OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 01 - Issue 12 (2024)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

RISK ASSESSMENT AND DETERMINATION OF ARSENIC AND HEAVY METALS IN FISH SPECIES COLLECTED FROM FISH PONDS OF KANDHKOT SINDH PAKISTAN: AN IMPACTFUL STUDY

Farkhanda Zaman Dayo
Institute of Chemistry, Shah Abdul Latif University, Khairpur Mirs' Sindh, Pakistan ****Corresponding author***
farzam.chemist@gmail.com
Mushtaque Ali Jakhrani
Institute of Chemistry, Shah Abdul Latif University, Khairpur Mirs' Sindh, Pakistan
DOI: *https://doi.org/10.71146/kjmr148*

Article Info

Abstract

Assessing risks and quantifying arsenic and other toxic metals in species of fish are essential for protecting public health and ensuring the safety of seafood consumption. Using a microwave digestion technique and inductively coupled plasma optical emission spectroscopy, this study provides the first report of toxic metal concentration in two fish species found in Pakistan, Cirrhinus Mrigala (Mrigal) and Labeo Rohita (Rohu) fish species caught from Muhamadani and Baloach ponds from District Kandhkot, Sindh, Pakistan. The sediment, water, bioaccumulation factor, contamination factor, pollution load index, estimated daily intake, and Total Hazard Quotient as of potential health hazard of various heavy and toxic metals in these fish i.e., Fe, Cu, Zn, Cr, Cd and As were computed using appropriate formulas and modules. Comprehensive ways were taken to study the toxicity of these metals so that their effects on the consumers could be interpreted. The collection of fish organ samples (muscles, gills, liver) for quantitative heavy metal content as well as water and sediment from the ponds collected.

The analysis showed that metals were more accumulated in sediments and water with pollution load index for total heavy metals of sampling stations being greater than 1.9. The bioaccumulation factors for Fe, Cu, Zn, Cr, Cd, and As in fish organs, based on sediment and pond water, were 0.70, 0.30, 0.10, 0.35, 0.14, 0.10 and 0.60, 0.20, 0.01, 0.14, 0.12, 0.11 in both ponds respectively. The contamination factors were determined as 1.4 and 1.6 for Fe, 1.8 and 1.5 for Cu, 1.5 and 1.3 for Zn, 0.8 and 0.7 for Cr, 2.4 and 1.7 for Cd, and 4.1 and 4.4 for As in both ponds. Targeted Health Quotients (THQ factor) Fe, Cu, Zn, Cr, Cd and As recorded for this study were 0.11, 0.07, 0.03, 0.05, 0.03, 0.02 and 0.09, 0.06, 0.02, 0.07, 0.06, 0.01 respectively in both ponds which indicates a very low health hazard to human consumers in the study area.

The estimated daily intake and THQ values for studied metals i.e., Fe, Cu, Zn, Cr, Cd, and As ranked in order as Cr > As > Cd > Fe > Cu > Zn. Both the estimated daily intake and THQ values in samples of fish were lower than 1, which indicates a little risk to humans in the studied area. However, the elevated toxic metal content in sediments and water is likely due to the excessive waste, poor drainage infrastructure, and the overuse of poultry manure, which contribute to increasing pollution levels in these fish ponds in Kandhkot, Sindh, Pakistan.

Keywords: *Heavy metals, Labeo rohita fish, Cirrhinus mrigala fish, Fish ponds, Kandhkot city, ICP-OES*

Introduction

The growth in fish consumption around the world has been attributed to its therapeutic and dietary importance. Fish is one of the important sources of essential minerals, good quality protein, vitamins, and poly unsaturated fats. Due to its importance in human diets, it is of utmost important that fish is free of unsafe amounts of toxic metals that can impact human health. It is possible to derive a variety of nutrients from fish are healthful to human, and the USDA recommends eating fish at least twice a week, which will provide heart-healthy omega-3 fatty acids (American Association of Heart and Diseases) (Mozaffarian et al., 2003; Peter, Chopra, & Jacob, 2013).

