

# ANALYSIS AND HUMAN HEALTH RISK FROM SELECTED HEAVY METALS IN WATER, SEDIMENTS AND FRESHWATER FISH (*LABEO ROHITA*, *CYPRINUS CARPIO*, *GLYPTOTHORAX PUNJABENSIS*) COLLECTED FROM THREE RIVERS IN DISTRICT CHARSADDA, KHYBER-PAKHTUNKHWA, PAKISTAN

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**Abstract:** Water, sediments and freshwater fishes (*Labeo rohita*, *Cyprinus carpio*, *Glyptothorax punjabensis*) were collected from three rivers namely Jindi, Khyali (Swat) and Kabul river in District Charsadda Khyber Pakhtunkhwa Pakistan. The samples were analyzed for the concentrations of seven heavy metals i.e. cobalt, chromium, copper, manganese, nickel, antimony and zinc. The metal concentration data in fishes was used to evaluate health risks for the people of Charsadda. Health risks associated with the intake of these metals were evaluated in terms of dietary intake, target hazard quotients (THQs), hazard index (HI) and target cancer risk (TR). The other metals concentrations in water samples were higher than the WHO permissible limits except copper and Zn. The THQ values for all the metals were calculated to be below 1 except for Cu and Sb. This suggested that there is no significant health hazards if a single metal from one species of fish is ingested. The target cancer risk for Ni in case three fish species was calculated to be higher than the accepted risk level. To both carcinogenic and non-carcinogenic consequences the inhabitants of the area may be exposed if they consume contaminated fishes. Also there is dire need of monitoring the metal level in the fishes in this area.

**Keywords:** Kabul river, fishes, human health risk, Charsadda

## 1. INTRODUCTION

The discharge of effluents into the lakes due to rapid urbanization and industrialization is a big concern worldwide now days (Akan et al., 2012). A number of toxic pollutants are added to the aquatic environment. Due to their action and persistence in biological amplification, heavy metals are particularly more dangerous (Erdogru & Erbilir 2007, Waqar 2006, Honggang et al., 2010, Babatunde et al., 2012). Atmospheric deposition, mining wastes and erosion of the geological matrix are the main sources through which metals can enter the aquatic environment. Even at quite low concentrations most of the toxic metals are harmful to the living organisms but some are biologically essential and natural constituents of aquatic ecosystems, they can cause hazards at very high concentrations (Abida et al., 2008, Bahnasawy et

al., 2011). For the last few decades the determination of heavy metal levels in the marine environment and contamination of public food particularly fish has been gained a considerable interest (Alkkaahemet al., 2013).

Under favorable conditions sediments in rivers, lakes, and oceans acts as a sink for various pollutants like pesticides and heavy metals and also play a vital role in the remobilization of contaminants in aquatic environment (Öztürk et al., 2009). These contaminated sediments with toxic heavy metals in turn can threaten creatures i.e. worms, crustaceans and insects in the benthic environment, (Mason 1991). For the determination of state of pollution of aquatic ecosystem water and sediments are commonly used as indicators. Fish is a part of the human food, a rich source of essential amino acids and protein which play a proper role in the functioning of body tissues.

Fishes can accumulate trace metals and

pesticide residues in their bodies taken from water and through diet to an extent far greater than their concentration in water, sediments and food (Osman et al., 2007). The contamination of sea food like fishes with heavy metal has become an important concern worldwide due to their health hazards (Alhashemi et al., 2012, Pan & Wang 2012). Contaminants can enter the bodies of fishes through skin or gills and digestive tract via the food (Hussien & EL-Shafei 2015). In recent years fishes has been used as bio-indicators of the integrity of aquatic environmental systems (Opaluwa et al., 2012, Opaluwa & Umar 2010). It should be kept in mind that the fish species from these rivers are supplied to majority of other districts in this province. Keeping in view the toxicity of heavy metals, accurate information about their concentration in aquatic ecosystems is needed. Therefore, the present study was carried out to asses and compare heavy metals levels of water, sediments and fish species collected from three rivers in District Charsadda Khyber-Pakhtunkhwa Pakistan. Estimation of the risk level for the inhabitants of area associated with fish consumption was the aim of the study.

