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Utility of Fishes as Bio-indicators for Tracing Metal Pollution in the Cochin Backwaters, Kerala, India

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Abstract: Trace metal (Fe, Ni, Zn, Cu, Pb, Cd and Cr) concentrations in water and tissues (flesh, gills and liver) of two fishes, *Etroplus suratensis* and *Arius arius* from the Cochin backwaters were investigated during February 2017 to January 2018. Trace metals were analysed using an Atomic Absorption Spectrophotometer (AAS). The omnivorous species, *E. suratensis* showed a higher concentration of trace metals in tissues than the carnivorous species, *A. arius*. Bioaccumulation of metals in fish tissues showed higher levels in the liver, which was followed by gill and flesh maybe due to the different physiological roles of these organs in fish metabolism. Bioaccumulation factors (BAF) of metals calculated for the species, *E. suratensis* follows an order Fe > Zn > Ni > Cu > Cr > Pb > Cd whereas that for the species, *A. arius* follows an another order Fe > Zn > Cr > Cu > Ni > Pb > Cd. Comparatively higher BAF values in organs of fishes indicate the increased concentration of the soluble metal in the medium in which the fishes inhabit. Metal selectivity index (MSI) and tissue selectivity index (TSI) are better tool for explaining bioaccumulation of trace metals in fishes. The results of the present study suggest the potential use of fishes, *E. suratensis* and *A. arius* as bioindicators for tracing metal pollution in the estuarine environment of Cochin.

Keywords: Trace metal, Bio-indicators, Fishes, Metal selectivity index, Tissue selectivity index, Cochin backwaters

Monitoring and analysis of trace metals in the environmental compartments of aquatic systems are necessary for pollution assessment and control (Elzwayie et al 2017). For a meaningful assessment of environmental impacts of metal pollution, measurement of metal concentrations in selected aquatic species of the resident biota in an ecosystem is necessary. Fishes are considered one of the most significant bio-indicators in aquatic systems to estimate metal pollution (Authman 2008, Al-Kahtani 2009). As fishes are located at the top of the aquatic food chains, the accumulated metals in their various tissues may pass to human beings through consumption of fish which may cause chronic or acute diseases (Al-Yousuf et al 2000). Hence, it is necessary to estimate the bioaccumulation of trace metals in fishes for monitoring water pollution and assessing potential risks associated with consuming the contaminated fish concerning human health (Ural et al 2012). The bioaccumulation of toxic metals in fishes has been a matter of public health concern since fish forms a part of the diet for a majority of the human population. The bioaccumulation of toxic contaminants in fish is caused by its uptake from ambient medium and through its diet (George et al 2012). Many factors depend on the retention of trace metals in the fish's body, such as the speciation of the metal concerned, the physiological mechanism for regulation, homeostasis and detoxification of the metal. Depending on the structure and function, the degree of accumulation of trace metals in fish tissues may vary. Generally, metabolically active tissues like gills, liver and kidneys have a higher accumulation of trace metals than the other tissues like muscles and skin (Hazrat et al 2019). The mechanism by which trace metal accumulates in tissues of different fish species appears to be a complex biochemical phenomenon that was not fully explored (George et al 2021). The accumulation of trace metals in fish tissues partly depends on metal concentrations in water and the duration of exposure. Moreover, environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon play a significant role in the metal accumulation and toxicity to fish. The present study aims to evaluate and quantify the bioaccumulation of trace metals (Fe, Ni, Zn, Cu, Pb, Cd and Cr) in tissues (flesh, liver and gills) of two species of fishes, *Etroplus suratensis* and *Arius arius* collected from the Cochin Back waters.

