Toxicology Reports 12 (2024) 168–177

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Health effects of heavy metals in meat and poultry consumption in Noakhali, Bangladesh

Akibul Islam Chowdhury [a,b], Mohammad Rahanur Alam [a,c,*]

[a] Department of Food Technology and Nutrition Science, Noakhali Science and Technology University, Noakhali, Bangladesh
[b] Department of Nutrition and Food Engineering, Daffodil International University, Dhaka, Bangladesh
[c] Department of Nutrition, University of Tennessee, Knoxville, TN, USA

ARTICLE INFO

Handling editor: Prof. L.H. Lash

Keywords:
Food safety
Heavy metals
Meat
Poultry
Atomic Absorption Spectrophotometry
Health Risk Assessment

ABSTRACT

This study examined the quantities of heavy metals (Cd, Cr, Pb, Ni, Fe, and Cu) in commercially available meat, poultry, and game products in Noakhali, Bangladesh, and their potential health effects, as heavy metal contamination poses a significant food safety risk to human health Atomic Absorption Spectrophotometry was used to analyze heavy metals, and the health risk assessment was based on Estimated Daily Intake (EDI), Targeted Hazard Quotient (THQ), Total THQ, and Total Carcinogenic Risk (TCR). Most samples exceeded Maximum Allowable Concentrations (MAC) for heavy metals. The EDI value of Cd, Pb, and Cr for duck liver, goat liver, and pigeon brain, were higher than the Maximum Tolerable Dietary Intake (MTDI). Children had 1.28 times higher HI values than an adult. The calculation of THQ of all elements in adults and children was in the order of Cu; Pb; Ni; Cr; Cd; and Fe. The calculated TTHQ values were in the range of 0.051 to 1.988 and 0.047 to 3.975 for adults and children, respectively. The TCR values for Cd in poultry liver, brain, and meat, Sonali chicken, cow, pigeon, duck, and goat liver were higher than the reference value for adults and children, suggesting a potential cancer risk. The average exposure to lead leads to an increase in blood pressure by 0.47 mmHg and a decrease in IQ score by 1.94 points. The present study reveals the need to determine strong relationships between heavy metal exposure and food supply.

1. Introduction

Heavy metal contamination is a global food safety concern, as it can occur in a variety of food products, including meat, fish, dairy, fruits, and vegetables [1,2]. Dietary heavy metal exposure can have negative impacts on human health [3]. Heavy metals such as lead, cadmium, and mercury can accumulate in the body over time [4,5], and exposure to high levels can lead to developmental delays, behavioral and learning problems, kidney damage, cancer, osteoporosis, and damage to the nervous, immune, and cardiovascular systems [5–7]. Heavy metals in foods accumulated from environment can cause human health risks and estimation of potentil health risk associated with heavy metals on the human population over a certain period of time is one of the widely used method [8,9].

Heavy metal contamination in meat and poultry in Bangladesh is a major concern. The primary sources of heavy metal contamination in meat and poultry in Bangladesh are believed to be from the feed, as well as from the environment. Contaminated feed can be a result of the use of pesticides, fertilizers, and industrial waste in agriculture, and also from contaminated water used to grow crops [10,11]. The environment, including the soil, air, and water, can also be contaminated with heavy metals due to industrial activities, mining, and inadequate waste management [12]. However, even though animal meat supplies 30–40% of the country's protein needs [13], there is a paucity of published material in Bangladesh on the topic of heavy metals and the associated health risk in meat. Studies have found that meat and poultry products in the country can contain elevated levels of heavy metals. One study found that the levels of lead, cadmium, and chromium in chicken samples were higher than the permissible limits set by the World Health Organization (WHO) [14]. Another study reported that the higher levels of copper, cadmium, and chromium were higher in cattle and chicken [15].

The Noakhali region of Bangladesh is prone to heavy metal contamination due to a combination of factors. One primary reason is the widespread use of pesticides and fertilizers in agriculture, which can contaminate soil and water with heavy metals such as cadmium and lead [16]. Additionally, the region is known for its large shrimp farming

* Correspondence to: Department of Food Technology and Nutrition Science, Noakhali Science and Technology University, Noakhali 3814, Bangladesh.
E-mail address: rahanur.ftns@nstu.edu.bd (M.R. Alam).

https://doi.org/10.1016/j.toxrep.2024.01.008
Received 27 October 2023; Received in revised form 30 December 2023; Accepted 13 January 2024
Available online 17 January 2024

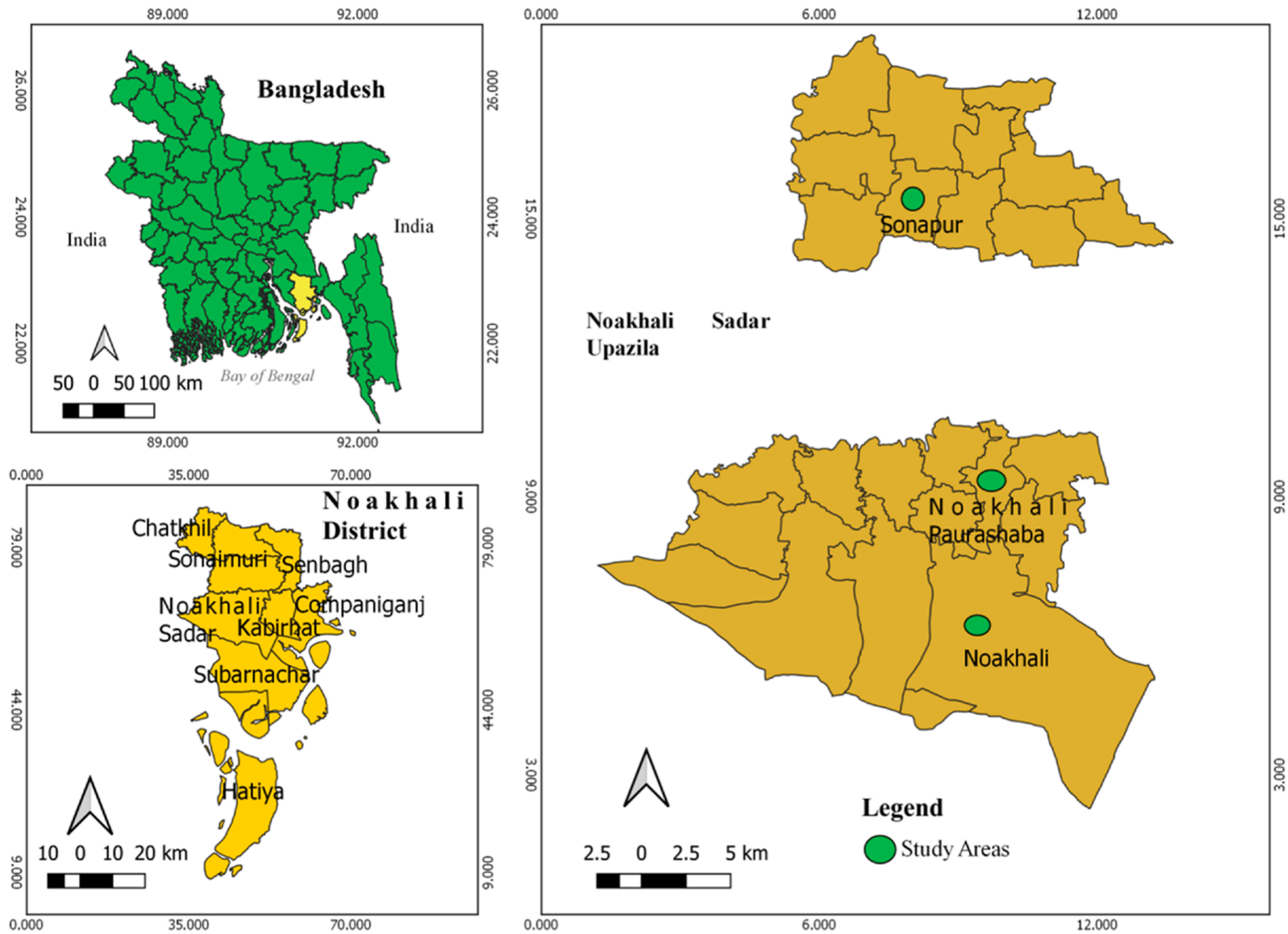

Fig. 1. Map of the study area, Noakhali, Bangladesh.

