



Heavy metal pollution in poultry feeds and broiler chickens in Bangladesh

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ABSTRACT

The poultry industry poses a significant threat of heavy metal poisoning for the people of Bangladesh. The research was performed to assess the levels of heavy metals in chicken feed as well as other consumable sections of poultry fowl, and to determine the possible health hazards implicated. The levels of seven metals were evaluated in sixteen commercially available poultry feeds and in three edible portions of chicken obtained from several local markets in Rajshahi city. The metal concentrations were investigated via an atomic absorption spectrophotometer following the wet digestion method. The amount of Cr, Cd, Pb, Cu, Mn, Ni, and Fe in poultry feeds were observed from 0.03 to 12.85 mg/kg, 0.01–1.64 mg/kg, 0.15–4.21 mg/kg, 2.65–45.83 mg/kg, 22.63–188.85 mg/kg, 0.09–2.64 mg/kg, and 0.54–41.01 mg/kg, respectively. In broiler chickens, the concentrations were determined from 0.87 to 3.15 mg/kg, 0.01–0.05 mg/kg, 0.19–1.09 mg/kg, 0.96–3.78 mg/kg, 4.45–23.53 mg/kg, 0.07–0.56 mg/kg, and 2.70–92.32 mg/kg, respectively. With the exception of Cu, Mn, and Fe, most heavy metal concentrations in chickens exceeded the highest allowed concentration set by FAO/WHO. The estimated EDI, THQ and TTHQ numbers for all metals examined were found to be below MTDI, indicating that consumption of chicken meat poses noncarcinogenic risk to individuals. Comparatively, ILCR associated with Cd and Pb are around the safety threshold, but Cr exceeds the permissible range and poses a significant risk.

1. Introduction

The issue of environmental contamination by heavy metals is becoming increasingly prominent because of the detrimental effects it is having on people worldwide. Over the past century, industrialization has rapidly expanded. This has heightened the urgency for the careless exploitation of Earth's natural resources and exacerbated the problem of environmental contamination on a worldwide scale [1]. Metallic materials will soon be abundant in both terrestrial and marine habitats. Heavy metal pollution has arisen as a result of anthropogenic activity, specifically the mining, smelting, foundry, and other metal-based industries. Numerous places, such as garbage dumps, landfills, excrement, animal and poultry dung, runoffs, automobiles, and road construction, can leach metals [2].

Poultry meat is crucial for meeting the demand for high-quality animal protein and food security. The global poultry industry has grown due to increased consumer demand for chicken meat. Advancements in production have reduced international food shortages [3]. The

development of efficient chickens for meat and eggs has made animal-based proteins more accessible in established and rising economies. Improvements in genetics and nutrition have led to efficiency increases. Poultry meat is a superior source of protein due to its abundance of easily absorbable amino acids and rich content of essential minerals like phosphorus, iron, zinc, iodine, and vitamin B12 [4].

Animals can absorb environmental components and metallic substances, leading to heavy metal pollution in the food chain. Poultry feed, which contains small amounts of these pollutants, can cause weight loss, organ dysfunction, and mortality in chickens, posing a significant threat to the biosphere [5]. Heavy metal deposits in animals and birds accumulate in tissues, causing harmful effects. Distribution is influenced by ingested amounts, exposure duration, breed, and age [6]. Chicken consumption requires further attention due to bioaccumulation and potential harm. Contamination in poultry feed is a significant global concern that is worsened by environmental degradation.

The World Health Organization (WHO) has voiced legitimate apprehension over the incidence of heavy metals in broiler meat and the

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potential hazards it poses to human health. The health hazards including neurological impairments, developmental impairments in children, hypertension, and significantly altered enzyme function [7]. Cadmium is very hazardous to all bodily systems, and it has a tendency to build up in the kidneys and liver. This accumulation can lead to renal malfunction, bone harm, prostate cancer, and genetic mutation [8]. Chromium displays several oxidation states, functions as an essential trace element, and has a substantial impact on nutrition. Chromium (III) functions as a cofactor for insulin, playing a vital part in the metabolic processes of glucose, lipids, and proteins. Nevertheless, the hexavalent form of chromium is known to be carcinogenic [9]. The other heavy metals which come from poultry feeds and broiler chickens including lead (Pb), nickel (Ni), iron (Fe) also pose some detrimental threats to human health [10].

Rajshahi is an expanding urban center which accommodates three prominent educational institutes in Bangladesh, whose students depend solely on poultry meat as their principal source of protein [11]. It is imperative to assess and address food safety concerns in the region to protect public health and ensure food security. This study is particularly significant as it comprehensively evaluates heavy metal contamination in both poultry feeds and broiler meat within a specific geographic context of Rajshahi City, Bangladesh. While previous research has often focused on isolated components, such as either feed or edible meat portions, this study integrates these aspects to present a holistic risk assessment.

The purpose of this research is to analyze the concentrations and safety levels heavy metals like manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), cadmium (Cd), lead (Pb), and chromium (Cr) in available 8 different brands of poultry feeds and broiler meat collected from 8 different chicken markets of Rajshahi city, Bangladesh using atomic absorption spectroscopy (AAS) as well as estimated daily intake (EDI), target hazard quotient (THQ), and incremental lifetime carcinogenic risk (ILCR) health risk assessment.

2. Materials and methods

2.1. Study area and sample collection

Rajshahi is renowned for its cleanliness and abundant greenery. The area of Rajshahi City Corporation is 95.56 square kilometers. It is situated at Latitude: 24° 22' 26.40" N and Longitude: 88° 36' 4.10" E. The study area is situated in multiple locations within the Rajshahi City Corporation. The following locations are: Shaheb Bazar, Court Bazar, Kashiadanga, Bornali, Noudapara, Vodra, Binodpur, and Katakhal.

Samples were collected to analyze heavy metal contamination in poultry feeds and broiler chickens. Poultry feed samples, representing eight distinct commercial brands, included two types—starter and grower varieties—from each brand. This study additionally surveyed eight broiler chickens from eight different markets in Rajshahi City using popular feed brands. From each chicken, three edible parts—muscle, liver, and gizzard—were collected for heavy metal analysis. The weight and age of the chickens, that were taken for analysis was between 1 and 1.4 kg and 30–35 days. Fig. 1 displays the geographical layout of the study area, highlighting the position of the sampling site.

