



Bioaccumulation of Heavy Metals in *Marcia recens*, an Edible Bivalve of Ashtamudi Lake, a Ramsar Site (1204), India

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Article Info	ABSTRACT
Article type: Research Article	As a RAMSAR site (no.1204), Ashtamudi Lake has very productive and significant ecosystem services. Currently, the lake is being threatened by severe pollution stress, especially with heavy metals. Heavy metal pollution is a great concern of matter as it enters in the bioaccumulation and bio-magnification processes of the aquatic food chain. The present study seasonally investigated the bioaccumulation of seven heavy metals (Pb, Zn, Fe, Cu, Cr, Cd and Co) in the body tissues of an edible bivalve, <i>Marcia recens</i> , from three sampling stations (viz. Neendakara, Kureepuzha and Asramam) of the lake during the study period in 2019. The results showed that, as per the standard permissible limits (FAO/USEPA, 1989), some of the metal accumulations were extremely high in the bivalve tissues. Highest Metal Pollution Index was observed in station II. There were significant spatial and temporal variations in the accumulation of heavy metals in the the examined bivalve. Two way ANOVA analysis also reveals a statistically significant differences ($p<0.5$), in the heavy metal accumulation in the bivalve, among the stations as well as seasons. The continual exposure to even a relatively low levels of these metals by regular consumption of contaminated bivalves, may entail adverse health issues. Implementation of appropriate scientific and sustainable conservation strategies will ensure the health of the estuaries and the sustainability of bio-resources.
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INTRODUCTION

Ashtamudi Wetland System (8°56'46.18" N and 76° 33' 16.33E), a Ramsar site in Kollam District of Kerala State, also known as the gate way to the back waters of Kerala. It is one of the major estuarine wetlands in the tropics and the second largest backwater system of Kerala State. The lake is highly significant for its hydrological functions and biodiversity. Ashtamudi lake is currently getting polluted day by day due to offshore anthropogenic activities such as discharge of waste effluents, urban runoff, land encroachment, unscientific dredging, fuel leakage from mechanized boats, combustion of damaged boats, dumping organic and inorganic wastes (Surya lekshmi & Mophin, 2017; Chinnadurai et al., 2016). This loads heavy metal inputs into the lake which can pose serious threats to the aquatic biota and its consumers (Jiang et al., 2018). Deteriorating water conditions severely affect its inhabitants and the health of consumers.

The shell fishery has great importance in the Ashtamudi Lake. The bivalves caught from the lake support food security as a cheap food source. Bivalves have been identified as better

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adapted sentinels (da Silveira Fiori *et al.*, 2018). Characteristic features of bivalves such as filter feeding habit (Ruiz *et al.*, 2018), larger size, sedentary nature, low metabolism, tolerance to toxic exposure, wide distribution, relative ease of collection and species identification along with simple and inexpensive analytical as well as quantification method are the factors contributes for its fitness as a biomonitoring system (Jorgenson, 1996; Szefer, 2002; Waykar & Deshmukh, 2012; Zuykov *et al.*, 2013; Szefer, 2002).

Bivalves provides an overall cumulative-temporal information over the time on the current pollutant status of the living environment (Erk *et al.*, 2018). This signifies an immediate implementation of various conservation strategies to protect this lake ecosystem. The present study focuses on the heavy metal contamination on an edible bivalve *Marcia recens*, one of the export quality clams of the lake.

MATERIALS AND METHODS

Three sampling stations were selected in and around Ashtamudi wetland area (Fig.1) for the present investigation. Station I, Neendakara bar mouth (8° 56'09"N and 76° 32'45"E), where large scale mechanized boats/ trawler traffic oil spillage poses the major pollution. Besides inorganic pollution, mining in certain areas were also noticed in this station. Station II Kureepuzha (8° 55'10"N and 76° 33'58"E), is the place near to the Municipal Waste dumping area of Kollam District. Station III, Asramam (8° 55'50"N and 76° 35'04"E), is at the vicinity of boat jetty, where the untreated effluents from various hospitals and waste discharge from many establishments such as, KSRTC bus station, government boat jetty, private house boats, Corporation slaughter house, urban park, unscientific dredging, hospital discharges (extensive biomedical pollution) and dumping of constructional debris were reported as the major issue.