MATERIAL AND METHODS

Study area: The Cochin backwaters, extending between (Lat. 9º 30´-10º 10´ N and Long.76º15´-76º 25´E) are located at the northern part of the Vembanad Lake which forms a complex network of the shallow brackish water body. This backwater system is subjected to inputs of various organic as well as inorganic contaminants from its neighbouring hinterlands. The estuary was polluted by trace metals loaded from industrial, agricultural and municipal sources (Jayasooryan 2015, George et al 2016, Lallu 2017). The main source of metal pollution to the central part of the estuary seems to be due to the processing of metal-containing minerals (ores) at the FACT plant, paints and pigments that are used at the shipyard and ports (George et al 2016). The southern part of the estuary is subjected to metal pollution due to land runoff from Kuttanad agricultural fields that make use of excessive fertilizers and pesticides. Three seasonal conditions viz. pre-monsoon (PrM), monsoon (M) and post-monsoon (PoM) were prevailing in the study region. For this study five stations were selected from central to the southern part of Cochin backwaters based on the geographic features, anthropogenic activities and the inflow of pollutants from the different sources (Fig. 1).

Sample collection: A bimonthly water and fish samples were collected from Cochin backwaters during February 2017 to January 2018. This work was conducted in Zoology Research Centre, St. Stephens College, Pathanapuram, India. Water samples for the analysis of dissolved trace metals were collected using a pre-sterilized plastic bucket and were stored in plastic bottles that are pre-sterilized with 1N HCl. Water samples taken in plastic bottles were fixed with 1ml of 70% HNO_3 and were preserved at about 4°C in a

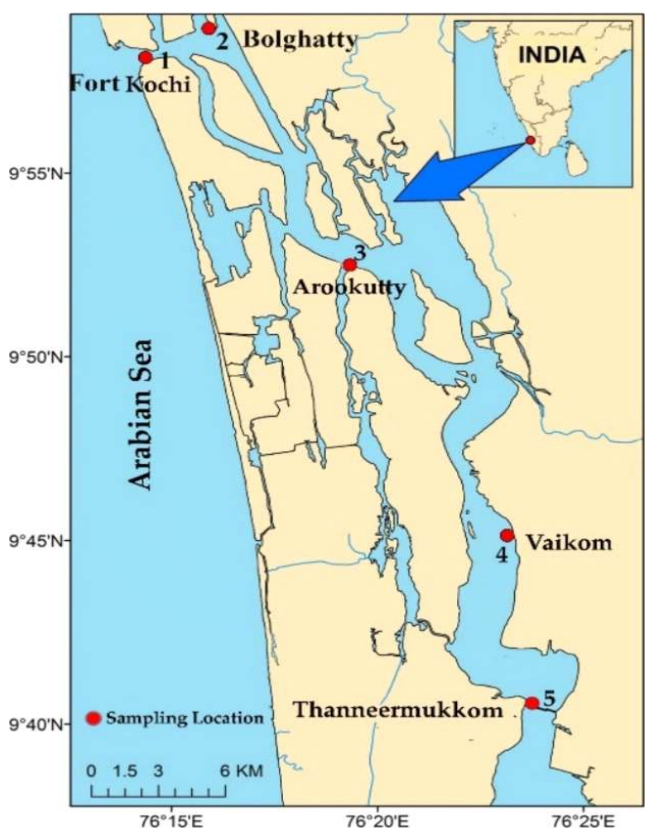

Fig. 1. Sampling locations

refrigerator till analysis in a Graphite Furnace AAS. Two species of fishes, *E. suratensis* (locally called Karimeen) and *Arius arius* (Koori) in the Cochin backwaters were collected directly from the local fishermen. The selected species are economically important and abundant in the study area. Eighty samples of fishes (40 samples of *E. suratensis* and 40 samples of *A. arius*) were collected from the sampling area. Immediately after the collection, fish samples were washed thoroughly with distilled water to remove mud or other fouling substances and were put in a clean polythene bag. They were preserved in iceboxes packed with ice in order to maintain the freshness and were transported to the laboratory. In the laboratory, fish samples were weighed and recorded for their total length. The flesh, liver and gills of the fish samples were dissected and washed thoroughly with distilled water. The organs were dried in an oven at 65 °C. The dried samples were powdered using mortar and pestle and were stored in a vacuum desiccator.

