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Analysis of heavy metals in some commercially important fishes of Kathmandu Valley, Nepal

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Abstract

Since the most of the fish consumed in the Kathmandu Valley are brought from commercial farms in the Terai region of Nepal and some from India, the quality characteristics of such fishes are foremost importance. Therefore, this study was carried out to provide the information on heavy metal concentrations in the muscles of nine commercial fish species available in the markets of Kathmandu Valley and to make people aware of the possible health risk associated with their consumption. The concentrations of Lead (Pb), Arsenic (As), Cadmium (Cd), Zinc (Zn), Manganese (Mn) and Chromium (Cr) were determined in the muscles of *Catla catla, Pampus species, Puntius chola, Eutropiichthys vacha, Pampus chinensis, Clarias batrachus, Labeo bata, Labeo rohita* and *Mystus tengara.* The levels of heavy metals were measured by atomic absorption spectrophotometry after digestion of the samples using the heating digester. There were significant variations among heavy metal levels in the muscles of the nine fish species, and no fish types had the highest levels of more than two metals. First, three maximum mean concentrations of potentially toxic elements were found 16.75 µg/g in *Puntius chola,* 12.13 µg/g in *Eutropiichthys vacha*, 11.63 µg/g in *Catla catla* for lead; 1.45 µg/g in *Puntius chola*, 1.35 µg/g in *Pampus* species and 1.15 µg/g in *Mystus tengara* for cadmium; and 1.01 µg/g in *Eutropiichthys vacha*, 0.76 µg/g in *Pampus* species, 0.65 µg/g in *Pampus chinensis* for arsenic. Similarly, first three maximum mean concentrations of essential elements were found 9.88 µg/g in *Puntius chola,* 7.63 µg/g in *Clarias batrachus*, 5.75 µg/g in *Catla catla* for manganese; 89.75 µg/g in *Clarias batrachus*, 68.37 µg/g in *Catla catla*, 65.38 µg/g in *Puntius chola* for zinc; and 14.63 µg/g in *Clarias batrachus*, 13.13 µg/g in *Pampus* species, 10.50 µg/g in *Labeo rohita* for chromium. The results showed that the heavy metals were found to be higher mean concentrations in the sample of *Puntius chola*, then in *Catla catla, Pampus* species and *Clarias batrachus* respectively; whereas they were found to be lower mean concentration in *Eutropiichthys vacha, Pampus chinensis, Labeo bata, Labeo rohita* and *Mystus tengara* correspondingly. The total mean concentration of manganese, lead and chromium in all fish samples was found to be higher than the limits permitted by the Food and Agriculture Organization (FAO) of the United Nations/World Health Organization (WHO) whereas the mean concentration of zinc and cadmium was found lower than the permissible level of FAO/WHO, National Health and Medical Research Council (NHMRC) and Malaysian Food and Regulations (MFR) guidelines. Similarly, the total mean concentration of cadmium was also found to be lower than the permissible level of Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Boletin Oficial del Estado (BOE), NHMRC, European Community Regulation (EU) and MRF in all fish samples. The total mean level of lead was found lower than BOE guidelines. Likewise, the total mean level of chromium was found slightly exceeded over limits suggested by the European Economic Community (EEC). This study showed that all the fish samples examined were found to contain some heavy metals above the FAO/WHO standard levels which may cause problems on the human health. At last, this research work suggests that fish consumers should always bear in mind that standards cannot provide a margin of safety when they are not enforced locally.

Introduction

All over the world, fish is considered an excellent source of nutrient for people, particularly as a source of protein. It is often assumed to be healthier than chicken and other varieties of meat. Therefore, fish consumption has increased among an increasingly health-conscious population. The nutritional values of the fish are mainly due to the content of high-quality protein and high content of two types of omega-3 polyunsaturated fatty acid, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in different species (Clarkson, 2002; Domingo *et al*., 2007). EPA plays the vital role in preventing the coronary heart diseases, reducing arrhythmias, thrombosis (Kinsella *et al*., 1990; Oomen *et al*., 2000; Kris-Etherton *et al*.,

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2002) lowering the plasma triglyceride level (Harris, 2004; Ismail, 2005) and preterm delivery (Anderson and Wiener, 1995; Daviglus *et al.*, 2002).

