Research Article

Determination of heavy metal concentrations in six species of fresh water fish sold in Quetta city, Pakistan

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Abstract

Current study was carried out to determine heavy metals in six species of fish i.e. *Oreochromis mosambicus*, *Cyprinus carpio*, *Tor putitora*, *Cirrhinus mrigala*, *Labeo rohita* and *Ctenopharyngodon idella,* belonging to class Cyprinidae, collected from the fish markets of Quetta city, Balochistan. Concentrations of Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Cadmium (Cd) Lead (Pb), Chromium (Cr) and Nickle (Ni) were determined in Liver, Heart, Gills and Muscles of selected species by using atomic absorption spectrophotometer. Concentration of Fe was higher than the World Health Organization (WHO) limits in all species except *Ctenopharyngodon idella.* Concentration of Zn was within the limits except *Cyprinus carpio.* Concentration of Mn, Cu, Cd and Pb were below the limits set by WHO. Cr and Ni were not detected in any species. The concentrations of heavy metals in edible part (muscles) of all species were below the WHO limits. So, current study reveals that the fish of selected species are safe for human consumption.

Keywords: Atomic absorption spectrophotometer*;* Cyprinidae; Heavy metals; Quetta; WHO

Introduction

Fish are an important aquatic source of food, which are found in aquatic ecosystems. Fish are also used as a good indicator of heavy metals and various other elemental contaminations [1]. Aquaculture is one of the newest fields of fisheries for food production [2]. As a nutrition, fish is very

important for human beings because it contains not only proteins but also omega-3 polyunsaturated fatty acids of two types, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA). For regular development, Omega-3 (n-3) fatty acids are very essential, where they prevent preterm delivery, stroke and heart disease by reducing cholesterol levels [3]. Fish also perform an essential role in human health because they contain minerals and vitamins [4].

Fish are considered to be nutritive, due to the presence of lipid soluble vitamins, important proteins and polyunsaturated fatty acids. From marine and freshwater environments, fish usually accumulate pollutants, which may be harmful for human health, and therefore, this information is essential for safety. Because of their accumulative behavior and toxicity, metals released into the oceanic water can destruct both marine ecosystems and diversity of marine life [5]. The primary route for heavy metal consumption or dissolved pollutants in fish is via their gills or through ionic interchange across the membranes or transport across biological membranes. The second route is through consumption of sediment particles or food which is then transported through the mouth [6]. Heavy metals like manganese, zinc, nickel, iron, copper and chromium are essential and vital elements, subsequently they play a significant function in organic or biotic body systems. Heavy metals are essential for the regular metabolic activity in fish and are found in the aquatic food, in different sediments and water [7]. Lead, Arsenic and Cadmium are termed as non-essential elements, and are poisonous even in minute quantities [8]. Fishes play a vital role in aquatic food web and chain which are very sensitive to pollution. Fishes mostly inhabit freshwaters such as lakes, streams, and rivers. If these water systems are polluted

with trace and heavy metals, fishes migrate to the areas where there is minimum contamination in order to survive [1].

Quetta is the Capital of Balochistan [9]. District Quetta is divided into rural and urban population. According to the fishermen, fish available in Quetta mostly come from Karachi, Sukkur, Naseerabad and Jafarabad. Some of them are farmed fishes, but most (about 70%) are from Indus River. Fishes are available in Quetta market throughout the year but people mostly use fish from September to April because in the winter season the fish production increases and Quetta experiences severe winters, consumption of fish increases in these days.

Materials and methods

Fish availability

The present study was conducted on freshwater fish which were collected from local fish market of Quetta city. It is worth noting that a wide variety of edible fish are available in Quetta city which are transported from different areas of Pakistan, as mentioned earlier, and are available throughout the year (except in June and July when fishing is prohibited).

