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# Distribution and Bioaccumulation of Essential and Toxic Metals in Tissues of Thaila (*Catla catla*) from a Natural Lake, Pakistan and Its Possible Health Impact on Consumers

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**Abstract:** Although fish are often recommended as a component of a healthy diet, the environmental accumulation of heavy metals in many fish species has been of considerable concern for those weighing the nutritional health benefits against adverse toxic outcome of excess intake of toxic metals. This study aimed to determine the concentration of essential and toxic metals in the tissues of *Catla catla* in Mangla Lake and to assess the possible risk to the consumers. Fifty samples of *Catla catla* were collected from Mangla Lake, Mirpur, Azad Jammu and Kashmir, Pakistan and analyzed for eighteen metals including essential and trace metals. The measured range concentrations (µg/g, wet weight) in muscle tissues, in decreasing order, were: K (955–1632), Ca (550–2081), Na (449–896), Mg (129–312), Zn (61.2–215), Fe (11.6–26.8), Sr (2.60–9.27), Pb (1.72–7.81), Se (1.55–3.55), Co (0.12–4.08), Mn (1.04–4.33), Ni (0.69–3.06), Cu (0.88–2.78), Cr (0.45–1.88), As (0.67–1.58), Cd (0.28–0.56), Hg (0.17–0.57) and Li (0.12–0.38). The metal concentrations found in this study were comparatively higher than those reported in literature. A majority of the metals exhibited higher accumulation in gills compared with those in scales and muscles. Mean levels of Pb, As, Co, Mn, Cd, Cr and Zn in *Catla catla* muscle were found to be exceeding the international permissible limits for the safe human consumption. The condition factor (K), as an indicator of fish health status, indicated that *Catla catla* of Mangla Lake are in good health condition. The metal pollution index (MPI) of gills (27.9), scales (12.5) and muscle (7.57) indicated low contamination. Moreover, human health risk was evaluated using estimated weekly intake (EWI) and daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and target cancer risk (TCR). Estimated weekly and daily intake values for As, Cd, Cr, Hg, Ni and Pb were higher than provisional permissible tolerable weekly intake and permissible tolerable daily intake while THQ for As, Cd, Cr, Hg, Pb, Se and Zn was higher than 1. The THQ for As, Hg and Pb was several folds higher than 1, indicative of lifetime non-carcinogenic health risks to the consumers. The hazard index indicated cumulative risk, which greatly increased with increasing fish consumption. Target cancer risk indicated that the people eating the *Catla catla* from Mangla Lake were exposed to As, Cd, Cr, Ni and Pb with a significant lifetime carcinogenic risk. In summary, consumption of *Catla catla* from this lake was found to be associated with an increased lifetime risk to the general health of the consumers.

**Keywords:** *Catla catla*; Mangla Lake; risk assessment; metal pollution index; coefficient of condition



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## 1. Introduction

Fish is an important component of the human diet and generally known as a vital component of a well-balanced diet [1–5]. Fish consumption worldwide has been increasing in the last few decades due to the nutritional and preventive benefits. In addition to being a good source of low-fat high-quality protein, fish also provides omega-3 fatty acids, vitamins such as A, D, and B, calcium and phosphorus and minerals, such as iron, zinc and potassium [6–12]. It lowers the blood pressure, prevents cardiovascular diseases, diabetes, cancer, reduces Alzheimer's disease and dementia, normalizes cholesterol levels, reduces LDL (low-density lipoprotein) cholesterol, and increases HDL (high-density lipoprotein) cholesterol. The American Heart Association recommends eating fish at least two times per week as part of a healthy diet [5,9,13–21]. Regardless of their known health benefits, fish and other seafood may represent a risk to human health due to the bioaccumulation and biomagnification of contaminants through the food chain. Fish reside near the top of the aquatic food chain; therefore, they accumulate pollutants from the consumption of other aquatic organisms. Along with essential metals, toxic metals are also accumulated in fish tissues. Thus, the presence of toxic heavy metals in fish can lower their overall nutritional benefits [9,15,22–28].

Toxic metal contamination of aquatic environments and aquatic diets is a serious environmental issue worldwide [5,27,28]. Industrialization, agricultural activity, rapid urbanization, atmospheric deposition, geological weathering, etc. have resulted in aquatic environmental pollution [29–32]. Heavy metals, originating from natural as well as anthropogenic sources, continually enter the aquatic environment and pose serious threats to human health due to the tendency of metals to bioaccumulate and to persist due to metals being unable to be metabolized or chemically consumed [30,33–38]. Some metals are essential (e.g., Cu, Fe, Zn), while others (e.g., Cd, Pb, Hg) are non-essential yet can compete with essential metals for transport and utilization and are highly toxic to organisms [39]. It is well recognized that essential elements, i.e., Fe, Cu, Ni, Na, K and Zn, are vital in the biological systems and have important functions but very high intakes can cause adverse health problems [40]. Furthermore, metals like Cd, Hg and Pb have no biological role and, hence, they are toxic even at low levels [10,25].

Metal pollution levels of the aquatic environment can be assessed by analyzing water, sediments, flora and fauna for metal content [30,41–43]. In many studies, fish are employed as bioindicators of aquatic contamination [15,44,45]. Fish are good bioindicators of the effects of metals because some metals disturb important metabolic processes involved in reproduction, immune system, ecological degradation and pathological changes in tissues [44], and consumption of such metal-contaminated fish can cause adverse health effects, particularly for sensitive populations such as pregnant women and young children [44,46,47]. These bioindicator species collect metal contaminants from their environment with the passage of time and pass accumulated contaminants through the food chain to top predatory fish and higher trophic–level organisms, including fish-eating sea birds, sea mammals and human consumers [46,48–50]. Many factors, including both intrinsic species differences (trophic status, feeding strategy, age, gender, body size and phase of sexual reproduction) and extrinsic environmental conditions (the chemical form of the metal, contamination severity, and occurrence of other contaminants, and associated water quality conditions, such as salinity, temperature, pH, etc.), affect the accumulation of metals in fish [51–55]. Therefore, tissue metal levels can be used to give an integrated view of environmentally available metals, potential effects on the fish itself and on the potential adverse health risk to the consumer [3,56,57].

Currently, human health risks through consumption of contaminated aquatic food are generally recognized as a major health issue, and this concern is increasing globally [5,58–60]. Furthermore, the intake of toxic metal-contaminated food can reduce vital nutrients in the body, causing detrimental health effects; therefore, the risk assessment of these metals is a very crucial matter [61–63]. The analysis of metal content in fish can be extended to the assessment of potential effects to human health through the use of risk analysis. This can gauge the extent of the hazards to human health and begin to estimate the scope of the environmental metal pollution problem [10,64]. This method is typically based on the target hazard quotient (THQ). In addition, biometric parameters such as condition factor (CF) are used as indicators of metabolic status in fish which is highly dependent on water quality [65]. CF also provides information on habitat quality and health condition of fish populations [66,67]. Thus, this study was planned to gauge the risk assessment associated with *Catla catla* consumption supplied to local markets in Pakistan from Mangla Lake. *Catla catla* is one of the well-known fish found in the Mangla Lake and one of the popular fish varieties found in the local market almost available throughout the year. *Catla catla* marketing depends on domestic markets. In local markets, it is sold fresh. *Catla catla*, a surface-feeder, usually feeds on phytoplankton, zooplankton, small insects, and crustacean. April to late July is its spawning period. The carp, including *Catla catla*, an important aquatic food, is known as a protein major source from Pakistani aquatic bodies. Due to their high commercial demand and higher economic importance, all these species are cultured in priority base [68]. According to Mirza et al. [69], the total commercial fish production from the Lake had an average total catch of 441.26 mt/year in 2010, which was 130 mt higher as compared with the catches during the previous year, and their demand was increasing annually.