On the other hand, water organisms such as fish, can become contaminated with toxic metals during their life span by polluted water or localized ecological conditions, environmental pollution, sediments, food, salinity and metabolism (Odoemelam, Wemedo, Akani, & Douglas, 2023). Aquatic ecosystems that are contaminated with toxic metals have become an international concern over the last two to three decades because metals are persistent and pose a risk to humans and other life forms (Elhag Elhussien, 2018). Fish contamination with metals poses a health risk when these fish are consumed, and these metals can persist for long durations in river and pond sediments and in water which has been verified in various samples (Wong, von Keyserlingk, Richards, & Weary, 2014).
Fish samples serve as a crucial tool for assessing water body contamination levels too, especially regarding metal pollutants. The bioaccumulation of the toxic metals in aquatic-ecosystems has raised global concern among researchers, as toxic metal atoms pileup into the water and enter the food web, potentially posing serious health risks to humans (Mawia Hassan, Aisha Abd, Alaa, & Rash, 2018). Increased metal levels directly correlate with heightened health risks for humans and aquatic life, often due to contaminated aquatic environments (Hamed, Alim, Babiker, & Saad, 2015). Metals may enter water bodies through various human activities, such as shipping, industrial or domestic wastewater discharge, atmospheric deposition, landfill leaching, fertilizer and pesticide use, harbor activities, and natural processes like as weathering due geological changes over time (Hamed et al., 2015; Mohamed & Osman, 2014).

This study evaluated essential trace metal elements in the samples of fish by help of monitoring toxic effects, bioaccumulation through water and sediment analysis. To this end, the maximum and minimum levels of the contamination factor (CF), targeted health quotient (THQ), pollution load index (PLI), and bioaccumulation factor (BAF) were measured in two well-known fish species, Cirrhinus mrigala (Mrigal) and Labeo rohita (Rohu), fish species caught from Muhamadani and Baloach ponds from District Kandhkot, Sindh, Pakistan.

2.Materials and Methods

2.1. Study area

Kandhkot is a major city which is located at Kashmore Tehsil of Sindh, Pakistan. Its coordinates are as 28° 14' 38.26" N (latitude), and 69° 10' 56.46" E (longitude). The fish production at Kandhkot is supported by both natural lakes and man-made fish ponds operated by local farmers (Fig. 1.). There are over 500 fish ponds in Kandhkot and its surrounding areas. Consequently, fish farming plays a vital role in the social and economic life of the local community and contributes significantly to the country's GDP through the export of fish and fish products.

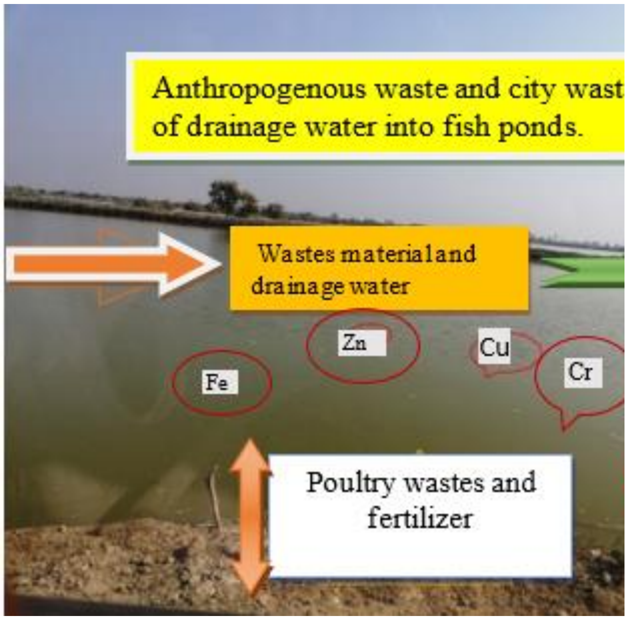

Fig. 1. Map of Study area (district Kashmore @ Kandhkot).

2.2. Collection of Fish Samples

Two fish species, Cirrhinus mrigala (Mrigal) and Labeo rohita (Rohu) fish species caught from Muhamadani and Baloach ponds with the assistance of expert fishermen. The fish samples kept at 4° C in iceboxes and then transported towards the laboratories of Institute of Chemistry, Shah Abdul Latif University Khairpur. At the point of receiving the samples, it was put on an electronic balance and weighed. Next, it was cutting of specific tissues which included the muscle belly, the gills, and the liver using sterilized cutting differential instruments.

