubiana *et al.*, 2006), and physiological condition (Fischer, 1983; Soto *et al.*, 2000) have been extensively studied. Seasonal variation in the tissue weight of bioindicator organisms can significantly affect metal concentrations by simply diluting or concentrating the animal's total metal body burden (Lobel and Wright, 1982).

Farrington *et al.*, (1983) conclusively showed that pollutants enter the body of mussels as they filter water through their gills for respiration and feeding or in case of inorganic pollutants such as metals, through facilitated diffusion, active transport or endocytosis. In addition to the gills, the mantle, foot, kidney and hepatopancreas are predicted to be major sites of metal uptake due to their large surface areas, thus cleaning the aquatic habitat. They are widespread and have a long-life span. As sessile, filter-feeding and low metabolism organisms they may take up and accumulate pollutants to levels well above those present in the surrounding waters or sediment (Philips, 1990). These properties has capable bivalve to be recognized as water pollution indicators (Goldberg, 1975).

Nair and Nair (1986) showed that seasonalities in bioaccumulation of trace metals such as copper, cadmium, iron, manganese, zinc and mercury in the oyster, *Crassostrea madrasensis* in Cochin backwaters in the west coast. The distribution and association of trace metals in soft tissues and byssus of Mytilus edulis from the east coast of Kyushu Island, Japan has been studied by Szefer*et al*., (1997). The distribution of heavy metals in the different soft tissues such as mantle, muscle, ligament, foot, remainder, gill and digestive gland of mussels was studied by Soto *et al*., (1995). Szefer (1999) *et al*., studied the distribution and relationships of metals in molluscs and associated sediments from the Gulf of Aden, Yemen.

Salanki (1986) reported a good bioindicator is one which shows the earliest responses to the pollutants allowing indicating the presence and predicting the consequences of adverse anthropogenic effects. The organisms in the ecosystem are collected for analysis of biological responses to pollutant exposure, suitable bioindicator organism have following characters *viz.*, (1) it can accumulate elevated levels of pollutants without death; (2) it has sufficient abundance and wide distribution for the repetitious sampling and comparison; (3) it lives in a sessile style, thus definitely indicating the local pollution; (4) its life is long sufficient for the comparison between various ages; (5) it has suitable target tissue or cell for the further research at microcosmic level; (6) easy sampling and raising in the lab; (7) it occupy the main position in food chain; well dose-effect relationship can be detected in it.

Soto *et al*., (1995) showed that metal/shell-weight indices provide a reliable tool to assess metal bioavailability in coastal waters to sentinel molluscs. It offers a low noise-to-signal ratio and a high sensitivity, and is easily calculated. Additionally, by simply using the weight calculated for biometric estimations in previous 'Mussel Watch' monitoring programmes, or by weighing stored shells, most of the previous records on metal bioavailability, based on the conventional use of metal concentrations, can be transformed into metal/shell-weight indices for comparisons.

According to Connell et al., (1999) the accumulation of metals into organism’s cells is largely dependent on their ability to pass through the cell membrane, despite the metal route of entry. Uptake can take place through a number of carrying pathways; include the passive diffusion of neutral metal species across the membrane, active transport through major ion channels, endocytosis and facilitated diffusion of metals. Gundacker (2000) reported that Zebra mussel accumulates high amounts of toxic heavy metals and is extensively used as a bio-monitoring organism. Several monitoring studies have employed mussels as bioindicator to assess the spatial and temporal trends of contaminants in the environment.

Topcuoglu (2003) said that the analysis of heavy metal content in whole soft body tissues of bivalves as bio-indicator gives some information about the degree of metal pollution in the environment. It was found that metals concentration in benthic organisms is directly correlated with concentrations of metals present in sediments and water. Metal concentration in sediments and water decreased after a period of time, but concentration in benthic animals remained relatively unchanged.

Ali *et al.*, (2005) studied the concentration of trace metals such as (Zn, Mn, Cu, Cr, Cd) in water, sediment, benthos and some common fish species from lake Qarun, Egypt. Bioaccumulation factor values showed that the trend of accumulation of most metals was as follows: Mollusc >Crustacean>Annelids>Solea species> Mugil species >Tilapia species.