The main objectives of the present work were: (i) to evaluate metal levels: arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr) and zinc (Zn) in muscle, gills and scales of *Catla catla* from Mangla Lake, Mirpur Azad Kashmir, Pakistan, (ii) to compare metal accumulation in muscle, gills and scales of *Catla catla*, (iii) to compare metal data against international standards as well as worldwide reported levels in various reservoirs, (iv) to evaluate the possible sources of metals by principal component analysis and cluster analysis, (v) to assess the estimated daily/weekly intake (EDI/EWI), target hazard quotient (THQ), hazard index (HI) and target cancer risk (TCR) in *Catla catla* for the purpose of gauging the potential health risk to consumers.

## 2. Materials and Methods

### *2.1. Study Area*

Mangla Lake, one of the important freshwater resources in Pakistan (Figure 1), was built on the Jhelum River near Mirpur, Azad Jammu and Kashmir, Pakistan. Four rivers (two perennial and two non-perennial) were the feeding source to the Mangla Lake. This Lake has many positive impacts including agriculture growth, hydropower, water supply and fish farming. Fish species (*Cyprinus carpio*, *Catla catla, Wallago attu* etc.) having high market value are cultured on commercial basis. The Mangla Lake major contributions to population are that water is used for irrigation, hydroelectric production, domestic/drinking purpose and recreational activities. The untreated urban runoffs, poultry waste, industrial waste and agricultural runoffs around the Lake are the major pollution sources [70,71].

### *2.2. Sampling*

Fifty samples of *Catla catla* were collected by a standard procedure with the help of fishermen from Mangla Lake, using protocols designed by the USEPA 2003 [72]. Fish samples collected during sampling were stored immediately in an ice box for transportation to the chemical laboratory. Total length (mm) and body weight (g) were measured prior to dissection of muscle, gills and scales. Fish samples were washed with deionized water, bisected into muscle, scales and gills, put in clean dry polyethylene bags and stored at −20 °C until further metal analysis [3,32,73].

### *2.3. Sample Preparation and Analysis*

Fish samples were defrosted, weighed (wet weight) and dried at 102 °C for 12 h, then weighed again (dry weight) after cooling to room temperature. The dried fish tissues (1.000 ± 0.001 g) were digested with 5 mL concentrated nitric acid, 1 mL HCl and 8 mL deionized water in an appropriate digestion vessel using a closed-vessel microwave digestion system. The digestion vessel was wrapped and placed in the microwave oven system according to the manufacturer's instructions ensuring all safety considerations. The vessel was heated for at 300 W for 5 min; followed by 5 min at 600 W, 5 min at 900 W and 10–15 min at 1200 W, respectively, until a light yellow and clear solution was obtained and the vessel was allowed to cool for 5 min each time before further processing. The cooled digested solution was filtered through filter paper, and the final volume was adjusted to 50 mL [73–77]. A reagent blank without a sample was also prepared using the same procedure.

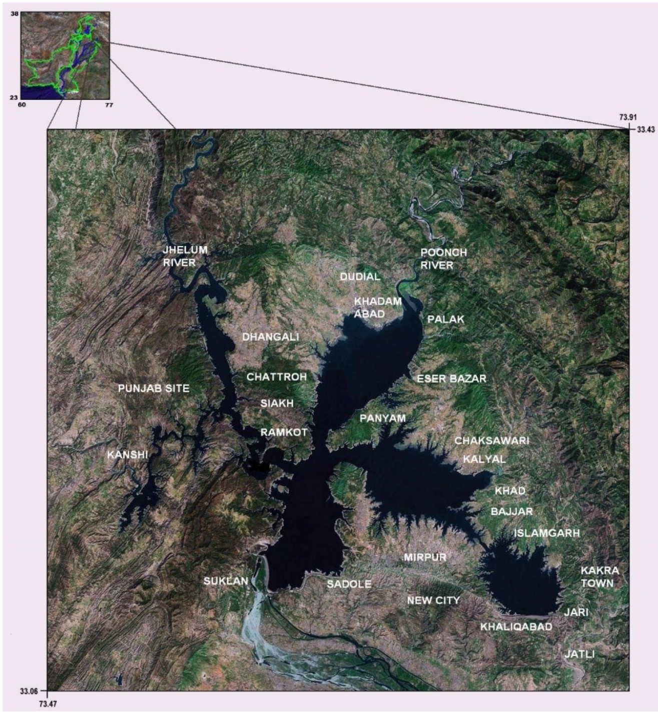


**Figure 1.** Location map of the study area.

Samples were analyzed using flame atomic absorption spectroscopy for the metals Ca, Co, Cu, Fe, K, Li, Mn, Mg, Na, Sr and Zn (Shimadzu AA 670, Kyoto, Japan) and by inductively coupled plasma mass spectrometry (ICP-MS) for the metals As, Cd, Cr, Hg, Ni, Pb and Se, using an Agilent ICP-MS (7500ce, USA), operated in the helium collision mode for Cr, Ni, As and Se (to eliminate interference from polyatomic species) and in standard mode for Cd, Hg and Pb. The RF power was 1500 W, and the isotopes measured were 75As, 114Cd, 52Cr, 201Hg, 60Ni, 208Pb and 82Se. All the measurements were recorded in triplicate. Quantification of the metals in fish samples was done by the calibration line method maintaining optimum analytical conditions [78]. Instrument settings were as recommended in the manufacturer's manual.

Quality control and quality assurance of fish tissue digestion and analysis were monitored using blanks and certified standard reference material (SRM 1946; Lake Superior Fish Tissue). Three standard reference samples were analyzed with every 20 samples for quality assurance, and three blanks were analyzed with each round of digestion to monitor contamination during extraction and in reagents and digestion vessels. Recoveries of the selected metals ranged from 92% to 105% of the certified value. All the chemicals used throughout the study were of analytical reagent grade. Deionized water was used to prepare all the reagents and standards. All glassware was soaked over-night in 10% ($v/v$) nitric acid, rinsed with distilled and deionized water and oven dried before use during this study [3,32,79].

### *2.4. Calculation of Accumulation Levels*

The metal pollution index (*MPI*) was calculated to estimate total accumulation of the metals in the various tissues. The metal pollution index (*MPI*) was calculated by the following equation [80–82]:

$$MPI = (M1 \times M2 \times M3 \times \ldots\ldots Mn)^{1/n} \quad (1)$$

where *Mn* is the concentration of an element *n* in the sample (µg/g). *MPI* describes the overall quality of environmental components with respect to metals, and has shown to be suitable tool in assessing pollution level.

