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Analysis of Accumulated Heavy Metal Concentrations in Various Body Parts of Chillapi (*Oreochromis mossambicus*) Fish from Ujjani Reservoir of Maharashtra, India

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Abstract The heavy metal contamination is creating devastating effects on ecosystem and environment and eventually hazardous to human health. Ujjani is one of the largest reservoir in Maharashtra state. It receives water from different metropolitan cities harboring numerous industries. Moreover, anthropogenic activities pollute water and fish fauna of the reservoir. In the present study, the concentrations of heavy metals such as Iron (Fe), Copper (Cu), Zinc (Zn), and Manganese (Mn) were analyzed from various tissues namely muscles, liver, gill, and fin of Chillapi fish. The variation in level of metals was found with respect to tissue types and body weight groups. The concentrations of Fe were ranged between 15.94 to 91.56µg/g, Cu concentration was between 1.88 to 48.88µg/g, Zn concentration was between 25.72 to 84.2µg/g and Mn was recorded between 2.12 to 28.48µg/g in Chillapi fish. Fe and Cu have recorded highest in the liver, whereas peak of Zn and Mn was detected in fins. Furthermore, the minimal metal concentrations were observed in muscle samples of all different weight groups. It shows that Fe, Zn, Cu concentration in all four tissues was within the limit, whereas the Mn level was found to be exceeding the permissible limit as prescribed FAO/WHO, 1989. The presence of heavy metals higher than normal level in fish bodies is a clear indicator of biomagnifications. Furthermore, THQ and HI values were within limit for all studied metals in fish but if consumed in excess amount may cause toxicity in humans. Therefore, daily intake of fish should be strictly monitored to avoid excess intake of these heavy metals. Our study signifies the importance of addressing the heavy metal contamination issue to avoid health related problems.

Keywords Ujjani Reservoir, Heavy Metals, Chillapi, *Oreochromis mossambicus*, AAS, World Health Organization, Biomagnifications

1. Introduction

The pollution of fresh water resources is the major concern globally. The discharge of pollutant through anthropogenic activity has been increased tremendously and creating terrible consequences on quality of water [1-3]. However, the activities like rapid growth of industries, extensive use of fertilizers, pesticides in agriculture and mining has increasing discharge of heavy metals in aquatic environments [4]. Heavy metal contamination leads to devastating effect on the ecosystem and the environment [5, 6]. Heavy metals are predominantly natural trace components of the aquatic environment and also act as micronutrients which are required in limited quantity for growth of living organism, but their level has continuously rising due to anthropogenic activities which is lethal to aquatic environment [7]. Above mentioned sources of pollution alters the physiochemical properties of aquatic habitat, and quality and quantity of fish stocks [7 - 9]. It is well known fact that heavy metals accumulate in the sediment surface, benthic living organisms and these metals exhibit the increasing trend in the concentration through biomagnifications process in the food chain. Fishes are often top choice among all aquatic foods for consumers and frequently susceptible for accumulation of large amount of metals present in water [10]. Presence of organic contaminants are less hazardous to the environment because their toxicity reduces with time, but heavy metals cannot be degraded and their concentration increases through bioaccumulation and biomagnifications processes, and they persistent in nature [11].

Fish has rich nutritional value. It contains high proteins, amino acids, vitamins, minerals, and polyunsaturated fatty acids and also an important source of essential heavy metals. Heavy metals like Iron, Copper, Zinc, and Manganese are essential to normal growth as a micronutrient, whereas some other metals such as Arsenic, Cadmium, Mercury and Lead have no role in biological systems and presence of these metals is hazardous to the environment [12-14]. Fish has dietary importance, bioaccumulation of essential as well as nonessential heavy metals in fish leads to a serious adverse effect on a human health [15]. Numerous researchers have reported that heavy metal accumulation results in disturbance in normal cellular activities, oxidative damage to biological macromolecules, DNA and proteins [16]. The prime symptoms of toxicity of metals are intellectual disability in children, dementia, central nervous system disorders, kidney diseases, liver diseases, insomnia, emotional instability, depression and vision disturbance [16, 17]. Moreover, several researchers have studied and found that, metal accumulation also leads to cancer disease [18].