Shah *et al*., (2005) showed that in the aquatic environment, heavy metals in dissolved form are easily taken up by aquatic organisms where they are strongly bound with sulfhydril groups of proteins and accumulate in their tissues. Fish absorb dissolved or available metals and can therefore serve as a reliable indication of metal pollution in an aquatic ecosystem. Tench (*Tincatinca*) is considered a good test organism for heavy metal contamination because of its feeding behavior and bottom feeding habits.

Sidoumou et al., (2006) studied the heavy metal concentration (Cd, Zn and Cu) in mollusks from the Senegal cost. Bivalve mollusc *Cardita ajar* and the oyster *Crassostrea gasar* present higher Cd concentration respectively 6.82 ± 0.54 and 13.77 ± .80 µg Cd/g (dry wt) than two other species (*Dosimia isocardia*: 3.88 ± 0.31 µg/g and *Perna perna* 2.37 ± 0.22 µg/g). Cu and Zn concentration are in range of the published values for *Crassostrea gasar, Perna perna* collected elsewhere in Africa. The results suggest that Cd may be present in high concentration in Senegalese waters where upwelling occurs.

Sivaprasad, P. S., (2007) study concluded that V. cyprinoides possess the capabilities for accumulating heavy metals and OCPs from the surrounding medium. However, the bioaccumulation potential of the animal was found to vary with contaminants, seasons, levels in the surrounding medium and other unknown intrinsic and extrinsic factors. Therefore, the black clam can be used as sentinel organism in monitoring heavy metal and pesticide pollution in inland waters of Vembanad Lake systems. The residual limits of all the toxic contaminants are within the safe limits, and therefore the bivalve clam from Cochin backwater is safe and suitable for human consumption and for aquaculture as nutrient rich wet feed.

Sivaperumal *et al.,* (2007) analysed the heavy metal concentration in fish, shellfish and fish products in and around the Cochin area. The concentration ranges of Cd, Cr, Zn, Cu, Mn were<0.07-

1,<0.05-3.65,<0.6-165,<0.15-24 <0.08-9.2. This study showed that different metals were present in the samples within the maximum residual levels. However, in general, they are safe for human consumption.

Gabre *et al.,* (2008) said that Contamination of bivalve shellfish (e.g., oysters, clams, mussels and cockles) is a major food safety concern, so suppliers and retailers need to be sure that the products they sell are safe. Bivalves respond to changes in concentrations of contaminants in water, and they integrate contaminants from the water. Shellfish contamination is caused, among other things, by the discharge of chemical substances such as metals, pesticides and organochlorine compounds from industrial and municipal treatment processes. Contaminated mollusc shellfish (oysters, clams) may cause illness in humans.

Deshmukh (2013) observed that the digestive glands accumulated highest concentration of all metals as compared to other studied organs in three bivalve species, this indicated that digestive glands was the potential biomonitoring organ of heavy metal pollution in these bivalve species. He also said that mobility of heavy metals is related to their solubility in water and once transferred to the environment this metal gets accumulated and migrates through the food chain. The concentration of metals is more in the sedentary organisms of aquatic ecosystem. The bioaccumulations of metals by aquatic organisms cause a serious problem to human populations. Consumption of aquatic food highly contaminated with heavy metals causes health effect to human being.

Anu *et al*., (2014) concluded that Cochin backwater is presently facing serious challenges because of heavy metal contamination. The concentrations of heavy metals in the water as well as sediment at different locations in the backwater system are consistent with the local industrialization levels. Northern part of the estuary is found to be the most polluted compared to other part of the estuary. Heavy metal levels in some aquatic organisms exceeded the safety limit. Therefore, it is necessary to give more attention to accumulation of heavy metals in organisms as far as the seafood industry and public health is concerned. More precise environmental protection measures should be taken to control the discharge heavy metals from anthropogenic sources.

A number of species of mytilid mussels are important aquaculture species. Mytilids typically have a high fecundity, rapid growth rate, are filter feeders and are tolerant of a wide range of environmental conditions, all attributes that make them ideal for aquaculture. However, these features also make them potential invasive species. *Mytella strigata* clearly fits into this category. It is tolerant of a wide range of salinities, between 2 and 40 psu (Yuan *et al*., 2010; Rice *et al.*, 2016).

Puccinelli *et al.,* (2016) assessed if the proximity to urban centres influenced the dietary regime of marine benthic filter feeders. They found that mussels from urbanised sites had fatty acid signatures enriched with a greater proportion of polyunsaturated fatty acids (PUFA), indicative of exposure to large food availability and quality. As human concentration associated to urbanisation promotes nutrient input into the sea, it may also enhance primary production and thus the amount of PUFA in phytoplankton. He also conclude that urbanisation increases the availability of PUFA for benthic filter feeders and therefore nourishing and suitable food accessibility. This could explain why mussels in urban areas reach a greater-size.