### *2.5. Health Risk Assessment*

#### 2.5.1. Coefficient of Condition

The coefficient of condition (*K*) in fish samples was calculated for each sample by using the formula equation [83–85]

$$K = 100 \times W/L^3 \quad (2)$$

where *W* is weight in grams, and *L* is body length in centimeters.

#### 2.5.2. Assessment of Daily and Weekly Intakes

The potential health risk of the studied metals to consumers was measured using calculation of weekly and daily intake according to the following equation [82,86]:

$$EDI = (C \times IR)/BW \quad (3)$$

$$EWI = EDI \times 7 \quad (4)$$

where *C* is the mean metal concentrations in muscle tissue (µg/g, wet weight), *IR* is the ingestion rate (250 g/day/person), and *BW* is the human body weight (70 kg) [5,82,86,87].

#### 2.5.3. Assessment of Target Hazard Quotient (*THQ*), Hazard Index (*HI*) and Target Cancer Risk (*TCR*)

Target hazard quotient (*THQ*) is an assessment method to assess the possible non-carcinogenic risks to humans from pollutant intake [87,88]. The method to estimate *THQ* was provided in the USEPA Region III Risk-Based Concentration Table [89]

$$THQ = \left(C_{fish} \times IR \times 10^{-3} \times EF_r \times ED_{tot}\right)/(RfD \times BW_a \times AT_n) \quad (5)$$

where $C_{fish}$ is the mean metal level in fish (µg/g, wet weight); *IR* is the fish ingestion rate (0.250 kg/day) [87]; *EFr* is the exposure frequency (365 days/year); *EDtot* is the total exposure duration (70 years); *RfD* is the reference dose for the specific constituent (µg/g/day); *BWa* is the body weight, adult (70 kg); and *ATn* is the averaging time, non-carcinogens (*EDtot* × 365 day/year) [82,90,91].

The hazard index (*HI*) can be expressed as the sum of the hazard quotients for all selected metals [89]

$$HI = THQ_1 + THQ_2 + \ldots + THQ_n \quad (6)$$

where *THQ* is the target hazard quotients for *n* selected metals.

Target cancer risk (*TCR*) is used to assess the carcinogenic risks to the inhabitants from fish consumption [88]. The method to estimate *TCR* was also provided in the USEPA Region III Risk-Based Concentration Table [89]:

$$TCR = \left(C_b \times IRF \times 10^{-3} \times CPS_o \times EF_r \times ED_{tot}\right)/(BW_a \times AT_c) \quad (7)$$

where $CPS_o$ is the carcinogenic potency slope, oral (μg/g/day) − 1; $AT_c$ is the averaging time, carcinogens (70 × 365 days). Since $CPS_o$ values were not available for all the selected metals, the *TCR* of As, Cd, Cr, Ni and Pb were thus calculated only to indicate the lifetime carcinogenic risk to the populations [92].

### 2.6. Statistical Analysis

Statistical analysis can be used to measure the complex ecotoxicological processes by indicating the relationship and interdependency among the variables and their relative weights [93]. Standard statistical analyses (minimum, maximum, mean, median, standard deviation) were used to analyze the heavy metal data in fish. The most common multivariate statistical methods, principal component analysis (PCA), and cluster analysis (CA) were used to determine the relationship among metals in aquatic environments and their possible sources. A $p < 0.05$ was considered to be statistically significant. Statistical software package (STATISTICA-Version 5.5, Tulsa, OK, USA) was used for multivariate statistical analyses of the fish metal data [94]. Ward's method was used in cluster analysis, and results were shown in dendrogram while Varimax rotation methods were performed in principal components analyses. PCA was performed using principal component extraction with eigenvalue > 1 [95].

## 3. Results and Discussion

### 3.1. Biometric Data for *Catla catla*

The biometric data (length—L, weight—W, coefficient of condition—K) for *Catla catla* were measured. Ranges and mean levels (in bracket) of L, W and K were 29.7–46.0 cm (36.3 cm), 473–988 g (629 g) and 1.00–1.80 (1.33), respectively (Table 1). The coefficient of condition (K) has noteworthy roles in fishery management and reflects physical and biological condition, and energy level of the fish. This parameter can be influenced by variations feeding conditions, parasitic infections and physiological factors, and is used to assess the overall fish healthiness or robustness. The higher the K values, the healthier the fish [83–85,87]. Therefore, our data indicate that *Catla catla* was in relatively good health within in the waters of the Lake.

**Table 1.** Values of biometric parameters in *Catla catla*.

| | Weight (g) (W) | Length (cm) (L) | Coefficient of Condition (g/cm$^3$) (K) |
|---|---|---|---|
| Min | 473 | 29.7 | 1.00 |
| Max | 988 | 46.0 | 1.80 |
| Mean | 629 | 36.3 | 1.33 |
| Median | 588 | 35.5 | 1.30 |
| SD | 143 | 4.20 | 0.26 |

### 3.2. Metal Concentrations in Tissues of *Catla catla*

Determination of metal levels in fish tissue is very important for management authorities and is a major concern for human health. In the current study, statistical data for the tissue contents of the 18 metals (μg/g) in the muscle of *Catla catla* are shown in Table 2. These data are compared with reported levels in literature (Table 3). On average, the measured levels of As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr and Zn in muscle tissue were found to be 1.04, 944, 0.40, 2.08, 1.05, 1.57, 18.1, 0.33, 1254, 0.24, 187, 2.07, 639, 1.86, 4.09, 2.59, 5.10, 110 μg/g, respectively. Among the eighteen metals, the four electrolyte metals K, Ca, Na, and Mg form the group with highest contents. Of the remaining metals, Fe, Cu, Zn, Mn, Se, Co, Ni, Cr are essential but can become toxic at higher levels, and Pb, Cd, Hg, As are toxic even at low levels associated with environmental contamination [96,97].

Ca, K, Mg and Na are the major, most dominant and vital elements and found in all animal tissues. Ca and Mg are important in bones and for teeth formation while Na and K play important role in nerve impulses transmission and electrolyte balancing [97,98]. The average levels of Ca, K, Mg and Na in *Catla catla* were 944, 1254, 187and 639 µg/g, respectively. Their elements levels were higher than concentrations reported by Kalyoncu et al. [99] and found lower compared to Mogobe et al. [98].

Selenium is a well-known antioxidant/anti-inflammatory agent and an important constituent of various selenoproteins that avoid damage from free radicals and reactive oxygen species [100,101]. Selenoprotiens are important in the cellular defense of carcinogenesis, cardiovascular disease and inflammation [102,103]. Selenium levels varied from 1.55 to 3.55 µg/g in muscles with an average level of 2.59 µg/g. Average levels of selenium were higher than levels reported by Alam et al., 2002 [104], Schenone et al., 2014 [105], and Qin et al., 2015 [76].