industry, and the use of antibiotics and other chemicals in shrimp ponds can contribute to heavy metal contamination in the surrounding areas [17]. Furthermore, the Noakhali region is near the coast, making it vulnerable to sea level rise, salinity intrusion, and coastal erosion, which can contaminate the soil and water with heavy metals [18]. The concentrations of heavy metals in coastal areas like Noakhali are higher due to submarine groundwater discharge [19]. A recent study reported heavy metal pollution in Mechna river estuaruy, Noakhali coast [20]. However, there is few studies in Bangladesh concerning heavy metals in commonly meat, poutry and their edible offal and the potential health risk assessment and maximum of papers evaluated heavy metals in those areas which are situated in different industrial or river zone. Moreover, estimation of carcinogenic and non-carcinogenic risk from the consumption of heavy metals in meat, poultry and edible offals have not been studied in Noakhali region early.

The objective of this study was to determine the levels of Cd, Cr, Pb, Ni, Fe, and Cu in Fresh meat, poultry, and game collected from the Noakhali district and assess the carcinogenic and non-carcinogenic effects of these metals from dietary intake. For the first time, this study also evalutes the effects of heavy metals in systolic blood pressure and IQ score in Bangladeshi population.

2. Methods

2.1. Study area and sample collection

The study focused on the areas located on the sothern part of Bangladesh e.g. Noakhali district of Bangladesh (N22°49'28.7", E91°6′6.24"). A total of 63 samples of seven (7) varieties of animals including muscle, brain, and liver were collected from three different local markets of that district, namely Maijdee Bazar, Pouro bazaar, and Sonapur Bazar from January to April 2022. The global positioning system was used to mapping the sampling positions using QGIS software (v-3.10.2) (Fig. 1).

2.2. Sample analysis

All samples were cleaned with distilled water and stored at − 20 °C in a refrigerator before starting drying. The sample were then dried in oven at 60 °C for 24 h and grinded for digestion [21]. Wet digestion of the collected sample was carried out using a 1:4 concentrated mixture of HNO_3 (69% conc.) and H_2S0_4 (97% conc.). The mixture was heated with 0.5 g dehydrated homogenized sample from 130 °C to 170 °C using a thermostat-controlled heating block. The pre-digested samples were further digested by adding 2 ml of H_2O_2 (30% conc.) and heated again to ensure organic matters free solutions. Following filtration, the eluate was diluted to 50 ml with deionized water.

2.3. Instrumental analysis

The heavy metal determination was performed with a PerkinElmer Inc. PinAAcleTM 900 H Atomic Absorption Spectrometer (AAS) with a single beam and deuterium background correction. Limits of Detection and instrumental conditions during heavy metals analysis were

Table 1
Concentrations of heavy metals in foodstuffs (meat, brain, and liver) commonly consumed by Bangladeshi populations in Noakhali region.

Foodstuffs		Scientific name	Heavy Metals (mg/kg fw)					
			Cd	Cr	Pb	Ni	Fe	Cu
Brain	Poultry (n = 9)	*Gallus gallus domesticus*	0.40 ± 0.024	1.62 ± 0.29	7.54 ± 2.54	4.47 ± 0.40	131.31 ± 1.41	98.11 ± 2.86
	Cow (n = 9)	*Bos primigenius*	0.46 ± 0.019	1.29 ± 0.28	4.62 ± .85	10.64 ± 0.14	97.35 ± 0.63	88.0 ± 0.72
	Sonali Chicken (n = 9)	*Gallus gallus domesticus*	0.39 ± 0.021	0.95 ± 0.008	5.59 ± 1.81	8.58 ± 0.39	152.41 ± 3.33	120.56 ± 1.32
	Pigeon (n = 9)	*Columba livia*	0.19 ± 0.047	4.38 ± 0.48	4.45 ± .31	5.47 ± 0.24	150.43 ± 0.33	98.65 ± 0.15
	Quail (n = 9)	*Coturnix coturnix*	0.04 ± 0.023	.92 ± 0.05	3.07 ± 2.29	4.32 ± 0.26	112.76 ± 0.59	161.45 ± 1.77
	Duck (n = 9)	*Anas platyrhynchos*	0.28 ± 0.019	2.38 ± 0.05	3.25 ± 1.11	5.55 ± 0.05	125.15 ± 2.50	106.02 ± 1.48
	Goat (n = 9)	*Capra aegagrus hircus*	0.26 ± 0.021	1.82 ± 0.06	3.11 ± 1.77	4.35 ± 0.22	112.32 ± 0.36	176.26 ± 2.03
Muscle	Poultry (n = 9)	*Gallus gallus domesticus*	0.16 ± 0.005	0.46 ± 0.018	3.13 ± 1.01	5.48 ± 0.16	64.60 ± 2.63	83.07 ± 1.66
	Cow (n = 9)	*Bos primigenius*	0.48 ± 0.005	1.22 ± 0.05	4.83 ± 1.01	5.29 ± 0.05	114.72 ± 1.03	92.57 ± 0.66
	Sonali Chicken (n = 9)	*Gallus gallus domesticus*	0.06 ± 0.021	.96 ± 0.016	1.91 ± 1.00	7.39 ± 0.22	120.35 ± 1.17	88.67 ± 0.26
	Pigeon (n = 9)	*Columba livia*	0.13 ± 0.022	2.39 ± 0.27	5.91 ± .94	3.55 ± 0.24	167.43 ± 0.39	99.48 ± 0.58
	Quail (n = 9)	*Coturnix coturnix*	BDL	1.39 ± 0.05	1.64 ± 1.32	4.27 ± 0.08	92.36 ± 0.21	87.96 ± 1.42
	Duck (n = 9)	*Anas platyrhynchos*	0.11 ± 0.022	1.40 ± 0.06	1.92 ± .88	5.09 ± 0.28	112.06 ± 2.14	93.34 ± 0.56
	Goat (n = 9)	*Capra aegagrus hircus*	0.31 ± 0.025	3.15 ± 0.13	2.05 ± 1.33	7.74 ± 0.18	128.04 ± 0.97	94.25 ± 0.28
Liver	Poultry (n = 9)	*Gallus gallus domesticus*	0.65 ± 0.017	0.82 ± 0.23	7.05 ± .73	2.72 ± 0.12	94.26 ± 2.04	77.51 ± 1.67
	Cow (n = 9)	*Bos primigenius*	2.40 ± 0.008	1.32 ± 0.11	10.54 ± 2.23	41.42 ± 0.27	203.71 ± 2.23	86.51 ± 0.34
	Sonali Chicken (n = 9)	*Gallus gallus domesticus*	1.45 ± 0.007	1.27 ± 0.014	5.15 ± 2.22	6.34 ± 0.1	191.48 ± 0.68	109.98 ± 0.67
	Pigeon (n = 9)	*Columba livia*	0.69 ± 0.075	3.81 ± 0.26	2.79 ± 1.42	2.71 ± 0.41	1650.60 ± 4.51	101.82 ± 0.06
	Quail (n = 9)	*Coturnix coturnix*	0.78 ± 0.023	3.21 ± 0.12	4.88 ± 1.16	3.22 ± 0.38	227.99 ± 0.46	103.35 ± 0.37
	Duck (n = 9)	*Anas platyrhynchos*	15.98 ± 0.87	2.51 ± 0.34	7.48 ± 1.64	3.64 ± 0.38	824.25 ± 1.63	173.72 ± 0.61
	Goat (n = 9)	*Capra aegagrus hircus*	1.81 ± 0.024	3.71 ± 0.19	81.87 ± 29.95	3.26 ± 0.34	611.55 ± 1.69	473.99 ± 2.81
	MAC		0.1[a]	1[a]	0.1[a]	0.5[a]	NA	0.1[b]

Cd, Cadmium; Cr, Chromium; Pb, Lead; Ni, Nickel; Fe, Iron; Cu, Copper; BDL, below detection limit; MAC, maximum allowable concentration
[a] JECFA 2005 [60]
[b] JECFA 2012 [61]

calculated by following European Commission Guidelines (Supplementary table 1) [22]. Pb, Cd, Cr, and Ni were quantified by graphite furnace, while Fe and Cu were quantified by flame technique.

