Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **7(2),** 42-48, February **(2017)** *Res. J. Chem. Sci.*

Assessment of trace elements in some commonly consumed fish species marketed in Kathmandu, Nepal

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Available online at: www.isca.in, www.isca.me
Received 12th December 2016, revised 7th February 2017, accepted 12th February 2017

Abstract

In recent years, the consumption of fish in Nepal has been increasing largely due to its high nutritional value. However, it could bring serious health impacts due to heavy metals in elevated quantity since bioaccumulation of metals in aquatic inhabitants enter into human body through food chain. In this study, levels of Pb and Cd were determined by Atomic Absorption Spectrophotometer (AAS) in a total of 20 fish samples of four fish species viz., Buhari (Wallago attu), Mugree (Clarias batrachus), Catla (Catla catla) and Rohu (Labeo rohita) marketed in Kathmandu. Four various organs (liver, flesh, intestine and gills) of each fish species were analyzed as potential sites for accumulation of these toxic metals. The overall concentration ranges considering all the organs for the metals analyzed in mg kg^{-1} (dry basis) were Pb (0.02 – 0.47) and Cd (0.01 – 0.29) for Buhari (Wallago attu), Pb (0.02 – 1.29) and Cd (0.04 – 0.24) for Mugree (Clarias batrachus), Pb (0.02 – 0.11) and Cd (0.01 – 0.22) for Catla (Catla catla) and Pb (0.02 – 1.10) and Cd (0.01 – 0.39) for Rohu (Labeo rohita). The results also revealed variation in metal concentration in various organs of the fish species. All the fish samples except Buhari (Wallago attu) recorded higher accumulation of Pb and Cd in gills. An estimation of Pb and Cd weekly intake through the fish consumption was also investigated. The results showed that the concentrations of Pb and Cd in these fish species did not exceed the maximum permitted limits set forth by FAO/WHO indicating that they are safe for human consumption. However, a regular monitoring and assessment of toxic contaminants in fish marketed in Kathmandu is needed to help safeguard the health of humans and environment as well.

Keywords: Lead, Cadmium, Fish, Weekly intake, Kathmandu.

Introduction

Fish is one of the widely distributed animals in aquatic environment and important food source for human body. It is, therefore consumed by a large population of the world. The fish consumption is beneficial to human health particularly due to the presence of essential ω-3 polyunsaturated fatty acids (PUFA), vitamins, proteins and other valuable mineral compounds. These constituents in fish has offered health beneficiaries against coronary heart disease, reducing blood clotting arrhythmia and thrombosis and as well as lowering plasma triglyceride levels[1,2].

Fish is regarded as a bio-accumulator of inorganic and organic pollutants[3]. They can absorb heavy metals (HMs) through epithelial or mucosal surface of their skin, gills and gastrointestinal tract. Heavy metal contamination in freshwater fish is a recognized environmental problem and is mainly by the anthropogenic activities. The anthropogenic sources include the increasing urbanization and population growth, industrial and agricultural activities, exploration and exploitation of natural resources as well poor environmental regulations[4]. The regular monitoring of elements such as Hg, Cd, Pb, As, Cu, Zn, Fe and Sn in fish, are therefore important[5].

Methyl-mercury, Pb and Cd pose a serious risk in children and adults through the dietary in elevated concentrations. In addition to chronic and acute diseases, these toxic metals also damage brain and kidneys including carcinogenic actions[2,6]. Besides, Pb may cause learning disabilities, impaired protein and hemoglobin synthesis and shorten the life span of RBC which leads to severe anemia in children[7]. The renal failure in human, calcium loss in bone and malfunction of peripheral and cranial nervous system are some of the toxic effect of cadmium[2].

Citing the toxicity of heavy metals on human, the WHO, FAO and the US Food and Drug Administration (US FDA) have set forth limits of these toxic metals on the human dietary intake. Besides, the joint FAO/WHO committee on Food Additives has also estimated values for provisional tolerable weekly intake (PTWI) for safety consumption by human beings[8].