2.2. Preparation and analysis of sample

The feed samples were dried at 110°C to remove moisture, then crushed into a fine powder. The resulting fine powder was stored in desiccators in the dark until digestion. The ten meat samples underwent a series of steps including washing, cutting into pieces, drying in a continuous oven at 80°C for 48 hours, grinding into a fine powder, and storing in polythene bags. The samples were then labeled, kept cool and dry, and stored until acid digestion. The amount of moisture of the samples was assessed based on their fresh and dry weights. Initially, a quantity of 2 g of the sample was measured and transferred to a beaker. The beaker was then placed inside a muffle furnace, with a watch glass

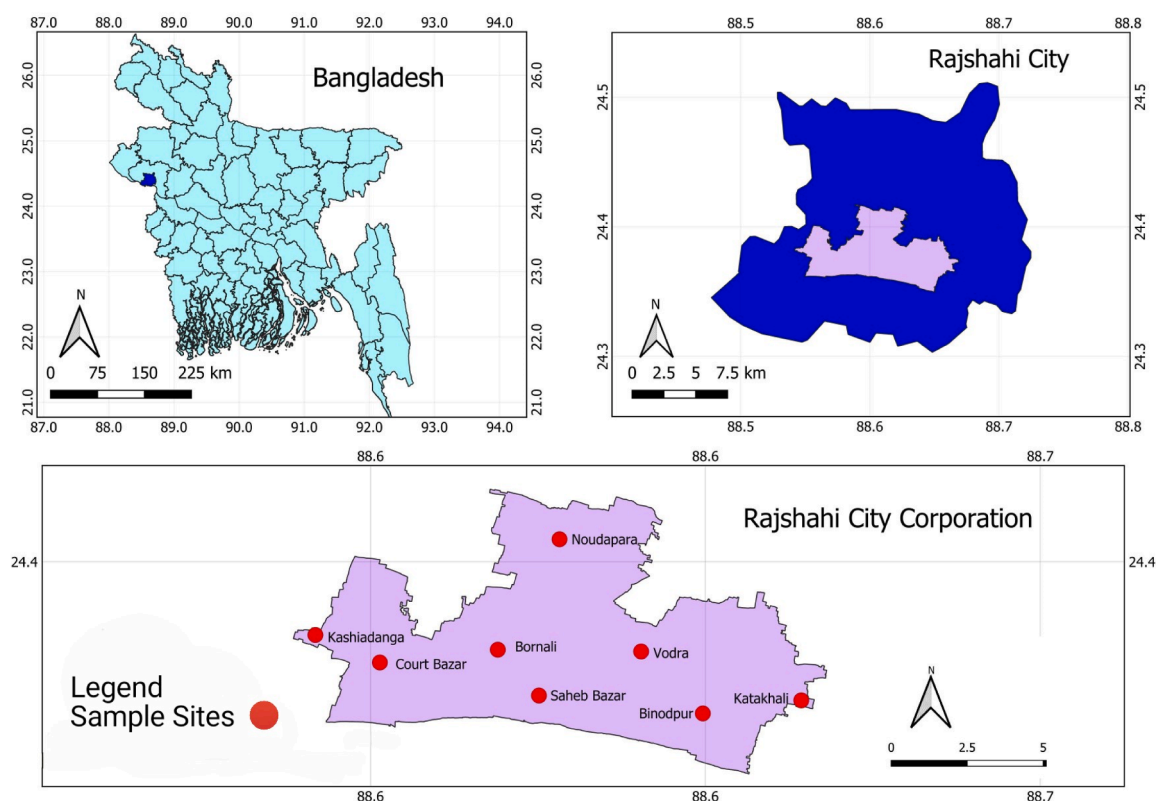


Fig. 1. Sample collection area.

covering it, leaving a small opening. The furnace temperature was set to 110°C and maintained at that level for approximately one hour. Next, adjust the temperature to 200°C, 300°C, and 400°C consecutively, maintaining each temperature for a duration of 30 minutes. Finally, the temperature was increased to 600°C and maintains it for a period of 6 hours. Subsequently, the furnace was halted and left to cool down to the ambient temperature.

Thereafter, the beaker was extracted from the furnace and a precise amount of 5 mL of concentrated nitric acid, in a 1:1 ratio, was cautiously introduced to immerse the sample. The beaker was positioned on a hot plate and subjected to heat until the vapor dispersed, reaching a temperature of about 120°C. Afterwards, a volume of 5–10 mL of concentrated HNO₃ was introduced, and the beaker was put on a hot plate until the solution became colorless. If the solution was not readily comprehensible, an additional 5 mL of concentrated HClO₄ was introduced and the mixture was further heated on a hot plate as long as it either became colorless or a transparent solution with color emerged. Subsequently, the solution was chilled and placed into a validated 50 mL volumetric flask. The flask was filled to the designated level with deionized water and rapidly agitated. If any solid residue was present, the solution was filtered using filter paper. Finally, the samples were analyzed through an atomic absorption spectrometer (SHIMADZU AA-6300, Shimadzu Europe, Kyoto, Japan). The equipment was fitted with a specialty bulb composed of a certain metal. The instrument underwent calibration using manually generated reference solutions of the corresponding heavy metals, together with a drift blank. Stock solutions containing metals at a concentration of 1000 parts per million (ppm) were obtained from Kanto Chemical Co. Ltd. in Tokyo, Japan. The solutions were diluted in order to acquire the necessary concentrations for the purpose of calibrating the device.

3. Health risk assessment

A human Health Risk Assessment (HRA) is employed to evaluate the probability and severity of adverse health impacts on humans who may come into contact with chemicals or other potentially hazardous substances in their environment. An HRA is a specific health survey that aims to assess an individual's health risks and overall quality of life [12]. This research aims to evaluate the likelihood of carcinogenic and non-carcinogenic risks to individuals by ingesting contaminated broiler chickens, specifically as a result of contact with heavy metals. The analysis will consider factors such as EDI, THQ, and ILCR.