2.4. Quality control/quality assurance

Quality control was performed using blank samples of each analysis. All samples were measured in triplate and presented as mean and standard deviation (SD). The results of the validation of procedures were summarized in supplementary table 2. The mean recoveries of heavy metals were found in the range of 91.7–101.5%, and the correlation coefficient (R^2) of metals ranged from 0.995 to 0.998. The analytical detection limits of metals were Pb= 0.005321 mg/kg, Cd= 0.002134 mg/kg, Cr= 0.001569 mg/kg, Fe= 0.005391 mg/kg, Cu= 0.002153 mg/kg, Ni= 0.001328 mg/kg. Necessary precautions are taken to prevent contamination of samples and nitric acid (1%) was used to clean and rinse all glass materials.

2.5. Health risk assessment

The Health risk associated with heavy metal-contaminated meat consumption was assessed in terms of Estimated Daily Intake (EDI) of metals, Target Hazard Quotients (THQ), Hazard Index (HI), and Target Cancer Risk (TCR) according to the standards of The US Environmental Protection Agency [23].

Estimated daily intake (EDI): EDI is measured in mg/kg body weight/day [24]. To estimate EDI, the average metal content in each sample was calculated and multiplied by the respective consumption rate. Daily intake rate was determined by the following Eq. (1):

$$\mathrm{EDI} = \frac{MC \times IR}{BW} \tag{1}$$

Where MC is the metal concentration in the meat, liver, and brain (mg/kg wet weight), and IR (kg/day/person) is the ingestion rate of the sample, which is taken as 7.54 g/day for beef, 0.55 g/day for mutton, 17.33 g/day for chicken/duck/others (quail, pigeon), for an adult individual of 60 kg (adult) body weight respectively according to "Report of the household income and expenditure survey 2016 [25]. For children, the average body weight was 30 kg [26], and the ingestion rate is 3.1 g/day for beef, 0.3 g/day for mutton, 8.3 g/day for chicken, 1.7 g/day for duck, quail, and pigeon [13]. The Bangladeshi population commonly consumes the liver of animals, especially children, and so, for accurate estimation, the ingestion rate of liver tissue and brain of animals was estimated as 3 g/day [26].

2.6. Non-carcinogenic risk

Target hazard quotient (THQ): THQ is an estimation of the risk level (non-carcinogenic) due to pollutant exposure (Eq. 2). THQ was calculated as per USEPA Region III Risk-based Concentration Table [27] and in Wang et al. [28].

$$THQ = \frac{EF \times ED \times FIR \times CM}{BW \times AT \times RfD} \times 10^{-3} \tag{2}$$

Where EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years for non-cancer risk in this study), as used by [29], FIR is the food ingestion rate (g/person/day) (BBS, 2016), CM is the heavy metal concentration in meat, liver, and brain (mg/kg, w/w), BW is the average body weight (bw) (adult: 60 kg and children: 30 kg) and AT is the average exposure time for non-carcinogens (EF×ED) (365 days/year for 70 years (i.e. AT=25,550 days). The oral reference dose (RfD) of the metal (an estimate of the daily exposure to which the human population may be continuously exposed over a lifetime without an appreciable risk of deleterious effects) are based on 0.001, 0.003, 0.04, 0.02, and 0.004 (mg/kg-BW/day) for Cd, Cr, Cu, Ni, and Pb, respectively [27,30,31]. According to the guidelines of the Chinese Nutrition Society (CNS), the RfDs for Fe is 0.667 mg/kg-BW/ day [32,33].

Total target hazard quotient (TTHQ): TTHQ for an individual from THQs is expressed as the sum of the hazard quotients (Eq. 3) [29].

$$TTHQ = THQ(\mathrm{Cr}) + THQ(\mathrm{Pb}) + \mathrm{THQ(Cd)} + \mathrm{THQ(Ni)} + \mathrm{THQ(Fe)} + \mathrm{THQ(Cu)} \tag{3}$$

Where TTHQ≤ 1 is safe, TTHQ> 1 is hazardous.

Hazard index (HI): HI is assessed to estimate the overall potential for non-carcinogenic health risks from consuming more than one metal.

Table 2
Non-carcinogenic (THQ and TTHQ) health risks of trace elements due to consumption of brain, muscle, and liver in Bangladesh.

Foodstuffs	THQ													TTHQ	
		Cd				Cr		Pb		Fe		Cu			
		Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
Brain	Poultry	0.021	0.041	0.023	0.047	0.102	0.205	0.010	0.021	0.009	0.019	0.125	0.249	0.291	0.583
	Cow	0.022	0.044	0.019	0.038	0.055	0.109	0.026	0.053	0.007	0.015	0.109	0.218	0.238	0.477
	Sonali chicken	0.020	0.040	0.016	0.032	0.080	0.160	0.022	0.044	0.011	0.023	0.152	0.303	0.300	0.602
	Pigeon	0.011	0.021	0.068	0.136	0.060	0.120	0.014	0.027	0.065	0.023	0.123	0.246	0.341	0.574
	Quail	0.003	0.005	0.016	0.032	0.029	0.059	0.010	0.020	0.049	0.017	0.200	0.400	0.307	0.533
	Duck	0.014	0.029	0.039	0.078	0.039	0.079	0.014	0.029	0.055	0.019	0.131	0.263	0.294	0.498
	Goat	0.014	0.028	0.031	0.063	0.037	0.073	0.010	0.020	0.002	0.017	0.219	0.438	0.312	0.638
Muscle	Poultry	0.047	0.046	0.042	0.040	0.219	0.209	0.075	0.072	0.028	0.027	0.599	0.574	**1.012**	0.969
	Cow	0.059	0.049	0.051	0.042	0.141	0.116	0.033	0.027	0.022	0.018	0.289	0.238	0.595	0.489
	Sonali chicken	0.020	0.020	0.095	0.091	0.132	0.127	0.105	0.101	0.052	0.049	0.642	0.615	**1.047**	**1.003**
	Pigeon	0.042	0.008	0.218	0.043	0.452	0.089	0.055	0.011	0.013	0.014	0.721	0.141	**1.501**	0.306
	Quail	< 0.001	< 0.001	0.129	0.025	0.058	0.011	0.061	0.012	0.007	0.008	0.640	0.126	0.895	0.182
	Duck	0.036	0.007	0.138	0.027	0.168	0.033	0.074	0.014	0.008	0.009	0.673	0.132	**1.097**	0.223
	Goat	0.003	0.003	0.009	0.010	0.004	0.004	0.004	0.004	0.009	0.002	0.026	0.024	0.051	0.047
Liver	Poultry	0.033	0.066	0.016	0.032	0.081	0.163	0.007	0.014	0.007	0.014	0.095	0.190	0.239	0.479
	Cow	0.120	0.240	0.021	0.043	0.132	0.265	0.103	0.207	0.015	0.031	0.108	0.216	0.501	**1.001**
	Sonali chicken	0.073	0.145	0.021	0.042	0.052	0.105	0.016	0.032	0.0143	0.029	0.137	0.274	0.314	0.627
	Pigeon	0.037	0.073	0.066	0.132	0.029	0.059	0.007	0.014	0.123	0.247	0.127	0.254	0.390	0.780
	Quail	0.039	0.079	0.052	0.104	0.061	0.122	0.008	0.016	0.017	0.034	0.129	0.259	0.307	0.614
	Duck	0.774	**1.548**	0.045	0.090	0.093	0.185	0.009	0.019	0.062	0.124	0.217	0.435	**1.201**	**2.402**
	Goat	0.091	0.182	0.059	0.119	**1.192**	**2.383**	0.009	0.017	0.046	0.092	0.591	**1.181**	**1.988**	**3.975**
Hazard Index							**13.223**								

