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Research Article

Bioaccumulation and Health Risk Assessment of Heavy Metals in Some Fish Species Available in Local Fish Markets of Kathmandu, Nepal

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Abstract

In this study, concentrations of Cd, Cr, Mn, Pb, and Zn were determined by flame atomic absorption spectrophotometer (FAAS) in edible muscle tissues of a total of 25 fish samples belonging to five fish species *viz.*, *Hypophthalmichthys nobilis* (Bighead), *Wallago attu* (Buhari), *Catla catla* (Catla), *Clarias batrachus* (Mugree), and *Labeo rohita* (Rohu) marketed in Kathmandu, Nepal and evaluated potential health risks for adults using USEPA deterministic approaches. The average metal accumulation in all fish species followed the order of Zn (27.89) > Mn (5.36) > Pb (2.37) > Cr (1.12) > Cd (0.44) mg/kg, exceeding the FAO/WHO guidelines except for Zn. Likewise, the sum of average metal concentrations ($\sum_5$HM) followed the descending order of *C. catla* (49.61) > *C. batrachus* (44.49) > *W. attu* (34.51)> *H. nobilis* (32.10) > *L. rohita* (25.18) mg/kg. The correlation matrix showed significant correlations among some HMs indicating their common sources of origin in the examined fish species. The estimated daily intakes (EDIs) of HMs were lower than the maximum tolerable daily intake (MTDI). The target hazard quotients (THQs) of a single element in all fish species were less than 1.0 while the total target hazard quotients (TTHQs) in *C. batrachus*, *C. catla*, and *H. nobilis* exceeded the safe limit of 1.0 suggesting a potential non-carcinogenic risk. Moreover, the target cancer risks (TRs) of Cd and Cr were higher than the acceptable risk limit (10^{-4}) in all fish species suggesting that their consumption might pose a lifetime cancer risk for adults. Therefore, the study recommends regular monitoring of HMs in commercial fish to ensure the safety of consumer health.

Keywords: Bioaccumulation, Fish species, Heavy metals, Health risk, Kathmandu.

Introduction

Fish is often considered a vital source of nutrients for the human body. The health benefits of fish have encouraged a large population of the world for its wide consumption. Although it constitutes a small part of food intake in comparison to other products, fish is a great source of omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Domingo *et al.*, 2007). These are incredibly important for our body and brain. EPA plays a vital role in preventing coronary heart diseases and reducing arrhythmias and thrombosis (Oomen *et al.*, 2000), lowering the plasma triglyceride level (Harris, 2004; Ismail, 2005) and premature delivery (Daviglus *et al.*, 2002). Besides, fish is highly rich in many important nutrients, including high-quality protein, iodine, vitamins, and minerals. Despite its nutritional value, the consumption of fish brings many times a hazardous concern for human consumers.

Heavy metals from natural and anthropogenic sources continuously enter the aquatic environment where they pose a serious threat to ecological as well as human health. They are characterized by their non-biodegradative, long persistent, bio-accumulative nature and have potential biomagnification or are not easily metabolized (Rahman *et al.*, 2012). Such metals accumulate in the ecological food chain through uptake at the primary producer level and then through consumption at consumer levels. Metals such as Fe, Ni, Cu, Zn, and Mn are essential in trace amounts that play an important role in biological systems but a higher concentration of these metals becomes toxic to biota and their environment. On the contrary, Pb, Cd, and Hg are toxic even at low concentrations and have no known role in biological systems (Niroula *et al.*, 2022; Sharma *et al.*, 2022).

Fish is regarded as a bio-accumulator of inorganic and organic pollutants (King & Jonathan, 2003). They can absorb heavy metals (HMs) through the epithelial or mucosal surface of their skin, gills, and gastrointestinal tract. Contamination of freshwater fish with heavy metals (HMs) is, therefore a recognized problem in terms of food safety. This situation has arisen as a result of the rapid growth of population, increased urbanization, expansion of industrial activities, exploration, and exploitation of natural resources, and the extension of irrigation and other modern agricultural practices (Ibok *et al.*, 1989). Some of the toxic effects of heavy metals on fishes and aquatic invertebrates include a reduction of developmental growth, an increase in developmental anomalies, a reduction of survival especially at the beginning of exogenous feeding, or even cause the extinction of an entire fish population in polluted reservoirs (Authman *et al.*, 2013). The World Health Organization as well as the Food and Agriculture Organization of the United Nations state that monitoring eight elements in fish (Hg, Cd, Pb, As, Cu, Zn, Fe, and Sn) is obligatory and monitoring of others is suggested. Chromium and Ni are known to cause a variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema, and tumors, while a high intake of Cu can cause adverse health problems such as liver and kidney damage (Tuzen, 2009). Lead has been associated with pathological changes in organs and the central nervous system, leading to decrements in intelligence quotients (IQ) in children. Cadmium is toxic to the cardiovascular system, kidneys, and bones (Fang *et al.*, 2014) while inorganic As is a human carcinogen (Banerjee *et al.*, 2011; Anawar *et al.*, 2002).

The commercial production of fish is one of the main economic activities in Nepal that offers many profitable opportunities for farmers. Nepal has the potential for fish farming in more than 6000 rivers (Agri-farming, 2021). Fish farming has therefore become one of the major national priority agricultural practices and the Terai is the leading region for fish production in the country. The fish produced by farmers in the Terai region and the peri-urban areas of the central city areas are largely consumed everywhere including Kathmandu Valley. However, commercial production of fish in Nepal is still inadequate to fulfill the increasing demand for consumers. Hence, thousands of tons of fish and its products are imported every year from neighboring countries like India and Bangladesh (Agri-farming, 2021). Moreover, the consumption of uncontaminated or healthy fish has become a matter of concern for all Nepalese populations on account of food safety and human health. A periodical examination of the level of heavy metals in consumed fish is, therefore very important so that the safety of fish protein supplied to the consumers could be ensured.

