



REVIEW ON HEAVY AND TRACE METAL STUDIES IN VEMBANAD LAKE ECOSYSTEM: SPECIAL REFERENCE TO COCHIN BACKWATERS

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ABSTRACT

Vembanad Lake is facing gross pollution problem mainly due to the release of untreated effluents from industries and domestic sectors. The increasing concentrations of pesticides, heavy metals and trace metals in Vembanad Lake reached hazardous levels, which may cause major health risk to humans through the trophic transfer. In this review paper, a sincere effort is made to bring forward the various studies on heavy and trace metals, conducted in the Vembanad Lake especially in Cochin backwater system, by various researchers during the past few decades. A number of metals are present in higher concentrations in various areas of the estuarine region. Copper (Cu), Zinc (Zn), Lead (Pb) and Mercury (Hg) are the most reporting metals from the backwater system. Selenium (Se) and Nickel (Ni) are the least found trace metals.

Keywords: Trace metals; heavy metals; trophic transfer; pollution; Vembanad Lake; Cochin estuary.

1. INTRODUCTION

There are about 19 Ramsar sites in India. Of these, Vembanad Lake, Ashtamudi Lake and Sasthamcotta Lake are the three Ramsar sites present in Kerala state [1,2]. The Vembanad Lake and Ashtamudi Lake correspond to the backwater ecosystems in India. Major port activities in Kerala are through the

Vembanad Lake and principally through the Cochin backwater [3,4,5]. The Vembanad Lake is facing various pollution problems [6]. Various human activities, industrial effluents from nearby factories, pesticides and chemicals from the agricultural lands, heavy and trace metals from various sources, port activities and aquatic tourism adversely affect the backwater ecosystem [7, 8]. They cause deprivation in

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the water quality that lead serious health hazards to aquatic organisms and finally humans who depends on the particular lake water [9, 10, 11, 12]. We know that heavy metals are toxic to the environment as well as to animals in very low concentrations whereas, trace metals are needed in very trace amounts for the physiological and metabolic functioning of organisms.

Water quality can be determined by analyzing the surface water [13, 14, 15, 16]. Vembanad Lake and Cochin backwater systems are getting polluted day by day by the increasing level of heavy and trace metals. These enriched metals, due to industrialization [15, 17] and urbanization, are dissolved in water and deposited in the sediments [18, 19] and are absorbed by the aquatic plants and accumulated in the aquatic biota. Since fish are the main food source of humans, it is very important to check the quality of water [20] because the contaminants like heavy and trace metals primarily affects the water quality. Rivers like Pampa, Achencovil, Manimala, Muvattupuzha, Meenachil and Periyar joins with the Vembanad Lake and flows their water to the lake [21]. The river water may contain various pollutants and chemicals that deposit in the lake water.

The effluents that contaminate Vembanad aquatic system comes from chemical industries and other manufacturing units like food, drugs, paper, rayon, textiles and rubber. In addition, various other sources like Cochin shipyard and port releasing high quantities of waste oil, metals, and also wastes from paper mills highly contaminates the water body [22]. As pollutants enter an environment, they undergo a variety of physical and biogeochemical processes that lead to either their degeneration or sometimes their ultimate deduction. The persisting chemicals which do not undergo breakdown cause serious environmental problems [23]. Heavy and trace metals are such a group of chemicals which can accumulate easily and so their toxic effects are transmitted to various trophic levels [24, 25, 26]. When these metals present in the water, they deposit in the sediments, accumulate into the flora and fauna of the aquatic ecosystem and finally to humans. All trace metals, naturally occurring in the biosphere have special role in various functions of life. Although they are essential for life, are toxic at sufficiently high concentrations [27, 28, 16].

2. METALS IN WATER

Reports have shown that Cu and Zn were the major trace metals found in higher concentration whereas Se and Ni were the least found metals in Vembanad Lake. The presence of trace metals in the aquatic life were depends on their concentration in water and

bottom sediments [29, 30]. Analysis of metal concentration in Cochin backwaters found Iron (Fe), Manganese (Mn) and Copper (Cu) at high levels in the river mouth [31] and observed an increasing trend in the concentrations of Zinc (Zn) and Nickel (Ni) [32]. The industrial effluent discharge site of Cochin backwaters showed enrichment of Cu during monsoon season and Zn during pre monsoon season [33]. The Cochin backwater considered as one of the most polluted estuaries in India [34].

Dissolved and particulate trace metals in the Cochin estuary showed Cu, Zn and Cadmium (Cd) with high seasonal variation, Ni and Pb in negligible concentration and Fe found to be removed from the dissolved state [35]. The salinity played an important role in the precipitation of particulate matter and heavy metals with respect to estuarine mixing. The assessment of heavy metal pollution status of Vembanad Lake revealed some areas of the backwater system were beyond the standard limits. The distribution and risk assessment of trace metals in Vembanad Lake showed the surface water with distribution of trace metals in the order $Zn > Pb > Cu > Cd > Hg$ and the bottom water with $Zn > Pb > Cu > Hg > Cd$ [12]. Trace metal studies in the surface waters of Vembanad Lake detected high concentrations of Cu and Zn [19].

Seasonal variation of heavy metals in Cochin estuary and adjoining Muvattupuzha and Periyar rivers showed high concentration of Fe ($311.88 \mu\text{g/l}$) and Cu ($25 \mu\text{g/l}$) during pre monsoon in Cochin estuary and lowest concentration in Muvattupuzha river. The concentration of Zn was maximum in Periyar river ($147.46 \mu\text{g/l}$) and minimum in lake region ($25.6 \mu\text{g/l}$). The Pb concentration was maximum in lake water ($64 \mu\text{g/l}$) and minimum in Muvattupuzha river [15].

Ramasamy and his co-workers analyzed total and methyl mercury in the Vembanad backwater system and concluded that the concentration of total mercury (THg) and methyl mercury (MHg) in surface water samples were 31.8 ng/l and 0.21 ng/l respectively whereas the bottom water samples with total mercury (THg) 206 ng/l and methyl mercury (MHg) 1.22 ng/l [36]. The bottom waters of Cochin estuary was highly polluted with Hg when compared with the surface waters [13]. The trace metals (Ni, Pb, Zn and Mn) with their distribution and partitioning behavior in the Cochin backwater column were found influenced by various factors like, the presence of a salinity barrier across the backwater system and use of pesticides and chemical fertilizers in the agricultural land near the water system [37].

Water quality in south western parts of Cochin estuary was analyzed and observed that the concentration of

trace elements in the Eloor industrial belt was higher than other parts of the Vembanad Lake [38]. Analysis of dissolved and particulate metals in overlying waters of Cochin backwater system observed Cu and Cd concentrations constantly co-varied, whereas, the metals Zn, Ni and Pb co-varied on seasonal basis [39]. Studies on assimilative capacity of dissolved nutrients, trace metals, etc from Cochin estuary and inshore waters of Cochin revealed that total suspended solids and Cd had reach dangerous levels while Pb had arrive at levels of warning [40]; whereas in the case of Cochin inshore waters, Cd reached critical level while Cu and Pb at the level of warning [41].