In the present study, the status of fish resources of Ujjani reservoir and its interaction with heavy metals has been studied. Ujjani reservoir is a terminal Reservoir on the river Bhima. It has 14500 Sq. km catchment area in the Pune district. In the Western Zone of the Ujjani reservoir, the Bhima river enters and receiving water from the river tributaries such as Mula, Mutha, Ghod, Pawana, Kukadi, Indrayani and Vel. The catchment of the Ujjani reservoir has extreme physiographic and agro-climatic variations. In the last few decades, industrial, urban and agriculture development in the catchment area of the Ujjani reservoir led to the degradation of the water quality. Untreated or inadequately treated sewage, industrial and agriculture waste water generated from Pune and Pimpri Chinchwad urban and rural areas have been directly discharged into Bhima River and its tributaries, and finally it reaches to Ujjani reservoir and creates heavy pollution of reservoir water and aquatic environment [19-20].

Oreochromis mossambicus commonly known as Chillapi is very famous species of the Ujjani reservoir. It shares maximum fish stock in the local and state fish market. Water pollution in the Ujjani reservoir leads to degradation of fish quality. The presence of contaminants in the water habitat leads to bioaccumulation and biomagnifications into fish and finally it enters in food chain. Therefore, this study was targeted to assess the impact of water pollution on Chillapi fish with reference to bioaccumulation and human health hazards of selected heavy metals. Bioaccumulation of heavy metals such as Fe, Cu, Zn and Mn was determined in different tissues like liver, gill and fin of Chillapi fish.

2. Material and Methodology

2.1. Sampling Site Description and Sample Collection

The study has been performed on the Chillapi fish species of Ujjani reservoir. The Ujjani reservoir (Topo sheet No. 47 N/4; Latitude 180 04’ 24" N and Longitude 750 07’ 15" E) is located in the district of Solapur (Taluka-Madha). Its catchment area is about 14856 Sq. km. This reservoir receives water by several rivers such as Mula, mutha and Bhima. The river Bhima originate from Bhimashankar hills located in Western hills of Maharashtra. Later, these rivers pass through metropolitan and industrialized cities named as Pune, Pimpri Chinchwad and Chakan. Finally, the river water is cached in Ujjani reservoir. Here, we have chosen the sampling locations in between Bhigwan (start point of reservoir) and Bhimanagar (end point of reservoir) villages. The Chillapi fish samples were collected during January 2017 to April 2017, with the help of fishing net and brought to the laboratory. These samples were divided into five categories based on their weight, approximately 150g, 300g, 450g, 600g, and 750g. These specimens were dissected to obtain the tissues such as muscle, liver, gill and fin. The whole fish, as well as different dissected tissues were thoroughly washed with tap water followed by distilled water and excess water was removed by bloating with Whatman filter paper.

2.2. Sample Preparation for Analysis

Fish tissues (muscles, liver, gills and fins) of each weight group were weighed (1g) and digested with mixture of 10 ml of concentrated HNO_3 /H_2O_2 (3:1) on hot plate, initially for 1 hour at room temperature, then for 1 hour at 40^o C followed by 3 hours at 140^{o}C. Subsequently, samples were cooled at room temperature. Later, samples were diluted with distilled water to final volume 50 ml and filtered through Whatman paper grade 42 [21, 22], and stored at 4^{o}C in the refrigerator for further analysis.

2.3. Determination of Heavy Metals in Fish Tissue

The concentrations of Fe, Cu, Zn and Mn were determined in muscle, liver, gill and fin samples on Atomic Absorption Spectrophotometer (Sistronics, Model AAS - 263) by using standard protocols [23].

2.4. Statistical Analysis

Data collected were subjected to statistical analysis. Mean, Standard Deviation and Standard Error of the database were calculated. The average values (mean ± standard error) were compared.

2.5. Health Risk Assessment for Fish Consumption

The values of heavy metal accumulation in fish tissues were used to calculate the estimated daily intake of metals (EDI), target hazard quotients (THQ) and hazard index (HI) separately for adult male and female individuals.

2.6. Estimated Daily intake of Metals (EDI)

EDI is measured in (mg/kg body-weight/day) [24].

$$EDI = Mc \times IR / Bw$$

Where, Mc is the metal concentration in the fish muscle (mg/kg dry weight). IR is the ingestion rate, which is considered as 19.5 × 10^{-3} kg/day [25, 26] and this consumption rate was used in health-risk assessment. The hypothesis of an adult ingestion rate of fish over a life time is an assessment of actual fish consumption. BW is an average body weight of Indian male (57 kg) and female (50 kg) [27].