Worldwide, there are many studies associated with risk assessment of HMs exposure in fish species. Islam *et al.* (2016) in their study observed non-carcinogenic risk for Cd and Pb in some fish species but lifetime cancer risk (TR) for Pb. Aytekin *et al.* (2019) reported that the toxic metal (Cd and Pb) levels detected in muscle tissues of some fish species were not within safe limits for human consumption. Kortei *et al.* (2020) evaluated human health risks from heavy metals (As, Hg, Pb, and Cd) exposure through fish consumption and showed no significant non-carcinogenic risk to humans but likely to cause adverse effects during a person's lifetime from As and Cd. Tahity *et al.* (2022), based on some risk parameters like EDI, THQ, and CR, concluded Barramundi fish as safe for human consumption as they were within the safe limits. Similarly, Hossain *et al.* (2022) reported that their studied fish species were safe for consumers from non-carcinogenic health risks even upon exposure to Pb, Cd, Cr, Ni, Cu, and Zn. In the context of Nepal, there are very limited studies on the heavy metal content and health risk assessment of commercial fish available in local fish markets. The study is very important on account of the toxic nature of heavy metals as their harmful effects on individuals, populations, or the ecosystem could be monitored easily. Therefore, the main aims of this study were to evaluate the levels of heavy metal contents in some most commonly consumed fish species marketed in Kathmandu inhabited by over 1.5 million people, and to assess both the non-carcinogenic and carcinogenic health risks of metals for adults as a receptor group through the consumption of fish. The concentrations of studied heavy metals and their estimated daily intakes (EDIs) were also compared against the FAO/WHO recommended maximum permissible levels allowed in fish.

Materials and Methods

Sample Collection and Processing

In total, twenty-five fish samples belonging to the five most commonly consumed fish species by the local population of Kathmandu were randomly selected for the study purpose. The scientific as well as local names of the selected five species were identified as *Hypophthalmichthys nobilis* (Bighead), *Wallago attu* (Buhari), *Catla catla* (Catla), *Clarias batrachus* (Mugree), and *Labeo rohita* (Rohu). Five replicates of each species were purchased and collected from different local fish markets in Kathmandu on different dates during August-September, 2022 with a view to observing possible variations in the elemental concentrations. After in-situ measurements of weights and lengths, all collected samples were immediately packed up in clean polythene bags, labeled, and kept in ice boxes for transport to the Environmental Science lab, Padmakanya Multiple Campus, Kathmandu. In the laboratory, the samples were carefully washed first with tap water and then rinsed with de-ionized water. The edible parts of fish (muscle tissues) were cut into small pieces after removing scales (if any) and then oven-dried at 70-80 ºC for several hours to attain constant weight (Islam *et al*., 2016). The dried samples were crumbled and pulverized with a porcelain mortar and pestle. All the processed samples were stored in well-labeled clean and dry airtight zip-lock plastic bags and stored in desiccators until further chemical treatment.

Chemical Reagents

All reagents used for the purpose of chemical analysis were of analytical grade. The standard solutions (1000 ppm) for Cd, Cr, Mn, Pb, and Zn were certified and purchased from E. Merck, Germany. These solutions were used to prepare calibration standards by diluting carefully to the required concentrations with de-ionized water. Concentrated nitric acid (65%), and hydrogen peroxide (30%) were of analytical grade and used without further purification. All glassware and plastic vessels used for the experimental purpose were treated with dilute (1:1) nitric acid for 24 h and rinsed well with deionized water before use. Deionized water was used for all purposes throughout the experiment.

Sample Preparation

Acid digestion procedure was followed for the quantification of heavy metals in the muscle tissue of fish samples (Arian *et al*., 2008). Accordingly, 1.0 g of the dried and pulverized sample was taken in a digestion tube. The sample was treated with an acid mixture of HNO_3 (10 mL) and H_2O_2 (5 mL) for the decomposition of muscle tissue. The mixture was placed in a chemical hood overnight so as to assist in complete dissolution and prevent foaming during the subsequent digestion process. The sample was digested at 80 ºC for 2-3 hours using a block digestor (Hanon SH420F Kjedahl). Further, 10 mL of HNO_3 was added and digestion continued till the solution became completely clear. After complete digestion and sufficient cooling, the digested solution was filtered into a 50 mL volumetric flask using Whatman 42. The digestion tube was rinsed with de-ionized water and transferred the solution into the same volumetric flask. The final volume was adjusted with the same water through continuous swirling. Solutions of all other samples were prepared in an analogous way. The blank (without sample) was prepared in the same manner as the samples.

Instrumental Analysis and Quality Control

The concentrations of investigated heavy metals in the digested solutions were determined by Flame Atomic Absorption Spectrophotometer (Shimadzu AA-6800) using an air-acetylene flame (Welz, 1985). The working standard solutions of the metals were prepared in series by appropriate dilution of the respective reference metals. The calibration curves were constructed at specific wavelengths for detecting the metals under study. All instrumental parameters were followed as per the specifications of the manufacturing company. Accordingly, hollow cathode lamps were used for Cd, Cr, Mn, Pb, and Zn with their operating wavelengths of 228.8, 357.9, 285.2, 283.3, and 213.9 nm respectively. The cathode lamp currents were 8.0, 10.0, 8.0, 10.0, and 10.0 mA for Cd, Cr, Mn, Pb, and Zn respectively, and 0.5 nm slit widths for all metals except Zn with 1.0 nm. The instrument was operated according to the procedure recommended by the manufacturer.

Three samples were selected from each fish species for the spike analysis of each analyte. A known quantity of analyte was added to 1.0 g of selected samples. For validation, precision, and accuracy of the analytical technique of the instrument, standard reference materials (SRM) traceable to NIST manufactured by Merck, Germany was used to prepare fortified samples. The fortified samples were treated as per the sample and the heavy metal level in the fortified samples was identified by FAAS. The average percent recovery for Cd, Cr, Mn, Pb, and Zn was 98.0, 98.8, 99.0, 99.2, and 98.6 with standard deviations of 1.6, 2.5, 2.8, 2.0, and 3.1 % respectively. The calculated detection limits were 6.0, 10.0, 20.0, 9.0, and 10.0 µg/L for Cd, Cr, Mn, Pb, and Zn respectively.

Health Risk Assessment

To assess possible health risks (non-carcinogenic and carcinogenic) associated with the ingestion of heavy metals through fish consumption, the estimated daily intake (EDI) of heavy metals, target hazard quotient (THQ), total target hazard quotient (TTHQ), and target cancer risk (TR) were calculated.