3. METALS IN SEDIMENTS

Metals are feebly dissolved in water. They are adsorbed on the particulate matter in the water body and finally settled as sediments. Trace metal levels in the sediments of Cochin backwater system revealed that Cu and Zn concentrations vary with areas and seasons. The sediments in the area of effluent discharge showed high deposition of Cu during monsoon and Zn during pre-monsoon. During post monsoon and pre monsoon there occurred enrichment in the level of Zn in the downstream of effluent discharge site [33]. The fine grained sediments in the estuarine region were poor in their Mn and Co contents and enriched with Ni and Cu contents when compared with the fine grain sediments of other parts of the Vembanad Lake [42].

The studies on distribution and contamination of heavy metals in the sediments of Vembanad Lake observed a decreasing trend in the concentration of the metals Hg, Pb, Cu, Cd, Zn, Mn, Fe, Cr, Al and Ni from north to south of the lake [43]. Nearly 260 liters of industrial effluents reached daily from the Kochi industrial belt to the Vembanad Lake and also various rivers that contribute high metal concentrations by reaching the lake [42, 44, 8, 43, 21]. The Cochin estuarine area were anthropologically contaminated with heavy metals and showed maximum concentrations of Zn, Pb, Cd, Cr, Ba, Fe₂O₃, MgO and P₂O₅ [45]. Bindu and co-workers reported heavy metal contamination (Cd, Zn, Pb and Ni) with anthropogenic influence in Cochin estuary [46].

Studies on heavy metal distribution[47, 45, 48, 6, 49] and contamination status in the sedimentary environment of Cochin estuary pointed out that the occurrence of Cu and Ni were by natural weathering process whereas the higher levels of Cd, Cr, Zn and Pb were due to anthropogenic activities [50, 46, 51]. Serious trace metal accumulation was exhibited in the sediments of the northern arm of the estuary [33, 50,

52]. Dipu and Kumar studied distribution of Hg and other trace metals like Pb, Cd, Cu, Zn, Cr and Mn in the sediments [53]. They observed high metal concentration in the sediments near the industrial belt. The enrichment of Cu, Ni, Co, Zn and Cd were observed in the vicinity of industrial area [54]. The presence of metals in the top 20 cm indicated the increase of contamination in the recent past. Cochin estuary showed high concentration of Hg in the sediments during summer season [55]. The industrial effluents are the major sources of metal enrichment [54, 10, 56, 46].

The surficial sediments of Cochin backwaters were analysed for the concentration of metals like Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn during monsoon and pre monsoon periods observed that, sediments were moderately polluted with Cu and Pb; moderately to somewhat heavily pollute with Zn and extremely polluted with Cd [51]. The increased level of metals during post-flood period indicated the accumulation of metals occurred in the sedimentary materials [39, 57, 58].

Using geochemical and statistical tools on the surface sediments of Vembanad Lake for the assessment of heavy metals observed enrichment of Al, Fe, Mn, Cr, Zn, Ni, Pb, Cu, Co and Cd. The analysis of enrichment factor and pollution load index showed severe and moderately severe enrichment of Cd and Zn, minor enrichment of Pb and Cr in the north estuary [48]. The concentration and partitioning behavior[59] of trace metals (Cd, Co, Cr, Cu and Fe) between exchangeable, reducible and organic/residual phases of the sediments in the southern upstream part of Cochin estuarine system were found significantly higher when compared to the metal concentration in the other part of the Cochin estuarine system [60].

The assessment of metal contamination in sediments of Vembanad wetland system showed high heterogeneity of sediments and the concentrations of metals Cd, As, Pb and Zn were high and exceeded the standard limits. The pollution load index, geoaccumulation index and enrichment factor studies [51, 61, 62, 63, 64] showed metal pollution was moderate to extremely high in the northern sites [65]. The enrichment of metals (Fe, Cu, Ni and Zn) observed towards the surface of the core sediments of Vembanad wetland system and the metals like Pb, Cd and Hg showed uniform distribution along the core. Vembanad Lake ecosystem expressed serious metal pollution due to increased metal deposition [6, 43]. Ecotoxicological impact assessment of heavy metals (Fe, Mn, Ni, Cu, Zn, Cd, Pb and Hg) in core sediments of Cochin estuary observed that the metal concentration was higher in surface layers than deeper

layers and the concentration of some metals exceeded the required limits [66].

The accumulation status of the metals (Cd, Cr, Co, Cu and Pb), enrichment factor and anthropogenic factor in the core sediments of Cochin estuarine system revealed that metal enrichment was heavy at northern region compared with southern region because of the high deposition of industrial effluents [67]. An environmental magnetic record of heavy metal pollution was observed in Vembanad lagoon. Several sediment cores were analysed and observed that the coarse magnetic grain size greatly affects the pollution of Vembanad lagoon. The concentrations of Fe, Mn, Cu, Zn, Ni and Cr were exhibited higher levels in the top layers of cores [68].

Studies on fractionation and accumulation of the metals Zn, Cd, Pb and Cu in the sediments of Cochin estuary showed high concentration of Zn and Cd and also Pb had the risk of bioaccumulation as a result of the presence of oxidisable and reducible fractions [69]. Metal concentrations in the sediments of Cochin estuary were increased by the anthropogenic inputs from the industries [10]. The enrichment of Cd, Cu, Pb and Zn in the estuary was caused by the accumulation of metals initiated with precipitation of iron complexes. The increased accumulation of metals in the estuarine system was due to weak flushing. Industrial effluents had lead to the present level of metal contamination in the estuary [10, 56, 70, 46]. Chemometric study on the accumulation of trace metals Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb, organic carbon, total nitrogen, sulphur, etc. in the sediments were analysed and observed the enrichment of Cd and Zn. Pre dominant spatial variation observed for the metals like Mg, Cr, Fe, Co, Ni, Cu, Zn, Cd and Pb whereas temporal variation with Mg only. The anthropogenic activities and industrial effluents were the major sources of trace metal enrichment in the Cochin estuary [11].

Chandran and his co-workers analysed the historical trend in trace metal enrichment of core sediments of Cochin estuary [12]. By the use of $^{210}\text{Po}/^{210}\text{Pb}$ isotope method the sedimentation rate was determined and the distribution pattern of metals observed in the order $\text{Zn} > \text{Cu} > \text{Pb} > \text{Cd}$. Increased trend of metal concentration from the bottom layer to successive upper layers indicated that the contamination issue of the lake system was mounted after the industrialization of 1940's. Temporal and spatial variations of heavy metals Cd, Pb, Cr, Ni, Co, Cu, Zn, Mn, Mg and Fe in the sediments of Cochin estuary were investigated for the anthropogenic pollution using multivariate statistical analysis and it was understood that the estuary was highly polluted with

Cd, Zn, Pb and Ni. The northern part of the estuary showed high enrichment of Zn and Cd [46].

