Concentration, source identification and potential human health risk assessment of heavy metals in chicken meat and egg in Bangladesh

Ullah AKM Atique (ullaha@msu.edu)
Michigan State University https://orcid.org/0000-0002-4545-2663
Shazia Afrin
Dhaka University
Mohammad Mozammal Hosen
Bangladesh Atomic Energy Commission
Maesha Musarrat
Bangladesh Council of Scientific and Industrial Research
Tania Ferdoushy
Dhaka University
Quamrun Nahar
Bangladesh Institute of Research and Rehabilitation in Diabetes Endocrines and Metabolic Disorders
Shamshad B. Quraishi
Bangladesh Atomic Energy Commission

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Abstract

Chicken meat and hen egg are very popular foodstuffs around the world and highly consumed as curry, fast food, processed food, etc. assuming a promising source of protein. In the present study, the concentrations of Pb, Cd, Cr, As, Hg, Mn, Fe, and Zn in nationally representative samples of chicken meat and hen egg were determined and found in the range of 0.03–2.73, 0.01–0.015, 0.025–0.67, 0.04–0.06, 0.01–0.015, 0.15–0.63, 2.50–38.6, and 1.02–19.4 mg/kg-fw, respectively. The results demonstrated that only Pb exceeded the maximum allowable concentration (MAC) for dietary food. Multivariate statistical analyses depicted that anthropogenic activities were the major source of heavy metals in the investigated foodstuffs. Human health risks associated with the dietary intake of these metals through the consumption of chicken meat and hen egg were evaluated in terms of estimated daily intake (EDI), non-carcinogenic risk of individual heavy metal by target hazard quotient (THQ), total target hazard quotient (TTHQ) for combined metals and carcinogenic risk (CR) for lifetime exposure. The calculated values of EDI, THQ, TTHQ, and CR were below their respective permissible benchmarks indicating the safe consumption of the investigated foodstuffs with respect to heavy metal contamination.

Introduction

Owing to various natural and anthropogenic interventions, metals and metalloids are ubiquitous in our environment and their concentration is on a constant rise resulting from various familiar activities all around like application of fertilizer and pesticides in the arable land, household and industrial waste disposal, sewage sludge, and smelter stacks from mills and factories etc. (Islam et al., 2015a). Depending upon their nature and the amount and the extent of exposure, presence of these metals particularly of heavy metals can cause both beneficial and harmful effects in human body and hence deserve attention (Shaheen et al., 2016a). According to literature, heavy metals have been classified into different groups including toxic (arsenic, cadmium, lead, mercury, chromium, nickel, etc.), probably essential (vanadium), and essential (copper, zinc, iron, manganese, selenium, and cobalt) metals (Munoz-Olivas and Camara, 2001). The non-biodegradable nature, long biological half-lives and the capability to be deposited in different body organs resulted from their high retention capacity has made the toxic metals most notorious (Jarup, 2003; Shaheen et al., 2016a).

Heavy metals have been reported to cause different carcinogenic, mutagenic, and teratogenic effects to human health (IARC, 1993; Pitot and Dragan, 1996; Radwan and Salama, 2006). For instance, lead has been associated with poor cognitive development and intellectual capability in children, increased blood pressure and cardiovascular disease in adults (Al-Hossainy et el., 2017). Cadmium causes kidney failure, reduced reproduction ability, hypertension, tumorous and hepatic dysfunction (Luckey and Venugopal, 1977; Al-Busaidi et al., 2011). Further, cadmium toxicity may lead to genotoxicity, endocrine disruption, oxidative damage, and ion regulatory disruption (Renieri et al., 2017). Chromium exposure may cause severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects, ultimately leading to death (Ullah et al., 2017). Long term arsenic exposure creates arsenicosis diseases

 r, nervous, renal, hepatobiliary, gastro-intestinal, and respiratory systems of the body (Tchounwou et al., 2003a). Inhalation or ingestion of mercury leads to gastrointestinal toxicity, neurotoxicity, and nephrotoxicity (Tchounwou et al., 2003b). On the contrary, iron, copper, zinc, and manganese are considered essential for human health although they may also turn to our health when exceed certain level. Altogether, toxicity from heavy metals is an important issue from the consideration of their bio-magnifications in the food chain, interaction with the body organs, bioaccumulation in the body, and toxicity to our health (Eisler, 1988). Thus, metallic intake to human body via different pathway e.g. intake of food stuffs requires critical review.

All around the world, chicken meat and their egg-based foodstuffs are very popular as a non-piscine protein source and widely consumed as curry, fast food, processed food, etc. These have been helping people every day from different walks of life to meet their hunger easily, at a nominal cost as well as giving them the access to a rich amount of dietary proteins, essential amino acids, minerals, vitamins, and essential trace elements regularly which are ultimately beneficial as well as necessary for a sound and safe health (Alturiqi and Albedair 2012). As an important source of many micronutrients such as iron, selenium, vitamins (e.g. A, B12, D), and folic acid which are either not present in plant-derived foods or have a poor bioavailability from them, chicken meat are often prescribed by doctors. As a protein-rich and low-carbohydrate product, it also contributes to a low glycemic index which is believed to help against obesity, diabetes development, and cancer (insulin resistance hypothesis). As an inevitable part of a balanced diet, chicken meat ensures adequate delivery of essential micronutrients and amino acids and is involved in regulatory processes of energy metabolism (Biesalski 2005; Bauchart et al., 2007; Cabrera and Saadoun 2014). Similar to meat, egg is also highly nutritious and mandatory for a healthy and balanced diet (Hashish et al., 2012). Thus, both chicken meat and egg are important nutrient for our health and development. As a developing country with a huge number of people deprived of balanced nutrition, chicken meat and hen egg are very important foodstuffs to the people of Bangladesh.

There have been a very few studies on the levels of trace elements in Bangladeshi chicken meat and poultry egg (Islam et al., 2015a; Islam et al., 2015b; Shaheen et al., 2016b). Moreover, there is no or very limited information on the heavy metal concentration in chicken meat and hen egg considering the variety of chicken and egg available in the country. Besides, the constantly rising anthropogenic and industrial pollutions stresses on the necessity of continuous monitoring of the presence of heavy metals in food samples. Thus, the objectives of the current study were set to measure the concentration of Pb, Cd, Cr, As, Hg, Mn, Fe and Zn in three most commonly consumed varieties of chicken meat and three varieties of hen egg in Bangladesh, identification of their probable sources, and assess the associated carcinogenic and non-carcinogenic risks from their intake.

Materials And Methods

Sample Collection and preservation

Dhaka is one of the most densely populated cities in the world with more than 20 M people. A total of 72 f

collected from four wholesale markets of Dhaka namely-

Kawran Bazar, Mirpur-1, New Market, and Mohammadpur Bazar. Food items are generally supplied to the other parts of Bangladesh from these wholesale markets of Dhaka. The most common three categories of chicken such as broiler, local, and sonali and three categories of hen egg namely layer, local, and organic were targeted in the present study. Organic egg samples were collected from four major shopping malls; Shapna, Agora, Meena Bazaar, and G – mart. Egg laid by the hens those are feed fortified foods is termed as organic egg. In order to avoid the cross contamination, samples were transported and stored in the laboratory in such a way that they were not in contact with any metal instruments. Global positioning system (GPS) was used to locate the sampling positions (Fig. 1).