The increasing fish consumption by a large portion of the population of Kathmandu has heightened the importance on assessment and regular monitoring of the quality of fish marketed in this city area. The study on heavy metals accumulation in fish is of particular interest because of the potential risk to humans who consume them. Despite, there are very limited studies available in literature regarding the assessment of heavy metals in fish marketed in Kathmandu. Hence, the objectives of current study were: (a) the determination of Pb and Cd levels accumulated in various organs (liver, intestine, flesh and gills) of four fish species *viz.,* Buhari (*Wallago attu*), Mugree (*Clarias batrachus*), Catla (*Catla catla*) and Rohu (*Labeo rohita*) marketed in Kathmandu, and (b) the estimation of weekly intake of these trace metals through the fish consumption.

Material and methods

Fish sample collection: Initially, local fish markets and departmental stores were surveyed to assess different types of fish species available in Kathmandu for consumption. A list of fish species marketed in Kathmandu is presented in Table-1. Among the available fish types, four most common ones preferred for consumption by a large population of Kathmandu as revealed by formal interviews were Buhari (*Wallago attu*), Mugree (*Clarias batrachus*), Catla (*Catla catla*) and Rohu (*Labeo rohita*) and hence selected for the purpose. These four species of fish were sampled randomly from five different locations (Khichapokhari, Kalimati, Chabahil, Dillibazar and Balaju,) in Kathmandu. A total of 20 samples (each four species from the above five different market sites) were collected during March 2016. The samples packed up in separate polythene bags were brought to the laboratory for further processing. They were stored in a refrigerator at -20^0 C till further processing. A brief description of each fish species is given in Table-2.

Sample treatment and processing: Each of the sampled fish was washed thoroughly with clean tap water first and then rinsed with distilled water. They were dissected one after another to collect the fish organs (liver, intestine, flesh and gills) separately after removing the scales. The dissected four various organs of each fish species were dried in a hot air oven at 105^{0}C for 24 hours till constant weight. Then, they were pooled and milled separately with a mortar and pestle, packed up in dry labeled plastic containers and finally stored in desiccators until digestion.

Sample digestion and analysis of Pb and Cd: Accurately 1.00 g of each dried organ was taken in acid washed crucible and ignited in muffle furnace at 500^{0}C till complete ash. The crucible was then cooled to room temperature and the ash was extracted with small volume of nitric acid (1:1). After cooling the digested sample, they were filtered using the Whatman No. 42. Finally, doubly distilled water was used to adjust the final volume of the filtrate to 50 ml.

Reagents and instrument: The certified standard solutions (1000 ppm) for Pb and Cd were obtained from Merck, Germany. Dilute (1:1) nitric acid prepared from the HNO_3 of analytical grade (E. Merck, Germany) was used to wash all the glassware apparatus and DD water for rinsing them before use.

Table-1: List of fish species available in fish markets of Kathmandu.

S.N.	Scientific Name	Common name
1.	*Labeo rohita*	Rohu
2.	*Clarias batrachus*	Mugree
3.	*Heteropneustes fossilis*	Singya
4.	*Channa punctatus*	Hele macha
5.	*Channa gachua*	Hele macha
6.	*Catla catla*	Catla
7.	*Wallago attu*	Buhari
8.	*Pampus species*	Pamplet
9.	*Puntius chola*	Pothi
10.	*Eutropiichthys vacha*	Bachawa
11.	*Pampus chinensis*	Rup Chanda
12.	*Labeo bata*	Mahur (Walking catfish)
13.	*Mystus tengara*	Tengra

Quantification of Pb and Cd in each of the similarly extracted samples was performed by Atomic Absorption Spectro-photometer (AAS) using air acetylene flame at Aastha Scientific Research Service, Pvt. Ltd., Maitidevi, Kathmandu. The AAS of model novaAA305 manufactured by Analytikjena, Germany was used for the purpose following strictly all the instrumental parameters as recommended by the manufacturer.