3.1. Estimated Daily Intake (EDI)

The amount of metallic substances consumed via diet is determined by the metal concentration in food and the daily food intake. The ability of an individual to tolerate pollutants may also be influenced by their body weight [13]. The EDI can be determined or calculated in the subsequent manner [14],

$$EDI = \frac{C \times F_{IR}}{BW} \quad (1)$$

In this case, C represents the average quantity of heavy metals in the samples being studied, F_{IR} indicates the daily rate at which food is consumed (measured in grams per person per day), and BW refers to the weight of the body of a person. The detrimental effect of heavy metals to humans is contingent upon their daily consumption [15]. The Food Ingestion Rate (F_{IR}) for adults in Bangladesh is 17.4 g per person per day. The data was collected from the "Report of the Household Income and Expenditure Survey 2010," which was published in 2010 [16]. The reference concentration for the examined heavy metals in food was taken from WHO and FAO standards. The values are presented in Table 1 alongside the suggested maximum tolerable daily intake (MTDI).

Table 1

Maximum Permissible level established by WHO/FAO alongside Maximum Daily Intake recommendations.

Metals	MPL (mg/kg)	MTDI (mg/kg)
Cr	1.0 ^a	0.0028 ^d
Cd	0.05 ^b	0.00066 ^e
Pd	0.3 ^b	0.003 ^e
Cu	10 ^c	0.166 ^d
Mn	100 ^a	0.028 ^d
Ni	0.5 ^b	0.0015 ^d
Fe	180 ^a	0.80 ^d

MPL= Maximum Permissible level; MTDI=Maximum Tolerable Daily Intake.

^a WHO/FAO [17]

^b WHO/FAO [18]

^c WHO/FAO [19]

^d DRI (2001) [20]

^e RDA (1989) [21]

3.2. Target Hazard Quotient (THQ)

The THQ is a measure of exposure to a toxic substance compared to the reference dosage, defined as the maximum quantity at which no negative health effects are anticipated. The trace element being tested specifies the reference dosage [22,23]. A THQ value less than 1 indicates an exposure level that is inferior to the reference dose. This indicates that the daily exposure of this magnitude is improbable to have any obviously detrimental effects on the people being exposed. In essence, a THQ score under 1 indicates that the detrimental effects are minimal. Whenever the THQ value surpasses 1, there exists a potential of non-carcinogenic consequences. to develop. The likelihood of this event grows as the THQ value rises [24]. Calculations are conducted assuming an integrated USEPA risk analysis [25]. Calculation techniques for THQs are [26],

$$THQ = \frac{E_F E_D F_{IR} C}{R_{FD} W_{AB} T_A} \times 10^{-3} \quad (2)$$

$$\Sigma THQ = THQ_{Cr} + THQ_{Cd} + THQ_{Pb} + THQ_{Cu} + THQ_{Mn} + THQ_{Ni} + THQ_{Fe} \quad (3)$$

E_F denotes the frequency of exposure, which amounts to 365 days annually. E_D denotes the length of exposure, which spans 70 years, corresponding to the typical lifespan [27]. The F_{IR} is a metric that quantifies the daily food intake of a person, measured in grams. The variable C denotes the concentration of metal in food, quantified in micrograms per gram. R_{FD} stands for recommended daily intake of metal by oral ingestion, articulated in milligrams per kilogram of body weight per day. The Reference Doses (R_{FD} s) for Pb, Cd, Cr, Fe, Cu, Ni, and Mn are 0.0035, 0.001, 0.003, 0.7, 0.04, 0.02, and 0.14 mg/kg BW/day, respectively [28]. W_{AB} refers to the mean body weight, which is typically 60 kg for adults [29]. The T_A is the cumulative exposure time for non-carcinogens is determined by multiplying the number of exposure years by 365 days per year, assuming a study period of 70 years [30].

3.3. Incremental Lifetime Carcinogenic Risk (ILCR)

Carcinogenic risk (CR) indicates the likelihood of an individual acquiring cancer due to potential lifetime exposure to a substance that may induce cancer [31]. The carcinogenic analysis is conducted by assessing the potential cancer risks linked to heavy metal exposure through the calculation of ILCR [32,33].

$$ILCR = CDI \times CSF \quad (4)$$

$$\Sigma_{i=1}^n ILCR = ILCR_1 + ILCR_2 + \dots + ILCR_n \quad (5)$$

The variable n which refers to the heavy metals that is responsible for causing cancer when chicken meat is consumed. The equation given

calculates the possible cancer risks and the total cancer risk linked to the ingestion of carcinogenic heavy metals (Cr, Cd, and Pb) present in the studied meat samples. CDI stands for chronic daily intake, which is the amount of a chemical carcinogen consumed every day, quantified in milligrams per kilogram of body mass. It quantifies the typical amount of daily exposure to the chemical carcinogen over the course of an individual's lifespan. The CDI value was computed using the following equation,

$$CDI = \frac{EDI \times EF_R \times ED_{TOT}}{T_A}$$

(6)

Where E_{FR} represents the frequency of exposure, which is 365 days per year. E_{TOT} represents the length of exposure, which is 70.7 years, the average lifetime for Bangladesh. The cancer slope factor (CSF) represents the risk associated with a lifetime average exposure of 1 mg/kg BW/day and is particular to the pollutant in question. The suggested CSF values by USPEA are 0.5 for Cr, 0.0085 for Pb, 0.84 for Ni, and 0.38 for Cd [34]. A cancer risk linked with a certain heavy metal is considered insignificant if the ILCR is below 1×10^{-6} , and may be ignored. On the other hand, if the ILCR is more than 1×10^{-4} , it is regarded harmful and the risk of cancer is considered significant [35,36].

4. Results and discussion

4.1. Concentration of Heavy Metals

The Table 2 presents the metal concentration in sixteen chicken feed options in Rajshahi city. It also provides the standard deviation, relative standard deviation, minimum and maximum values for the metals Mn, Cd, Cu, Fe, Ni, Cr and Pb. Next, the levels of these metals in chicken feed are compared to the maximum permissible limit established by WHO/FAO. Fig. 2 presents a comparison of the average concentrations of metals in various chicken body parts sourced from different markets.

4.2. Chromium (Cr)

The investigation established that the concentration of Cr in the analyzed chicken feed samples ranged from not detected (ND) in

samples S-5, S-6, S-7, and S-8, to a maximum of 12.8521 mg/kg in sample S-16. The average value of Cr measured was 3.8253 mg/kg. The maximum allowable concentration of Cr in foodstuffs is 1 mg/kg [17]. Analysis of the data in Table 2 reveals that S-1, S-2, S-9, S-10, S-11, S-13, S-14, S-15, and S-16 display concentrations above the acceptable limit, accounting for 56.25 % of the total number of samples. Excluding these samples, the remaining 43.75 % have Cr values far below the permissible threshold. The range of Cr concentration (mg/kg) in various chicken organs was 0.869–2.271 in muscles, 0.947–3.157 in the liver, and 0.875–2.839 in the gizzard across eight samples. The mean values of Cr concentration were ranked as follows: liver > gizzard > muscle, with respective concentrations of 1.839 mg/kg, 1.808 mg/kg and 1.595 mg/kg.