Estimated daily intake (EDI) of heavy metals: The estimated daily intake (EDI) is a method commonly used for the identification of the number of pollutants consumed daily (Vrhovnik *et al.*, 2013). The EDI of potentially toxic elements (PTE) is directly proportional to the concentrations of PTE in the food and daily food consumption. Furthermore, human body weight has an important impact on the tolerance to contaminants (Vrhovnik *et al.*, 2013). In this study, the EDI of heavy metals through fish consumption was measured using a metal concentration in the sample, daily consumption rate, and body weight. The EDI was calculated using Eqn. 1 (Shaheen *et al.*, 2015; Chary *et al.*, 2008).

$$EDI = \frac{FIR \times C}{BW} \quad \text{(Eqn. 1)}$$

Where, *EDI* is the estimated daily dietary intake of each heavy metal from fish (mg/day/kg body weight); *FIR* is the daily intake of fish (g/person/day), *C* is the metal concentration in fish (mg/kg), and *BW* is the average body weight assuming 60 kg for adults. Similarly, the daily consumption rate of fish was assumed 50 g for adults on a wet weight basis in this study (Shakya *et al.*, 2017).

Non-carcinogenic health risk

The methodology for the estimation of non-carcinogenic risk was applied in accordance with the provision of USEPA Region III's Risk-based Concentration Table (USEPA, 2010).

Target hazard quotient (THQ)

The non-carcinogenic risk for each individual metal through fish consumption was assessed by the target hazard quotient (THQ), which is "the ratio of a single metal exposure level over a specified time period to a reference dose (R*f*D) for that metal derived from a similar exposure period". Eqn. 2 was used for estimating THQ as follows:

$$THQ = \mathrm{C} \times \frac{EFr \times ED \times FIR}{RfD \times BW \times AT} \times 10^{-3} \quad \text{(Eqn. 2)}$$

Where, *THQ* is the target hazard quotient; *C* is the metal concentration in fish (mg/kg); *EFr* is the exposure frequency (365 days/year); *ED* is the exposure duration (70 years); *FIR* is the fish ingestion rate (50 g/person/day); *RfD* is the oral reference dose (mg/kg/day); *BW* is the average body weight of a person (kg) and *AT* is the averaging time for non-carcinogens (365 days/year × *ED*). The oral reference doses are based on 1.0×10^{-3}, 3.0×10^{-3}, 0.14, 3.5×10^{-3}, and 0.3 mg/kg/day for Cd, Cr, Mn, Pb, and Zn, respectively (USEPA, 2010).

Total target hazard quotient (TTHQ)

In order to assess the overall potential for non-carcinogenic effects from more than one heavy metal, a total target hazard quotient (TTHQ) is used. Since different pollutants can cause similar adverse health effects, TTHQ is calculated as the sum of target hazard quotients (THQs). The index is based on the Guidelines for Health Risk Assessment of Chemical Mixtures of the US Environmental Protection Agency (USEPA, 1989) using Eqn. 3 as follows:

$$Total\ THQ(TTHQ) = \sum THQ \quad \text{(Eqn. 3)}$$

In the event of $TTHQ \leq 1$, adverse health effects would be unlikely to occur. However, potential non-carcinogenic effects would occur when $TTHQ > 1$ as this indicates that there is a significant non-carcinogenic risk that is posed to human health (Wang *et al.*, 2005).

Target cancer risk (TR)

Incremental lifetime cancer risk or target cancer risk (TR) is the lifetime probability of an individual developing any type of cancer due to carcinogenic daily exposure to a contaminant over a lifetime. Eqn. 5 was used for estimating the target carcinogenic risk factor (lifetime cancer risk) (USEPA, 1989) as follows:

$$TR = \mathrm{C} \times \frac{EFr \times ED \times FIR \times CSFo}{BW \times AT} \times 10^{-3} \quad \text{(Eqn. 4)}$$

Where, *TR* represents the target cancer risk or the risk of cancer over a lifetime; *AT* is the averaging time for carcinogens (365 days/year × *ED*); and *CSFo* is the oral carcinogenic slope factor from the Integrated Risk Information System US Environmental Protection Agency (USEPA, 2010) database. The *CSFo* for Cd, Cr, and Pb are 6.3, 0.5, and 8.5×10^{-3} $(mg/kg/day)^{-1}$ respectively. The slope factors for Mn and Zn are unavailable since these elements are less likely to cause carcinogenic risk. The acceptable range of TR is 10^{-6} to 10^{-4}. If the risk exceeds the range, this implies that carcinogenic risks exist and the potential carcinogenic effect would likely occur (Wu *et. al.*, 2015). Chemicals for which the risk factor falls below 10^{-6} may be eliminated from further consideration as a chemical of concern.

Statistical Analysis

In this study, an IBM-PC computer was used for the storage and processing of all data. The statistical parameters including mean, range, frequency, percentage, and standard deviation were calculated using Excel spreadsheets wherever applicable. Inter-metal correlations were analyzed using Pearson's correlation coefficient along with the significance test.

Results and Discussion

Heavy Metals (HMs) Concentration in Fish Species

In the present study, a total of 25 fish samples belonging to the five most commonly consumed fish species *viz.*, *H. nobilis*, *W. attu*, *C. catla*, *C. batrachus*, and *L. rohita* available in local fish markets of Kathmandu were analyzed for levels of heavy metal contaminants. Some general characteristics of these fish species are summarized in Table 1.

Table 1. General characteristics of the collected fish species (Mean ± SD; n=5)

Fish species	Local name	Length (cm)	Weight (kg)	Type/ habitat	Feeding habit
H. nobilis	Bighead	47.60 ± 2.07	1.11 ± 0.11	Freshwater fish	Plankton and detritus feeder
W. attu	Buhari	41.80 ± 2.86	1.08 ± 0.10	Freshwater catfish	Carnivorous
C. catla	Catla	32.60 ± 2.97	0.95 ± 0.08	Freshwater carp fish	Planktivorous
C. batrachus	Mugree	25.80 ± 1.30	0.69 ± 0.06	Benthic/ walking catfish	Omnivorous
L. rohita	Rohu	41.60 ± 1.14	0.93 ± 0.08	Freshwater fish	Herbivorous

Table 2: Mean concentrations of heavy metals (mg/kg dw) in muscle tissues of fish species (mean ± SD, n=5).