Bimonthly collection of triplicated samples of the bivalve, *Marcia recens* (Fig. 2) from each sampling station were conducted during the study period (Feb- Dec 2019) and were stored in sterile polythene bags with the help of local fisherman. The samples were transported to laboratory. The bivalves were grouped into different sizes (adult, 35-40 mm shell length; intermediate, 30-35 mm shell length and juvenile, 20-25 mm shell length). In order to increase resolution and reduce analytical load, pooling of ten individual clams of nearly equal length from the three size groups have been done (Martincic *et al.*, 1987).

Tissue samples were oven dried (80-85° C, overnight), cooled and ground in a clean mortar and pestle. Approximately 0.5g of powdered samples (weighed to an accuracy of 0.001g) were acid digested (8ml high purity HNO₃ and 1 ml of 30% H₂ O₂). Triplicate digestion were made for each sample. Samples were properly filtered (Whatman Filter No.1) and allowed to ramp in the microwave digestion system (Model No. MS20443DB; 1050W) as per U.S. EPA methods (1993). After digestion, allow the vessel to cool and the samples were quantitatively transfer to clean volumetric flasks and diluted with de-ionized water. The analytical solutions were transferred to a clean polypropylene centrifuge tubes for storage (at 5 ° C) until ready for the analysis of metals.

The samples were analysed on a ICP-Optimal Emission Spectrometer (Thermo Fischer iCAP 7200 DUO). Samples were compared to respective standard curve to determine the ppb of each metal in the digested solution. The wavelength used for the detection and measurement of each metal Pb, Zn, Fe, Cu, Cr, Cd, and Co were 220.353, 213.856, 259.940, 324.754, 205.560, 214.438 and 228.616 respectively.

Metal Pollution Index, indicates the pollution load (Lafabrie *et al.*, 2008) of the study system. The metal pollution index (MPI) was calculated according to the following equation (Usero *et al.*, 1997):

$$\text{MPI} = (\text{Cf}_1 \times \text{Cf}_2 \dots \dots \dots \text{Cfn})^{1/n}$$

where Cf_n is the concentration of metal n in the sample.

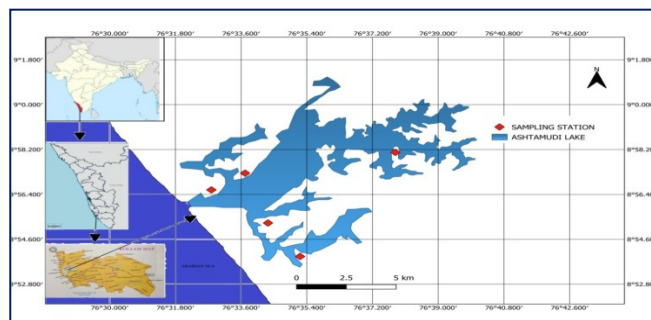


Fig. 1. Study site: Ashtamudi lake.



Fig. 2. *Marcia recens*.

Heavy metal concentration in the soft tissues of the bivalve were expressed in means (ppb). The data were analysed in Microsoft Excel (version.2010). Two way ANOVA were carried out to compare the significant differences in the seasonal as well as station-wise metal accumulation.

RESULTS AND DISCUSSION

The **station – wise analysis** showed that Fe was the highest accumulated metal in all the sampling stations during all the seasons. Cd was the lowest accumulated metal in Station I (Neendakara). In the case of Station II (Kureepuzha) and Station III (Asramam), Co was the least concentrated metal in the bivalve samples.

Seasonal analysis of average accumulation of heavy metal (ppb) in *M.recens* among the three sampling stations are shown in table 1. Seasonal analysis revealed that Fe was the most accumulated metal throughout the seasons. Co was the least accumulated metal in Station II (Kureepuzha) and station III (Asramam) during monsoon and pre- monsoon respectively. In the case of post-monsoon, Cd (37.15ppb) showed the lowest concentration in Station I (Neendakara) (Table 1).