**Table 2.** Statistical summary of selected metal distribution (µg/g) and metal pollution index (MPI) in various tissues of *Catla catla*.

| | | As | Ca | Cd | Co | Cr | Cu | Fe | Hg | K | Li | Mg | Mn | Na | Ni | Pb | Se | Sr | Zn | MPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Muscles | Min | 0.67 | 550 | 0.28 | 0.12 | 0.45 | 0.88 | 11.6 | 0.17 | 955 | 0.12 | 129 | 1.04 | 449 | 0.69 | 1.72 | 1.55 | 2.60 | 61.2 | 3.73 |
| | Max | 1.58 | 2081 | 0.56 | 4.08 | 1.88 | 2.78 | 26.8 | 0.57 | 1632 | 0.38 | 312 | 4.33 | 896 | 3.06 | 7.81 | 3.55 | 9.27 | 215 | 12.7 |
| | Mean | 1.04 | 944 | 0.40 | 2.08 | 1.05 | 1.57 | 18.1 | 0.33 | 1254 | 0.24 | 187 | 2.07 | 639 | 1.86 | 4.09 | 2.59 | 5.10 | 110 | 7.57 |
| | Median | 1.01 | 769 | 0.40 | 2.15 | 1.06 | 1.41 | 17.5 | 0.30 | 1249 | 0.25 | 171 | 1.63 | 619 | 1.93 | 3.72 | 2.71 | 4.78 | 101 | 7.20 |
| | SD | 0.24 | 458 | 0.07 | 1.18 | 0.44 | 0.52 | 4.06 | 0.11 | 162 | 0.08 | 48.8 | 0.96 | 110 | 0.67 | 1.74 | 0.54 | 1.76 | 43.8 | |
| Gills | Min | 0.71 | 4817 | 0.36 | 3.08 | 2.34 | 2.47 | 58.3 | 0.21 | 711 | 0.76 | 716 | 17.7 | 1306 | 1.33 | 5.93 | 2.77 | 30.4 | 1463 | 15.4 |
| | Max | 1.17 | 8288 | 1.39 | 14.0 | 10.1 | 5.26 | 132 | 1.59 | 1119 | 2.43 | 1383 | 31.7 | 2110 | 12.8 | 24.8 | 4.41 | 59.4 | 2180 | 40.7 |
| | Mean | 0.93 | 6520 | 0.83 | 7.89 | 5.84 | 4.03 | 94.3 | 0.81 | 896 | 1.62 | 976 | 25.3 | 1698 | 6.55 | 13.1 | 3.64 | 44.1 | 1785 | 27.9 |
| | Median | 0.92 | 6409 | 0.84 | 8.24 | 5.88 | 4.02 | 98.4 | 0.82 | 898 | 1.73 | 936 | 25.9 | 1692 | 8.15 | 12.6 | 3.64 | 43.3 | 1788 | 28.4 |
| | SD | 0.11 | 890 | 0.30 | 3.29 | 2.10 | 0.82 | 23.7 | 0.43 | 106 | 0.51 | 176 | 3.63 | 197 | 4.23 | 5.42 | 0.42 | 7.73 | 189 | |
| Scales | Min | 0.12 | 3867 | 0.54 | 1.82 | 0.87 | 1.89 | 20.7 | 0.05 | 126 | 0.71 | 364 | 8.68 | 485 | 0.39 | 3.56 | 1.51 | 19.5 | 209 | 6.78 |
| | Max | 0.33 | 5632 | 1.21 | 8.50 | 6.76 | 3.56 | 44.8 | 0.23 | 488 | 1.68 | 571 | 17.0 | 980 | 5.06 | 10.9 | 2.92 | 38.6 | 561 | 19.2 |
| | Mean | 0.21 | 4781 | 0.82 | 4.94 | 3.17 | 2.57 | 31.5 | 0.12 | 281 | 1.01 | 463 | 12.8 | 719 | 2.60 | 6.74 | 2.23 | 29.0 | 355 | 12.5 |
| | Median | 0.19 | 4812 | 0.79 | 5.71 | 2.73 | 2.47 | 31.2 | 0.10 | 287 | 0.93 | 464 | 13.0 | 705 | 2.42 | 6.64 | 2.23 | 29.2 | 344 | 12.2 |
| | SD | 0.06 | 496 | 0.18 | 2.23 | 2.03 | 0.45 | 6.88 | 0.05 | 109 | 0.27 | 54.4 | 2.22 | 132 | 1.41 | 1.96 | 0.37 | 4.79 | 91.1 | |
| Tolerable and permissible levels of selected metals in the muscles of fish | | | | | | | | | | | | | | | | | | | | |
| USEPA (1983) | | - | - | - | - | 8 | 120 | - | - | - | - | - | - | - | - | 4 | - | - | 480 | - |
| MAFF (2000) | | - | - | 0.2 | - | - | 20 | - | - | - | - | - | - | - | - | 2 | - | - | 50 | - |
| WHO (2000) | | 1 | - | 0.5 | 0.5 | 0.15 | 30 | 109 | 0.5 | - | - | - | 1 | - | 30 | 0.5 | - | - | - | - |
| FAO (2000) | | 1 | - | 0.5 | 0.5 | - | 30 | 180 | 0.5 | - | - | - | 0.5 | - | 55 | 2 | - | - | 30 | - |
| EC Regulation (2006) | | - | - | 0.1 | - | - | - | - | 0.5 | - | - | - | - | - | - | 0.3 | - | - | - | - |

Fish is major source of iron for humans. It is an integral component of hemoglobin, myoglobin, ferritin, hemosiderin and many enzymes; it also plays a vital role in overall brain functioning [106,107]. Iron deficiency causes anemia, while excess intake may cause a variety of adverse health effects, such as cancer, diabetes, liver and heart diseases [108–111]. In the present study, Fe levels ranged from 11.6 to 26.8 µg/g. The mean level of Fe (18.1 µg/g) in muscle was higher than most of the reported values in literature [29,76,87,99,104,112,113]. The measured metal levels were also compared with the permissible levels (109 µg/g and 180 µg/g as per WHO and FAO guidelines, respectively [114,115], which revealed that the mean level of Fe in muscles was lower than the recommended limits and, thus, is of no risk for consumers.

Lithium can take part in some important biological processes in the human body such as, functioning of enzymes, hormones, vitamins and in female reproductive systems [116,117]. Its deficiency has been found to be associated with high rates of suicides, homicides and drug cases [118]. In this study, Li ranges from 0.12–0.38 µg/g, which is higher than values reported by Schenone et al., 2014 [105] and Qin et al., 2015 [76] but lower than that by Kalyoncu et al., 2012 [99].

Strontium is considered a non-essential element. However, excess consumption may result in adverse health effects such as bone growth problems in children, hard tissues, hypocalcaemia, anaemia and cancer [96,97,119,120]. In the current study, the range of Sr was observed to be 2.60–9.27 (µg/g) with an average of 5.10 (µg/g). Average levels of Sr were noted to be lower than those reported by Kalyoncu et al., 2012 [99] and Schenone et al., 2014 [105].