Fish species	Statistical parameters	Heavy metals					$\sum_5$HM
		Cd	Cr	Mn	Pb	Zn	
H. nobilis	Mean	0.22	0.67	5.63	2.51	23.07	32.10
	Min.	0.17	0.55	4.55	1.56	18.99	25.82
	Max.	0.28	0.81	7.23	3.86	28.33	40.51
	SD	0.04	0.11	1.02	0.95	3.92	6.04
W. attu	Mean	0.30	0.87	3.85	1.71	27.78	34.51
	Min.	0.19	0.71	2.33	0.94	22.65	26.82
	Max.	0.45	1.08	4.77	2.18	32.30	40.78
	SD	0.10	0.16	0.94	0.50	3.86	5.56
C. catla	Mean	0.84	1.73	7.26	3.10	36.68	49.61
	Min.	0.51	1.34	5.98	2.87	29.56	40.26
	Max.	1.05	2.05	9.21	3.56	42.45	58.32
	SD	0.20	0.30	1.21	0.27	5.17	7.15
C. batrachus	Mean	0.70	1.79	5.94	3.86	32.20	44.49
	Min.	0.40	1.25	4.44	2.83	29.80	38.72
	Max.	0.91	2.18	7.89	4.89	36.65	52.52
	SD	0.21	0.39	1.27	0.78	2.71	5.36
L. rohita	Mean	0.13	0.55	4.14	0.65	19.71	25.18
	Min.	0.07	0.42	3.05	0.45	17.77	21.76
	Max.	0.19	0.65	5.67	0.87	21.05	28.43
	SD	0.05	0.10	1.10	0.17	1.25	2.67
Mean of all samples		0.44	1.12	5.36	2.37	27.89	37.18
*MAC (FAO/WHO, 2002)		0.10	1.00	5.0	0.50	40.00	-

*Maximum allowable concentration (MAC) of metals in fish

Although there are several works of literature presenting the elemental concentrations in various tissues such as the liver, kidneys, gills, intestine, and muscles of fish (Aytekin *et al.*, 2019; Venkateswarlu & Venkatrayulu, 2020; Shakya *et al.*, 2017), in the present study only muscle tissues (edible portion) were considered for determination of heavy metals with a view to assessing their risks on human health. It is because the fish muscle is the most important part to be consumed by humans. The statistical parameters of Cd, Cr, Mn, Pb, and Zn (mg/kg dw) in the fish species are summarized in Table 2. Results revealed that the overall mean concentrations of five HMs in the muscle tissues of all fish species were found in the decreasing order of Zn > Mn > Pb > Cr > Cd accounting for about 75, 15, 6, 3, and 1% of $\sum_5$HM respectively. These overall mean concentrations were higher than the maximum allowable concentration (FAO/WHO, 2002). except for Zn. The results of the present study are consistent with the studies conducted by Aytekin *et al.* (2019), Tahity *et al.* (2022), and Hossain *et al.* (2022) who also reported similar order of metal accumulation in their studied fish species with Zn and Cd as the highest and lowest levels respectively. In all species, the highest levels of Zn may be attributed to the role of this metal in the enzymatic and respiratory processes of fish (Aytekin *et al.*, 2019). Non-essential metals such as Cd, and Pb do not have any function for fish's metabolism and are not regulated by the organism.

Results also showed that the sum of mean concentrations of all five HMs i.e., $\sum_5$HM was the highest in *C. catla* (49.61 mg/kg) followed by *C. batrachus* (44.49 mg/kg), *W. attu* (34.51 mg/kg), *H. nobilis* (32.10 mg/kg) and *L. rohita* (25.18 mg/kg). Similarly, the $\sum_5$HM was found in the range of 40.26 – 58.32, 38.72 – 52.52, 26.82 – 40.78, 25.82 – 40.51, and 21.76 – 28.43 mg/kg for *C. catla*, *C. batrachus*, *W. attu*, *H. nobilis* and *L. rohita* respectively. It was also found that the muscle tissues of each fish species accumulated HMs in the decreasing order of Zn > Mn > Pb > Cr > Cd. Besides, the examined fish species showed their bioaccumulating capacity for each HM in the following order:

Zn: *C. catla* > *C. batrachus* > *W. attu* > *H. nobilis* > *L. rohita*

Mn: *C. catla* > *C. batrachus* > *H. nobilis* > *L. rohita* > *W. attu*

Pb: *C. batrachus* > *C. catla* > *H. nobilis* > *W. attu* > *L. rohita*

Cr: *C. batrachus* > *C. catla* > *W. attu* > *H. nobilis* > *L. rohita*

Cd: *C. catla* > *C. batrachus* > *W. attu* > *H. nobilis* > *L. rohita*

It is well known that metals can be bioaccumulated in fish tissues (Van der Oost *et al.*, 2003) and the present study showed different HM levels in different species. These differences in metal levels of the species may be attributed due to different biotopes, metabolic activity, and eating habits (Aytekin *et al.*, 2019). In addition, the magnitude of bioaccumulation is a function of age, species, and trophic transfer (Spry & Wiener, 1991).

Among the species, two fish species (*C. catla* and *C. batrachus*) showed the highest accumulation of Zn and Mn levels in their muscle tissues while *L. rohita* showed the lowest levels of all HMs except Mn. *C. batrachus* is benthic species and is likely to have higher metal concentration than fish inhabiting the upper water column because they are greatly exposed to the sediments and their greater uptake of HMs from zoobenthics (Yi *et al.*, 2011). The finding of the present study is in agreement with Aytekin *et al.* (2019) who also reported a higher accumulation of HMs in benthic species such as *P. semiculatus* and *S. solea*. *C. catla* is a freshwater fish and accumulated higher metal concentrations which might be due to its exposure to polluted ponds, streams, lakes, or rivers (Bae *et al.*, 2020). Therefore, the metal concentration is largely controlled by the habitat, feeding habits, metal accumulation capacity, and organism type (Agah *et al.*, 2009). Besides, the concentrations of metals may vary with age and body weight within the same species (Qiu *et al.*, 2011).

