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FIRST REPORT ON THE SCANNING ELECTRON MICROSCOPIC INVESTIGATION OF THE GILLS OF YELLOW FOOT CLAMS FROM ASHTAMUDI LAKE WITH REFERENCE TO HEAVY METAL POLLUTION

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ABSTRACT
The aim of present work was to evaluate and compare the bioaccumulation of selected heavy metals – Cadmium, Chromium, Copper, Lead and Zinc in the gills of yellow foot clams collected from polluted and reference sites of Ashtamudi Lake and thereby its predominant effects on histological moieties. The order of accumulation of heavy metals in gills of clams from polluted sites - site 1 and site 2 is Zn (79.33 mg/kg) >Pb (11.56 mg/kg) > Cr (4.99 mg/kg) > Cu (4.53 mg/kg) > Cd 1.35 mg/kg) and Zn (21.77 mg/kg) > Cr (4.13 mg/kg) > Cu (2.71 mg/kg) >Pb (1.68 mg/kg) > Cd (1.01 mg/kg) respectively; whereas from site 3 (reference site) the order is Zn (8.80 mg/kg) > Cu (1.53 mg/kg) > Cr (0.15 mg/kg) >Pb (0.07 mg/kg) > Cd (0.008 mg/kg. Predominant histological alterations such as clubbing and fusion of gills, degeneration, necrosis, atrophy, abnormal gill morphology, oedema etc. were observed in the gills from polluted sites; whereas gills from reference site showed a normal morphology. The findings of the present study revealed the burden of heavy metals in the gills of edible yellow foot clams.

***Keywords**: Gills, Yellow Foot Clams, Heavy Metals, Scanning Electron Microscope*

INTRODUCTION
Aquatic pollutants seriously affect the ecological status of backwaters and rural estuaries in Kerala. Ashtamudi Lake, the second largest backwater in Kerala is prone to several kinds of toxic aquatic pollutants. Rapid industrialization, tourism activities without proper effluent and waste treatment facilities, intensive coconut husk retting, oil and excreta release from house boats together with encroachment are the major ecological threats which affects Ashtamudi Lake. Of the several threats, heavy metal pollution is one the crucial problem faced by Ashtamudi Lake. Studies of heavy metal toxicity in the water, sediment and fish samples of Ashtamudi Lake have been reported by many (Geetha, 1997; Bhavan and Geraldine, 2000; Suma *et al.*, 2012; Razeena., 2014; Lekshmi and Sherly, 2018). The toxic metals usually penetrate and concentrated on different tissues of organisms especially through skin and gills (Ahmed *et al.*, 2014). Among the various organs, the gills of shellfishes are considered the main site of entry to aquatic pollutants as they are in direct contact with the external medium to perform gaseous exchanges and ionic regulations. In order to promote its normal functions the gills have linked sites, which link to toxic pollutants with differentiated charges, triggering, mechanical responses, and toxic effects to the organism (Roberts, 1989). For the present study the gills of edible molluscs, yellow foot clams - *Paphiamalabarica* was targeted in order to analyze the severity of heavy metal contamination in them.
Molluscs which forms the major constituents of aquatic food chain when exposed to alarming levels of metals in a polluted aquatic ecosystem tends to take these metals up from their direct environment. Studies especially on the changes in the histological structure can probably use as a reliable tool for

determining the action of different toxicants on the body of organisms. Histological analysis using electron microscopic techniques have been widely used as diagnostic tools in order to ascertain the morphological changes in the tissues of fishes due to the chronic exposure to various heavy metals (Sivakumar *et al.*, 2015; Arian *et al.*, 2016; Smriti *et al.*, 2019). The higher level of toxic heavy metals caused adverse effects on aquatic organisms, at cellular or molecular level and results in the alteration of histological structure (Kavitha *et al.*, 2010; Nurnadia *et al.*, 2011).

