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Human health risk assessment of heavy metals in major carp (*Labeo rohita*) of Mahananda river in Northern India

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Abstract

This study determined the levels of heavy metals (Cr, Cu, Ni, Zn, Cd, and Pb) in muscle tissues of *Labeo rohita* from river Mahananda and estimated the consumption rate limits and health risk posed by fish ingestion. The metals followed the magnitude order of Zn > Cu > Pb > Ni > Cr > Cd. The levels of studied metals were below the permissible limits set by WHO (1995) and USFDA (1993), while Cd and Pb had a mean value of 0.64 ± 0.017 and 1.135 ± 0.013 µg/gw respectively, which was above the FAO (1983) and FAO/WHO (1989) guidelines. The estimated tolerable weekly intake (ETWI) was 10 times below the provisional tolerable weekly intake (PTWI) sea by JECFA (2003).To estimate human health risk target hazard quotient (THQ), hazard index (HI) and target cancer risk (TCR) were calculated and discussed. THQ for individual and combined metals were lower than one representing no non-carcinogenic risk to consumers. The TCR of Cr, Ni, Cd, and Pb for intake fish was $7.3x10^{-7}$, 1.07 $x10^{-7}$, 1.85 x 10^{-7}, and 6.67 x 10^{-7} respectively was below the acceptable carcinogenic risk (10^{-6} -10^{-4}) set by USEPA (2010) showing no carcinogenic risk to consumers. The correlation matrix indicated positive and significant correlations among studied metals, establishing chemical affinity. Significant relationships were found between metal levels in fish with weight and length. Relative risk showed that potential health risk could be attributed only to Cd (45.05%) level. The study concluded that consumption of the muscle tissues of *L. rohita* may not pose a health risk to human health at the levels of the analyzed metals, but should be consumed moderately to prevent bioaccumulation of the metals especially Cd.

Keywords fish consumption, fish muscle, health risks assessment, heavy metals

Introduction

Heavy metals are dangerous due to their toxicity, persistence in the environment, and bioaccumulation in nature [1-2]. They enter an aquatic ecosystem from natural and anthropogenic sources. Anthropogenic activities such as municipal and industrial effluent, sewage sludge [3-4], atmospheric deposition [5], and agricultural activities [6] continuously increase the amount of heavy metal in the aquatic ecosystem, which deteriorates water quality and danger to human health and aquatic organisms [2]. Due to non-degradation, heavy metals are deposited and assimilated in water, sediment and body of aquatic organisms leading pollution in the aquatic ecosystem [7]. When organisms are exposed to high metal levels in an aquatic environment they can absorb metals directly from the environment, contaminated water, and food, and thus, accumulate them in their tissues, further they can enter

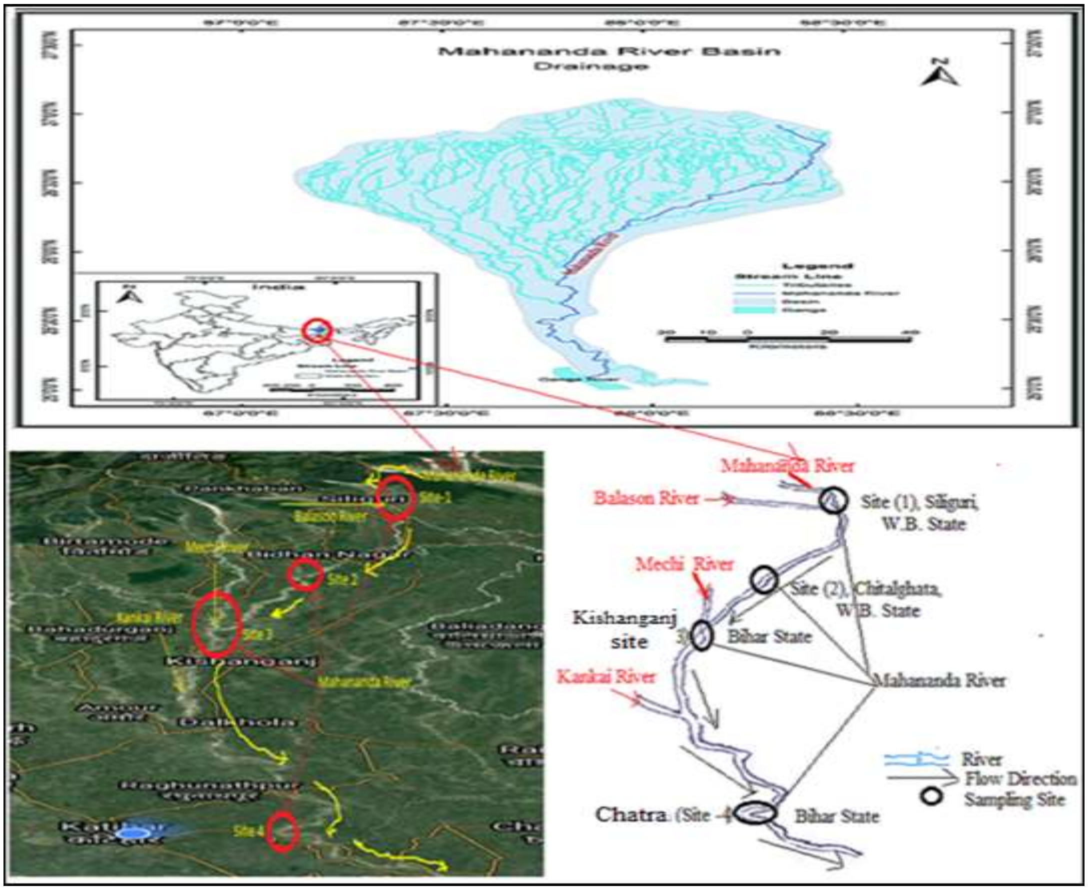

Figure 1. Map showing sampling sites and satellite map of Mahananda River in Northern India