2.3. Experimental reagents and Standards

All the desired chemical reagents in this work were analytical grade. Standard of metals Fe, Cu, Zn, Cr, Cd, and As were prepared in three stock solutions of one thousand milligrams per liter through dissolving these metals with nitric acid and then were further diluted by help of deionized water. The standard solutions of all samples had an acid concentration that was same as that used in preparing the digested samples. The sediment and water samples were also taken from Muhamadani and Baloach fish ponds. The solutions filtered using Whatman-42 filter paper. Instrument Inductively Coupled Plasma Optical Emission Spectrophotometer (ICPOES) was used in the processing of samples. All of the background correction was done with the assistance of a deuterium lamp (Saad, Nada, Nada, & Nada, 2021).

Sediment digestion was done with help of a hotplate and a domestic microwave operated at 1000 W having a capacity of 32 L (Samsung, Japan), followed by an ultrasonic bath having a a capacity of 1.9 L (Branson, USA). Digestion tubes, all glassware, polyethylene bottles, and Teflon vessels were soaked for 24 hours in nitric acid (10%) and were rinsed multiple times with a Milli-Q water before use (Hamed et al., 2015).

2.4. Metals in Sediment Samples

2.4.1. Hotplate digestion:

In this research work, metals from reference materials were recovered by altering the acid digestion process through the use of acid mixtures. The compositions of five acid mixtures were of HNO_3-H_2O (1:1), HNO_3 to H_2SO_4 (1:1), HNO_3-HCl in a 1:3 proportion as aqua regia, HNO_3-HCl in 3:1 ratio as reverse aqua regia, and HNO_3-H_2O_2 (1:1). Accordingly, three kinds of sediment samples were treated with each of the acid mixtures on three different occasions (Saad et al., 2021).

For the sediment samples, 4 grams of sediment were treated with 10ml of the mixture, and the samples were placed in a hot plate at 120°C temperature for three hours duration. Solid digests were then prepared by dissolving the samples in water to get 25ml final volume. These solutions were subjected to AAS and ICP-OES techniques. Two ICP techniques were utilized: Atomic Absorption Spectroscopy (AAS); and Inductively Coupled Plasma Optical Emission Spectrophotometer (ICPOES)

In order to evaluate whether microwave digestion increased metal recovery, three of the most efficient acid blends on hot plates were subjected to microwave digestion. For that particular acid mixture, the power output and the timeframe of heating of the microwave oven was calibrated. Each acid mixture was used to digest standard sediment samples in triplicate. The

Teflon vessels used for the digestions were pressurized and had venting features.

Water samples collected from designated sampling sites to measure pH, electrical conductivity, total dissolved solid, chlorides and alkalinity. For elemental analysis, water samples in triplicate were stored in trace element-free polypropylene bottles, pre-soaked in 10% HNO_3 solution. Two to three drops of concentrated HNO_3 were added to each sample for preservation.

In addition, recovery of metals was also evaluated using high intensity ultrasonic digestion treatment followed by additional hotplate treatment employing the acid combinations that produced the highest yield recovery in the previous studies.

2.5.Estimated daily intake (EDI)

The mean concentration of metals ingested through fish consumption and the body weight of the consumer, determines the estimated daily exposure to metals. The metals estimated daily intake may be carried out as follows (Islam & Hoque, 2014):

$$\text{EDI} = \frac{\text{Daily Intake / Metals}}{\text{Body Weight}}$$

Daily intake = daily fish consumption × mean concentration of metals in fish (Hosseini Alhashemi, Sekhavatjou, Hassanzadeh Kiabi, & Karbassi, 2012).

2.6. Bio-accumulation factor (BAF)

It represents the transfer of trace elements from sediment to fish. The BAF is the ratio of concentration in fish tissue to concentration in dry sediment samples which was calculated by:

$$\text{The bioconcentration factor} = \frac{[\text{M}]\text{fish}}{[\text{M}]\text{sediment}}$$

2.7. Pollution Load Index (PLI)

The PLI is created from concentration factors (CFs). Each CF is determined as a ratio, using the concentration of a specific metal and its corresponding background level. A PLI for any location is determined using the following formula: taking the product of the individual CF of each metal analyzed then taking the nth root of the derived number. The PLI formula is based on the method developed by (Rabee, Hassoon, & Mohammed, 2017):

$$CF = \frac{C\ metal}{C\ background\ value}$$

$$PLI = \sqrt[n]{CF1 \times CF2 \times CF3 \times \ldots..\times CFn}$$

A PLI value greater than 1 suggest pollution, while its lower value suggest no pollution.