Fresh samples were washed with distilled water and the edible parts of the chicken were chopped into small pieces with a cleaned stainless-steel knife. Small pieces of the chicken samples and the edible parts of the egg samples were homogenized separately using a food processor having a plastic container with stainless-steel bottom blade. The blended chicken samples were air dried in order to remove the extra water from the samples and about 200 g of test portions were stored at − 20°C until analysis. The blended egg samples were oven dried at 105°C to attain constant weight. The moisture contents of the egg samples were calculated from the fresh and dried weight of the samples. The dried samples were then homogenized with a porcelain mortar and pestle and were also stored at − 20°C until analysis.

Acid digestion of samples

1 g of each homogenized blended chicken and 2 g of each egg powder samples were pre-digested with 6 mL of concentrated HNO_3 (69%, Sigma-Aldrich) by slow heating. The solutions were evaporated to near dryness. Then the pre-digested samples were further digested with 2 mL H_2O_2 (30%, Sigma-Aldrich) and again evaporated to near dryness in order to ensure the residues obtained after digestion were free from organic matters, which otherwise act as impurities in metal analysis. The residue of the chicken and egg samples was simultaneously filtered through Whatman filter paper (number 41) and diluted to a final volume of 10 mL and 20 mL, respectively.

Analysis of heavy metals

Pb, Cd, and Cr were quantified by using an atomic absorption spectrophotometer (AA280Z, Varian) equipped with graphite furnace (GTA 120) and an autosampler (PSD 120). The chemical modifiers used for the quantification of Pb and Cd were phosphoric acid (Merck, Darmstadt, Germany) and ammonium phosphate monobasic (Merck, Darmstadt, Germany), respectively. As and Hg were measured by hydride generation-atomic absorption spectrophotometry (HG-AAS) and cold vapour-atomic absorption spectrophotometry (CV-AAS) techniques, respectively using a Varian AA240FS atomic absorption spectrophotometer equipped with a hydride vapour generator (VGA 77). Hydrochloric acid (Merck, Germany), sodium hydroxide (BDH), and sodium borohydride (Acros Organics, USA) were used as reductant for the quantification of As and Hg. Moreover, for the As analysis, digested samples were pretreated with ascorbic acid (Merck, Germany) and potassium iodide (Merck, Germany). A flame atomic absorption spectrophotometer (FAAS) (Varian AA-240) with air-acetylene flame was used in order to analyze Mn, Fe, and Zn in the samples. The instrumental conditions during the analysis of heavy metals and the method quantification limits calculated following the European Commission guideline (EC, 2002) are summarized in **Table S1**. The stock NIST standard solutions of 1000 mg L^{-1} were diluted to the required times using 1% (w/w) suprapur grade nitric acid (Merck, Darmstadt, Germany) daily in order to prepare the working calibration standards. The reagents and chemicals used in the sample preparation and analysis were of analytical grade and used without further purification. All the solutions were prepared using de-ionised water with resistivity > 18 MΩ/cm produced using an E-pure system (Thermo Scientific, USA). The glassware and containers were cleaned by soaking into 20% nitric acid for at least 24 h and rinsed three times with deionized water prior to use

Quality control program

We are maintaining both internal and external quality control programs routinely in the Analytical Chemistry Laboratory in order to maintain the ISO/IEC 17025 accreditation of the laboratory. As a part of the internal quality control program, the quality control charts are constructed with the values of quality control standards for different heavy metals in order to check the accuracy of the data obtained daily in the laboratory. The laboratory is also participating in a good number of proficiency testing per year provided by the international proficiency testing providers as a part of external quality control program and securing the required satisfactory scores. Moreover, for the validation of the analytical procedure, a certified reference material NIST CRM 1566a (Oyster Tissue) was analyzed and the obtained results were summarized in **Table S2**. The mean recoveries of the analyzed heavy

metals were found in the range of 93.5-104.5% indicating the fitness of the method for analysis of heavy metals in the foodstuffs. Reagents blank determinations were used to correct the instrument readings.

Statistical analysis

All samples were analyzed in triplicate and the mean concentrations with standard deviations were used to represent the data. The statistical analyses were performed using the statistical package, SPSS (IBM SPSS Statistics 23). The statistically significant level of differences among the heavy metals were tested using t-test. Multivariate methods in terms of Pearson correlation and principal component analysis (PCA) were performed in order to find out the distribution of heavy metals in the foodstuffs. The PCA was performed using varimax-normalized rotation on the data set using Ward’s method. Other calculations were performed by Microsoft Excel 2019.

Calculations

Estimated daily intake of heavy metals

The estimated daily intakes (EDIs) of heavy metals through the consumption of chicken meat and egg were calculated using the formula (Shaheen et al., 2016b):

EDI = (FIR × MC)/BW

where, FIR is the food ingestion rate (g/person/day) for Bangladeshi population, MC is the mean metal concentration in the foods samples (mg/kg fw), and BW is the body weight of the consumer. The average consumption of chicken meat and egg by an individual of 60 kg body weight is 17.33 g and 13.58 g, respectively according to "Report of the household income and expenditure survey 2016" (BBS, 2018).

Non-carcinogenic risk

The non-carcinogenic risk of heavy metals due to the consumption of food is typically assessed from the target hazard quotient (THQ) which were calculated using the formula (FAO/WHO, 2011):

$$THQ = (EFr \times ED \times FIR \times MC) / (RfD \times BW \times AT) \times 10^{-3}$$

where, EFr is the exposure frequency (365 days/year), ED is the exposure duration (70 years equivalent to the average human life time), FIR is the food ingestion rate (g/person/day), MC is the metal concentration in food samples (mg/kg-fw), RfD is the oral reference dose (mg/kg-bw/day), BW is the body weight of the consumers (adult: 60 kg), and AT is the averaging time for non-carcinogens (365 days/year × number of exposure years, assuming 70 years).

Reference dose (RfD) states to a quantity that a consumer can be unceasingly exposed to this level for a long period without being affected. Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended RFDs are 0.0035 mg/kg-bw/day for Pb, 0.00083 mg/kg-bw/day for Cd, 0.0083 mg/kg-bw/day for Cr, and 0.00057 mg/kg-bw/day for Hg (Zheng et al., 2020). According to the guidelines of Chinese Nutrition Society (CNS), the RfDs for Mn is 0.183 mg/kg-bw/day and for Fe and Zn is 0.667 mg/kg-bw/day (Zheng et al., 2020).

If the $THQ < 1$, the exposed consumers are unlikely to experience any adverse health risk, while if the $THQ \geq 1$, there is a potential health risk (Wang et al., 2005), and associated interventions and protecting initiatives are required to be taken.

