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Heavy Metals Uptake by *Oreochromis Mossambicus* of Budhasagar Pond

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Abstract

Food consumption is a major source through which humans as well as other animals are exposed to toxic heavy metals. Many reported studies have confirmed that contamination of heavy metals via the food chain can cause human health risk because of their toxicity, long persistence, bioaccumulation, and biomagnification. Heavy metals disrupt cellular events including growth, proliferation, damage-repairing processes, and apoptosis.Studies have widely used fishes as a bioindicator of metal pollution in the aquatic ecosystem for advantages discussed in the previous sections. Studying heavy metals presence in fishes has another advantage. Since we eat fish, it also tells us about possible health risks it poses for us to consume contaminated fish.We measured the presence of heavy metals, namely, mercury, lead, cadmium, and chromium in gills, livers, and muscles of Oreochromis mossambicus of Budhasagar pond using Target Hazard Quotient (THQ) and Hazard Index (HI). An HI of >1 indicates health risks, that <1 indicates a safe level of exposure. HI was recorded at safe levels in gills, livers, and muscles for both adults and children. Similarly, THQ too was found within the safe limits of 1 for each of the four heavy metals across the study period.

Keywords: *Oreochromis mossambicus, heavy metals contamination, Budhasagar pond*

Introduction

Even though two-third of the Earth's surface is covered with water, only 1% of this is fresh water. This freshwater is mainly found in ponds, rivers, lakes, and groundwater. These sources have served and supported evolving needs of the human civilization for millennials. Unfortunately, the latest evolution in the human civilization that took place in the form of industrial revolution in the 18thcentury has come to threaten the freshwater security for the whole human civilization as well as various animal and plant kingdoms. Researchers have attempted to bring attention to this growing insecurity and have predicted water insecurity to be the biggest crisis of the 21^{st} century.

Water pollution is one of three major pollutions threatening the environment today. It is a broad term defined by the presence of unwanted contaminants in water and includes seawater as well as freshwater contamination. Seawater pollution threatens ocean and sea habitats such as seawater plants and fishes. Disposal of plastic at beaches and oil spills are two major sources of seawater pollution. Freshwater contamination is due to three primary sources: domestic, agriculture, and industrial. Disposal of domestic waste and sewage discharge into rivers and ponds pollute these freshwater sources. Agricultural runoffs containing chemical fertilizers and discharge of untreated industrial waste are major and most lethal sources of freshwater contamination.

The presence of heavy metals such as iron, lead, and cadmium in agriculture runoffs and industrial water make contaminated freshwater unsafe for human use. Heavy metals are elements with an atomic density of more than 4 g/cm^3 (Nriagu, 1988). Some of the major sources of heavy metals pollution of freshwater bodies include sewage discharge, agricultural runoffs, battery industry, metal electroplating, chrome plating, tanning and leather industry, and dyes industries (Farmakiand Thomaidis, 2008).

While the human body needs certain heavy metals such as iron, zinc, chromium, copper and manganese up to a limited extent for proper functioning of organs, other heavy metals such as mercury and lead are non-essential and even the slightest presence of these metals in the human body can prove to be lethal (Unger, 2002). International and national authorities such as the European Union and the United States Environmental Protection Agency have defined permissible limits for the presence of different heavy metals in food products (see Table 1). Above these permissible limits, the food is considered unsafe for human consumption (Nolan, 2003; Young, 2005).

Table 1 Permissible limits of different heavy metals in drinking water

Heavy metals	USEPA (mg/L)	Indian Standards (mg/L)
Cadmium (Cd)	0.005	0.01
Chromium (Cr)	0.05	0.05
Iron (Fe)	0.3	0.3
Lead (Pb)	0.05	0.1
Manganese (Mn)	0.05	0.1
Mercury (Hg)	0.002	0.001
Nickel (Ni)	0.1	0.05

Source: Gautam et al. (2016)

Certain properties of heavy metals make them more toxic to consume than other contaminants. First of all, consumption of heavy metals such as mercury and lead or even the essential ones like iron and zinc, if taken in a dose more than a certain limit, can lead to deadly diseases such as cancer (Unger, 2002). Secondly, heavy metals tend to accumulate as they pass from one stage to another in the food chain. This phenomenon is known as bioaccumulation and results in an increased concentration of heavy metals in tertiary-level fishes which makes them unsafe for human consumption.