The presence of toxic heavy metal in fish, however, not only impairs the health of the consumers, but also obstructs the positive effects of beneficial compounds. Therefore, levels of contaminants in the aquatic ecosystem, particularly in fish, and their potential effects on the fish themselves or the organisms that consume them, including top-level receptors, including people, are of great interest today (Kornekova *et al.*, 2006; Orak *et al*., 2005). Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, etc.), probably essential (nickel, vanadium, cobalt) and essential (copper, zinc, iron, manganese). Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when their intake is excessive.

The aquatic environment is heavily degraded due to various anthropogenic activities that cause domestic and industrial effluents (Kamaludeen *et al*., 2003). Particularly river, lakes, and the reservoirs (aquatic ecosystem) located near industrial and urban areas are the potential target for the disposal of the environmentally harmful elements like organic and inorganic contaminants. Heavy metals are of particular concern due to their non-biodegradable nature and their detrimental effect to the human health. These heavy metals have a high ability to bio-accumulate in various organs and muscle tissue of fish (DeForest *et al*., 2007).

The gradual awareness about the beneficial role of the fish in the people's health has raised the demands of fish in the market, which is the actual scenario in the context of Kathmandu Valley. Nepal actually imports fish from India, its closest south neighbour because the country is not self-sufficient in the production of fish. Every year, thousands of tons of fish are consumed in the market of almost 2.5 million inhabitants in Kathmandu (The World Bank, 2013). In this way, the fish has provided an excellent source of nutrient for the people of Nepal. Unfortunately, there is not enough information on heavy metal content of commercial fish available in the fish-markets of Kathmandu valley. Therefore, the aim of this study was to determine the levels of heavy metal contents found in the more popular fish species consumed in the Kathmandu valley. These concentrations were then compared against the recommended maximum levels allowed in fish and other food stuff. In addition, the quality of the fish for human consumption was assessed.

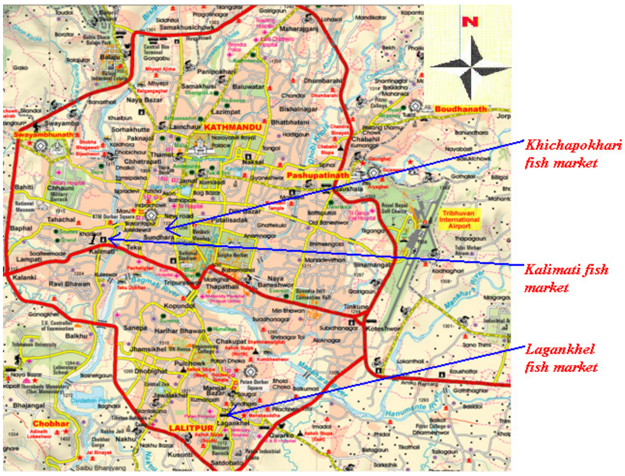

Figure 1. Map showing the sites of fish sampling from different fish shops of Kathmandu valley

Materials and Methods

Sample collection

Nine different fish species (36 samples) were purchased from the various fish-shops in the markets of Kathmandu Valley on November, 2012 as shown in Figure 1. These fish samples were identified by the help of Dr. Bibhuti Ranjan Jha, Associate Prof. Dept. of Environmental Science. These fish samples were Katle *(Catla catla)*, Pamplet (*Pampus* sps.) and Bachawa *(Eutropiichthys vacha)* collected from Lagankhel fish market; Pothi machha *(Puntius chola)*, Rup Chanda *(Pampus chinensis)*, Mahur or Walking catfish *(Clarias batrachus)* from the Kalimati fish market, and Bata *(Labeo bata)*, Rohu *(Labeo rohita)* and Tengra *(Mystus tengara)* from Khichapokhari fish market. Before keeping in the ice-cold box, individual fish were washed with distilled water and packed into the pre-cleaned plastic bags and carried to the Instrumental Analysis laboratory of Kathmandu University (KU) and finally kept frozen at -4^{o}C until further analysis.