the food chain and extend the problem to humans [8]. Aquatic organisms, especially fishes are in the food basket of human as they are one of the best sources of the high level of unsaturated acids and low levels of cholesterol and also have a high level of many essential nutrients, including high quality of proteins, iodine and various vitamins and minerals [9]. According to the literature, it contains omega-3 fatty acids, which are beneficial for humans who obtain from their diet, which have potential health benefits, such as helping prevent cardiovascular disease, prevent and treat depression, reduced risk of type -1 diabetics, prevent asthma in children and protect vision in old age and also improve sleep quality [10]. Various researches have shown the adverse effect of heavy metals on human health, such as renal failure, cardiovascular diseases, liver damage, and even death [11-12]. Fishes are on top of the aquatic food chain therefore, they can accumulate a significant amount of heavy metals [13]. Bioaccumulation of heavy metal in freshwater fish depends upon the various factors such as fish characteristics and external environmental factors. The retention of heavy metal in the body of an organism depends on many factors such as the speciation of the metal concerned and the physical mechanism developed by the organism for the regulation, homeostasis, and detoxification of the heavy metal. The degree of bioaccumulation in different tissues of fish is generally different depending on the active tissue like liver, gills, and kidneys have a higher accumulation of the heavy metal than other tissues such as skin and muscles [14]. The low-level of heavy metal in muscle is particularly important because muscles are the main part of the fish and directly influence human health [14-15].

The risks of heavy metals are mainly divided into non-carcinogenic and carcinogenic effects. The U.S. EPA Region III Risk-Based Concentration Table (US EPA, 2011) presents methods for estimating the non-cancer risk (THQs) and the target cancer risk (TR). The USEPA developed the acceptable guideline

Table 1. Sampling sites at Mahananda River with their geographical coordinate

Site	Name	District	State	Latitude	Longitude	Elevation (ft.)
1	Siliguri	Darjeeling	West Bengal	26.703765 N	88.413772 S	400
2	Chitalghata	Uttar Dinajpur	West Bengal	26.481197 N	88.262439 S	239
3	Kishanganj	Kishanganj	North Bihar	26.099847 N	87.939582 S	173
4	Chatra	Katihar	North Bihar	25.491435 N	87.725059 S	105

values for THQs and TR, 1 and 10^{-6}-10^{-4}, respectively. The risk linked with the carcinogenic influences of target metal is conveyed as the additional probability of contracting cancer over a lifetime of 70 years. Several studies have been conducted on the risk assessment of heavy metals [16-19]. However, the regional survey of heavy metals in fish and an assessment of their risk to the general population is lacking. In the present study, the concentrations of Cr, Cu, Ni, Zn, Cd, and Pb in freshwater fish *Labeo rohita* inhabiting the heavy metal-laden Mahananda River were determined. The potential risks (non-carcinogenic and carcinogenic) of heavy metals by consuming contaminated fish for adults were also estimated.

Methodology

Description of the study area

The River Mahananda is one of the major rivers of northern India; it originates 6Kms north of Kurseong in the Darjeeling district of West Bengal in the Himalayan range, at the elevation of 2062 meters. It has a powerful role in regulating overall economy of the catchment area, such as Darjeeling, Uttar Dinajpur, and Maldah districts of West Bengal and Kishanganj, Purnea and Katihar districts of Bihar in more than one way. The River Mahananda starts its 360 Kms long journey to the Ganga out of which 324Kms are in India and 36Kms are in Bangladesh. The total drainage area of this river is 24,753 Sqkms, of which 5,293 Sqkms are located in Nepal, 6,677 Sqkms in West Bengal, 7,975 Sqkms in Bihar and rest is located in Bangladesh, where it finally joins the Ganga (Padma) near Godagarigh at in Nawabganj District. The River Mechi and Kankai flow through Nepal and form the boundary between India and Nepal and then flow through the Indian state of Bihar to join the Mahananda in Kishanganj district and join the Ganga near Chowkia Paharpur in the Katihar district (Figure 1).

Siliguri is only city of Darjeeling district of W.B., which located in the stretch of this river. According to Siliguri Municipal Corporation, Siliguri produces more than 350 tons of solid wastes daily. In this zone, the river receives an enormous amount of discharge municipal sewage and other effluents as well as dumping of solid wastes round the year. River Mahananda flows through Chitlghata village located in Chopra tehsil of Uttar Dinajpur district of W.B. It is perennial but in monsoon time, carries a huge amount of polluted water from the township and also receives effluents from agricultural runoff from catchment areas. There are environmental issues in pollution of the river in Kishanganj town of Kishanganj district of Bihar and discriminate due to municipal, domestic wastes and industrial effluents of many large, medium and small enterprises. Chatra village is situated at the bank of a river in Pranpur tehsil of Katihar district of Bihar. About 60% of solid wastes produced from domestic uses and municipal areas from the nearest township are dumped daily in the river. The river also receives an excessive amount of fertilizers and pesticides from surrounding vegetation derived through runoff from the surface of the soil. The positions of sampling locations with their coordinates are presented in Table 1.