Mercury (Hg)

Some heavy metals are also more toxic than others. Consumption of mercury even in the slightest amount can result in nervous system disorder such as Minimata. The Minimata disease was unknown until 1952 when consumption of local fish and shellfish contaminated with mercury compounds lead to the rapid spread of the disease at Japanese coasts and erupted into an epidemic (Takeuchi, 1968; Vandecasteele and Block, 1991). Mercury is also the only common metal that exists in the liquid form at room temperature. It is found mainly in compound forms in nature. Consumption of mercury can lead to disruptions in the nervous and excretory systems disrupt brain activities, damage DNA aberrations and male sperm, lead to miscarriages and birth defects in pregnant women, and develop allergic reactions like rashes and headache.

Lead (Pb)

Burning of petroleum products is the primary source of lead pollution. Lead emissions from petrol engine contain lead bromides, oxides, and chlorides. Smaller particles pollute the air while bigger particles fall and pollute soil and water bodies. Corrosion of pipelines and pesticide runoffs from agricultural fields may as well contaminate freshwater bodies with lead which may then bioaccumulate through various stages of food chain, eventually entering the human body either through air or via consumption of contaminated fish or water (Jarup, 2003). Use of contaminated water in agricultural field can also result in contaminated vegetables, fruits, and agricultural produce.

Lead is one of the four most toxic heavy metals for human consumption. It can disrupt the biosynthesis of haemoglobin and anaemia, increase blood pressure, result in kidney failure, miscarriage, damage brain activities, affect the fertility of men, and result in behavioural changes in children (Duruibe et al., 2007). Lead is also capable of transplacental penetration and could damage the nervous system of an unborn body as well (Jarup, 2003).

Cadmium (Cd)

Industrial as well as natural phenomenon can result in cadmium pollution of water. The phenomenon whereby rocks weather and break into sand release cadmium into the environment which may then runoff into water bodies (Hagino, 1961). Industrial waste such as nickel-cadmium batteries too contain cadmium untreated discharge of which into water bodies contaminate these sources of freshwater (Nordberg et al., 2007). Cadmium tends to enter the human body via consumption of cigarettes and fish and sea animals (Bernard, 1986). Diarrhoea, bone disease, reproductive dysfunction, and psychological disorders are some of the adverse health effects of lead consumption (Sethi et al., 2006).

Chromium (Cr)

Chromium is radiant, brittle, dim color metal. The human body requires it to perform certain vital functions like catabolism of fat and carbohydrate and to control the optimum level of glucose and blood pressure (Anderson, 1989). It is found in two forms in the environment - trivalent and hexavalent. Hexavalent chromium is not found naturally, it is produced by different industrial activities. Hexavalent chromium is loosely dissolved in water and leached into underground water. Sometimes underground water may contain a higher level of chromium (Sullivan, 1969; Towill, 1978).Some industries are directly responsible for releasing chromium into the environment. Industrial establishments like steel, leather tanneries, textile dyeing, printing, photography, and chrome electroplating are mainly responsible for this. Chromium accumulates in aquatic flora and fauna and tend to bioconcentrate at the higher levels of the chain (Hantson, 2005; Adeniyi and Yusuf, 2007; Gupta et al., 2009; Raphael et al., 2011).

Consumption of chromium above the permissible limit of 0.05 mg/L could result in allergic reactions such as rashes, nose irritations, nose bleeding and stomach disorders like ulcers, respiratory complications, nephrological and liver damage, genetic aberrations, and lung cancer (Braver, 1985; Cohen, 1993; Geller, 2001).

Iron (Fe)

Iron contamination of freshwater are generally due to mining activities. Iron pyrites (FeS_2) found in the coalfield releases iron by weathering and bacterial action. Mining and oxidation of iron pyrite results in the production of sulphuric acid and the formation of Ferrous iron (Fe^{2+}) (Smith et al., 1973).Different types of iron ores like hematite and magnetite when comes in contact with acidic water releases ferrous and ferric ion.Ferrous ion (Fe^{2+}) is considered more toxic to fish than the ferric ion (Fe^{3+}) (Decker et al., 1978).

Iron plays a vital role as a part of enzymes such as catalase and cytochrome, and most importantly, as a part of hemoglobin and myoglobin. It is commonly found in all freshwater environments (Livingstone, 1963; Forstner et al., 1979). High iron content in water causes corrosion and rust formation of pipelines and can be toxic at high concentrations (Theis et al., 1974). Iron's ability to transfer electrons means that it can form free radicals; it can convert hydrogen peroxide into free radicals. Free radicals can damage the structure of the cell and ultimately kill the cell (Crichton et al., 2002).