THQ, target hazard quotient; TTHQ, total target hazard quotient, HI, hazard index
Values in bold indicate THQ≥ 1 and TTHQ> 1
THQ< 1, no health risk; THQ ≥ 1 indicates potential health risk
TTHQ< 1 indicates safe and TTHQ> 1 indicates hazardous

$$HI = TTHQ(food_1) + TTHQ(food_2) + \ldots\ldots\ldots\ldots + TTHQ(food_{23})$$

Target cancer risk (TCR): TCR was used to indicate carcinogenic risks. The method to estimate TCR is also provided in USEPA Region III Risk-Based Concentration Table [29]. The model for estimating TCR was shown as follows (Eq. 4):

$$TCR = EDI \times CPSo \tag{4}$$

Where EDI is estimated daily intake (mg/ kg/ day), CPSo is the carcinogenic potency slope for oral route of 0.0085 (mg/kg bw/day)$^{-1}$ for Pb, 6.3 (mg/kg bw/day)$^{-1}$ for Cd, and 0.5(mg/kg bw/day)$^{-1}$ for Cr and TA is the averaging time of carcinogens (365 days/year for 70 years), as used by USEPA (2011) [29,33,34].

2.7. Estimation of health effects

There is no direct calculation to estimate the effects of heavy metal exposures on health due to the lack of human dose-response functions between heavy metal concentration and human illness rate. However, according to JECFA methodology [35], there is link that concerns Pb exposures with increased systolic blood pressure in adults and decline IQ level in children. In this present study, we calculated the effect of lead intake on systolic blood pressure in adult population and on IQ level in children based on JECFA approach [35]. According to JECFA approach, 1 mmHg systolic blood pressure increased due to ingestion of 1.3 μg/kg bw/day of lead (Pb) in adult and 1 IQ point was decline due to ingestion of 0.6 μg/kg bw/day of lead [35].

2.8. Statistical analysis

Data collected were presented as mean and standard deviation and were tested for normality and homogeneity of variance prior to analysis. One-way analysis of variance (ANOVA) ($p < 0.05$) was performed to assess whether heavy metals varied significantly between animals. Pearson correlation and Principal Component Analysis (PCA) were performed to get detailed information about the distribution of heavy metals and their similarities and dissimilarities in the samples. All statistical calculations were performed with SPSS 23.0 Inc., Chicago, IL, USA, for Windows.

3. Results and discussion

3.1. Concentration of heavy metals in commonly consumed animal tissues

The concentrations of heavy metals (Cd, Cr, Pb, Ni, Fe, and Cu) from different varieties of animal tissues are presented in Table 1. Concentration of heavy metals varies among different species of animals.

Cadmium is one of the most common toxic metals that occurs naturally in soil and is transmitted to food via soil-plant-animal or soil-water-animal routes [36]. A statistically significant difference ($p < 0.05$) in Cd levels was found between meat and liver as well as between brain and liver in this study (Supplementary table 3**)**. The highest Cd concentration in an animal's brain was found in a cow's brain and lowest in a quail brain which is higher than some reported studies [37,38]. In the case of muscle meat, the concentrations of Cd were higher than MAC except for the Sonali chicken muscle meat (Table 2). The results of Cd levels in meat in this study were higher than those found in [38–45] and lower than those obtained in [37,46–49]. Cadmium levels in different animals' livers were also higher than previously reported findings [37,40,43,45–50].

Chromium is an essential metal for our diet as it helps to maintain the blood glucose level of our body by making the function of insulin efficient [51]. However, Cr toxicity affects the function of different enzymes like catalase, peroxidase, and cytochrome oxidase [52]. There were significant differences observed amongst brain, meat, and liver for Cr concentrations (Supplementary table 3**)**. The highest mean concentration of Cr levels was observed in the pigeon brain (4.8 mg/kg/fw), and the lowest was found in poultry muscle (0.46 mg/kg/fw). The observed concentrations of Cr among the maximum food samples were higher than MAC level (Table 1). The higher level of Cr in poultry muscle may

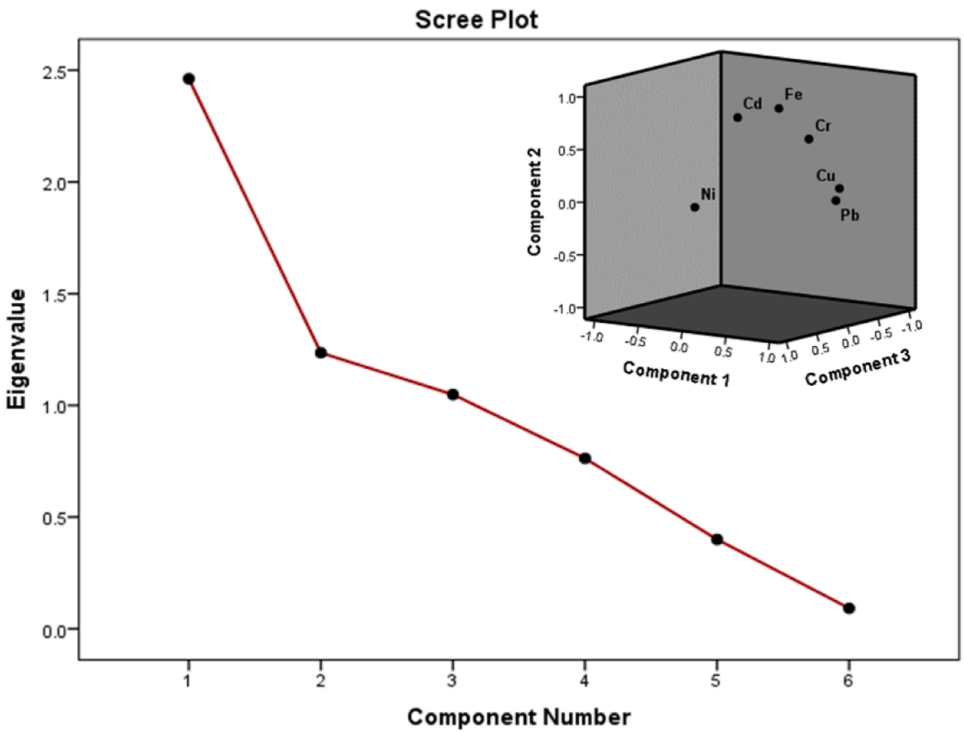

Fig. 2. : Principal component analysis (PCA) of heavy metals by scree plot and a three-dimensional plot showing loadings for heavy metals.

be due to the use of feed from tannery waste which contains an elevated level of Cr [53]. However, these concentrations of Cr were lower than some of the previous literature [14,37,41,47].

Heavy metals accumulate in the brain, especially Pb, which easily pass the blood-brain barrier and accumulate, leading to damage to the central nervous system. In the present study, the highest concentration of lead (Pb) was found in the poultry brain (7.54 ± 2.54) and lowest in the quail brain (0.04 ± 0.023). The concentration of Pb in different animal's brains and muscle were higher than MAC (Table 1) and other reported studies [15,37–47]. However, the concentrations of Pb found in goat and duck muscle were lower than in some studies [48,54]. The concentration of Pb in animal's liver was in order pigeon > quail > Sonali chicken > cow > duck > poultry > goat, and the concentrations of Pb were higher than other studies report [37,38,40,45–49,54].

Nickel, the metallic element mostly used for industrial purposes, can show some adverse health effects like immunologic, neurologic, reproductive, carcinogenic, and allergic reactions depending on the route of exposure (inhalation, oral or dental) [55]. The mean concentration of nickel in the analyzed animal's foodstuffs was in order brain > muscle > liver. The highest mean concentration was found in cow liver (41.42 mg/kg fw), and the lowest Ni level was found in pigeon liver (2.71 mg/kg fw). All of the mean Ni concentrations among animal's brains, muscles, and liver were higher than MAC (Table 1) and the findings of previously reported literatures [14,15,38,40,41,47] and lower than those found in [46].