It has been stated that experience to more than one pollutant may result in additive and/or interactive effects (Hallenbeck 1993). Thus, in the present study, the cumulative health risk of heavy metals for individual food stuff was also evaluated by summing THQ value of individual metal and expressed as total THQ (TTHQ) as follows:

$$TTHQ \text{ (individual food stuff)} = THQ_{\text{toxicant 1}} + THQ_{\text{toxicant 2}} + \ldots\ldots THQ_{\text{toxicant n}}$$

The larger the value of TTHQ, the higher the level of concern.

Carcinogenic risk

Carcinogenic risk (CR) of a carcinogen shows an incremental probability of a consumer to develop cancer over the lifetime exposure to that carcinogen. Cancer risk over a lifetime exposure to Pb, Cd, Cr and As was calculated using the equation as follows (USEPA, 1989; USEPA, 2006; Islam et al., 2015b):

$$CR = (EF \times ED \times FIR \times MC \times CSFo)/(BW \times TA) \times 10^{-3}$$

where, CSFo is the carcinogenic slope factor of 0.0085 $(mg/kg/day)^{-1}$ for Pb, 6.3 $(mg/kg/day)^{-1}$ for Cd, 0.5 $(mg/kg/day)^{-1}$ for Cr, and 1.5 $(mg/kg/day)^{-1}$ for inorganic As set by USPEA (USPEA, 1989; Ullah et al., 2017; Zheng et al., 2020).

In general, CR value lower than 1.0E-06 is considered to be negligible, above 1.0E-04 is considered unacceptable, and lying between 1.0E-06 and 1.0E-04 is considered an acceptable range (USEPA, 1989; USEPA, 2010).

Results And Discussion

Concentrations of heavy metals in chicken meat and hen egg

The concentrations of Pb, Cd, Cr, As, Hg, Mn, Fe, and Zn were determined in three varieties of chicken meat (broiler, local, and sonali) and three varieties of hen egg (layer, local, and organic) collected from four wholesale markets of Dhaka, Bangladesh and summarized in **Table S3** and **Table S4**, respectively. The average concentrations of the studied heavy metals in the selected samples were presented in Table 1. The mean, median, 1.5 IQR, 25th, and 75th percentiles values for the studied heavy metals were represented in Fig. 2. Relatively wide variations were observed in the metal concentrations of the foodstuffs even within the same group. The concentrations of heavy metals in chicken meat and hen egg obtained in this study were compared to other reported data studied in Bangladesh and other regions and were presented in Table 2.

Table 1
Heavy metals in chicken meat and egg commonly consumed by Bangladeshi population [mean ± SD, (range)][a].

Food items	Heavy metals (mg/kg fw)							
	Pb	**Cd**	**Cr**	**As**	**Hg**	**Mn**	**Fe**	**Zn**
Chicken meat								
Broiler	0.59 ± 0.65 0.03–1.21	0.01 ± 0.00 0.01–0.015	0.17 ± 0.07 0.08–0.26	0.04 ± 0.00 0.04–0.06	0.01 ± 0.00 0.01–0.015	0.31 ± 0.20 0.15–0.56	19.2 ± 10.3 5.40–28.6	9.80 ± 0.84 9.03–10.9
Local	0.64 ± 0.83 0.03–1.84	0.01 ± 0.00 0.01–0.015	0.18 ± 0.06 0.14–0.27	0.04 ± 0.00 0.04–0.06	0.01 ± 0.00 0.01–0.015	0.39 ± 0.20 0.15–0.63	17.8 ± 10.3 4.38–27.4	13.1 ± 4.77 8.92–19.4
Sonali	1.02 ± 1.18 0.03–2.73	0.01 ± 0.00 0.01–0.015	0.32 ± 0.26 0.07–0.67	0.04 ± 0.00 0.04–0.06	0.01 ± 0.00 0.01–0.015	0.29 ± 0.18 0.15–0.51	13.6 ± 8.61 2.5–20.6	8.20 ± 5.03 1.02–12.8
MAC	0.1[b]	0.1[b]	1.0[c]	0.1[b]	0.05[d]	6.5[e]	NA	100[e]
Hen egg								
Layer	0.10 ± 0.06 0.03–0.16	0.01 ± 0.00 0.01–0.015	0.03 ± 0.01 0.025–0.05	0.04 ± 0.00 0.04–0.06	0.01 ± 0.00 0.01–0.015	0.15 ± 0.00 0.15–0.20	12.9 ± 7.32 6.82–22.4	10.1 ± 1.50 7.96–11.4
Local	0.24 ± 0.31 0.03–0.68	0.01 ± 0.00 0.01–0.015	0.03 ± 0.02 0.025–0.06	0.04 ± 0.00 0.04–0.06	0.01 ± 0.00 0.01–0.015	0.15 ± 0.00 0.15–0.20	18.3 ± 6.17 11.6–24.7	11.7 ± 1.01 10.5–12.8
Organic	0.06 ± 0.03 0.03–0.09	0.01 ± 0.00 0.01–0.015	0.025 ± 0.00 0.025–0.03	0.04 ± 0.00 0.04–0.06	0.01 ± 0.00 0.01–0.015	0.15 ± 0.00 0.15–0.20	25.7 ± 8.88 19.7–38.6	12.4 ± 2.02 10.9–15.4
MAC	0.1[b]	0.1[b]	1.0[c]	0.1[b]	0.05[d]	6.5[e]	NA	100[e]

[a]Note: undetected results were expressed as ½ LOD.

[b] JECFA (2005)

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Food items	Heavy metals (mg/kg fw)							
	Pb	Cd	Cr	As	Hg	Mn	Fe	Zn
[d] USDA (2014)								
[e] WHO (1998)								

Table 2
Comparison of heavy metals concentrations ((mg/kg fw) in chicken meat and hen egg with the reported values in the literatures.

Food items and region	Heavy metals (mg/kg fw)								References
	Pb	Cd	Cr	As	Hg	Mn	Fe	Zn	
Chicken meat									
Saudi Arab	2.72	0.46	N/A	N/A	0.004	7.97	66.33	10.37	Alturiqi et al., 2012
Nigeria	0.215	0.016	N/A	N/A	N/A	0.266	N/A	1.57	Oforka et al., 2012
Bangladesh	0.090	0.020	2.4	0.09	N/A	N/A	N/A	N/A	Islam et al., 2015b
Bangladesh	0.17	0.030	1.4	0.032	N/A	N/A	N/A	N/A	Islam et al., 2015a
Bangladesh	0.37	0.23	2.17	0.43	N/A	N/A	N/A	N/A	Shaheen et al., 2016b
Bangladesh	0.75	0.01	0.223	0.04	0.01	0.33	16.87	10.37	This study
Hen egg									
Egypt	0.07	0.003	N/A	0.01	N/A	0.37	25.35	18.96	Hashis et al., 2012
Chili	0.041	< 0.0008	N/A	0.019	0.003	N/A	N/A	N/A	Mun˜oz et al., 2005
Bangladesh	0.20	0.037	1.5	0.23	N/A	N/A	N/A	N/A	Islam et al., 2015b
Bangladesh	0.24	0.022	1.4	0.087	N/A	N/A	N/A	N/A	Islam et al., 2015a
Bangladesh	0.28	0.30	1.34	0.3	N/A	N/A	N/A	N/A	Shaheen et al., 2016b
Bangladesh	0.13	0.01	0.028	0.04	0.01	0.15	18.97	11.4	This study