Investigation of the concentration of a number of metals like Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in a recently deposited sediments in Cochin backwaters before, during and after the flood conditions showed increase in the concentration of metals Cd (0.6-1.8 ppm), Cr (20-35 ppm), Ni (20-45 ppm) and Pb (12-18 ppm) near the site of industrial discharge. The enrichment of metals observed during post monsoon periods [39]. Spatial diversity of trace metals like Fe, Mn, Cu, Cr, Pb, Sr, Zn, Co, Cd and Ni in the surficial sediments of Cochin estuary showed enrichment of metals in the middle estuarine sediments and correlations were observed between metals Cu, Cd, Co, Zn, Ni and Fe and Mn [64]. Studies on hydride forming toxic metals such as Hg, As, Sb and Se in the surficial sediments of Cochin estuarine system showed the presence of these element in the order $\text{Hg} > \text{Sb} > \text{Se} > \text{As}$. The industrial effluents, municipal sewage and fluvial inputs were the major sources of metal contamination in the estuarine system.

The sediment floor with alarming levels of Hg was reported in the Mulavukkad region of Cochin estuary [71]. Various surveys on heavy metals (Fe, Mn, Cu, Ni, Zn and Cr), texture and organic carbon in suspended particulates and bed sediments of tropical perennial Muvattupuzha river and adjoining Vembanad estuary observed that the estuarine sediments showed 3-4 times increase in organic carbon than that in the river sediments. Fe, Mn and Zn reported higher in bed sediments than in suspended particulates whereas Cu, Ni and Zn enriched in particulate phases [72]. Significant spatial variations in the geochemical parameters and characteristics of redox elements Fe and S in the sandy stations of Cochin estuary indicated that the texture of the sediment was the main factor influencing the sediment geochemistry [73]. Cadmium with high pollution status in some areas of Vembanad Lake was observed [74].

4. METALS IN AQUATIC FAUNA

There are many studies that have revealed the bioaccumulation of metals in aquatic fauna, as a result of the metal contamination in both water and sediment. Analysis on concentrations of trace metals like Fe, Mn, Co, Ni, Cu, Zn, Cd, Cr and Pb in two species of Mysids (*Mesopodopsis orientalis* and *Rhopalophthalmus indicus*) showed species specific bioaccumulation with high metal concentration in *R. indicus* than *M. orientalis* [75]. Species specific and organ specific bioaccumulation was also observed in the muscle, gill and liver of some pelagic fish and

demersal fish of Cochin waters during the monsoon, pre monsoon and post monsoon periods [76]. The liver appeared to be the main target tissue for trace metal accumulation and muscle had the lowest level. With the increase in population, the rate of discharge of domestic effluents and industrial effluents into the backwater has also increased. The contamination of estuarine water adversely affected the production of estuarine dependent fishery resources [9].

Concentration of trace metals (Fe, Co, Ni, Cu, Zn, Cd and Pb) in the muscle, gill and liver of three fishes from Cochin backwaters during monsoon, pre monsoon and post monsoon periods [77] observed higher metal bioaccumulation in liver (average ranges: Fe 706.72 – 1114.58 µg/g; Co 30.90–52.23 µg/g; Ni 11.59–39.18 µg/g; Cu 33.19 – 44.70 µg/g; Zn 200.82– 403.40 µg/g; Cd 3.49 – 4.37 µg/g; and Pb 2.11 – 3.03 µg/g) than muscle (average ranges: Co 12.50 – 28.05 µg/g; Ni 4.45 – 6.63 µg/g; Cu 5.12 – 6.16 µg/g; Zn 48.97 – 144.50 µg/g; Cd 1.17 – 2.35 µg/g and Pb 1.01 – 1.95 µg/g) and gill (average ranges: Fe 21.43 – 35.73 µg/g; Co 6.73 – 14.83 µg/g; Ni 17.79 – 30.53 µg/g; Cu 89.02 – 246.79 µg/g; Zn 2.01 – 3.50 µg/g; and Cd 1.32 to 2.07 µg/g). The trace metal concentrations of Fe, Co, Ni, Cu, Zn, Cd and Pb in the muscles of *Villorita cyprinoides* from Cochin backwaters were investigated during the pre monsoon, monsoon and post monsoon periods [78] observed seasonal average range of metals as Fe (18,532.44–28267.05), Co (23.25–3758), Ni (10.56–19.28), Cu (3.58–11.35), Zn (48.45–139.15), Cd (1.06–1.50) and Pb (3.05–4.35). Seasonal variations of metals As, Cd, Cr, Hg, Pb and Zn found in *V. cyprinoides* [79]. The bivalve mollusc *Sunetta scripta* from inshore waters of Cochin estuary recorded high Cu concentration in smaller ones than larger ones [80].

Studies on the impact of man-made hydrological barrage (Thanneermukkom barrage) on the concentration of Fe, Zn, Cu, Co, Cr, Mn, Ni, Pb in mesozooplankton of Kochi backwaters showed that, during pre-monsoon the concentration of metals in mesozooplankton was high in upstream due to closure of the barrage and during post monsoon, metal concentration was lower when compared with other seasons [81]. Quantisation of specific metals like Cr, Cd, Pb, Zn and Cu in the bivalve *Villorita* species from Vembanad Lake and observed zinc concentration was high when compared with other metals and maximum concentration observed during pre-monsoon whereas minimum was during the other two seasons [82]. The influence of dissolved heavy metals (Cr, Mn, Fe, Ni, Cu and Zn) on the community structure of *Cyanobacteria* in Cochin estuary showed Zn as the major pollutant and if it is not controlled,

may accelerate eutrophication and other ecological constraints [83].

Concentration of six heavy metals investigated in some fish from Cochin estuary showed that Cd and Pb were present in the alimentary canal of three fish species whereas Cu, Zn and Fe were found in all the fish species studied. Fe was the most abundant metal present in the fish samples [23]. Studies showed the concentration of THg was four-five times higher than the normal limits of human consumption (0.5 mg/kg dry wt) in many fish species [36]. The presence of Cu (0.033 ppm), Mn (5.3 ppm), Zn (17.21 ppm), Mg (154.1 ppm), Hg (0.0035 ppm) and Cr (15.8 ppm) were observed in the Tank Goby *Glossogobius giuris* from the brackish water of Vembanad Lake [84]. The concentration of Fe in the hepatic tissue of *Eetroplus suratensis* reported high (11.29 ± 0.39 ppm) from the region of Vembanad lake [85]. Reports point out that the liver, gills, kidneys and muscles of some fish highly accumulate Pb and Cd from the surrounding [86].

