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Carcinogenic and non–carcinogenic health risk of heavy metal via the consumption of freshwater fish from Gogabil Lake, Northern India

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Abstract

In this research, concentrations of heavy metals were determined in water and muscle tissues of fish species collected from Gogabil Lake. The level of Cr, Cu, Cd and Pb in water samples was found below the water quality standard set by European Commission (EU) while in muscle tissue of fish species was observed from ranges as follows 0.38 ± 0.02 - 1.256 ± 0.021,1.246 ± 0.011 - 7.846 ± 0.015, 0.36 ± 0.01 - 1.353 ± 0.011 and 1.14 ± 0.01 - 2.346 ± 0.015 respectively. The level of analysed metal in muscle tissues was recorded higher than the limit set by Federal Environmental Protection Agency (FEPA) and EU. The value of estimated tolerable daily intake and estimated tolerable weekly intake was significantly below the oral reference dose (R_{fD}) and provisional tolerable weekly intake respectively, suggested that consumption of studied fish do not pose serious health effect. The target hazard quotient (THQ) and total THQ for studied metals were < 1 and maximum allowable monthly (MCR_{mm}) except Cd was observed >16. Cancer risk value for Cr was found $>10^{-5}$. Therefore, consumption of analyzed fish species could pose mild non-carcinogenic and carcinogenic effect on health regarding specially Cd and Cr respectively.

Keywords: Gogabil Lake**,** water, fish, heavy metal, health risk, non–carcinogenic and carcinogenic effect

Introduction

Pollution of the aquatic environments is one of the serious environmental problems in the World [1-4]. Among the several pollutants, heavy metals/metalloids are known as the most usual environmental pollutants. Owing to their toxicological effects, non-biodegradable nature, bioaccumulation and biomagnifications in the food chain and high persistence, they are dangerous pollutants in the aquatic ecosystems [5-7].

Heavy metals diffuse to aquatic environment from different natural and anthropogenic sources. In the light of the extreme human activity, natural sources of heavy metals are usually of little importance [8]. Anthropogenic activities such as sewage sludge industrial, domestic wastes [1,9-11], atmospheric pollutants [12] and agricultural runoff [13] entering the water bodies are one of the prime sources of heavy metal toxicity, which deteriorate water quality and pose danger to human health and aquatic organisms [14]. Low levels of some heavy metals essential for the development and growth, but some of them are non essential and toxic for living organism. If an essential metal concentration exceed above permissible level, it will be toxic [15,16]. The levels of heavy metal in water, based on some chemical and physical parameters such as redox potential, temperature, pH, salinity, dissolved oxygen, ionic strength and conductivity [1820853457, 7, 17,38]

Fish is an important part of a healthy human diet, which contains a large amount of nutrients that are not normally found in other food sources. It is an excellent source of high level of unsaturated acids and low levels of cholesterol and also has high levels of many important nutrients, including high quality of proteins, iodine and various vitamins and minerals [15, 18-20]. Various investigators reported that, it contains two essential omega-3 fatty acids (DHA and EPA), which are beneficial for humans, 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 and also prevent blood clots, improve blood pressure, stabilize heart rhythms and reduce the risk of a heart attack [21, 22]. Even though fish consumption provides numerous health benefits, however it contains certain environmental pollutants, such as toxic metals, PCBs, pesticides, dioxins and PAHs, which may cause adverse effects on human health [23] such as renal failure, cardiovascular diseases, liver damage and even death [24,25].

Potential human health risks of heavy metals/metalloids from fish consumption can be carcinogenic and non-carcinogenic [26-28]. Therefore, the USEPA has developed numerous risk assessments for the study of both carcinogenic and non-carcinogenic effect of metals. According to Bassey and Chukwu (2019), [29] health risk assessment divides chemical substances in two classes, carcinogenic and non-carcinogenic. It is presumed that the non-carcinogenic substances have a threshold limit and as such are to think with no adverse health effects at a dose below the threshold level. Whereas carcinogenic substances are presumed to have no successful threshold limits. Such presumption suggests that the risk of cancer developing over time with exposures to the chemicals is at low doses. The target hazard quotient (THQ) and hazard index (HI) have been used to determine the human health risks of non-carcinogenic metals [30]. Cancer Potency slope, oral (CPS_o) have been developed for estimating lifetime cancer risks (CR) associated with carcinogenic metals [30-32]. In addition, the USEPA (2000), [33] recommends that the maximum allowable daily (DCR_{lim}) and monthly (MCR_{mm}) fish consumption limits should be used to determine carcinogenic and non-carcinogenic effects of metals [26, 27].

Metal levels in fishes depend upon on the various factors like ecological need, age, size, sex, life cycle and life history, feeding behavior, swimming pattern and geographical location season of capture and physico-chemical parameter of water [15, 20, 34, 35, 90]. The degree of bioaccumulation in different tissues of fish is generally different depending in the active tissue as liver, gills, and kidney have higher accumulation of the heavy metal than other tissues such as skin and muscles [36]. Heavy metals can enter in three different ways via body surface, gills and digestive tract [37,38] .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 [39,40].

Gogabil Lake is one of the largest wetlands of Bihar, and is connected with two major Rivers Mahananda and Ganga. It receives large amount of wastes through multifarious human activities. The lake provides level of the local economic activities such as fish production and irrigation system. There are potential irrigable lands around the lake for the cultivation of rice, maize and vegetables for food security. Excessive use of fertilizers, pesticides in the cultivation resulted in elevated metals concentration in the lake. There are no published papers available to study about trace metal concentration in the fish species of the Gogabil Lake.

Therefore, main goals of the present study were to do with human health risk assessment for carcinogenic and non-carcinogenic metals associated with consumption of fish species living in the Gogabil Lake. The results were then compared to the levels of metals with published data from International guideline and Word's River to detect whether the heavy metal contamination levels in fish of the Lake exceed the safe consumption permissible limit. Thus the present study will be quite helpful for both inhabitants in taking protective measure and Government officials in reducing heavy meals contamination.