Field sampling

Fish samples

A total of 40 fish samples were collected at the four sampling sites from the Mahananda river during the year 2018-2019 using a multifilament, nylon gillnet with help of local fishermen and immediately preserved on ice in an ice chest and then ice-packed fish species were transferred to the laboratory. After identifying all fishes, total length 30.5±0.1 to 36.7±0.1 cm and total weight 450.2±0.1 to 607±0.1g were recorded and kept frozen at -20°C. About 3g of the epaxial muscle on the dorsal surface, from each sample, were dissected using a stainless instrument by applying the method of [20] and put into Petri plate to dry at 120°C until reaching a constant weight.

Sample preparation for heavy metal analysis

The dried muscle tissues were digested by the method described by [20]. In this method, one gram of each sample was digested separately with $HClO_4$ and HNO_3 in the ratio 1:1 followed by sulphuric acid, and the mixture was heated at 200°C for 30 minutes. After complete digestion, each digested mixture was cooled at room temperature and then transferred into 50 mL volumetric flask. Distilled water was added to it to fill up to the mark and analyzed for Cr, Cu, Ni, Zn, Cd, and Pb using Atomic Absorption Spectrophotometer.

Tolerable rate limit

Estimated tolerable daily intake (ETDI)

The Estimated tolerable daily intake (ETDI) was calculated by using the following equation [21].

$$ETDI = \frac{E_D \times E_F \times F_{IR} \times C_F \times M_c}{BW_a \times AT_n} 10^{-3}$$

where ED is the exposure duration (30 years for non-cancer risk as used by USEPA 2011[22]; EF is the exposure frequency (365 days/year); FIR is the ingestion rate of fish, 19.5 g/day for Indian [23-24]; CF is the conversion factor (0.208) to convert fresh weight dry weight [25]; MC is the metal concentration in the muscle tissue of fish (µg/g dry weight basis); BWa is the average body weight (53.3 kg) of Indian male which is taken as 57 kg and that of female as 50 kg [26] and ATn is the average exposure time for non-cancerous ($E_F \times E_D$= 365 x 30 days as used by USEPA, 2011 [22, 25].

Estimated tolerable weekly intake (ETWI)

The Estimated tolerable weekly intake (ETWI) was estimated by using the equation described by USEPA, 2000 [27], and applied by Miri et al., [28].

$$ETWI = \frac{M_C \times C_R}{BW_a}$$

Where C_R is the fish consumption rate, 0.160 kg of fish consumption per week was set by FAO, [29] and other parameters have been already defined.

Percentage of provisional tolerable weekly intake (% PTWI)

The % PTWI was calculated by using the following equation described by Yeh et al., [30].

$$\% PTWI = \frac{ETDI}{PTWI} \times 100$$

Where provisional tolerable weekly intake (PTWT) is reference dose set by the Joint FAO/WHO Expert Committee on Food Additive (JECFA, 2003) [31] and ETWI is the estimated tolerable weekly intake in mg/kg bw/week.

Daily consumption rate limit of Fish (DCR_{lim})

To assess the non-carcinogenic effect of the contaminants, the daily consuming rare limit of fish was evaluated by using the following equation [28]

$$DCR_{lim} = \frac{R_{fD} \text{ x } BW_a}{M_c}$$

Where R_{fD} is the oral reference dose The value of R_{fD} in mg/kg bw/d for Cr, Cu, Ni, Zn, Cd, and Pb was applied from Integrated Risk Information System (USEPA, 2011) [32]. Other parameters have been defined previously. Based on the carcinogenic effect of the contaminants, the daily intake rare limit of fish contaminated with heavy metals was also evaluated by using the following equation [28]

$$DCR_{lim} = \frac{ARL \text{ x } BW_a}{CPS_o \text{ x } M_c}$$

Where ARL is the maximum acceptable individual lifetime risk level (10-5 was used for ARL [33], CPSo is the carcinogenic slope, oral in mg/kg bw /d taken from Integrated Risk Information System (USEPA, 2011) [32]. Other parameters have been discussed previously.

Monthly consumption rate limit of Fish (MCR_{mm})

To assess the maximum acceptable intake rate limit of fish contaminated with heavy metals in terms of meal per month (MCR_{mm}) was also obtained by using following equation described by [34] and developed by [28]

$$MCR_{mm} = \frac{DRC_{lim} \text{ x } T_{AP}}{MS}$$

Where, DCR_{lim} is daily consumption rate limit of fish in non-carcinogenic and carcinogenic effects. TAP stands for the average time period (30.44 days/month) and MS is the meal size (0.227 kg fish/meals) [34]

Metal Pollution Index (MPI)

Metal Pollution Index (MPI) as a mathematical model, used to calculate the total metals accumulation level in fish tissues and calculated using the equation described by Usero et al., [35]

$$MPI = (C_1 \text{ x } C_2 \text{ x -----------x } C_{fn})^{1/n}$$

Where C_f is the concentration of the metal n in the sample

Health risk assessment for fish consumption

Target hazard quotient (THQ)