2.7. Target Hazard Quotient (THQ)

To assess the human health risk from consuming the fish contaminated with heavy metals, the target hazard quotient (THQ) was calculated as per USEPA Region III Risk-Based Concentration [28]. The THQ is an estimate of the non-carcinogenic risk level rises due to pollutant exposure and calculated by the following equation:

$$THQ = Mc \times IR \times 10^{-3} \times EF \times ED / RfD \times BW \times ATn$$

Where, THQ is non-carcinogenic risk and is dimensionless. EF is the exposure frequency (365 days/year). ED is the exposure duration (67 years) (life expectancy of Indian male = 65 years approximately and for Indian females is 68) years approximately. Therefore, an average of two extremes have been taken. RfD is the reference dose of an individual metal (mg/kg/ day) [29] (Table 2). ATn is the averaging time for non-carcinogens (365 days/year × ED) [28].

2.8. Hazard Index (HI)

To evaluate the overall potential health risk posed by more than one metal, THQ of every metal is summed up and is known as hazard index (HI). The HI can be calculated by the sum of the target hazard quotients of each metal [29].

$$HI = THQ\ Fe + THQ\ Cu + THQ\ Zn + THQ\ Mn$$

3. Results

In this study, we have determined concentration of various metals in Chillapi fish tissues in different weight groups by the Atomic Absorption Spectrophotometer (AAS). The observed concentration of different metals in various tissue types and weight groups is shown as bellow in Table 1. Also the values of heavy metal accumulation in fish tissues were used to calculate the estimated daily intake of metals (EDI), target hazard quotients (THQ) and hazard index (HI) separately for adult male and female individuals (Table 2).

Table 1. Heavy metal Concentrations (µg/g wet weight) in M (muscle), L (liver), G (gill), F (fin) tissue of Chillapi with respect to body weight. Values are Mean ± S.E., (n = 5 for each weight groups)

Body weight groups	Tissue types	Fe	Cu	Zn	Mn
150g ±10g	M	58.08 ± 4.53	11.58 ± 2.77	43.82 ± 4.79	2.6 ± 0.41
	L	91.56 ± 7.28	24.32 ± 3.58	44.58 ± 12.18	10.12 ± 2.72
	G	75.52 ± 6.52	23.08 ± 2.49	39.68 ± 3.66	2.4 ± 0.59
	F	60.88 ± 8.30	12.2 ± 4.79	54.2 ± 11.42	11.42 ± 2.65
300g ±10g	M	54.22 ± 3.63	2.64 ± 0.50	48.06 ± 2.14	2.12 ± 0.49
	L	78.03 ± 9.95	20.88 ± 7.14	45.98 ± 13.12	7.22 ± 2.18
	G	79.98 ± 2.40	16.9 ± 5.60	45.46 ± 3.77	5.98 ± 2.16
	F	72.18 ± 7.60	6.48 ± 2.38	84.2 ± 9.39	8.52 ± 2.63
450g ±10g	M	33.86 ± 6.53	3.76 ± 0.86	34.74 ± 3.70	2.16 ± 0.45
	L	67.64 ± 15.7	37.68 ± 9.28	33.44 ± 0.84	3.66 ± 0.75
	G	55.6 ± 11.81	4.7 ± 0.30	38.74 ± 3.08	3.58 ± 0.71
	F	53.4 ± 5.11	4.524 ± 0.70	66.28 ± 3.44	23.28 ± 3.52
600g ±10g	M	48.66 ± 4.19	3.76 ± 0.82	32.04 ± 2.25	7.46 ± 2.82
	L	90.56 ± 23.7	48.88 ± 8.8	29.16 ± 2.53	13.3 ± 1.57
	G	58.38 ± 3.55	3.76 ± 0.48	27.88 ± 3.51	7.52 ± 1.79
	F	49.54 ± 5.87	3.7 ± 0.92	61.24 ± 3.52	28.48 ± 5.92
750g ±10g	M	18.7 ± 5.55	1.88 ± 0.53	25.72 ± 3.85	3.42 ± 0.88
	L	40.26 ± 5.41	48.76 ± 14.62	37.8 ± 7.90	7.88 ± 1.07
	G	35.52 ± 2.22	2.2 ± 0.51	26.22 ± 4.77	5.1 ± 1.36
	F	15.94 ± 5.06	2.08 ± 0.57	56.60 ± 17.50	22.66 ± 5.37