## 2.EXPERIMENTAL

### 2.1Materials and methods

#### 2.1.1 Description of the study area

The district Charsadda is the largest district of Khyber Pakhtukhwa, Pakistan based on the area (996km<sup>2</sup>) and lies between 34e030 and 34e380 north latitudes and 71e280 and 71e530 east longitudes. Malakand District lies on its north and Nowshera

and Peshawar districts on the south, Mardan District on the east and Mohmand Agency on the west. Charsadda has a total population of approximately 1 million. Charsadda, Tangi, and Shabqadar are the three tehsils administratively in district Charsadda. Farming handmade weaving cloth (Khamta), embroidery, leather shoes (Chappal) and small scale industries like brown sugar industries are the main earning sources of the people of Charsadda (Sardar et al., 2013). Three rivers namely Jindi, Khyali (Swat) and Kabul river passing through Charsadda which provides water for irrigation, domestic use and for drinking purposes. Biodiversity of fishes and other biota is present in these rivers. It is the main fishing source for the local residents (Fig. 1).

#### 2.1.2 Collection of samples

River water samples were collected in pre-rinsed plastic bottles with 0.01N nitric acid and kept in freezer. With the help of plastic spoon sediment samples were collected from those points where the water samples were taken. Sediment samples were air dried and kept in plastic containers. A number of three fish species (*Labeo rohita*, *Cyprinus carpio*, *Glyptothorax punjabensis*) were collected from various spots in the river Jindi, Khyali and Kabul rivers respectively. The fish samples were put in sterile polythene bags and taken to the laboratory. The samples were washed with tap water to remove dirt and stored at about -10°C. Then the samples were dissected with sterile scissors and kept in ample bottles, labeled and kept for digestion and analysis of heavy metals.

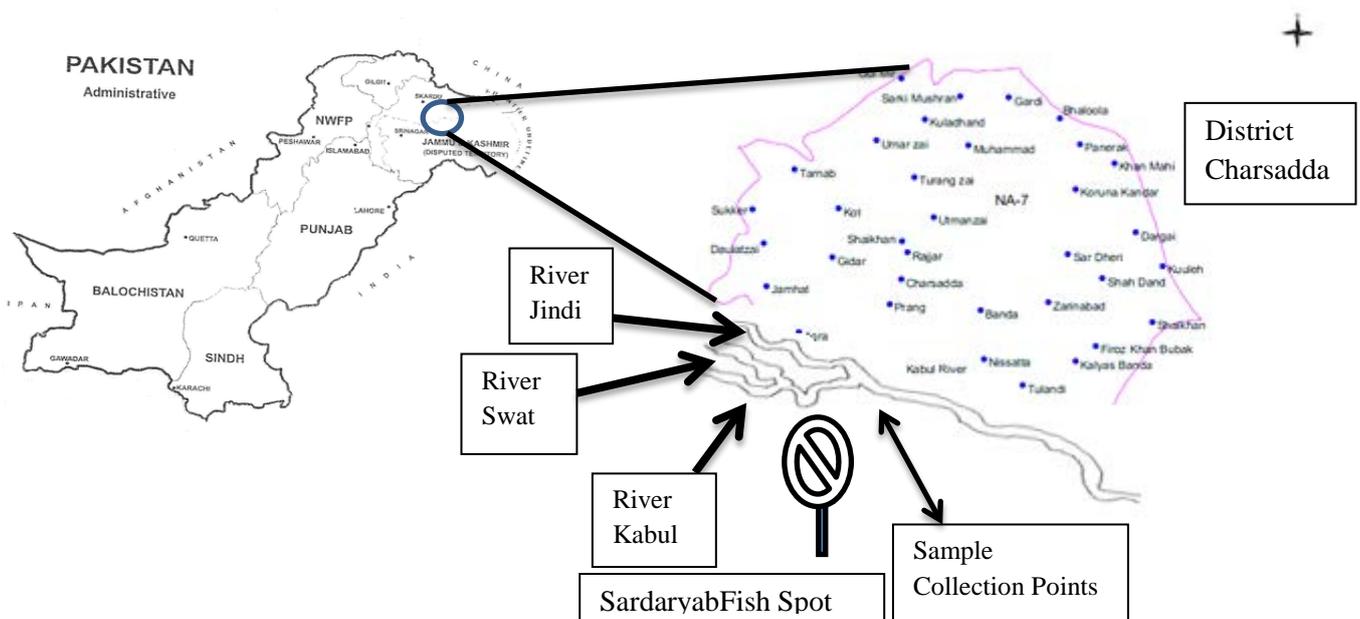


Figure 1. Location map of rivers and sample collection points in District Charsadda.