The concentrations of Cr in the analyzed chicken feed samples were higher than the range reported by Islam et al. (2016), which was 1.03–2.26 mg/kg [37]. Iqbal et al. determined in their study, that the average level of Cr in chicken feed was 2.36 mg/kg [38]. The findings for Cr concentrations in chicken organs were consistent with those reported by Hossen et al., in which the content of Cr in various organs of chickens ranged from 0.857 to 3.550 mg/kg [39]. Cr is utilized in the production of dyes, paints, leather tanning, brick firing, and metallurgy to provide resistance against corrosion and a lustrous appearance. Because of its use of fertilizers and wastewater irrigation in agriculture has widespread applications. As a result, individuals are exposed to these substances through many means, including inhalation, ingestion, and skin contact [40]. Recently, tannery waste, due to its elevated protein content, has been utilized in the production of poultry feed in Bangladesh. A significant concentration of Cr, a hazardous substance, is typically present in tannery effluents [41]. Cr forms can be identified based on their degree of toxicity. Cr (III) is not carcinogenic and is a required nutrient for humans. However, if the levels of Cr reach a particular threshold, it can lead to adverse health effects. Cr (IV) poses significant risks to human health, including the potential to induce skin rashes, respiratory and cardiovascular issues, as well as kidney and liver damage, and even cancer [42].

Table 2
Heavy metals concentration (mg/kg) in different poultry feed.

Sample ID	Concentration of Heavy metals in different feed Samples (mg/kg)						
	Cr	Cd	Pb	Cu	Mn	Ni	Fe
Sample-1	6.0159 ± 0.0001*	0.7121 ± 0.0016*	ND	2.6543 ± 0.0002*	23.6802 ± 0.0005*	0.4531 ± 0.0005*	1.5089 ± 0.0002*
Sample-2	5.3067 ± 0.0004*	ND	ND	10.9226 ± 0.0004*	62.0349 ± 0.0013*	0.2793 ± 0.0006*	1.0972 ± 0.0001*
Sample-3	0.0747 ± 0.0003*	1.6442 ± 0.0007*	ND	7.1499 ± 0.0007*	52.9845 ± 0.0006*	0.3388 ± 0.0003*	2.2122 ± 0.0006*
Sample-4	0.0349 ± 0.0004*	ND	ND	45.8333 ± 0.0008*	188.8572 ± 0.0006*	0.2644 ± 0.0003*	1.7814 ± 0.0003*
Sample-5	ND	0.3040 ± 0.0008*	0.2542 ± 0.0003*	7.9461 ± 0.0004*	64.9800 ± 0.0006*	ND	0.5483 ± 0.0002*
Sample-6	ND	0.0547 ± 0.0003*	0.2490 ± 0.0006*	5.9561 ± 0.0003*	117.7788 ± 0.0005*	ND	0.6424 ± 0.0003*
Sample-7	ND	ND	0.1791 ± 0.0004*	8.8252 ± 0.0002*	75.4106 ± 0.0010*	ND	2.7874 ± 0.0007*
Sample-8	ND	ND	0.1548 ± 0	19.8751 ± 0.0004*	142.1178 ± 0.0017*	ND	1.5884 ± 0.0005*
Sample-9	7.9392 ± 0.0001*	0.0149 ± 0.0002*	1.1996 ± 0.0005*	34.0467 ± 0.0003*	102.5385 ± 0.0013*	0.0945 ± 0.0001*	39.1338 ± 0.0017*
Sample-10	7.9432 ± 0.0002*	0.0647 ± 0.0002*	0.8017 ± 0.0002*	11.5986 ± 0.0006*	102.0717 ± 0.0003*	0.1643 ± 0.0003*	20.4432 ± 0.0011*
Sample-11	4.7023 ± 0.0002*	0.0248 ± 0.0004*	4.2162 ± 0.0005*	4.3551 ± 0.0003*	23.5863 ± 0.0005*	2.6438 ± 0.0009*	2.9662 ± 0.0005*
Sample-12	0.0699 ± 0.0002*	0.0399 ± 0.0001*	1.0294 ± 0.0001*	12.5337 ± 0.0010*	98.0859 ± 0.0007*	2.0439 ± 0.0007*	3.1334 ± 0.0004*
Sample-13	3.1508 ± 0.0001*	0.1592 ± 0.0004*	ND	2.9467 ± 0.0002*	22.6381 ± 0.0001*	ND	41.0104 ± 0.0019*
Sample-14	4.9451 ± 0.0004*	0.1096 ± 0.0003	ND	15.3738 ± 0.0021*	124.5613 ± 0.0006*	ND	37.6071 ± 0.0015*
Sample-15	8.1704 ± 0.0002*	0.2043 ± 0.0001*	1.4207 ± 0.0002*	10.3240 ± 0.0001*	60.5134 ± 0.0012*	0.3040 ± 0.0002*	27.5074 ± 0.0013*
Sample-16	12.8521 ± 0.0008*	0.0499 ± 0.0002*	1.4035 ± 0.0003*	27.3176 ± 0.0004*	96.4185 ± 0.0011*	0.2347 ± 0.0003*	2.3126 ± 0.0003*
Minimum	ND	ND	ND	2.6543	22.6381	ND	0.5483
Maximum	12.8521	1.6442	4.2162	45.8333	188.8573	2.6438	41.0104
Average	3.8253	0.2114	0.6817	14.2287	84.8911	0.4263	11.6425
SD	4.0396	0.4226	1.0848	12.0499	45.9378	0.7707	15.6629
%RSD	105.6027	199.9	159.13	84.6872	54.1138	180.8	134.53
MPL	1	0.05	0.3	10	100	0.5	180
(WHO/FAO)							

Values are expressed as mean ± standard deviation of triplicate determinations (n = 3); ND = Not Detected; * - p value at < 0.001 was used to indicate Significant differences between samples. SD= Standard Deviation, RSD= Relative Standard Deviation, MPL= Maximum Permissible level (Table 1).