Studies on the toxic effects of Cu, Zn, Cr, Cd, Ni and Pb in *Eetroplus suratensis* and also the analysis of geo accumulation index and contamination factor indicated that the pollution was moderate to heavy in the Cochin estuarine system [87]. Copper and zinc showed chronic toxicity values 0.23 mg/l and 2.005 mg/l respectively. The influence of heavy metals on *Cyanobacteria* in the nutrient rich Cochin estuary observed that Zn as the major pollutant [83]. Their conclusion was that, since the *Synecococcus sps* as the dominant *cyanobacteria* in Cochin estuary and the Zn pollution may interrupt the community structure of *Cyanobacteria* which can lead to involuntary alterations in the functioning of estuarine ecosystem.

Analysis of various heavy metals like Zn, Cd, Pb, Cu and Hg among various edible fish of Cochin backwaters showed higher concentrations of metals in kidney and liver of most fishes. The Hg concentration in muscle was 1.6 mg/kg dry weight, higher than the approved limit [88]. The Hg concentration in the Oyster *Crassostrea madrasensis* (Pestron) from Cochin backwaters showed high concentration in smaller oyster than large ones especially in summer season [55]. Studies showed free living protozoans as pollution indicators possessing the property of heavy metal uptake [89]. The analysis of the lake water showed high concentration of heavy metals and thus the researchers suggested the possibility of using these ciliates for the bioremediation of aquatic pollutants in the lake. The toxicity effect of Copper on the estuarine clam *Villorita cyprinoides* from Cochin estuary showed acute value 0.858 mgL^{-1} and chronic value 0.373 mgL^{-1} and as a result of copper exposure there occurred alterations in the physiological and

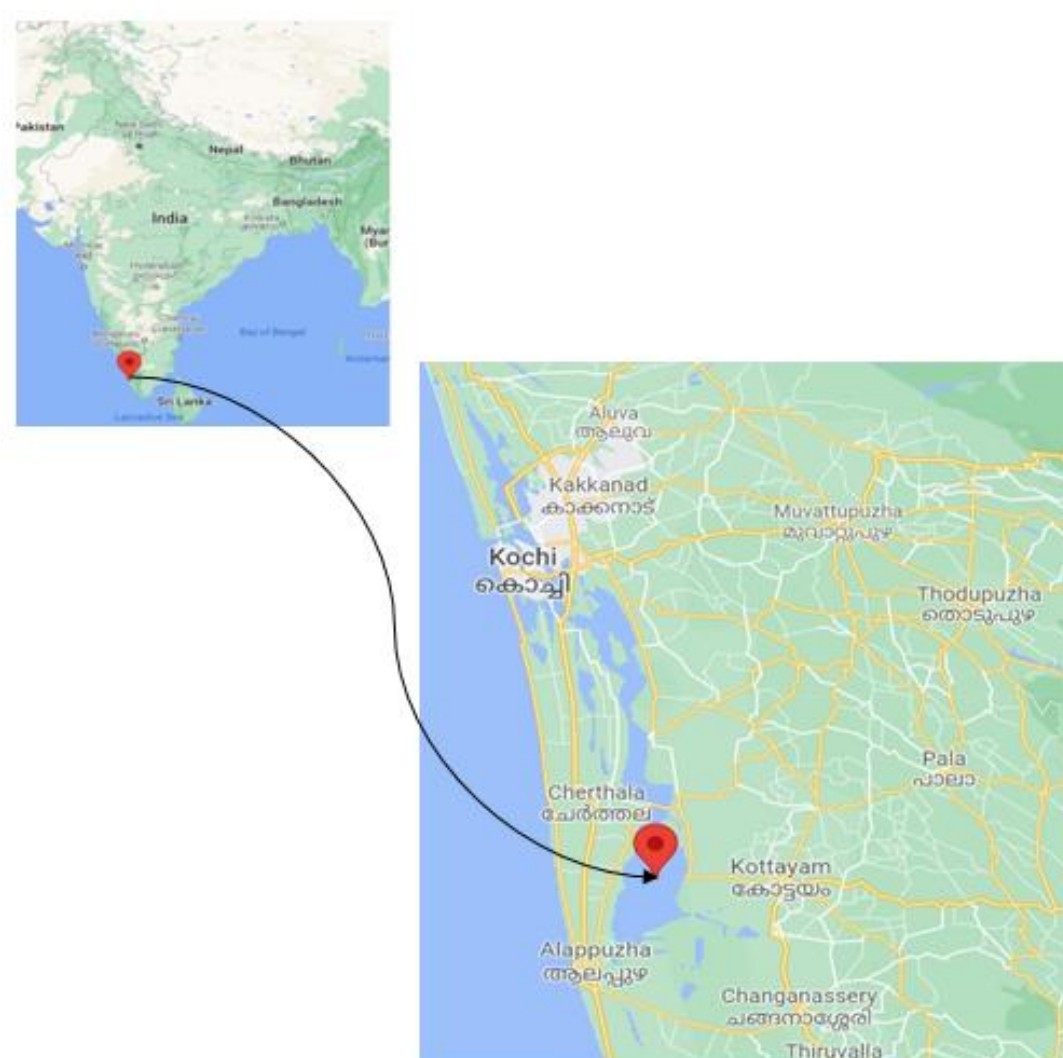


Fig. 1. Location of Vembanad Lake, Kerala

metabolic behaviour of the bivalve [90]. The impact of heavy metals in *V. cyprinoides* observed that the concentration of Cu was maximum during monsoon and post monsoon, and minimum during pre-monsoon in Cochin backwaters [91]. As the black clam (*V. cyprinoides*) an important fishery of Vembanad lake, various factors like effluents from shrimp processing factories, swarming of weeds and frequent dredging were adversely affected the sustainability of this fishery [92]. The evaluation of bioavailability of trace metals (Cu, Cd, Zn, Pb, Ni) to the bivalve mollusc showed that the enrichment factors reflected the enhanced or reduced vulnerability of the biota to metal concentrations [93]. Mysids showed species specific metal bioaccumulation in Cochin estuary [75]. The concentration of Zn and Pb detected from shrimp species around Cochin estuary were within the limits of permissible level for human consumption prescribed by World Health Organization [94].

5. HISTOLOGICAL CHANGES OBSERVED IN THE LIVER, MUSCLE, GILL, KIDNEY AND BRAIN OF SOME FISHES EXPOSED TO METALS

Accumulation of heavy and trace metals in tissues of various organisms lead to numerous alterations in the morphology, physiology and reproduction. It also causes various histopathological changes. Major histological changes observed in some fishes by various studies are given below.