The target hazard quotient (THQ) is usually applied to show the risk of non-carcinogenic effects, for each individual metal through fish consumption, which was calculated as per US EPA Region III Risk-Based Concentration Table (USEPA, 2011).The equation used for estimating THQ was as –

$$TCR = \frac{E_F \text{ x } E_D \text{ x } F_{IR} \text{ x } C_F \text{ x } M_c}{R_{fD} \text{ x } BW_a \text{ x } AT_n} \text{ x } 10^{-3}$$

or

$$THQ = \frac{ETDI}{R_{fD}}$$

Where ETDI is the estimated tolerance daily intake in mg/kg bw/day and R_{fD} is an oral reference dose. The value of RfD for Cr, Cu, Ni, Zn, Cd and Pb are 0.003, 0.04, 0.02, 0.3, 0.001 and 0.004 mg/kg bw/day respectively set by USEPA, 2011.

Hazard index (HI)

Hazard index is combined toxic effect of heavy metals on human health was estimated from THQs can be expressed as the sum of hazard quotients:

$$HI = \sum_{n=1}^{n=5} THQ = THQ_{(Cr)} + THQ_{(Cu)} + THQ_{(Zn)} + THQ_{(Cd)} + THQ_{(Pb)}$$

Target cancer risk (TCR)

The carcinogenic effect of consuming metal-contaminated fish was determined from target cancer risk (TCR). The method to estimate TCR was also provided in US EPA Region III Risk-Based Concentration Table (USEPA, 2011). The equation for calculating TCR was given as follows:

$$TCR = \frac{E_F \text{ x } E_D \text{ x } F_{IR} \text{ x } C_F \text{ x } C_m \text{ x } CPS_O}{BW_a \text{ x } AT_C} \text{ x } 10\text{-}3$$

or

$$TCR = ETDI \text{ x } CPSo$$

Where ATC is the average time for carcinogens (365 days/year x 67 years), since in India the average life expectancy for males is 65 years (approx.) and for females is 68 years (approx.), therefore an average of two extremes have been taken for carcinogenic averaging time (http://countryeconomy.com/demography/life-expectancy/india). CPS_O is the carcinogenic potency slope, oral. The value of CPS_O Pb, Cd, Ni, and Cr are $8.5 \text{x} 10^{-3}$, $3.8 \text{x} 10^{-3}$, $1.7 \text{x} 10^{-3}$ and $41 \text{x} 10^{-3}$ mg/kg/day as provided USEPA, 2011, while the other parameters have been defined previously. The US Environmental Protection Agency set an acceptable lifetime carcinogenic risk of 10^{-5} [25]

Relative risk/ Percentage Relative risk

Relative risk (RR) of contaminants for both carcinogens and non- carcinogens effect can be helpful for deciding the most harmful contaminants, which can be calculated by applying the following equation described by [33]

$$RR = M_c / R_{fD}$$

Where Mc and RfD are already explained above. Human health effects through fish consumption should increase with an increase in the relative risk.

Fulton's condition factor (K)

Fulton's condition factor (K) has been used as an indicator of health in fishing biology studies. It was estimated according to Htun-Han, [36] as per the formula given below:

$$K = \frac{W \times 100}{L^3}$$

Where, W=weight of fish (g), L=Length of fish (cm). K is the Condition Factor or Coefficient of Condition, W is the weight of the fish in grams, L is the total length of the fish in centimeters. Bamham and Baxter [37] classified 'K' as if K= 1.60 excellent conditions, trophy-class fish; K= 1.40 a good, well-proportioned fish; K= 1.20 a fair fish, acceptable to many anglers; K= 1.00 a poor fish, long and thin; K= 0.80 extremely poor fish, big head and narrow, thin body.

Results and Discussion

The concentration of Cr ranged from 0.81-0.84 µg/gdw with mean value 0.827±0.103 µg/gdw, which is very close to 0.84±0.05 µg/gdw reported by [38] in *L. rohita* from river basin of Ganga, India, and 0.83±0.001 µg/gdw reported by [39] in different fish species from Taihu lake, China. However, the level of Cr observed in this study is significantly higher than 0.15 µg/gdw set by FEPA, 2003[40] and below than 12-13 mg/kg recommended by USFDA, 1993[41] and also below than 18.84 ± 1.70 µg/gdw in same fish species from Buriganga, Bangladesh as reported by [42]. Cr is biologically essential for the metabolism of carbohydrate and amino and nucleic acid synthesis. However, when accumulated at high levels, it can cause serious trouble and diseases. When concentration reaches 0.1 mg/g or 100 ppm body weight, it can cause death. A high level of Cr at sampling point may be due to agricultural runoff, paints used in boats, and leaching from rocks in the study area [43].

