Bioaccumulation of Heavy Metals in Fresh Water Fish, (*Cyprinus carpio*) Netted from Kabul River, Shah Alam Khyber Pakhtunkhwa Pakistan

Syed Salman[*], Ihtesham Ul Haq[†], Ye Fanfan[‡], Sahibzada Muhammad Jawad[§], Saeed Ullah[**], Sharbat Khan Nafees[††], Shahzad Shoukat[‡‡6]

Abstract

The current study evaluated the selected heavy metals, zinc (Zn), Nickel (Ni), Chromium (Cr), Copper (Cu), Lead (Pb), Cadmium (Cd), and Arsenic (Ar) in the intestine, gills, and muscles of a freshwater fish species Cyprinus carpio. The level of each heavy metal was recorded in the intestine, gill, and muscle by using the Spectrophotometer. The intestine showed the highest metallic burden followed by muscles and gills. By taking the average, it is found that the metals can be represented by the following concentrations which are present in the organs studied. It can be seen that the sort of metal accumulation in fish body was Cr > Ni > Zn >Pb> Cd>Cu>Ar with the average values of 3.18, 2.73, 1.52, 1.25, 0.95, 0.77 and 0.76 ppm respectively. Further the comparison of the present data with the Maximum Permissible Limits of international standards provided by the Food and Agriculture Organization (FAO), it can be seen that the levels of Zinc, Nickel, Copper, and Arsenic are within the normal range with the average values of 1.52 ppm, 2.73 ppm, 0.77 ppm, and 0.76 ppm respectively. While the values of Chromium (3.18 ppm), Lead (1.25 ppm), and Cadmium (0.95) are higher than permissible limits that are 1 ppm, 0.5 ppm, and 0.05 ppm respectively.

Key Words: Bioaccumulation, Heavy metals, Atomic Absorption Spectrophotometer, *Cyprinus carpio*

Introduction

Over the last few decades, Heavy metal pollution of natural aquatic resources due to industrial, residential, and other anthropogenic activities has become a major concern. (Waqar *et al*., 2013). The destructive effects of heavy metals on aquatic creatures can be exposed

[*] Faculty of Biological, Chemical and Environmental Science and Technology, Dalian University of Technology, China, Email: syedsalman@mail.dlut.edu.cn
[†] Government Primary School Wahid Garhi Circle dawoodzai Peshawar KPK
[‡] Faculty of Biological, Chemical and Environmental Science and Technology, Dalian University of Technology, China
[§] Department of Zoology Islamia College University Peshawar, KPK-Pakistan
[**] Department of Bioscience Universiti Technologi Malaysia (UTM)
[††] Department of Biology Shaikh Zayed University Khost Afghanistan
[‡‡] Department of Immunology, Postgraduate School, Universitas Airlangga, Surabaya, Indonesia

through toxicity tests that allow a dose-response relationship to be established (Akhter *et al*., 2008; Javed, 2013). This enables us in expecting and limiting harmful chemical flows into water bodies, as well as anticipating acute damage to aquatic wildlife. (APHA, 2005). Metal bioaccumulation patterns must be observed due to the obvious negative effects metals had upon aquatic life. This will show the extent of metal accumulation across time and space, as well as a risk assessment for human health (Ladipo *et al*., 2012). Amongst aquatic creatures, fishes are more vulnerable to the aquatic food chain as they collect a huge amount of heavy metals (Chezhian *et al*., 2010). The blood act as a vehicle to carry the chemicals to the various part of the fish body such as liver, bones, kidney, and gills (Dural, 2007). It has been proven that arsenic has the most hurtful chemical having a carcinogenic and genotoxic effect (Chen *et al*., 2005). The intake of arsenic through drinking water can trigger chromosomal defects in human beings (Wen-Chien *et al*., 2001). It is also used for pharmaceutical products, pesticides, veterinary products, and coloring agents that make people more exposed to them (Bohrer *et al*., 2005). Nickle is another heavy element frequently considered as a genotoxic, immunotoxic, and oncogenic effect against livelihood animals (Kasprzak *et al*., 2003). The building up of *Ni* element is responsible for the metabolic alteration, lipid peroxidation, and content of metal in various tissue of an organism (Funakoshi *et al*., 1996: Chakrabarti and Bai, 1999). Even though Ni toxicity in humans is well-known, the mechanism of toxicity in fish is poorly understood. (Pane *et al*., 2005). In the same context, fishes obtained an important micronutrient Zinc from the aquatic environment (Wood, 2001). Meanwhile, the consumption of a high amount of *Zn* can adversely affect the fish body (Hayat *et al*., 2007). Pakistan freshwater lakes have been contaminated by numerous contaminants, including heavy metals. High concentration of *Fe, Zn, Cu, Cr,* and *Co* while *Ni*, *Pb,* and trace elements were observed at low concentration as compared.