Applications: The AAS was firstly assembled with hollow cathode lamps along with a deuterium background corrector and then warmed up for nearly 30 minutes. An air-acetylene flame was used during analysis. Background correction measurements were made by means of non-absorbing lines. The calibration curve was checked virtually for linearity and replication. A series of the standard solutions of each specific metal was prepared and the instrument calibrated with the prepared solutions as per the manufacturer's recommendations. The analytes were passed through the column maintaining a flow rate of 2 mL/min. The doubly distilled water was used for dilution of the solutions whenever required.

Calibration standards: Three point calibration standards of appropriate concentration were prepared for each element from the stock standard solution of 1000 ppm traceable to NIST manufactured by Merck, Germany. Successive dilution of the stock solution was carried out at the order of 10^{-1} to obtain the calibration standard of linear range. These standards were used to calibrate the AAS while analyzing the samples.

Analysis data of fortified samples (Recovery test): At least three organ samples were selected from different fish species for the spike analysis of each analyte. A known quantity of analyte was added to 1.000 g of selected samples. Reference metal standards traceable to NIST manufactured by Merck, Germany were used to prepare fortified samples. Fortified samples were treated as per the sample and heavy metal level in the fortified samples was identified by AAS. The average percent recovery for Pb and Cd were 94.8 and 92.6 respectively.

Reagent blank: Nitric acid (10 ml) was added to a clean acid washed beaker. The beaker was partly covered with watch glass and was slowly evaporated to until the volume was reduced to 1-2 ml. Then the beaker was cooled and interior of the beaker was rinsed with small volume of distilled water. A volumetric flask with a 25 ml capacity was taken for the reagent blank and the final volume adjusted up to the mark with the distilled water. This reagent blank was used to exclude potential error on measurement caused by the reagents.

Calculation: The working formula below was used to calculate the metal concentration:

$$\text{Concentration of metal, } \mu g/g = \frac{\text{Observed conc. (ppm) *Vol. of sample prepared (mL)}}{\text{Wt. of sample (g)}}$$

Note: 1µg/g is equivalent to 1 mg/kg.

Weekly intake of metal (WIM): The weekly intake of Pb and Cd through fish consumption was calculated by the following equation[9].

Weekly intake of metal (µg/kg body weight) = C_{metal} x $W_{fish\ intake}$ / $B_{average\ weight}$

Where: C_{metal} = mean metal concentration in fish, $W_{fish\ intake}$ = weekly intake of fish, $B_{average\ weight}$ = average body weight.

The average weekly fish intake rate was calculated by conducting a survey where 100 people having average body weight of 60 kg were asked for their daily intake of particular fish species from the sampling area.

Associated health risk after WIM analysis was studied comparing against FAO/WHO permissible guideline values[10].

Statistical evaluation: Data processing and statistical analysis were carried out in IBM-PC computer using EXCEL spreadsheets. Descriptive statistics (mean, range, standard deviation etc.) was performed after the elemental analysis. Pearson's correlation coefficient was used to evaluate the correlation between the metals.

Results and discussion

The present study was performed to investigate Pb ad Cd accumulation in four commercially important fish species *viz.*, Buhari (*Wallago attu*), Mugree (*Clarias batrachus*), Catla (*Catla catla*) and Rohu (*Labeo rohita*) marketed in Kathmandu because the level of such inorganic contaminants in these commercially important fish has been rarely investigated in a developing country like Nepal. A total of 20 fish samples were analyzed for the metal accumulation in four various organs (liver, intestine, flesh and gills) of each species (Table-2).

Levels of Pb and Cd in fish organs: Table-3 shows mean and range of heavy metal concentration in various organs of the fish samples. The overall ranges of the metals comprising all the organs of the fish samples are as follows: Pb (0.02 – 0.47 mg kg^{-1}) and Cd (0.01 – 0.29 mg kg^{-1}) for Buhari (*Wallago attu*), Pb (0.02 – 1.29 mg kg^{-1}) and Cd (0.04 – 0.24 mg kg^{-1}) for Mugree (*Clarias batrachus*), Pb (0.02 – 0.11 mg kg^{-1}) and Cd (0.01 – 0.22 mg kg^{-1}) for Catla (*Catla catla*) and Pb (0.02 – 1.10 mg kg^{-1}) and Cd (0.01 – 0.39 mg kg^{-1}) for Rohu (*Labeo rohita*). This result revealed the maximum range of Pb concentration in Mugree (*Clarias batrachus*) and Cd in Rohu (*Labeo rohita*). The significant correlation (r = 0.732) between Pb and Cd show a common source of the metal exposure (water), possibly waste disposals from anthropogenic activity.