Research Geography and Objectives

The research has been carried out with fish samples from the Budhasagar pond situated in the Indian state of Chhattisgarh. Budhasagar is a man-made perennial pond and is filled with water year-round. It is also connected with the municipal sewage of the Baldeobagh town. It also used to carry the industrial waste of a cotton mill in the town. The mill shut a couple of years ago, however. The pond serves the bathing and washing needs of town residents and fishing culture has also been continued since it was first started in 1961. Given the fact that the pond is sewage-fed and once served the disposal of a cotton mill, it is imperative to think that the pond and its fish habitats may be contaminated with heavy metals. We collected samples of *Oreochromis Mossambicus* from the pond across three seasons in 2016-17 and measured the concentration of heavy metals in their gills, livers, and muscles.

Literature Review

The toxicity of heavy metals in fish was studied by Khangarot and Ray (1987). Kureishy and D'Silva (1993) carried out experiments on *Pernaviridis, Villoritacypriniides*, and *Oreochromis mossambicus*and found cadmium, lead, and mercury accumulation in this fishes.Hepatic, renal, and gill histopathological abnormalities were seen in fish treated with copper, cadmium, and mercury by Manoj and Ragothaman (1999). Heavy metals have been studied in freshwater*Channa punctatus* by Shukla et al. (n.d.) and Gupta and Dua (2002). The effect of heavy metals on *Cirrhinusmrigala* was studied by Sharma and Jain (2004).

Heavy metal levels in soil, water and fish from the sewage-fed pond were examined by Pandey et al. (1995). The build-up of nickel, copper, and cadmium in fish tissues was also investigated by Balasubramanian et al. (1997) in a sewage-fed pond. The iron, lead, nickel, chromium, zinc, manganese, and cadmium levels discovered in tissues of Yamuna River fish were attributed to fertiliser and chemical industry effluent, according to Ajmal et al. (1985).Fertilizer, agricultural ashes, industrial effluents, and rubbish contaminated the Cauvery River, and the researchers found high levels of heavy metal in the fish. To find out how much heavy metal is in fresh water, scientists have done a variety of tests. Fish muscle from home sewage and industrial effluent was investigated by Nayaka et al. (2009).

Heavy metals in tissues of *Mystusvittatus* were studied by Rao and Patnaik (2000). Maiti and Banerjee (2002) and Vinodhini and Narayanan (2008) conducted a similar study in Kolkata’s various freshwater bodies in *Cyprinus carpio* fish. Heavy metal build-up in fish tissues were studied by Begum et al. (2009b) in Bangalore's Madivala Lake. As the muscle is the most consumed and most heavily contaminated section of fish, Gupta et al. (2009) examined the contamination of *Aorichthysaor* and *Channa punctatus* to check if they were infected. Other studies investigated the presence of heavy metals in freshwater fish tissues and tested them up to the maximum allowable level (Gupta et al., 2002; Shrivastava and Sohani, 2002; Chandrasekhar et al., 2003; Chakraborty et al., 2003; Raja et al., 2009).

Heavy metals in *Oreochromis mossambicus*

Fishes are great bioindicators of metal contamination of a water body as they appear at higher trophic levels allowing for metals to accumulate (Palanichamy and Baskaran, 1995). Since fishes are also eaten by humans, investigating the contamination in fishes also allows us to measure the health risks posed by contamination simultaneously.

James (1990) examined individual as well as combined effects of the heavy metals' contamination on *Oreochromis mossambicus'* respiratory and behavioural responses, their oxygen consumption, and opercular movements. Chatterjee et al. (2006) found that metal concentrations were lowest in muscle and highest in liver in *Oreochromis mossambicus* sampled from coastal waters of Kolkata. Dye et al. (2007) studied the histological changes in the livers of *Oreochromis mossambicus* following exposure to cadmium and zinc. Dye et al. found that longer exposure affected results.

Oreochromis species had a higher metal contamination index value in Malaysian aquaculture ponds where they were compared to Penaeus monodon species (Mokhtar, 2009). Hossein et al. (2015) looked at the build-up of heavy metals in fish tissues from Egypt's Nile River and concluded that species like *Oreochromis* are suitable candidates for bio-monitoring pollution since they can tolerate the harsh circumstances of the ecosystem. Noorjahan and Jamuna (2015) used *Azolla Microphylla* as a biodegradation agent in their study of sewage wastewater treatment and repurposed the treated water for aquaculture. To treat sewage water in a sustainable and environmentally friendly manner while also promoting aquaculture, researchers used *Oreochromis mossambicus* farmed fish.