Iron is the most crucial element for living creatures due to its support in the respiratory process [56]. The free radical formation is most common due to iron toxicity that may cause DNA damage leading to initiate cancer [57]. In the present study, the highest mean concentration of Fe was found in pigeon liver, followed by the descending order of duck liver > goat liver > quail liver > cow liver > Sonali chicken liver > pigeon muscle > Sonali chicken brain > pigeon brain > poultry brain > goat muscle > duck brain > Sonali chicken muscle > cow muscle > quail brain > goat brain > duck muscle > cow brain > poultry liver > quail muscle > poultry muscle. There was a statistically significant difference ($p < 0.05$) observed between meat and liver and brain and liver (Supplementary table 3**)**. There is no MAC value for Fe in foods. However, the concentration of Fe found in the present study was higher than those reported by [38–40] and lower than those reported by [47, 54].

The highest mean concentration of trace element Cu was found in goat liver (473.99 mg/kg fw), and the lowest concentration of Cu was in poultry liver (77.51 mg/kg fw). Statistically significant differences ($p < 0.05$) among brain, muscle, and liver for Cu concentrations were found in the present study (Supplementary table 3). The mean concentrations of Cu in the muscle, liver, and brain were much higher than in MAC. The concentrations of Cu in these foodstuffs were also higher than some previously reported findings [14,15,37,38,41–43]. However, the concentrations of Cu in duck liver, cow liver and goat meat were lower than the findings reported in previous studies [40,49,54].

The present study findings showed different concentrations of heavy metals in different types of animal's meat and offals. The differences in concentration of heavy metals are due to the age of the animal, feed, bioaccumulation process, and sex as these factors affects the accumulation of heavy metals in muscle, liver, kidney and brain [58,59].

3.2. Source identification

3.2.1. Multivariate analysis

By calculating a summary index, the Pearson correlation coefficient is a potential tool for assessing the strength of linear association between the pairs of variables [62]. Consequently, the Pearson product-moment correlation coefficients for the metal-to-metal correlation data that were significant at the 99% and 95% confidence levels were assessed (Supplementary table-4). At a 99% confidence level, the pairs of Fe-Cr (0.523) and Pb-Cu (0.889) displayed strong and significant correlations, and the pairs of Cd-Fe (0.382), Pb-Cr (0.327) and Cr-Cu (0.358) showed weak and significant correlations, while Cu displayed a weak correlation with Fe (0.268) at 95% confidence level. The strong connections provided evidence in favor of the theory that the sources of the metals might be comparable. From the analysis, we suppose that Fe-Cr-Pb-Cu were accumulated in animal body from same sources. In

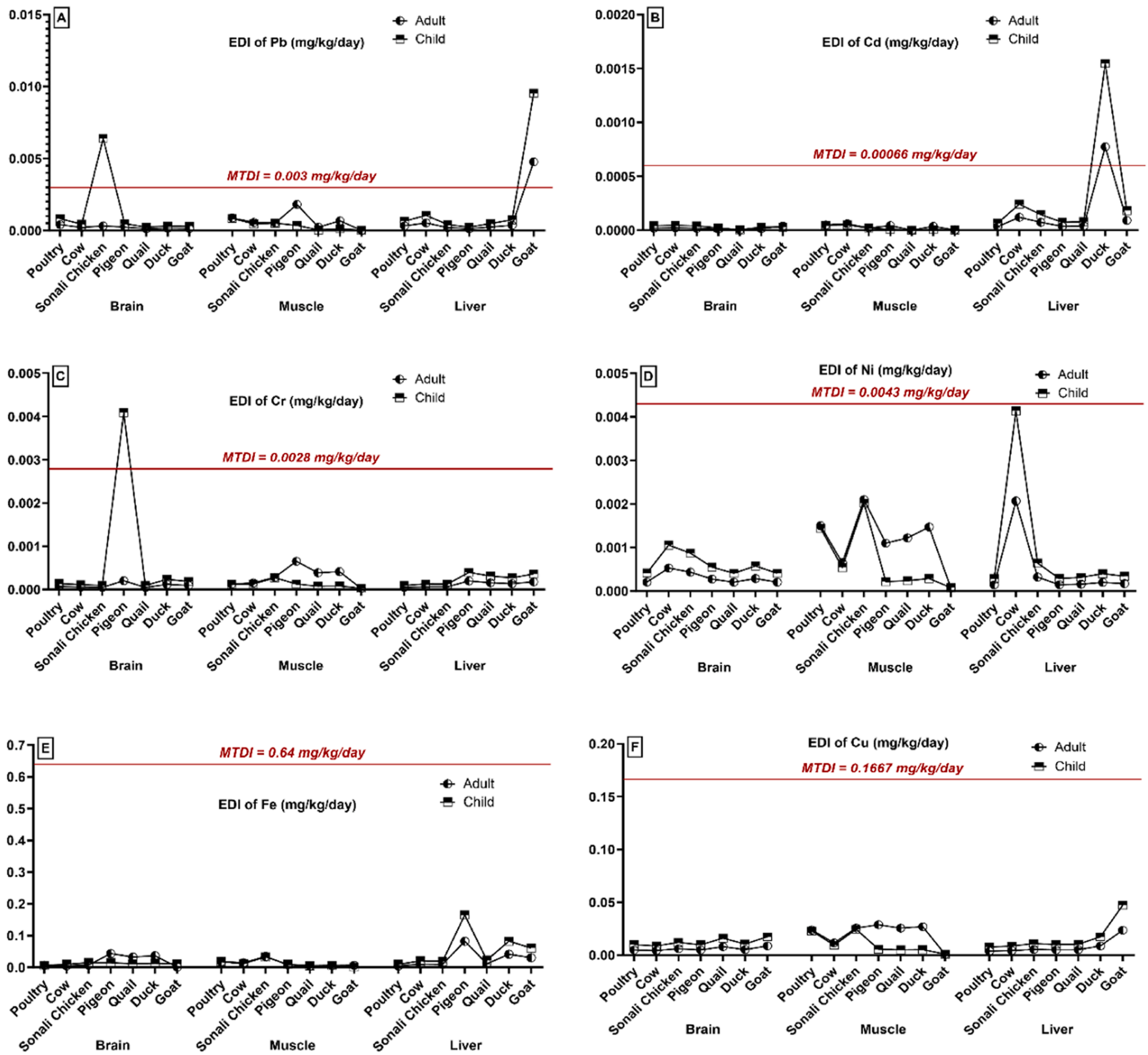

Fig. 3. : Estimated Daily Intake (EDI) of Cd (A), Cr (B), Pb (C), Ni (D), Fe (E), Cu (F) from commonly consumed brain, meat (muscle), and liver according to maximum tolerable dietary intake (MTDI) based on the data established by WHO/FAO joint committee for food additives and JECFA.

Bangladesh, most of the livestock and poutry were farming using artificial feeds which may contain heavy metals that can affect our food chain [63,64]. Artifical feeds usually contaminated with harmful elements such as Pb, Cr, Cd and As [65], however, there is limited data about heavy metals in feeds in Bangladesh context.

3.2.2. Principal component analysis

The principal component analysis (PCA) on meat and edible offal data utilizing varimax-normalized rotation was performed to observe the relationship of cluster variables in simple ways [66]. A large number of variables are reduced into a new set of reduced variables based on their mutual dependence, which is the PCA's most significant contribution. A significant number of PCs was observed using a scree plot depicted in Fig. 1. According to the results, three eigenvalues greater than one account for 78.95% of the total variance. Supplementary Table 5 includes the computation of commonalities, percent of the total variance, and cumulative percent of the variance. While PC2 accounted for 20.75% of the total variation and exhibited the highest loadings for Fe, Cd, and Cr, indicating that they originated from the same origins, PC1 revealed the highest loadings for Cu and Pb, explaining more than 40.7% of the total variance. With a variance of 17.42%, the final significant factor revealed that Ni had the highest loadings among the materials with different sources. To understand the relationship among metals, a three-dimensional plot of the PCA loadings was presented in Fig. 2.