The discharge of untreated industrial effluents is the primary source of water pollution in Pakistan, resulting in significant levels of pollution in both surface and groundwater. Even though the number of metals has been recorded at a critical level in many industrial receiving waters. To date, only a few studies have been conducted on freshwater toxicity due to heavy metals and their effects on fish species. In order to conserve indigenous fauna, it is necessary to understand their sensitivity and inherent potentials for metal uptake and buildup in their natural habitats (water bodies).

Materials and Methods

Study Area

Our sampling location for this study was near Shah Alam river (a tributary of the Kabul river) in the Charsadda district of Khyber Pakhtunkhwa province in Pakistan. The Kabul river enters from Afghanistan to Pakistan's Khyber Pakhtunkhwa province (Figure 1). It branches off to three rivers in the Charsadda district (including the Shah Alam river). Then it joins the Indus river about 40 km downstream of our sampling location. Shah Alam river alone is the home of 13 fish species (CIDA and WAPDA 1996). It branches off the Kabul river near Kandir Landi at Daudzai. receives all the sewage from Peshawar *via* the Ganda Arab and Budni Nalla, as well as sewages from 30 surrounding villages. We selected the Shaha Alam river as our sampling location because it is downstream from various industrial and domestic effluent discharge sites. Hence one would expect it to be a more polluted segment of the Kabul river.

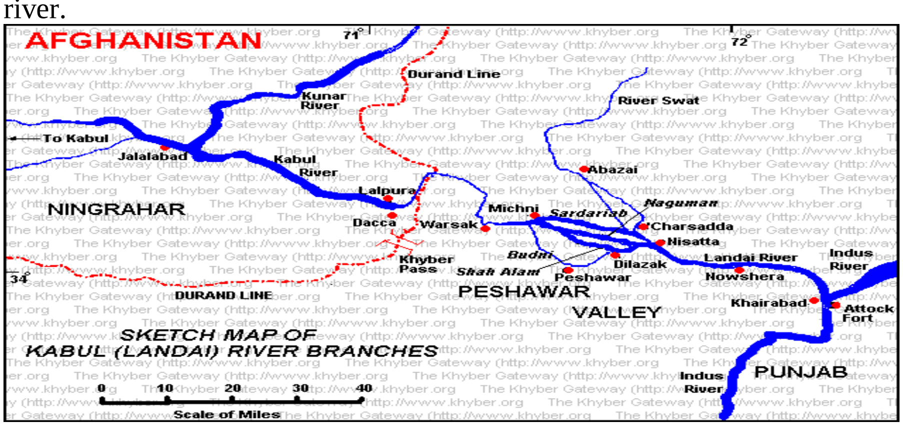

Figure 1. Map of the study area in river Kabul (Google researchgate.net)

Sampling

Eight different samples of the same fish species *C. carpio* were collected from the Kabul river Shah Alam Peshawar Khyber Pakhtunkhwa Pakistan. They were instantly moved to the icebox and brought to the laboratory of Zoology at Qurtuba University Peshawar for measurements, dissection, and acid digestion. The remaining experimental work linked to metal analysis was implemented at Abdul Wali Khan University Mardan KP.

Procedure

The acid ingestion of the samples was performed to calculate the heavy metals in the samples. The acid consumption is used primarily to prepare the samples for the atomic absorption procedure. Eight common carp (*C. carpio*) samples, ranging in size from 26 -34.5cm, were purchased at random from the Kabul river Shah Alam Peshawar Khyber Pakhtunkhwa Pakistan. All samples were cleaned in distilled water and stored in an icebox until they were taken to the Qurtuba University Peshawar Zoology Laboratory for dissection and acid consumption. The whole length of each fish was measured using China tape to determine its size.

Bioaccumulation of Heavy metals in Fish Organs; The fish samples were again cleaned with distilled water following morphometric measures and divided into the clean working glass by a stainless steel knife to remove a mixture of tissues. The sterilized polythene bags were removed and unlocked for a heavy portion of gills, muscles, and intestines. For further analysis, bags were then frozen at -20°C.

For further inquiry gills, muscles, and intestine samples were melted washed in distilled water, blotted with blotting papers, and shifted to 100mL volumetric flasks. Each flask was washed with distilled water and dried for 20 minutes in the oven at 60°C before transfers the gills, muscles, and intestine, Then the volumetric flasks were shifted to a known weight of each organ. Some changes were made in the process according to Yousafzai and Shakoori, 2008.