Manganese is an essential metal and required in a trace amount for the processing of carbohydrate, protein and cholesterol for animals and plants. It is associated with bone formation and also present in many enzymes. Its deficiency causes severe skeletal and reproductive abnormalities [87,121,122]. The mean manganese level in fish muscles was found to be 2.07 µg/g, which was higher than those reported by Iqbal and Shah, 2014 [87], Schenone et al., 2014 [105], Qin et al., 2015 [76], Hao et al., 2013 [112], Alam et al., 2002 [104], Mogobe et al., 2015 [98], whereas the Mn level in all samples (100%) was higher than the international permissible limits [114,123].

Copper is an essential nutrient for human health. Cu is an essential part of several enzymes and required for hemoglobin synthesis and strengthening bones; nevertheless, high intake causes liver and kidney damage [40,97,121,122,124,125]. In the present study, Cu was found in all the tested fish samples and the concentrations ranged from 0.88 to 2.78 µg/g with an average of 1.57 µg/g. Similarly, the average level of Cu in fish muscle was higher than those reported in literature [29,76,87,98,104,105,113,126]. The mean level of Cu was observed to be lower than the international permissible limits [114,115,123,127], indicating no risk to consumers.

**Table 3.** Comparison of measured average metal levels (µg/g) in the muscles of *Catla catla* and other reported levels worldwide.

| As | Ca | Cd | Co | Cr | Cu | Fe | Hg | K | Li | Mg | Mn | Na | Ni | Pb | Se | Sr | Zn | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.04 | 944 | 0.40 | 2.08 | 1.05 | 1.57 | 18.1 | 0.33 | 1254 | 0.24 | 187 | 2.07 | 639 | 1.86 | 4.09 | 2.59 | 5.10 | 110 | Present Study |
| - | 132.4 | 2.27 | 2.8 | 13.48 | 1.6 | 12.33 | - | 295 | 23.19 | 202.2 | 9.94 | 467 | 1.57 | 2.41 | - | 10.77 | 24.27 | [99] |
| - | - | 0.745 | 4.917 | 2.389 | 1.293 | 9.835 | - | - | - | - | 0.487 | - | - | 8.796 | - | - | 24.23 | [87] |
| 0.27 | - | <0.01 | 0.058 | 2.23 | 1.35 | 52.1 | - | - | 0.046 | - | 1.58 | - | 1.04 | 1.3 | 2.28 | 22.9 | 20.8 | [105] |
| 0.090 | - | 0.014 | BDL | 0.173 | 0.241 | 7.12 | 0.010 | - | 0.013 | - | 0.1 | - | 0.165 | 0.181 | 0.23 | 1.15 | 6.62 | [76] |
| - | - | 0.006 | 0.01 | | 1.26 | 12.31 | 0.76 | - | - | - | - | - | - | 0.02 | - | - | 30.6 | [29] |
| 0.166 | - | 0.011 | - | 0.4 | - | 15.9 | 0.073 | - | - | - | 1.95 | - | - | 0.047 | - | - | 24.4 | [112] |
| 0.095 | - | 0.009 | 0.005 | 0.067 | 0.249 | 2.729 | - | - | - | - | 0.307 | - | 0.041 | 0.031 | 0.3 | - | 5.433 | [104] |
| - | - | 0.84 | 0.23 | 0.365 | 0.52 | 0.85 | - | - | - | - | - | - | 0.08 | 0.22 | - | - | 0.92 | [113] |
| - | 4130 | - | - | - | 0.2 | 41.5 | - | 2490 | - | 350 | 0.6 | 860 | - | - | - | - | 16.3 | [98] |
| - | - | 0.02 | 0.1 | 0.05 | 0.66 | - | 0.04 | - | - | - | - | - | 0.16 | 0.06 | - | - | 3.42 | [126] |

Zinc, an essential metal for human health, is an important component of cells, enzymes, and cofactors. It is one of the most important trace and microelements for the human body and involved in many biological functions. It has a protective effect against cadmium and lead toxicities [40,97,125,128]. The concentration of Zn in fish muscle in this study (61.2–215 µg/g: 110 µg/g) is higher in those reported elsewhere (0.92–30.6 µg/g) (Table 3). The average Cu concentration (110 µg/g) in tissue was higher than tolerable and permissible levels of zinc in the muscle of fish given by FAO, 2000 [115] and MAFF, 2000 [123] but lower than the limit set by USEPA, 1983 [127].

Cobalt, an essential part of vitamin B12, has also been involved in the production of red blood cells and is necessary for proper thyroid function [87,97]; however, high levels of cobalt may cause lung damage, hair loss, bleeding and even death [122]. The measured levels of Co in fish muscle varied from 0.12 to 4.08 µg/g. Average Co concentration (2.08 µg/g) is lower than levels reported by Kalyoncu et al., 2012 [99] and Iqbal and Shah, 2014 [87] but higher than those by Alam et al., 2002 [104], Monroy et al., 2014 [29], Schenone et al., 2014 [105], Qin et al., 2015 [76], Kumar et al., 2020 [113] and Storelli et al., 2020 [126]. The measured levels of Co were also higher than the international permissible limits [114,123].

Chromium, which is commonly measured as a pollutant, is considered as a vital element for carbohydrate and fat metabolism in humans and its hexavalent form causes carcinogenicity, respiratory and reproductive disorders [63,129–131]. The measured levels of Cr in fish muscle varied from 0.45 to 1.88 µg/g with an average concentration of 1.04 µg/g. Chromium was detected in almost all the muscle samples, with the highest concentration being 1.88 µg/g, which is within the limits of 8 µg/g by USPEA [127] but higher than 0.15 µg/g by WHO [114]. The average level of Cr in fish muscles in this study is higher than reported values by Hao et al., 2013 [112], Qin et al., 2015 [76], Alam et al., 2002 [104], Kumar et al., 2020 [113] and Storelli et al., 2020 [126] but lower than that from Karacaoren Dam (Turkey) by Kalyoncu et al., 2012 [59] and Rawal Lake (Pakistan) by Iqbal and Shah, 2014 [87].

Nickel is a ubiquitous element in nature and essential for normal growth of many species of microorganisms and plants and several species of vertebrates [132]. It is required in low concentrations in the living organisms but elevated levels may result in carcinogenic effects [133]. Nickel concentrations in this study ranged from 0.69–3.06 µg/g in fish muscle. However, the mean Ni level (1.86 µg/g) was well within limits set by WHO, 2000 [114] and FAO, 2000 [115] but higher than those all worldwide reported values (Table 2) in fish muscle. The high level of Ni in the *Catla catla* of Mangla Lake indicates a potential negative impact for fish consumption.

Mercury is a non-essential, carcinogenic metal that is capable of accumulating in higher organisms, due to a strong tendency of biomagnification. Mercury is also well known to be a neurotoxin when in the methylated form, and can cause other adverse health problems [44,131,134,135]. The minimum and maximum mercury levels in *Catla catla* muscles were noted as 0.17 and 0.57 µg/g, respectively. The European Commission Regulation (2006) [136] as well as WHO (2000) [114] and FAO (2000) [115] set the limit of mercury levels to be 0.50 µg/g which was higher than our mean level found in *Catla catla*. Similarly, the average mercury level (0.33 µg/g) in fish muscle is lower than those reported by Monroy et al., 2014 [29] while higher than those by Qin et al., 2015 [76], Hao et al., 2013 [112] and Storelli et al., 2020 [126].