The acclimation trial, done by Rice *et al*. (2016) showed that some mussels survived to salinity 60, but were inactive, and all died when salinity reached 65. These salinity shock and acclimation trials suggest that charru mussels may be best suited to Philippine waters during and after the monsoonal rainy seasons when salinities are routinely below 35, and may be spread among different estuaries via larval transport during the monsoonal rainy season. On the basis of these criteria, charru mussels may be a potential species for aquaculture complementing the culture of the native *Perna viridis*, a species with a higher preferred salinity that is traditionally cultured in the dry season.

Vallejo *et al*., (2017) suggested that the invasion of M. strigata in the Philippines was mediated by ballast water discharge from ships or biofouling on the hulls of shipping boats. Mussel M. strigata can survive both in marine environments- e.g., salinities higher than 25 ppt and in estuarine environment-e.g., salinities as low as 5 ppt (Rice *et al.*, 2016). Furthermore, this species can acclimate to gradual changes in salinity, further increasing its potential to invade a wide variety of habitats (Yuan *et al.,* 2010; Rice *et al.,* 2016). Thus, the establishment of this invasive species in the bay may not only have adverse impacts on the local species that may be displaced but will also have socioeconomic implications.

Khan *et al*., (2018) study indicated that high concentrations of Cd, Cr, Cu, Fe, Mn Ni, Pb, and Zn in Kabul River. The study highlights the capability of heavy metals for bioaccumulation in the tissues of freshwater mussels and reveals histopathological alterations in mussel tissues. The study elaborates on vulnerability of riverine ecosystems to low levels of perturbation, that is, heavy metal pollution. It shows occurrence of bioaccumulation at permissible levels of pollutants.

Khayatzadeh and Abbasi (2010) concluded that every pollution in the aquatic environment which impacts physiology, development, growth or survival of fish, affects human that, at the top of the food chain, consume fish. The accumulation of heavy metals in the tissues of organisms can result inchronic illness and cause potential damage to the population. Aquatic animals have often been used in bioassays to monitor water quality of effluent and surface water. The development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants.

Azizi, G *et al*., (2018) study revealed that many different species of bivalve mussels have been used in attempts to monitor the concentrations of heavy metals in the the coastal environment of many countries. The genus *Mytilus* is capable of acting as an efficient time-integrated indicator of heavy metal over a wide variety of environmental conditions. It is well known that metal concentration levels in *Mytilus* spp. are not only the result of their bioavailability in the environment. Biotic and abiotic factors are acting. *Mytilus* spp. provides a global view of the health status of the coastal environment.

Biju *et al*.,(2019) study revealed that the rapidly increasing biomass of *M. strigata* in Ashtamudi Lake, a Ramsar site in Kerala, had wiped out the green mussel (*Perna viridis*) populations and their massive settlement in many areas has replaced the dominant clam species (short-neck clam or yellow-foot clam, *Marcia recens*) and backwater oysters *Magallana bilineata* and *Saccostrea cuccullata* populations, which support the livelihoods of hundreds of fishers in the area, besides damaging the aquaculture systems. The increasing populations of *M. strigata*, coupled with its ability to survive across the salinity gradients from the mouth of the estuary to the river discharge zone in the tail end, may have long-term implications on biodiversity and ecosystem services of the lake. He further revealed that the abundance of invasive mussels in the lake bottom not only reduce the availability of clam resources and impact their livelihoods, but also increase the labour of local fishers as they have to spend more time removing the invasive mussels from their harvest. The density of *M. strigata* in Ashtamudi Lake is higher than the maximum density recorded from other areas of India such as Vembanad Lake. The black clam *Villorita cyprinoides* is present in the low salinity regions of Ashtamudi Lake, and the invading mussels have impacted black clam fisheries. Field observations indicate that they have also replaced the non-commercial gastropods.

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MATERIALS AND METHODS

STUDY AREA

Kerala lies in the southern corner of peninsular India, has 3,36,000 hectares of inland water area of which the back water system consisting of estuaries of the rivers. The study is based on Kochi backwaters.