### 2.1.3 Sample treatment

To 250.0 ml of water sample 5ml of concentrated hydrochloric acid was added and then evaporated to 25.0ml. The concentrate was taken in flask and diluted to 50ml mark with de-ionized water. 15ml of nitric acid, 20ml perchloric acid and 15ml hydrofluoric acid was added to exactly 5.0 g of prepared sediment sample and placed on a hot plate for 3hours. When cooled the digest was filtered into a 100ml flask and diluted to the mark with distilled water.

Scales and skin were removed from fishes and the muscle tissues and gills were chopped into small pieces, ground well till homogeneity. Exactly 4 g of each sample was taken in a crucible and kept in the muffle furnace at the temperature of 550°C for 3 hours till ash was obtained. The resulting ash was dissolved in 10 ml of 20% HCl and filtered into 100 ml flask and diluted to 50ml with distilled water (Umar & Opaluwa 2010).

### 2.1.4 Mineral analysis

The concentration of cobalt, chromium, copper, manganese, nickel, antimony and zinc were determined in water, sediments and fish samples using Atomic Absorption Spectrophotometer (PerkinElmer Analyst PinAAcle™ 900T equipped with an AS-900 graphite furnace auto sampler and with deuterium background corrector) in Advanced Research laboratory, Department of Chemistry, Bacha Khan University Charsadda Khyber Pakhtunkhwa Pakistan. The instrumental conditions for different elements are given in table-1.

Table 1. Instrumental conditions for the investigated elements

Element	Acetylene (Lmin <sup>-1</sup> )	Air (Lmin <sup>-1</sup> )	Wavelength (nm)	Slit width (nm)
Co	2.50	10	240.7	0.2
Cr	2.50	10	357.9	0.7
Cu	2.50	10	324.8	0.7
Mn	2.50	10	279.5	0.2
Ni	2.50	10	232.0	0.2
Sb	2.50	10	217.6	0.2
Zn	2.50	10	213.9	0.7

Precession and accuracy of the analysis were ensured through repeated analysis of the samples against certified reference materials.

## 2.2 Health risk estimation

### 2.2.1 Estimated daily intake (EDI)

The estimated daily intake (EDI) of heavy metals (Co, Cr, Cu, Mn, Ni, Sb and Zn) were

evaluated according to the average concentration of each heavy metal in fish and the respective ingestion rate. The following relation was used to estimate EDI for respective metal.

$$EDI = \frac{Mc \times IR}{Bw \times 10^{-3}}$$

Where Mc stands for metal concentration, IR is the ingestion rate estimated to be 10g/person/day, and Bw is the average adult body weight which was taken as 70Kg.

### 2.2.2 Target hazard quotient (THQ)

For the estimation of risk level (non-carcinogenic) due to pollutant exposure target hazard quotient (THQ) is used. In order to estimate human health risk from consuming metal-contaminated fish, the target hazard quotient (THQ) was calculated as per USEPA Region III Risk-Based Concentration Table (Nazir et al., 2015). The following relation was used for estimating THQ

$$THQ = \frac{(M_c \times C_f \times IR \times EF \times ED)}{(RfD \times BW \times ATn)} \times 10^{-3}$$

Where EF is the exposure frequency (365 days/year), Cf is the conversion factor (00.208), ED is the exposure duration (30 years for noncancer risk as used by USEPA 2011), RfD is the reference dose of the metal and ATn is the average exposure time for noncarcinogens (EF×ED) (365 days/year for 30 years (i.e., ATn=10,950 days).

### 2.2.3 Hazard index(HI)

The hazard index (HI) is basically the sum of THQs of each metal and is calculated as

$$HI = THQ_{Co} + THQ_{Cr} + THQ_{Cu} + THQ_{Mn} + THQ_{Ni} + THQ_{Sb} + THQ_{Zn}$$

### 2.2.4 Target cancer risk(TR)

Target cancer risk (TR) is used to indicate carcinogenic risks. The following relation was used to estimate TR.

$$TR = \frac{M_c \times IR \times 10^{-3} \times CPSo \times EF \times ED}{BW \times ATc}$$

where CPSo indicates the carcinogenic potency slope, oral (mg/kg bw/day), and ATc is the averaging time, carcinogens (365 days/year for 70 year as used by USEPA (Aremu et al., 2011). Since CPSo value was known for Ni only so TR was calculated for Ni intake (Giri & Singh2014).