Cadmium is a toxic metal of high concern, has no essential biological activity in humans, and its exposure is mostly through the consumption of contaminated food and not from drinking water. Cadmium ions are well known to be complexed by metallothionein proteins, thereby limiting the toxicity [125,137]. In our study, the Cd levels in muscle varied from 0.28 to 0.56 µg/g with an average value 0.40 µg/g. Maximum permissible level for fish is 0.5 µg/g set by WHO (2000) [114] and FAO (2000) [115]. This indicated that Cd levels in the fish from Mangla Lake were below the limits set WHO (2000) [114] and FAO (2000) [115], while were above the limits set by 0.2 µg/g (MAFF, 2000) [123] and 0.1 µg/g (EC regulation, 2000) [136]. Similarly, the average value of Cd was well above the reported values by Alam et al., 2002 [104], Hao et al., 2013 [112], Monroy et al., 2014 [29], Schenone et al., 2014 [105], Qin et al., 2015 [76] and Storelli et al., 2020 [126], while lower than reported values by Kalyoncu et al., 2012 [99], Iqbal and Shah, 2014 [87] and Kumar et al., 2020 [113].

Lead is a persistent, non-essential metal with no known biological function in humans. Lead has been associated with various cancers, liver and kidney damage, and can lead to impaired neurological development in children [138–140]. In the present study, Pb was found in all the analyzed fish samples and the concentrations ranged from 1.72 to 7.81 µg/g with an average value 4.09 µg/g. Similarly, the average level of Pb in fish muscle from Mangla Lake is higher than those reported in literature: 2.41 µg/g [99], 1.3 µg/g [105], 0.181 µg/g [76], 0.02 µg/g [29], 0.047 µg/g [112], 0.031 µg/g [104], 0.22 µg/g [113] and 0.06 µg/g [126]. The mean level of Pb was observed to be higher than the international permissible limits [114,115,123,127,136], indicating a potential risk to people eating *Catla catla*.

Arsenic is a well-known carcinogen, affects the central and peripheral nervous systems, disrupts the heart rhythm, and can cause melanosis and hyperkeratosis [131]. The highest concentration of arsenic was found in fish muscle, with a maximum value of

1.58 μg/g. Compared with published data, the average value of As was well above the reported values by Schenone et al., 2014 [105], Qin et al., 2015 [76], Hao et al., 2013 [112] and Alam et al., 2002 [104]. The average arsenic concentration (1.04 μg/g) is higher than maximum permissible level for fish (1 μg/g) set by WHO (2000) [114] and FAO (2000) [115], suggesting an increased risk to the fish consumers.

Descriptive statistics for selected metal levels (μg/g) in the gills and scales of *Catla catla* are also shown in Table 2. The mean level in gills for Ca is highest (6520 μg/g), followed by Zn (1785 μg/g), Na (1698 μg/g), Mg (976 μg/g), K (896 μg/g) and Fe (94.3 μg/g), whereas Se (3.64 μg/g), Li (1.62 μg/g), As (0.93 μg/g), Cd (0.83 μg/g), and Hg (0.81 μg/g) exhibited relatively lower concentrations. Overall, the metal contents showed the following decreasing order based on average levels in the gills samples: Ca > Zn > Na > Mg > K > Fe > Sr > Mn > Pb > Co > Ni > Cr > Cu > Se > Li > As > Cd > Hg. Average concentrations of As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr and Zn in the scales were measured at 0.21, 4781, 0.82, 4.94, 3.17, 2.57, 31.5, 0.12, 281, 1.01, 463, 12.8, 719, 2.60, 6.74, 2.23, 29.0 and 355 μg/g, respectively. Among the selected metals, Ca, Na, Mg, Zn and K were the dominant contributors, while Li, Cd, As and Hg were the minor components in the scales of *Catla catla*. Ranking in decreasing order, the mean metal contents of scales were: Ca > Na > Mg > Zn > K > Fe > Sr > Mn > Pb > Co > Cr > Ni > Cu > Se > Li > Cd > As > Hg. To compare the total metal accumulation in the different tissue, the metal pollution index (MPI) was calculated (Table 2). Gills showed a higher MPI mean value (27.9), followed by scales (12.5) and muscle (7.57). This result clearly indicates that each tissue has a different capacity of accumulation, and higher metal accumulation was observed in gills as compared with scales and muscle. According to Jamil et al. (2014) [141], when MPI in the tissues ranged from 5 to 10, the contamination is low, while the MPI index values that varied between 2 and 5 suggests a very low contamination, and MPI < 2 means that not contaminated [142]. MPI value in this study was higher than 5, indicating low contamination in tissue following decreasing trends: Gills > Scales > Muscle.

### 3.3. Comparison of Metal Levels in Fish Tissues

*Catla catla* is a surface feeder and they are also omnivorous in nature. Metal levels assessment in fish tissue reflects the exposure of fish to these metals in the aquatic environment. Fish accumulate metals through respiration, adsorption, and ingestion. Different tissues have different accumulating capacities of metals, which may be due to differences in metabolic activity, changes in environmental pollution and functions of organs [143,144]. Furthermore, metal levels in different fish species might be due to different ecological requirements, metabolisms, age, size, and length of the fish, their habitats and feeding patterns [9,145].

Metal level comparisons in muscle, gills and scales of *Catla catla* are shown in Figure 2. An examination of the data document that the mean levels of Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Na, Ni, Pb, Sr and Zn were comparatively higher in the gills, showing the following decreasing order among the three tissues: gills > scales > muscles. However, average concentrations of As and K were highest in muscle tissue, followed by gills with the lowest metal content in scales. In addition, Hg and Se exhibited the highest concentrations in the gills but lowest in the scales, giving the overall order of: gills > muscles > scales. In general, the gills showed elevated metal levels over the other tissues. The gills are considered a greater metal accumulator than muscles due to metal complexation with mucus [146]. Another factor is that the gills are the interface of metal ion exchange from the surrounding water and the fish circulatory system, and the tremendous surface area of this organ maximizes metal transport and thus metal accumulation. Therefore, the metal contents accrued in the gills were mainly concentrated from water [9,147,148].