Materials and Methods

Study area

Gogabil Lake is the first largest freshwater lake in Bihar, and lies in between $25^0$22.5263 North latitude and $87^0$41216.63 East longitudes. It covers an area of about 60 km^2, out of which about 20 km^2 is purely lake throughout the year. The lake is directly linked to Ganga and Mahananda River and during the peak of rainy season and flood; the lake communicates to these rivers through channels and tributaries. Figure 1 showing location and Figure 2 showing satellite map of Gogabil Lake.

Collection of water and fish samples

About 500 mL water samples were collected at 0.5 meter below the water surface, filtered in pre cleaned bottle and preserved by adding 5 mL of 20% HNO_3 to it and then packed in ice bath (4^0C) and brought to laboratory for further digestion APHA (2005), [41]. A total 35 fish samples were collected at the three sampling sites from the Gogabil Lake, Northern India, during year 2019-2020 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, average length 20.3 to 29.21 cm and average weight 136.41 to 395.20g were recorded and kept frozen at -20^0C. About 3g of the epaxial muscle on the dorsal surface, from each sample were dissected using stainless steel instrument by applying the method of Atta et al. [42] and put in to Petri plate to dry at 120^0C until reaching a constant weight.

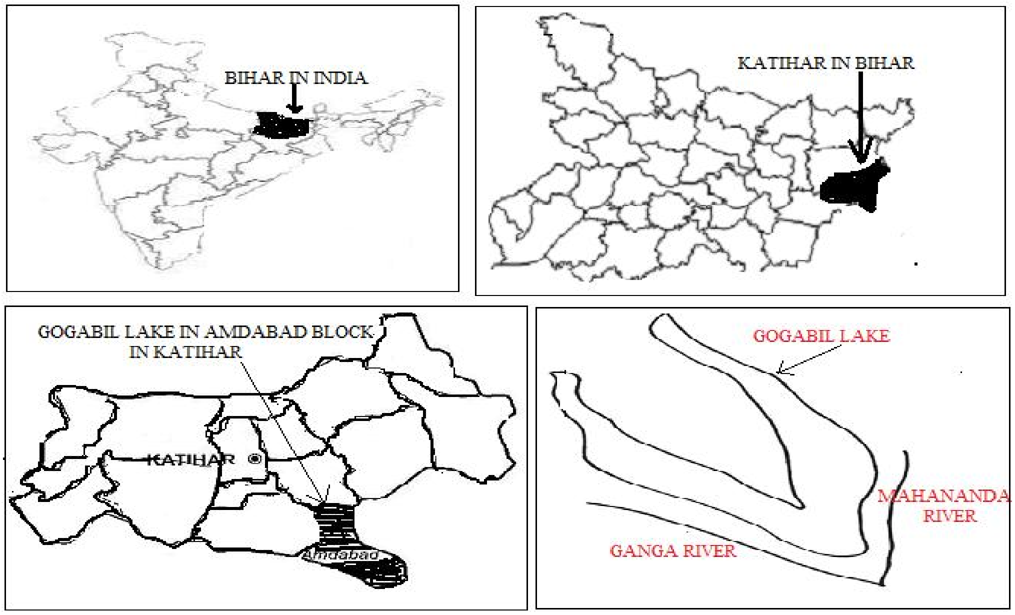

Fig. 1 Location map of Gogabil lake, Katihar, India

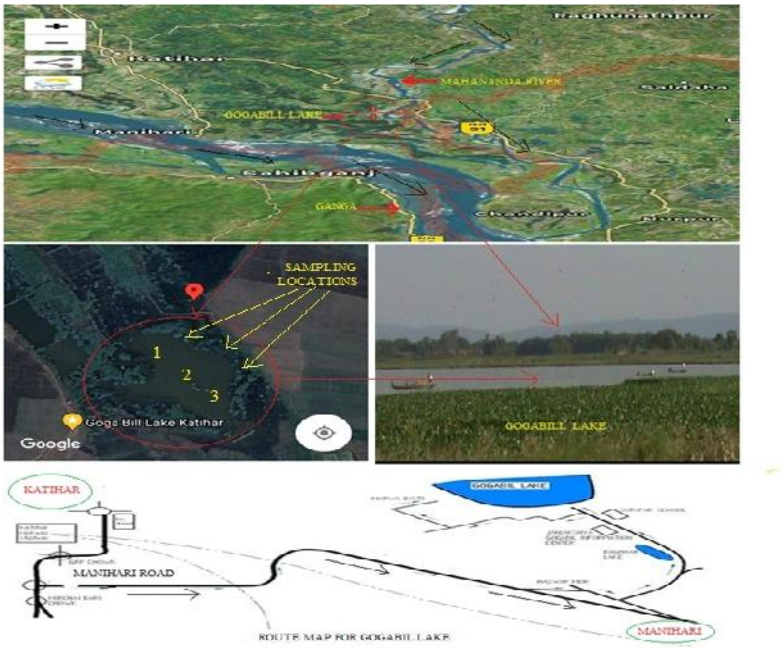

Fig. 2 Satellite map of Gogabil Lake, Katihar, India (Taken from maps, Google.com)

Sample preparation for heavy metal analysis

100 mL water sample was taken in conical flask and 5 mL of concentrated HCl acid was added to it and then heated on the hot plate for two hours at 105^{0}C to 25 mL. The concentrated water sample was then transfer into 100 mL volumetric flask and distilled water was added to fill up to the mark and then analyzed for Cr, Cu, Cd and Pb using Atomic Absorption Spectrophotometer. The dried muscle tissues were digested by the method described [42]. 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^{0}C for 30 minutes. After complete digestion, each digested mixture was cooled at room temperature and then transferred in to 50 mL volumetric flask. Distilled water was added to it to fill up to the mark and analyzed for Cr, Cu, Cd, and Pb using Atomic Absorption Spectrophotometer.