5.1 Gills

Fish gill is the first organ to which any pollutant comes into contact. The studies of Pandey et al., [95] on the effects of exposure to multiple trace metals on histological features of gills in *Channa punctata* Bloch showed spiking and fusion of secondary

lamellae, formation of club shaped filaments, vacuolization and necrosis of filament epithelium in the interlamellar regions. Lead and Cadmium exposed gills of *Cyprinus carpio communis* showed disintegration and fusion of primary lamellae, extensive vacuolization with disruption of epithelial lining, hyperplasia of branchial arch, vacuolization and congestion of blood vessels [96]. The gills of *Catla catla* showed morphological changes and exhibited alterations in the epithelia, haemorrhages and circulatory anomalies [97]. The histological study on *Ctenopharyngodon idella* showed alterations in the gills as epithelial lifting, club gill filaments, gill bridging, curling filaments, swelling and fusion of cells and cellular necrosis [98].

5.2 Muscles

The exposure to Pb and Cd in *Cyprinus carpio communis* lead to the thickening and separation of muscle bundles with severe intramuscular oedema [96]. The muscle tissues of freshwater cyprinid, *Labeo rohita* (Hamilton) collected from highly contaminated area (with As, Cr, Cd, Mn and Pb) showed shortening and elongation of muscle bundles [99]. The studies of Shah and co-workers [98] observed the fish muscle tissues with necrosis, inflammation and degeneration of muscle fibres, edema of muscle bundles and lesions.

5.3 Liver

The fish exposed with Pb and Cd exhibited hepatic degeneration and severe necrosis [96]. The histological changes occurred in the liver of *Tilapia nilotica* due to heavy metals were cloudy swelling, prominent coagulative necrosis, vacuolar and hydropic changes of the hepatocytes [100]. The freshwater fish *Labeo rohita* showed hepatic lesions like severe necrosis, cytoplasmic degeneration, melano-macrophage centres, infiltration of leukocytes, pyknosis and nuclear degeneration [99]. The histopathological examination of *Oreochromis niloticus* exposed to heavy metals like Pb, Cu and Ni showed liver with cytoplasmic vacuolation, necrosis and sinusoid dilation [101].

5.4 Brain

Brain cells with neuronal cell degeneration, swelling of pyramidal cells, vacuolization and dystrophic changes were observed in the fish exposed with Pb and Cd [96]. The histological changes observed in the heavy metals treated brain of *Catla catla* were degeneration of granular and molecular layer, vacuolization and necrosis of the brain cells [97]. The

analysis of heavy metals polluted carp brain exhibited meningitis and gliosis [100]. The brain tissues of zebrafish, *Danio rerio* with Cd toxicity showed different extents of granule cell loss, degeneration of purkinje cells, aggregation area of gliosis and many areas of necrosis [102].

5.5 Kidney

Due to the accumulation of Zn, the histopathological changes exhibited in the kidney of fish *Channa punctatus* were highly expanded renal tubule, necrosis, loss of cellular integrity of renal tubules, dilation, oedema and hypertrophied nuclei of renal tubules [103]. The heavy metals accumulated kidneys of *Tilapia* appeared as hyaline casts, interstitial nephritis, renal necrosis and mononuclear cells infiltration [100]. The freshwater fish *Labeo rohita* exposed with metals As, Cr, Cd, Mn and Pb showed renal alterations like edema, irregular diameters, degeneration and atrophy of renal tubules [99]. The histopathological examination of the effects of heavy metals Pb, Cu and Ni on the kidney of *Oreochromis niloticus* exhibited congestion, necrosis and dilation in bowman capsule space [101].

6. CONCLUSION

Vembanad Lake, the most important fresh water lake, is facing critical pollution problem because of metal contamination. The trace metal concentrations in the water, sediment and different aquatic organisms are mainly depends on the industrial effluents, agricultural wastes, waste waters from the surrounding localities, etc. Most of the aquatic organisms possess high metal concentration that exceeds the safety limit. Metal pollution is found to be higher in the northern region of the Cochin estuary. There occurs more accumulation of heavy metals in organisms through trophic transfer. Therefore it is important to provide awareness regarding the effect of industrial effluents being discharged to the aquatic system.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sruthy S, Ramasamy EV. Microplastic pollution in Vembanad Lake, Kerala, India: the first report of microplastics in lake and estuarine sediments in India. Environmental Pollution. 2017;222:315-322.

2. Chandrasekar V, Gopal SM, Vidhyavathi A, Jayanthi C, Sathy R, Gopal N. Recreational value of Vembanad lake in Kerala using individual travel cost method. *International Journal of Current Microbiology and Applied Sciences*. 2020;9(6):3280-3291.
3. Khan RA, Nandi NC, Roy MKD, Radhakrishnan C. Faunal diversity of Vembanad lake:an over view. *Wetland Ecosystem Series*. 2009;10:1-24.
4. Anu PR, Jayachandran PR, Sreekumar PK, Nandan SB. A review on heavy metal pollution in Cochin backwaters, southwest coast of India. *International Journal of Marine Science*. 2014;4(10):92-98.
5. Asha CV, Cleetus RI, Suson PS, Nandan SB. Ecosystem analysis of the degrading Vembanad wetland ecosystem, the largest Ramsar site on the south west coast of India-measures for its sustainable management. *Regional Studies in Marine Science*. 2016;8(3):408-421.
6. Harikumar PS, Nasir UP, Rahman MPM. Distribution of heavy metals in the core sediments of a tropical wetland system. *International Journal of Environmental Science and Technology*. 2009;6(2):225-232.
7. Vincy MV, Rajan B, Kumar AP. Water quality assessment of a tropical wetland ecosystem with special reference to backwater tourism, Kerala, South India. *International Journal of Environmental Science*. 2012;1(5):62-68.
8. Safoorabeevi KH, Devadas V. Impact of tourism on Vembanad lake system in Alappuzha district. *International Journal of Research*. 2014;1(5):542-551.
9. Gopalan UK, Vengayil DT, Udayavarma P, Krishnankutty M. The shrinking backwaters of Kerala. *Journal of Marine Biological Association of India*. 1983;25(1,2):131-141.
10. Balachandran KK, Raj CML, Nair M, Joseph T, Sheeba P, Venugopal P. Heavy metal accumulation in a flow restricted tropical estuary. *Estuarine, Coastal and Shelf Science*. 2005;65(1-2):361-370.
11. Deepulal PM, Kumar TRG, Sujatha CH, George R. Chemometric study on the trace metal accumulation in the sediments of the Cochin estuary- southwest coast of India. *Environmental Monitoring and Assessment*. 2012;184:6261-6279.
12. Chandran MSS, Ramasamy EV, Mohan M, Sruthi SN, Jayasooran KK, Augustine T, Mohan K. Distribution and risk assessment of trace metals in multifarious matrices of Vembanad lake system, peninsular India. *Marine Pollution Bulletin*. 2019;145:490-498.
13. Ouseph PP. Dissolved, Particulate and sedimentary mercury in the Cochin estuary, southwest coast of India. *Coastal and estuarine studies (book series). Estuarine Water Quality Management: Monitoring, Modelling and Research*. 1990;36.
14. Sandor Z, Csengeri I, Oncsik MB, Alexis MN. Trace metal levels in freshwater fish, sediment and water. *Environmental Science and Pollution Research*. 2001;8(4):265-268.
15. Kumar AA, Dipu S, Sobha V. Seasonal variation of heavy metals in Cochin estuary and adjoining Periyar and Muvattupuzha rivers, Kerala, India. *Global Journal of Environmental Research*. 2011;5(1):15-20.
16. Sobha V, Pournami P, Santhosh S, Hashim KA. Assessment of heavy metal pollution status of Vembanad lake – a case study, Kerala. *Scientia Acta Xaveriana*. 2011;2(1):49-57.
17. Udayakumar P, Chandran A, Jose JJ, Rajesh BR, Babu KN, Ouseph PP. Seasonal dynamics of dissolved metals in surface coastal waters of southwest India. *Bulletin of Environmental Contamination and Toxicology*. 2011;87:662-668.
18. Murty PSN, Veerayya M. Studies on the sediments of Vembanad lake, Kerala state:part III- distribution and interpretation of bottom sediments. *Indian Journal of Marine Sciences*. 1974;3:16-27.
19. Muhamed AP, Leela E, Meenakumari B. Trace metal pollution in estuaries of south India. *Asian Journal of Water, Environment and Pollution*. 2008;5(2):63-69.
20. Riley JP, Chester R. *Introduction to marine chemistry*. Academic press, London and New York. 1971.
21. Haldar R, Khosa R, Gosain AK. Impact of anthropogenic interventions on the Vembanad lake system. *Water Resources and Environmental Engineering*. 2018;9-29.
22. Namboodiri MMT, Pakshirajan K. Sustainable and green approach of chitosan production from *Penicillium citrinum* biomass using industrial waste water as a cheap substrate. *Journal of Environmental Management*. 2019;240(15):431-440.
23. Rajamany-Amma V. The distribution and partition of some of the trace metals in sediments and waters of the coastal environment. Ph. D thesis. Cochin University. 1994.
24. Martin S, Griswold W. Human health effects of heavy metals. *Environmental Science and Technology Briefs for Citizens*. 2009;15:1-6.
25. Fatima M, Usmani N, Hossain MM. Heavy metal in aquatic ecosystem emphasizing its