Table 2. Estimated daily intake (EDI), Target hazard quotient (THQ) and Hazard index (HI), Reference dose (mg/kg/day) values of metals via consumption of fish *Chillapi fish*

Heavy Metals	EDI (mg/kg body-weight/day)		THQ		HI		RfD (mg/kg/day)
	Male	Female	Male	Female	Male	Female	
Fe	0.019	0.022	0.027	0.031	0.21	0.24	0.7
Cu	0.004	0.005	0.12	0.13			0.04
Zn	0.014	0.017	0.049	0.056			0.3
Mn	0.003	0.003	0.022	0.024			0.1

3.1. Iron (Fe)

The Fe concentrations was observed in the different *Chillapi* fish tissues in an order such as liver, gill, fin, followed by muscle except 750±10g fish. The highest Fe level was detected in liver tissue compared to other tissue types in all weight groups. The lowest Fe concentration was detected in muscles than other tissue types in all weight groups. A concentration of Fe was highest in 150g weight of fish than other weight groups. Overall, it was observed that concentration of Fe decreases as fish body weight increases (Table 1, Fig. 1.A).

3.2. Copper (Cu)

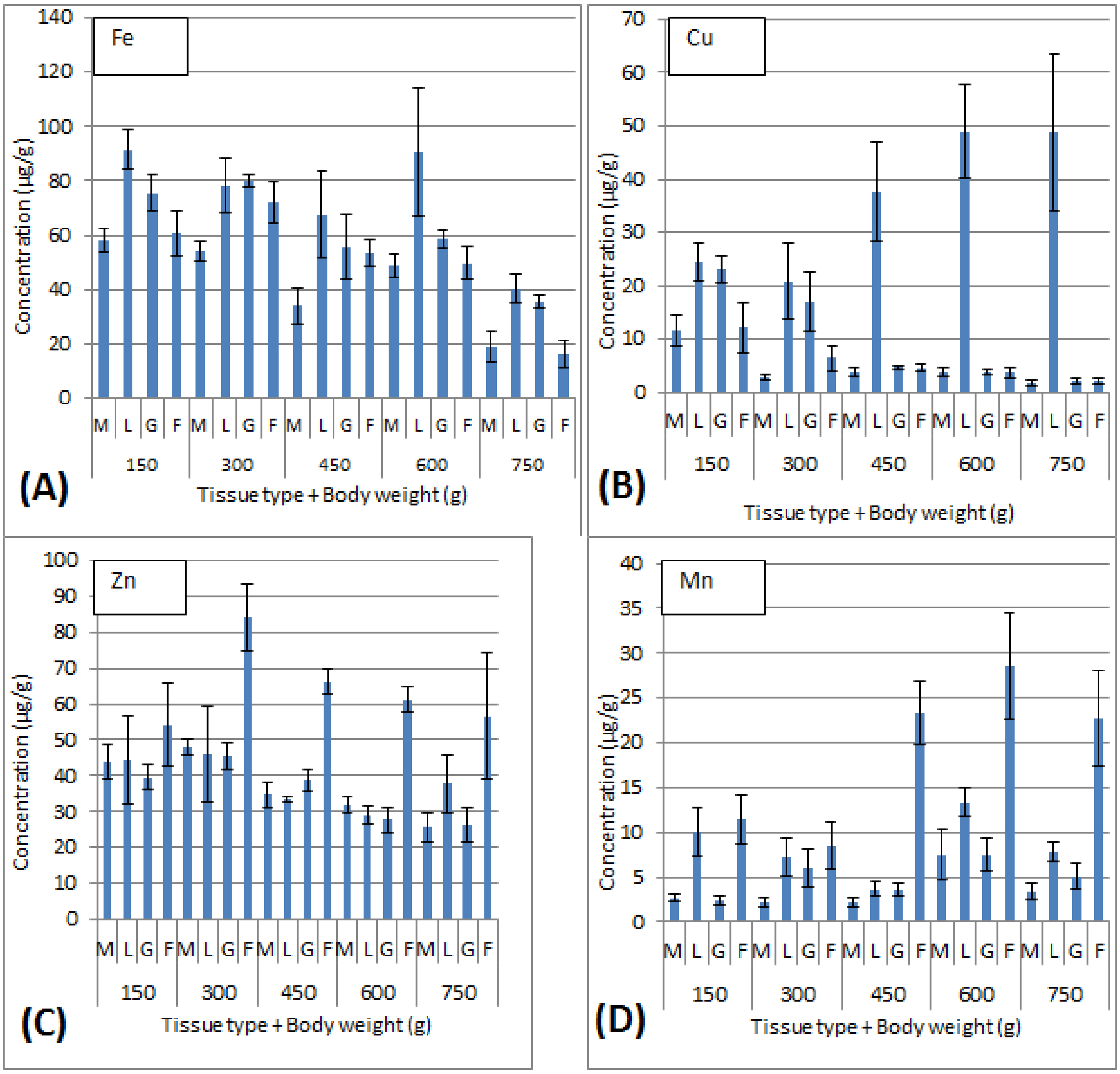
As the fish weight rises the accumulation of Cu in liver showed increasing trend in all weight groups (Table 1, Fig.1.B). Moreover, it was also observed that level of Cu in muscle, liver, and gill of 150g weight group fish was more than all weight groups. Overall, in muscle, gill and fin the Cu concentration was decreased as fish body weight increased (Table 1, Fig.1.B).