The mean concentration of Cu in this study was 3.84±0.014 µg/gdw. The maximum concentration of 3.84 µg/gdw was recorded at site 1 and site 2 (W.B. region) and minimum concentration 3.81 µg/gdw was observed at site 3 (Bihar region) of Mahananda River. The level of Cu observed in this study is close to 0.15 - 4.42 µg/gdw in different fish species from Geriyo lake, Nigeria as reported by [44]and good agreement with 3.88 ± 0.15 µg/gdw in same fish species from river basin of Ganga as reported by [38]. The level of Cu recorded in this study are below the maximum permissible limit (MPL) set by FAO(1983), FAO/WHO (1989), WHO (1995 [45-47] and also below than 18.77 ± 2.18, 3.97-7.51, 41.36 ± 0.38, 6.39 - 25.66 and 6.99 -16.18 µg/gdw as reported by [42,48-51] respectively as shown Table 2. Cu is an essential element for the formation of hemoglobin and some enzymes in human, however, high intake can result in damage to the liver and kidneys [52]. The high level of Cu in the studied fish may be due to domestic, agricultural, and industrial wastes and also due to increased boating activities in the study areas.

The Ni concentration ranged from 0.82-0.86 µg/gdw with a mean value 0.83 ±0.019 µg/gdw. The highest level 0.86µg/gdw was found at the Siliguri site of W. B. and lowest 0.82µg/gdw was recorded at Kishanganj and Chatra sites of Bihar region of the river. The recommended dietary allowance for Ni has not been established but the estimated maximum guideline set by USFDA [41] for Ni is 70-80 µg/gdw. The level of Ni found in this study is significantly below the USFDA guideline and also below than 6.64±0.024

and 58.98 ±0.06 µg/gdw as reported by Ahmed et al., [42]and Jajed and Usmania [49] respectively, however, close to 0.14-1.09 µg/gdw in *Cyprinus carpio* from Aras Dam lake, Iran as reported Farsani et al., [50]. Although Ni is considered an essential element for plants and some animals but shows a carcinogenic effect when consumed in a high amount. Forti et al., [53] reported as Ni normally occurs at very low levels

Table 2. Heavy metal concentration (µg/g dw) in the muscle of *L. rohita* of Mahananda River with International guideline and Word 'River

Statistics	Cr	Cu	Ni	Zn	Cd	Pb	Reference
Range	0.81-0.84	3.81-3.84	0.82-0.86	25.16-25.31	0.62-0.66	1.12-1.15	Present study
Mean ± SD	0.827 ± 0.13	3.84 ± 0.014	0.83 ±0.019	25.23 ± 0.066	0.64 ± 0.017	1.135 ±0.013	
Median	0.82	3.825	0.825	25.23	0.63	1.13	
varicnce	1.68 x10-4	1.96 x10-4	3.61 x10-4	4.36 x10-4	2.89 x10-4	1.69 x10-4	
% CV	1.57	0.365	2.29	0.26	2.66	1.145	
Maximum Permissible Limits (MPL) International guideline							
FAO	-----	30	-----	30	0.05	0.5	FAO(1983)
FAO/WHO	-----	30	-----	40	0.5	0.5	FAO/WHO (1989)
WHO	-----	30	-----	100	1	2	WHO (1995)
FEPA	0.15	1.3	-----	30	0.5	2	FEPA (2003)
EC	-----	0.1	-----	>100	0.05	0.3	European Commission (2006)
USFDA `	12-13mg/kg	-----	70-80	-----	-----	-----	USFDA (1993)
World 'River							
India	-----	3.97-7.51	BDL -8.5	31.71 -92.7	-----	-----	Kumar and Mukherjee (2011)
Bangladesh	18.84 ± 1.70	18.77 ± 2.18	6.64 ±0.024	251.69 ± 18.17	0.04 ± 0.00	6.98 ± 0.23	Ahmed et al., (2016)
India	0.84 ± 0.05	3.88 ± 0.15	-----	25.26 ± 2.04	0.65 ± 0.10	1.12 ± 0.03	Maurya et al., (2019)
India	-----	41.36 ± 0.38	58.98 ±0.06	186.19 ± 0.02	-----	-----	Javed and Usmania (2016)
Ghana	-----	0.02 - 0..56	-----	0.01`5-0.019	0.007-0.019	0.054-0.085	Kwaansa- Ansaha et al., (2019)
Nigeria	-----	0.15 - 4.42	-----	4.17 - 10.18	0.30 - 0.54	0.11 - 8.44	Bawuro et al., (2019)
Iran	-----	6.39 - 25.66	0.14- 1.09	9.11- 31.06	0.15 - 0.75	0.18 - 1.19	Farsani et al., (2019)
China	0.83 ± 0.001	0.037 ±0.002	-----	-----	0.042 ±0.001	0.87± 0.003	Rejeshkumar and Li (2018)
India	0.147 - 0.633	6.99 -16.18	-----	9.8 - 21.03	0.137 - 0.473	0.41 - 0.623	Kumar et al., (2020)