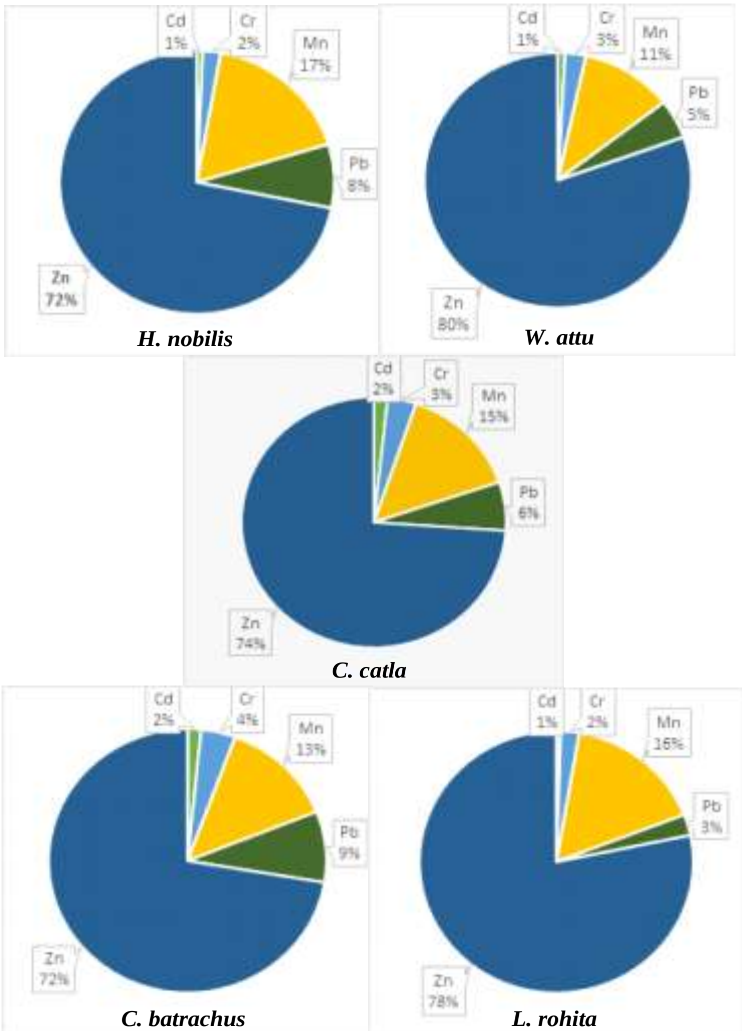

Fig. 1. Pie chart showing percent distribution of individual metal to a total mean of $\sum_5$HM in fish species.

Fig. 1 depicts the metal distribution profile in the studied fish species. Results showed variation in terms of metal distribution in muscle tissues of each fish species. Accordingly, a range of 72 – 78, 11 – 17, 3 – 9, 2 – 4, and 1 – 2 % for Zn, Mn, Pb, Cr, and Cd respectively was observed indicating Zn as the main domain metal in all fish species. Of $\sum_5$HM among the fish species, *W. attu* demonstrated a comparatively higher accumulation of Zn (80%) in its muscle tissue. Similarly, a maximum of Mn (17%) was measured in *H. nobilis* while Pb (9%), and Cr (4%) were recorded as maximum in *C. batrachus*. However, all fish species measured Cd not exceeding 2% in their muscle tissues.

Table 2 shows that the mean concentration of Cd ranged from 0.13 mg/kg (*L. rohita*) to 0.84 mg/kg (*C. catla*), and measured an overall mean concentration of 0.44 mg/kg. The concentration of Cd in this study was found comparatively higher than those reported in many other countries (Table 3) except Santa Marta/Colombia (Pinzón-Bedoya *et al.*, 2020) which showed a Cd level of 12.40 mg/kg. The observed mean concentrations of Cd in this study were higher than the FAO/WHO (2002) recommended safe limit of 0.1 mg/kg indicating that these species might pose health hazards. Variations in the levels of Cd in fish muscle tissues may be attributed to its contamination in the environment from events of agricultural practices such as land preparation, spraying crops with agrochemicals for the purposes of controlling weeds as well as pests, and some other activities that wash off and empty into the water bodies. Phosphate-based fertilizers as well as other types contain an average of 13.4 μg/g of Cd that gets deposited on farmlands when applied annually (Ntiforo *et al.*, 2012). Besides, substantial amounts of Cd are also released into the environment through weathering of rocks and manufacturing and mining activities (Rahman *et al.*, 2012). Cadmium is a toxic metal with no known biochemical importance to humans. It is poorly absorbed into the body, but once absorbed is slowly excreted, like other metals, and accumulates in the kidney causing renal damage (Tchounwou *et al.*, 2012). Diseases such as renal failure, osteoporosis, lung cancer, and increased blood pressure could arise from the consumption of fish with high a concentration (> 0.05 mg/kg) of Cd and these will pose hazards to humans (Olsson *et al.*, 2005). Eisler (1985) reported an adverse effect of cadmium with dietary levels of 0.1 ppm.

The overall mean concentration of Cr in the fish species was found to be 1.12 mg/kg, where the mean concentration ranged from 0.55 mg/kg (*L. rohita*) to 1.79 mg/kg (*C. batrachus*) (Table 2). Except for *C. catla* and *C. batrachus*, the mean concentrations of Cr in all fish species were lower than the guideline value of 1.0 mg/kg set by FAO/WHO (2002). However, predators or scavengers would be at risk from the consumption of *C. catla* and *C. batrachus* since these two species measured levels of Cr above the permissible limit. Table 3 shows lower Cd contents in fish species in cities/countries like Dhaka/Bangladesh (Akter *et al.*, 2021), Dalmai/Iraq (Al-Sulttani *et al.*, 2022), and Aba/Nigeria (Chima *et al.*, 2022) compared to the present study. Chromium (VI) is a non-essential and toxic element whereas Cr (III) is an essential trace element, although it can cause toxicity in high doses. The presence of Cr (III) in the diet is of great importance due to its active involvement in lipid metabolism and insulin function (Ahmed *et al.*, 2015). Chromium (VI) and its compounds have been reported as carcinogens that also help develop cancer cells in humans and animals as stomach tumors, and cancer in the bladder, kidney, bone, thyroid, testicles, larynx, and lungs (Ahmed *et al.*, 2013). Anthropogenic sources of Cr emission in surface waters are municipal wastes, laundry chemicals, paints, leather, road runoff, agricultural runoff, etc., (Dixit & Tiwari, 2008). Ingestion of Cr or taking it through gills and its accumulation in fish tissues and liver may occur at higher temperatures in fish (Ahmed *et al.*, 2013).