3.3. Zinc (Zn)

It was observed that among the all parts of fish tissues Zn accumulation was highest in fin. Level of Zn showed descending trend in fish tissue as fin followed by liver, muscle and gill in all weight groups. It was observed that there is less difference in Zn accumulation among the liver, gill and fin. In case of 300g of fish Zn content was highest than other weight group of fish. Overall, in all tissue types except liver the Zn showed decreasing trend in concentration as the body weight of fish increases (Table 1, Fig.1.C).

3.4. Manganese (Mn)

In all weight groups of Chillapi fish the accumulation of Mn was highest in fin and lowest in muscle. Furthermore, accumulation of Mn was observed in descending order in tissues as fin, liver, gill and muscle respectively. Accumulation of Mn was seen highest in all tissues of 600g weight group of fish than other weight groups (Table 1, Fig.1.D).



Description: Concentrations of Fe (A), Cu (B), Zn (C) and Mn (D) in M (muscle), L (liver), G (gill) of 150 ±10 g, 300 ±10 g, 450 ±10 g, 600 ± 10 g, 750 ±10 g body weight fish. Mean and standard errors are compared (Mean ±SE), n = 5, concentration in (µg/g wet weight).

Figure 1. The concentration of heavy metals in various body weight groups and tissue types of Chillapi fish

3.5. Human Health Risk Assessment

In this study, we have estimated daily intake (EDI), Target hazard quotient (THQ) and Hazard index (HI) values (Table 2). EDI values were lower than the respective reference doses. Among the metals, THQ was highest for Cu followed by Zn > Cu >Fe > Mn for both male and female individuals. The values of HI were estimated for both females (0.24) and males (0.21).

4. Discussion

In this study, we have investigated metal contents and its distribution pattern in different fish tissues (muscle, liver, gill and fin) with respect to fish body weight. Generally, microelements are beneficial to human being in adequate quantity, while its more abundance in fresh water bodies causes deleterious effects. In general, among the metals, Fe content is observed to be higher than other metals in fish [30]. The excess intake of Fe through fish food in humans causes symptoms like constipation, nausea, diarrhea, and vomiting. Gastric and esophageal ulceration are associated with chronic exposure of Fe in human beings. Here, in our work, the concentration of Fe in Chillapi was observed bellow the prescribed standard of FAO/WHO, 1989 (100 PPM) [31]. The decrease in element concentration with increasing body weight was observed in many marine organisms [32]. The study found that heavy metals contamination is higher in well-irrigated agriculture areas [33] and reported fertilizers and heavy metals cause pollution of water bodies and fish.

The Studies carried out on *Clarias gariepinus* [34], *Tinca tinca* and *Selda Tekin* [36] reported the highest accumulation of Fe in the liver and least in muscle. We also observed similar pattern as [34, 35] with respect to Fe accumulation in liver and muscle. The high concentration of Fe in the liver may be due to iron-containing enzymes and extensive vascular system, as the hemoglobin interacts with the Fe [36].

To acquire further insight into other metal contents, we determined Cu level and it was more accumulated in liver. It was noticed that except liver, accumulation of Cu in other fish parts under study bellow 30 PPM, the limit prescribed by FAO/WHO, 1983 [37]. Although Cu is essential, its higher dose can be harmful to human health. Long term exposure to Cu higher than normal level causes nausea, vomiting, stomach cramps or diarrhea in human beings [38]. Researchers also reported the highest Cu accumulation in the liver of fishes *Labeo umbratus* [39] *Oncorhynchus mykiss, Cyprinus carpio* [40]. Even in system high content of Cu level, fish muscles have poor accumulation properties [41].