Meanwhile, during ingestion, 5 mL nitric acid (55%) and 2 mL perchloric acid (70%) was added to each flask instead of 10 mL nitric acid (55%) and 5 mL perchloric acid and then were kept airtight overnight. The next day, each bottle received a second dose of 5 ml of nitric acid (55%) and 3 ml (70%) of perchloric acid. The flasks were then placed on the hot plate and digested at 200-250°C to provide a clear and transparent solution. After brown smoke, the dense white fumes from the flasks were a sign that the intake procedure was completed. This approach took around 20 minutes to use, rather than the 3 to 4 hours reported by Van Loon, 1980. After ingestion samples were frozen and diluted to 10mL with nano pure water by cleaning of the ingestion flasks. All the samples were properly filtered and moved to sterile plastic bottles.

After acid digestion, Atomic Absorption Spectrophotometer was used to run the samples. The flames were used as air acetylene for the atomic absorption spectrophotometer, and the flame was set into an atomic absorption spectrophotometer for every heavy metal analysis. A standard solution for each heavy metal first aspires for the preparation of 26

Standard heavy metal curvatures into the atomic absorption spectrophotometer, then samples are sucked and the absorption of selected heavy metals is observed.

Atomic absorption Spectrophotometer was used for the determination of the concentration of the following heavy metals: Copper (Cu), Zinc (Zn), Nickel (Ni), Chromium (Cr), and Lead (Pb) Cadmium (Cd) arsenic (As) in the gills, muscles, and intestine. Samples of each fish. Each sample was examined in triplicate. The concentration of each heavy metal was reported in ppm (Parts per Million).
Data obtained was analyzed and the results were stated as mean ± S.E.

Statistical Methods of Mean of Data

It is the representative part of the whole data, denoted by X. The data collected from six samples was noted 3 times and then the average value was calculated. The mean was calculated by the following formula to evaluate the representative value for each sample.
X (Mean) = £ x 4 n Where,
X = mean of observation **£ x=** sum of observations **n =**number of observation

Results

By taking the average, it was found that the concentration of metals accumulation in the fish body was Cr > Ni > Zn >Pb> Cu>As

Table 1: Average values of heavy metals detected in Cyprinus carpio fish organs collected from river Kabul in May, June, July & August, 20??

ORGANS	Copper	Nickel	Lead	Chromium	Cadmium	Zinc	Arsenic
Intestine	0.813625	2.337	1.0225	3.62125	0.984125	1.892	0.81625
Gills	0.675375	3.30375	1.26325	2.66125	1.16375	1.08475	0.691875
Muscles	0.85475	2.5675	1.584125	3.26	0.70525	1.58325	0.79875
AVERAGE	0.77125	2.736083	1.25456	3.18083	0.9510416	1.52	0.7689583

Discussion

Nickel (Ni): Nickel concentrations were highest in the gills of all the fish samples. Our result indicates that the concentration of nickel was in the order Gills>Muscles>Intestine. Nickel level of 0.7pg/g is considered potentially harmful to fish and marine birds that feed on them (Lemly, 1993). 2.3 µg/g or higher levels of nickel may cause impairment in the reproduction of fishes (Baumann and May 1984). The maximum permissible limits of FAO for Nickel are 0.05-5.5 ppm. Neither of the samples approached the concentration levels in this investigation. Thus, nickel concentrations do not represent an indulgence in the consumption of these fish species in the entire fish species.

Copper (Cu): The observation revealed that a high amount of copper was accumulated in the muscle of all samples of fishes, while Gills showed the least accumulation. The distribution of copper was in the order of muscles>intestine>gills in the entire fish sampled. Copper can merge with other contaminants such as ammonia, mercury, and zinc to create an additive toxic effect on fish (Rompala *et al.,* 1984). The acceptable limit for Cu set by FAO (1983) is 30 ppm, which exceeds the accumulation levels obtained in the present study.

Lead (Pb)): Lead was significantly accumulated in a higher amount in gills, livers, stomach, skin, and flesh tissue of whol fish species investigated. The concentration of lead accumulation was in the following order muscles>gills>intestine, resulting in highly toxic to aquatic organisms, especially fish (Rompala et al., 1984). Delay in embryonic development, inhibited reproduction, inhalation of growth, increased mucous formation, neurological difficulties, enzyme inhalation, and renal malfunction are some of the biological impacts of sub-lethal amounts of lead (Rompala *et al.,* 1984). Lead concentrations in the muscles, intestine, and gills of all the fish samples were above the 0.5ppm limit (FAO, 1983)

