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Seasonal variation in accumulation of metals in selected tissues of the Ribbon fish, *Trichiurus lepturus c*ollected from Chaliyar River, Kerala, India

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Abstract
The healthy condition of an aquatic ecosystem depends upon the physico-chemical and biological characteristics, which usually fluctuate with season and degree of pollution. The present investigation was carried out to assess the seasonal variations in bioaccumulation of metals (Cr, Mn, Fe, Cu, Zn and Pb) in selected tissues (Gill, Liver, Kidney and Muscle) of the freshwater fish *Trichiurus lepturus* collected from Chaliyar River, Kerala state. The results showed variation in accumulation of metals in different seasons. Gills accumulated maximum quantities in monsoon period followed by post monsoon, summer and pre monsoon period respectively. Liver, kidney and muscle accumulated maximum in monsoon and post monsoon periods. The order of accumulation was Fe > Zn >Mn> Cu > Cr >Pb. From this study it is clear that the Chaliyar River is polluted due to the discharge of industrial effluents, sewage run off from the villages as well as gold mining activities in the bank of the river by tribal people. The entry of metals into the river system reflects the pollution by industries which may adversely affect the fish population. Hence, to maintain a healthy biodiversity and abundance of fish, educate locals about the negative impact of pollution with sewage, fertilizers, pesticides and other chemicals.

Keywords: Chaliyar River, *Trichurus*, Ribbon fish, bioaccumulation

1. Introduction

During last few decades, there has been considerable concern over heavy metals contamination of aquatic environment and the potential health threat to public potable water sources. The non-degradable and persistent nature of the metal ions results in longer exposure and accumulation of these substances in the aquatic flora and fauna. This would result in deterioration and disturbance of aquatic ecosystem. Heavy metal accumulation in the aquatic environment could result in toxicity to both aquatic life and human. Edible fish present in aquatic bodies form an important group of organism as heavy metal once accumulated in fish tissues could act as a potential carrier of metal ion along the food chain. At the end, directly or indirectly the metal ion in the aquatic medium reaches to the man. Hence several studies involving bioaccumulation of heavy metals have been conducted in fishes [1] found in river streams generally receiving industrial effluents containing toxic heavy metals and organic pollutants. Accumulation of metals (Cu, Zn, Cd, Ni, Cr, Pb) in different tissues viz., blood, gill, gut, liver, muscle, kidney, ovary and gonad etc., have been extensively investigated in various fishes [2-7]. Most of these studies report metal accumulation indicating preference of the tissues for some metals over the others. The characterization of the accumulation of metals into different organs has proven to be a representative measure of the heavy metal exposure and is used to monitor the bioavailability of these metals. Gills and livers are considered most important for assessing metal accumulation. Since gill is in direct contact with metal present in the water, the concentrations of metals in gills reflect the concentration of metals in water.

There are two main routes of heavy metals exposure [8]: (1) The primary route of intake of these chemicals in fish species is via gills or transport of dissolved contaminants in water across biological membranes and ionic exchange etc. (2) The secondary route is through ingestion of food or sediment particles with subsequent transport across the gut [9]. Studies show that gastrointestinal route is the most important route of uptake [10, 11]. Heavy metals are known to distort the structural or biological functions of biomolecules [12]. Since metals act as endocrine disruptors, they can interfere with metabolism, synthesis, and transport of hormones or receptors [13]. The aquatic microflora or microfauna, which constitute the food for fish species, accumulate these metals in their living cells from the environment. The fish consume them and gradually get enriched with heavy metals via bioaccumulation. Their concentration in turn increases into the predators and finally into the human beings resulting into onset of various types of disease syndromes. After a careful review of the existing literature; it appears that not much attention has been paid towards studies on the accumulation of heavy metals in riverine system which includes water, sediment and aquatic fish species in the Indian context. Hence, the present study has been conducted to understand the seasonal variation in metal accumulation in selected tissues of the fish *Trichiurus lepturus* of Chaliyar River, Kerala, India.