Metal-wise analysis showed that Zn and Co was mostly concentrated in the bivalve tissue of Station I (Neendakara). Pb and Cd was observed to the highest level in the *M.recens* of Station II (Kureepuzha), whereas Fe, Cu and Cr were in Station III (Asramam). The cumulative load of heavy metal accumulations in the tissues of *M.recens* are indicated by the Metal Pollution Index (MPI) values. The highest and the lowest Metal Pollution Index (MPI) were obtained in Station II (Kureepuzha), during pre-monsoon (238.29ppb) and post-monsoon (9.21ppb) period (Table1).

The statistical significance of spatial and temporal differences in the heavy metal accumulation of the bivalve tissue were analysed using two- way ANOVA. The results indicate that, spatial

Table 1. Seasonal variation of heavy metal (ppb) distribution in *Marcia recens* collected from three sampling stations in Ashtamudi lake during the period February to December 2019.

Pre-monsoon								
	Pb	Zn	Fe	Cu	Cr	Cd	Co	MPI
Neendakara	226.275	14553.7*	41226.6#*	467.875*	248.283*	29.6167	112.817*	69.263
Kureepuzha	527.167*	1561.42	15926.7	246.217	146.667	58.75*	93.5833	238.293#
Asramam	129.167	704.167	8116.67	133.333	63.75	30	8.75	148.919
Monsoon								
Neendakara	258.75	4166.67*	628958	1687.08*	1109.58	34.375	36.25	112.071
Kureepuzha	64.1667	798.333	12065	722.5	61.25	54.5833*	23.75	130.389
Asramam	787.5*	3427.08	646565#*	908.75	3511.25*	31.25	216.25*	13.826
Post-monsoon								
Neendakara	85.6167	13208.8*	22860.2	578	259.117*	37.15*	113.675*	19.626
Kureepuzha	208.333*	7425	46525#*	3362.5*	762.5	BDL	BDL	9.211
Asramam	NA	NA	NA	NA	NA	NA	NA	NA

BDL- Below Detection Limit

NA-Specimen is Not Available from the site at the time of sampling.

MPI-Metal Pollution Index

*The peak of each metal in each season (from top to bottom in the table)

The highest accumulated metal in each season

and temporal differences in the metal concentrations was statistically significant ($p < 0.05$) in the bivalve tissue. During all seasons, the spatial differences in the metal accumulation was quite significant among the three stations.

Analysis of Correlation studies on each metal in the respective sampling stations showed that in Station I, Cd and Fe, Cd and Cu, Cd and Cr shows positively correlated (green); Cd and Zn, Co and Cd (green) were negatively correlated (Table 3). In Station II, no very large bond of relationship were not found among the metals except Fe and Zn, Cu with Zn and Fe, Cr with Zn and Fe and Cu, Cd with all the metals except Co and Pb, (Table 4) whereas in Station III, Cd shows medium correlation (green) with the metals except Co, while all the other metals were strongly (light Brown) Correlated with each other and shows positive relation among them (Table 5). The indications of the strength of association between the metals in each station is shown in Table 6.

COMPARITIVE ANALYSIS OF MANIFOLD CONCENTRATION OF EACH METAL IN THE SAMPLES

Zn and Pb

The analysis of the Manifold Concentration (MC^1 and MC^2) showed the increasing fold of each metal with respect to the standard guidelines on food safety by the WHO/USEPA/FAO(PIS^1), and Provisional Maximum Tolerable limit for Daily Intake($PMTDI/JECFA$) (PIS^2) (Table 2.).

During monsoon, Cr level exceeds the standard permissible limits of WHO/USEPA/FAO in Station I (1109.58ppb) and in Station II (3511.25ppb) (Table 1 and 2). As per the guidelines of Provisional Maximum Tolerable limit for Daily Intake ($PMTDI/JECFA$), Lead (>25ppb), Cadmium (>0.8ppb) and Zinc (>1000ppb; except Station II), content in the tissue are found to be far exceed (Table 1 and 2). While analysing the seasonal Manifold Concentrations (MC^1 and MC^2) of Pb, Cr and Cu in Station II and III were >1 times than the PIS^1 level of WHO/USEPA/FAO limits. Likewise, Zinc and Lead were many times higher than the PIS^2 level of $PMTDI/JECFA$ in Station I, during pre-monsoon, monsoon and post-monsoon (Table 2).