in the environment and it may cause deleterious effects on pulmonary, like lung inflammation, fibrosis, emphysema, and tumors. The Zn content ranged between 25.16-25.31µg/gdw with the highest 25.31 µg/gdw at site 1 (upstream), whereas lowest level 25.16 µg/gdw was found at site 4 (downstream) of the river. The range of Zn observed in this study is quite low when compared with values reported in the muscle of same fish species 31.71-92.7 µg/gdw from Kolkata wetland, India as reported by [48], 251.69 ± 18.17 µg/gdw from Buriganga, Bangladesh as reported by USFDA [42] and in different fish species 186.19 ± 0.02 in *M. armadas* from Kaismpur canal, India as reported by Javed and Usmania [49]. However this range is very close to 25.26 ± 2.04 µg/gdw in *Cyprinus carpio* from Aras Dam lake, Iran as reported [50], 9.8 - 21.03 µg/gdw in *H. fossilis* from Gogabil lake, India as reported by Kumar et al., [51] and good agreement with 25.26 ± 2.04 in *L. rohita* from Ganga, India as reported by Maurya et al., [38]. The range of Zn concentration is significantly below the Maximum Permissible Limits (MPL), International guideline (Table 2). Zn is a cofactor in several enzyme systems including carbonic anhydrase found in red blood cells [14]. It has been found to have a low toxicity effect in man however; the prolonged consumption of large doses can result in some health complications such as fatigue, dizziness, and neutropenia [54]. Its toxicity to fish can be greatly influenced by both water hardness and pH. Zn is a potential toxicant to fish, which causes ion regulation, disturbances, disruption of gill tissue, and hypoxia [55]. The sources of Zn in the study area may be geological rock weathering or human activities such as industrial and domestic wastes water discharges.

The levels of Cd varied from 0.62-0.66 µg/gdw and the maximum value was found at site 1 (Siliguri) and the minimum level was recorded at site 2 (Chitalghata). The amount of Cd found in the muscle of *L rohita* exceeded the permissible limit set by regulatory bodies as shown Table 2 and exceedingly higher than 0.04 ± 0.00, 0.007- 0.019, 0.042±0.001, 0.137-0.473 µg/gdw reported by Ahmed et al., [42], Kwaansa-Ansaha et al., [57], Rejeshkumar and Li [39] and Kumar et al., [51] respectively in muscle of same and different fish species. However, the mean value of Cd observed in this study is approximately the same as the value of 1.12 ± 0.03 as reported by Maurya et al., [38]. Cd is the non-essential and most toxic heavy metal which is widely distributed in the aquatic environment and earth's crust. High concentrations of Cd lead to chronic kidney dysfunction, inducing cell injury and death by interfering with calcium regulation in biological systems in man, fish, and other aquatic organisms Ezemonye et al., [58]. Consumption of fish with a high concentration of Cd (> 0.05 mg/kg) could pose threat such as lung cancer, osteoporosis, and increased blood pressure in humans [59]. In the study points, Cd enters into the freshwater by disposal of industrial, municipal and household waste, and also agricultural runoff.

The states of Pb in the muscle of *L. rohita* ranged from 1.12-1.15 µg/gdw having an average value of 1.135±0.013 µg/gdw. The maximum concentration was found at the Siliguri site followed by Chatalghata, Chatra and Kishanganj site of river. Results showed that the values of Pb are higher than the guideline of FAO (1983), FAO/WHO (1989), and EC (2006) but lower the guideline of WHO (1995) and FEPA (2003) (Table 2). The range of Pb obtained in this study is much higher than the value of 0.054-0.085 µg/gdw in fish from Asafo market, Ghana, 0.87± 0.003 µg/gdw in fish from Taihu lake in China and 0.41 - 0.623 µg/gdw in fish from Gogabil lake, India reported by Kwaansa- Ansaha et al., [57], Rejeshkumar and Li [39] and Kumar et al., [51] respectively, however approximately same result results were reported by several researchers. For example 0.18 - 1.19 µg/gdw and 1.12 ± 0.03 µg/gdw as reported by Farsani et al., [50] and Maurya et al., [38] respectively. Pb enter in water system through runoff, industrial and sewage waste streams, The high concentration of Pb in experimental regions may be due to prolonged agriculture, textile poultry form, industrial and other activates near to the study points. Pb as being potentially hazardous and toxic to most forms of life. Lead deplete sulfhydryl containing antioxidants and enzymes in the cell hence increasing reactive oxygen species (ROS) production leading to various dysfunctions in lipids, proteins, and DNA[60]. The result of the present study can be a basis for predicting severe chronic Pb poisoning via the consumption of *L. rohita* from River Mahananda.

Tolerable rate limit

The calculated ETDI, ETWI, and %PTWI are presented in Table 3. The highest daily intake was recorded for Cu (2.922 x 10^{-4} mg/kg bw/d), while the highest weekly intake was observed for Zn (7.573 x 10^{-2} mg/kg bw/w). The value of ETDI was recorded in the decreasing order of Cu > Zn > Pb > Ni ≈ Cr > Cd, while the value of ETWI was found in the order of Zn > Pb > Ni ≈ Cr > Cd > Cu. The highest value of % PTWI was noted for Cd (27.44 %) and sequence of the studied metals was Cd >> Pb >Cr > Ni >> Zn >> Cu. Based on the results obtained from this study ETDI of each studied metal was lower than PTDI set by FAO/WHO

Joint Expert Committee on the Food Additive, 2003. Consequntly, the presence of studied metals in the muscle of *L. rohita* from Mahananda River not considered to pose any serious human health risk after consumption The calculated ETDI values were comparable with previous studiesfor *L. rohita* from Kolkatta wetland, India [48], from River Ganga, India [38] and different fish species for *M. armatus* from Kasimpur canal, India [49], and *H. molitrix* form Chah Nime lake Iran [28].