Fish sample collection

Total 18 (three from each species) commercial fish were purchased from local fish market of Quetta. Six species i.e. *Labeo rohita* (Rohu), *Cirrhinus marigala* (Mori), *Cyprinus carpio* (Gulfam), *Tor putitora* (Mahaseer), *Ctenopharyngodon idella* (Grass carp) and *Oreochromis mosambicus* (Tilapia, Daya) were selected. The fish samples were kept in cool ice boxes and moved to the Zoology Department, University of Balochistan.

Identification of fish species

Fish were identified to confirm the selected species through illustrations and using keys provided by Talwar and Jhingaran [10], and Jayaram [11].

Preparation of sampling for heavy metal detection

In the laboratory fish were dissected by using plastic scissors and knives to separate the organs (liver, heart, gill, and muscles). These samples were dried separately in the oven at 105 ^{o}C for 3 hours. The dried tissues of fish were collected and reduced into powder form by using pestle and mortar. 1gm powdered samples were taken in a 100 ml beaker and 10 ml of Nitric acid (67%) was added. Afterwards, the samples were kept in a fume hood for digestion at room temperature for 24 hours.

Then 4 ml perchloric acid ($HClO_4$) was added to the samples and kept at 60 ^{o}C on a hot plate until samples were reduced to 1 ml residue. The residue was cooled and diluted with deionized water up to 50 ml and then filtered through whatman (41) filter paper [12]. To make the volume up to hundred ml (stock solution), appropriate de-ionized water was added and kept in volumetric flask until analyzed by Atomic absorption spectrophotometer (Perkin- Analyst 800 JAPAN) [13-15].

Statistical analysis

The results obtained were subjected to statistical evaluation. The evaluated parameters were mean, standard deviation (SD) and standard error mean (SEM) by using SPSS (16) software.

Results

The results indicate the differences in sensitivity to metals among the different organs of same fish.

***Oreochromis mosambicus* (Tilapia)**

The concentration of Fe was higher in the liver as compared to the limits set by WHO. Mn, Cd, Zn and Cu were detected in all organs except Cu which was not detected in muscles and their values were below the limits of WHO. Pb was found only in the gills and its value was below the WHO limits (Table 1).

Table 1. Concentration of trace and heavy metals in the *Oreochromis mossambicus*

Metals	Fe	Mn	Zn	Cu	Cd	Pb	Cr	Ni
Liver (ppm $+$SEM)	2.156$+$ 0.001155	0.067$+$ 0.000577	0.635$+$ 0.000577	0.025$+$ 0.000577	0.022$+$ 0.006351	ND	ND	ND
Heart (ppm +SEM)	0.27$+$ 0.005774	0.065$+$ 0.000577	0.493$+$ 0.000577	0.026$+$ 0.000577	0.013$+$ 0.000577	ND	ND	ND
Gills (ppm +SEM)	0.638$+$ 0.000577	0.126$+$ 0.000577	1.263$+$ 0.000577	0.009$+$ 0.001155	0.052$+$ 0.000577	0.047$+$ 0.000577	ND	ND
Muscle (ppm +SEM)	0.065$+$ 0.000577	0.064$+$ 0.000577	0.408$+$ 0.000577	ND	0.018$+$ 0.000577	ND	ND	ND
WHO Limit (ppm)	1	2.5	5	0.5	0.2	1.5	0.5	0.5

Values are Mean $\pm$ SEM of the concentration of heavy metals in *Oreochromis mossambicus,* ND= not detected, ppm=part per million.

***Cyprinus carpio* (Gulfam)**

The highest mean concentration of Fe was detected in the liver. The highest concentration of Zn was found in the gills, which was above the WHO limit. Concentration of Pb and Mn were below the limits of WHO. Cu was found in liver only. Ni and Pd were not reported in any selected organ (Table 2).