Chromium (Cr): Chromium is usually not collected in fish, hence even in developed parts of the world there have been minimal levels of Cr (Moore and Ramamoorthy, 1984). The mean concentration of *Cr* found in the fish muscle samples from the Mediterranean coastal waters was 2.1pg/g. Still lower averages of muscles *P. barberinus* (0.4pg/g.) and 0.6pg/g Scarus variegatus were also recorded from the Gulf of Aqaba. (Wahbeh and Mahasneh, 1987). The Gills of the investigated fish had the greatest quantities in the current study, whereas the gut had the second-highest values. Another study of Yousafzai, (2004) reported that in *L. dyocheilus* and *W. attu*, the gills were the site of greatest Cr accumulation. The examination made in muscles of *Oreochromis mossambicus* and *Cyprinus carpio* (Jabeen and Chaudhry, 2009; Jabeen and Chaudhry, 2010) showed the maximum concentration of Cr. Wahbeh, and Mahasneh, (1987) investigated the following pattern of distribution of Cr in *L. rohita* gills>kidney >liver >skin >muscles, whereas in the present study the maximum accumulation of Cr was observed in the gills. The permissible limit set for Cr by FAO, (2003) is 1.0 ppm.

Zinc (Zn): In the present investigation, the highest zn level was observed in the *Caprinus carpio* intestine. However, the highest accumulation was seen in other organs such as gills of *C. punctatus* (Vineeta et al., 2007), testes of *O. niloticus*, and *L. niloticus* (Charbonneau and Nash, 1993). Accumulation of metals reported in our present study for muscles and gills of *Caprinus carpio* is comparable to *O. niloticus* (Charbonneau and Nash, 1993) and *C. punctatus* (Murugan et al., 2008) respectively. In our study, the least accumulation of Zn was seen in the gills of *C naziri*. The permissible limit for Zn set by FAO, (1983) is 30 ppm from which the values observed during this study were less. Zinc was detected in all the fish samples and the highest concentrations were observed in the Intestine followed by muscles and gills. Fishes can intake and accumulate zinc from their food and water (Eisler, 1993). Though zinc is a vital element, however, at high concentrations, it can adversely affect the fish by introducing low growth levels, mortality, and reproductive impairment (Sorenson, 1991). Zinc can interact with other elements and generate unfavorable effects such as antagonistic, additive, or synergistic effects (Baumann and May 1984). Within this area of River Kabul, zinc doesn't seem to be a pollutant threat to fish.

Cadmium (Cd): The highest concentrations of Cadmium were found in the gills of all the fish samples. As a result of these researches, the concentration of nickel was in the order gills >intestine> muscles.

Cadmium is largely deposited in fish's major organ tissues instead of muscles and flesh (Moore and Ramamoorthy, 1984). In the present investigation, Cd in flesh was shown to have the lower level whereas the highest mean values in gills and livers were detected. Cadmium is a non-essential metal that can be hazardous to most fish and fauna, especially freshwater. In the gills and liver, the greatest levels were observed.

The lowest quantities in flesh were found in the tissues of all the species investigated. The NCBP content of 2.1µg/g is not greater than that found, which is deemed harmful to fish and predators (Schmitt and Brumbaugh, 1990; Robertson *et al.*, 1991).

The permissible limits set for Cd by FAO, (1983) is 0.05. The values for Cadmium in the current study are also above the maximum permissible limits of FAO that is 0.05ppm. This study's samples all approached these levels of concern. As a result, the fishes' Cadmium levels are over the typical permitted limits.

Arsenic (As): The highest concentrations of Arsenic were observed in the intestine of all the fish samples. Our result suggested that the concentration of nickel is in the order Intestine > Muscles > Gills.

The values for Arsenic in the current study are also above the maximum permissible limits of FAO that is 1.4ppm. All the samples in this study are in the normal range. Comparing the present data with the Maximum Permissible Limits (MPL) of international standards provided by the Food and Agriculture Organization (FAO), 1983, it can be seen that the levels of Zinc, Nickel, and Copper are within the normal range with the average values of 1.65 ppm, 6.93 ppm, and 0.096 ppm respectively. While the values of Chromium (2.27 ppm) and Lead (0.926 ppm) are higher than permissible values 1 ppm and 0.5 ppm respectively.

Table 2. Comparison between FAO standards and observed heavy metal concentration in Cyprinus carpio

Sr. No	Metals	Observe value	FAO Limit
1	Copper	0.77125	30
2	Lead	1.25456	0.5
3	Nickel	2.736083	0.05-5.5
4	Chromium	3.18083	1
5	Zinc	1.52	30
6	Cadmium	0.9510416	0.05
7	Arsenic	0.7689583	1.4

Conclusion

The overall burden of heavy metals was highest in the Intestine followed by muscles and lowest in the Gills.The highest value of accumulation was shown by *Cr*, *Ni, Zn, Pb, Cu* and *As* respectively. Comparison of these metals with standards provided by FAO indicates that *Cu, Ni, Zn,* and *Ar* levels are in the normal range while *Cd, Cr,* and *Pb* are slightly higher than permissible limits.

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