Trace metal analysis: The aliquots of about 300 mg were digested for 3h at 80°C with 3ml conc. HNO_3 (65% Merck, Suprapure) in Teflon beakers. Additional nitric acid was added if the samples were charred and 1ml of conc. $HClO_4$ (Merck Suprapure) was added to make the solution clear and evaporated to near dryness. The digests were cooled and diluted to 25ml with deionised water and were kept in plastic vials. Trace metals were analysed using an Atomic Absorption Spectrophotometer (AAS) Perkin Elmer India Pvt. Ltd. (Model: Pinaacle 900H). All metal concentrations in tissues of fish species were reported in ppm, dry weight.

Bioaccumulation factors (BAF) or Concentration factors were calculated for each metal as the ratio between the metal concentrations in the organism's body to its concentration in the ambient medium. Bioaccumulation factors (BAF) were used in assessment models as they provide pollution scale-independent parameter (Karlsson et al 2002).

$$\text{BAF} = \frac{\text{The metal concentration in organism's body}}{\text{Metal concentration in the ambient medium (Water)}}$$

Metal Selectivity Index (MSI) was calculated as the percentage of absolute concentration of a particular metal in tissue to the total concentration of all metals in that tissue (Nair et al 2006). MSI gives the affinity of a particular metal to a particular organ or tissue and is used as a reliable index for risk assessment of that particular metal.

$$\text{MSI} = \frac{\text{Absolute concentration of a metal in a tissue}}{\text{Total concentration of all metals in that tissue}} \times 100$$

Tissue selectivity index (TSI) is the percentage of the ratio between the absolute concentration of a metal in tissue and the total concentration of that metal in all the tissues (Nair et al 2006).

$$\text{TSI} = \frac{\text{Absolute concentration of a metal in a tissue}}{\text{Total concentration of all metals in that tissue}} \times 100$$

RESULTS AND DISCUSSION

Trace metal concentrations in water: The concentrations of Fe, Ni, Zn, Cu, Pb, Cd and Cr in water ranged from 0.051 to 0.284 mg L^{-1}, 0.008 to 0.112 mg L^{-1}, 0.012 to 0.341 mg L^{-1}, 0.009 to 0.126 mg L^{-1}, 0.013 to 0.199 mg L^{-1}, 0.002 to 0.095 mg $L^{-1}$and 0.018 to 0.124 mg L^{-1} respectively. The mean concentration of trace metals in water (mg L^{-1}) follows a decreasing order: Fe > Zn > Pb > Cr > Cu > Ni > Cd (Table 1). The increased concentration of trace metals in the water might be due to the large quantities of solid wastes mixed with the wastes of factories, market and industrial wastes that either discharge directly into the water bodies or dumped in open land fill which are directed to the water bodies through the rainwater runoff (Elamin et al 2021). In Cochin backwaters, the dissolved phase is the most sensitive compartment to every change in hydrographical conditions of the water column due to its large surface area and shallow depth (1.5- 6 m) (Anu et al 2014).

Hence, seasonally the concentration of dissolved metals in the water column may vary due to variations in the magnitude of freshwater discharges from rivers, discharge of industrial effluents and domestic sewage, and leakage of pesticide and fertilizer residues from agricultural fields. Thus, the increased concentrations of metals in the water column due to anthropogenic activities act as a significant source to the increased metal levels in fishes of the Cochin backwaters (Hannibal et al 2006, George et al 2012).

Table 1. Trace metal concentration in water (mg L^{-1}) from the Cochin backwaters

Trace metals	Mean ± SD
Fe	0.284 ± 0.267
Ni	0.049 ± 0.025
Zn	0.093 ± 0.091
Cu	0.064 ± 0.033
Pb	0.079 ± 0.044
Cd	0.038 ± 0.025
Cr	0.066 ± 0.029

Trace metal concentrations in fishes: The trace metal concentrations (in ppm, dry weight) in flesh, gill and liver tissues of fishes, *E. suratensis* and *A. arius* were presented in (Table 2). Marked variations were found in the concentrations of trace metals in flesh, gill and liver of *E. suratensis* and *A. arius* that collected from the Cochin backwaters. In general, the studied fish species showed a higher concentration of metals in the liver with few exceptions and the lowest concentrations of metals were found in the flesh.