Research Methodology

Samples of *Oreochromis mossambicus*were collected across winter, summer, and post-monsoon seasons in 2016-17 and their gills, livers, and muscles were studied for presence of heavy metals. The concentration of select heavy metals were recorded in different organs of sample and results analysed using SPSS v. 26. The selection of *Oreochromis mossambicus* is based on the following criteria (Widdows, 1985; Adelman and Smith, 1976).

i) Edible status – Whether the fish is widely consumed by people.
ii) Availability – Whether the fish is available in the pond throughout the year.
iii) Omnivores – Sample fishes should be omnivores to allow bioaccumulation.
iv) Environment tolerability – Sample fishes should have greater tolerability to a wide range of environmental conditions.

Oreochromis mossambicus fulfils all the above criteria. They are widely eaten fish specimen across the state of Chhattisgarh. They also have an advantage over other fish species as they are prolific breeder and eat blue-green algae, insects, and weeds, therefore, contaminants found in these insects and organisms could also be observed in *Oreochromis mossambicus* (Jhingran, 1984). *Oreochromis mossambicus* are also found in abundance and throughout the year.

Target Hazard Quotient (THQ)

After computing the heavy metals concentration in fish samples, we compared the findings on two indices, e.g., the Target Hazard Quotient (THQ) and the Hazard Index (HI). THQ was developed by the United States Environmental Protection Agency in 1989 and is used for the assessment of potential non-carcinogenic threat associated with exposure to contaminants such as heavy metals in food. The THQ is a ratio of the determined dose of a pollutant to a reference dose level. It has a binary interpretation. A THQ value of < 1 indicates that the contamination is within safe permissible limits, whereas a THQ value of > 1 is indicative of potential risk (USEPA, 2010). One should take care in interpreting THQ as the values are additive but not multiplicative. That is, a THQ value of 20 does not indicate that the risk is tenfold of those at THQ value of 2. THQ doesn't measure risk but is indicative of the level of concern.

We computed the THQ values based on Chien et al.'s (2002) method as follows.

$$THQ = \frac{\mathrm{EF.ED.FIR.C}}{\mathrm{RFD.WAB.TA}} \times 10^{-3}$$

Where,

E_F is the exposure frequency measured per 365 days/year,

E_D refers to the exposure duration,

F_{IR} is the food ingestion rate (g/person/day),

C is the total concentration in food (mg/kg),

R_{FD} is the oral reference dose (Table 2),

W_{AB}refers to the average body weight (55kg for adults and 20kg for children), and

T_A is the averaging exposure time for non-carcinogens.

Table 2 USEPA Oral Reference Dose

Heavy metals	**R_{FD} value (mg/kg) (USEPA)**
Cadmium	0.001
Lead	0.004
Mercury	5×10^{-4}
Chromium	0.003
Iron	0.7

Hazard index (HI)

Hazard Index (HI) is based on EPA's guidelines for health risk assessment of chemical mixtures (USEPA, 1986). It is used to measure the overall risk hazard for non-carcinogenic effects posed by more than one heavy metal. HI is given by the sum of THQ as described in the below equation. An HI value of > 1 indicates potential health risks (USEPA, 1989).

$$HI = \sum_{i} \mathrm{THQi}$$

Heavy Metals Analysis

Inductively coupled plasma-optical emission spectrometry (ICP-OES) was used to examine fish samples for heavy metal contamination. The study focused on heavy elements like lead, cadmium, chromium, iron, and mercury. Using the methods provided in the American Public Health Association (2005) and the United States Department of Agriculture, samples were crushed and examined inductively with ICP-OES (Perkin Elmer, 2008). Glassware used in the experiment was rinsed with 10% (v/v) nitric acid and deionized water before the samples were digested. Sterilized surgical blades and scissors were used to defrost fish samples and remove tissues. Acid-washed petri dishes were used to oven-dry fish tissues at 80°C to a consistent weight. Desiccators were used to chill the fish samples. The fish tissues were ground into a fine powder and weighed after being homogenised with a mortar and pestle. Microwave digestion was used to breakdown fish tissues processed in Nitric acid (Table 3). 2 mL of 30% hydrogen peroxide were given to digests after digestion to reduce nitric acid vapours and speed up organic component digestion by raising the temperature (Dig-Acids, 2001).

Blanks are used to ensure that the analysis is authentic. Triplicate analyses of fish samples were performed to ensure high analytical quality. Digested fish samples were diluted in acid-washed standard flasks with 50ml of ion free water and filtered through a 0.45-m filter paper. ICP-OES was used to evaluate digested materials after they had been filtered and purified. Table 4lists the parameters for the Perkin Elmer Optima 4100DV ICP-OES system. Standardization was carried out by diluting 1000 mg/L stock solutions of multi-element standard solutions (Merck) (Mohammed, 2007). Heavy metal concentrations in sample fishes were measured in mg/L of dry weight. All tests were carried out in threes to ensure reproducibility and a detection limit of 0.01 mg/kg was used. The results indicated below detection limit (BDL).