Table-2: Brief description of fish samples for the present study (n=5; mean values).

S.N.	Species	Common name	Height (cm)	Length (cm)	Weight (gm)	Sampling Sites
1.	*Wallago attu*	Buhari	10 ± 2.5	40 ± 4.5	1100 ± 50.0	S_1-S_5
2.	*Clarias batrachus*	Mugree	5 ± 1.5	25 ± 3.0	650 ± 25.0	S_1-S_5
3.	*Catla catla*	Catla	17 5 ± 2.0	34 ± 2.5	1000 ± 80.0	S_1-S_5
4.	*Labeo rohita*	Rohu	15 ± 3.0	38 ± 4.0	930 ± 40.0	S_1-S_5

S_1= Khichapokhari, S_2 = Kalimati, S_3 = Chabahil, S_4 = Dillibazar, S_5 = Balaju.

Table-3: Concentrations of Pb and Cd (mg kg^{-1}, dry basis) in various organs of fish species (mean ± SD; n=5).

S.N.	Specimens	Heavy metal		Liver	Intestine	Flesh	Gills
1.	Buhari (*Wallago attu*)	Pb	Mean	0.44± 0.02	0.33±0.01	0.05±0.03	0.05±0.02
			Range	0.41-0.47	0.31-0.35	0.02-0.08	0.02-0.08
		Cd	Mean	0.13± 0.01	0.17±0.02	0.26± 0.2	0.19±0.25
			Range	0.01-0.15	0.13-0.19	0.22-0.29	0.16-0.23
2.	Mugree (*Clarias batrachus*)	Pb	Mean	0.13±0.01	0.05±0.02	0.08±0.02	1.27±0.02
			Range	0.10-0.17	0.02-0.08	0.05-0.09	1.22-1.29
		Cd	Mean	0.05±0.02	0.10±0.02	0.06±0.01	0.21±0.02
			Range	0.04-0.09	0.08-0.14	0.05-0.09	0.18-0.24
3.	Catla (*Catla catla*)	Pb	Mean	0.05±0.03	0.05±0.03	0.05±0.02	0.07±0.02
			Range	0.02-0.10	0.02-0.10	0.02-0.08	0.05-0.11
		Cd	Mean	0.17±0.02	0.07±0.02	0.02±0.01	0.18±0.07
			Range	0.14-0.18	0.04-0.09	0.01-0.03	0.14-0.22
4.	Rohu (*Labeo rohita*)	Pb	Mean	0.05±0.05	0.05±0.01	0.05±0.03	1.06±0.49
			Range	0.03-0.07	0.02-0.05	0.02-0.10	0.80-1.10
		Cd	Mean	0.02±0.01	0.02±0.01	0.05±0.02	0.30±0.06
			Range	0.01-0.04	0.01-0.04	0.01-0.09	0.22-0.39

Recommended maximum limits for fish[9]: Pb: 2 mg kg^{-1} and Cd: 1 mg kg^{-1}.

Buhari (*Wallago attu*) contained the highest level (0.44 mg kg^{-1}) of Pb in liver followed by intestine (0.33 mg kg^{-1}), while flesh and gills showed the least preferred site for Pb as they both recorded the same lowest levels (Table-3). The level of Pb accumulation in various organs of the same species followed the order as liver > intestine > flesh = gills. On the other side, the level of Cd followed almost reverse order as flesh > gills > intestine > liver with the highest mean concentration of 0.26 mg kg^{-1} (gills) and the lowest concentration of 0.13 mg kg^{-1} (liver). Among the organs of this species, liver and intestine showed comparatively higher accumulation of Pb than Cd, flesh and gills showed higher accumulation of Cd than Pb.