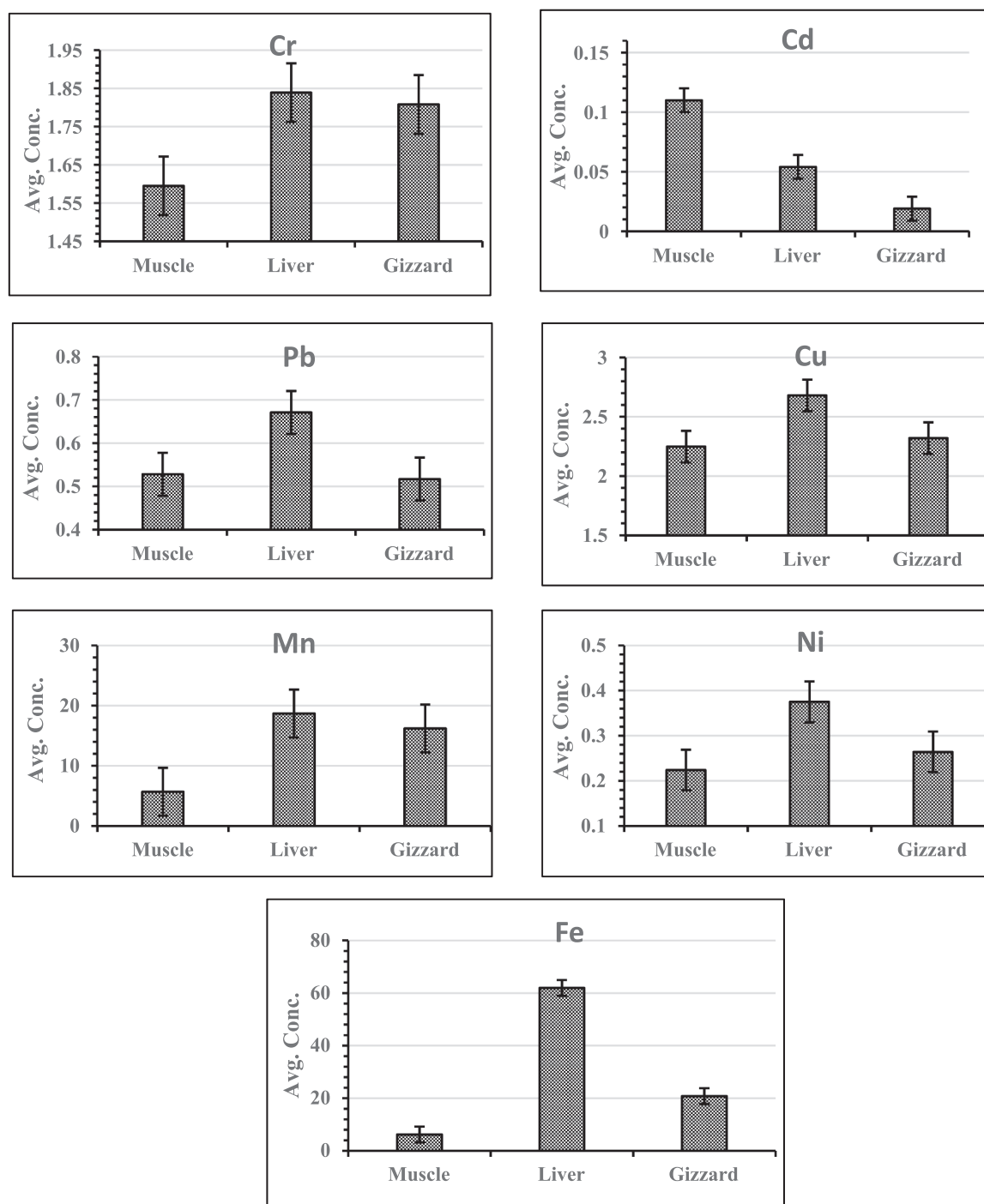


Fig. 2. Average concentrations (mg/kg) of metals in different body parts of the chicken.

4.3. Cadmium (Cd)

The study indicated that the chicken feed samples exhibited a variety of Cd concentrations, from non-detectable (ND) levels in samples S-2, S-4, S-7, and S-8, to a maximum concentration of 1.6442 mg/kg in sample S-3. The measured average concentration of Cd was 0.2114 mg/kg. The permissible limit for Cd in food is 0.05 mg/kg [18]. Considering the information in Table 2, it is evident that S-1, S-3, S-5, S-10, S-13, S-14, and S-15 that is 43.75 % of the total number of sample exhibit concentrations that exceed the permissible limit, rendering them unsafe for poultry. With the exception of these samples, the remaining 57.25 % samples contain a significantly lower concentration of Cd than the

acceptable level. Among the 8 samples, the ranges of levels of Cd were below the detection limit (BDL) to 0.035 mg/kg in the muscles, 0.019–0.109 mg/kg in the liver, and BDL to 0.055 mg/kg in the gizzard. The mean values of Cd concentration were ranked as follows: liver > gizzard > muscle, with respective concentrations of 0.054 mg/kg, 0.019 mg/kg and 0.011 mg/kg.

The results of feeds obtained from this research align with the findings of Imran et al., which revealed that the Cd levels in all the feed samples varied between 1.41 and 0.11 mg/kg [43]. Islam et al. reported that the amount of Cd in the branded feed samples was 1.329 mg/kg, while in the non-branded samples it was 1.328 mg/kg [44]. The average concentration of Cd in chicken liver and muscles, as determined by

Oforika et al. in Nigeria, was 0.0457 mg/kg and 0.0162 mg/kg, respectively [45]. Mottalib et al. found that the levels of Cd in chicken organs were within the allowed range [46]. These results are consistent with previous investigations. Diverse environmental sources, such as soil, lakes, sewage, fertilizers, and groundwater, are known to contain Cd. The Cd sources in chicken feed ingredients in Bangladesh comprise contaminated grains, oilseeds, fishmeal, animal by-products, water, and mineral additions [47]. Cd, when inhaled or swallowed, leads to dysfunction in the skeletal, cardiovascular, hepatic, renal, and respiratory systems. It also has the potential to induce cancer and genetic mutations [48,49].