The mean concentrations of Pb were found to be higher in chicken meat than hen egg which might be attributed to the high bioaccumulation of Pb in muscle tissues of animals. The highest concentration of Pb was measured in sonali chicken (mean: 1.02 mg/kg-fw, range: 0.03–2.73 mg/kg-fw) and the lowest concentration in organic egg (mean: 0.06 mg/kg-fw, range: 0.03–0.09 mg/kg-fw) (Table 1). The concentrations of Pb in all the tree categories of chicken meat were higher than the MAC of Pb in chicken meat (Table 1). The mean concentrations of Pb in chicken meat were higher than those obtained by Oforka et al. (2012), Islam et al. (2015a), Islam et al. (2015b), and Shaheen et al. (2016b) and lower than Alturiqi et al. (2012) (Table 2). The mean concentrations of Pb in egg samples were lower than those reported by Islam et al. (2015a), Islam et al. (2015b), and Shaheen et al. (2016b) and higher than Hashis et al. (2012) and Mun˜oz et al. (2005) (Table 2). The results of Pb content in egg were found higher than the MAC except in organic egg. The lowest concentration of Pb in organic egg can be attributed to the food habit of hen as they were feed simulated organic food. The high contents of Pb in almost all of the studied foodstuffs indicated sever contamination of Pb in the foodstuffs.

The mean concentrations of Cd in the selected foodstuffs were 0.01 mg/kg-fw which were below the MAC for Cd in foodstuffs (Table 1). Moreover, the results of Cd contents in chicken meat were lower than those reported by Alturiqi et al. (2012), Oforka et al. (2012), Islam et al. (2015a), Islam et al. (2015b) and Shaheen et al. (2016b) (Table 2). The measured mean concentrations of Cd in egg were also lower than those reported by Islam et al. (2015a), Islam et al. (2015b), and Shaheen et al. (2016b) and higher than Hashis et al. (2012) and Mun˜oz et al. (2005) (Table 2).

The highest mean Cr concentration was found in sonali chicken following the descending order of local chicken > broiler chicken > layer egg = local egg > organic egg. The mean concentrations of Cr in chicken meat and egg were lower than those reported by Islam et al. (2015a), Islam et al. (2015b), and Shaheen et al. (2016b). However, the concentrations of Cr in sonali chicken and organic egg showed statistically significant difference at 0.05 level (Fig. 2). Moreover, the mean concentrations of Cr in chicken meat and hen egg were below the MAC for Cr in foodstuffs (Table 1).

The mean concentrations of As in the selected foodstuffs were found as 0.04 mg/kg-fw. The calculated mean concentrations of As in chicken meat samples were lower than those of the previous studies conducted elsewhere by Islam et al. (2015b) and Shaheen et al. (2016b) and higher than reported by Islam et al. (2015a). The result of Cd contents in egg samples obtained in this study were lower than those obtained by Islam et al. (2015a), Islam et al. (2015b) and Shaheen et al. (2016b) and higher than those reported by Hashis et al. (2012) and Mun˜oz et al. (2005). As contents in both chicken meat and hen egg were below the MAC for As in foodstuffs indicating their safe intake in respect of As.

The mean concentrations of Hg in the selected foodstuffs were 0.01 mg/kg-fw. There is limited information on the report of Hg content in chicken meat and hen egg. Hg content in chicken meat was higher than that reported by Alturiqi et al. (2012). Moreover, hen egg contained higher amount of Hg than that reported by Mun˜oz et al. (2005). However, the results of Hg content in chicken meat and hen egg were below the MAC for Hg in foodstuffs indicating their safe consumption with respect to Hg.

The mean concentrations of Mn in chicken meat and hen egg were found 0.33 and 0.15 mg/kg-fw, respectively (Table 2). The concentrations of Mn in local chicken meat and hen egg were statistically significant difference at 0.05 level (Fig. 2). There is limited information on chicken meat and hen egg reported earlier. The results of Mn contents in chicken meat were higher than those reported by Oforka et al., 2012 and lower than reported by Alturiqi et al., 2012. Mn contents in egg in the present study were lower than those reported by Hashis et al., 2012. The mean concentrations of Mn in all the studied samples were below the MAC for Mn in foodstuffs.

The highest mean concentration of Fe was found in organic egg following the descending order of boiler chicken > local egg > local chicken > sonali chicken > layer egg. Statistically significant difference at level 0.05 was found between layer egg and organic egg. High content of Fe in organic egg could be due to the fact that simulated organic feed were provided to the hen lay organic egg. There is no or limited legislative value set for Fe in foodstuffs. Fe content in chicken meat and hen egg were lower than those reported by Alturiqi et al., 2012 and Hashis et al., 2012, respectively.

The highest mean concentration of Zn was in local chicken and the lowest concentration of Zn was in sonali chicken. Statistically significant difference at 0.05 level was found between the concentrations in broiler chicken and layer egg. Zn concentrations in chicken meat of the present study were in line with the study reported by Alturiqi et al., 2012. However, a lower value of Zn in chicken meat was reported by Oforka et al., 2012. Zn contents in egg of the present study were lower than the value reported by Hashis et al., 2012. The mean concentration of Zn in the studied foodstuffs was almost ten times lower than the MAC for Zn in foodstuffs indicating the safe consumption of the studied foodstuffs in respect to Zn contamination.

Multivariate statistical analysis

The Pearson correlation coefficient is a potential tool used to measure the strength of linear association between the pairs of variables by calculating a summary index (Sˇkrbic´ and Onjia, 2007). Hence, the metal to metal correlation data in terms of Pearson product moment correlation coefficients that were significant at 99% and 95% confidence level was evaluated and presented in Table 3. The pairs of Pb-Cr (0.862), Cd–As (1.000), Cd–Hg (1.000), As–Hg (1.000), and Fe–Zn (0.537) showed high and significant correlations at 99% confidence level. While Mn showed weak correlation with Fe (0.407) at 95% confidence level. The high correlations supported a hypothesis that the source of the metals might be analogous.

Table 3
Correlation between the heavy metals in chicken meat and egg samples.

	Pb	Cd	Cr	As	Hg	Mn	Fe	Zn
Pb	1							
Cd	.241	1						
Cr	.862**	.050	1					
As	.241	1.000**	.050	1				
Hg	.241	1.000**	.050	1.000**	1			
Mn	.277	− .124	.232	− .124	− .124	1		
Fe	− .182	− .302	− .222	− .302	− .302	.407*	1	
Zn	− .230	− .003	− .330	− .003	− .003	.194	.537**	1
**Correlation is significant at the 0.01 level (2-tailed).								
*Correlation is significant at the 0.05 level (2-tailed).								