- effect on tissue bioaccumulation and histopathology: a review. *Journal of Environmental Science and Technology*. 2014;7(1):1-15.
26. Perera PACT, Kodithuwakku SP, Sundarabarathy TV, Edirisinghe U. (2015). Bioaccumulation of cadmium in freshwater fish: an environmental perspective. *Insight Ecology*. 2015;4(1):1-12.
27. Luoma SN. Bioavailability of trace metals to aquatic organisms- a review. *Science of the Total Environment*. 1983;28(1-3):1-22.
28. Pillai VK. Studies on the hydrobiology and pollution of the Vembanad lake and adjacent waters. Doctoral thesis. ICAR. 1991.
29. Radwan S, Kowalik W, Kornijow R. Accumulation of heavy metals in a lake ecosystem. *Science of the Total Environment*. 1990;96(1-2):121-129.
30. Padmalal D, Maya K, Seralathan P. Geochemistry of Cu, Co, Ni, Zn, Cd and Cr in the surficial sediments of a tropical estuary, southwest coast of India: a granulometric approach. *Environmental Geochemistry*. 1997;32(1/2):85-93.
31. Sankaranarayanan VN, Stephen R. Particulate iron, manganese, copper and zinc in backwaters of Cochin backwater. *Indian Journal of Marine Sciences*. 1978;7:201-203.
32. Sankaranarayanan VN, Jayalakshmi KV, Joseph T. Particulate trace metals in Cochin backwaters: distribution of seasonal indices. *Indian Journal of Fisheries*. 1998;45(3):321-329.
33. Venugopal P, Devi KS, Remani KN, Unnithan RV. Trace metal levels in the sediments of the Cochin backwaters. *Mahasagar, National Institute of Oceanography*. 1982;15(4).
34. Menon NN, Balchand AN, Menon NR. Hydrobiology of the Cochin backwater system- a review. *Hydrobiologia*. 2000;430:149-183.
35. Ouseph PP. Dissolved and particulate trace metals in the Cochin estuary. *Marine pollution bulletin*. 1992;24(4):186-192.
36. Ramasamy EV, Jayasooryan KK, Chandran MSS, Mohan M. Total and methyl mercury in the water, sediment and fishes of Vembanad, a tropical backwater system in India. *Environmental Monitoring and Assessment*. 2017;189(130):
37. Unnikrishnan P, Nair SM. Partitioning of trace metals between dissolved and particulate phases in a typical backwater system of Kerala, India. *International Journal of Environmental Studies*. 2004;61(6):659-676.
38. Khurshid S, Basheer A, Zaheeruddin, Shabeer MU. Effect of waste disposal on water quality in parts of Cochin, Kerala. *Indian Journal of Environmental Health*. 1998;40(1):45-50.
39. Nair SM, Balchand AN, Nambisan PNK. Metal concentrations in recently deposited sediments of Cochin backwaters, India. *Science of the Total Environment*. 1990;97, 98:507-524.
40. Florence TM. Trace metal species in fresh waters. *Water Research*. 1977;11(8):681-687.
41. Kaladharan P, Krishnakumar PK, Prema D, Nandakumar A, Khambadkar LR, Valsala KK. Assimilative capacity of Cochin inshore waters with reference to contaminants received from the backwaters and the upstream areas. *Indian Journal of Fisheries*. 2011;58(2):75-83.
42. Murty PSN, Veerayya M. Studies on the sediments of Vembanad lake, Kerala state, part IV- distribution of trace elements. *Indian Journal of Marine Sciences*. 1981;10:165-172.
43. Binish MB, Kannan VM, Sruthy S, Sreedharan K, Ramasamy EV, Mohan M. Heavy metal distribution and contamination in the sediments of Vembanad lake, India: an ICP-MS approach. *Conference Paper (ICMS)*; 2017.
44. Mallik TK, Suchindan GK. Some sedimentological aspects of Vembanad lake, Kerala, west coast of India. *Indian Journal of Marine Sciences*. 1984;13:159-163.
45. Shajan KP. Geochemistry of bottom sediments from a river-estuary-shelf mixing zone on the tropical southwest coast of India. *Bulletin of the Geological Survey of Japan*. 2001;52:371-382.
46. Bindu KR, Deepulal PM, Gireeshkumar TR, Chandramohanakumar N. Evaluation of heavy metal enrichment in Cochin estuary and its adjacent coast: multivariate statistical approach. *Environmental Monitoring and Assessment*. 2015;187(519):1-23.
47. Padmalal D, Seralathan P. Geochemistry of Fe and Mn in surficial sediments of a tropical river and estuary, India- a granulometric approach. *Environmental Geology*. 1995;25:270-276.
48. Selvam AP, Priya SL, Banerjee K, Hariharan G, Purvaja R, Ramesh R. Heavy metal assessment using geochemical and statistical tools in the surface sediments of Vembanad lake, South west coast of India. *Environmental Monitoring and Assessment*. 2011;1-17.
49. Kumar RCS, Joseph MM, Kumar GTR, Renjith KR, Manju MN, Chandramohanakumar N. Spatial variability and contamination of heavy metals in the inter-tidal systems of a tropical environment. *International Journal of Environmental Research*. 2010;4(4):691-700.
50. George R, Martin GD, Nair SM, Thomas SP, Jacob S. Geochemical assessment of trace metal pollution in sediments of the Cochin