The average concentrations of Fe, Ni, Zn, Cu, Pb, Cd and Cr in *E. suratensis* were 290.39, 23.52, 84.85, 11.24, 8.33, 4.00 and 34.76 respectively in flesh, 820.89, 40.67, 252.94, 51.17, 28.29, 7.33 and 50.50 respectively in liver, 712.45, 45.75, 210.05, 24.50, 17.04, 7.86 and 46.10 respectively in gills (Table 2). The average concentrations of Fe, Ni, Zn, Cu, Pb, Cd and Cr in flesh of *A. arius* were 266.01, 21.20, 62.98, 9.37, 8.80, 3.51 and 19.02 respectively in flesh, 719.95, 31.00, 196.89. 45.24, 15.69, 6.76 and 47.45 respectively in liver and 610.06, 29.76, 166.03, 17.86, 19.30, 6.86 and 40.35 respectively in gills (Table 2). The average concentration of trace metals accumulated in *E. suratensis* follows an order: Fe > Zn > Cr > Ni > Cu > Pb > Cd for flesh, Fe > Zn > Cu > Cr > Ni > Pb > Cd for gill, and Fe > Zn > Cr > Ni > Cu > Pb > Cd for liver, respectively. In *A. arius* the trace metals accumulated follows an order: Fe > Zn > Ni > Cr > Cu > Pb > Cd for flesh, Fe > Zn > Cr > Ni > Cu > Pb > Cd for gill and Fe > Zn > Cr > Cu > Ni > Pb > Cd for liver, respectively (Table 2). Trace metal bioaccumulation in fish depends on the trophic level, size, food and feeding habits (Sankar et al 2006, George et al 2012, 2021). Dietary preferences, foraging behaviours and food web structure also influence metal bioaccumulation in fishes (Geldiay and Balik 2000). The concentration of trace metals detected in fish organs of *E. suratensis* and *A. arius* indicates different bioaccumulation

Table 2. Trace metal concentration in fishes (ppm) of the Cochin backwaters

Trace metals	*E. suratensis*			*A. arius*		
	Flesh	Liver	Gills	Flesh	Liver	Gills
Fe	290.39 ± 103.57	820.89 ± 345.66	712.45 ± 249.58	266.01 ±118.97	719.95 ± 188.80	610.06 ± 200.06
Ni	23.52 ± 9.15	40.67 ± 16.95	45.75 ± 14.12	21.20 ± 7.60	31.00 ± 11.25	29.76 ± 8.45
Zn	84.85 ± 43.97	252.94 ± 106.62	210.05 ± 91.85	62.98 ± 23.22	196.89± 68.42	166.03 ± 51.62
Cu	11.24 ± 7.25	51.17 ± 25.53	24.50 ± 15.31	9.37 ± 5.87	45.24 ± 21.20	17.86 ± 9.84
Pb	8.33 ± 5.00	28.29 ± 24.28	17.04 ±13.13	9.67 ± 4.35	15.69 ± 8.18	19.30 ± 9.44
Cd	4.00 ± 1.61	7.33 ± 4.34	7.86 ± 4.69	3.51 ± 1.52	6.69 ± 3.83	6.86 ± 3.64
Cr	34.76 ± 20.08	50.50 ± 20.84	46.10 ± 22.33	19.02 ± 9.32	47.45 ± 18.55	40.18 ± 20.81

potentials (Mahesh et al 2012). The interspecies variability in metal accumulation can be explained by species-specific differences in bioaccumulation dynamics.