### 2.2.5 Statistical analysis

The results obtained were subjected to statistical analysis. The data has been presented as grand mean with standard deviations (SD).

### 3. RESULTS AND DISCUSSION

#### 3.1 Heavy metals in water sediments and fishes

The mean concentrations and standard deviations of heavy metals (Co, Cr, Cu, Mn, Ni, Sb and Zn) in water samples collected from three rivers in the district Charsadda is given in table-2. In case of water samples collected from three rivers Co, Cr, Cu, Mn, Ni, Sb and Zn mean concentration was found in the range of 0.24 -1.13mg/Kg, 0.16-0.35mg/Kg, 0.01 - 0.08mg/Kg, 1.15-3.47mg/Kg, 0.38-1.59mg/Kg, 1.71-1.97mg/Kg and 0.22-1.49mg/Kg respectively.

In case of water samples from the three rivers the mean Co concentration was higher than the WHO permissible limit (0.04mg/L). The higher Co concentration was found in case of Jindi and Swat river water. This can be attributed to the anthropogenic intrusions, atmospheric deposition, agricultural activities, untreated urban, and industrial wastes. Cr was also found higher than the acceptable limit (0.05mg/L). Highest Cr concentration was found in the Kabul river water (0.35mg/L) which might have resulted from the effluent coming from the tannery industries which submerges with river Kabul. Mn concentration was also found higher than WHO permissible limit (0.1mg/L). Highest concentration beyond 3.00mg/L was found in the water samples of Jindi and Swat rivers.

This can be attributed to the discharge of effluents from pharmaceutical industries to all of these rivers (Ahmed et al., 2015, Sivaperumal et al., 2007, Jan et al., 2010). Ni concentration in all water samples was found highest as compared with the WHO permissible limit (0.07mg/L) and other metals. This can be attributed to the high concentration of Ni in sediments samples from three lakes as it has been reported that Ni forms complexes with the humic materials present in the lakes (WHO 2008). Sb concentration was also found higher than the WHO permissible limit (0.02mg/L).

The mean concentrations and standard deviation of heavy metals (Co, Cr, Cu, Mn, Ni, Sb and Zn) in sediment samples collected from three rivers in the district Charsadda is given in table-3. In the sediments samples Co, Cr, Cu, Mn, Ni, Sb and Zn concentrations was found in the range of 0.55 - 0.63mg/Kg, 0.19-0.38mg/Kg, 0.10-0.20mg/Kg, 2.87-3.88mg/Kg, 0.89-1.09mg/Kg, 4.34-5.16mg/Kg and 0.77-1.39mg/Kg respectively.

The mean concentrations and standard deviation of heavy metals (Co, Cr, Cu, Mn, Ni, Sb and Zn) in fish species collected from three rivers in the district Charsadda is given in table 4. In case of fishes Co, Cr, Cu, Mn, Ni, As and Zn concentrations ranged from 0.23-0.25mg/Kg, 0.47-0.62mg/Kg, 0.035-0.074mg/Kg, 0.820-0.852mg/Kg, 4.165-4.244mg/Kg, 0.704-0.993mg/Kg and 0.602-1.583mg/Kg respectively. Though the concentration of Ni was low in water samples. This can be attributed to slow bioaccumulation of Ni in fishes.

Table 2. Metal concentration (mg/L) in water samples collected from selected rivers in district Charsadda

Samples	Co	Cr	Cu	Mn	Ni	Sb	Zn
Kabul	0.55±0.006	0.25±0.028	0.12±0.010	2.87±0.007	0.89±0.018	5.16±0.126	0.77±0.006
Jindi	0.63±0.005	0.38±0.080	0.20±0.003	3.88±0.037	1.09±0.003	5.09±0.184	1.26±0.004
Swat (Khyali)	0.57±0.017	0.19±0.056	0.10±0.003	3.66±0.014	0.89±0.007	4.34±0.014	1.39±0.005

Table 3. Metal concentration (mg/Kg) in sediment samples collected from selected rivers in district Charsadda