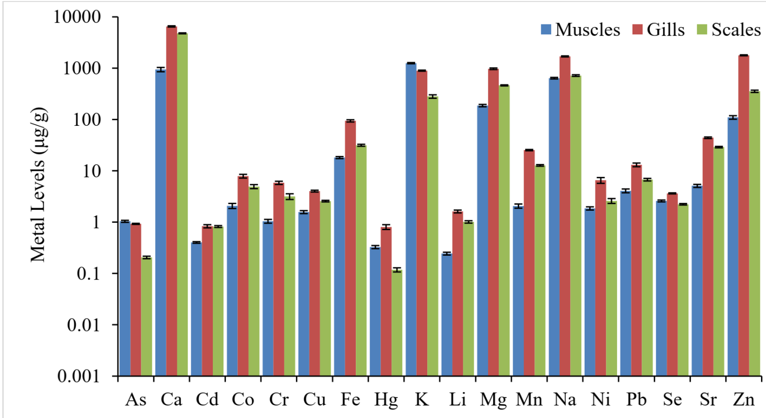


**Figure 2.** Comparison of average metal levels (µg/g ± SE) in the muscles, gills and scales of *Catla catla*.

### 3.4. Multivariate Analyses of Selected Metals in the Fish Species

Trace metals in sediments and fish may threaten the existence and survival of aquatic biota and human health; therefore, it is considered imperative to investigate and regulate the pollution sources. One of the most important aspects of the present study was source apportionment of metal pollutants in the aquatic system. For this purpose, principle component analysis (PCA) and cluster analysis (CA) were employed to assess the possible pollution sources in *Catla catla*. The PC loadings of selected metals in the fish samples are shown in Table 4, where five PCs were extracted with eigenvalues greater than one, together explaining more than 82% of cumulative variance of the data. The dendrogram of CA related to the metal levels in fish samples is shown in Figure 3, which exhibited four significant clusters of the studied metals. In the case of PCA, the first PC (PC1) showed the highest loadings for Cu, Fe, K, Mg, Na and Zn; PC2 exhibited highest loadings for Ca, Mn and Sr; PC3 revealed highest loadings for Co, Cr and Se; PC4 demonstrated highest loadings for Cd, Li and Ni, while last PC exhibited highest loadings for As, Hg and Pb.

**Table 4.** Principle component analysis of selected metals in *Catla catla*.

| | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 |
|---|---|---|---|---|---|
| Eigenvalue | 5.714 | 4.337 | 1.776 | 1.584 | 1.323 |
| Total Variance (%) | 31.74 | 24.10 | 9.869 | 8.799 | 7.347 |
| Cumulative Eigenvalue (%) | 5.714 | 10.05 | 11.83 | 13.41 | 14.73 |
| Cumulative Variance (%) | 31.74 | 55.84 | 65.71 | 74.51 | 81.86 |
| As | - | - | - | - | 0.689 |
| Ca | - | 0.843 | - | - | - |
| Cd | - | - | - | 0.806 | - |
| Co | - | - | 0.736 | - | - |
| Cr | - | - | 0.792 | - | - |
| Cu | 0.836 | - | - | - | - |
| Fe | 0.877 | - | - | - | - |
| Hg | - | - | - | - | 0.872 |
| K | 0.825 | - | - | - | - |
| Li | - | - | - | 0.898 | - |
| Mg | 0.769 | - | - | - | - |
| Mn | - | 0.805 | - | - | - |
| Na | 0.866 | - | - | - | - |
| Ni | - | - | - | 0.769 | - |
| Pb | - | - | - | - | 0.630 |
| Se | - | - | 0.837 | - | - |
| Sr | - | 0.893 | - | - | - |
| Zn | 0.871 | - | - | - | - |

Only main values are presented.

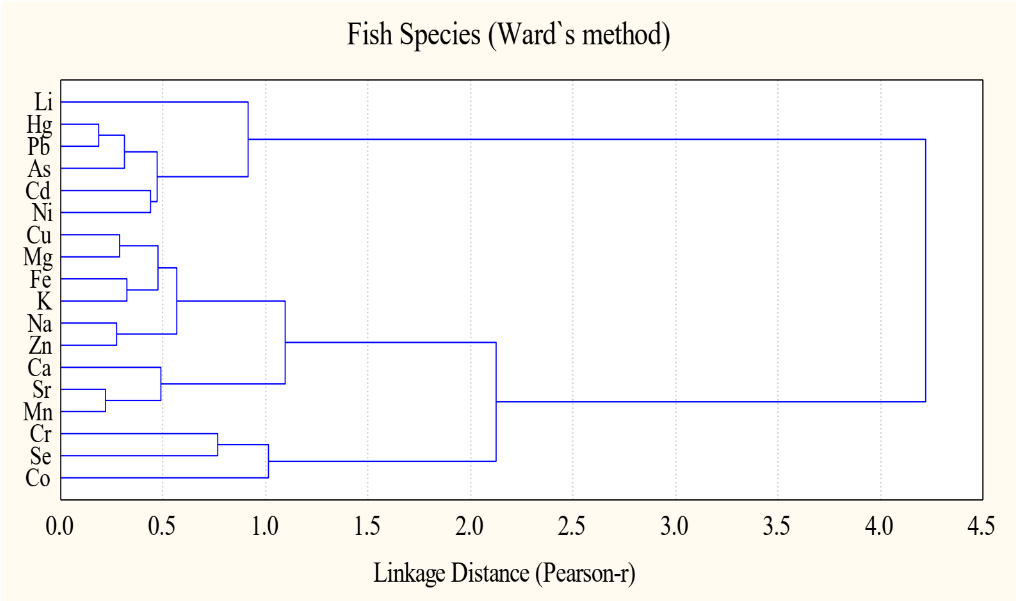


**Figure 3.** Cluster analysis of selected metals in *Catla catla*.

Similarly, in CA, the first cluster was composed of Hg–Pb–As–Cd–Ni–Li which showed that these metals were mostly contributed by the anthropogenic activities. Cd and Pb could be initiated from agricultural activities, fertilizers, pesticides and industrial emissions. In the past, Pb had been ascribed to the excessive use of gasoline as an additive [149]. The sources for As and Hg could be agriculture and industrial runoffs [150]. Moreover, Cd, Co and Pb might be linked with untreated sewage sludge, domestic/ agricultural wastes and boating activities [151–153]. Likewise, the second cluster consisted of Cu–Mg–Fe–K–Na–Zn and the third cluster was comprised of Ca–Sr–Mn. The metals in these two clusters were mainly contributed by natural inputs/lithogenic sources. The last cluster contained Cr–Se–Co, which were mostly contributed by mixed sources (anthropogenic as well as lithogenic contributions). Co, Cr and Ni may be from anthropogenic inputs such as fertilizers, limestone, excavation activities and manure [71,154,155]. Overall, CA results were in very good agreement with PCA findings and both multivariate methods revealed significant contamination inputs in the water Lake.

### *3.5. Potential Health Risk Assessment*

A fish diet has many health benefits, but consumption of fish contaminated with toxic substances may actually threaten the health of the consumers [3,90]. Risk assessment measures the impacts of toxic substances exposure on human health, and has been defined by the USEPA among other government entities. Risk assessment for heavy metals is estimated by estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and target cancer risk (TR). These parameters, which were presented by US-EPA for the potential health risk estimation, depend on various factors (amount of contaminant intake, exposure frequency and duration, average body weight and oral reference dose (RfD) [156]. Human health risk assessments associated with the consumption of *Catla catla* were also evaluated and are shown in Table 5.