The study area, Kochi backwaters, is a shallow brackish water which lies between $09^{0}40$'-$10^{0}12$'N and $76^{0}15$'-$76^{0}25$'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 in the state of Kerala, India. It connects the tourist destinations Mattancherry, Fort Kochi, Palluruthy and Willingdon Island. Cochin Fisheries Harbour, located at Thoppumpady, is one of the major fishing industries in the state. Cheppanam is a small village near Panagad in Ernakulam District. It is a calm and quite place with some beautiful backwater view. Panangad, is a small suburban village located in Kochi, a part of Kerala state in India. It is a small village grown to a suburban that lies in the skirts of Kochi Bypass. Panangad is one of the suburban villages that make up the urban agglomeration of Kochi.

Palluruthy is part of the water bound west Kochi, lying westward to the Kochi mainland. It comprises the regions Thoppumpady, Perumpadappu, Edakochi, Mundamveli and Kumbalanghi. The place is famous for its backwaters. Chellanam is on a narrow landform about 10 km in length. There was a canal (azhi) passing through this place to the sea which later closed naturally and formedin a place called Andhakaranazhi in Alappuzha district. Chellanam harbour, one of the finest picturesque harbours in Kochi is located at south chellanam. Chellanam harbour is one of the main fishing centres in Kochi. Most of the people make their living from fishing and agriculture. Fishermenwork at deep-sea and fresh-water fishing, using the latest technologies.

Kallencherry is a place in Kumbalangi, an island village in the outskirts of Kochi city in the state of Kerala, India. 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.

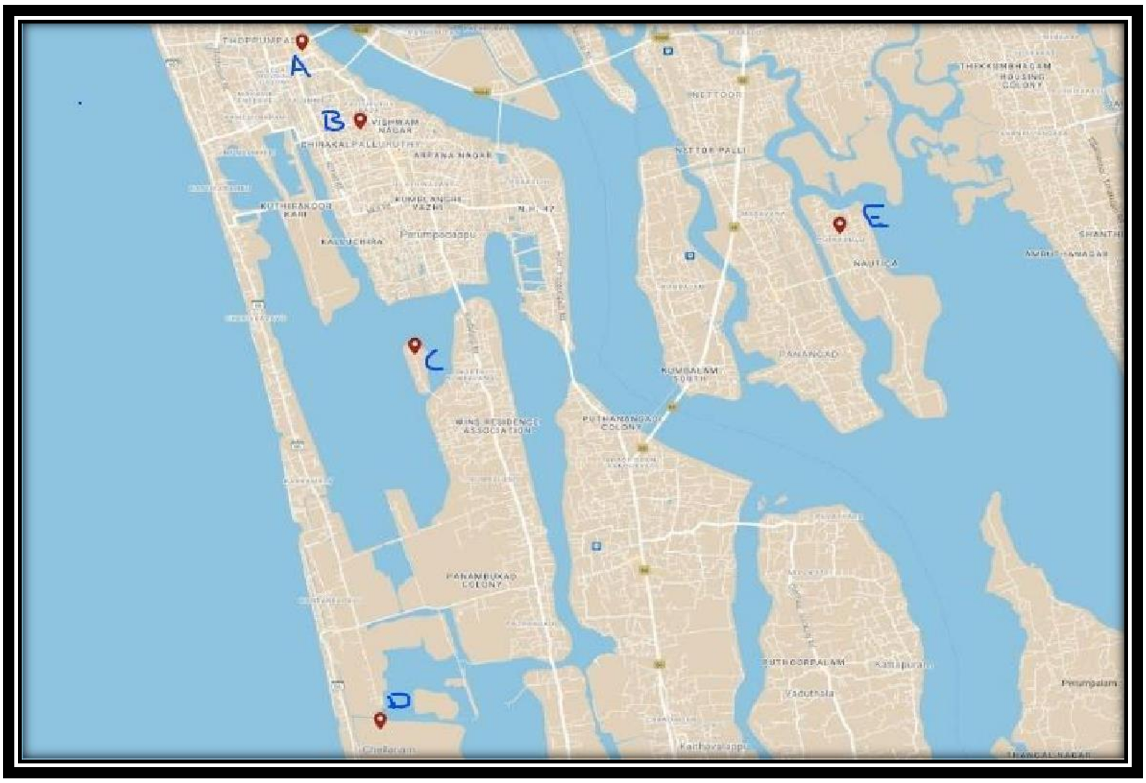

FIGURE 1: STUDY AREA

A-Thoppumpady **B-Palluruthy** **C- Kallencherry** **D- Chellanam** **E-Cheppanam**

SAMPLE COLLECTION

Sample collection was done with the help of local fisherman. For this study, total five samples of *Mytella strigata* from two sites-Four samples from seaward side and one sample from riverine side are analysed for heavy metal contents. In addition to this, two samples of common edible clams (*Villorita cyprinoides*) were collected from two different sites, where clam fisheries is the main occupation. The collected samples brought to the laboratory and placed in the refrigerator for preservation.