The principal component analysis (PCA) using varimax-normalized rotation was subsequently carried out for factor loadings in each metal. The most important significance of the PCA is to reduce a large number of variables into a new set of reduced variables based on their mutual dependence (Manzoor et al., 2006). The significant number of PCs was identified using a scree plot (Fig. 3) in order to recognize the structure of the underlying parameters. The results showed that three eigen values greater than 1 explained more than 87% of the total variance (Fig. 3). The calculated factor loadings, the cumulative percentage of variance, and the percentages of total variance explained by each factor were shown in **Table S5**. The first factor showed the highest loadings for Cd, As, and Hg, explained more than 41.46% of the total variance, so they were mainly derived from common sources. The second factor accounted for about 25.51% of the total variance which showed the highest loadings of Pb and Cr, indicating that they originated from the same origins. The last significant factor with a variance of (20.15%) showed the highest loadings for Mn, Fe, and Zn with its sources differing from the other materials. It appeared that factors 1 and 2 were from anthropogenic resources and human activities (Saeedi et al., 2012). However, factor 3 might be due to metabolic processes in chicken as these elements are essential to animals although they may be toxic beyond their required concentrations (Sˇkrbic´ and Onjia, 2007). A three-dimensional plot of the PCA loadings was illustrated in Fig. 3 (inset) and the relationships among the heavy metals were readily understood. The relations among the heavy metals based on the first three PCs agreed well with the correlation study (Table 3).

Human health risk assessment

Among the eight heavy metals (Pb, Cd, Cr, As, Hg, Mn, Fe, and Zn) analyzed in the present study, the concentrations of seven heavy metals except Pb in the tested chicken meat and hen egg samples did not exceed the legislative limits set by various countries/agencies. However, the exposure dose of chemical contaminants can affect the potentiality of toxicity. Thus, combining the estimated concentration of heavy meats in the foodstuffs and the respective food consumption rate was considered as a potential tool to evaluate the benefits to risk.

As a first attempt of evaluation of human health risk, daily dietary intake of each metal through the consumption of chicken meat and hen egg was estimated and summarized in **Table S6**. The highest mean value of EDI was calculated for Fe (4.87E-03 mg/kg-bw/day) while the lowest mean value for Cd and Hg (2.89E-06 mg/kg-bw/day) (**Table S6**) due to the consumption of chicken meat. From the consumption of hen egg, the highest mean EDI was measured for Fe (4.39E-03) while the lowest value was measured for Cd and Hg (2.31E-06) (**Table S6**). The EDI results were compared with the respective maximum tolerable daily intake (MTDI) of individual heavy metal suggested by Joint FAO/WHO Expert Committee on Food Additive (JECFA) for Pb, Cd, Cr, As, and Hg and China National Standards (CNS) for Mn, Fe, and Zn (Zheng et al., 2020). Figure 4 clearly demonstrated that EDI values for all the analyzed heavy metals were below the respective MTDI levels indicating that it was unlikely to experience adverse health effects from exposure to the targeted heavy metals.

The non-carcinogenic risk of the selected heavy metals due to the consumption of chicken meat and hen egg was calculated based on target hazard quotient (THQ) and total target hazard quotient (TTHQ) and summarized in **Table S7**. The mean highest THQ was measured for Pb due to the consumption of both chicken meat (0.0619) and hen egg (0.0088) while for the both categories samples the lowest value was calculated for Mn (0.0005) and (0.0002) for chicken meat and hen egg, respectively (**Table S7**). The cumulative health risks by summing the health risks of eight investigated heavy metals was also evaluated as TTHQ. The mean value of TTHQ was found 0.0944 and 0.0302 for the consumption of chicken meat and hen egg, respectively. Both the THQ and TTHQ values did not exceed the threshold value of 1 (Fig. 5). The results indicated that the consumers would not experience any potential significant health risk during an entire lifetime. However, the present study demonstrated that only chicken consumption and hen egg consumption contributed about 10% and 3%, respectively to the threshold limit.

The carcinogenic risks (CRs) derived due to the dietary intake of Pb, Cd, Cr, and As were calculated as these heavy metals may promote both non-carcinogenic and carcinogenic risk depending upon their exposure dose. The CRs of Pb, Cd, Cr, and As due to the consumption of chicken meat and hen egg were calculated and summarized in **Table S8**. The mean CR values of Pb, Cd, Cr, and As due to the consumption of chicken meat were found 1.84E-06, 1.82E-05, 3.23E-05, and 1.73E-05, respectively, while those for the consumption of hen egg were 2.62E-07, 1.46E-05, 3.28E-06, and 1.39E-05, respectively. Generally, CR value lower than 1.0E-06 is considered to be negligible, above 1.0E-04 is considered unacceptable, and lying between 1.0E-06 and 1.0E-04 is considered an acceptable range (USEPA 1989, [illegible] risk of Pb, Cd, Cr, and As due to the consumption of chicken meat and hen egg were negligible to acceptable range (Fig. 6). This result suggested that it was unlikely to experience any carcinogenic risk of Pb, Cd, Cr, and As due to the consumption of the studied foodstuffs.

Conclusion

The concentrations of Pb, Cd, Cr, As, Hg, Mn, Fe, and Zn in three varieties of chicken meat and three varieties of hen egg samples collected from one of the mostly densely populated cities in the world, Dhaka, Bangladesh were determined and potential human health risk was assessed in terms of EDI, THQ, TTHQ, and CR. The metal concentrations were found below the maximum allowable concentration (MAC) in the foodstuffs except Pb in chicken meat. Pb concentration in chicken meat was found eight times higher than the MAC. However, the EDIs of heavy metals was below the maximum tolerable daily intake (MTDI). The calculated THQ and TTHQ values were less than 1 indicating the consumers would not experience any noncarcinogenic risk due to the consumption of the foodstuffs. The CRs of Pb, Cd, Cr, and As were within the acceptable range. The estimated human health risk assessment clearly revealed that chicken meat and hen egg could be a potential source of safe protein for the consumers with respect to heavy metals contamination. However, this study recommends that an attempt is required to estimate the organic contaminants and antibiotic residues in the foodstuffs in order to assess the collective health risk.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: The authors declare that they don't have any known conflict of interest to declare.

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Authors' Contributions: AKMAU analyzed the samples, interpreted the data and wrote the manuscript, SA collected, prepared and analyzed the samples, MMH analyzed the samples, MM analyzed the data and wrote the manuscript, TF, QN and SBQ supervised the works. All authors read and approved the manuscript.