2.8. Toxic Risk Assessment (THQ)

The Targeted Health Quotient (THQ) is most suitable for estimating risk factors related to human health of the local population related to overconsumption of toxic elements through food. However, THQs for toxic elements may be computed as function of a certain equation:

$THQ = EF \times ED \times FI \times MCRfd \times BW \times AT \times 10 - 3$ (Huang et al., 2007).

3.Results and Discussion

The values of iron, copper, zinc, chromium, cadmium and arsenic while using sediment, water, BAF, CF, PLI, EDI and THQ values in the samples from the Muhamadani and Baloach fish ponds, are summarized in Table 1. The greatest accumulation of Fe in sediments collected from both ponds was 26.6 and 25.1 mg/kg, and it is extremely higher than the accepted amount recommended by FAO/ WHO. Average concentrations of copper, zinc, chromium, cadmium and arsenic were 2.71 and 2.2 mg/kg, 1.32 and 1.11 mg/kg, 0.61 and 0.64 mg/kg, 0.43 and 0.54 mg/kg 0.03 and 0.05 mg/kg respectively. However, concentrations of chromium and cadmium were greater than the recommended values provided by the WHO. The concentrations of Fe, Cu, Zn, Cr, Cd and As in the water from the Muhammadani pond respectively were 18.6 mg/L, 2.5 mg/L, 0.42 mg/L, 0.41 mg/L, 0.37 mg/L and 0.03 mg/L. In the Baloach pond, however, these were 13.9 mg/L, 1.7 mg/L, 0.31 mg/L, 0.44 mg/L, 0.35 mg/L and 0.04 mg/L respectively in the waters of both ponds and these quantities are within the recommended limits except iron and chromium.

Muhamadani Pond (P1) and Baloach Pond (P2)														
Metals	Sediment mg/kg		Water mg/l		BAF Factor		CF Factor		PLI Factor		EDI Factor		THQ Factor	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Fe	26.6	25.1	18.6	13.9	0.7	0.6	1.4	1.[illegible]	1.9	1.8	0.08	0.07	0.11	0.09
Cu	2.71	2.2	2.51	1.72	0.3	0.2	1.8	1.5	1.4	1.4	0.003	0.002	0.07	0.06
Zn	1.32	1.11	0.42	0.31	0.1	0.009	1.5	1.3	1.7	1.6	0.001	0.001	0.03	0.02
Cr	0.61	0.64	0.41	0.44	0.35	0.14	0.8	0.7	2.6	1.7	0.004	0.002	0.05	0.07
Cd	0.43	0.54	0.37	0.35	0.14	0.12	2.4	1.7	2.3	1.5	0.001	0.004	0.03	0.06
As	0.03	0.05	0.03	0.04	0.10	0.11	4.1	4.4	2.1	1.2	0.005	0.007	0.02	0.01

Table. 1. Different statical factors of metals in water, and sediment of Muhamadani and Baloach fish pond.

Bioaccumulation factor was less than 1 in all samples taken from the two ponds and such figure indicates very low uptake of these metals by the fish population. Factors such as CF, PLI, EDI, and THQ also indicated that Muhammadani pond had relatively higher concentrations of iron, copper, zinc, cadmium, and arsenic than Baloach pond where the levels of iron, copper, and zinc were already above the permissible levels. Further there was pollution load index (PLI) greater than 1 in all the stations of both ponds with the exception of chromium. This suggests that the Kandhkot ponds face environmental concentrations were 12.5-18.7 mg/kg for Fe, 0.33-0.74 mg/kg for Cu, 0.04-0.049 mg/kg for pollution and industrial waste contamination, which poses a risk if trace elements further exceed allowable limits. THQ values for each metal via fish consumption in the order: Fe > Cu > Cr > Cd > Zn > As.