Table 3 Microwave digestion program used for fish (Source: USDA, 2008)

S. no.	Temp. (^{o}C)	Time (min.)	Power (Watt.)
1	25-96	20	1000
2	96	30	1000
3	180	10	1000
4	180	10	1000

Table 4 Summary of the operational parameter setting used for the ICR-OES

Characteristics	Instrument condition
RF Generator	Fully solid-state generator. Operating frequency ~40 MHz
RF Power	Adjustable power between 750 to 1300 Watts
Spray chamber	Scott type

Nebulizer	Cross flow
Plasma gas flow	15 L/min
Auxiliary gas flow	L/min
Nebulizer gas flow	0.60 L/min

Results and Discussion

Table 5, 6 and 7 list the season-wise heavy metals concentrations in gills, livers and muscles of *Oreochromis Mossambicus*, respectively. Figures 1, 3 and 5 indicate the min., max., and other descriptive values of recorded across three seasons for the concentration of mercury, cadmium, chromium, and lead in gills, livers, and muscles, respectively. Figures 2, 4 and 6 respectively indicate the descriptive stats for iron concentration in gills, livers and muscles.

In general, higher concentrations of heavy metals were found in gills except for iron which shows a much greater concentration in livers than gills. On the other hand, muscles had the least concentration for heavy metals across the three seasons for all five metals. Mercury was the only heavy metal (out of the five selected heavy metals) which was not detected at all in summer and post-monsoon samples. A slight amount of mercury from 0.05 to 0.151 mg/Kg was detected in all three organs during the winter season. Cadmium was not detected in gills during the post-monsoon and in muscles in summer as well as post-monsoon seasons.

Table 5 Heavy metals concentration in gills

	Summer	**Post Mon**	**Winter**
Mercury	BDL	BDL	0.148
Lead	3.94	2.98	2.88
Cadmium	0.614	BDL	0.04
Chromium	1.08	2.4	3.77
Iron	336	347	286

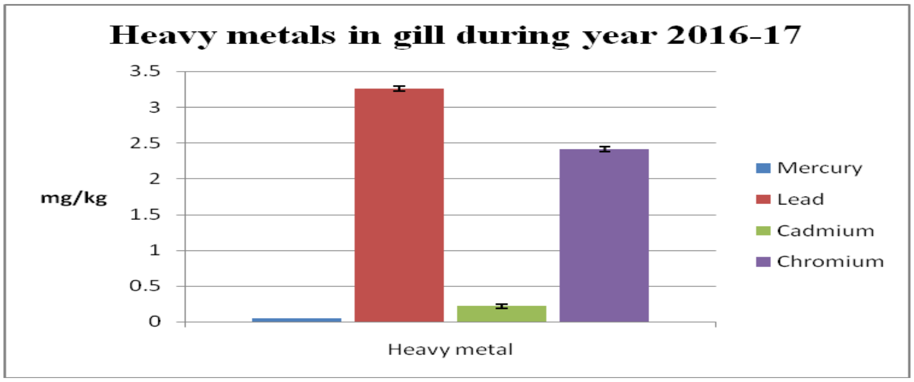

Figure 1

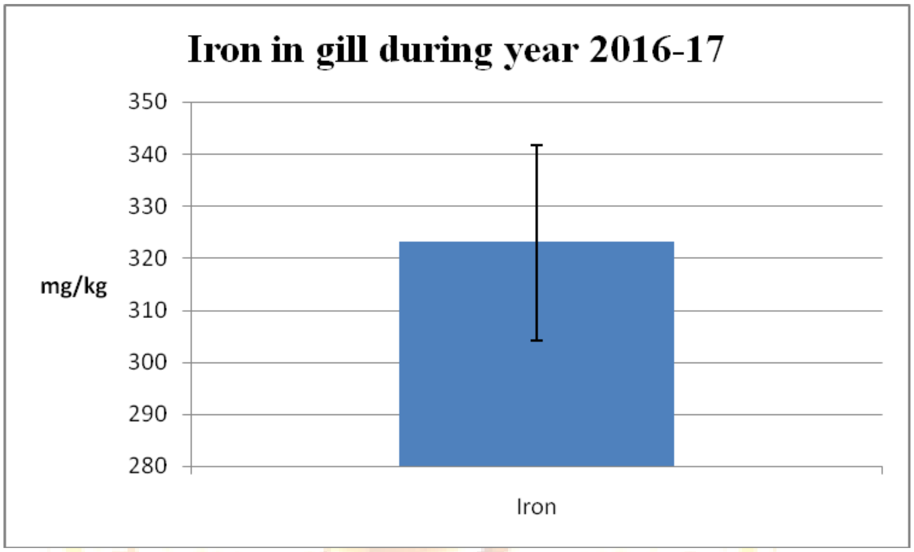

Figure 2

Table 6 Heavy metals concentration in livers

	Summer	**Post Mon**	**Winter**	**Mean**
Mercury	0	0	0.147	0.049
Lead	4.36	3.38	2.85	3.53
Cadmium	0.044	0.85	0.04	0.31
Chromium	5.91	1.72	3.74	3.79
Iron	6649	9351	1784	5928