In general, the concentration of trace metals was higher in the omnivorous species, *E. suratensis* when compared to the carnivorous species, *A. arius.* The concentration of Fe, Zn, Cu and Cd in organs of *E. suratensis* follow an order liver > gill > flesh while the Ni, Pb and Cr follow another order gills > liver > flesh. Similarly in *A. arius*, the Fe, Zn, Cu Pb, Cd and Cr concentration in the organs follow an order liver > gill > flesh, while, the Ni follow another order gills > liver > flesh. The omnivorous fish species, *E. suratensis,* showed higher concentrations of metals when compared to the carnivorous fish species, *A. arius.* This may be due to the feeding of *E. suratensis,* in both benthic and pelagic zones of the backwaters. *E. suratensis,* is a bentho-pelagic species and hence can feed on both benthic and pelagic food chains which enhances its trace metal accumulation behaviour when compared to the benthic species, *A. arius* (Yousafzai et al 2012, Siraj et al 2014, Obasohan 2008).

Bioaccumulation factor (BAF): The degree to which bioaccumulation occurs can be expressed as bioaccumulation factor and is evaluated in relation to the concentration of the soluble metal in the medium (water) in which the fishes inhabit. The concentration factor of the elements in the species *E. suratensis* and *A. arius* are in the order: Fe > Zn > Ni > Cu > Cr > Pb > Cd and Fe > Zn > Cr > Cu > Ni > Pb > Cd respectively. Of the fishes examined, *E. suratensis* showed a higher range of bioaccumulation factors for all the metals in the liver (Table 3). The bioaccumulation factor of elements in liver of the species *E. suratensis* and *A. arius* follows the order: Fe > Zn > Ni > Cu > Cr > Pb > Cd and Fe > Zn > Cr > Cu > Ni > Pb > Cd, respectively. In general, the higher values of BAF found in plankton followed by zoobenthos, predator fish and herbivorous fishes which is mainly depending up on the organism placed in the food chain, their feeding behaviour, hydrology and age of the organism (Culioli et al 2009, Tao et al 2012, Pantelica et al 2012). In the present study comparatively higher BAF values were observed in organs of studied fishes (Table 3), indicate the increased concentration of the soluble metal in the medium (water) in which the fishes inhabit. Many other studies reported the highest BAF in the fish organs and the tissues as Fe and Zn followed by Cu, Pb Cd and As (Uluturhan and Kucuksezgin 2007, Ayotunde et al 2012, Nwani et al 2010, Farombi et al 2007). Most metals showed a decreasing BAF in higher chains of food web, from phytoplankton to zooplankton, then fish (Tiphaine et al 2019). The BAFs of many elements in a given type of organism are much lower in marine waters compared to the freshwater environments, whereas the opposite is true for some elements.

Metal selectivity index (MSI) and Tissue selectivity index (TSI): Metal selectivity index (MSI) measures the affinity of a species to accumulate a particular metal in that tissue or organ of the body and is calculated for all the metals. MSI values (%) for different organs of fishes (Table 4). In *E. suratensis,* MSI values follows an order: Fe > Zn > Cr > Ni > Cu > Pb > Cd in flesh and gills but in liver it follows another order: Fe > Zn > Cr > Ni > Cu > Pb > Cd. In *A. arius,* MSI values were in the order: Fe > Zn > Ni > Cr > Cu > Pb > Cd in flesh, Fe > Zn > Cr > Cu > Ni > Pb > Cd in liver and Fe > Zn > Cr > Ni > Cu > Pb > Cd in gills. In both fishes, Fe and Cd showed the highest and lowest MSI values, respectively. The relative tissue occupying capacity of metal in a particular tissue is known as Tissue Selectivity Index (TSI). The tissue selectivity of trace metals in fishes is determined by the bioavailability of metals in the environmental matrix, feeding behaviour and metabolic activities. The TSI values (%) for different organs of fishes are given in (Table 5). In *E. suratensis*, the TSI values for the metals Fe, Zn, Cu, Pb and Cr follow an order: liver > gill > flesh whereas for the metals Ni and Cd it follows another order: gills > liver > flesh. In *A. arius* the TSI values for the metals Fe, Ni, Zn, Cu and Cr follow an order liver > gill > flesh whereas for the metals Pb and Cd it follows another order: gills > liver > flesh.