- backwaters. *Environmental Forensics*. 2016;17(2):156-171.
51. Martin GD, George R, Shaiju P, Muraleedharan KR, Nair SM, Chandramohanakumar N. Toxic metals enrichment in the surficial sediments of a eutrophic tropical estuary (Cochin backwaters, southwest coast of India). *The Scientific World Journal*. , 2012;1-17.
52. Salas PM, Sujatha CH, Kumar CSR, Cheriyan E. Heavy metal distribution and contamination status in the sedimentary environment of Cochin estuary. *Marine Pollution Bulletin*. 2017;119(2):191-203.
53. Dipu S, Kumar AA. Distribution of mercury and other trace metals in the sediments of Cochin estuary (a Ramsar site), Kerala, India. *Environmental Monitoring and Assessment*. 2013;184(12):1-11.
54. Priju CP, Narayana AC. Heavy and trace metals in Vembanad lake sediments. *International Journal of Environmental Research*. 2007;1(4):280-289.
55. Rajendran N, Kurian CV, George V. Mercury concentrations in *Crassostrea madrasensis* (Preston) from Cochin backwater. *Journal of Marine Biological Association of India*. 1987;29(1&2):237-243.
56. Balachandran KK, Laluraj CM, Martin GD, Srinivas K, Venugopal P. Environmental analysis of heavy metal deposition in a flow restricted tropical estuary and its adjacent shelf. *Environmental Forensics*. 2006;7(4):345-351.
57. Chapman PM, Wang F. Assessing sediment contamination in estuaries. *Environmental Toxicology and Chemistry*. 2001;20:3-22.
58. Purandara BK, Venkatesh B, Choubey VK. Sediment transport and sedimentation in a coastal ecosystem- a case study. *Materials and Geoenvironment*. 2011;58:289-302.
59. Kunhikrishnan Nair C. Chemical partitioning of trace metals in sediments of a tropical estuary. Ph. D thesis. Cochin University; 1992.
60. Unnikrishnan P, Velukkutty B, Gopinath A, Nair SM. The impact of a salinity barrier on the partitioning of heavy metals in sediments of a tropical backwater system. *Chemical speciation and bioavailability*. 2006;18(4):153-167.
61. Alagarsamy R, Zhang J. Geochemical characterization of major and trace elements in the coastal sediments of India. *Environmental Monitoring and Assessment*. 2010;161(1-4):161-176.
62. Nair CK, Balchand AN, Chacko J. Sediment characteristics in relation to changing hydrography of Cochin estuary. *Indian Journal of Marine Sciences*. 1993;22(1):33-36.
63. Nair CK, Balchand AN, Nambisan PNK. Heavy metal speciation in sediments of Cochin estuary determined using chemical extraction techniques. *Science of the Total Environment*. 1991;102:113-128.
64. Jayasree P, Nair SM. Spatial diversity of trace metals in recent sediments of Cochin estuary (India). *Toxicological and Environmental Chemistry*. 1995;51(1-4):243-254.
65. Manoj MC, Thakur B, Uddandam PR, Prasad V. Assessment of metal contamination in the sediments of Vembanad wetland system, from the urban city of southwest India. *Environmental Nanotechnology, Monitoring and Management*. 2018;10:238-252.
66. Harikumar PS, Nasir U. Ecotoxicological impact assessment of heavy metals in core sediments of a tropical estuary. *Ecotoxicology and Environmental Safety*. 2010;73(7):1742-1747.
67. Manju P, Nair, Akhil PS, Sujatha CH. Toxic metal distribution in the core sediment of Cochin estuarine system (CES). *International Journal of Environmental Research*. 2014;8(1):133-138.
68. Narayana AC, Ismaiel M, Priju CP. An environmental magnetic record of heavy metal pollution in Vembanad lagoon, southwest coast of India. *Marine Pollution Bulletin*. 2021; 167.
69. Jayasooryan KK, Ramasamy EV, Chandini PK, Mohan M. Fractionation and accumulation of selected metals in a tropical estuary, south-west coast of India. *Environmental Monitoring and Assessment*. 2021;193(4):220.
70. Balachandran KK, Reddy GS, Revichandran C, Srinivas K, Vijayan PR, Thottam TJ. Modelling of tidal hydrodynamics for a tropical ecosystem with implications for pollutant dispersion (Cochin estuary, South west India). *Ocean Dynamics*. 2008;58:259-273.
71. Shylesh Chandran MS, Jayasooryan KK, Jose SK, Mohan M, Ramasamy EV. Mercury in the core sediment of Vembanad backwater- an implication towards anthropogenic contamination. *Lake 2010: Wetlands, Biodiversity and Climate Change*. 2010;1-9.
72. Padmalal D, Seralathan P. Heavy metal content in suspended particulates and bed sediments of a tropical perennial river and estuary, central Kerala, India. *Journal of Geological Society of India*. 1993;42(4):349-355.
73. Renjith KR, Chandramohanakumar N. Geochemical characteristics of surficial sediments in a tropical estuary, south-west India. *Chemistry and ecology*. 2007;23(4):337-343.