Even though many have investigated effects of heavy metal pollution on the mortality of aquatic animals (Arafa *et al.*, 2015; Celine *et al.*, 2015; Rajalakshmi, 2015) very little is known about the histological changes in the tissues of aquatic organisms on exposure to toxic heavy metals from Ashtamudi Lake. Hence, the present study was emphasized on alterations in the histological structure of gill tissues of edible yellow foot clams of Ashtamudi Lake with special reference to heavy metal toxicity in them.

MATERIALS AND METHODS

Ashtamudi Lake, (lat-76°.32' and 76°.41 E ; long- 8°.52' and 9°.02' N) the second largest (area of 61.42 Km^2) and deepest (maximum depth of 6.4 m at the confluence zone) wetland ecosystem of Kerala is situated in the Kollam district. As the name implies 'Ashtamudi' Lake is planimetric in shape, which is divided into eight principal arms. Of these Kureepuzha, Perumon and West Kallada regions of Ashtamudi Lake were selected as site 1, 2 and 3 respectively for the present study. The effluents from Parvathy Mills, Milma Dairy, KSRTC workshop, municipal waste dump site and many small scale industries are the major sources of heavy metals in this region (Razeena, 2014) The Aluminum Industries Ltd., Kerala Ceramics Ltd., Kerala Electrical and Allied/Engineering Company and Techno Park are the major industries discharging effluents at site 2, the Perumon region (Razeena, 2014; Gireesh Kumar, 2016). The region of West Kallada Lake which is not much disturbed with anthropogenic interferences and urbanization is selected as the third and reference site.

Yellow foot clam (*Paphia malabarica)* otherwise known as short neck clam is the most widely distributed and continuously exploited clam for local consumption as well as for export. Yellow foot clams of Ashtamudi Lake was the first fishery in India to be MSC certified (Wakamatsu and Wakamatsu, 2017). Shell is large and thick with concentric and strong close set ridges. The front and hind margins are narrowly rounded and the ventral margins almost straight. The striation is almost parallel to the ventral margin and corresponding eduntation is noted in the hind margin. Pallial sinus 'U' shaped and lunate is short and depressed (FAO, 1984). Yellow foot clams (360 numbers of about 1 to 2.5 cm shell length and 0.250 to 0.700 gm weight) were collected from each study site for one year. Gills of the selected species were carefully removed for heavy metal and histological analysis.

Heavy metals such as Cadmium, Chromium, Copper, Lead and Zinc were selected for the present study. Heavy metal analysis in the gills of yellow foot clams were carried out using an atomic absorption spectrophotometer (AAS, Pinnacle 900H) as described by APHA (2012). Metal concentrations were calculated in mg/kg.Data obtained was generalized and the results were expressed as mean ± standard deviation. Statistical analysis of data was performed using SPSS statistical program (Package-22, registered). Significant differences between heavy metal components of the candidate species from various sites, determined using One- Way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test.

For electron microscopic investigation, gills of clams were cut into 1mm diameter and were initially fixed in 4% Glutaraldehyde solution for 24 hours. Then multiple rinsing in Cacodylate buffer with pH 7.2 was carried out. Post fixation was done in 1% Osmium Tetroxide solution for 2 hours. The sections after dehydrated in a graded series of Ethanol were dried by the critical point method. Dried tissues were mounted on stubs using carbon tape and were viewed and photographed with a scanning electron microscope (JEOL Model JSM – 6390 LV) operating on 30 kV at STIC, CUSAT, Kochi.

RESULTS AND DISCUSSION

Statistical results of ANOVA showing the heavy metal accumulation in the gill of clams is shown in table 1. The results of the One Way Analysis of Variance (ANOVA) showed that selected heavy metals for the present study such as Cadmium (F = 77.57), Chromium (F = 46.58), Copper (F = 18.37), Lead (F = 71.21) and Zinc (F = 112.76) were found to be different among each other with respect to the study sites. The mean difference was found significant at 1% ($p < 0.01$). The results of the Fisher's LSD Post hoc multiple comparisons further reveals that the site 1, 2 and 3 significantly differ among each other with respect to the heavy metals Cadmium and Zinc. For the heavy metal Chromium, site 1 and 2 significantly differ from site 3. In the case of heavy metals Copper and Lead site 1 significantly differ from site 2 and 3.