Condition factor (K)

The condition factor of a fish indicates physical and biological facts and fluctuations by interaction among feeding conditions, parasitic infections, and physiological factors [61]. The values of condition factor 'K' recorded in the present study was 1.406 (K>1) indicating robustness or well being of studied fish [37] and this was in agreement with earlier K value in the range of 1.094-1.235 [62], 1.05-1.89 [63], 1.04±0.01-1.61±0.04 [57].

Metal Pollution Index (MPI)

The sequence of cotenants of heavy metals in the muscle of *L. rohita* was as following Zn > Cu > Pb > Ni > Cr > Cd. The results revealed that the accumulation of Zn was accumulated higher than other metals. The MPI value was computed to normalize and compare the whole metal contamination and observed 1.09 (Table 3). *L. rohita* is relatively larger in weights and Cacador et al., [64] reported that the larger fish species showed lower values of MPI (always < 3) in comparison to other species having lower weights. Therefore, the weight is also a reliable factor affecting the MPI [65].

Table 3. Estimated values of ETDI, ETWI, %PTWI, MPI, K and standard values of R_{fD} and PTW

Element	R_{fD} (mg/kg bw/d)	ETDI (mg/kg bw/d)	ETWI (mg/kg bw/w)	PTWI (mg/kg bw/d)	% PTWI	Metal Pollution Index (MPI)	Condition Factor (K)
Cr	0.003	0.629×10^{-4}	2.461×10^{-3}	0.0233[b]	10.56	1.908	1.406
Cu	0.04	2.922×10^{-4}	1.153×10^{-2}	3.5[c]	0.33		
Ni	0.02	0.632×10^{-4}	2.491×10^{-3}	0.035[b]	7.12		
Zn	0.3	1.919×10^{-4}	7.573×10^{-2}	7.0[c]	1.08		
Cd	0.001	0.487×10^{-4}	1.921×10^{-3}	0.007[b]	27.44		
Pb	0.004	0.864×10^{-4}	3.407×10^{-3}	0.025[b]	13.63		

Source a Alipour et al., 2015, Source b Miri et al., 2017, Source c Kwaansa-Ansaha et al., 2019

Human Health Risk Assessment

Based on the non-carcinogenic and carcinogenic risk, daily intake rate limit and meal size of fish intake per month for both non-carcinogenic and carcinogenic risk are presented in Table 4.The daily intake rate limit for consumption of contaminated fish regarding non-carcinogenic effect of studied metals range between 0.0118-1.284 kg/day, and the maximum value was recorded for Ni and the minimum value was noted for Cu. Regarding carcinogenic effect, daily consumption rate limit of Cr, Ni, Cd, and Pb in contaminated fish was 0.0157, 0.3777, 0.2192, and 0.055 kg/day respectively. Therefore, this was expected that there was not to cause any adverse effect to consumers [28, 57]. Similarly, as shown Table 4 the meal size for non-carcinogenic effect of consumption of contaminated fish ranged from 1.587-172.23 meal/month and the highest value was observed for Ni and the lowest value was recorded for Cu. The carcinogenic value was recorded in the order of Cd > Pb > Cr > Ni. A similar trend of daily intake limit and meal per month based on non-carcinogenic and carcinogenic effect was reported by Miri et al., [28] and Kwaansa- Ansaha et al., [57].

The values of THQ and TCR for studied metal are presented in Table 4.The maximum THQ value was found for Cd followed by Cr, Pb, Cu, Zn, and Ni. The results revealed that the value of THQ of each metal was less than 1 and the sum of THQs of all studied metals (HI = 0.11021) was also less than 1. Therefore, as per the guideline of USEPA, 2011 consumption of muscle tissues of *L. rohita* has no potential significant health risk to consumers. Maruya et al., [38] reported that THQ value of heavy metals in *L. rohita* in the following order Pb >, Cu > Zn > Cr > Cd and the combined value of THQs was 0.4808 and

Kumar and Mukherjee, [48] also reported the value of HI in L. rohita was 0.66 which are above than the present study. The average value of target cancer risk was calculated for Cr, Ni, Cd, and Pb, and the highest value 7.34 x10-7 mg/kg/d was found for Cr and lowest value 1.074 x10-7 mg/kg/d was observed for Ni. As given

Table 4. Estimated values of non-carcinogenic and carcinogenic risk, THQ, TR, RR, and standard values of CPSo

Element	Non-carcinogenic risk		Carcinogenic risk		CPSo	THQ	HI= $\sum_{n=1}^{n=6}$ THQ	TCR	RR	% RR
	DCR_{lim}	MCR_{mm}	DCR_{lim}	MCR_{mm}						
Cr	0.1933	25.92	0.0157	2.108	$41x10^{-3c}$	$2.306\ x10^{-2}$	0.11021= 1.102 $x10^{-1}$	$7.34x10^{-7}$	276	19.42
Cu	0.0118	1.587	-----	-----	------	$73.04x10^{-4}$		--------	96	6.75
Ni	1.284	172.23	0.3777	1.34	$1.7x10^{-3b}$	$31.58x10^{-4}$		$1.074x10^{-7}$	41.5	2.92
Zn	0.6313	84.651	-----	-----	-----	$63.98x10^{-4}$		--------	84.1	5.92
Cd	0.0833	11.17	0.2192	29.39	$3.8\ x10^{-3b}$	$4.87x10^{-2}$		$1.85x10^{-7}$	640	45.05
Pb	0.1878	25.19	0.055	7.375	$8.5\ x10^{-3a}$	$2.16x10^{-2}$		$6.67x10^{-7}$	284	19.98