74. Shibinmol PA, Raveendran, Ranjitha, Sujatha CH. Elucidation of contaminant-induced toxic responses in the biota of lake Vembanad, Kerala, India. *Human and Ecological Risk Assessment*. 2015;21(6):1576-1592.
75. Biju A, George R. Trace metal dynamics in Mysids from the Cochin estuary. *Environmental Forensics*. 2021;22(1-2):56-62.
76. George R, Biju A, Martin GD, Gerson VJ. Distribution and concentration of trace metals in tissues of pelagic and demersal fishes from the coastal waters of Cochin. *Environmental Forensics*. 2021;22(3,4). DOI:10.1080/15275922.2021.1907820.
77. George R, Martin GD, Nair SM, Chandramohanakumar N. Biomonitoring of trace metal pollution using fishes from the Cochin backwaters. *Environmental Forensics*. 2012;13(3):272-283.
78. George R, Martin GD, Nair SM, Chandramohanakumar N. Biomonitoring of trace metal pollution using the bivalve mollusc, *Villorita cyprinoides* from the Cochin backwaters. *Environmental Monitoring and Assessment*. 2013;185:10317-10331.
79. Sivaprasad PS. Bioaccumulation of heavy metals and pesticides in *Villorita cyprinoides* (Hanley) (Pelecypoda:Corbiculidae) from Cochin backwaters, Kerala. Doctoral thesis. 2007.
80. Pillai VK, Valsala KK. Seasonal variations of some metals in bivalve mollusc *Sunetta scripta* from Cochin coastal waters. *Indian Journal of Marine Sciences*. 1995;24:113-115.
81. Arunpandi N, Jyothibabu R, Jagadeesan L, Parthasarathi S, Albin, KJ, Pandiyarajan RS. Impact of large hydraulic barrage on the trace metals concentration in meso-zooplankton in the Kochi backwaters, along the southwest coast of India. *Marine Pollution Bulletin*. 2020;160(2).
82. Raveenderan Ranjitha H, Sujatha C. Quantization of specific trace metals in bivalve, *Villorita cyprinoides* var *cochinensis* in the Cochin estuary. *Indian Journal of Geo-Marine Sciences*. 2011;40(3):424-429.
83. Anas A, Jasmin C, Sheeba VA, Gireeshkumar TR, Nair S. Heavy metals pollution influence the community structure of Cyanobacteria in nutrient rich tropical estuary. *Oceanography*. 2015;3(1):1-8.
84. Dana EB, Jinoy VG, Mathew S. Assessment of nutritional quality in the tissue of euryhaline fish tank goby *Glossogobius giuris*, hamilton 1822 caught from Vembanad lake, Kerala, India. *International Journal of Fisheries and Aquatic Studies*. 2019;7(3):213-218.
85. Pettamanna A, Raghav D, Nair RH. Hepatic toxicity in *Etroplus suratensis* (Bloch 1790):an economically important edible fish in Vembanad fresh water lake, Kerala, India. *Bulletin of Environmental Contamination and Toxicology*. 2020;105:565-571.
86. Afshan S, Ali S, Ameen US, Farid M, Bharwana SA, Hannan F, Ahmad R. Effect of different heavy metal pollution on fish. *Research Journal of Chemical and Environmental Sciences*. 2014;2(1):74-79.
87. Xavier NDD, Nandan SB, Jayachandran PR, Anu PR, Midhun AM, Mohan D. Chronic effects of Cu and Zn on the fish *Etroplus suratensis* (Bloch, 1970) by continuous flow through (CFT) bioassay. *Marine Environmental Research*. 2019;143:141-157.
88. Mohan M, Deepa M, Ramasamy EV, Thomas AP. Accumulation of mercury and other heavy metals in edible fishes of Cochin backwaters, Southwest India. *Environmental Monitoring and Assessment*. 2012;184:4233-4245.
89. Radhakrishnan R, Jayaprakas V. Free living protozoans as bioindicators in Vembanad lake, Kerala, India, an important Ramsar site. *International Journal of Fisheries and Aquatic Studies*. 2015;2(3):192-197.
90. Jayachandran PR, Nandan SB, Anu PR, Don Xavier ND, Vaisakh K, Midhun AM, Mohan D. Toxicity effect of Copper on *Villorita cyprinoides* gray, 1825 (Black clam):A major clam fishery resource of Cochin backwaters, Southwest coast of India. *Biodiversity and Evaluation: Perspectives and Paradigms Shifts*. 2015;259-263.
91. Priyalekshmi A. Heavy metal loading and its impact on *Villorita cyprinoides* (Hanley) along the estuaries of south west coast of India. PhD thesis, Central institute of fisheries education, Versova, Mumbai; 2001.
92. Laxmilatha P, Appukkuttan KK. A review of the black clam (*Villorita cyprinoide*) fishery of the Vembanad lake. *Indian Journal of Fisheries*. 2002;49(1).
93. Babukutty Y. Studies on the inter-compartmental exchange of trace metals in an estuarine system. Doctoral thesis, Cochin University; 1991.
94. Chandini PK, Shaji S, Shivalingam R. A study on the accumulation of heavy metals on the sediments and shrimps of Cochin estuary. *Indian Journal of Scientific Research*. 2018;18(2):92-96.
95. Pandey S, Parvez S, Ansari RA, Ali M, Kaur M, Hayat F, Ahmad F, Raisuddin S. Effects of exposure to multiple trace metals on biochemical, histological and ultra structural

- features of gills of a fresh water fish, *Channa punctata* Bloch. Chemico-biological Interactions. 2008;174(3):183-192.
96. Patnaik BB, Howrelia H, Mathews T, Selvanayagam M. Histopathology of gill, liver, muscle and brain of *Cyprinus carpio communis* L. exposed to sublethal concentration of lead and cadmium. African Journal of Biotechnology. 2011;10(57):12218-12223.
 97. Bose MTJ, Ilavazhahan M, Tamilselvi R, Viswanathan M. Effects of heavy metals on the histopathology of gills and brain of freshwater fish *Catla catla*. Biomedical and Pharmacology Journal. 2013;6(1).
 98. Shah N, Khan A, Ali R, Marimuthu K, Uddin MN, Rizwan M, Rahman KU, Alam M, Adnan M, Jawad SM, Hussain S, Khisroon M. Monitoring bioaccumulation (in gills and muscle tissues), hematology and genotoxic alterations in *Ctenopharyngodon idella* exposed to selected heavy metals. Biomed Research International; 2020. DOI: 10.1155/2020/6185231.
 99. Kaur S, Khera KS, Kondal JK. Heavy metal induced histopathological alterations in the liver, muscle and kidney of fresh water cyprinid, *Labeo rohita* (Hamilton). Journal of Entomology and Zoology Studies. 2018;6(2):2137-2144.
 100. Zeitoun MM, Mehana EE. Impact of water pollution with heavy metals on fish health:overview and updates. Global Veterinaria. 2014;12(2):219-231.
 101. Mahboob S, Al-Khanim KA, Al-Balawi HF, Al-Misned F, Ahmed Z. Toxicological effects of heavy metals on histological alterations in various organs in Nile tilapia (*Oreochromis niloticus*) from freshwater reservoir. Journal of King Saud University-science. 2020;32(1):970-973.
 102. Al-sawafi AGA, Wang L, Yan Y. Cadmium accumulation and its histological effects on brain and skeletal muscle of zebrafish. Journal of Heavy metal Toxicity And diseases. 2017;2(1,2):1-6.
 103. Gupta P, Srivastava N. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus* (Bloch). Journal of Environmental Biology. 2006;27(2):211-215.