Table 1: Analysis of variance (One Way ANOVA) of heavy metals in the gill of yellow foot clams comparing study sites

	Study sites				
Heavy metals (mg/kg)	**Site 1 (Mean ± SD)**	**Site 2 (Mean ± SD)**	**Site 3 (Mean ± SD)**	**F value comparing study sites**	**P Value**
Cadmium	1.35 ± 0.24^{a}	1.01 ± 0.40^{b}	0.00 ± 0.02^{c}	77.57	< 0.01*
Chromium	4.99 ± 1.72^{a}	4.13 ± 1.43^{a}	0.15 ± 0.29^{b}	46.58	< 0.01*
Copper	4.53 ± 1.78^{a}	2.71 ± 0.97^{b}	1.53 ± 0.57^{b}	18.37	< 0.01*
Lead	11.56 ± 4.24^{a}	1.68 ± 1.21^{b}	0.07 ± 0.17^{b}	71.21	< 0.01*
Zinc	79.33 ± 20.11^{a}	21.77 ± 6.67^{b}	8.80 ± 0.77^{c}	112.76	< 0.01*

** = p < 0 .01, The mean difference is significant at 1% level; SD – Standard deviation; $^{a, b, c}$. Means*

Table 2: Comparing the heavy metal analysis in the gill of yellow foot clams with international standards

Heavy metals	**FAO limits (mg/kg)**	**Present study - mean values (mg/kg)**			**Inference**
		Site1	**Site 2**	**Site3**	
Cadmium	1	1.35	1.01	0.00	Site 1 and 2 above permissible limit
Chromium	0.2	4.99	4.13	0.15	Site 1 and 2 above permissible limit
Copper	30	4.53	2.71	1.53	All sites below permissible limit
Lead	1	11.56	1.68	0.07	Site 1 and 2 above permissible limit
Zinc	40	79.33	21.77	8.80	Site 1 above permissible limit

within rows with differing subscripts are significantly different using Fisher's LSD post hoc test.

Comparison of heavy metal analysis in the gills of clams with international standards (FAO, 2012) is shown in table 2. The maximum permissible limit of Cadmium in fresh water clams according to FAO is 1 mg/kg. Gills of selected clams from site 1 and 2 with their mean values 1.35 mg/kg and 1.01 mg/kg were above the prescribed limit of international standard limit. At site 3 the mean value was 0.00 which was below the limit. For Chromium, the allowable limit is 0.2 mg/kg. Site 1 and 2 with their respective mean values 4.99 mg/kg and 4.13 mg/kg respectively, were found to be much greater than the FAO prescribed limit. For site 3 the value was 0.15 mg/kg which was below the limit. The maximum permissible limit of Copper in fresh water clams according to FAO is 30 mg/kg and gill of three study sites were safe with respect to this limit. The mean values of gill were 4.53 mg/kg, 2.71 mg/kg and 1.53 mg/kg for site 1, 2 and 3 respectively. The maximum allowable limit for Lead is 1 mg/kg. Site 1 with a mean value of 11.56 mg/kg and site 2 with a mean value of 1.68 mg/kg were found to be beyond this limit. Site 3 with a mean value of 0.07 mg/kg is found to be below the limit. The permissible limit for Zinc is 40 mg/kg and the gill sample of site 1 with a mean value of 79.33 mg/kg exceeds the limit. The mean values for site 2 and 3 were 21.77 mg/kg and 8.80 mg/kg respectively which were below the limit.