Table 2. Concentration of trace and heavy metals in the *Cyprinus carpio*

Metals	Fe	Mn	Zn	Cu	Cd	Cr	Ni	Pb
Liver (ppm $\pm$ SEM)	1.036$\pm$ 0.000577	0.058$\pm$ 0.000577	1.806$\pm$ 0.000577	0.063$\pm$ 0.000577	0.029$\pm$ 0.001155	ND	ND	ND
Heart (ppm + SEM)	0.191$\pm$ 0.001155	0.075$\pm$ 0.000577	0.328$\pm$ 0.000577	ND	0.033$\pm$ 0.000577	ND	ND	ND
Gills (ppm +SEM)	0.572$\pm$ 0.000577	0.14$\pm$ 0.005774	10.92$\pm$ 0.017321	ND	0.077$\pm$ 0.001155	ND	ND	ND
Muscle (ppm $\pm$SEM)	0.052$\pm$ 0.001732	0.052$\pm$ 0.001732	0.262$\pm$ 0.000577	ND	0.026$\pm$ 0.000577	ND	ND	ND
WHO Limit (ppm)	1	2.5	5	0.5	0.2	0.5	0.5	1.5

Values are Mean $\pm$ SEM of the concentration of heavy metals in *Cyprinus carpio,* ND= not detected, ppm=part per million.

Tor putitora (mahaseer)

Iron (Fe) was reported in all the organs of fish except muscles. The highest concentration of Fe was in gills. Fe was detected in liver and heart. Zn, Mn and Cu were detected in the entire selected organ and found within the WHO limits. Cd and Pd were detected in all selected organs and their concentration was below the limits suggested by WHO. Ni and Cr were not detected in any organ (Table 3).

Table 3. Concentration of trace and heavy metals in *Tor putitora*

Metal	Fe	Mn	Zn	Cu	Cd	Pb	Cr	Ni
Liver (ppm $\pm$ SEM)	1.252$\pm$ 0.001	0.126$\pm$ 0.001	0.971$\pm$ 0.001	0.091$\pm$ 0.002	0.037$\pm$ 0.001	0.164$\pm$ 0.001	ND	ND
Heart (ppm $\pm$ SEM)	1.445$\pm$ 0.000577	0.364$\pm$ 0.000577	1.268$\pm$ 0.000577	0.103$\pm$ 0.000577	0.041$\pm$ 0.000577	0.195$\pm$ 0.000577	ND	ND
Gills (ppm $\pm$ SEM)	4.211$\pm$ 0.000577	1.437$\pm$ 0.000577	1.701$\pm$ 0.000577	0.052$\pm$ 0.000577	0.052$\pm$ 0.000577	0.454$\pm$ 0.000577	ND	ND
Muscle (ppm $\pm$ SEM)	0.086$\pm$ 0.000577	0.09$\pm$ 0.00577	0.214$\pm$ 0.000577	ND	0.04$\pm$ 0.005774	0.512$\pm$ 0.000577	ND	ND
WHO Limit (ppm)	1	2.5	5	0.5	0.2	1.5	0.5	0.5

Values are Mean $\pm$ SEM of the concentration of heavy metals in *Tor putitora,* ND= not detected, ppm=part per million

Cirrhinus mrigala (mori)

Highest quantity of Fe was found in the heart and its value was above the recommended WHO limits (1ppm). Cu was reported in all selected organs except in muscles. Cd, Mn, Zn were reported in all the samples and found below the recommended WHO limits. Ni was reported only in gills of the fish. Cr and Pb were not found in any of the selected organs (Table 4).