3.3. Health risk assessment

3.3.1. Estimated daily intake (EDI)

The health risk assessment of the population was estimated by the value of EDI for both adults and children and depicted in Fig. 3. The EDI of trace elements (Cd, Cr, Pb, Ni, Fe, and Cu) was compared with

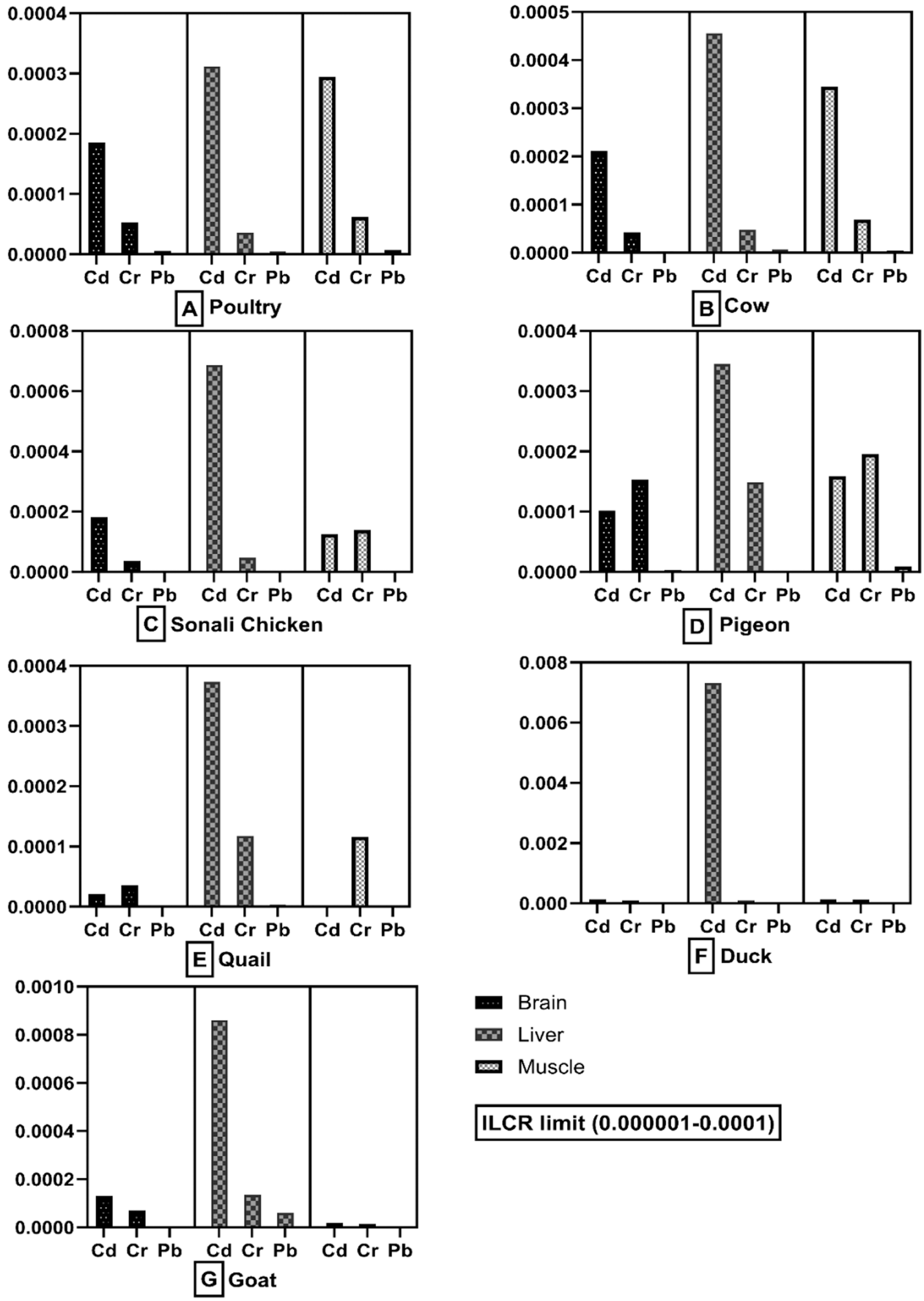

Fig. 4. **:** Calculation of total carcinogenic risk (TCR) in meat, liver, and brain by consumption of heavy metals according to USEPA. (A) Poultry, (B) Cow, (C) Sonali chicken, (D) pigeon, (E) Quail, (F) Duck, and (G) Goat.

maximum tolerable daily intake (MTDI) (Figs. 3a, 3b, 3c, 3d, 3e, 3f), which indicates no adverse health effects after consuming foods [67]. The EDI of adults from consuming different animal muscles, liver, and brain were shown in the descending order of Fe > Cu > Ni > Pb > Cr > Cd, and for children, it was of Fe > Cu > Pb > Ni > Cr > Cd. The value of Cd and Pb through consumption of duck liver and goat liver, respectively, were above the permissible limit for both adults and children, whereas the levels of Pb and Cr from the consumption of sonali chicken brain and pigeon brain respectively, were also above the MTDI value for children. The EDI revealed that brain and liver are more responsible to create health risk than muscle meat among Noakhali region of Bangladesh.

3.3.2. Targeted hazard quotient (THQ) and total targeted hazard quotient (TTHQ)

The non-carcinogenic health risk was assessed in terms of targeted hazard quotients (THQ), and total targeted hazard quotients (TTHQ), summarized in Table 2. The estimated THQ value of Cd was greater than 1 for children through consuming duck liver, whereas the THQ value of Pb was above 1 for both adults and children and for Cu for child through the consumption of goat liver. Furthermore, the THQ in adults and children found that Cu> Pb>Ni>Cr>Cd>Fe. However, the value of THQ does not provide a quantitative probability of experiencing adverse health effects. It only indicates the level of risk due to exposure [15]. Considering all elements, the TTHQ value was estimated in the range of 0.051 to 1.988 and 0.047 to 3.975 for adults and children, respectively. The value of TTHQ is helpful to assess and understand the combined risk of different foods for human health. In the present study, TTHQ in children was almost two times higher than in adults, especially relating to poultry brain and liver, cow muscle and offal, Sonali chicken liver, pigeon muscle, and liver, and brain and liver of duck, goat, quail likely due to children consuming comparatively more muscle and edible offal than adults.

The hazard index (HI) was calculated to assess the non-carcinogenic risk of multiple elements by consuming one or more food items (Table 2). HI values by consuming foodstuffs were 13.22 and 17.005 for adults and children, respectively. The contribution of Cu to HI value was the highest for both adult and child.

3.3.3. Total carcinogenic risk (TCR)

The target carcinogenic risks (TCRs) derived from Cr, Cd, and Pb consumption was calculated because ingestion of these compounds may result in both non-carcinogenic and carcinogenic consequences depending on the exposure amount (Fig. 4). The TCR values from exposure of Cd were found in the range of 9.46E-05 to 7.59E-04 and 2.06E-05 to 9.75E-03, whereas for Cr, it was 1.4E-05 to 3.3E-04 and 1.5E-05 to 2.04E-04, and for Pb, it was 1.3E-07 to 4.05E-05 and 1.37E-07 to 8.1E-05, for adults and children, respectively (Figs. 4a, 4b, 4c, 4d, 4e, 4f, 4g). In general, TCR values below 1.0E-06 are regarded as negligible, those above 1.0E-04 are unacceptable, and those falling between 1.0E-06 and 1.0E-04 are considered as falling within an acceptable range [68,69]. The estimation showed that the carcinogenic risk (TCR) of Pb due to consumption of muscle, liver, and brain was within the negligible range (<1.0E-4) to the acceptable range (1.0E-6 to 1.0E-4), whereas the TCR of Cd for both adults and children were within unacceptable range (>1.0E-4) due to consumption of liver, brain, and muscle of poultry, Sonali chicken, cow as well as liver of pigeon, duck, and goat. In the present study, it was suggested that children are more susceptible to toxic elements from dietary intake of foods. Exposure to heavy metals especially Pb, Cd, and Cr may associated with several types of cancer risk such as ovarian cancer, lung cancer, prostate or testicular cancer, renal cancer and bladder cancer [9]. Although mechanism of progessing cancer from heavy metals via food remains unclear, foods are one kind of main source heavy metals found in human body [70].