FIGURE 2A

FIGURE 2B

FIGURE 2A and 2B- Sample collection of *Mytella strigata*

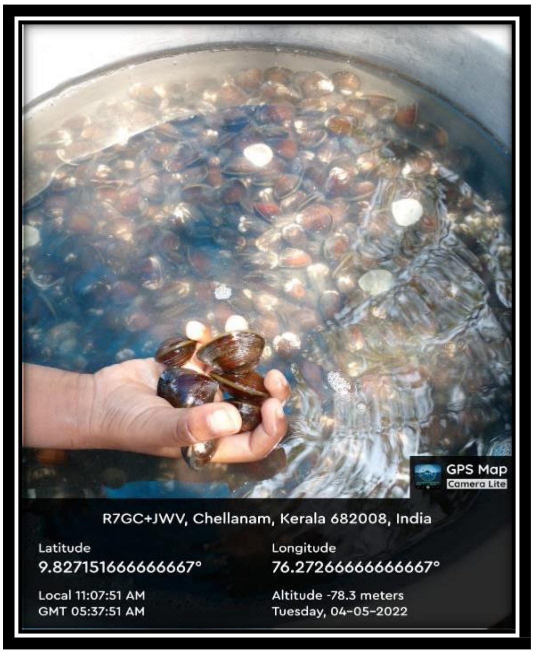

FIGURE 3A

FIGURE 3B

FIGURE 3A and 3B-sample collection of *Villorita cyprinoides*

SAMPLE ANALYSIS

Sample Preparation

Prior to the analysis, the shells of the mussels were removed with the help of scalpels and the tissues were extracted. The flesh of mussels was dried in an oven separately at a temperature around 65-80^{0}C for two days, so as to ensure all the moisture content is removed. The samples werethen ground at a time using a mortar and pestle to fine powder. The powdered material then transferredto different sample bottle and each one labelled accordingly. The estimation of different metals was done using EDXRF spectroscopy (X -ray fluorescence spectroscopy) at Bhabha Atomic Research Centre, University of Mumbai.

Energy Dispersive X-ray Fluorescence (EDXRF)

It is one of two general types of X-ray Fluorescence techniques used for elemental analysis applications. It’s a powerful, non-destructive technique for measuring elemental composition from magnesium (Mg) to uranium (U), from parts per million to 100%. EDXRF is a convenient technology to screen all kinds of materials for quick identification and quantification of elements with little or no sample preparation

Principle

In EDXRF spectrometers, all of the elements in the sample are excited simultaneously, and an energy dispersive detector in combination with a multi-channel analyzer is used to simultaneously collect the fluorescence radiation emitted from the sample and then separate the different energies of the characteristic radiation from each of the different sample elements. Resolution of EDXRF systems is dependent upon the detector, and typically ranges from 150 eV – 600 eV. The principal advantages of EDXRF systems are their simplicity, fast operation, lack of moving parts, and high source efficiency

FIGURE 4- Heat dried samples

FIGURE 5- samples are powdered with the help of mortar and pestle.

FIGURE 6- Powdered samples are transferred to sample bottle for analysis

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RESULTS

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 study areas. The results indicated 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.

Table 1: Heavy metal concentration in PPM in *Mytella strigata and Villorita cyprinoides*

SAMPLE	STUDY AREA	PPM					
		As	Cd	Mn	Pb	Zn	Fe
Mytella strigata	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