Table 1: Fish species ecological characterizes

Scientific name	Common name	Feeding habit	Biotype complex	No. of Samples	Length (mean) in cm	Weight (mean) in g	Condition factor (K)
C. mrigala	Mringal	Vegetation, phytoplankton, zooplankton and detritus feeder	Bottom feeder	6	25.3 - 30.6 (29.21)	365.4-398,87 (395.2)	1.58
L. rohita	Rohu	Herbivorous, phytoplankton, zooplankton	Flowing and standing water	8	22.4 - 29.78 (28.85)	350.2 - 396.7 (390.5)	1.62
C. catla	Catla	Mainly omnivorous	Surface and mid water feeders	9	20.5 – 28.45 (27.75)	349 8 – 394.5 (360.4)	1.68
M. tengara	Tengara	Carnivorous	Middle bottom feeder	6	20.32 - 25.27 (21.33)	150.4 - 160.8 (156.32)	1.61
N. Chitala	Moya	Carnivorous and predator nature	Deep and clean water	6	18.5 - 22.7 (20.3)	134.7 - 142.6 (136.41)	1.63

Data Processing

Estimated tolerable daily intake (ETDI)

The ETDI was calculated by using the following equation described by Song et al. [43].

$$\mathrm{ETDI} = \frac{\mathrm{Ed} \times \mathrm{Ef} \times \mathrm{Fir} \times \mathrm{Cf} \times \mathrm{Mc}}{\mathrm{BWa} \times \mathrm{ATn}} \times 10^{-3}$$

where Ed is the exposure duration (30 years for non cancer risk as used by USEPA (2011), [44]; Ef is the exposure frequency (365 days/year); Fir is the ingestion rate of fish, 19.5 g/day for Indian [45,46]; Cf is the conversion factor (0.208) to convert dry weight fish to wet weight ; 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 [47] and ATn is the average exposure time for non-cancerous (Ef x Ed= 365 x 30 days as used by USEPA (2011), [44].

Estimated tolerable weekly intake (ETWI)

The ETWI was estimated by using equation described by USEPA, 2000 [33].

$$\mathrm{ETWI} = \frac{\mathrm{Mc} \times \mathrm{Cr}}{\mathrm{BWa}}$$

Where Cr is the fish consumption rate, 0.160 kg of fish consumption per week was set by FAO (2016), [48] 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. [49].

$$\%\,\mathrm{PTWI} = \frac{\mathrm{ETWI}}{\mathrm{PTWI}} \times 10$$

Where provisional tolerable weekly intake (PTWI) is reference dose set by the JECFA (2003), [50]. As per JECFA, (2003) [50] guideline PTWI of 0.0233, 3.5, 0 .007 and 0.025 mg/kg bw/w for Cr, Cu, Cd and Pb were taken in this study.

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

To assess non-carcinogenic effect of the contaminants, the daily consuming rate limit of fish was evaluated by using following equation described by USEPA (2000), [33].

$$\mathrm{DCR\ lim} = \frac{\mathrm{RfD} \times \mathrm{BWa}}{\mathrm{Mc}}$$

Where RfD is the oral reference dose. The value of RfD in mg/kg bw/d for Cr, Cu, Cd and Pb was applied from Integrated Risk Information System USEPA (2012), [51]. Other parameters have been defined previously.

Based on the carcinogenic effect of the contaminants, the daily intake rare limit of was also evaluated by using following equation described by Miri et al. [52] and applied by Kwaansa-Ansha et al. [53].

$$\mathrm{DCR\ lim} = \frac{\mathrm{ARL} \times \mathrm{BWa}}{\mathrm{CPSo} \times \mathrm{Mc}}$$

Where ARL is the maximum acceptable individual lifetime risk level (10^{-5} was used for ARL according Yu et al. [26], CPSo is the carcinogenic potency slope, oral in mg/kg bw /d taken from Integrated Risk Information System USEPA(2012), [51]. Other parameters have been discussed previously.

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

To assess the maximum acceptable intake rate limit of fish in term of meal per month (MCR_{mm}) was also obtained by using following equation described by Shakeri et al. [54].

$$\mathrm{MCRmm} = \frac{\mathrm{DCRlim} \times \mathrm{Tap}}{\mathrm{Ms}}$$

Where, DCR_{lim} is daily consumption rate limit of fish in non-carcinogenic and carcinogenic effect. Tap stands for the average time period (30.44 days/month) and M_S is the meal size (0.227 kg fish/meals) was applied as according USEPA (2000), [33] and Shakeri et al. [54] respectively.

Metal Pollution Index (MPI)

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. [55].

$$\mathrm{MPI} = (C_{f1} \times C_{f2} \times \text{-----------} \times C_{fn})^{1/n}$$

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

Fulton’s condition factor (K)

K has been used as an indicator of health in fishing biology studies. It was estimated according to Htun-Han (1978), [56].

$$K = W\,x 100 / L^3$$

Where, W=weight of fish (g), L=Length of fish (cm).

Health risk assessment

Target hazard quotient (THQ)

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 described by Kumar and Mukherjee, 2011[57] and modified by Adebiyi et al. [58].

$$THQ = \frac{Ed\,x\,Ef\,x\,Fir\,x\,Cf\,x\,Mc}{BWa\,x\,ATn\,x\,RfD}\,x\,10^{-3}$$

$$\text{Or,}\quad THQ = \frac{ETDI}{RfD}$$

Where, ETDI is the estimated tolerance daily intake in mg/kg bw/day and R_{fD} is oral reference dose. The value of R_{fD} for Cr, Cu, Cd and Pb are 0.003, 0.04, 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:

$$HI= \sum_{n=1}^{n=4} THQ = [THQ_{(Cr)} + THQ_{(Cu)} + THQ_{(Cd)} + THQ_{(Pb)}]$$

Target cancer risk (TCR)

The carcinogenic effect from 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 applied by Shaheen et al. [59] and modified by Adebiyi et al. [58].

$$TCR = \frac{Ed\,x\,Ef\,x\,Fir\,x\,Cf\,x\,Mc\,x\,CPSo}{BWa\,x\,ATc}\,x\,10^{-3}$$

$$\text{Or,}\quad TCR = \sum_{n=1}^{n=4} [ETDI\ x\ CPSo]$$

Where AT_C 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 for Pb, Cd and Cr are $8.5x10^{-3}$, $3.8x10^{-3}$and $41x10^{-3}$ mg/kg/day as provided USEPA (2012), [51] while the other parameters have been defined previously. The US Environmental Protection Agency set an acceptance lifetime carcinogenic risk of 10^{-5}.