3.4. Health effects of heavy metals

According to the result, the average intake of lead (Pb) from meat and offal consumption in adults is about 0.61 μg which leads to increased systolic blood pressure about 0.47 mmHg in adults. The IQ score is declined to about 1.94 due to ingestion of 1.16 μg Pb on average in children.

4. Conclusion

The article focused on the levels of heavy metals in commonly consumed animal's edible tissues (muscle, brain, and liver) and determined the health risk in terms of EDI, THQ, TTHQ, and TCR. The maximum edible tissues contained heavy metals that exceeded the maximum allowable concentrations (MAC) and indicated potential health risks. However, a maximum of edible tissues had low EDI than MTDI values. PCA and multivariate analysis showed the sources of heavy metals in food and a strong correlation between the metals. In terms of health risk assessment, the present study found that children are more susceptible to developing cancer compared with adults. The present study does not include other foodstuffs (rice, vegetables, fish, pulses, ground vegetables etc) which are other sources of metals and can also cause cancer among people of Noakhali region. Thus, this study suggested that the Government of Bangladesh routinely monitors the contamination levels of hazardous heavy metals and metalloids in foodstuffs to enforce regulatory limits and assess the risk of long-term exposure. Determination of the effects of the geographical distribution of heavy metals and feedings practices in meat and edible organs, including assessment of hazardous elements in feeds and foodstuffs, is also recommended.

Ethics approval

The study was classified as exempt according to the institutional ethics committee of the Noakhali Science and Technology University. All methods were performed in accordance with the relevant guidelines and regulations.

Funding

This work was elevant g. Funding supported by the NSTU research grant 2021–2022 by the Research cell of Noakhali Science and Technology University (NSTU/RC-FN-01/T-22144).

CRediT authorship contribution statement

Alam Mohammad Rahanur: Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. **Chowdhury Akibul Islam:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

We would like to express our gratitude to the Research cell of Noakhali Science and Technology University.

Submission declaration and verification

The study is reported in accordance with ARRIVE guidelines. The Authors hereby consent to publish this research article. This article has not been published or submitted elsewhere for publication. The authors also declare that this work does not libel anyone or violate anyone's copyright or common law rights.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2024.01.008.