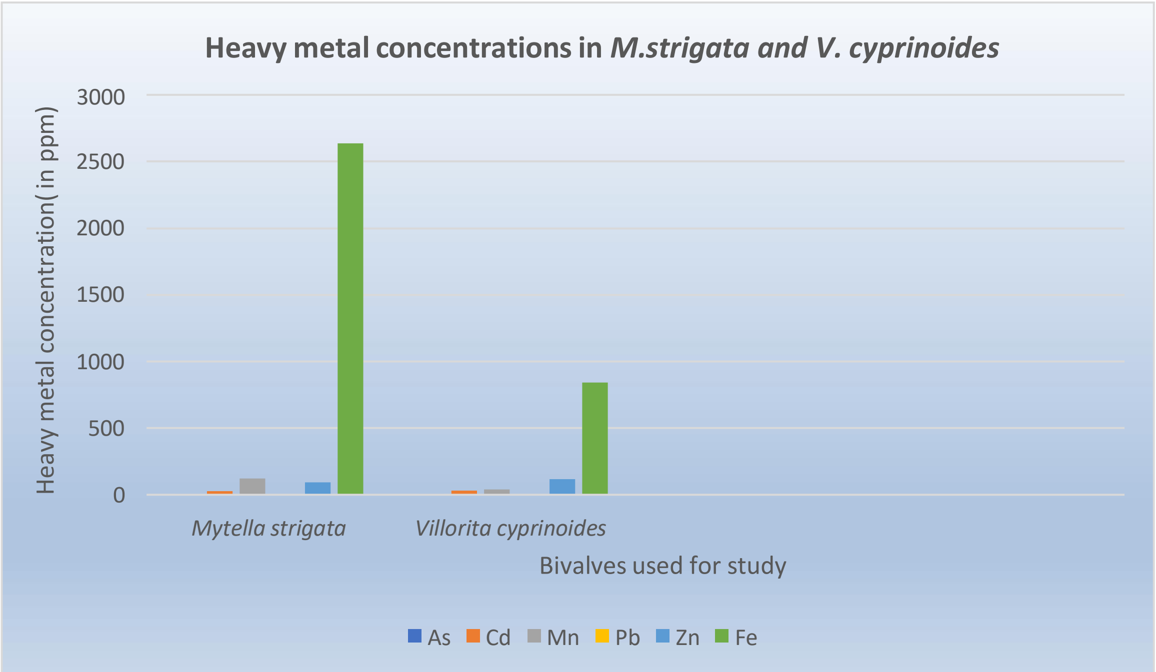

Figure 7: Graph showing the heavy metal concentration in *Mytella strigata* and *Villorita cyprinoides*

DISCUSSION

Aquatic environment is treated with greatest carelessness in terms of pollution. This attitude is apparently because of the prevailing impression that water bodies have enormous capacity for uptake of pollutants without causing noticeable hazards. A class of pollutants deserving special attention in all environments including aquatic environment is heavy metals. Continuous heavy metal monitoring is encouraged to effectively analyses the risk & impact of heavy metals on environment and on the general public's welfare. 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 bio accumulative 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 m various seafood products especially in bivalves from different parts of the world including India (Kureishy, 1985, Lakshmanan, 1988, Krishnakumar et al, 1990b, 1998, Prema *et al*., 2006, Sivaperumal *et al*., 2007).

Cd, Pb, As, Fe, and Zn were analysed in the soft tissues of the mussels from the Kochi backwaters. The primary concentrations of the investigated trace metals (PPM) in the mussels are given in Table 1. The patterns of accumulation of heavy metal studied in two bivalves – *Mytella strigata* (Charru mussel) and *Villorita cyprinoides* (Black clam). It was observed that different species of bivalves showed 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, 0.8 µg /g, 121.8 µg /g ,4 µg /g, 91.4 µg /g respectively. While in *Villorita cyprinoides* the presence of Pb is not detected and the highest concentrate metal found in them is Fe (843 µg /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.

Variability in metal body concentrations between closely related species are mainly caused by interspecific differences in the biokinetics of uptake, elimination, species-specific ability/capacity to accumulate trace metals and different physiological rates such as pumping, filtration, respiration and species-specific digestive physiology and absorption rate of a metal across gut epithelium. Both physiological/ biochemical responses, metal geochemistry, differences in metal efflux rates might be responsible for the differences in metal bioaccumulation as observed in the experimental bivalve species.

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 flowing 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* is 1.5 ppm 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 (3182.5ppm) and Mn (111.5ppm). The permissible limit of metal concentration of Pb by WHO is2ppm 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 7ppm, which is higher than the permissible limit of metal by WHO. Among all the samples under study the highest amount of Fe reported is 2990.5ppm and it was reported in this area. The concentration of Mn recorded in this area was 93ppm, 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 85ppm ,34ppm,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 higherlevels 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(136ppm), Fe (182), Mn(28ppm) and Cd(24ppm) 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* sample the highest concentration of Fe (1504ppm), cd (34ppm) and Mn (50ppm) were detected in this area. Compared to the sample 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 (4ppm) and is present only in one of the samples collected from Thoppumpady 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, bio accumulative 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.