Metals which are essential for the growth and metabolism of the aquatic organisms become toxic to the body when exceeds a specific limit (Authman *et al.*, 2015). In the present study the order of accumulation of heavy metals in the gill of yellow foot clams from site 1 is Zn (79.33 mg/kg) > Pb (11.56 mg/kg) > Cr (4.99 mg/kg) > Cu (4.53 mg/kg) > Cd 1.35 mg/kg). With respect to site 2 the order is Zn (21.77 mg/kg) > Cr (4.13 mg/kg) > Cu (2.71 mg/kg) > Pb (1.68 mg/kg) > Cd (1.01 mg/kg). The order of heavy metal accumulation of site 3 is Zn (8.80 mg/kg) > Cu (1.53 mg/kg) > Cr (0.15 mg/kg) > Pb (0.07 mg/kg) > Cd (0.008 mg/kg). Study confirms that gills of clams from site 1 and 2 were found to bioaccumulated with heavy metals when compared with the reference site (site 3). Similar results with heavy metal accumulation have been reported in tissues of mangrove crab, collected from polluted sites of Ashtamudi Lake (Remadevi and Abdul, 1995). Bioaccumulation status of heavy metals in different fish species such as *Liza parsia* (Razeena, 2014), *Etroplus suratensis* (Geetha, 1997), *Scylla serrata* (Ramadevi and Abdul, 1995) *and Paphia malabarica*(Wilfred, 1993) collected from the polluted regions of Ashtamudi Lake further supports the result of the present study.

Investigation under Scanning Electron Microscope (SEM) revealed that gill structure ofclams from site 3 are composed of interlamellar and interfilamentous junctions (Figure 1). The entire gill has the appearance of a perforated, leaf like organ (Figure 2). Several predominant histological alterations has been noticed in the gill of *P. malabarica* collected from site 1 and 2 while viewing through SEM. Fibrous growth like observation (Figure 3), clubbing and fusion of gill (Figure 4) degeneration, necrosis, atrophy (Figure 5) and accumulation of heavy metal deposition (Figure 6) were clearly observed on the surface of gill lamellae collected from site 1. Extensive necrosis, fusion of gill lamellae (Figure 7), curling of the tip of gill lamellae (Figure 8), splitting of gill lamellae, abnormal gill morphology (Figure 9), oedematic surface and fibrous growth like observation (Figure 10) were the predominant histological alterations observed on the gill lamellae from site 2.