2. Materials and Methods

2.1 Study Area: Chaliyar is the fourth largest river in Kerala and drains a very extensive tract of the Wynad ghats and Nilgiri mountains. This is the only main stream in Malabar which brings in considerable portion of waters from the crest of the Ghat mountain ridge. Its two main branches viz., Punnapuzha and Chola river rise respectively in the Kunda mountains on the Nilgiri plateau and on the lower ranges of the south east Wynad. The two streams after receiving many large feeders (Korapuzha, Kalakkupuzha) unite in the midst of the Nilambur Government Teak plantations and then flow on, receiving several important feeders from the north and south, to their outlet into the sea at Beypore. Total length of the river is nearly 152km. four different stations were selected on the river namely: sampling station 1 (S1) is Chungathara, station 2 (S2) is Edavanna, station 3 (S3) is Vazhakkad and station 4 (S4) is Beypore. (Fig 1.).

Samples were collected on every month from January 2013 to December 2014 (Two years). The months were divided into different seasons such as Summer (January, February and March); Pre Monsoon (April, May and June); Monsoon (July, August and September) and Post Monsoon (October, November and December). The average of monthly data was taken for representing the seasonal data.

Fig 1: Showing the four study areas in the Map of Chaliyar River

2.2 Bioaccumulation of metals in fish tissues: The commonly available fish, ribbon fish *Trichiurus lepturus* was collected on every month and dissected to separate the organs (gills, kidney, liver and muscle) following FAO methods. The separated organs were dried at 120 ˚C in petri dishes until reaching a constant weight. The separated organs were placed in digestion flasks and ultrapure concentrated nitric acid and hydrogen peroxide (1:1 v/v) (SD fine chemicals) were added. The digestion flasks were then heated to 130 ˚C until all the materials were dissolved. Digest was diluted with double distilled water appropriately and Cr, Mn, Fe, Cu, Zn and Pb was assayed. Atomic absorption spectrophotometer (Perkin Elmer) using element- specific hollow cathode lamps in default condition, by flame absorption mode was used to approximate the metal concentration. Metal standards recommended by Perkin Elmer were used for checking the sensitivity of the instrument and calibration.

2.3 Statistical Analysis: The data obtained were subjected to standard statistical analysis. One way analysis of variance (ANOVA) followed by Duncan's multiple range test [14] was performed to determine whether the parameters altered significantly by different seasons.

3. Result and Discussion

The concentration of metals (Cr, Mn, Fe, Cu, Zn and Pb) in selected tissues (Gill, Liver, Kidney and Muscle) of the freshwater fish *Trichiurus lepturus* collected from Chaliyar River at different seasons for a period of two years are given in table 1 and 2. The fish tissues showed variation in accumulation of metals in different seasons. Gills accumulated maximum quantities in monsoon period followed by post monsoon, summer and pre monsoon period respectively. Liver, kidney and muscle accumulated maximum in monsoon and post monsoon periods. The order of accumulation was Fe > Zn >Mn> Cu > Cr >Pb.

Table 1: Seasonal Variations in metal concentrations in tissues of the fish *Trichiurus lepturus* (µg /g dry weight) collected from Chaliyar River for January 2013 to December 2013