References

[1] R. Martí-Cid, et al., Dietary intake of arsenic, cadmium, mercury, and lead by the population of Catalonia, Spain, Biol. Trace Elem. Res. 125 (2) (2008) 120–132.
[2] S. Khan, et al., Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China, Environ. Pollut. 152 (3) (2008) 686–692.
[3] S.M. Abd-Elghany, et al., Health risk assessment of exposure to heavy metals from sheep meat and offal in Kuwait, J. Food Prot. 83 (3) (2020) 503–510.
[4] X. Wang, et al., Spatial analysis of heavy metals in meat products in China during 2015–2017, Food Control 104 (2019) 174–180.
[5] L. Jarup, Hazards of heavy metal contamination (Find this article online), Br. Med Bull. 68 (2003) *167–182* (Find this article online).
[6] J.M. Goodrich, et al., Methylmercury and elemental mercury differentially associate with blood pressure among dental professionals, Int. J. Hyg. Environ. Health 216 (2) (2013) 195–201.
[7] M. Rajaee, et al., An investigation of organic and inorganic mercury exposure and blood pressure in a small-scale gold mining community in Ghana, Int. J. Environ. Res. Public Health 12 (8) (2015) 10020–10038.
[8] M. Kovochich, et al., Review of techniques and studies characterizing the release of carbon nanotubes from nanocomposites: implications for exposure and human health risk assessment, J. Expo. Sci. Environ. Epidemiol. 28 (3) (2018) 203–215.
[9] P. Aendo, et al., Carcinogenic and non-carcinogenic risk assessment of heavy metals contamination in duck eggs and meat as a warning scenario in Thailand, Sci. Total Environ. 689 (2019) 215–222.
[10] M.S. Islam, et al., Assessment of trace metals in foodstuffs grown around the vicinity of industries in Bangladesh, J. Food Compos. Anal. 42 (2015) 8–15.
[11] A. Taiwo, et al., Carcinogenic and non-carcinogenic evaluations of heavy metals in protein foods from southwestern Nigeria, J. Food Compos. Anal. 73 (2018) 60–66.
[12] K. Sardar, et al., Heavy metals contamination and what are the impacts on living organisms, Greener J. Environ. Manag. Public Saf. 2 (4) (2013) 172–179.
[13] BBS, Report of the household income and expenditure survey 2010.. 2011, Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning: Bangladesh.
[14] M. Mottalib, et al., Assessment of trace metals in consumer chickens in Bangladesh, J. Health Pollut. 8 (20) (2018).
[15] N. Shaheen, et al., Health risk assessment of trace elements via dietary intake of 'non-piscine protein source'foodstuffs (meat, milk and egg) in Bangladesh, Environ. Sci. Pollut. Res. 23 (8) (2016) 7794–7806.
[16] B. Yargholi, S. Azarneshan, Long-term effects of pesticides and chemical fertilizers usage on some soil properties and accumulation of heavy metals in the soil (case study of Moghan plain's (Iran) irrigation and drainage network), Int. J. Agric. Crop Sci. 7 (8) (2014) 518.
[17] M. Sajjaduzzaman, N. Muhammed, M. Koike, *Mangrove plantation destruction in Noakhali coastal forests of Bangladesh: a case study on causes, consequences and model prescription to halt deforestation.* Int J Agric, Biol 7 (5) (2005) 732–734.
[18] P. Liu, et al., Accumulation and ecological risk of heavy metals in soils along the coastal areas of the Bohai Sea and the Yellow Sea: A comparative study of China and South Korea, Environ. Int. 137 (2020) 105519.
[19] M. Luo, et al., Pollution assessment and sources of dissolved heavy metals in coastal water of a highly urbanized coastal area: The role of groundwater discharge, Sci. Total Environ. 807 (2022) 151070.
[20] M.A.M. Siddique, et al., Assessment of heavy metal contamination in the surficial sediments from the lower Meghna River estuary, Noakhali coast, Bangladesh, Int. J. Sediment Res. 36 (3) (2021) 384–391.
[21] J. Binkowski, The effect of material preparation on the dry weight used in trace elements determination in biological samples, Fresenius Environ. Bull. 21 (7a) (2012) 1956–1960.
[22] EC, European Commission. Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off J Eur Communities. 657/EC, L 221/8–L 221/36, 2002.
[23] USEPA, D.J.H.H.E.M.P.A., *Risk assessment guidance for superfund.* 1989.
[24] S. Bo, et al., Assessing the health risk of heavy metals in vegetables to the general population in Beijing, *China* J. Environ. Sci. 21 (12) (2009) 1702–1709.
[25] BBS, *Report of the household income and expenditure survey 2016*. 2016, Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning,: Bangladesh
[26] T. Zeinali, F. Salmani, K. Naseri, Dietary intake of cadmium, chromium, copper, nickel, and lead through the consumption of meat, liver, and kidney and assessment of human health risk in Birjand, Southeast of Iran, Biol. Trace Elem. Res. 191 (2) (2019) 338–347.
[27] EPA, U., *Integrated Risk Information System-Database (IRIS)*. 2007.
[28] X. Wang, et al., Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish, Sci. Total Environ. 350 (1-3) (2005) 28–37.
[29] USEPA, *Regional Screening Level (RSL) Summary Table: November 2011*. 2011.
[30] M. Harmanescu, et al., *Heavy metals health risk assessment for population* via *consumption of vegetables grown in old mining area; a case study: Banat County, Romania.* Chemistry Central, Journal 5 (1) (2011) 1–10.
[31] USEPA, *Risk Based Screening Table. Composite Table: Summary Table 0615*. 2015.
[32] O, C.S, *Dietary nutrient intake for Chinese residents*. 2013.
[33] L. Zheng, et al., Exposure risk assessment of nine metal elements in Chongqing hotpot seasoning, RSC Adv. 10 (4) (2020) 1971–1980.
[34] A.A. Ullah, et al., Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh, Toxicol. Rep. 4 (2017) 574–579.
[35] F. Joint, W.E.C.o.F. Additives, and W.H. Organization, Safety evaluation of certain food additives and contaminants: prepared by the Seventy-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), World Health Organization, 2011.
[36] F. Zhu, et al., Health risk assessment of eight heavy metals in nine varieties of edible vegetable oils consumed in China, Food Chem. Toxicol. 49 (12) (2011) 3081–3085.
[37] N. Baqar, M. Manzoor, M. Qadir, Accumulation of heavy metals in edible organs of different meat products available in the markets of lahore, pakistan, Pak. J. Sci. Ind. Res. Ser. B. Biol. Sci. 58 (2) (2015) 92–97.
[38] E. Kalisińska, et al., Using the Mallard to biomonitor heavy metal contamination of wetlands in north-western Poland, Sci. Total Environ. 320 (2-3) (2004) 145–161.
[39] A. Ullah, et al., Concentration, source identification, and potential human health risk assessment of heavy metals in chicken meat and egg in Bangladesh, Environ. Sci. Pollut. Res. 29 (15) (2022) 22031–22042.
[40] E.T. Ogbomida, et al., Accumulation patterns and risk assessment of metals and metalloid in muscle and offal of free-range chickens, cattle and goat in Benin City, Nigeria, Ecotoxicol. Environ. Saf. 151 (2018) 98–108.
[41] M.S. Islam, et al., The concentration, source and potential human health risk of heavy metals in the commonly consumed foods in Bangladesh, Ecotoxicol. Environ. Saf. 122 (2015) 462–469.
[42] A. Begum, S. Sehrin, Levels of heavy metals in different tissues of pigeon (Columba livia) of Bangladesh for safety assessment for human consumption, Bangladesh Pharm. J. 16 (1) (2013) 81–87.
[43] J. Torres, et al., Trace element concentrations in Raillietina micracantha in comparison to its definitive host, the feral pigeon Columba livia in Santa Cruz de Tenerife (Canary Archipelago, Spain), Arch. Environ. Contam. Toxicol. 58 (1) (2010) 176–182.
[44] S.-S. Chen, et al., *Trace Elem. Heavy Met. Poult. Livest. meat Taiwan.* Food Addit. Contam.: Part B 6 (4) (2013) 231–236.
[45] E.O. Njoga, et al., Detection, distribution and health risk assessment of toxic heavy metals/metalloids, arsenic, cadmium, and lead in goat carcasses processed for human consumption in South-Eastern Nigeria, Foods 10 (4) (2021) 798.
[46] W.S. Darwish, et al., Metal contamination in quail meat: residues, sources, molecular biomarkers, and human health risk assessment, Environ. Sci. Pollut. Res. 25 (20) (2018) 20106–20115.
[47] S.A. Abduljaleel, M. Shuhaimi-Othman, A. Babji, Assessment of trace metals contents in chicken (Gallus gallus domesticus) and quail (Coturnix coturnix japonica) tissues from Selangor (Malaysia), J. Environ. Sci. Technol. 5 (6) (2012) 441–451.
[48] I. Mariam, S. Iqbal, S.A. Nagra, *Distribution of some trace and macrominerals in beef, mutton and poultry.* Int. J. Agric, Biol 6 (5) (2004) 816–820.
[49] C. Okoye, J. Ugwu, Impact of environmental cadmium, lead, copper and zinc on quality of goat meat in Nigeria, Bull. Chem. Soc. Ethiop. 24 (1) (2010).
[50] D.-H. Nam, D.-P. Lee, T.-H. Koo, Factors causing variations of lead and cadmium accumulation of feral pigeons (Columba livia), Environ. Monit. Assess. 95 (1) (2004) 23–35.
[51] R.A. Anderson, Chromium in the prevention and control of diabetes, Diabetes Metab. 26 (1) (2000) 22–28.
[52] K. Nath, et al., Effect of chromium and tannery effluent toxicity on metabolism and growth in cowpea (Vigna sinensis L. Saviex Hassk) seedling, Res. Environ. Life Sci. 1 (3) (2008) 91–94.
[53] T. Mahmud, et al., Estimation of chromium (VI) in various body parts of local chicken, J. Chem. Soc. Pak. 33 (6) (2011) 339.
[54] P. Aendo, et al., Pb, cd, and cu play a major role in health risk from contamination in duck meat and offal for food production in Thailand, Biol. Trace Elem. Res. 198 (1) (2020) 243–252.
[55] K.K. Das, et al., Primary concept of nickel toxicity–an overview, J. Basic Clin. Physiol. Pharmacol. 30 (2) (2019) 141–152.
[56] J. Albretsen, The toxicity of iron, an essential element, Veterinary Med.-Bonn. Springs then edwardsville 101 (2) (-, 2006) 82.
[57] G. Bhasin, H. Kauser, M. Athar, Iron augments stage-I and stage-II tumor promotion in murine skin, Cancer Lett. 183 (2) (2002) 113–122.
[58] M. Rudy, Chemical composition of wild boar meat and relationship between age and bioaccumulation of heavy metals in muscle and liver tissue, Food Addit. Contam. 27 (4) (2010) 464–472.
[59] M.Hd.M. Garcia, et al., Sex-and age-dependent accumulation of heavy metals (Cd, Pb and Zn) in liver, kidney and muscle of roe deer (Capreolus capreolus) from NW Spain, J. Environ. Sci. Health, Part A 46 (2) (2011) 109–116.

[60] JECFA, *Codex General Standard for Contami nants and Toxins in Food and Feeds. In: 64th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*, JECFA/64/CAC/RCP 49–2001. 2005.
[61] JECFA, *Working document for information and use in discussions related to contaminants and toxins in the GSCTFF. Joint FAO/WHO Food Stand ards Programme, Codex Committee on Contaminants in Foods, Sixth Session, Maastricht, the Netherlands, CF/6 INF/1, 2012*. 2012.
[62] B. Škrbić, A. Onjia, Multivariate analyses of microelement contents in wheat cultivated in Serbia (2002), Food Control 18 (4) (2007) 338–345.
[63] M.A. Rahman, et al., Assessment of the quality of the poultry feed and its effect in poultry products in Bangladesh, J. Bangladesh Chem. Soc. 27 (1-2) (2014) 1–9.
[64] M.M. Haque, et al., Probabilistic health risk assessment of toxic metals in chickens from the largest production areas of Dhaka, Bangladesh, Environ. Sci. Pollut. Res. 28 (37) (2021) 51329–51341.
[65] C. Tao, et al., Heavy metal content in feedstuffs and feeds in Hubei province, China, J. Food Prot. 83 (5) (2020) 762–766.
[66] C.A. Mertler, R.A. Vannatta, Advanced and multivariate statistical methods: Practical application and interpretation, Taylor & Francis, 2016.
[67] F. Joint, Summary and conclusions of the 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), FAO/WHO, Rome, 2003.
[68] EPA, A., *Risk assessment guidance for superfund. Volume I: human health evaluation manual (part a)*. 1989, EPA/540/1–89/002.
[69] USEPA, Risk-based concentration table, U.S. Environmental Protection Agency, Washington DC, 2010.
[70] P. Aendo, et al., Comparison of zinc, lead, cadmium, cobalt, manganese, iron, chromium and copper in duck eggs from three duck farm systems in Central and Western, Thailand, Ecotoxicol. Environ. Saf. 161 (2018) 691–698.