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 following equation described by Yu et al. [26]

$$RR = Mc / RfD$$

Where Mc and R_{fD} are already explained above. Human health effect through fish consumption should increase with an increase in the relative risk.

Results and Discussion

Metal ions concentration in water

Seasonal variation of levels of heavy metals in the water is present in Figure 3. The mean concentrations of Cr, Cu, Cd and Pb were found 0.06 ± 0.013, 0.477 ± 0.117, 0.065 ± 0.005 and 0,139 ± 0.032 mg/Lrespectively. Heavy metal concentrations in water samples are in the following order Cu > Pb > Cr > Cd in summer and Cu > Pb > Cd > Cr in winter and rainy seasons. Significant variations of heavy metals were found with higher values in summer followed by winter and rainy. The mean concentration of studied metals were significantly higher than the heavy metal levels of various rivers, Kosi [60], Ghaghra [61], Kali

[62], Gomati [63] and Ganga [64]. The level of Cu was lower and concentrations of Cd, Cr and Pb were higher than WHO joint FAO/WHO (2011), [65] during all three seasons.

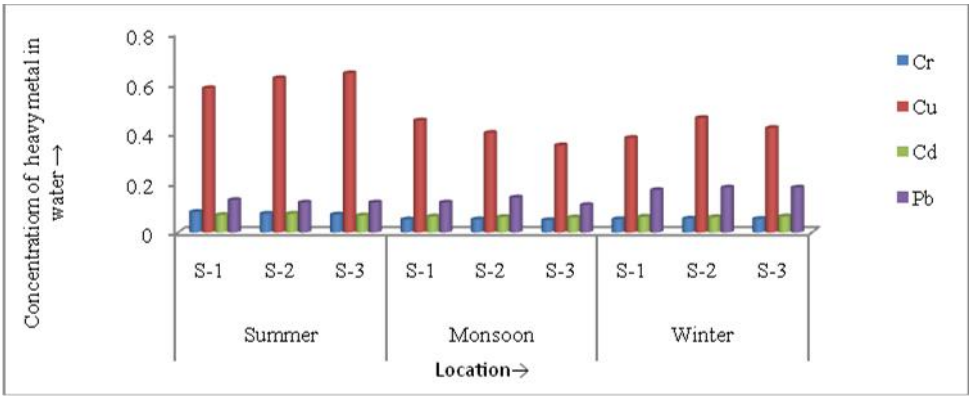

Fig. 3 Seasonal variation of heavy metal concentration in water (mg/l) at three different sites of Gogabil Lake

Difference between three sampling seasons with higher concentration of metals during summer, may be explained by metrological conditions. The summer season’s combined effect of increased vaporizations and decreased rainfall may lead to higher concentration. Similar trends were reported by various authors **[** 10,16,66,67]. Moreover reverse result was observed in rainy season might be due to rainfall effect which caused increase in the lixiviation process and continue to the dilution of heavy metals during wet season [68].

Metal ions concentration in fish tissues

The levels of studied heavy metals observed in the muscle tissues of studied fish at three different sites are shown in Figure 4 and their mean concentrations, variances, percentage of coefficient of variance, skewness and kurtosis are presented in Table 2.The coefficient of variance (CV) was applied to know about the degree of variability of heavy metal levels in the muscle tissues of studied fish species. The CV of Pb (31.30%) and of Cr (40.1%) suggested that their concentration were moderate variability and high CV > 50% was found for Cd (58.23%) and Cu (71.69%), which revealed that their concentration vary significantly in the studied fish tissues[53,69]. The skewness value of the studied heavy metals was less than 1, suggested that these concentrations were not positively skewed in the direction of low concentration. However the kurtosis values of all studied metal contents >>1 especially for Cu (2.187) and Cd (2.052) indicating that the concentrations of these metals in muscle tissues of fish are strongly positive packed with some extremely high values on the data sets [70].The condition factor (K) recorded in the present study was greater than 1, indicating robustness or well being of studied fish [71]. The MPI value was computed to normalize and compare the whole metal contamination and observed 1.269 (Table 4). Cacador et al. [72] 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 [73].

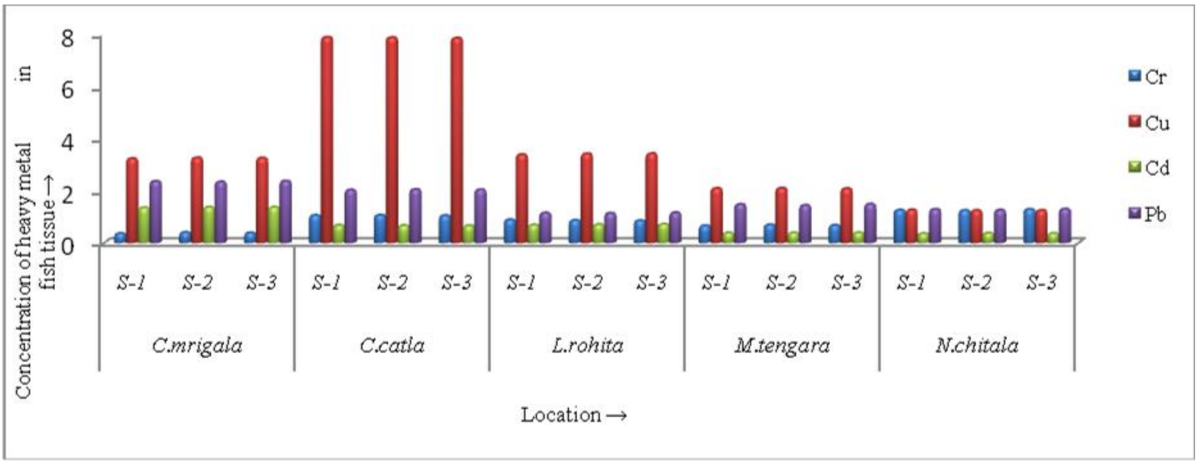

Fig. 4 Heavy metal concentrations (μg/g dw) in different fish species at three different sites of Gogabil Lake