In Mugree (Clarias batrachus), the mean level of Pb was found to be 0.13, 0.05, 0.08 and 1.27 mg kg^{-1} in liver, intestine, flesh and gills respectively. On the other side, the Cd level was found to be 0.05, 0.10, 0.06 and 0.20 mg kg^{-1} in liver, intestine, flesh and gills respectively. In both the cases, gills contained the highest levels of Pb and Cd while the intestine recorded the lowest concentration for Pb and liver for Cd.

In Catla (Catla catla), the concentration of Pb in liver, intestine and flesh was found to be same (0.05 mg kg^{-1}) except gills which showed slightly higher level (0.07 mg kg^{-1}). For Cd, the concentration in various organs followed the order as gills > liver > intestine > flesh with gills accumulating 0.18 mg kg^{-1} and flesh accumulating 0.02 mg kg^{-1}. Gills showed the preferred site for Pb and Cd accumulation in this species.

Rohu (*Labeo rohita*) also followed the same pattern of Pb accumulation in its organs like that of Catla (Catla catla). While this species exhibited the same level of Pb in liver, intestine and flesh, comparatively higher Pb concentration (1.06 mg kg^{-1}) was recorded in gills. Similarly, the accumulation of Cd in this species was also found to be comparatively higher in gills (0.30 mg kg^{-1}) like that of Pb accumulation. While liver and intestine showed the same Cd level, flesh recorded slightly higher Cd level compared to these two organs. Of these two inorganic contaminants, Pb demonstrated comparatively higher accumulation in almost all the organs of the species than that of Cd.

The physiological role of each organ of a fish may be responsible for the accumulation of different levels of Pb and Cd. These metals are assimilated by fish organism via two routes: digestion and absorption through the gill[11]. In addition, biological habitat, temperature, salinity, chemical state, pH, dissolved oxygen, water transparency etc.,[12] as abiotic factors may influence the metal accumulation in fish tissue together with biotic ones such as species, sex, body mass, age, physiologic conditions, nourishment sources and feeding habit[2]. Therefore, it becomes difficulty in predicting a rate of metal bioaccumulation in fish tissue. However, the higher metal concentrations in the metabolically active gills of almost all the fish samples could be attributed to the metal complexion with the mucus. That virtually makes impossible to remove metals from the gill lamellae[13]. Furthermore, the adsorption of metals onto the gills surface as the first target for pollutants in water could also contribute in the total metal levels of the gills.

Khail and Faragallah[13] and Marzouk[14] also reported similar results with higher metal concentration in gills of some fish species. Deb and Fukushima[15] also confirmed gills as the potential site for higher metal accumulation. In the study conducted by Ishaq *et al*[16], the highest concentration of heavy metals were found in gills of Tilapia zilli and Clarias gariepinus. However, the present study revealed that the mean values of Pb and Cd levels (Table-3) in various fish organs did not exceed the maximum permissible limits as set by FAO/WHO[10]. Hence it may be confirmed that these fish species are safe for human consumption.

Estimated weekly intake of metal: Flesh (muscle) is the main edible part of fish for human consumption and hence, used in the present study for risk assessment. For Pb and Cd, the weekly intake of such contaminants through fish consumption was estimated based on PTWI (provisional tolerable weekly intake) values set forth by JECFA[9]. Formal interviews conducted during the present study showed that the average consumptions of fresh fish per person per day was 50 g. Therefore, in weekly intake estimation, a 50 g of flesh per person per day and the adults having an average of 60 kg body weight were used. This amount is appropriate in accordance with the United States Environmental Protection Agency (US EPA), which recommends a fish intake of 340 g per person per week, a quantity equivalent to 49 g per person per day[17].