4.4. Lead (Pb)

Among the most dangerous heavy metals is undoubtedly Pb. It does not provide any advantages for animals or people. Pb values in the gathered feed samples range from non-detected (ND) (S-1, S-2, S-3, S-4, S-13, and S-14) to 4.2162 mg/kg (S-11). The average value of Pb observed was 0.6817 mg/kg. The allowable limit for Pb in food is 0.3 mg/kg [18]. The quantities of S-9, S-10, S-11, S-12, S-15, and S-16, constituting 37.5 % of the total samples, were found to exceed the allowed limits, rendering them unsafe for poultry. Excluding these samples, the other 62.5 % showed Pb amounts much below the permissible threshold. Across 8 samples, there were differences in the Pb levels in the muscles (0.289–0.915 mg/kg), liver (0.266–0.1009 mg/kg), and gizzard (0.175–0.907 mg/kg). The mean concentrations of Pb were ordered in the following order: liver > muscle > gizzard, with respective concentrations of 0.671 mg/kg, 0.528 mg/kg and 0.517 mg/kg.

The feed results are higher than the concentration found from the study of Sarker et al. which was between 0 and 0.729 mg/kg [50]. Igwemmar et al. reported that the average concentration of Pb in poultry feed was 2.31 ± 1.23 mg/kg [51]. The levels of chicken organs deviated from the published values by Mathaiyan et al., both in lower and higher magnitudes. Where the levels of Pb in the breast, liver, neck, and kidney varied from 0.5633 to 4.9577 mg/kg, 0.6475–1.7798 mg/kg, 0.0048–1.2690 mg/kg, and 0.0017–4.1546 mg/kg, respectively [52]. The levels of Pb in consumable components of chicken ranged from 0.185 to 0.480 mg/kg, according to Benamirouche et al. [53]. Pb is utilized in batteries, construction, munitions, electronics, pigments, glass, ceramics, and previously as a fuel additive, however its application is becoming limited due to toxicity issues. It enters chicken feed through contaminated ingredients, such as grains, fishmeal, and water, as well as from environmental sources like lead battery waste [54].

Pb has the ability to build up in the liver and bones, and it can also have an impact on several bodily systems, such as the cardiovascular, neurological, hematological, reproductive, and adrenal systems [55].

4.5. Copper (Cu)

The concentrations of Cu in the studied feed samples ranged from 2.6543 mg/kg (S-1) to 45.8333 mg/kg (S-4). The obtained mean level of Cu was 14.2287 mg/kg. The highest amount of Cu that can be found in food is 10 mg/kg [19]. It is evident that S-2, S-4, S-8, S-9, S-10, and S-16, constituting 37.5 % of the entire sample, have concentrations that exceed the permissible level, making them unsafe for poultry. With the exception of these, 62.5 % of the remaining samples have Cu concentrations below the acceptable range. Cu values in the 8 chicken samples varied from 0.962 to 3.41 mg/kg in the muscles, 1.534–3.783 mg/kg in the liver, and 0.965–3.446 mg/kg in the gizzard. The average amounts of Cu were arranged in the subsequent order: liver > gizzard > muscle, with respective amounts of 2.682 mg/kg, 2.327 mg/kg, and 2.248 mg/kg.

The concentration of chicken feeds significantly exceeds the value reported in the study Chowdhury et al., which was at the range of only 0.554–1.317 mg/kg [56]. The observed value of Cu in chicken organs is

below the studied level of Alturiqi and Albedair, 2.31–7.79 mg/kg. and above the level of Elsharawy and Elsharawy, 0.15–1.16 mg/kg [57,58]. Cu contamination in chicken feed can result from various sources, including excessive copper in mineral supplements, by-products like fish meal or meat and bone meal, and grains grown in copper-rich soil. Environmental factors such as contaminated water, soil, or the use of Cu-based fungicides can also contribute. Additionally, cross-contamination during feed manufacturing or storage in Cu-based containers may increase Cu levels [40]. Cu is a crucial component for numerous enzymes in our body. However, levels above certain limitations, they might lead to liver and renal diseases [59].

4.6. Manganese (Mn)

The investigation revealed that the Mn concentrations in poultry feed samples ranged from 22.6381 mg/kg (S-13) to 188.8572 mg/kg (S-4). The average content of Mn was 84.89 mg/kg. Mn in food is permitted up to a level of 100 mg/kg [17]. It has been shown that the concentrations of S-4, S-6, S-8, S-9, S-10, and S-14 that is 37.5 % of the total samples exceed the permissible limits, rendering them unsafe for poultry. With the exception of these samples, the remaining 62.5 % samples exhibit Mn concentrations below the safe threshold. The Mn levels in the muscles, liver, and gizzard varied between 4.452 and 7.951 mg/kg, 16.047 and 23.533 mg/kg, and 11.783 and 18.219 mg/kg, respectively, throughout the 8 chicken samples. The mean values of Mn concentration were ranked as follows: liver > gizzard > muscle, with corresponding values of 18.682 mg/kg, 16.199 mg/kg, and 5.608 mg/kg.

The value of the Mn found in chicken feed is way higher than the value from Imran et al., in which the maximum mean level of Mn was determined to be 0.57 ± 0.2 mg/kg [43]. The study by Igwemmar et al. reported Mn levels of 99.57 ± 15.63 mg/kg, which closely aligns with the findings of our study [51]. The Mn content in chicken organs in this study was significantly higher compared to the values collected by Falandysz, where the following average values were found for the chicken's muscle, liver, and kidney: 0.11–0.27 mg/kg in muscle, 0.73–3.3 mg/kg in liver, and 0.90–1.9 mg/kg in kidney [60]. The levels were also very low obtained by Oforika et al., where the Mn content for chicken gizzard, liver, and muscle was measured to be 0.1265 ± 0.0096 , 0.4150 ± 0.0283 , and 0.2657 ± 0.1068 (mg/kg) respectively [45]. Mn is a vital trace mineral in poultry feed, facilitating bone formation, enzymatic activity, and eggshell integrity [61]. Mn contamination in crops like grains and soybeans can be caused by mining operations, natural deposits, irrigation with contaminated water, and excessive application of substandard manganese premixes [62]. Mn is crucial for maintaining good health as it promotes bone growth, regulates metabolism, and facilitates the function of the enzymes that perform antioxidant activities [63]. However, an excessive quantity of it can be detrimental to one's health as it can lead to neurological harm, oxidative stress, liver toxicity, respiratory problems, disruption of other essential nutrients, and impact the neurodevelopment of children [64].