Sample preparation

The fish samples were thoroughly washed with distilled water to remove any adhering contaminants, and they were put on dissection tray and thawed at room temperature. They were dissected using a knife and forceps, and the intestine, guts and bones were removed. Then the only muscle portion was taken in a clean Petri dish and dried in an oven at 120± 2^{o}C for 48 hours at which time the weight was constant weight. Before acid digestion, the dried muscle was ground to a fine powder using a mortar and pestle, and stored in a deep freezer (-4^{o}C) prior to analysis. The digestion procedure was carried out as described by Kotze *et al.* (2006). 1 gram of each the powder sample was mixed with 20 ml distilled water and

Table 1. Mean concentration ± standard deviations Mn, Zn, Pb, As, Cd and Cr (µg/g dry wt.) in muscle tissues of nine different fish species collected from fish- markets of Kathmandu valley

Locations	Fish Species	Mn	Zn	Pb	As	Cd	Cr
Lagankhel fish market	*Catla catla* (A)	5.75±2.50	68.37±38.17	11.63±3.07	<ND	0.79±0.48e	9.50±4.30
Lagankhel fish market	*Pampus species* (B)	5.63±1.25	26.88±6.25	9.50±2.48	0.76±0.50	1.35±0.31	13.13±3.17
Kalimati fish market	*Puntius chola* (C)	9.88±0.85	65.38±6.88	16.75±2.22	0.58±0.20	1.45±0.34	8.13±4.59
Lagankhel fish market	*Eutropiichthys vacha* (D)	5.63±1.75	64.88±46.67	12.13±4.97	1.01±0.60	0.61±0.18	10.00±3.92
Kalimati fish market	*Pampus chinensis* (E)	0.68±0.21	25.38±8.33	4.50±2.86	0.65±0.27	0.49±0.26	9.50±1.68
Kalimati fish market	*Clarias batrachus* (F)	7.63±1.89	89.75±34.59	6.13±2.06	<ND	0.53±0.33	14.63±2.56
Khichapokhari fish market	*Labeo bata* (G)	0.51±0.13	35.75±27.90	4.75±1.19	0.58±0.15	0.80±0.25	7.75±5.24
Khichapokhari fish market	*Labeo rohita* (H)	3.63±1.11	23.13±2.32	6.63±2.29	<ND	0.76±0.27	10.50±3.54
Khichapokhari fish market	*Mystus tengara*(I)	4.63±1.60	20.63±0.85	4.13±1.93	0.58±0.21	1.15±0.42	9.75±3.52
Overall mean conc.		4.89±3.02	46.68±25.51	8.46±4.33	0.69±0.17	0.88±0.35	10.32±2.23
Detection limit (mg/L)		0.05	0.02	0.01	0.005	0.003	0.02

Note : A = Katla (Catle) ; B = Pamplet ; C = Pothi D = Bachawa; E = Rup Chanda; F = Mahur (Walking catfish); G = Bata; H = Rohu; I = Tengra. ND= Not detected (Below detection limit).

digested using 2:1 mixture of conc. HNO_3 (55%) and conc. $HClO_4$ (70%) [Analytical grade from Merk-India] in 100 ml Erlenmeyer flask on a heating digester (200 to 250°C) until a clear solution was obtained and the volume reduced to approximately 15 ml. This solution was then filtered using Whatman no. 1 filter paper into the volumetric flask and diluted to 50 ml. The samples thus prepared were analyzed using Atomic Absorption Spectrophotometer (Model: Thermo Electron Corporation, Type: M5 Mk 2 Aa system, S. No.: GE 650255, S. Series, AA Spectrometer with Graphite furnace, UK). To draw the calibration curves, the different standards of the various salt solutions were used.

Sample analysis

The concentrations of heavy metals Cd, As, Pb, Mn, Cr and Zn were measured by using atomic absorption spectrophotometry (Thermo Electron Corporation). Results were expressed as parts per million, which were changed into micro-grams per gram (µg /g dry wt. of muscle tissue). The analytical procedure was checked using suitable reference material available in Aquatic Ecological Center, KU.