The mean concentration of Cr ranged from 0.38 ± 0.02 - 1.256 ± 0.021μg/g dw and the maximum value 1.28 μg/g dw was recorded in *N. chitala* at site 3 and minimum value 0.36 μg/g dw was found in *C. mrigala* at site 1 of the lake. The level of Cr observed was higher than 0.15 μg/g dw recommended by FEPA (2003) [74**]** but significantly below than the value of 12-13 μg/g dw as set by USFDA (1993) [75].The mean concentration of Cu ranging between 1.246 ± 0.01 -7.846 ± 0.015 μg/g dw. The maximum value of 7.86 μg/g dw was found in *C. catla* species at site 1, whereas minimum value of 1.246 ± 0.011μg/g dw was recorded in *N chitala* at site 2 and 3, which was significantly below the maximum permissible limit (MPL) 30 μg/g dw set by FAO (1983), FAO/WHO (1989) [76,77] and WHO (1995) [78] and exceed the 1.3 and 0.1 μg/g dw as guided by FEPA (2003 [74]) and EC (2006) [79] respectively. The level of Cd varying between 0.36 ± 0.01 (*N. chatila*) to 1.353 ± 0.01 (*C. mrigala*) which was higher the 0.05 μg/g dw recommended by FAO (1983) [76] and EC (2006) [79] but the level of Cd was below in *M. tengara* (0.38 μg/g dw) *and N. chitala* (0.36 μg/g dw) than 0.5 μg/g dw set by FAO/WHO (1989) [77]and FEPA (2003) [74]. The Pb content ranging from 1.14 ± 0.01- 2.346 ± 0.015 μg/g dw. highest value (2.36 μg/g dw) was recorded at site 3 in *C. mrigala* species and lowest value (1.13 μg/g dw) was observed at site 2 in *L. rohita* which was higher than MPL (0.5 μg/g dw) as set by FAO (1983) [76] and FAO/WHO (1989) [77].The level of Pb was also exceed in *C. mrigala* and *C. catla* species than the guidance of WHO (1995) [78] and FEPA (2003) [74] (Table 3).

Table 2: Statistics of heavy metal concentration (μg/g dw) in the muscle of five fish species from Gigabil Lake

Fish Species	Cr	Cu	Cd	Pb
C. mrigala	0.38 ± 0.02	3.233 ± 0.015	1.353 ± 0.011	2.346± 0.015
C. catla	1.05 ± 0.01	7.846 ± 0.015	0.66 ± 0.01	2.03 ± 0.01
L. rohita	0.863 ± 0.015	3.393 ± 0.021	0.693 ± 0.015	1.14 ± 0.01
M. tengara	0.67 ± 0.02	2.08 ± 0.01	0.38 ± 0.00	1.46 ±0.026
N. chitala	1.256 ± 0.021	1.246 ± 0.011	0.36 ± 0.01	1.273 ± 0.021
Variance	3.132 x 10^{-4}	2.25 x 10^{-4}	1.09 x 10^{-4}	3.082 x 10^{-4}
% of CV	40.1	71.69	58.23	31.30
Skewness	-0.1699	0.9497	0.8703	0.3667
Kurtosis	1.491	2.187	2.052	1.2136

CV= Coefficient of variance

The level of Cr observed in this study was significantly below compared with those of Kali River in India, Buriganga in Bangladesh, Barekese Reserver in Ghana [62, 80,81] and higher in the Gogabil Lake, Mahananda River, Godilam River in India [3, 40, 82**]**, however very close to 0.28 ± 0.03 - 1.08 ± 0.06 µg/g dw as reported by Maurya et al., [36] in Ganga River, India (Table 3). Cr is biologically essential for the metabolism of carbohydrate and amino and nucleic acid synthesis [83]. 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. 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 [62,84].The Cu content recorded in this study was approximately same as value of 7.05 µg/g dw in Benian River, Negeria [85] and 0.58 ± 0.09 - 7.87 ± 2.58 in in Ganga River, India [36] but lower than the value found in Gogabil lake, Kali River, in India [3, 62], Buriganga in Bangladesh [80], Ara Dam Lake in Iran [10] . However higher than value observed in Mahananda River in India [40], Asafa market, Ghana [53], Gariyo Lake, Neigeria [20] and Taihu Lake, Chana [86]. The high levels of Cu in the muscle tissues of studied fishes may be due to domestic waste, agricultural and industrial wastes and also due to increased boating activities, recurrent usage of antifouling paint, oil dropping from boat and commercial fishing in the study area. Cu is an essential element for the formation of haemoglobin and some enzymes in human [87]; however, high intake can result in damage to liver and kidneys [88].The amount of Cd found in this study was below the value reported by Maruyra and Malik, (2016) [62] in Kali River, India and also reported by Gyimah et al. [81] in Barekese Reserver, Ghana but approximately same as 0.32 ± 0.07 - 1.32 ± 0.32 as reported by Maruya et al.[36] in Ganga River, India. The level of Cd recorded was significantly higher than value obtained in Gogabil Lake, India; Buriganga, Bangladesh; Asafa market, Ghana; Ara Dam Lake, Iran; Gariyo lake, Neigeria; Taihu lake, Chana (Table 3). In the study points Cd enter into the fresh water by disposal of industrial, municipal and household waste and also agricultural runoff. Cd is the non-essential and most toxic heavy metal which is widely distributed in aquatic environment and earth's crust. The range of Pb obtained in this study is much higher than the value of 0.06 ± 0.007 - 0.085 ± 0.01µg/gdw in fish from Asafo market, Ghana; 0.35 ± 0.02 - 1.20 ± 0.05µg/gdw in fish from Taihu lake in China ; 0.41 ± 0.01 - 0.623 ± 0.025µg/gdw in fish from Gogabil lake, India and 0.18 ± 0.03 - 0.93 ± 0.08 µg/gdw in Ara Dam Lake, Iran reported by Kwaansa- Ansaha et al.[53], Rejeshkumar and Li (2018), [15], Kumar et al. [3] and Farsani et al. [10] respectively, however approximately same result (1.12 ± 0.03 - 2.37 ± 0.21 µg/gdw) was reported by Maurya et al. [36]. Several researchers reported higher value of Pb compare to our findings, for example 1.776 ± 0.015 - 8.236 ± 0.247 µg/gdw, in Mahananda River [4], 15.28 ± 0.99 µg/gdw in Kali River in India [62], 1.77 ± 0.1 - 6.95 ± 0.23 µg/gdw Buriganga, in Bangladesh [80] and 36.04 µg/gdw in Benian River, Negeria [85]. Pb enter 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 farm, industrial and other activities 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 [89].