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Appendix A. Supplementary data

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Figures

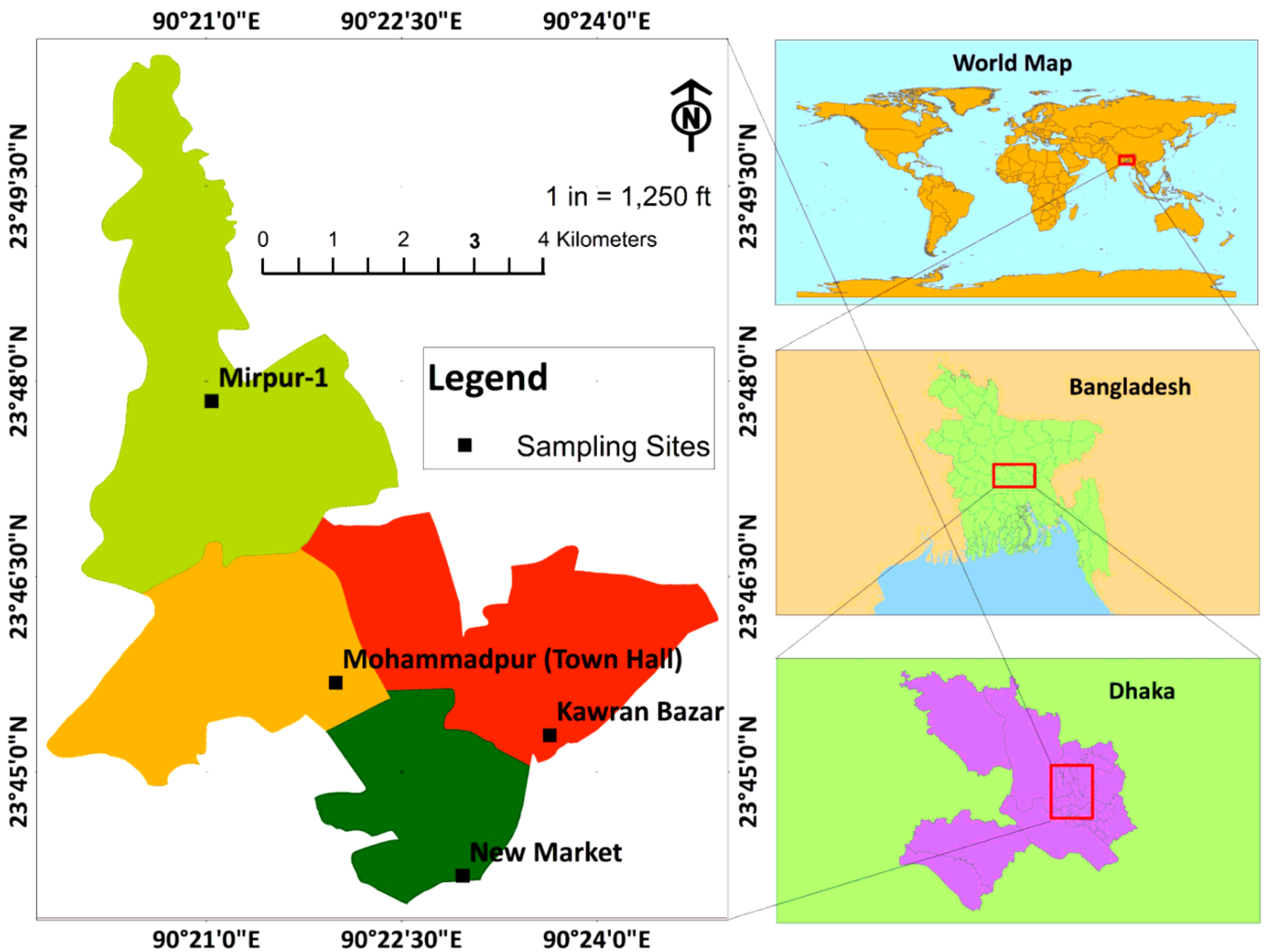

Figure 1

Map of the study area, Dhaka, Bangladesh. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

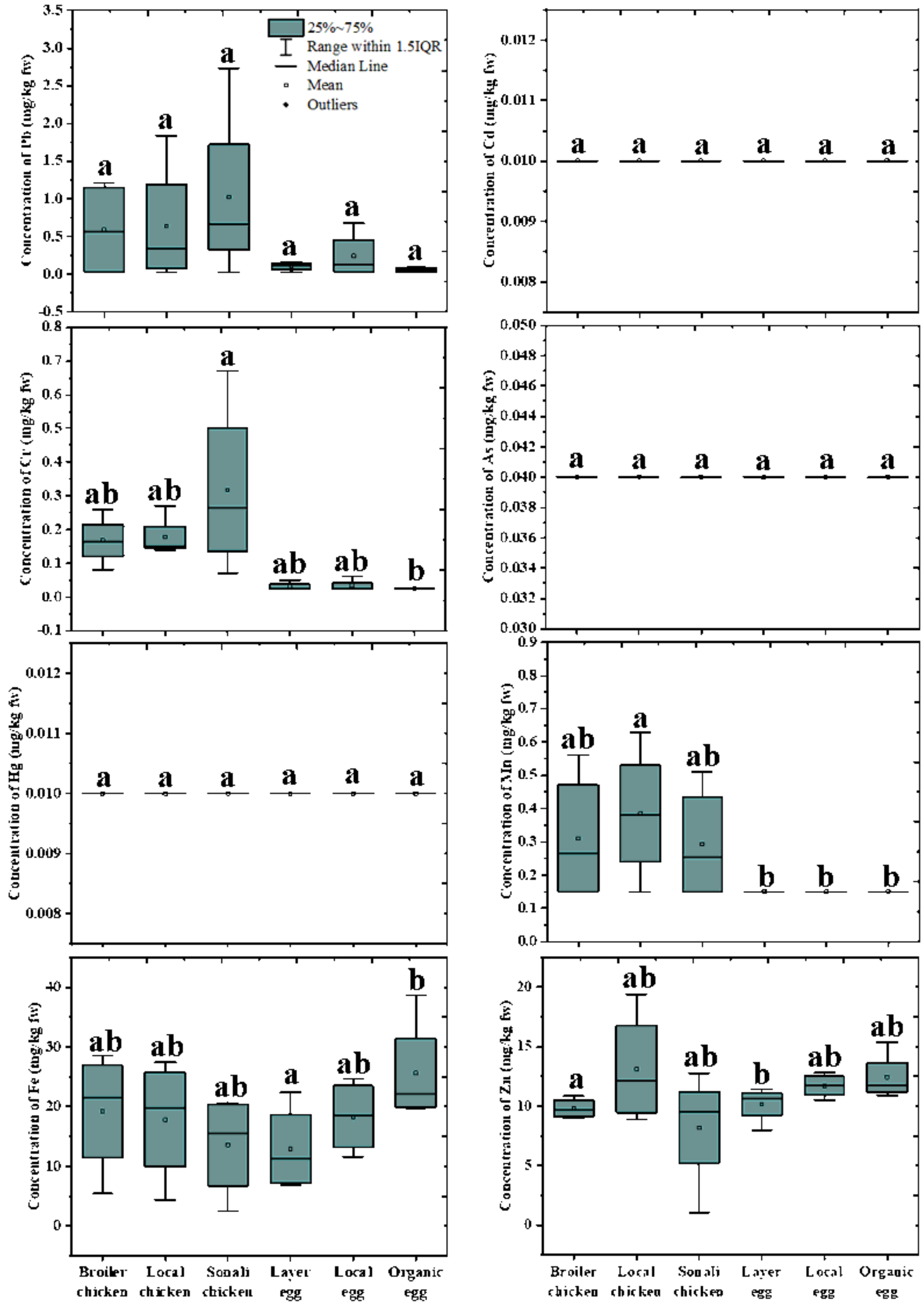

Figure 2

Box-whisker representation indicating the distribution of heavy metals in chicken meat and hen egg collected from Dhaka, Bangladesh. Letters a and b indicate statistically significant difference at 0.05 level.