Results

This study was carried out to investigate heavy metal concentrations in the muscle of nine commercially important fish species available in fish-markets of Kathmandu Valley because the level of heavy metals in such commercial fish has been rarely investigated. A total of 36 fish samples was analyzed for levels of heavy metal contaminants. The levels of these contaminants vary significantly from species to species. Thus, the data were combined for the inter-specific comparisons which showed that there were inter-specific differences in levels of all heavy metals. No single type of fish species was consistently higher for several metals. Our results on heavy metals present in the fish tissues from the Kathmandu Valley were compiled and presented in the Table 1. A comparative study of mean concentrations (Mean±SD) of Mn, Zn, Pb, As, Cd and Cr (µg/g dry wt.) in the muscle tissues of different fish species available in the fish-markets of Kathmandu Valley were also clearly shown in Figure 2. *Puntius chola* had the highest mean value of lead, cadmium, and manganese; *Eutropiichthys vacha* had the highest mean value of arsenic, and *Clarias batrachus* had the highest mean value of zinc and chromium. The mean values varied among the species by an order of magnitude for most heavy metals, except arsenic, cadmium and chromium. The levels of arsenic in *Catla catla, Clarias batrachus* and *Labeo rohita* were below detection limits of the instrument. Nevertheless, the levels of arsenic in other fish species were also very low. Overall, the mean values of heavy metals in most cases are considerably lower than the standard permissible level except Mn, Pb and Cr. Low Cd, As and Zn levels in fish muscle

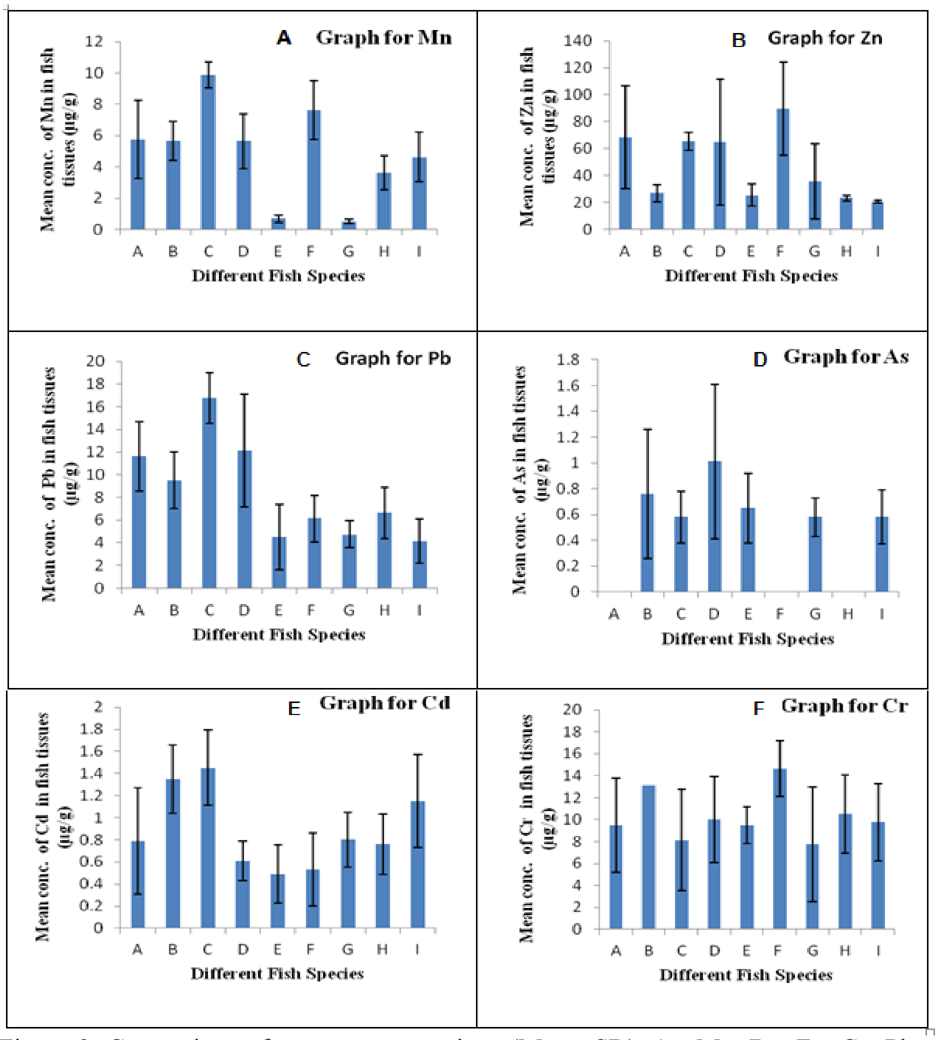

Figure 2. Comparison of mean concentrations (Mean±SD). A - Mn, B - Zn, C - Pb, D - As,E- Cd and F-Cr (µg/g dry wt.) in the muscle tissues of different fish species available in the fish- markets of Kathmandu valley