Figure 1: Gill of *P.malabarica* from site 3

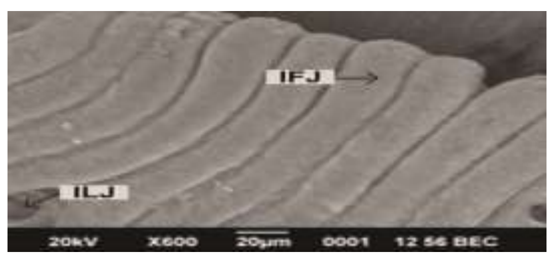

Figure 2: Gill of *P.malabarica* from site 3

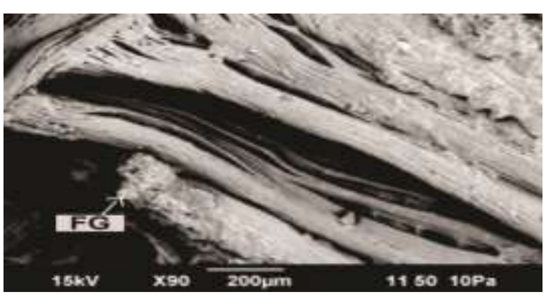

Figure 3: Gill of *P. malabarica* from site 1

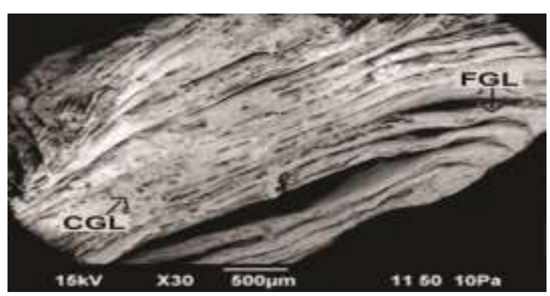

Figure 4: Gill of *P. malabarica* from site 1

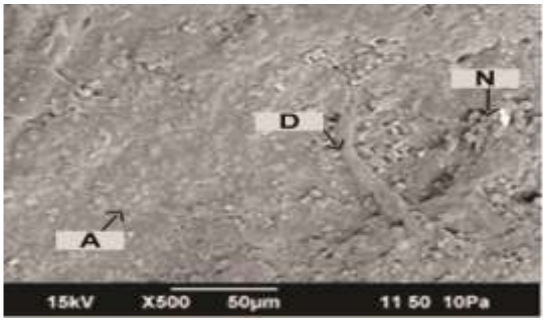

Figure 5: Gill of *P.malabarica* from site 1

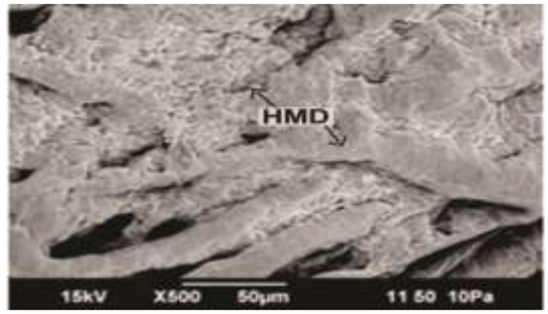

Figure 6: Gill of *P.malabarica* from site 1

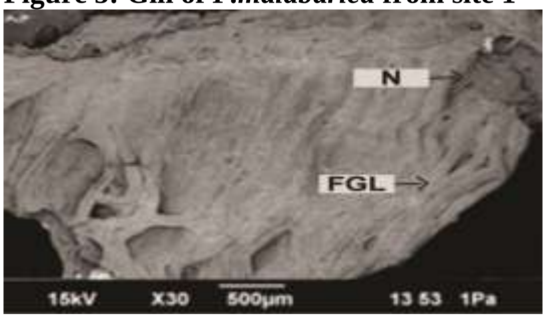

Figure 7: Gill of *P.malabarica* from site 2

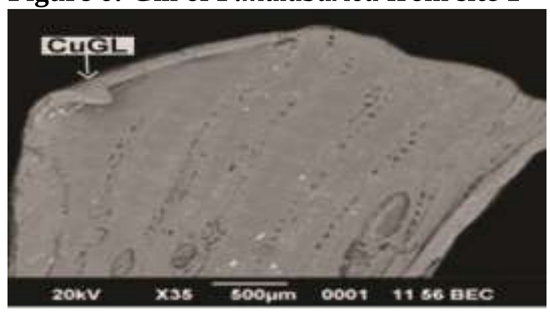

Figure 8: Gill of *P. malabarica* from site 2

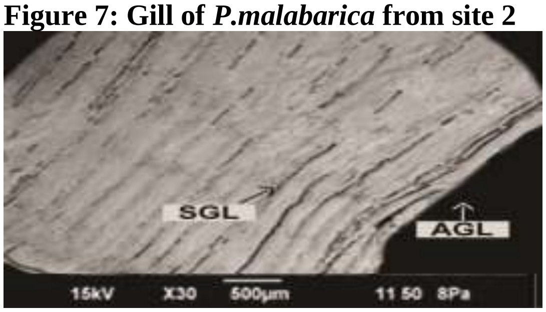

Figure 9: Gill of *P.malabarica* from site 2

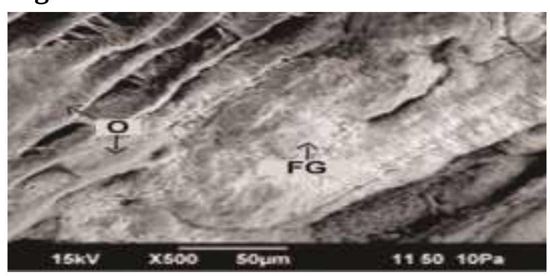

Figure 10: Gill of *P.malabarica* from site 2

ILJ – Interlamellar Junction; IFJ – Inter Filamentous Junction; FG – Fibrous Growth; FGL – Fusion of Gill Lamellae; CGL – Clubbing of Gill Lamellae; A - Atrophy; D- Degeneration; N- Necrosis; HMD – Heavy Metal Deposition; N – Necrosis; FGL – Fusion of Gill Lamellae; AGL – Abnormal Gill Lamellae; CuGL – Curly Gill Lamellae; SGL – Splitting of Gill Lamellae; O – Oedema; FG – Fibrous Growth