Table 3. Bioaccumulation factors for trace metals in fishes from Cochin backwaters

Trace metals	*E. suratensis*			*A. arius*		
	Flesh	Liver	Gills	Flesh	Liver	Gills
Fe	1014.74	2868.57	2489.64	929.56	2515.83	2131.83
Ni	478.43	827.13	930.41	431.14	630.60	605.24
Zn	908.80	2709.12	2249.69	674.56	2108.74	1778.30
Cu	174.66	795.04	380.56	145.52	702.91	277.51
Pb	105.88	359.73	216.67	111.92	199.58	245.50
Cd	105.64	193.87	207.69	92.75	178.59	181.30
Cr	526.74	765.10	698.53	288.23	718.95	611.43

Table 4. Metal selectivity index (%) of fishes collected from Cochin backwaters

Fishes	Organ	Fe	Ni	Zn	Cu	Pb	Cd	Cr
E. suratensis	Flesh	63.53	5.15	18.56	2.46	1.82	0.87	7.61
	Liver	65.58	3.25	20.21	4.09	2.26	0.59	4.03
	Gills	66.98	4.30	19.75	2.30	1.60	0.74	4.33
A. arius	Flesh	68.05	5.42	16.11	2.40	2.25	0.90	4.87
	Liver	67.73	2.92	18.52	4.26	1.48	0.64	4.46
	Gills	68.53	3.34	18.65	2.01	2.17	0.77	4.53

Table 5. Tissue selectivity index (%) off fishes collected from the Cochin backwaters

Fishes	Organ	Fe	Ni	Zn	Cu	Pb	Cd	Cr
E. suratensis	Flesh	15.92	21.40	15.49	12.94	15.52	20.83	26.46
	Liver	45.01	36.99	46.17	58.88	52.73	38.22	38.44
	Gills	39.07	41.61	38.34	28.18	31.76	40.95	35.10
A. *arius*	Flesh	16.67	25.86	14.79	12.92	20.09	20.49	17.81
	Liver	45.11	37.83	46.23	62.43	35.83	39.46	44.42
	Gills	38.22	36.31	38.98	24.65	44.08	40.05	37.77

The TSI reveal that the accumulation of trace metals in the flesh of fishes was lowest when compared to the gill and liver. This is maybe due to the different physiological roles of these organs in fish metabolism (Merciai et al 2014). When compared to muscle, the gill and liver in fishes serve for respiration and metabolism and are considered as target organs for trace metal contaminant accumulation (Nair et al 2006). This is associated to the fact that food and water are the main routes of contaminant assimilation as it is directly linked with the metabolism and respiration (Reddy et al 2007). Trace elements assimilated from food that contaminated with wastewater effluents are ingested in fish body and are transported through the blood and gets incorporated in various tissues at variable degrees (Kojadinovic et al 2007, George et al 2021). When compared to the liver and gill, the low concentration of metals in the muscle of fish species may be the low metabolic activity and/or low levels of metal-binding proteins in the muscle. Unlike other tissues, the liver accumulates high concentrations of metals, irrespectively of the uptake route and is considered as an indicator of water pollution by trace metals since their concentrations accumulated in this organ are often proportional to those present in the aquatic environment. Consequently, the high level of metals accumulation in the fish liver followed by gill and muscle highlights an environmental indication of water pollution due to the persistent exposure of trace metals loaded from the industrial effluent discharges, agricultural run-off and domestic sewage inputs (George et al 2012, Robin et al 2012). Hence metal concentrations in organs of fishes could be used as an index to estimate the level of pollution in aquatic ecosystems (Karadede-Akin and Unlu 2007).

CONCLUSION

The accumulation of trace metals in fish tissues varied among species. The omnivorous species, *E. suratensis* accumulated a higher concentration of trace metals in fish tissues than the carnivorous species, *A. arius*. The liver and gill of fishes showed higher concentrations of metals when compared to the flesh tissue due to higher metabolic activity of the liver and gill when compared to muscle. Bioaccumulation of trace metals in aquatic life, especially fishes have possible detrimental effects and direct toxic effect on human life and hence have environmental concern worldwide.

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