Table 3: Comparison of heavy metals in muscle tissue of analyzed fish spices with International guidelines and other studies in the world

Location	Cr	Cu	Cd	Pb	Reference
Gogabil lake, India	0.38 ± 0.02 - 1.256 ± 0.021	1.246 ± 0.01- 7.846 ± 0.015	0.36 ± 0.01- 1.353 ± 0.01	1.14 ± 0.01- 2.346 ± 0.015	Present study
Mahananda River, India	--------	0.853 ± 0.05- 2.313 ± 0.31	ND	1.776 ± 0.15 - 8.236 ± 0.247	Kumar et al., 2020b, [4]
Kali River, India	20.39 ± 0.65	30.66 ± 0.92	30.39 ± 0.21	15.28 ± 0.99	Maurya and Malik, 2016, [62]

Godilam River, India	0.56 ± 0.021	0.40 ± 0.115	0.64 ± 0.022	0.34 ± 0.011	Ambedkar & Muniyan,2012, [82]
Mahananda River, India	0.81- 0.84	3.81 -3.84	0.62 - 0.66	1.12 - 1.15	Kumar et al., 2020c, [40]
Ganga River, India	0.28 ± 0.03 - 1.08 ± 0.06	0.58 ± 0.09 - 7.87± 2.58	0.32 ± 0.07 - 1.32 ± 0.32	1.12 ± 0.03 - 2.37 ± 0.21	Maurya et al., 2019, 36]
Gogabil lake, India	0.147 ± 0.01 - 0.633 ± 0.035	0.699 ± 0.02- 16.18 ± 0.246	0.137± 0.01- 0.473 ± 0.006	0.41 ± 0.01 - 0.623 ± 0.025	Kumar et al., 2020a, [3]
Buriganga, Bangladesh	44.33 ± 1.35 - 18.84 ± 1.72	5.9 ± 0.5 - 18.77 ± 2.18	0.01 ± 0.00 - 0.04 ± 0.00	1.77 ± 0.1 - 6.95 ± 0.23	Ahmed et al ., 2016, [80]
Asafa market, Ghana	--------	0.058 ± 0.05 -0.156 ± 0.04	0.007 ± 0.001 -0.019 ± 0.008	0.06 ± 0.007 - 0.085 ± 0.01	Kwaansa-Ansah et al.,2019, [53]
Ara Dam Lake, Iran	--------	6.39 ± 0.78 - 15.36 ± 0.52	0.15 ± 0.03 - 0.46 ± 0.09	0.18 ± 0.03 - 0.93 ± 0.08	Farsani et al., 2019, [10]
Gariyo lake, Neigeria	--------	0.1 5- 4.42	0.30 - 0.54	0.01 -8.44	Bawuro et al., 2019, [20]
Taihu lake, Chana	0.12 ± 0.03 - 0.22 ± 0.06	0.39 ± 0.02 - 1.42 ± 0.07	0.02 ± 0.01 - 0.11±1.16	0.35 ± 0.02 - 1.20 ± 0.05	Rajeshkumar et al.,2018, [16]
Siston region, Iron	0.17	--------	0.15	0.23	Mir et al., 2017, [52]
Benian River, Negeria	--------	7.05	0.98	36.04	Ezemonye et al., 2019, [85]
Barekese Reserver Ghana	5.68 ± 1.13 - 7.00 ± 1.50	--------	6.0 2±1.03 - 11.05 ± 7.85	0.80 ± 0.25	Gyimah et al ., 2019, [81]
Taihu lake, Chana	0.06 - 0.18	0.25 - 0.97	0.01 -0.07	0.08 - 0.43	Tao et al., 2012, [86]
Maximum Permissible Limits (MPL) International Guideline					
FAO	--------	30	0.05	0.5	FAO (1983), [76]
FAO/WHO	--------	30	0.5	0.5	FAO/WHO (1989), [77]
WHO	--------	30	1	2	WHO (1995), [78]
FEPA	0.15	1.3	0.5	2	FEPA (2003), [74]
EC	--------	0.1	0.05	0.3	EC (2006), [79]
USFDA	12-13	--------	--------	--------	USFDA (1993) [75]

Length and weight play an important role in bioaccumulation of heavy metals in fish tissues [90]. Length was considered as the basic measure, since it is less likely to be subjected to major fluctuations than weight which is highly influenced by changes in proximate composition of muscles tissues [91]. Among the five commercial fish species *C. mrigala, C. catla,* and *L. rohita,* accumulated the highest level of almost all four studied metals, this is due to higher biomass (large length/weight). Similar results of large fish tend to accumulate higher amount of heavy metals reported by Maurya & Malik (2016), [62], Karunandhi et al. [91], Maurya et al. [36]. The lowest level of metal was recorded in *N. chatala* ans *M. tengera* might be due to their smaller body size which reduces the bioaccumulation of metal concentration through body surface action [36, 90]. The maturity of fish which was measured by fish length influenced the accumulation of heavy metal [93] as mature fish accumulated higher metals than immature fish [94]. This is due to fish with a constant growth rate that inhibit continuous polluted habitats stabilize the accumulation of heavy metals [94]. The result of this study reveals that there is positive correlation between fish size (length/weight) with metal concentration in most cases, moreover negative correlation found only in the level of Cr (Table 6). In spite of these studied there are no definite relationship between heavy metal concentration and fish size. Metal concentration in fish has been found to reach a steady state after a certain age [95].