Table 3. Comparison of HMs concentration (mg/kg) in fishes in this study with previous studies in different cities/countries.

Cities/ Countries	Cd	Cr	Mn	Pb	Zn	References
Kathmandu/ Nepal	0.44	1.12	5.36	2.37	27.89	Present study
Dhaka/Bangladesh	0.02	0.14	1.41	0.26	6.93	Akter *et al.* (2021)
Santa Marta/Colombia	12.40	-	-	37.84	7.18	Pinzón-Bedoya *et al.* (2020)
Asafo/Ghana	0.01	-	0.02	0.07	0.02	Kwaansa-Ansah *et al.* (2019)
Olsztyn/Poland	-	-	0.08	-	5.13	Luczynska *et al.* (2022)
Cairo/Egypt	0.02	-	-	0.08	-	Sanou *et al.* (2021)
Dalmaj/Iraq	0.31	0.23	-	6.70	22.46	Al-Sulttani *et al.* (2022)
Mazandaran/Iran	0.01	-	-	0.10	3.11	Aski *et al.* (2023)
Aba/Nigeria	0.41	0.23	-	0.22	-	Chima *et al.* (2022)

There are remarkably few studies on manganese associated with the muscle tissues of fish and hence is of importance in this study. Manganese is an essential element for the healthy life of organisms having a wide range of biological applications, but ingesting excess amounts of the metal can bring various disorders in humans (Burger & Gochfeld, 1996). Manganese is involved in the synthesis and activation of many enzymes and in the regulation of the metabolism of glucose and lipids in humans. The toxicity of Mn in humans leads to a syndrome called manganism which involves both psychiatric symptoms and features of Parkinson's disease (Dobson *et al.*, 2004). In the present study, the overall mean concentration of Mn was observed to be 5.36 mg/kg with a range from 3.85 mg/kg (*W. attu*) to 7.26 mg/kg (*C. catla*). The mean concentrations of Mn in fish species except (*W. attu* and *L. rohita*) were higher than the MAC value of 1.0 mg/kg recommended by FAO/WHO (2002). Manganese contents, as evident from Table 3, in this study were comparatively higher than those reported from cities/countries like Dhaka/Bangladesh (Akter *et al*., 2021), Asafo/Ghana (Kwaansa-Ansah *et al*., 2019) and Olsztyn/Poland (Luczynska *et al*., 2022). The environmental contamination of Mn includes anthropogenic sources such as municipal wastewater discharges, sewage sludge, mining, and mineral processing, emissions from alloy, steel, and iron production, combustion of fossil fuels, and, to a much lesser extent, emissions from the combustion of fuel additives (Honeyman & Santschi, 1988; Cima, 2019).

Lead is a serious cumulative body poison that enters into the body system through the air, water, and food and cannot be removed by cooking fish (Sharma *et al*., 2007). In the present study, Pb was found accumulating in all studied fish species where the mean concentration ranged from 0.65 mg/kg (*L. rohita*) to 3.86 mg/kg (*C. batrachus*). The overall mean concentration was found to be 2.37 mg/kg. Lead in all studied fish species was found to be higher than the safe limit of 0.5 mg/kg set by FAO/WHO (2002), indicating that these species were contaminated with Pb and might pose risk. While comparing with other studies (Table 3), Pb content in the present study was lower than those reported values from Santa Marta/Colombia (Pinzón-Bedoya *et al*., 2020) and Dalmaj/Iraq (Al-Sulttani *et al*., 2022) but higher than other cities/countries. Lead in muscle tissues of the fish samples could be due to anthropogenic activities including mining, chemical, and metal processing industries, refineries, and urban runoff (Vidmar *et al.*, 2017). Lead, like that of Cd, is a non-essential element that is associated with neurotoxicity, nephrotoxicity, and a variety of other health disorders (García-Lestón *et al.*, 2010).

From the quantitative five HMs, Zn had the highest concentration in muscle tissues of all studied fish species. The overall mean concentration of Zn was 27.89 mg/kg in the present study, where the mean concentration was found in the range between 19.71 mg/kg (*L. rohita*) to 36.68 mg/kg (*C. catla*). The observed Zn levels in all fish species were lower than the maximum allowable concentration of 1.0 mg/kg set by FAO/WHO (2002). Like Cr and Mn, this study also showed a higher Zn level in studied fish species compared to all other cities/countries (Table 3). Zinc is an essential micronutrient for living organisms and acts as a catalyst for about 300 enzymes in aquatic creatures. Therefore, a relatively high level of Zn is required to balance certain biological functions. Zn is involved in many metabolic events in humans, and its deficiency can lead to loss of appetite, the inhibition of growth, skin changes, and immunological abnormalities (Tuzen, 2009).

Correlation analysis is an important tool in HM analyses for evaluating the inter-relationship of paired data (Bradford *et al.*, 1996) that provides substantial information about sources of origin and pathways of HMs in the environment (Rodriguez *et al.*, 2008). In this study, a correlation matrix was calculated for the analyzed metals in fish species in order to identify the common sources of metals. Table 4 showed the inter-metal correlation in fish samples, using the Pearson correlation coefficient. Significant and positive correlations were found between Cd and Cr ($r = 0.760$), Cd and Pb ($r = 0.722$), Cd and Zn ($r = 0.851$), Cr and Pb ($r = 0.716$), Cr and Zn ($r = 0.678$), and Pb and Zn ($r = 0.644$) at $p < 0.05$, suggesting the possibility of contamination from a common source. Manganese, on the other hand, showed a positive but comparatively weak correlation with other metals. The results of inter-metal correlation in this study were found in line with Khanom *et al.* (2020) but slightly deviated from the findings of Poudel *et al.* (2016) who observed a strong and significant inter-relationship of Mn with Pb and Zn but not with Cd and As. The correlations among the studied metals may result from the similar accumulation behaviour of the metals in the fishes and their interactions (Rejomon *et al.*, 2010). Besides, it may also be indicative of similar biochemical pathways along with many other environmental factors and feeding habits (Jezierska & Witeska, 2006).