Metal toxicity in human

Cadmium: Cadmium is widely known to be a highly toxic non–essential heavy metal and it does not have a role in biological process in living organisms. Thus, even at its low concentration, cadmium could be harmful to living organisms (Tsui and Wang, 2004). Literature supports the view that, Cadmium which often accumulates in the human body via food negatively affects several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system (Chouba *et al.*, 2007). Other damages that have been observed include reproductive, and development toxicity, hepatic, hematological and immunological effects.

Manganese: Manganese is an essential trace element for both animals and man; necessary for the formation of connective tissues and bone, growth, carbohydrate and lipid metabolism, embryonic development of the inner ear, and reproductive function. From literature, symptoms of manganese toxicity in man include dullness, weak muscles, headaches and insomnia.

Zinc: Although Zn is an essential requirement for good health, excess Zn can be harmful. Excessive absorption of Zn suppresses Cu and Fe absorption. Acute adverse effects of high Zn include nausea, vomiting, diarrhoea, loss of appetite, abdominal cramps, metallic taste, kidney, stomach damage and headaches. Intakes of 150-450 mg of Zn per day have been associated with such chronic effects as low copper status, altered iron function and reduced levels of high levels of high-density lipoproteins.

Arsenic: Itis a ubiquitous element that is detected at low concentrations in virtually all environmental matrices. Contamination with high levels of arsenic is of concern because arsenic can cause a number of human health effects. Several epidemiological studies have reported a strong association between arsenic exposure and increased risks of both carcinogenic and systemic health effects (Tchounwou *et al.*,2003)

Iron: As iron is so very essential for our body and to know that it can have some toxic effects is perhaps difficult to believe. However, people do suffer from iron poisoning that may lead to multiple health disorders. Children suffer serious health troubles due to iron overdoseon overdose can affect nervous system and in rarest of cases may lead to Coma.Iron is considered to be bad for your heart health and causes low blood pressure in adults.

CONCLUSION

Heavy metal gets accumulated in each trophic level and its concentration gets higher in each level. Aquatic organisms are the one which get accumulate such heavy metals, so its necessary to check its concentration in the environment. Kochi is a coastal area so the people prefer to consume more seafoods than others. Since the mussels are rich source of calcium and iron. So, the biomonitoring of heavy metals is very important as far as human health and aquatic life is concerned. The mussels diet shows potent antioxidant activity in all animal groups.

The following were the conclusions drawn from the study:

- Heavy metal concentration differs between different species of mussels. Among *M. strigata* and *V. cyprinoides* the concentration of heavy metals is found to be greater in *M. strigata*. This is may be due its high resistance and bioaccumulation capacity towards heavy metals.
- The presence of *M. strigata* was reported in 2018 in Kochi backwaters and it cause great threat to the native mussels. But during the sample collection there is a decline in the population density of *M. strigata*. It may be related to chronic exposure to metals like As, Pb, Cd, Mn and Fe.
- The heavy metal like Zn, Cd and Mn are present above the permissible level in *V. cyprinoides.* So, the study indicates that the mussels that are collected from this area is not safe to eat. The continuous exposure to such heavy metals result in various health issues in humans.
- In this study, Palluruthy and Thoppumpady, was more polluted than other areas under study. Since it is highly populated area, it poses great threat to those who reside here.
- Almost all metals found in the mussel samples, from one or the other study area were above the maximum permissible limits as per the WHO/FAO guideline standards comparison. High concentrations of some heavy metals measured in the mussel tissues in these water bodies were attributed to probable high influx of metals as a result of pollution from the municipal and industrial wastes, market waste, septic waste etc.

In the present situation, where health concern regarding heavy metal contamination becomes a global concern, it is necessary to conduct more extended studies regarding the topic. But due to lack of time the present study is conducted focusing on only small area and only few samples and metals were analysed. Further extension of study areas, number of samples, study extension to different mussel species, study of bioaccumulation in higher tropic level organism etc will help us to understand about the sources and effects of heavy metal contamination in these areas. It is very necessary for the safety of those who directly depend on this water body and its fish for livelihood.

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