Table-4 shows the estimated weekly intake values for each fish species and compared against PTWI. If the mean concentrations of Pb and Cd recorded in the present study (Table-3) are representative of contaminants in the fish species under investigation and consumed by the subjects then the estimated weekly intake would be 0.29 (Pb) and 1.52 (Cd) µg/kg body weight for Buhari (*Wallago attu*), 0.47 (Pb) and 0.35 (Cd) µg/kg body weight for Mugree (Clarias batrachus), 0.29 (Pb) and 0.12 (Cd) µg/kg body weight for Catla (*Catla catla*) and 0.29 (Pb) and 0.29 (Cd) µg/kg body weight for Rohu (*Labeo rohita*) (Table-4).

The estimated weekly intake of such metal contaminants in the test species suggests that the consumption of such fishes does not pose a health risk as the values are well below the provisional tolerable weekly intakes (PTWIs) (Table-4) set by JECFA[9]. PIWI is expressed on a weekly basis and highlights a long-term exposure risk for metal contaminants that may accumulate in the human body.

Morgano *et al*[19] reported 1.08, 1.33, 0.88 and 0.59 µg kg^{-1} week body $weight^{-1}$ for Pb in Tuna, Salmon, Snook and Porgy fish types respectively while they estimated 0.08, 0.05, 0.04 and 0.05 µg kg^{-1} week body $weight^{-1}$ respectively for Cd in the same fish types. While Falco *et al*[18] in their study estimated the daily intake of Cd (1.34 µg/day) and Pb (2.48 µg/day) by the population of Catalonia through marine species, Marti-Cid *et al*[20] published some differentiated results on the dietary intakes of Cd, Hg, and Pb from an earlier study in 2000. The variation of metal levels in analyzed fishes was found to be increased in case of Pb daily intake, while intakes of Cd and total Hg decreased. However, intake levels remained below the respective PTWIs, indicating low risk for human health. The fish species and the estimated dietary intake of metals in the present study are, however not in consistent with the above reported values.

Table-4: Heavy metal, estimated weekly intake and PTWI values for intake of 50 g fish per person based on mean values found in samples (flesh).

Species	Mean heavy metal (mg/kg)	Estimated weekly metal intake (µg /kg body weight)[a]	PTWI[b,9]
Buhari (*Wallago attu*)	Pb = 0.05 Cd = 0.26	0.29 1.52	25 7
Mugree (*Clarias batrachus*)	Pb = 0.08 Cd = 0.06	0.47 0.35	25 7
Catla (*Catla catla*)	Pb = 0.05 Cd = 0.02	0.29 0.12	25 7
Rohu (*Labeo rohita*)	Pb = 0.05 Cd = 0.05	0.29 0.29	25 7

[a]Weight for adults (older than 17 years): 60 kg, [b]Unit: µg kg^{-1} week body $weight^{-1}$;

Conclusion

The Pb and Cd levels in the widely consumed fish species (Buhari, Mugree, Catla and Rohu) in Kathmandu were determined and assessed their quality by comparing the metal levels in samples against maximum permitted levels stipulated by FAO/WHO. The results from this study suggested that differences in the concentrations of Pb and Cd existed among the fish samples as well as within the various organs of the same fish species. All the four fish species assessed in the present study have shown Pb and Cd levels below the maximum permitted limits set forth by FAO/WHO.

In addition, the results from the estimated weekly intake of Pb and Cd suggested that they did not exceed the provisional tolerable weekly intake (PTWI) values. This indicates that the selected fish species for the study purpose are well safe for consumption. However, it is utmost important to know the origin and sources of fish intended for commercial purpose, since the aquatic environment, fish feed, catching place, age etc., are among many other factors that determine quality of the marketed fish. These factors may significantly influence on the levels of inorganic contaminants in fish. Moreover, regular monitoring and routine assessments on the quality of fish species and seafood marketed in Kathmandu are needed to help safeguard the health of humans and environment as well.

Acknowledgements

We are grateful to Research Management Committee (RMC), Padma Kanya Multiple Campus, Tribhuvan University, Kathmandu, Nepal for the financial support. We are also thankful to Aastha Scientific Research Service Pvt. Ltd., Maitidevi, Kathmandu for providing service for AAS analysis of fish samples.

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