It was reported that Zn accumulation is highest in the liver of *Clarias gariepinus* [39], *Channa punctatus* [42] and least in the integument of *Channa punctatus* [43]. Muscles of Chillapi show the least accumulation of Zn than other tissues under study. It is due to the de-loading ability of fish muscles and the transfer of Zn from muscles to other organs [42, 44]. The accumulation of Zn in tissues of every weight groups showed below the standard value 100 PPM, permissible limit set by FAO/WHO,1989 [31]. Physiological mechanisms in the body regulate Zn metabolism in many organisms [45]. Although Zn is an essential element, high concentration than the permissible limit is hazardous to human beings and other animal health ATSDR, 2005 [44] and causes anemia, damage to pancreas and decrease in the level of high- density lipoprotein (HDL) cholesterol. However, studies on fishes such as *Oreochromis mossambicus* [46], *Tinca tinca* [35], *Clarias gariepinus* [34], reported the highest accumulation in gills than muscles.

The content of Mn observed in all fish tissues under the study was several folds higher than the maximum permissible limits (1 PPM) of Mn set by FAO/WHO, 1989 [31]. Laboratory test with animals has shown that a high level of Mn causes tumor development, abortions, brain damage in animals.

In order to study the risk assessment of heavy metals for human beings by consumption of fish is estimated using several parameters such as estimated daily intake (EDI), target hazard quotient (THQ and hazard index (HI). These parameters are influenced by intake amount of contaminant, exposure frequency and duration, average body weight, and oral reference dose (RfD). THQ is referred as a dimensionless quantity and it is a ratio between concentration of heavy metals in food and RfD. The maximum THQ limit value should not exceed 1, otherwise it can cause potential non carcinogenic risks to exposed population. It is also important to note that THQ is not a measure of risk, however it imitates the level of concern [47, 48].

In the present study, all four heavy metals showed THQ values less than 1 in all fish samples. Moreover, the THQ values for all concerned heavy metals were comparatively higher in females than males. If the ratio of EDI of heavy metal to its RfD is equal to or less than the RfD then there will be a minimal risk. The ratio obtained for Fe, Cu, Zn and Mn, were less than 1, indicating that there is no possible health hazard. THQ deals with single heavy metal, but fish accumulates multiple heavy metals, so it becomes essential to calculate hazard index (HI). HI is the numerical sum of all the THQs. As similar to THQ, HI also should not exceed the limit of 1 [49, 50], if it exceeds the limit then it is an alarming condition for human health. Adult females were found to be more susceptible to heavy metal risks than males [51]. We have observed that HI values were within the limit for all metals. However, if fish consumption is in excess quantity can cause bioaccumulation and several health hazards to human beings.

5. Conclusions

The Fe and Cu exhibited more accumulation in liver, whereas Zn and Mn showed highest accumulation in fin tissues. Overall, Fe, Cu, Mn and Zn were accumulated less in the muscle tissue. Among the all metals under study Fe accumulation was highest while Cu was lowest in all weight groups. In our study, the EDI, THQ and HI values for individual element were lower than 1, indicating that there was no health risk for consumers due to intake of individual heavy metal through fish consumption. However more daily intake of fish can lead to accumulation of heavy metals and cause deleterious effects.

The effluent released from the urban, industrial and agriculture area contains heavy metals. The discharge of effluent into the river reaches finally to the reservoir and declines the water qualities. Fishes are affected by the accumulation of these metals and concentrate indifferent tissues through the food chain. The high concentration of heavy metals in fish tissues than permissible limits may cause an adverse effect on human health. So, wastewater should be treated before discharged into the water bodies for the safety of aquatic habitat and human health. To avoid epidemics of fishes from such contaminated water, fishes should be screened by food agencies before they reached to the humane. In this study results, indicate that the Ujjani reservoir has a higher pollution load and it was due to the discharge of industrial, agriculture and domestic wastewater which reaches to the reservoir body from its catchment area.

Further studies are suggested particularly to correlate disease patterning in population hosted near the bank on the Ujjani reservoir. Continuous monitoring should be normal practice to know the quality of water and fish resources. So we can avoid further negative consequences on the aquatic ecosystem and human health.

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