Along with the coastal areas, estuarine systems are also the most exposed regions to chemical pollution (Al meida & Soares, 2012). The Ashtamudi estuarine system, have immense

Table 2. Manifold Concentration level of each metals in *Marcia recens* collected from three sampling stations in Ashtamudi lake during the period February to December 2019.

PMTDI *Provisional Maximum Tolerable Limit for Daily Intake		<i>Marcia recens</i>						
Reference Value		Pb	Zn	Fe	Cu	Cr	Cd	Co
PIS¹WHO/USEPA (mg/kg)		2	100	-	30	1	1	0.5-1
PIS¹(ppb)		2000	100000	-	30000	1000	1000	500-1000
PIS²PMTDI/JECFA (mg/kg)		0.025	1	-	0.5	1.2	0.008	-
PIS²(ppb)		25	1000	-	500	1200	0.8	-
Pre-Monsoon								
Neendakara	MC ¹	0.113	0.14555	-	0.0155	0.248	0.029	0.112-0.225
	MC ²	9.051	14.55	-	0.93	0.206	3.7	-
Kureepuzha	MC ¹	0.263	0.01561	-	0.082	0.146	0.058	0.093-0.187
	MC ²	21.088	1.561	-	0.492	0.122	7.343	-
Asramam	MC ¹	0.065	0.00704	-	0.0044	0.063	0.03	0.0087-0.017
	MC ²	5.166	0.704	-	0.266	0.053	3.75	-
Monsoon								
Neendakara	MC ¹	0.129	0.0416	-	0.056	1.109	0.034	0.036-0.072
	MC ²	10.34	4.166	-	3.37	0.924	4.29	-
Kureepuzha	MC ¹	0.032	0.00798	-	0.2408	0.061	0.054	0.023-0.047
	MC ²	2.566	0.798	-	1.44	0.051	6.82	-
Asramam	MC ¹	0.394	0.03427	-	0.0302	3.511	0.0312	0.216-0.432
	MC ²	31.5	3.427	-	1.817	2.926	3.906	-
Post-Monsoon								
Neendakara	MC ¹	0.042	0.13208	-	0.019	0.259	0.037	0.113-0.22
	MC ²	3.424	13.2	-	1.156	0.215	4.64	-
Kureepuzha	MC ¹	0.104	0.07425	-	1.12	0.762	LESS	LESS
	MC ²	8.332	7.425	-	6.725	0.635	LESS	-
Asramam	MC ¹	-	-	-	-	-	-	-
	MC ²	-	-	-	-	-	-	-

PIS¹ Permissible International Standards(FAO/WHO, 2004/USEPA,1993)

PIS² Permissible International Standards(PMTDI/JECFA;EPA,2008)

MC¹ * Manifold concentration in relation with PIS¹

MC² * Manifold concentration in relation with PIS²

1ppb= 0.001mg/kg or 1mg/kg=1000ppb

1ppb=0.001 µg/g

significance due to its worthfull services to various sectors such as in fishing communities, inland fisheries, coconut husk retting, recreation and inland navigation. The present study reveals that the bivalve *M.recens* from the sampling stations of the Ashtamudi lake was moderately to heavily contaminated with the heavy metals (Pb, Zn, Fe, Cu, Cr, Cd and Co).

The present study showed that the maximum level of heavy metal accumulation in the bivalve was observed during monsoon and pre-monsoon period. Harboursing of mechanized boats and oil spillage poses major pollution threat to the Station I (Neendakara bar mouth). This may be attributed to the highest accumulation of metals such as Zn, Cu, Fe, Cr and Co during

Table 3. Seasonal comparison of Correlation between the seven studied metals in Station I.