Samples	Co	Cr	Cu	Mn	Ni	Sb	Zn
Kabul	0.24±0.005	0.16±0.116	0.01±0.008	1.15±0.009	0.38±0.013	1.48±0.041	0.22±0.006
Jindi	1.13±0.015	0.35±0.044	0.08±0.005	3.47±0.043	1.57±0.005	1.97±0.687	1.49±0.030
Swat (Khyali)	1.02±0.022	0.30±0.083	0.05±0.005	3.224±0.020	1.59±0.012	1.71±0.142	1.327±0.009
WHO	0.04	0.05	2.0	0.1	0.07	--	3.0
USEPA	--	0.1	1.3	0.05	0.7	--	5.0
Pak-EPA	---	0.05	2.0	0.5	0.02	0.02	5.0

Table 4. Metal concentration (mg/Kg) in fish species collected from selected rivers in district Charsadda

Samples	Co	Cr	Cu	Mn	Ni	Sb	Zn
<i>Labeo rohita</i> ,	0.25±0.005	0.48±0.120	0.07±0.007	0.85±0.003	4.24±0.015	0.99±0.074	1.58±0.005
<i>Glyptothorax punjabensis</i>	0.23±0.013	0.62±0.227	0.05±0.001	0.82±0.003	4.18±0.013	0.82±0.022	0.60±0.002
<i>Cyprinus carpio</i> ,	0.24±0.011	0.47±0.087	0.03±0.004	0.83±0.005	4.16±0.020	0.70±0.041	0.85±0.002
WHO/FAO	---	0.2	0.5	0.1-0.5	0.5-0.6	---	5

Due to toxicity and potential human carcinogenicity in recent years Sb has received increasing attention. Freshwater prawn (*Macrobrachium caledonicum*) has been reported to have accumulated high concentration of Ni that was 1.00 mg/Kg. In the present study, highest concentration of Sb (0.99mg/Kg) was accumulated by *Labeo rohita*.

Zinc, is an essential micronutrient for both animals and humans. IT act as a cofactor for nearly 300enzymes in all marine organisms. Certain biological functions are regulated and maintained by Zn. Electrolyte imbalance, nausea, anemia, and lethargy are caused by excessive intake of Zn. In all the fish samples Zinc concentration was found within WHO/FAO permissible limit (5.0 mg/Kg). The highest concentration (1.58mg/Kg) was found to have been accumulated by *Labeo rohita*.

Cobalt is an integral part of vitamin B-12 and essential nutrient for human beings and also enhancing thyroid function. The deficiency of Co causes anemia while excessive intake of Co has been found to cause congestive heart failure and polycythemia (Jan et al., 2010). Co concentration was found nearly the same in all fish samples. There are no established criteria for the permissible level of Co in food commodities. The results indicated that *Cyprinus carpio* has accumulated higher concentration of Cr (0.62mg/Kg). Chromium concentration found in the present study was above the permissible limit of approximately 0.2 mg/Kg dry wt as recommended by FAO.

Copper concentration in human body increases due to consumption of metal contaminated fishes. Mostly Cu is added to macrobenthic fauna by surrounding industrial activities. The Cu concentration was found within WHO/FAO permissible limits (0.5mg/Kg) in all the fish samples.

Manganese is an essential element for both animals and plants. Skeletal and reproductive abnormalities in mammals has been found due to Mn deficiency. The maximum permissible limit for Mn is in fish tissues is 0.1-0.5mg/Kg. Mn concentration was found higher than the permissible limits in all the fish species (*Labeo rohita*, *Cyprinus carpio*, *Glyptothorax punjabensis*) owing to the higher Mn concentration in the water samples.

Natural sources, as well as food processing industries are the major sources of Ni in human beings (NAS-NRC National Academy of Sciences-National Research Council). High intake of Ni can cause cancer of the lungs and nasal cavity. All the fish samples have been found to have accumulated higher concentration of Ni in their bodies than the WHO/FAO permissible limit (0.5-0.6mg/Kg).

### 3.2 Estimated daily intake (EDI)

Dietary exposure approach is a reliable tool for the investigation of population's intake levels of bioactive compounds, nutrients and contaminants. The data obtained for the intake can be used to examine a specific element of interest (Mantoura et al., 1978, Ahmed et al., 2015).

Through this study, we have estimated the dietary intake and dietary exposure to seven trace elements through consumption of fish. The Estimated Daily Intake (EDI) of heavy metals (Co, Cr, Cu, Mn, Ni, Sb and Zn) were evaluated according to the concentration of each heavy metal in each food and the respective consumption rate. The EDI of the studied metals from consumption of fish are given in table 5. The mean values of EDI showed the same descending order of Ni =Mn= Sb=Zn > Co > Cu.