Source: Miri et al., 2017, Markmanuel and Jnr 2016, Mohammadi et al., 2019, DCR_{lim} in kg/day, MCR_{lim} in meals/month, CPSo in mg/kg bw/day, TCR in mg/kg/day[7]

in Table 4, the values of TCR for studied metals were below the acceptable carcinogenic risk (10-6 to 10-.4 mg/kg/d) set by USEPA, 2010, so, the target cancer risk value of Cr, Ni, Cd and Pb was within the acceptable range. The TCR value of Ni (5.4 x 10-4) in *L. rohita* was reported by Kumar and Mukherjee, [48], for Pb (1.57 x10-7) in H. molitrix was reported by Miri et al., [28], and 6.75 x10-5 in seven commercial fish from Asafo market, Ghana was reported by and Kwaansa- Ansaha et al., [57] and for Cd (2.9 x10-3 – 9.8 x 10-4) was reported by Markmanuel and Jan [66] in snails commonly consumed in Nigeria.

Relative risk

The non-carcinogenic relative risk (RR) value for the consumption of contaminated *L. rohita* for the studied metals was of the order: Cd > Pb ≈ Cr > Cu > Zn > Ni The contribution of Cd was 45.05%, while that of Cr was 2.92%. The highest concern of *L. rohita* consumption for north Bihar and W.B. population is related to Cd. Miri et al., [28] assessed the RR posed by non-carcinogenic of Pb, Cd, Cr, and Ni in the order of Cd > Pb > Cr > Ni and maximum and minimum RRs value was reported to be 46.25% for Cd and 2.53% for Ni and Adebiyi et al., [67] also reported RRs values of metals in the order: Cd > Cr > Cu > Pb > Fe > Zn > Mn, and the contribution of Cd is 42.09 %, while that of Mn is 0.44 %.

Statistical analysis

The correlation matrix among heavy metal contents and their correlation with weight and length of *L. rohita* are shown in Table 5. Cr shows a significantly positive correlation with Cu, Ni, Zn, Pb, weight, and length but a low relationship with Cd (0.075). The correlation matrix reveals that Cu has a high level of positive correlation with Pb and moderate degree with Ni, Zn, length, and weight, though it is adversely related to Cd (-0.0003). From the results, it was noticed that Ni was strongly positively related to Zn, Pb, length, and weight but moderately with Cd. Zn demonstrates a high level of positive correlation relation with Pb and a moderate level with length and weight except for Cd. Cd and Pb both show positive relationship with weight and length of fish but they show weakly positive relationship with each other.

Thus, there is a significant positive correlation between heavy metal levels in fish with length and weight except for Cd. Miri et al., [28] recorded that a positive relationship among heavy metals (Pb, Cd, Cr, and Ni) concentrations in muscle tissue and with length and weight of *H. molitrix* fish, which are in agreement with the finding of the present study. Yi and Zhang [68] also found that there is a positive co-relationship between fish sizes and heavy metal (Pb, Zn, Cu, Hg Cd, and Cr) concentrations in most species but negative relationship between Cr and Hg concentrations and size of catfish. However Chatta et al., [69] reported a significant positive relationship between levels of Pb and Cd in four farmed Carp fish species, but Cr did not show a correlation with Pb and Cd and Liang et al., [70] also found that there was no positive

Table 5. Correlation matrix between heavy metal contents and their correlation with weight and length of *L. rohita*

Heavy Metal	Cr	Cu	Ni	Zn	Cd	Pb	Weight	Length
Cr	1							
Cu	0.9128	1						
Ni	0.8865	0.6225	1					
Zn	0.8408	0.6029	0.9002	1				
Cd	0.075	- 0.0003	0.4897	0.1998	1			
Pb	0.9989	0.9128	0.8865	0.8408	0.0755	1		
Weight	0.7426	0.5623	0.8288	0.5058	0.6259	0.7426	1	
Length	0.7458	0.6166	0.7773	0.4485	0.5318	0.7458	0.9906	1

relationship between concentrations of heavy metal (Cr, Pb, Zn, and Cu) and length of three farmed fish species (red snapper, snub nose pompano, and orange-spotted grouper.

Conclusion

This study was conducted to evaluate the levels of some selected potential toxic metals in the muscle tissues of *L. rohita* and their associated health risks to consumers. It was found that there was only a little concern with Pb, Cd and Cr levels in studied fish since their mean concentrations were slightly higher than the international guidelines of the FAO, FAO/WHO and the EC. The ETWI of the studied metals through fish consumption was below the PTWI values. The results of this study also revealed that the risk of the non-carcinogenic adverse effect is not high for consumers. In this study, the highest risks to human and aquatic health were contributed by Cd followed by Pb and Cr. The carcinogenic risk of Pb, Cd, Cr, and Ni in the fish was below 10^{-6}, which was acceptable. Correlation matrix indicated a significant relationship between the analyzed metals which suggested similar sources or genetic origin for the metals. Therefore, it may be concluded that the main risk for human health can be related to the amount of Cd followed by Pb and Cr and it is recommended that the *L. rohita* fish should be consumed at moderately amount. Finally, it is suggested that to continue the monitoring of heavy metal levels in fish species which is highly consumed in northern India.

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