normally indicate that no artificial contamination has occurred. First, three maximum mean concentrations of potentially toxic elements were found 16.75 µg/g in *Puntius chola*, 12.13 µg/g in *Eutropiichthys vacha*, 11.63 µg/g in *Catla catla* for lead; 1.45 µg/g in *Puntius chola*, 1.35 µg/g in *Pampus* species and 1.15 µg/g in *Mystus tengara* for cadmium; and 1.01 µg/g in *Eutropiichthys vacha*, 0.76 µg/g in *Pampus* species, 0.65 µg/g in *Pampus chinensis* for arsenic. Similarly, first three maximum mean concentrations of essential elements were found 9.88 µg/g in *Puntius chola*, 7.63 µg/g in *Clarias batrachus*, 5.75 µg/g in *Catla catla* for manganese; 89.75 µg/g in *Clarias batrachus*, 68.37 µg/g in *Catla catla*, 65.38 µg/g in *Puntius chol*a for zinc; and 14.63 µg/g in *Clarias batrachus,* 13.13 µg/g in *Pampus* species, 10.50 µg/g in *Labeo rohita* for chromium. Calculation of the overall mean concentrations of Zn, Cr, Pb, Mn, Cd and As in the muscles of the nine fish species gave the following results: Zn (46.68±25.51 µg/g), Cr (10.32±2.23 µg/g), Pb (8.46±4.33 µg/g), Mn (4.88±3.02 µg/g), Cd (0.88±0.35 µg/g) and As (0.69±0.17 µg/g) which leads to the following ranking: Zn > Cr > Pb > Mn > Cd > As. The metal levels in the muscles of the most of the fish species follow a similar ranking, except for few fish species.

Discussion

Like in other organisms, heavy metals are not destroyed by humans (Castro-Gonzeza and Méndez-Armentab, 2008). Instead, they tend to accumulate within the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans. Therefore, the heavy metals are among the pollutants which received the most attention and are considered to be in the most dangerous category of pollutants (Hassaan *et al*., 2007). An early example of an environmental problem due to heavy metal occurred in 1952, in the vicinity of the Japanese fishing harbour of Minamata. This disease (Minimata disease) was a

Table 2. Maximum acceptable levels of heavy metals in fish muscle (µg /g dry wt.) according to international standards and range of concentrations found in the muscle of commercial fishes from fish-market of Kathmandu valley

Organization	Mn	Zn	Pb	As	Cd	Cr	Reference
FAO/WHO	1.00	100	4.00	-	1.00	2.00	FAO (1983), WHO 1989).
CEFAS, BOE	-	-	-	-	5.00	-	CEFAS(1997), BOE(1991)
NHMRC	-	750	-	-	10.00	-	NHMRC(1987)
EEC	-	-	-	-	-	8.00	EEC(1979)
EC,2001	-	-	4	-	1.00		European Community
BOE	-	-	25.00	-	-	-	BOE (1991)
MFR	-	100	-	-	1.00		MFR(1985)
Range of metals in the present study	0.51-9.88	20.63-89.75	4.13-16.75	ND-1.01	0.49-1.45	7.75-14.63	Present study

* FAO/WHO, 1984. ND, not detected

result of consuming fish and shrimp contaminated by methyl mercury and non- organic mercury from the wastewaters discharged by Chlor-alkali factories. Another example is the Ita-Ita Disease in Fugawa, Japan in 1955 (Dural *et al*., 2007). Arsenic is very interesting because most studies deal with inorganic arsenic, yet the arsenic in fish is mainly organic (Eisler, 1994), making it difficult to examine the effects. Adverse effects of cadmium can occur in fish with dietary levels of 0.1 ppm (Eisler, 1985). Whole body burdens of cadmium in fish adversely affected at levels of 1.0 ppm in their diet (Eisler, 1994).