### 3.3 Health risk estimation

Actually the health risk assessments are used to express the excess probability of contracting cancer over a lifetime of 70 years and are based on assumptions. The target hazard quotient (THQ) values estimated for heavy metals have been presented in table 6. The acceptable guideline value for THQ is 1 (USEPA 2011). The THQ values estimated for different heavy metals were less than 1 in case of all three fish species.

This indicated no obvious noncarcinogenic health risk from intake a single heavy metal through consumption of the selected fish species. The highest THQ value was estimated for Cu followed by Sb contaminated in all the three fish species. Though the THQs for single metal ingestion for the other metals were within the acceptable limit. *Cyprinus carpio* showed the highest health risk among three fish species followed by *Glyptothorax punjabensis*.

Table 5. Estimated dietary intake (EDI) (mg/day) of heavy metals due to consumption of fish

Fishes	Co	Cr	Cu	Mn	Ni	Sb	Zn
<i>Labeo rohita</i>	4.3029E-05	8.2029E-05	1.26857E-05	1.4E-04	7.27E-04	1.7E-04	2.63E-04
<i>Cyprinus carpio</i>	3.9771E-05	1.0729E-04	8.40E-06	1.44E-04	7.17E-04	1.4E-04	1.03E-04
<i>Glyptothorax punjabensis</i>	4.1486E-05	7.98857E-05	6.00E-06	1.42E-04	7.14E-04	1.2E-04	1.45E-04

Table 6. Target hazard quotient (THQ) for different heavy metals and their hazard index from consumption of three fish species collected from three different rivers in Charsadda

Heavy metals	RfD(mg/Kg)	Target Hazard Quotient(THQ)		
		<i>Labeo rohita</i>	<i>Cyprinus carpio</i>	<i>Glyptothorax punjabensis</i>
Co	0.02	2.14E-01	1.98E-01	2.69E-01
Cr	0.009	1.85E-01	2.40E-01	1.79E-01
Cu	0.02	6.32E-00	4.18E-00	2.99E-00
Mn	0.003	1.09E-01	1.05E-01	1.06E-01
Ni	0.14	2.54E-03	2.50E-03	2.49E-03
Sb	0.001	4.20E-00	3.51E-00	3.00E-00
Zn	0.005	3.38E-01	1.28E-01	1.81E-01
Hazard Index(HI)		1.14E+01	8.36E+00	6.73E+00
Target Cancer Risk for (Ni)		1.67E-05	1.65E-05	1.64E-05

The HI for *Cyprinus carpio* was 8.36 while in case of *Glyptothorax punjabensis* 6.73 and for *Labeo rohita* was 1.14. This indicates that continuous and excessive intake of this fish especially *Cyprinus carpio* could result in chronic non-carcinogenic effect. The TR values was estimated for Ni only due to the CPSo value availability. The TR values for Ni was 1.67E-05 in case of *Labeo rohita*, 1.65E-05 for *Cyprinus carpio* and 1.64E-05 for *Glyptothorax punjabensis*.

Excessive consumption over a long time period might cause carcinogenic effect as the TR values were higher than the acceptable guideline value of 10-6 (USEPA 2011). This can be concluded that these fish species are safe for man consumption, the probability of contracting cancer is present for continuous consumption longer time or whole life.

#### 4. CONCLUSION

The investigated heavy metals concentration varied to a greater extent in all the water, sediments and fish samples. All water samples were found to have high metal concentration than the permissible limits except Mn and Zn.

The high input of these metals in these rivers can be attributed to the inclusion of industrial and house hold effluents. Owing to high concentration of metals in water samples the sediments and fishes also accumulated high concentration of metals. Individual heavy metal's THQ values were less than 1 for all three fish species indicating no any noncarcinogenic health risk. The highest THQ values were estimated for Cu followed by Sb contamination in all the three fish species. *Cyprinus carpio* showed the highest health risk among three fish species followed by *Glyptothorax punjabensis*. The HI for *Cyprinus carpio* was 8.36 while in case of *Glyptothorax punjabensis* 6.73 and for *Labeo*

*rohitawas* 1.14. So it can be concluded that continuous and excessive intake of fish *Cyprinus carpio* could result in chronic non-carcinogenic effect.

For investigation of potential health risks from dietary metal exposure constant monitoring of heavy metals is needed in all food commodities. The present study provide valuable information to the general public about the consumption of contaminated fishes.

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