Health Risk Assessment

The human diet is the most common ingestion pathway to toxic pollutants. Fish has a strong potential for accumulating a number of pollutants including metal and fish muscle is the most important part for human consumption. Therefore, the muscle part of fish is explored more than other organs for risk assessment due to heavy metals (Iqbal & Shah, 2014).

*Estimated daily intake (EDI) of heavy metals (HMs)**:***

In this study, the EDIs of heavy metals were evaluated on the basis of the average concentration of each heavy metal in fish muscles, consumption rate, and body weight. The EDIs of studied heavy metals through the consumption of fish are shown in Table 5. Besides, the EDIs in muscle tissues of each fish species were also compared with some existing standards (MTDI) for human consumption as set by joint FAO/WHO (1999).

Results showed EDI values in the descending order of Zn > Mn > Pb > Cr > Cd in all fish species. Among the fish species, *C. catla* demonstrated the highest EDIs for Zn (3.06 × 10^{-2}), Mn (6.05 × 10^{-3}) and Cd (7.00 × 10^{-4}) while *C. batrachus* estimated the highest daily intakes for Cr (1.49 × 10^{-3}) and Pb (3.22 × 10^{-3}). On the other hand, EDIs of all studied HMs (except Mn) were observed to be the lowest of all in *L. rohita*. However, the EDI values of HMs in each fish species including the total EDIs through fish consumption did not exceed the maximum tolerable daily intake (MTDI) limits set by joint FAO/WHO (1999), indicating that these fish species might pose a low risk to the consumers in the study area. The EDIs of studied HMs lower than MTDI limits also suggested a low consumption rate of fish by the adult population in Kathmandu. Though the EDI of the studied metals was lower than the MTDI, periodic surveillance is necessary to set up regulatory norms for the dietary intake of those fish species.

In the present study, the oral reference doses (R*f*D) are based on 1.0 ×10^{-3}, 3.0 ×10^{-3}, 0.14, 3.5 × 10^{-3}, and 0.3 mg/kg/day for Cd, Cr, Mn, Pb, and Zn, respectively (USEPA, 2010). A comparison of the EDI values (Table 5) against the R*f*Ds of respective HMs showed that the EDI values of all HMs were comparatively lower than the R*f*Ds in all tested fish species. EDIs lower than the R*f*Ds suggested that the targeted groups of people might experience low or no health effects (Aytekin *et al.*, 2019). However, determining an "acceptable limit" or an "unacceptable limit" based on doses less than the RDA or R*f*d is not a stable measurement technique (Baki *et al.*, 2018; Vu *et al.*, 2017). The New York State Department of Health (NYSDOH, 2007) has suggested that if the ratio of EDI/R*f*D is ≤ R*f*D, it is associated with minimum health risks. However, if it is >1-5 times higher than R*f*D, it is associated with low health risks. Similarly, if it is >5-10 times higher than R*f*D, it is associated with moderate health risks, and if it is >10 times higher than R*f*D, it is associated with high health risks. In the present study, the EDI/ R*f*D ratio for all fish species (calculation not shown) was found several times higher than the respective R*f*D, indicating potential health hazards in the long term.

Table 4: Inter-metal correlation in fish species

	Cd	Cr	Mn	Pb	Zn
Cd	1.000				
Cr	*0.760	1.000			
Mn	0.478	0.564	1.000		
Pb	*0.722	*0.716	0.556	1.000	
Zn	*0.851	*0.678	0.456	*0.644	1.000

*Correlation is significant at $p < 0.05$

Table 5. Estimated daily intake (EDI) of heavy metals (mg/day/kg bw) through consumption of fish.

Fish species	Estimated daily intake (EDI)				
	Cd	Cr	Mn	Pb	Zn
H. nobilis	1.83 × 10^{-4}	5.58 × 10^{-4}	4.69 × 10^{-3}	2.09 × 10^{-3}	1.92 × 10^{-2}
W. attu	2.50 × 10^{-4}	7.25 × 10^{-4}	3.21 × 10^{-3}	1.42 × 10^{-3}	2.32 × 10^{-2}
C. catla	7.00 × 10^{-4}	1.44 × 10^{-3}	6.05 × 10^{-3}	2.58 × 10^{-3}	3.06 × 10^{-2}
C. batrachus	5.83 × 10^{-4}	1.49 × 10^{-3}	4.95 × 10^{-3}	3.22 × 10^{-3}	2.68 × 10^{-2}
L. rohita	1.08 × 10^{-4}	4.58 × 10^{-4}	3.45 × 10^{-3}	5.42 × 10^{-4}	1.64 × 10^{-2}
Total	1.82 × 10^{-3}	4.67 × 10^{-3}	2.24 × 10^{-2}	9.85 × 10^{-3}	1.16 × 10^{-1}
*MTDI	0.03	0.20	11.00	0.21	60.00

*Maximum tolerable daily intake (Joint FAO/WHO, 1999)

Non-carcinogenic and carcinogenic health risk

The non-carcinogenic and carcinogenic risks through the consumption of fish by adults as a receptor group were assessed using target hazard quotient (THQ), total target hazard quotient (TTHQ), and target cancer risk (TR) parameters. The estimated THQs for non-carcinogenic risk for adult consumers are presented in Table 6. This health risk parameter was introduced by the USEPA (2010) for estimating the potential health risks caused by chemical contaminants over prolonged exposure (Zhong *et al.*, 2018).

Results revealed that the THQ value of each heavy metal decreased in the order of Pb > Cd > Cr > Zn > Mn for all fish species with few exceptions. Among the fish species, *C. batrachus* measured the highest THQ values for Pb (0.919) while *C. catla* for Cd (0.700). However, both the species measured comparable THQ values for Cr, Mn, and Zn. It was found that the THQ value of each metal was less than 1.0 in all tested fish species indicating that ingestion of a single metal through consumption of these species does not pose a significant potential health hazard (Islam *et al.*, 2016).