4.7. Nickel (Ni)

The Ni concentration in the collected feed samples ranged from below the detection range (S-5, S-6, S-7, S-8, S-13, and S-14) to 2.6438 mg/kg (S-11). The average concentration of Ni observed was 0.4263 mg/kg. Ni levels in food are allowed to be no more than 0.5 mg/kg [18]. All samples, except for S-11 and S-12, which constitute 12.5 % of the total, have Ni amounts much below the permissible limit. The amounts of Ni in the muscles ranged from below detection limit (BDL) to 0.421 mg/kg, 0.081–0.568 mg/kg in the liver, and 0.075–0.42 mg/kg in the gizzard across all 8 chicken samples. The average concentrations of Ni were ordered in the following order: liver > gizzard > muscle, with corresponding quantities of 0.375 mg/kg, 0.264 mg/kg, and 0.224 mg/kg.

The results of chicken feed align with the analysis performed by Igwemmar et al., which revealed that the average level of Ni in all the feed samples was 1.66 ± 0.82 mg/kg [51]. Korish and Attia found it in between 1.46 and 2.15 mg/kg [40]. The values of chicken organs are quite similar with the findings of Mottalib et al., where average Ni concentration was 0.398 mg/kg in the chicken's liver, and in the breast it was 0.298 mg/kg [46]. According to Naseri et al., Ni content in mg/kg for muscle varied from 0.166 to 7.166, for liver 0.010–0.321, and for gizzard 0.203–4.284 [34]. Ni contamination in chicken feed is primarily from industrial processes like electroplating, alloy fabrication, and chemical manufacturing, affecting grains like maize, wheat, soybean, and canola, and infiltrating feed through contaminated water and air [65]. An elevated concentration of Ni can lead to cancer, hepatotoxicity, immunotoxicity, allergic skin reactions, respiratory problems, and oxidative stress [66].

4.8. Iron (Fe)

The Fe concentration in the obtained feed samples ranged from 0.5483 mg/kg (S-5) to 41.0104 mg/kg (S-13). The average concentration of Fe detected was 11.6425 mg/kg. The allowable limit for Fe intake is 180 mg/kg [17]. It is notable that all the samples had Fe values significantly lower than the permissible threshold. The concentrations of Fe in the muscles varied from 2.701 to 10.271 mg/kg, in the liver from 34.55 to 92.317 mg/kg, and in the gizzard from 10.305 to 37.103 mg/kg across the 8 chicken samples. The average concentrations of Fe were ranked as follows: liver > gizzard > muscle, with respective concentrations of 61.955 mg/kg, 20.807 mg/kg, and 6.224 mg/kg.

The concentrations of Fe in chicken feed observed in this study are significantly lower compared to the levels reported by Chowdhury et al., where the range was 16.477 ± 0.001 – 108.392 ± 0.002 mg/kg [56]. The range collected from the study of Korish and Attia, was 120.3–142.9 mg/kg, which was even higher [40]. The values observed in chicken organs in this study are closely aligned with the findings reported by Benamirouche et al., where the span of Fe levels in the chicken's edible parts was between 28.536 and 88.306 mg/kg [53]. Whereas Badis et al. found a much higher Fe concentration, with 246.83 mg/kg being the maximum [67]. Fe is crucial for poultry nutrition, forming hemoglobin, myoglobin, and enzymes involved in oxygen transport and cellular energy production [68]. It is typically sourced in chicken feed from supplements such as iron sulfate, as well as from cereals, soybean meal, fish meal, and forage. Contamination may arise from apparatus such as iron mills, storage facilities, or water with elevated iron concentrations [69]. Though Fe is a much-needed element for our body system, elevated levels of Fe can lead to cardiac arrest and respiratory failure [70].

4.9. Health risk assessment

The calculated Health Risk Assessment (HRA) values for the factors EDI, THQ, and ILCR were derived based on the ingestion of broiler

chicken contaminated with metals. The estimated results were then compared to permissible threshold limits and evaluated to assess the potential health risks associated with them.

4.9.1. Estimated Daily Intake (EDI)

The estimated daily metal intake level is shown in Table 3 in comparison to the Maximum Tolerable Daily Intake (MTDI) limits. The EDI of various metals is determined from Eq. 1 using data from a 60 kg adult who eats 17.4 g of chicken meat on a daily basis.

As the obtained EDI value is below the suggested daily intake ranges for heavy metals set by WHO/FAO, the EDI values of heavy metals calculated from consuming chicken meat indicate that average consumption amounts of chicken meat do not constitute a health risk. The EDI findings in the present investigation align with the results provided by Morshdy et al. [71].

4.9.2. Target Hazard Quotient (THQ)

Table 4 displays the calculated Total Hazard Quotient (THQ) for 7 heavy metals that are contained in 17.4 g of chicken meat consumed by adults weighing 60 kg in Rajshahi City.

The bulk of the heavy metals found in chicken meat samples had THQ (Target Hazard Quotient) values less than 1, suggesting that eating chicken does not present a serious health risk when exposed to just one metal. The total target hazard quotient, or TTHQ, determined by applying Eq. 3, for the meat samples that were consumed, varied between 0.000235335 and 0.000343253, depending on the specific metals being analyzed. This information may be found in Table 4.

The research areas revealed TTHQ values (below 1), indicating that consuming chicken does not have a non-carcinogenic negative impact. The following order can be applied to the total amount of heavy metals found in samples of chicken meat that were consumed: Cr > Pb > Mn > Fe > Cu > Cd > Ni. Which corresponds to the results with Hossain et al. [54].

4.9.3. Incremental Lifetime Carcinogenic Risk (ILCR)

The calculated values for ILCR and the sum of ILCR (Σ ILCR) for Chromium, Cadmium, and Lead resulting from the consumption of 17.4 g of chicken meat by a 60 kg adult are displayed in Table 5. The value was derived using Eq. 4.

The present research shows, the individual ILCR values of Pb and Cd are all below 1×10^{-4} , indicating that they are tolerable and provide a very minimal risk for cancer. However, the ILCR readings for chromium were over the allowable limit in every sample. While chromium is less carcinogenic, it nevertheless poses certain health concerns.