Out of 35 fish caught 34.2% was carnivorous, 25.7% was omnivorous, and 22.8% was herbivorous and17.14% was phytoplankton and zooplankton fish species. The feeding habits of herbivorous, carnivorous and phytoplankton and zooplankton fish species were significantly different only for Cu ($p < 0.05$). The concentration of Cu was found to significantly higher in *C. catla* (omnivorous) fish species ($p < 0.05$). The accumulation of Cu in omnivorous fish may have been due to diversity of food intake. The level of Cr, Cd, and Pb in herbivorous fish was low compared to fish at other tropic levels. This tendency of herbivorous fish may be related to their feeding behavior and habitat. Hashim et al. [93] suggested that herbivorous fish, which are primary consumers, eat aquatic macrophytes, submerged land plants and filamentous algae. Being at a lower tropic level, herbivores do not have the variety of diet items found in carnivores and omnivores. Thus, biomagnifications in herbivorous fish is not as large as for secondary consumers [93]. Several researchers reported that high level of metal in fish is related to feeding habit of fish. A. Khalid (2004), [96] reported that *S. siganus* being an herbivore thus bio accumulate higher metal level in their muscle tissue than carnivore *S. sargus.* The suggestion of A. Khalid is an good agreement with present study as *C. catla* (omnivore) and *L. rohita* (herbivore) recorded higher level of metals (Cu > Pb > Cr > Cd) in muscle tissue compared to *M. tengara* and *N. chitala* (carnivore). M.A.M Abdollah (2008), [97] reported that high level of Pb and Zn in the muscle tissue of *S. aurita* (omnivore) collected from EL-Max Bay. Similarly high Cu and Zn level in muscle of *H. niloticas* (omnivore/herbivore) was reported by Bawuro et al. [20]. Findings of the present study are linked to feeding of the *C. mirgala* and *L. rohita* on phytoplankton because it is the probable biota component for Cu concentration [97]. Therefore finding of this research work is in good agreement with report of M. A.A. Abdollaha, (2008) [97] in which herbivore/omnivore (pelagic fish) reported higher metal concentration than carnivore (benthic fish).

Human Health Risk Assessment

Tolerable rate limit

The values of ETDI, ETWI and % PTWI in this study were calculated and presented in Table 4. The ETDI for Cr, Cu, Cd and Pb were found to be 0.00006, 0.0003, 0.00005 and 0.000012 mg/kg bw/d relatively. Among these values the highest value was recorded for Cu (2.7×10^{-4}mg/kg bw/d). The ETDI for studied metals were remarkably below than the R_{fD} values UPEPA (2012), [51], indicated that ETDI of the analyzed fish species might not have an adverse effect on consumers [58].

Table 4: Estimated values of ETDI, ETWI, % PTWI, MPI, and standard values of R_{fD} and PTWI

Element	R_{fD} [a] (mg/kg bw/d)	ETDI (mg/kg bw/d)	ETWI (mg/kg bw/w)	PTWI (mg/kg bw/w)	% PTWI	Metal Pollution Index (MPI)
Cr	0.003	0.642 x 10^{-4}	2.531 x 10^{-3}	0.0233[b]	108.56	1.269
Cu	0.04	2.705 x 10^{-4}	10.679 x 10^{-3}	3.5[c]	0.305	
Cd	0.001	0.525 x10^{-4}	2.069 x 10^{-3}	0.007[b]	29.56	
Pb	0.004	1.254 x 10^{-4}	4.949 x 10 $^{-3}$	0.025[b]	19.79	

Source [a] Alipour et al. 2015; Source [b] Miri et al. 2017; Source [c] Kwaansa-Ansaha et al. 2019

The value of ETWI was found in the order of Cd < Cr < Pb < Cu, while the % PTWI follows the order of Cu < Pb < Cd < Cu. These results showed that the ETWI for the investigated metals were lower than the PTWI values set by JECFA(2003), [50] for all the analyzed meals, indicated that the intake of all examined fish species not considered to pose any serious human health risk after consumption [40, 52, 53]. The calculated ETDI values were comparable with previous studies for *L. rohita* from Kolkatta wetland, India [57], from River Ganga, India [36] and different fish species for *H. molitrix* form Chah Nime lake Iran [52].

Non-Carcinogenic Effect

The Target Hazard Quotients (THQs) of metals through the consumptions of fish species from the Gogabil Lake are presented in Table 5. The results showed that the value of THQ and HI reflects the fact that exposure to studied metals in the examined fish species in adult consumers is lower than recommended threshold dose which is 1. Therefore, it can be concluded that human exposure to the analysed metals in the studied fish does not have a harmful effect during the lifespan. However Cadmium recorded the highest THQ value (5.245 x 10^{-1}) compared to the other detected metals in all examined fish species (Table 5). This study suggests a relatively higher potential health risk to humans via the consumption of Cd in the examined fish species. To assess the non-cancerous effects, daily consumption rate limit of fish /permissible limit (DCR_{lim}) and monthly consumption rate limit of Fish (MCR_{mm}) has been calculated for studied metals and in all examined fish species. Value of DCR_{lim} analysed metals in all examined fish species was more than 10^{-3} kg/ day (Table 5) and lowest value was observed for Cd (0.0773) and highest value was recorded for Cu(0.5989). The maximum permissible daily intake for all examined metals was less than the average daily consumption of fish (25g) which consistent with the results of the study by Varol et al. [98]. So that DCR_{lim} of fish throughout the life span that is not expected to have adverse non cancerous effects [40, 52, 53, 99]. The maximum allowable monthly consumption (MCR_{mm}) is also calculated to determine how many meals of these examined fishes can be used safely without undesirable non-cancerous effects per month. In this study, the observed MCR_{mm} for Cd in all fish species was < 16 whereas for Cr, Cu and Pb lead > 16 meals per month. As per USEPA (2000), [33] when MCR_{mm} of a meal is > 16 meals per month, it is safe to consume. Therefore, adults can consume more than 16 meals of such fish species based on the Cr, Cu and Pb metals. MCR_{mm} for Cd in examined fish species is less than 16 units, suggests non-cancerous harmful effects on the health of the consumer [99].