Tissues	Metals	Summer	Pre Monsoon	Monsoon	Post Monsoon	'F' value
Gill	Cr	2.5 ± 0.01 NS	2.8 ± 0.02 NS	3.2 ± 0.02*	2.5 ± 0.09 NS	16.18
	Mn	26 ± 1.15 NS	27 ± 1.20 NS	27.2 ± 1.00 NS	26 ± 0.78 NS	112.18
	Fe	35 ± 0.01 NS	36 ± 0.01 NS	41 ± 1.12*	39 ± 1.68*	164.32
	Cu	6.2 ± 0.02 NS	5.8 ± 0.01 NS	7.0 ± 0.02 NS	6.7 ± 0.02 NS	38.14
	Zn	12.7 ±1.00 NS	11.0 ± 0.98 NS	15.2 ± 0.01*	13.0 ± 1.10 NS	69.16
	Pb	1.61 ± 0.02 NS	1.58 ± 0.01 NS	2.18 ± 0.02*	1.98 ± 0.01*	14.15
Liver	Cr	7.12 ± 0.98 NS	7.0 ± 0.02 NS	7.19 ± 0.02*	7.18 ± 0.02*	48.28
	Mn	21.5± 0.01 NS	21.5 ± 0.75 NS	21.9 ± 0.01	21.0 ± 0.68 NS	112.56
	Fe	32.5 ± 1.10 NS	32.6 ± 0.01 NS	35.0 ± 1.68*	34.1 ± 0.01*	148.10
	Cu	24.2 ± 1.11 NS	24.8 ± 1.00 NS	28.15 ± 1.74*	27.13 ± 1.25*	138.42
	Zn	18.14 ± 0.01 NS	21.34 ± 0.01*	26.34 ± 0.01*	25.17 ±1.75*	116.24
	Pb	1.00 ± 0.00 NS	1.5 ± 0.01 NS	1.8 ± 0.01*	1.6 ± 0.01 NS	8.42
Kidney	Cr	1.61 ± 0.15 NS	1.68 ± 0.01*	1.75 ± 0.23*	1.65 ± 0.20 NS	10.16
	Mn	2.1 ± 0.01 NS	2.0 ± 0.02 NS	2.7 ± 0.01*	2.5 ± 0.01 NS	15.64
	Fe	20. 56 ± 1.15 NS	21.41 ± 1.00 NS	24.32 ± 1.14*	22.50 ± 1.08*	94.18
	Cu	21.2 ± 1.00*	19.14 ± 1.11 NS	22.5 ± 1.25*	21.0 ± 0.01*	100.15
	Zn	16.32 ± 1.20 NS	16.20 ± 0.01 NS	18.45 ± 1.00*	17.30 ± 1.13*	74.15
	Pb	1.28 ± 0.01 NS	1.30 ± 0.01 NS	2.15 ± 0.01*	1.92 ± 0.01*	12.10
Muscle	Cr	0.96 ± 0.01 NS	0.98 ± 0.01 NS	1.10 ± 0.02*	1.09 ± 0.02*	9.86
	Mn	1.0 ± 0.01 NS	1.02 ± 0.01 NS	1.68 ± 0.01*	1.45 ± 0.01*	12.14
	Fe	16.13 ± 1.0 NS	16.45 ± 1.01 NS	18. 16 ± 1.12*	18.0 ± 1.10*	76.00
	Cu	14.20 ± 1.0 NS	14.72 ± 1.14 NS	16.28 ± 1.14*	16.0 ± 1.10*	71.00
	Zn	14.18 ± 1.20 NS	14.10 ± 1.20 NS	16.24 ± 1.00*	16.0 ±1.22*	92.18
	Pb	0.96 ± 0.00 NS	0.98 ± 0.08 NS	1.50 ± 0.00*	1.00 ± 0.00*	6.19

Note: Values are expressed as mean ± SE; * $p \leq 0.05$ level.

Table 2: Seasonal Variations in metal concentrations in tissues of the fish *Trichiurus lepturus* (µg /g dry weight) collected from Chaliyar River for January 2014 to December 2014.