Finally, all of the broiler samples' combined cancer risk values (Σ ILCR or CCR) exceed the permissible threshold and pose a slight carcinogenic risk. Our data aligns with the analysis conducted by Kia et al. for the ILCR of Pb, while for Cd, there is a partial alignment [72]. Unlike our investigation, Kamaly and Sharkawy found that the values of Carcinogenic Risk for Pb, Cr and Cd were below acceptable limits and did not provide a danger of causing cancer [6].

Table 3

EDI based on consumption of 17.4 gm meat for average 60 kg weighted people in Bangladesh.

Sample Location	Estimated Daily Intake (EDI) (mg/kg-bw/day)						
	Cr	Cd	Pb	Cu	Mn	Ni	Fe
Shaheb Bazar	0.000455	0.0000139	0.000265	0.001002	0.004521	0.000131	0.001648
Court Bazar	0.000270	0.0000194	0.000205	0.000444	0.004933	0.000109	0.002074
Kashiadanga	0.000504	0.0000223	0.000111	0.001097	0.004700	0.000164	0.000783
Bornali	0.000658	0.0000316	0.000084	0.000715	0.004771	0.000119	0.001907
Noudapara	0.000482	0.0000101	0.000220	0.000906	0.005157	0.000137	0.002978
Vodra	0.000252	0.0000055	0.000155	0.000566	0.005283	0.000023	0.002335
Binodpur	0.000571	0.0000130	0.000083	0.000796	0.003417	0.000089	0.000902
Katakhal	0.000506	0.0000092	0.000099	0.000693	0.004797	0.000093	0.001811
Mean	0.000462	0.0000156	0.000152	0.0007773	0.0046973	0.0001081	0.001804
MTDI (Table 1)	0.0028	0.00066	0.003	0.1667	0.028	0.0015	0.80

Table 4

THQ based on consumption of 17.4 gm meat for average 60 kg weighted people.

Sample Location	Target Hazard Quotient (THQ)							Total THQ
	Cr	Cd	Pb	Cu	Mn	Ni	Fe	
Shaheb Bazar	0.0001518	0.000013	7.5814E-05	0.0000149	3.324E-05	6.568E-06	3.184E-05	0.000328216
Court Bazar	9.009E-05	0.000019	0.000058	1.826E-05	3.791E-05	5.495E-06	2.330E-05	0.000253083
Kashiadanga	0.0001681	0.000022	0.000031	2.498E-05	4.874E-05	0.00000823	2.193E-05	0.000326233
Bornali	0.0002195	0.000031	2.4194E-05	1.031E-05	3.518E-05	5.988E-06	1.643E-05	0.000343253
Noudapara	0.0001607	0.000010	6.3137E-05	2.065E-05	3.616E-05	6.887E-06	3.824E-05	0.000335993
Vodra	8.400E-05	0.000005	4.4328E-05	2.330E-05	4.506E-05	1.174E-06	3.194E-05	0.000235335
Binodpur	0.0001906	0.000013	2.3945E-05	6.996E-06	3.325E-05	4.480E-06	1.431E-05	0.000286667
Katakhali	0.000168	0.000009	0.0000284	1.549E-05	4.003E-05	4.683E-06	2.731E-05	0.000293911
Total	0.001233	0.000125	0.0003503	0.0001349	0.0003096	4.351E-05	0.0002053	0.002402693

Table 5

ILCR based on consumption of 17.4 gm meat for average 60 kg weighted people.

Sample Location	Incremental Lifetime Carcinogenic risk (ILCR)			Total ILCR
	Cr	Cd	Pb	
Shaheb Bazar	2.28E-04	5.28E-06	2.25E-06	2.35E-04
Court Bazar	1.35E-04	7.37E-06	1.74E-06	1.44E-04
Kashiadanga	2.52E-04	8.47E-06	9.44E-07	2.61E-04
Bornali	3.29E-04	1.20E-05	7.14E-07	3.42E-04
Noudapara	2.41E-04	3.84E-06	1.87E-06	2.47E-04
Vodra	1.26E-04	2.09E-06	1.32E-06	1.29E-04
Binodpur	2.86E-04	4.94E-06	7.06E-07	2.91E-04
Katakhali	2.53E-04	3.50E-06	8.42E-07	2.57E-04
Total	1.85E-03	4.75E-05	1.04E-05	1.91E-03

5. Conclusion

The purpose of this research is to assess the level of heavy metal contamination in the feeds of poultry and the accumulation of heavy metals in the chicken's edible sections that are eaten by humans directly and assessed the health risk in regard to EDI, THQ, and ILCR. While the results of the metal analyses are well under permissible limit for Cu, Mn, and Fe, the level of the other four heavy metals (Cr, Ni, Cd, and Pb) exceeds the maximum allowable level established by the WHO/FAO. According to the Health Risk Assessment, eating chicken meat has zero non-carcinogenic risks for the public, as all metal intake estimates are below the upper limit of what is considered tolerated each day. However, ILCR values for Cd and Pb are close to the safety limit where Cr is beyond the acceptable limit. Consequently, this research recommended that the Government of Bangladesh systematically oversees the contamination levels of harmful heavy metals and metalloids in food products to implement regulatory thresholds and evaluate the risks associated with prolonged exposure. Farmers and feed manufacturers in Bangladesh must cooperate to prevent heavy metal pollution in broiler feed. Enhanced legislation, sustainable agriculture methodologies, and research and development are essential. Farming producers have to get feed from reputable sources and use sound farming techniques. Feed manufacturers must emphasize superior raw resources, secure feed compositions, and contemporary processing technology. These actions will mitigate threats to human and animal health, augment animal production, bolster customer trust, and expand export possibilities for the Bangladeshi poultry sector.

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Author statement

We the undersigned declare that this manuscript is original, has not

been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRedit authorship contribution statement

Hasan Md. Mahfujul: Software, Methodology. **Salam Sayed M A:** Supervision, Formal analysis. **Sufal Md Tasif Amir:** Writing – review & editing, Formal analysis, Data curation. **Hassan Mohammad Tariqul:** Software, Methodology, Formal analysis. **Haque Md. Nazmul:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Hasan Tafsir:** Writing – review & editing, Software, Formal analysis, Data curation. **Shahriar Sha Md. Shahan:** Writing – original draft, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sha Md. Shahan Shahriar reports administrative support, equipment, drugs, or supplies, statistical analysis, and writing assistance were provided by University of Rajshahi. Sha Md. Shahan Shahriar reports a relationship with University of Rajshahi that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

No data was used for the research described in the article.

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