The average metal concentrations of Fe, Cu, Zn, Cr, Cd, and As in C. mrigala and L. rohita from the Muhammadani fish pond are presented in table 2. The liver of these species contained more copper, zinc, cadmium and arsenic than any other part of the body, whereas iron and chromium were deposited in the gills. In C. mrigala mean

Muhamadani fish Pond						
Fishes	*C*.mrigala			*L*.rohita		
Metals	Muscle (mg/kg)	Gills (mg/kg)	Liver (mg/kg)	Muscle (mg/kg)	Gills (mg/kg)	Liver (mg/kg)
Fe	12.5	18.7	17.3	19.6	18.3	15.8
Cu	0.33	0.62	0.74	0.73	0.96	0.85
Zn	0.04	0.042	0.049	0.047	0.038	0.039
Cr	0.58	0.62	0.87	0.57	0.67	0.52
Cd	0.18	0.23	0.25	0.49	0.43	0.51
As	0.002	0.001	0.003	0.002	0.003	0.001

Table. 2. Mean values of various metals in fish samples found from Muhamadani fish pond.

Zn, 0.58-0.87 mg/kg for Cr, 0.18-0.25 mg/kg for Cd, and 0.001-0.003 mg/kg for As, respectively. In the case of L. rohita, the values recorded were 15.8-19.6 mg/kg for Fe, 0.73-0.96 mg/kg for Cu, 0.039-0.047 mg/kg for Zn, 0.52-0.67 mg/kg for Cr, 0.43-0.51 mg/kg for Cd, and 0.001-0.003 mg/kg for As.

In fish samples, even though all toxic elements were present, their concentration was in the following order which was seen in all the species sampled: Fe > Cr > Cd > Cu > Zn > As levels. Iron, chromium and cadmium content from both species from the two ponds (Muhammadani and Baloach) were above the limits set by FAO / WHO which could be attributed to the effect of environmental pollutants, industrial or domestic discharge, drainage and feeds.

On a general note, the average copper, zinc, and arsenic concentrations of all fish samples analyzed were in compliance with the limits set by WHO and FAO in 2011. with regard to the elements pollution of aquatic organisms, this then is of concern due to the waste pollution from chemical industries, mining, and agriculture.

In the Baloach fish pond, the concentrations of Fe, Cu, Zn, Cr, Cd, and As in C, mrigala and L. rohita are given in table 3. Concentration of copper, and arsenic found to be higher in liver (C, mrigala), whereas, iron and chromium found to be higher in gills in the case of both species. The concentration of iron, copper, zinc, chromium, cadmium and arsenic in C. mrigala was 16.3-18.6 mg/kg, 0.81-0.94 mg/kg, 0.053-0.059 mg/kg, 0.23-0.78 mg/kg, 0.20-0.22 mg/kg and 0.001-0.004 mg/kg respectively. For L. rohita, they were 19.1-19.6 mg/kg, 0.46-0.55 mg/kg, 0.050-

Baloach Pond						
Fishes	*C*.mrigala			*L*.rohita		
Metals	Muscle (mg/kg)	Gills (mg/kg)	Liver (mg/kg)	Muscle (mg/kg)	Gills (mg/kg)	Liver (mg/kg)
Fe	17.4	18.6	16.3	19.1	19.6	19.3
Cu	0.91	0.81	0.94	0.52	0.46	0.55
Zn	0.059	0.053	0.057	0.052	0.050	0.055
Cr	0.23	0.78	0.27	0.51	0.79	0.74
Cd	0.22	0.20	0.21	0.45	0.37	0.48
As	0.002	0.001	0.004	0.003	0.002	0.002

Table. 3. Mean values of various metals in different fish samples of Baloach fish Pond.

0.055 mg/kg, 0.51-0.79 mg/kg, 0.37-0.48 mg/kg and 0.002-0.003 mg/kg respectively.

Five mean values of physicochemical parameters comprising of pH, EC, TDS, chloride, and Alkalinity for both ponds have been depicted in table 4. Mean ranges for the parameters for the Muhammadani pond were: pH (8.4-8.8), EC (2716-2846 µS/cm), TDS (1655-1989 mg/L), chloride (283-335 mg/L) and Alkalinity (643-742 mg/L). The Baloach pond results are pH (7.8-8.4), EC (2625-2762 µS/cm), TDS (1948-1962 mg/L), chloride (324-327 mg/L) and Alkalinity (723-735 mg/L). For pH, EC, and TDS rather high values have been recorded for the Muhammadani pond as against WHO recommendations, which did however apply to Alkalinity.

positive association suggests that these two metals may co-occur in the samples, potentially due to similar environmental or biological factors affecting their accumulation. The Cu-Chromium (Cr) correlation is 0.798, showing a moderately strong positive relationship. This association might also indicate that Cr and Cu have shared sources or similar behavior in the fish pond environment. Copper shows a moderately strong positive correlation with arsenic (As), with a coefficient of 0.696. This positive relationship may imply similar accumulation mechanisms for Cu and As in the samples. The Fe-Zn correlation is 0.785, which is moderately strong and also