In order to assess the overall potential for non-carcinogenic effects from more than one heavy metal, a total target hazard quotient (TTHQ) is used. This index is calculated as the sum of target hazard quotients (∑THQ). Potential non-carcinogenic effects would occur when the ∑THQ is equal to or higher than 1.0. In such a case, interventions and protective measures should be taken (Wang *et al.*, 2005). Table 6 shows the sum of THQs i.e., ∑THQ for HMs as a measure of non-carcinogenic effect. It was found that ∑THQ values for fish species were in the descending order of *C. batrachus* (2.12) > *C. catla* (2.06) > *H. nobilis* (1.07) > *W. attu* (1.00) > *L. rohita* (0.50). Accordingly, *C. batrachus* showed the highest ∑THQ value among all fish species. Since three fish species (*C. batrachus*, *C. catla*, and *H. nobilis*) exceeded the safe limit of 1.0, these species might pose a potential non-carcinogenic risk to the consumers in the study area. The remaining two species (*W. attu* and *L. rohita*) are, however safe for consumption as their ∑THQ values did not cross the limit.

Prolonged exposure to a specific carcinogen may lead to cancer, and the risk increases depending on the contact time. The risk associated with the carcinogenic effects of target metal is expressed as the excess probability of contracting cancer over a lifetime of 70 years (USEPA, 2010). Target carcinogenic risk (TR) denotes not only an estimation of the expected cancers but also represents the possibility of developing carcinogenic risks in an individual (NYSDOH, 2007). In the present study, the target carcinogenic risk (TR) derived from the intake of Cd, Cr, and Pb was calculated since these elements may promote both non-carcinogenic and carcinogenic effects depending on the exposure dose. The cancer slope factors of Mn and Zn are not available in the literature since they are non-carcinogenic in nature and hence are not included in the present study for the estimation of lifetime cancer risk. The estimated TR values of Cd, Cr, and Pb for the adult population from the consumption of tested fish samples are summarized in Table 7.

Table 6. Non-carcinogenic risk of heavy metals for adults through consumption of fish.

Fish species	Target hazard quotients (THQs)					∑THQ
	Cd	Cr	Mn	Pb	Zn	
H. nobilis	0.183	0.186	0.034	0.598	0.064	1.07
W. attu	0.250	0.242	0.023	0.407	0.077	1.00
C. catla	0.700	0.481	0.043	0.738	0.102	2.06
C. batrachus	0.583	0.497	0.035	0.919	0.090	2.12
L. rohita	0.108	0.153	0.025	0.155	0.055	0.50

Table 7. Carcinogenic risk of heavy metals for adults through consumption of fish.

Fish species	Target cancer risk (TR)				
	Cd	Cr	Mn	Pb	Zn
H. nobilis	1.16×10^{-3}	2.79×10^{-4}	-	1.78×10^{-5}	-
W. attu	1.58×10^{-3}	3.63×10^{-4}	-	1.21×10^{-5}	-
C. catla	4.41×10^{-3}	7.21×10^{-4}	-	2.20×10^{-5}	-
C. batrachus	3.68×10^{-3}	7.46×10^{-4}	-	2.74×10^{-5}	-
L. rohita	6.83×10^{-4}	2.29×10^{-4}	-	4.61×10^{-6}	-

Results revealed that TR values for Cd ranged from 6.83×10^{-4} (*L. rohita*) to 4.41×10^{-3} (*C. catla*), Cr from 2.29×10^{-4} (*L. rohita*) to 7.46×10^{-4} (*C. batrachus*) and Pb from 4.61×10^{-6} (*L. rohita*) to $2.74\times\ 0^{-5}$ (*C. batrachus*). In general, the excess cancer risks with TR value lower than 10^{-6} are considered to be negligible, cancer risks above 10^{-4} are considered unacceptable, and risks lying between 10^{-6} and 10^{-4} are generally considered an acceptable range (Wu *et. al.,* 2015). As might be evident from Table 7, the carcinogenic risk of these Cd, Cr, and Pb was in the unacceptable range (10^{-4}) to the acceptable range (10^{-6} to 10^{-4}). Specifically, TR values for Cd and Cr were higher than the acceptable risk limit (10^{-4}) in all tested fish species indicating that the consumption of these species exposed to both metals might pose a lifetime cancer risk. On the other hand, TR values for Pb were between the acceptable range of 10^{-6} and 10^{-4} in all fish species indicating no carcinogenic risks from their consumption. However, the potential health risk for the adult population from fish consumption should not be overlooked.

Conclusions

In conclusion, the present study revealed elevated concentrations of Cd, Cr, Mn, Pb, and Zn in muscle tissues of the five most commonly consumed fish species marketed in Kathmandu. The overall mean concentrations of HMs were found to exceed the FAO/WHO permissible levels except for Zn; however, the metal levels varied against the recommended limits if an individual fish species is taken into consideration. The extent of metal accumulation was found in the descending order of Zn > Mn > Pb > Cr > Cd in all fish species. The sum of mean concentrations of all five HMs in the fish species was found in the descending order of *C. catla* > *C. batrachus* > *W. attu* > *H. nobilis* > *L. rohita*. The positive and significant correlations ($p < 0.05$) among the metals in studied fish species indicate the possibility of contamination from a common source. The estimated daily intakes (EDIs) of HMs from fish consumption were lower than the maximum tolerable daily intake (MTDI), suggesting a low consumption of fish by the adult population in the study area. The target hazard quotient (THQ) from a single element would not pose any potential risk, whereas the sum of individual metal THQ in *C. batrachus*, *C. catla*, and *H. nobilis* exceeded the safe limit of 1.0 suggesting that these species might pose a potential non-carcinogenic risk to the consumers. Also, the estimation showed that the target carcinogenic risk (TRs) of Cd and Cr was higher than the acceptable risk limit (10^{-4}) in all studied fish species indicating that their consumption might pose a lifetime cancer risk. The entry of Cd and Cr in the food chain through fish consumption is, therefore a matter of health concern. However, constant monitoring of heavy metals is greatly recommended to reduce future risks of metal toxicity posed by the consumption of such fish species. The effectiveness of such measures would help ensure food quality as well as food safety for humans.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this paper.

Authors' Contribution

All authors have made equal contributions at every phase of the present work, including manuscript preparation, critical revision of the manuscript for important intellectual content, and final approval of the manuscript.

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