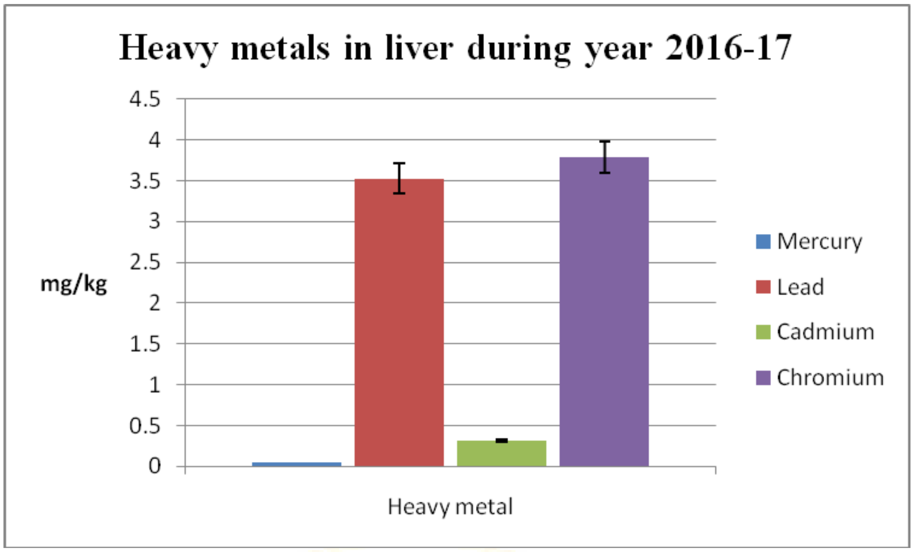

Figure 3

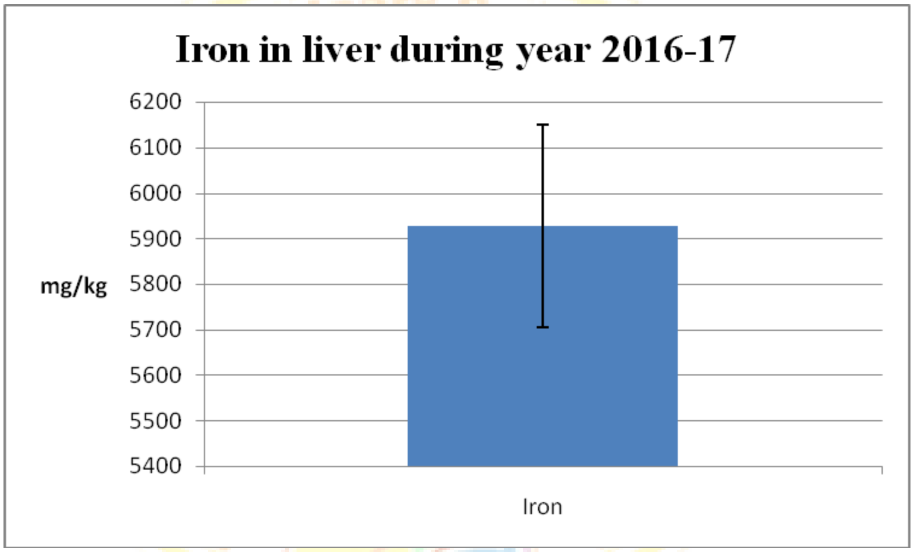

Figure 4

Table 7 Heavy metals concentration in muscles

	Summer	Post Mon	Winter	Mean
Mercury	BDL	BDL	0.418	0.139
Lead	2.83	0.96	0.343	1.377
Cadmium	BDL	BDL	0.015	0.005
Chromium	0.824	2.23	0.762	1.272
Iron	65.5	126	105	98.83