		Neendakara					
	Pb	Zn	Fe	Cu	Cr	Cd	Co
Pb	1						
Zn	-0.5494	1					
Fe	0.665086	-0.98937	1				
Cu	0.580406	-0.99929	0.994132	1			
Cr	0.636542	-0.99413	0.999293	0.997496	1		
Cd	-0.65862	-0.26689	0.123886	0.230495	0.161102	1	
Co	-0.6523	0.991694	-0.99986	-0.99583	-0.99979	-0.14073	1

Table 4. Seasonal comparison of Correlation between the seven metals in Station II.

		Kureepuzha					
	Pb	Zn	Fe	Cu	Cr	Cd	Co
Pb	1						
Zn	-0.10881	1					
Fe	-0.11177	0.999996	1				
Cu	-0.34928	0.969462	0.970188	1			
Cr	-0.10242	0.999979	0.999956	0.967864	1		
Cd	0.27448	-0.98575	-0.98625	-0.9969	-0.98465	1	
Co	0.849508	-0.61688	-0.61922	-0.79106	-0.61181	0.740486	1

Table 5. Seasonal comparison of Correlation between the seven studied metals in Station III.

		Asramam					
	Pb	Zn	Fe	Cu	Cr	Cd	Co
Pb	1						
Zn	0.999107	1					
Fe	0.989853	0.982966	1				
Cu	0.999851	0.99823	0.992156	1			
Cr	0.990541	0.98386	0.999988	0.99276	1		
Cd	0.653691	0.685077	0.539524	0.640544	0.543665	1	
Co	0.993073	0.987223	0.999692	0.994952	0.999802	0.560245	1

Table 6. The indications of the strength of association between each metal in each stations.

Strength of association	Coefficient,r	
	Positive(+ve)	Negative(-ve)
Small (Green)	0.1 to 0.5	-0.1 to -0.5
Medium (Red)	0.5 to 0.7	-0.5 to -0.7
Large (Light Brown)	0.71 to 1	-0.71 to -1

pre-monsoon, Zn and Cu during monsoon and Zn, Cr, Cd and Co during post-monsoon. The high tidal influences throughout the year and the prevailing salinity conditions may have the role to keep the metal level under the standard permissible levels.

In the case of Station II (Kureepuzha), discharges of municipal wastes, effluents from resorts, dumping of plastic wastes and flesh waste causes major pollution scenario (Fig. 3.). This may be laed to the highest accumulation of metals such as Cd and Pb during pre-monsoon, Cd alone in the monsoon and Pb, Fe and Cu during post-monsoon.



Fig. 3. Solid Waste disposal in Kureepuzha



Fig. 4. Dumping of wastes in Asramam

Station III (Asramam) showed the highest accumulation of metals such as Pb, Fe, Cr and during the monsoon period. Inputs of waste effluents from houseboats, K.S.R.T.C. bus station, hospitals, slaughter house leads to high organic and oil pollution in the area (Fig. 4.) Heavy rainfall during monsoon season results in leaching of effluents from municipal, agricultural and urban areas, acts as a major contributory factor towards the significant accumulation of heavy metal content ion the bivalves of the area, especially in Asramam and Kureepuzha regions. Anthropogenic activities such as urban, agricultural and industrial activities, waste discharges have led to the increase of chemical pollution in the aquatic systems (da Silveira Fiori et al., 2018; Esposito et al., 2018)

As the bivalves are benthic species, presence of heavy metals in the organic matter containing bottom sediments can enhance the metal accumulation in the bivalve tissues .

Several studies have pointed that highest metal accumulation during the monsoon season

may be coincided with the spawning period (June- November) of the bivalves, which enhances the protein and carbohydrate content (high affinity for metals) for the gonadal tissue generation, energetic storage and consumption (Latouche & Mix, 1982). The increased accumulation of heavy metals are contributed by the anthropogenic inputs such as industrial and urban effluents into the lake (Nagaraj Sitaram, 2014).