Gill structure of *Paphiamalabarica* collected from site 3 revealed a normal structure of eulamellibranch gill. While the observation on the gills from site 1 and 2 revealed abnormal morphology. Previous reports revealed significant morphological alterations in the gills and fins of *Penaeus monodon* collected from polluted regions of Ashtamudi Lake due to different kinds of aquatic pollutants, especially heavy metals (Sherly *et al.*, 2015). Chronic changes in the normal gill structure such as oedema, lifting of lamellar epithelia, lamellar degeneration and necrotic changes noticed in previous reports in the gills of different fresh water fishes due to exposure of different heavy metals (Bhavan and Geraldine; 2000; Selvanathan, 2013; Ahmed *et al.*, 2014; Chavan and Mulay, 2014; Authman *et al.*, 2015) further stabilizes the result of the present study.

CONCLUSION

The results of the present study revealed that the gills of yellow foot clams collected from site 1 and 2 are more contaminated with heavy metals, compared with reference site (site 3). Anthropogenic influences are seemed to the main sources of pollution of site 1 and 2. With respect to sites the decreasing order of heavy metal accumulation is site1 < site2 < site3. Bioaccumulation of heavy metals in turn alters and impairs the nutritional status of short neck yellow clams. The results clearly indicates the fact that molluscs inhabiting in the polluted regions are more prone to serious stress condition which is reflected from the alterations in their histological architecture. If the bioaccumulation of heavy metals occurs in an increasing proportion in the tissues of organisms it will further results in the deterioration of the organisms. Likewise yellow foot clams other organisms inhabiting the Lake has an equal chance for the accumulation of heavy metals in their tissues. These will in turn pave way for the deterioration of the aquatic organisms inhabiting the Lake. Stringent and immediate necessary actions should be taken up by the responsible authorities in order to safeguard the aquatic organisms and thereby the protection of Ashtamudi Lake as a whole from severe pollution.

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REFERENCES

Ahmed M, Parvin E, Islam MM, Akter MS, Khan MS and Mamun MH (2014). Lead and Cadmium induced histopathological changes in gill, kidney and lier tissues of freshwater climbing perch *Anabas testudineus* (Bloch, 1792). *Chemistry and Ecology*, **30**(6) 532-540.

APHA (2012). Standard methods for examination of water and wastewater. 22nd ed. American public health association/American water works association/water environment federation, Washington D.C., USA.

Arafa M, Afifi, SHH and Ali AT (2015). Investigating the oxidative stress of heavy metal's pollution in *ClariasGariepinus*, Egypt. *Journal of Chemical and Environmental Health*, **1**(1) 231-243.

Arian M, Filis M, Vesna D, Sladjana S, Jasmina KC, Bojan K andMirjana L (2016). Common barbel (*Barbusbarbus*) as a bioindicator of surface river sediment pollution with Cu and Zn in three rivers of the Danube River Basin in Serbia. *Environmental Science and Pollution Research*, **23**(7)6723-6734.

Authman MMN, Zaki MS, Khallaf EA and Abbas HH (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquatic Research Development*, **6** 4-13.

Bhavan PS and Geraldine P (2000). Histopathology of the hepatopancrease and gill of prawn *Macrobrachium malcolmsonii* exposed to endosulfan. *Toxicology* **50** 3331-339.

Celine HMS, Bhuvaneswari andAnandan R (2015). Biochemical and histopathological studies on lead nitrate induced toxicity in fresh water fish grass carp (*Ctenopharyngodon idella*). *European Journal of Experimental Biology*, **5**(11) 24-30.