Table 4. Concentration of trace and heavy metals in *Cirrhinus mrigala*

Metal	Fe	Mn	Zn	Cu	Cd	Ni	Cr	Pb
Liver (ppm $\pm$ SEM)	0.999$\pm$ 0.000577	0.112$\pm$ 0.000577	0.895$\pm$ 0.000577	0.197$\pm$ 0.000577	0.045$\pm$ 0.000577	ND	ND	ND
Heart (ppm $\pm$ SEM)	1.339$\pm$ 0.000577	0.218$\pm$ 0.000577	0.442$\pm$ 0.000577	0.132$\pm$ 0.000577	0.053$\pm$ 0.000577	ND	ND	ND
Gills (ppm $\pm$ SEM)	0.711$\pm$ 0.000577	0.338$\pm$ 0.000577	3.284$\pm$ 0.000577	0.103$\pm$ 0.000577	0.057$\pm$ 0.000577	0.001$\pm$ 0.000577	ND	ND
Muscle (ppm $\pm$ SEM)	0.101$\pm$ 0.000577	0.188$\pm$ 0.000577	0.262$\pm$ 0.000577	ND	0.051$\pm$ 0.000577	ND	ND	ND
WHO Limit (ppm)	1	2.5	5	0.5	0.2	0.5	0.5	1.5

Values are Mean $\pm$ SEM of the concentration of metals in *Cirrhinus mrigala,* ND= not detected, ppm=part per million

Labeo rohita (rohu)

Highest concentration of Fe was in the gills and it was above the WHO limit. Zn, Mn, Cd, Cu and Pd were detected in all organs and their concentrations were below the limits set by WHO. Cr and Ni were not found in any organs (Table 5).

Table 5. Concentration of trace and heavy metals in *Labeo rohita*

Metal	Fe	Mn	Zn	Cu	Cd	Pb	Cr	Ni
Liver (ppm +SEM)	0.204+ 0.000577	0.112+ 0.000577	1.365+ 0.000577	0.305+ 0.000577	0.059+ 0.000577	0.568+ 0.000577	ND	ND
Heart (ppm +SEM)	0.436+ 0.000577	0.135+ 0.000577	0.816+ 0.000577	0.078+ 0.000577	0.052+ 0.000577	0.741+ 0.000577	ND	ND
Gills (ppm +SEM)	5.701+ 0.000577	1.365+ 0.000577	3.726+ 0.000577	0.082+ 0.000577	0.081+ 0.000577	0.928+ 0.000577	ND	ND
Muscle (ppm +SEM)	0.353+ 0.000577	0.113+ 0.000577	0.666+ 0.00057	ND	0.06+ 0.005774	0.412+ 0.000577	ND	ND
WHO Limit (ppm)	1	2.5	5	0.5	0.2	1.5	0.5	0.5

Values are Mean $\pm$ SEM of the concentration of heavy metals in *Labeo rohita,* ND= not detected, ppm=part per million

Ctenopharyngodon idella (Grass carp)

Fe, Mn, Cd and Zn were detected in all organs and the values were below the limits of WHO. Cu was only detected in the liver and its concentration was below the WHO limits. Cr and Ni were not found in any organ. Pb was only found in liver and its concentration was within the WHO limits (Table 6).

Table 6. Concentration of trace and heavy metals in *Ctenopharyngodon idella*

Metal	Fe	Mn	Zn	Cu	Cd	Pb	Cr	Ni
Liver (ppm + SEM)	0.8+ 0.057735	0.267+ 0.000577	0.98+ 0.005774	0.001+ 0.000577	0.067+ 0.000577	ND	ND	ND
Heart (ppm + SEM)	0.191+ 0.000577	0.127+ 0.000577	0.581+ 0.000577	ND	0.07+ 0.005774	0.042+ 0.000577	ND	ND
Gills (ppm + SEM)	0.671+ 0.000577	0.301+ 0.000577	1.91+ 0.005774	ND	0.086+ 0.000577	0.003+ 0.000577	ND	ND
Muscle (ppm + SEM)	0.034+ 0.000577	0.074+ 0.000577	0.17+ 0.005774	ND	0.075+ 0.000577	0.076+ 0.000577	ND	ND
WHO Limit (ppm)	1	2.5	5	0.5	0.2	1.5	0.5	0.5

Values are Mean $\pm$ SEM of the concentration of heavy metals in *Ctenopharyngodon idella*, ND= not detected, ppm=part per million