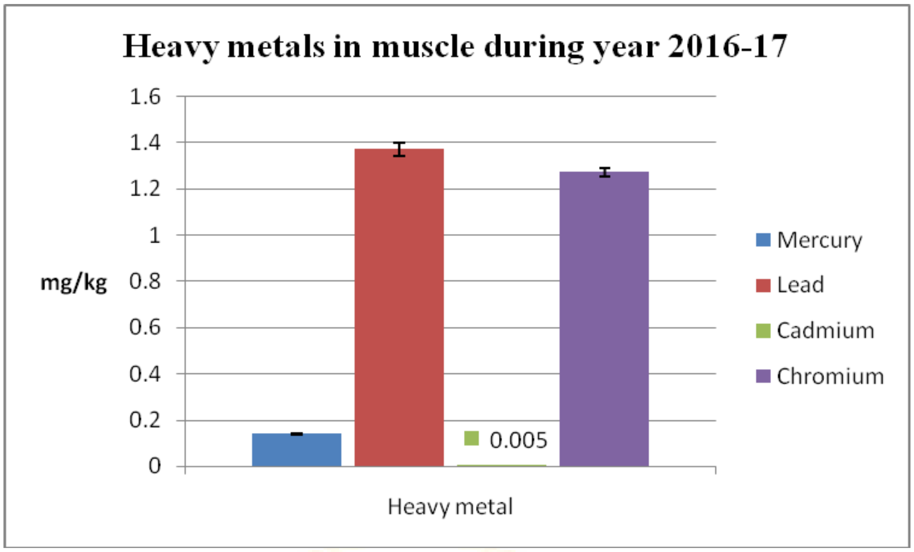

Figure 5

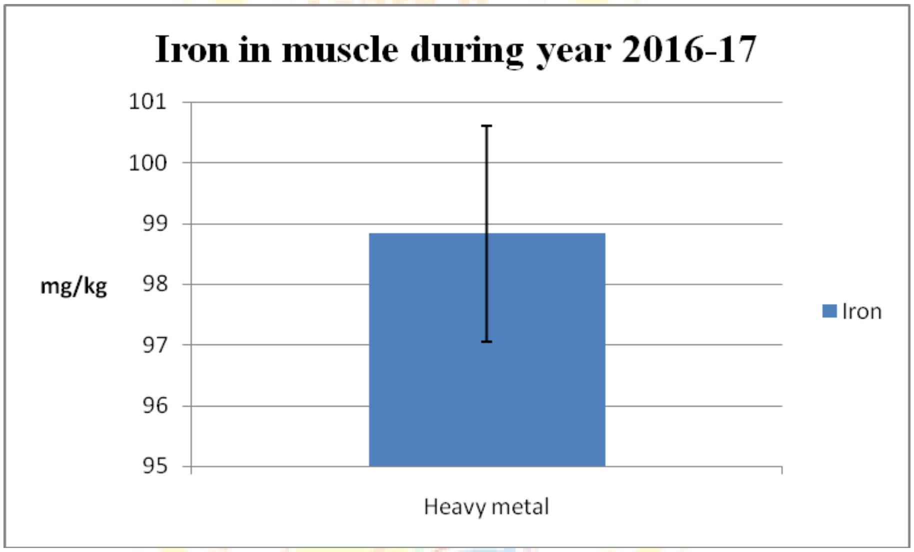

Figure 6

Potential Health Risks

We examined THQ and HI values to determine the health risks posed from the concentration of mercury, lead, cadmium, chromium and iron in *Oreochromis mossambicus* samples collected from Budhasagar pond. The previous section covered these indices. The previous section discussed safe levels of exposure for adults and children, measured in mg/Kg per day for each of the four heavy metals examined. There was 0.001 Cadmium in the air, 0.004 Lead in the soil, 0.0005 Mercury in the water, and 0.003 Chromium in the soil. Hazard index measures the health risk posed by multiple heavy metals exposure. The total hazard presented by all heavy metals, as determined by THQ, is what this represents. A HI value of > 1 should raise alarm as it indicates potential health risks to consumer of contaminated food.

Tables 8 and 9 respectively indicate the THQ and HI values for children. Tables 10 and 11 respectively indicate the THQ and HI values for adult consumers. One could observe from these tables that the THQ and HI values are well within permissible limits. That is, to say, it is safe to eat *Oreochromis mossambicus* of the Budhasagar pond.