Chavan VR and Muley DV (2014). Effect of heavy metals on liver and gill of fish *Cirrhina mrigala*. *International Journal of Current Microbiology and Applied Sciences* **3**(5) 277-288.

FAO (2012). (Food and Agriculture Organization) from heavy metal regulations faolex: http://faolex.org/docs/pdf/eri42405.pdf. 2003 [Retrieved in 2012].

FAO (1984). Species identification sheets for fishery purposes. Food and Agriculture Organization of the United Nations. Rome.

Geetha B (1997). Heavy metal pollution inAshtamudi estuarine system. *PhD Thesis*, University of Kerala. India.

Girish Kumar B (2016). Toxicity induced changes of Cu and Pb on the humoral and cellular factors on *Anabas testudineus* (Bloch, 1792) *PhD Thesis*, Cochin University of Science and Technology, India.

Kavitha C, Malarvizh A, Senthil KS and Ramesh M (2010). Toxicological effects of arsenate exposure on haematological, biochemical and liver transaminase activity in an Indian major carp, *Catlacatla. Food Chemistry Toxicology*, **48**(28) 48-54.

Lekshmi PV and Sherly WE (2018). An assessment of heavy metal pollution load of Ashtamudi lake with respect to the mangrove crab, *Scylla serrata. Eco Chronicle,* **4**(13) 203-208.

Nurnadia AA.Azrina A and Amin I (2011). Proximate composition and energetic value of selected marine fish and shellfish from the West coast of Peninsular Malaysia. *International Food Research. Journal*, **18**137-148.

Rajalakshmi P(2015). Effect of Lead nitrate on biochemical composition of blood and acid phosphatase activity in *Cyprinus carpio* (Linnaeus).*Current Biotica*, **9**(3) 256-262.

Razeena KL (2014). Ecobiological aspects of an estuarine fish *Lisa Parsia, PhD Thesis*, University of Kerala, India.

Remadevi V and Abdul APK (1995). Pollution status of Ashtamudi estuary need for management measures. Proceedings of the 7th Kerala Science Congress, State Committee on Science Technology and Environment.

Roberts RJ (1989). Fish pathology. 2nd ed., BalliereTindall. London.

Sandhya MS and Mayur S (2018). Effect of heavy metals Hgcl2 and Cdcl2 On glycogen activity of bivalve *Lamellidens marginali*, *Journal of Pharmacy,* **8**(8) 28-35.

Selvanathan J, Vincent S and Nirmala A (2013). Histopathology changes in fresh water fish *Clariasbatrachus*(linn.) exposed to Mercury and Cadmium. *International Journal of Life Science and Pharma Research,* **3**(2) 11-21.

Sherly WE, Lekshmi PV and Razeena KL (2015). Morphological alterations caused by pollution on gills and fins of *Penaeusmonodon*. *European Journal of Biomedical and Pharmaceutical sciences,* **2** 569-575.

Sivakumar R, Dhivya K and Grayson H (2015). An integrated use of histological and ultrastructural biomarkers in *Mugil cephalus* for assessing heavy metal pollution in east Berbice-Corentyne, Guyana. *International Journal of Bioassays,* **4**(11) 4541-4554.

Smriti B, Rajinder J, Sakshi V (2019). Scanning electron microscopic investigation on the scales of grass carp, *Ctenopharyngodonidellus*(Cuvier and Valenciennes) exposed to Lead nitrate. *International Journal of Fisheries and Aquatic Studies* **7**(3) 93-98.

Suma S, Manoj SV and Chithra PG (2012). Analysis of effluents discharged to Ashtamudi Lake from china clay industries**.** *International Journal of Chemistry Research,* **3** 0976-5689.

Wakamatsu M and Wakamatsu H (2017). Certifcation of small-scale fisheries. *Marine Policy* **77**(3) 97-103.

Wilfred J (1993). Distribution of trace metals in the Ashtamudi Estuary, Kerala with special reference to Molluscs. *PhD Thesis*, University of Kerala, India.