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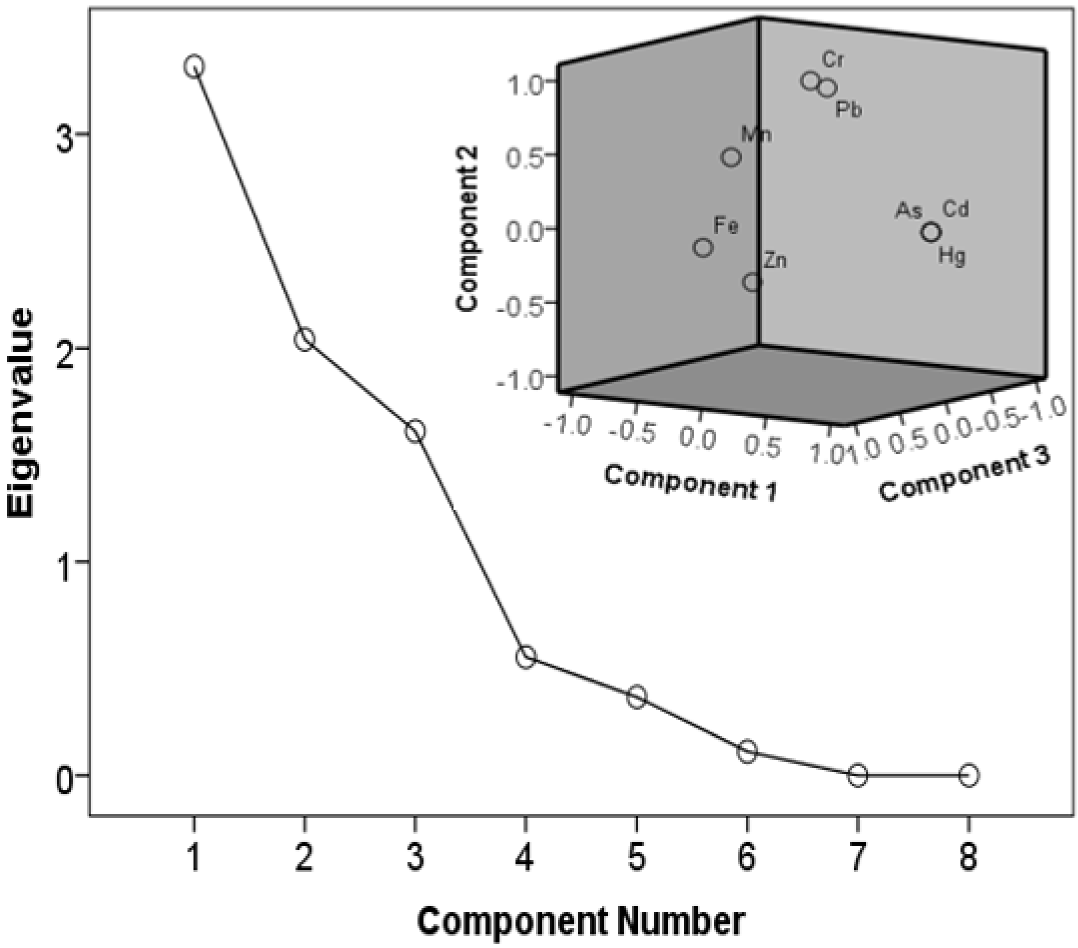

Figure 3

Principal component analysis of heavy metals by scree plot of the characteristic roots (Eigen values) (inset shows the three-dimensional plot of the PCA loadings demonstrating the relationships among the heavy metals).

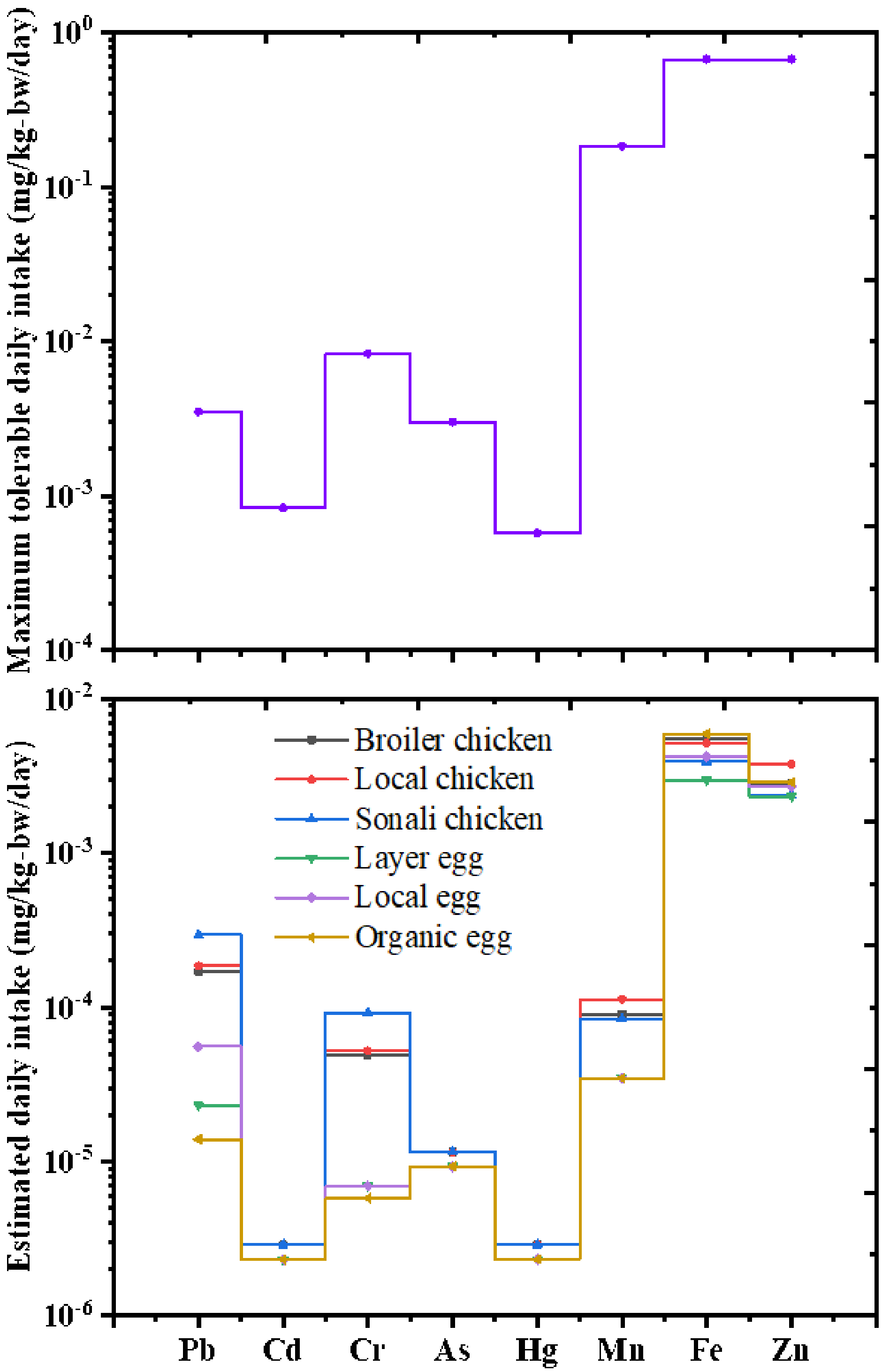

Figure 4

Estimated daily dietary intake of heavy metals and their comparison with the maximum tolerable daily intake. For more information see the Table S6 provided in supplementary materials.