Table 8 THQ (Children)

Metal	Gill			Liver			Muscle		
	Summer	Post Mon.	Winter	Summer	Post Mon.	Winter	Summer	Post Mon.	Winter
Mercury	0	0	0.070	0	0	0.069	0	0	0.197
Lead	0.232	0.176	0.170	0.257	0.199	0.168	0.167	0.056	0.020
Cadmium	0.145	0	0.009	0.010	0.201	0.009	0	0	0.003
Chromium	0.085	0.189	0.297	0.465	0.135	0.294	0.064	0.175	0.060
Iron	0.113	0.117	0.096	2.246	3.159	0.602	0.022	0.042	0.035

Table 9

Metal	Gill			Liver			Muscle		
	Sum.	Post Mon.	Wint.	Sum.	Post Mon.	Wint.	Sum.	Post Mon.	Wint.
HI	0.462	0.365	0.546	0.732	0.535	0.540	0.231	0.231	0.280

Table 10

Metal	Gill			Liver			Muscle		
	Summer	Post Mon.	Winter	Summer	Post Mon.	Winter	Summer	Post Mon.	Winter
Mercury	0	0	0.025	0	0	0.025	0	0	0.0718
Lead	0.084	0.064	0.061	0.093	0.072	0.061	0.060	0.020	0.007
Cadmium	0.052	0	0.003	0.003	0.073	0.003	0	0	0.001
Chromium	0.030	0.068	0.108	0.169	0.049	0.107	0.023	0.063	0.021
Iron	0.041	0.042	0.035	0.816	1.148	0.219	0.008	0.015	0.012

Table 11

Metal	Gill			Liver			Muscle		
	Sum.	Post Mon.	Wint.	Sum.	Post Mon.	Wint.	Sum.	Post Mon.	Wint.
HI	0.166	0.132	0.197	0.265	0.194	0.196	0.083	0.083	0.100

Conclusion

Food consumption is a major source through which humans as well as other animals are exposed to toxic heavy metals. Many reported studies have confirmed that contamination of heavy metals via the food chain can cause human health risk because of their toxicity, long persistence, bioaccumulation, and biomagnification. Heavy metals disrupt cellular events including growth, proliferation, damage-repairing processes, and apoptosis. Comparison of the mechanisms of action reveals similar pathways for these metals to toxicity including ROS generation, immunity weakening, enzyme inactivation, and oxidative stress.

On the other hand, some heavy metals have selective binding to certain macromolecules. The interaction of lead with aminolaevulinic acid dehydratase and ferro chelatase is within this context. Some toxic metals like chromium and cadmium cause genomic instability. Defects in DNA repair following the induction of oxidative stress and DNA damage by cadmium and chromium have been considered as the cause of their carcinogenicity. Mercury and lead, on the other hand, disrupts the functioning of human body in other ways. Mercury could cause thiol binding, inhibit glutathione peroxidase and enzymes, reduce aquaporins mRNA, and affect ROS production. Lead causes increased serum and inflammatory cytokines and a reduction in GSH, SOD, CAT, and GPx levels. The incidence of heavy metals poisoning remains considerable and requires preventive and effective treatment.

Studies have widely used fishes as a bioindicator of metal pollution in the aquatic ecosystem for advantages discussed in the previous sections. Studying heavy metals presence in fishes has another advantage. Since we eat fish, it also tells us about possible health risks it poses for us to consume contaminated fish. The present research was carried out at Budhasagar pond to investigate the presence of heavy metals in *Oreochromis mossambicus* and the health risks for humans upon the consumption of *Oreochromis mossambicus*. We investigated the health risks due to the consumption of contaminated *Oreochromis mossambicus*of the pond.

We measured the presence of heavy metals, namely, mercury, lead, cadmium, and chromium in gills, livers, and muscles of *Oreochromis mossambicus* of Budhasagar pond using THQ and HI. These indices were discussed in the previous section. In brief, a THQ of <1 is considered safe whereas a THQ of >1 poses health risks. Healthy limits of exposure, in mg/kg/day, for adults and children for all five studied heavy metals were mentioned in the previous section. These were 0.001 for Cadmium, 0.004 for Lead, 0.0005 for Mercury, and 0.003 for Chromium. Hazard index assesses the health risk posed from the combined exposure to multiple heavy metals. It is basically the sum of risk posed by all heavy metals measured in THQ. An HI of greater 1 is a cause of concern for it poses health risks for humans.

Between gills, livers, and muscles, it poses the most danger to human health of have a greater level of heavy metals presence in the muscles as this is the edible part in the fish. The presence of heavy metals in sample *Oreochromis mossambicus* of Budhasagar pond was found to be in controlled levels. That is, the consumption of *Oreochromis mossambicus* of Budhasagar pond does not pose health risks for adults or children and are safe to eat.

HIs were measured to assess health risks associated with exposure to the four heavy metals for adults and children. An HI of greater than 1 indicates potential health risks, that below 1 indicates a safe level of exposure. Across all three seasons, HI remained below the safe level of 1 in gills, livers, and muscles for both adults and children. Similarly, THQ too was found within the safe limits of 1 for each of the four heavy metals across the study period.

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