**Table 5.** Description of health risk assessment for selected metals in the muscles of *Catla catla* from Mangle Lake.

| | RfD/RDA (mg/kg/day, Wet Weight) | PTWI * | PTDI * | EWI | EDI | THQ | TCR |
|---|---|---|---|---|---|---|---|
| As | 0.0003 | 0.015 | 0.002 | 0.026 | 0.004 | 12.36 | 5.56E-03 |
| Ca | 13.33 | 99.33 | 14.19 | 23.59 | 3.370 | 0.253 | |
| Cd | 0.001 | 0.007 | 0.001 | 0.010 | 0.0014 | 1.438 | 8.63E-04 |
| Co | 0.06 | 0.42 | 0.06 | 0.052 | 0.007 | 0.124 | |
| Cr | 0.003 | 0.021 | 0.003 | 0.026 | 0.004 | 1.242 | 1.86E-03 |
| Cu | 0.04 | 3.5 | 0.5 | 0.039 | 0.006 | 0.140 | |
| Fe | 0.7 | 5.6 | 0.8 | 0.453 | 0.065 | 0.093 | |
| Hg | 0.0003 | 0.005 | 0.0007 | 0.008 | 0.001 | 3.900 | |
| K | 78.0 | 548.3 | 78.33 | 31.35 | 4.478 | 0.057 | |
| Li | 0.002 | 0.14 | 0.02 | 0.006 | 0.001 | 0.436 | |
| Mg | 5.83 | 40.83 | 5.833 | 4.675 | 0.668 | 0.115 | |
| Mn | 0.14 | 0.98 | 0.14 | 0.052 | 0.007 | 0.053 | |
| Na | 55 | 385 | 55 | 15.97 | 2.282 | 0.041 | |
| Ni | 0.020 | 0.035 | 0.005 | 0.046 | 0.007 | 0.331 | 1.13E-02 |
| Pb | 0.004 | 0.025 | 0.004 | 0.102 | 0.015 | 7.297 | 1.24E-04 |
| Se | 0.005 | 2.8 | 0.4 | 0.065 | 0.009 | 1.851 | |
| Sr | 0.6 | 4.2 | 0.6 | 0.127 | 0.018 | 0.030 | |
| Zn | 0.3 | 7 | 1 | 2.751 | 0.393 | 1.310 | |
| | | | | | HI | 31.07 | |

* Provisional permissible tolerable weekly intake (PTWI) in mg/week/kg body weight; Permissible tolerable daily intake (PTDI), in mg/day/kg body weight.

Average values of EWI and EDI for As, Cd, Cr, Hg, Ni and Pb were noted to be higher than the recommended provisional permissible tolerable weekly intake (PTWI) in mg/week/kg body weight; permissible tolerable daily intake (PTDI) values, while the remaining metals were within the recommended limits. According to the New York State Department of Health, 2007, if the EDI/ RfD ratio of heavy metal was equal to or less than the RfD, then the risk will be minimum. However, if it is >1–5 times the RfD, then risk will be low, if >5–10 times the RfD, then risk will be moderate; however, if it is >10 times the RfD, then the risk will be high. The EDI/ RfD ratio obtained for Cd, Cr and Zn was approximately 1.5-fold higher, two-fold for Se, four-fold for Pb and Hg and twelve times higher for As than their RfD values, indicating potential health hazard to the consumers, particularly for As; Hg and Pb registered moderate risk and Cd, Cr, Se and Zn manifested low risk, while EDI/ RfD ratios for the remaining metals were below one, thereby showing no risk.

Non-carcinogenic human health risks were also assessed for the consumer of *Catla catla* from Mangla Lake by using the THQ approach. THQ values higher than 1 would indicate a potential non-carcinogenic health risk to consumer health via contaminated fish consumption [90,157]. The measured THQ values for As, Cd, Cr, Hg, Pb, Se and Zn were higher than unity, indicating that the consumption of contaminated *Catla catla* may cause non-carcinogenic risks. The HI value was observed to be 31.07, thus exhibiting lifetime non-carcinogenic health risks to the consumers.

The carcinogenic risk for As, Cd, Cr, Ni and Pb related with fish consumption was also evaluated. Significantly higher values of target cancer risk (TCR) were obtained for As (5.56E-03), Cd (8.63E-04), Cr (1.86E-03), Ni (1.13E-02) and Pb (1.24E-04), which is considerably higher than the acceptable risk limit ($1 \times 10^{-6}$) [89], indicating that the consumption of *Catla catla* from Mangla Lake on a continuous basis is associated with lifetime carcinogenic risk.

## 4. Conclusions

In the present study, the concentrations of eighteen elements in *Catla catla* in Mangla Lake, consumed by the local and Punjab province population and their potential health risk were examined. On average, the measured levels of As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr and Zn in the muscles were found to be 1.04, 944, 0.40, 2.08, 1.05, 1.57, 18.1, 0.33, 1254, 0.24, 187, 2.07, 639, 1.86, 4.09, 2.59, 5.10 and 110 µg/g, respectively. The mean levels of most elements are higher in the gills than scales and muscles. The metal levels in muscles in this study were mostly higher than those reported in literature. The average concentration of As (in all samples), Mn (in all samples), Zn (in all samples) and Co (in 90% of the samples) was found to be higher than tolerable and permissible levels given by FAO, 2000 and WHO, 2000. Chromium levels were also above the limits set by WHO, 2000. Cd levels were above the limits (0.2 µg/g) set by MAFF (MAFF, 2000) and 0.1 µg/g EC (EC regulation, 2006). Mean levels of Pb were observed to be higher than the international permissible limits (USEPA, 1983; FAO, 2000; MAFF, 2000; WHO, 2000; EC regulation, 2006), indicating that fish is contaminated by these metals. Metal pollution index (MPI) in gills, scales and muscles revealed 27.9, 12.5 and 7.57, indicating low contamination in fish tissue. The PCA and CA indicated that As, Cd, Co, Cr, Hg, Li, Ni, Pb and Se in fish from Mangla Lake, Pakistan were likely to be from anthropogenic contamination. Average estimated weekly and daily intake for As, Cd, Cr, Hg, Ni and Pb were found to be higher than the recommended PTWI and PTDI values, while the remaining metals were within the recommended limits. EDI/ RfD ratios for As, Hg, Pb, Se, Cd, Cr and Zn indicated As as high risk, Hg and Pb as moderate risk and Cd, Cr, Se and Zn as low risk to consumers. THQ for As, Cd, Cr, Hg, Pb, Se and Zn was higher than 1, considered as the unsafe consumption level, indicating lifetime non-carcinogenic health risks to the inhabitants. Estimates of target cancer risk (TCR) suggest that people eating *Catla catla* from Mangla Lake were exposed to As, Cd, Cr, Ni and Pb contamination with a lifetime carcinogenic risk. Therefore, consumption of *Catla catla* in this study area was found to be unsafe from the health risk of non-carcinogenic and carcinogenic effects over a lifetime. We therefore recommend that further research in this area should be carried out to better understand the toxic metal effects on biotic factors of fish and to confirm the quality of foods for human health.

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