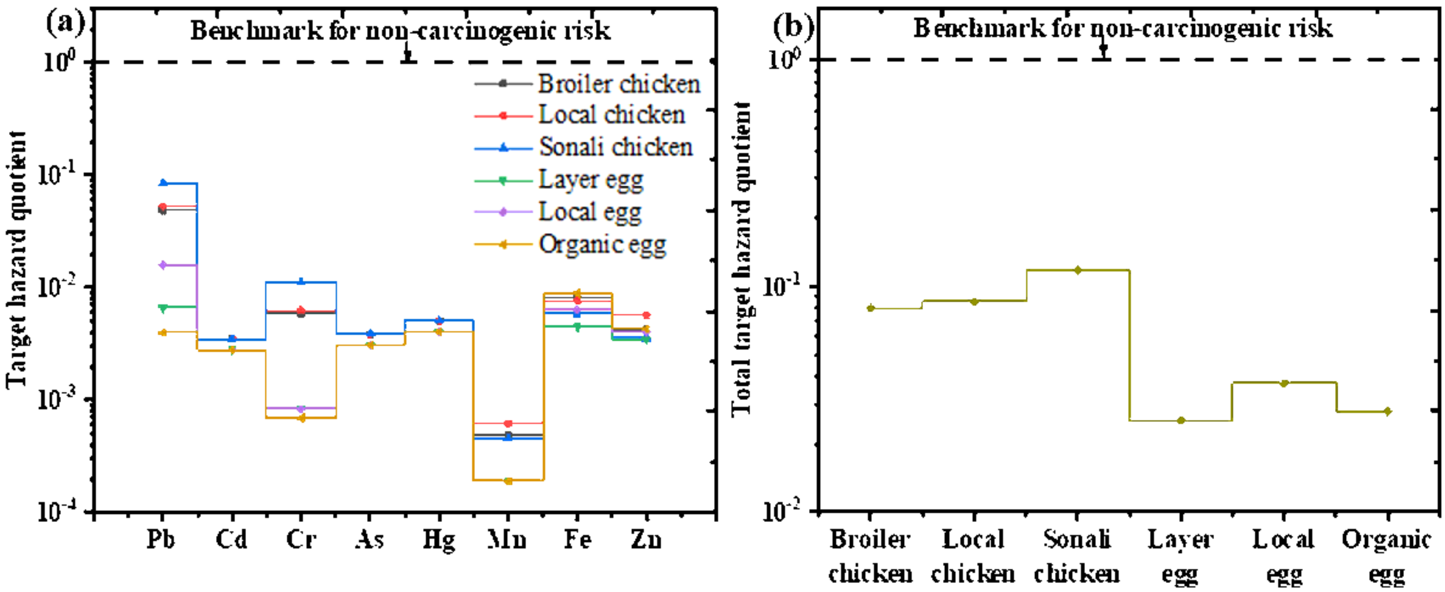

Figure 5

Non-carcinogenic risks (a) target hazard quotient (THQ) and (b) total target hazard quotient (TTHQ) due to dietary intake of eight heavy metals through the consumption of chicken meat and hen egg. For more information see the Table S7 provided in supplementary materials.

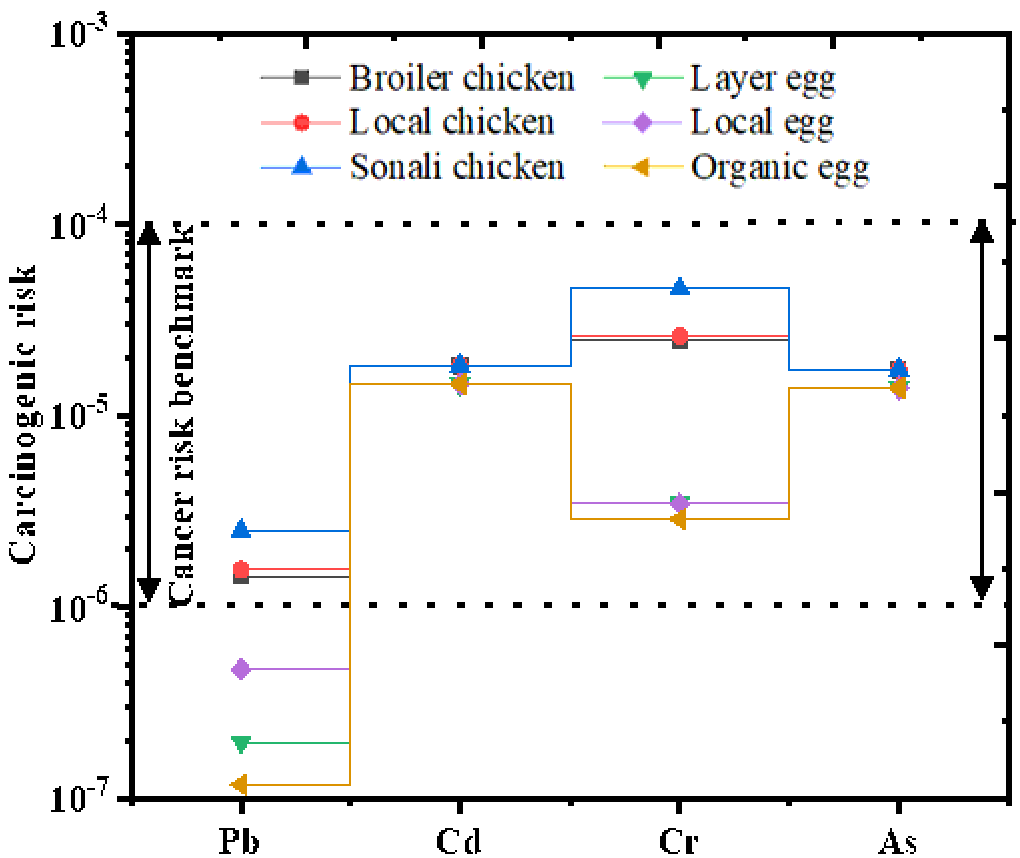

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Figure 6

Carcinogenic risks due to the dietary intake of carcinogenic heavy metals through the consumption of chicken meat and hen egg. For more information see the Table S8 provided in supplementary materials.

Supplementary Files

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