Levels of Cr in our commercial fish were slightly above the permissible limits, suggesting that predators or scavengers would be at risk from chromium if they ate them in the wild. Lead is a neurotoxin that causes behavioral deficits in vertebrates (Weber and Dingel, 1997) and can cause decreases in survival, growth rates, learning, and metabolism (Eisler, 1988; Burger and Gochfeld, 2002). Levels of 50 ppm in the diet can cause reproductive effects in some predators, and dietary levels as low as 0.1–0.5 ppm are associated with learning deficits in some vertebrates (Eisler, 1988). In this study, the levels of lead found to be in the range of 4.13-16.75 µg / g, suggesting that some sensitive predatory vertebrates may be impacted by the levels of lead in these fish. There are remarkably few studies on the dietary effects of manganese on predators or on the adverse effects associated with particular tissue levels on the organisms themselves. Of the metals examined during this study, manganese is in the most need of extensive laboratory and field studies. Although it is an essential trace element, it also exhibits toxicity (Burger and Gochfeld, 1996). Manganese and chromium are essential trace elements, although all can cause toxicity with high doses.

Standards and guidelines for humans

It is surprising that no uniform source of guidance or standards for most metal residues in fish tissue is available. There is no single reference for acceptable levels of most metals in marine or freshwater fish, whether self-caught or commercially. The maximum tolerable level of heavy metals in fish muscle given by different organizations and guidelines is depicted in the Table 2. The Environmental Protection Agency (EPA) has set arsenic tissue residues of 1.3 ppm fresh weight of freshwater fish as the criterion for human health protection. Although the EPA Integrated Risk Information System (IRIS) database does not specify values for fish or food in general, it can be understood by comparing the chronic oral reference doses.

From a public health perspective, people have several problems with making choices in markets about what fish to buy based upon available knowledge, which usually includes identification of species or at least type, and knowing which kinds of fish have low levels of contaminants. Except for methyl mercury (Burger and Gochfeld, 2004) and PCBs, this information is generally unavailable. As indicated above, the name from the fish for sale may even be misleading. The data from this paper suggested that some species have relatively low levels of contaminants of concern, such as lead, and cadmium. However, the same fish did not have either the highest levels of all metals or lower levels. We see that the risk information given to the public, which mainly deals with the risk from mercury (and PCBs), does not present a complete picture. Consumers should always bear in mind that standards provide a margin of safety only if they are enforced locally. Thus, we suggest that there is an urgent need for more information on contaminant levels in fish available at the fish market along with exact species identification, collection location, and growth method (farmed or wild-caught). Then data on heavy metal

Table 3. Pearson's moment correlation coefficients between the heavy metals

	Mn	Zn	Pb	As	Cd	Cr
Mn		0.654[b]	0.741[b]	0.142	0.473	0.310
Zn			0.509	0.432	-0.217	0.268
Pb				0.315	0.462	-0.183
As					-0.363	0.447
Cd						-0.147

Note: significant correlations at p<0.01(1%) are mark as a, and at p<0.05 (5%) are mark as b

contaminants in fish could allow people to make informed decisions about which fish to eat to reduce their risk from the contaminants.

Inter-Metal Relationship in fishes

Inter-metal correlations of fish species were assessed and presented in Table 3. The correlations between the different metals may result from the similar accumulation behaviour of the metals in the fishes and their interactions (Rejomon *et al.*, 2010). The significant correlations among metals may reflect a common source of occurrence and indicative of similar biochemical pathways for subsequent accumulation in the muscle tissue of fishes. In the present study, manganese is strongly correlated with zinc and lead. No other significant correlation was observed between the studying heavy metals.

Conclusion

The present study was carried out to provide information about the level of heavy metal contaminants in the muscle tissue of fish from the fish-markets of Kathmandu Valley and also to disseminate possible health hazards due to consumption of heavy metal contaminated fish. The results obtained from this study showed that the highest mean concentrations of metals in the muscles of the studied fish species were determined for zinc, and the lowest was for arsenic. There was no single type of fish that was consistently higher for all metals. The highest levels of metals were found in muscles of *Puntius chola, Clarias batrachus* and *Catla catla.* All fish samples exceeded the permissible limits of FAO/WHO guidelines for lead and chromium. Whereas, the rest of the fish samples showed the presence of the heavy metal contaminants at around or below the limits proposed by various international standards and guidelines. This indicates that none of the examined fishes are fully safe for human consumption, and people should be very conscious about the fish available in fish markets of Kathmandu Valley for frequent consumption. The current study also emphasized for a continuous monitoring of heavy metals in commercial fish of the Kathmandu Valley to ensure the prescribed worldwide limit.

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