Tissues	Metals	Summer	Pre Monsoon	Monsoon	Post Monsoon	'F' value
Gill	Cr	2.6 ± 0.01 NS	2.8 ± 0.02 NS	3.2 ± 0.02*	2.8 ± 0.09 NS	20.180
	Mn	27 ± 1.15 NS	27 ± 1.20 NS	27.6 ± 1.00 NS	27 ± 0.78 NS	110.12
	Fe	39 ± 0.01 NS	37 ± 0.01 NS	44 ± 1.12*	40 ± 1.68*	165.34
	Cu	6.8 ± 0.02 NS	6.8 ± 0.01 NS	7.4 ± 0.02 NS	7.0 ± 0.02 NS	40.16
	Zn	12.9 ±1.00 NS	12.0 ± 0.98 NS	15.2 ± 0.01*	15.0 ± 1.10 NS	74.15
	Pb	1.68 ± 0.02 NS	1.54 ± 0.01 NS	3.00 ± 0.02*	2.98 ± 0.01*	14.16
Liver	Cr	7.10 ± 0.96 NS	7.02 ± 0.01 NS	7.20 ± 0.02*	7.15 ± 0.02*	48.28
	Mn	22.5± 0.01 NS	22.5 ± 0.75 NS	24.9 ± 0.01	23.0 ± 0.68 NS	110.60
	Fe	34.5 ± 1.10 NS	33.6 ± 0.01 NS	36.0 ± 1.68*	36.1 ± 0.01*	148.15
	Cu	25.6 ± 1.11 NS	25.2 ± 1.00 NS	29.40 ± 1.74*	28.14 ± 1.25*	138.50
	Zn	19.14 ± 0.01 NS	22.30 ± 0.01*	27.34 ± 0.01*	26.10 ±1.75*	162.20
	Pb	1.05 ± 0.00 NS	1.51 ± 0.01 NS	1.80 ± 0.01*	1.62 ± 0.01 NS	10.40
Kidney	Cr	1.64 ± 0.15 NS	1.72 ± 0.01*	1.79 ± 0.23*	1.68 ± 0.20 NS	16.46
	Mn	2.4 ± 0.01 NS	2.4 ± 0.02 NS	3.1 ± 0.01*	2.8 ± 0.01 NS	18.65
	Fe	20.58 ± 1.15 NS	21.44 ± 1.00 NS	24.35 ± 1.14*	22.55 ± 1.08*	96.12
	Cu	21.5 ± 1.00*	19.19 ± 1.11 NS	22.9 ± 1.25*	21.6 ± 0.01*	106.14
	Zn	16.38 ± 1.20 NS	16.26 ± 0.01 NS	18.51 ± 1.00*	17.36 ± 1.13*	74.18
	Pb	1.68 ± 0.01 NS	1.60 ± 0.01 NS	2.50 ± 0.01*	1.95 ± 0.01*	12.16
Muscle	Cr	0.99 ± 0.01 NS	1.10 ± 0.01 NS	1.18 ± 0.02*	1.18 ± 0.02*	12.75
	Mn	1.5 ± 0.01 NS	1.07 ± 0.01 NS	1.68 ± 0.01*	1.45 ± 0.01*	12.14
	Fe	16.18 ± 1.0 NS	16.48 ± 1.01 NS	18. 26 ± 1.12*	18.5 ± 1.14*	78.00
	Cu	14.28 ± 1.0 NS	14.75 ± 1.16 NS	16.38 ± 1.16*	16.8 ± 1.14*	76.24
	Zn	16.14 ± 1.20 NS	15.14 ± 1.20 NS	16.28 ± 1.00*	16.8 ±1.25*	92.18
	Pb	1.06 ± 0.05 NS	1.95 ± 0.08 NS	2.10 ± 0.00*	1.98 ± 0.06*	6.19

Note: Values are expressed as mean ± SE; * $p \leq 0.05$ level.

In recent years the accumulation of heavy metals in aquatic systems has become a problem of great concern throughout the world. These metals may accumulate to a very high toxic levels and cause severe impact on the aquatic organisms without any visible sign. Increase in population, urbanization, industrialization and agricultural practices have further aggravated the situation [15]. The waste waters containing heavy metals produced by industries [16] are released directly into aquatic resources including rivers without any or with only partial pre-treatment. Heavy metals thus discharged persist in the aquatic bodies and bioaccumulate along the food chain. Metals present in the environment in minute quantities become part of various food chains through biomagnification and their concentration increases to such a level that may prove to be toxic to both humans and other living organisms. The bioaccumulation of hazardous heavy metals in fish species from different aquatic systems is related to their foraging habitats. Sedimentary fish species live in stagnant water in muddy streams, feed at bottom and exhibit higher levels of heavy metals.

Heavy metal pollution of river ecosystem has been reported by several authors [17-19]. Since fish are often the last link in aquatic food chains, the metal concentrations of many fish species have been analysed in relation to metal contents of aquatic environments [20-22]. Zinc (Zn), Copper (Cu), Iron (Fe), Cadmium (Cd) and Lead (Pb) were selected because most studies on heavy metal concentrations in fish dealing these metals. Some heavy metals, such as Cu and Zn are necessary in trace amounts for the functioning of biological systems [23, 24] while metals like Pb and Cd are known to interfere with the functioning of biological systems [25].

In the present study, the fish tissues showed variation in accumulation of metals in different seasons. Gills accumulated maximum quantities in monsoon period followed by post monsoon, summer and pre monsoon period respectively. Liver, kidney and muscle accumulated maximum in monsoon and post monsoon periods. The order of accumulation was Fe > Zn >Mn> Cu > Cr >Pb. In the present investigation, the pattern of accumulation of metals differs from tissue to tissue and metal to metal. This difference in accumulation may be attributed to the proximity of the tissue to the medium, the physiological state of the tissue, presence of ligands having an affinity to metals and /or to the role of the tissue in the detoxification process. From the point of view of proximity to toxicant of the various tissues analysed, gill is in direct contact with the medium, whereas liver, kidneys and muscle can accumulate through media effect (i.e. blood). One of the basic reasons for the gills to act as the primary site for accumulation, as observed in the present study, is its external position and its proximity to the medium, the highly branched structural organization of the gill and the resultant highly increased surface area, along with the large volume of water passing through the gill surface and the highly vascular physiological state and the relatively small biomass when compared to their surface area [26] make the gill a prime site for metal accumulation.