The declining trend in the rainfall and related surface runoff may results in the reduction of metal accumulation in the system during non-monsoon period. On the contrary, increased salinity induces high precipitation and coagulation of colloidal clay particles which results in rapid sedimentation and co-precipitation of metals (Bryan, 1976). This may be contributed to the highest Metal Pollution Index value reported from Station II (Kureepuzha) during pre-monsoon. The variation of heavy metals accumulation in the bivalve among the sampling stations may be due to the differences in the metal mobilization through organic matter, sediment and water as well as due to the tidal and salinity influence. The bioaccumulation rate of the heavy metals in the body of the bivalve, depends on the physiological conditions (size, age and weight) of the clams and environmental parameters including temperature, pH, salinity, oxygen concentrations (Jordaens, et al., 2006).

Heavy metal imposed risks to the human health

Even though, heavy metals are biologically vital (Suami et al., 2019), long term exposure beyond to a certain level, it starts to bioaccumulate faster than they are metabolized and this implies the significance for a greater concern. Each metal has specific effects on human health such as behavioural, physiological as well as other cognitive impacts. Several studies reveals the toxic effects of heavy metals as a hazardous carcinogenic compounds which can cause lethal metabolic and physiological interruptions (Piotr et al., 2015; Pais & Benton, 1997; Godt et al., 2006; Abou et al., 2001). The metal impacts on the consumer's health are depends on the specificity of the metal, affected body parts and duration of exposure (Denil et al., 2017; Schumann et al., 2002; Blackhima, 1970; Alexander, 1972; WHO; Massadeh et al., 2004; Guo et al., 2020). Cd and Pb are highly toxic even at low concentrations and can leads to intoxication, fertility problems, cellular and tissue damage, cell death, organ dysfunctions, cancer etc. to its consumers (Stankvoic & Jovic, 2012; Benavides et al., 2005; Nordberg, 2010; Pernia et al., 2018; Fernandez- Cadena et al., 2014).

Some benthic species exhibited well regulated mechanisms for excess metal accumulation such as storage in the hepatopancreas (Bootsma, et al., 1988) or excreted (Bharath, et al., 2019). Although the present study reveals the level of metal accumulation in the decreasing order Fe>Zn>Cr>Cu>Co>Cd, the lethality aspects of individual metal may vary. Toxicity also varies with the prevailing environmental conditions which can influence the chemical speciation of metals. The predominant factors responsible for deterioration of the Ashtamudi backwaters are the pollution and encroachment. If the discharge of aquatic pollutants, especially the heavy metals, into the Lake continue this scenario, it will definitely impose serious impediment to the survival of the aquatic biota as well as the sustainable and supportive nature of the estuarine system. This alarms an immediate call for the implementation of a proper and scientific control measures and strategies to counter this condition.

CONCLUSION

Although, bioaccumulation of heavy metals in the bivalve *M. recens*, didn't exhibit any remarkable seasonal fluctuations among the sampling stations but the area were moderately to heavily contaminated with the metals from various sources. Eventhough, bivalves are filter feeders, levels of metal accumulation in their body is an indication of metal contamination in the dependant medium. As a high proteinaceous foods, as other edible clams, *M. recens* also have demandable market and export values. Although, there was no apparent health issues

were evident to its consumers from being exposed to low level single metal contamination, the regular consumption of with high dose of multiple metal loaded bivalves can cause additive or interactive health effects.

As a Ramsar site (no.1204) with significant wetland features, temporal and spatial study about the current ecological status of the Ashtamudi Lake gains immense attention from the conservation point of view. Hence an appropriate scientific and legal measurements should be adopted to prevent further contamination of this estuarine system and thereby preventing the metal load into the bioaccumulation as well as biomagnification process.

Therefore, the observations made by the present study drew a cross section of heavy metal contamination status of the Ashtamudi Estuarine system. The study also recommends to develop and implement suitable methods or strategies for conservation of the lake ecosystem with immediate effect. A regulatory control measures and monitoring system can bring back the contamination levels within tolerable limits to a certain extent.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consents, misconduct, data fabrication and /or falsification, double publication and/or submission, and redundancy as being completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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