Relative risk

The non-carcinogenic relative risk (RR) value for the consumption of examined fish species for the studied metals was of the order: Cd > Pb > Cr > Cd (Table 5). The contribution of Cd was 50.22%, while that of Cu was 6.478%. The highest concern of examined fish species consumption for human is related to Cd. Therefore, this was expected that there was a non-cancerous harmful effect on the human health [40, 52, 58].

Table 5: Estimated values of non-carcinogenic and carcinogenic risk, THQ, TCR, % RR, and standard values of CPSo

Element	Non-carcinogenic risk		Carcinogenic risk		THQ	HI	TCR (mg/kg/day)	RR	% RR
	DCR_{lim} (kg/ day)	MCR_{mm} (meal/month)	DCR_{lim} (kg/ day)	MCR_{mm} (meal/ month)					
Cr	0.1895	25.41	0.0154	2.065	214.0×10^{-4}	$\sum_{n=1}^{n=4} THQ = 0.5840 = 58.4 \times 10^{-2}$	3.21×10^{-5}	281.3	20.48
Cu	0.5989	80.31	-----	-----	67.63×10^{-4}		--------	88.99	6.478
Cd	0.0773	10.36	0.2033	31.24	52.45×10^{-2}		1.99×10^{-7}	689.8	50.22
Pb	0.1700	22.79	0.0500	6.705	313.5×10^{-4}		1.07×10^{-6}	313.5	22.82

Source [a] Miri et al. 2017; Source [b] Markmanuel and Jnr, 2016; Source [c] Mohammadi et a l. 2019; CPSo in mg/kg bw/day; TCR in mg/kg/day

Carcinogenic effect

For carcinogenic effect, the daily intake rate limit (DCR_{lim}) and monthly consumption rate limit of Fish (MCR_{mm}) has been also calculated and presented in Table 5. Regarding carcinogenic effect DCR_{lim} of Cr, Cd and Pb in contaminated fish was 0.0154, 0.2033 and 0.050 kg/g respectively, and MCR_{mm} values ranged from 2.065 - 31.24 meal/month and the maximum value was recorded for Cd and the minimum value was observed for Cr. A similar trend of DCR_{lim} and MCR_{mm} based on carcinogenic effect was also reported by Miril et al. [52], Kwaansa-Ansah et al. [53] and Kumar et al. [40]. Based on the carcinogenic risk of the calculated ETDI for carcinogenicity, cancer risk was calculated using the respective carcinogenic potency slope, oral (CPS_o) of individual metal and the results presented in Table 5. Cancer risk regulation set by the USEPA, 2011 ranged from 1.0×10^{-6} to 1.0×10^{-4} .The results of the study revealed that the cancer risk for Cd and Pb through the consumption of studied fish species were less than the USEPA, (2011) guidelines. However, consumption of Cr from all studied fish species could pose carcinogenic effect to humans being at a mild risk as their cancer risk value was very close (0.321×10^{-4}). Therefore, the potential risk to health for those who are exposed by Cd by using fish should not be overlooked.

Correlation matrix analysis

Correlation matrix shows the strength of linear relationship between any two variable on a scale of -1 to +1. In the research work, the raw data was used in calculating the correlation coefficient using the Microsoft Excel computation software package (Table 6). The result revealed that except Cr, all three metals show positive correlation with each other (Cu/Cd, Cu/Pb, Cd/Pb) as well as with length and weight (Cu/length, Cu/weight, Cd/length, Cd/weight, Pb/length, Pb/weight) but show negative correlation with K. Strong and significant positive relationship exist between length and weight. Strong positive relationship suggested similar chemical affinity; genetic origin and common background level in the samples, whereas negative relationship could suggest that metals originate from different sources and have non chemical similarity.

Table 6: Correlation matrix of analysed metals in the examined fish species

Heavy metal/Size/K	Cr	Cu	Cd	Pb	Length	Weight	Constant factor (K)
Cr	1						
Cu	0.095	1					
Cd	- 0.727	0.213	1				
Pb	- 0.538	**0.501**	**0.761**	1			
Length	- 0.453	**0.594**	**0.779**	0.504	1		
Weight	- 0.411	**0.606**	**0.766**	0.492	**0.998**	1	
Constant (K)	0.518	- 0.014	-0.452	- 0.8	0.065	0.085	1

Conclusion

In the present study the levels of Cr, Cu, Cd and Pb in analyzed fish consumed by North Indians and their health risk regarding non-carcinogenic and carcinogenic was investigated. The results disclosed that the concentrations of Cr, Cu, Cd and Pb in the muscle tissue of studied fish were slightly higher than MPL set by FEPL and EU standards. The ETDI and ETWI of the studied metals through fish consumption was less than the RfD and PTWI respectively. The THQ and HI was less than 1 and the cancer risk was also less than the set tolerable limit. The health risk assessment showed that exposure to the studied metals not pose a risk to the health of consumers. However, based on the results obtained for DCR_{lim}, MCR_{mm} and relative risk among the analyzed metals, the main risk for human health can be related to the amount of Cr followed by Cd. Due to the possible accumulation of this metal to toxic levels, it is recommended that, moderate amount of intake is advisable to prevent non-carcinogenic and carcinogenic risk to consumers in future and also to continue the monitoring of heavy metal levels in the considered fish..

Competing interest statement

The authors declare that they have no competing interests

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