Discussion

Since ages, fishes have been used by humans and animals as a primary source of food. Fishes contain all those nutrients in their organs which are essential for our growth. Due to the presence of various minerals and metals, fishes are an excellent nutritional source [16]. The study showed a varied tendency of the different organs of the fish to accumulate heavy and trace metals. It was known through the present study that different organs of the fish stored metals in different concentrations depending on the variety and species of the fish, and this was due to the different feeding habits, behaviors, and living [17, 18]. Mn, Zn, Cu and Fe are counted in the group of essential and trace metals, and are compulsory for

normal functioning of many metal-containing enzymes and maintaining cellular function. Depending on their absorption, they may leave beneficial or harmful impact on aquatic animals i.e. fish [19]. Liver of the fish contained highest concentrations of heavy metals [21] [20]. Liver plays a significant function in detoxification and breakdown of metals. Metallothioneins which are mostly found in the liver of fish are responsible for storing and detoxifying of metals in fish [22]. Muscles of the fish were found the least metal storing organs according to the results of the present study. Accumulation of some of the metals like Cu, Mn, Fe, and Zn were found mainly in the liver and gills of the selected species. On the other hand, Pb and Cd were found mostly in the heart and the gills. The accumulation pattern of Pb and Cd differed between species where the concentration of metals fluctuated between the gills and liver. The assembling of essential and trace metals in the liver is related to the function in metabolism [23]. Fe is the main constituent of protein in hemoglobin, mainly deposited in livers of fish and reprocessed in the formation of RBCs. Higher accumulation of Fe in the livers of fish shows its significant physiological and metabolic role in the fish [24]. In present study iron was found in highest concentration in liver and heart of the fish. Presence of heavy metals in fishes potentially affects fish consumers. These heavy metals accumulate in fish tissues and lead to serious health problems. Because of this, the problem of trace and heavy metal pollution in fish has received great attention [25]. Heavy metals cause suppression of immune system, carcinogenesis and mutagenesis [26]. The toxicity of Cd and Pb is well known [27]. Cd is not considered as essential element for humans and known to be an element harmful for the organs such as liver, lungs and kidneys [28]. It is known that Cd accumulates in both kidneys and liver. Half-life of this heavy metal in human body is between 10-40 years [29]. Exposure to high concentrations of Cd can affect immune and central nervous system, and may cause fertility disorders and different types of cancers. Pb affects normal growth and causes intellectual retardation in children. In adults it causes hypertension and cardiac diseases [30]. Copper is an essential metal which produces lethal effects such as liver cirrhosis and dermatitis when taken in higher amounts [31]. At low concentrations, Copper causes vomiting, diarrhea, nausea, and headaches. At excessive levels of deposition, copper affects the proper functioning of kidneys and liver [32, 33].

In present study Pb was detected in *Oreochromis mosambicus*, *Tor putitora*, *Labeo rohita* and *Ctenopharyngodon idella* whereas Cd was detected in all the samples, and their concentrations were below the WHO limits. Pb was not detected in *Cirrhinus mrigala* and *Cyprinus carpio.* Ni and Cr were not detected in any sample. Current study reveals that the concentration of Pb and Cr were below the limits set by WHO, so these species are safe for human consumption.

Conclusion

It is deduced from the present study that the monitoring of the concentration of heavy metals in different tissues of fish is necessary. As it was observed that the concentration of heavy metals in edible part (muscles) was within the recommended limits of WHO, the higher concentration in nonedible parts should also be monitored in order to protect the aquatic ecosystems and to reduce its indirect impact on human health.

Authors' contributions

Conceived and designed the experiments: Wali Mohammad Achakzai, Shafi Muhammad, Shagufta saddozai, Performed the experiments: Yasmeen Malik, Safia

Mustafa, Analyzed the data: Abdul Qadir, Nisar Ahmad, Contributed reagents/ materials/ analysis tools: Iftikhar Ahmed Baloch, Wrote the paper. Yasmeen Malik

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