Parameters	WHO limits	Muhamadani Pond	Baloach Pond
pH	6.5-8.5	8.4-8.8	7.8-8.4
E.Cµs/cm	2500	2716- 2846	2625-2762
TDS mg/l	1000	1655- 1989	1948-1962
Chlorides mg/l	250	283- 335	324-327
Alkalinity mg/l	1000	643-742	723-735

Table. 4 Physicochemical parameters of water in Muhamadani fish pond and Baloach fish pond.

The Pearson correlation in table 5 shows relationships among six trace elements (Fe, Cu, Zn, Cr, Cd, and As) in samples from fish ponds. The correlations are assessed on a scale from -1 to 1, where value close to range of ±1 indicates a strong Pearson correlation, and values close to 0 suggest little or no correlation. Here's an analysis of each relationship: The correlation coefficient between copper (Cu) and iron (Fe) is 0.875, which indicates is a strong positive correlation which is significant at the 0.01 level ($p < 0.01$). This means that as Cu levels increase, Fe levels tend to increase as well. This strong association may reflect similar accumulation pathways or sources in the environment. The correlation between Cu and zinc (Zn) is 0.785, describing a moderately strong positive correlation. This statistically significant. This positive relationship indicates that higher Fe concentrations might be associated with higher Zn levels in the samples. The Pearson correlation between Fe and As is 0.838, a strong positive correlation, suggesting that both elements might be influenced by common environmental factors or sources in the ponds. While Cadmium (Cd) shows weak correlations with all other elements and is negatively correlated with Cu (-0.489) and Fe (-0.689). The negative correlations suggest that Cd behaves differently in the environment compared to Cu and Fe, possibly due to differing sources or

accumulation factors. However, the correlation between arsenic (As) and chromium (Cr) is negative at -0.322, indicating that as As levels increase, Cr levels tend to decrease, though this relationship is relatively weak and may not be significant.

iron, copper, and zinc were below 1. Concentrations of these metals were in the series Fe > Cu > Zn. Bioaccumulation factors (BAF) for

Pearson Correlation		Cu	Fe	Zn	Cr	Cd	As
Cu	-	1	-	-	-	-	-
	N	5	-		-	-	-
Fe	-	0.875*	1	-	-	-	-
	N	5	5		-	-	-
Zn	-	-	0.785*	1	-	-	-
	N	-	5	-	-	-	-
Cr	-	0.798*	0.411	-	1	-	-
	N	5	5	-	5	-	-
Cd	-	-0.489	-0.689	-	-0.136	1	-
	N	5	5	-	5	5	-
As	-	0.696*	0.838*	-	0.261	-0.322	1
	N	5	5	-	5	5	5

*** Correlation is significant at the 0.01 level.**

Table. 05. Pearson correlation of Cu, Fe, Zn, Cr, Cd and As metals in different specimens of both species of fish collected from Muhamadani and Baloach fish ponds

4.Conclusion

The study's conclusion is that the concentration of iron exceeds that of the maximum allowable level according to FAO/WHO limits while that of copper and zinc was with in recommended value. In C. mrigala, the residual concentration of trace elements was in this order: Fe > Cu > Zn, which is also noted for L. rohita. Water samples were also found with high levels of iron and copper and zinc did not exceed the permissible limits. Values of estimated daily intake (EDI) of majority of elements were less than 1.0 and targeted health hazard quotients (THQ) were in the series Fe > Cu > Zn.

5. Recommendations

The government agencies should develop quality standards for the feed, water, and sediment used in production of fish ponds and ensure that there is monitoring to prevent abuse. The current study also provides evidence to justify the practice of regularly monitoring and biomonitoring metal contents in fishes which are edible and fishes caught from the ecosystems used for sports. A simple analysis of the problem for heavy metals in the environment may not provide an overview of the risks to the consumer.

6. Acknowledgment

The authors are gratefully acknowledging the financial support from Institute of Chemistry, Shah Abdul Latif University, Khairpur Mir’s, Sindh, Pakistan during the period of sampling and throughout the research activities.

7. Conflicts

The authors declare no conflict of interest.

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