Even though liver and kidneys do not come into direct contact with the medium, the accumulation pattern in them followed more or less the same pattern as that of gills. Kidneys are next to gill quantity-wise in the accumulation of metals. One of the main reasons attributed to the increased presence of metals in these organs is their capacity to accumulate metals brought by blood from other parts of the body and induce the production of the metal binding protein, metallothionein, which is believed to play a crucial role against the toxic effects of heavy metals by binding them [27]. Moreover, the gill and the liver, along with kidney, are the main sites of metallothionein production and metal retention [28]. This may be yet another main reason for the enhanced presence of metals in the gills, kidneys and liver. In addition, all these tissues are rich in the metal binding-SH groups [29] and therefore it is not surprising that the metal ions are complexed in these organs.

According to Kent [30] the liver and kidneys are involved in the detoxification and removal of toxic substances circulating in the blood stream. Moreover, liver and kidneys, being the major organs of metabolic activities including detoxification [28], metals might also be transported into these organs from other tissues, including gills and muscle, for the purpose of subsequent elimination. Such transportation might lead to higher rates of accumulation in these two organs. The possibility of such detoxification/elimination-related mobilization of accumulated metals may be one reason for the intermittent reductions in the quantity of accumulated metals in gills. Further, unbound metals, such as cadmium and mercury, can be reabsorbed by active transport mechanism in the cells of the proximal convoluted tubule, and once they are in the cells they bind to metallothionein, resulting in their accumulation [28]. All these observations justify the possibility of transporting the trace amounts of cadmium from the various tissues to kidneys. Of all the tissues investigated in the present study, the muscle accumulated the lowest level of metals. This finding confirmed the existing reports [32, 33]. In the light of all these observations, and also from the results of the present study, it may be inferred that the residue of metals present in the fish muscle may not be exactly correlated to its concentration in the ambient water medium. Various reasons may be attributed to the lower rate of bioaccumulation of metals in muscle. First of all, the muscle does not come into direct contact with the water medium. Another probable reason may be the fact that even though muscle is the most valued edible tissue, it is not an active site for detoxification and therefore transport of metals from other tissues to muscle (as in the case of liver and kidney) does not seems to arise.

Considerable variations in the heavy metals were observed in all the samples studied with maximum accumulation during monsoon season and minimum during summer seasons under investigation. These metals exert sublethal effect on aquatic organisms and predators adversely influence their reproduction and behavior [34]. Metals are generally precipitated at alkaline pH in the form of insoluble oxides and carbonates. It has been proved that lethality increases as oxygen concentration decreases. Increase in temperature also increases toxicity due to depletion in dissolved oxygen, increase in energy demand causing rise in respiration rate in the organism, which leads to rapid assimilation of wastes [35].These elements are known to produce adverse effects on aquatic biota and human health. Effects of these metalsin fish include reduction of growth and reproductive capacity, swimming imbalance and inability to capture the prey [36].These heavy metals penetrate through the mucous membrane of the bronchia, from where they move in body via circulatory system and accumulate in liver and kidney [11]. Their absorption through intestinal tract depends upon pH, rate of movement through the tract and presence of other materials. High concentration of Pb, Cr and Ni in sediment and the fish tissues such as liver muscles and gills of common carp, *Cyprinus carpio* [38] and *Cnesterodon decemmaculatus* (Poeciliidae) as well as in the body of crab *Zilchiopsis collastinensis* (Decapoda) [38] has been reported specially in areas close to industries [39]. Although, accumulation of Cr was not significant they are important in maintaining proper function of biological systems working. Bottom dwelling fishes are found to exhibit higher concentration of heavy metals than pelagic fishes [40]. The increase in concentration of metals in fish could be mainly due to metal contaminated diet which comes from discharge of effluents into rivers from different industries and other sources in the form of particulate sand solutions [41]. Though Cr is not present in significant amount, it has been reported that could be known to be highly toxic because it can replace Zn in some enzymes and may cause diseases. The results from present study showed that the metal concentrative ability depends on the nature and proximity of organs to the water medium. The information obtained from this study could be useful for environmental agencies to monitor aquatic system and finally to the management of human health practices.

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