



# Investigating Heavy Metals Contamination Level in Poultry Feed and Layer Chicken Eggs and Its Associated Health Risk in Bangladesh

Afrose Sultana Chamon\*, M.N. Mondol, Sayada Kowka Batul Jannat Tajnin

Department of Soil, Water and Environment, University of Dhaka, Bangladesh

\*Corresponding Author

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## ABSTRACT

**Background:** Heavy metals contamination in poultry products are increasing public health concern in Bangladesh, due to rapid industrialization, contaminated irrigation water, and the use of low-quality feed ingredients and tannery waste contribute to metal accumulation in eggs.

**Objectives:** This study evaluated the concentrations of toxic and essential heavy metals in collected Layer feed samples and Layer eggs from major poultry-producing areas of Savar and Dhamrai, Bangladesh, and assessed the potential human health risks associated with their consumption.

**Methodology:** Five commercial and non-branded layer feed samples and eighteen egg samples from two farms were analyzed. Samples underwent acid digestion ( $\text{HNO}_3\text{-HClO}_4$ ), followed by metal quantification using AAS and ICP-MS. Health risks were assessed using Average Daily Intake (ADI), Hazard Quotient (HQ), and Hazard Index (HI) models.

**Key Results:** Feed samples showed metal concentrations ranging as follows: Ni ( $3.95\text{--}5.66\text{ mgkg}^{-1}$ ), Cd ( $0.11\text{--}0.33\text{ mgkg}^{-1}$ ), Pb ( $0.44\text{--}2.83\text{ mgkg}^{-1}$ ), Cr ( $0.00\text{--}13.48\text{ mgkg}^{-1}$ ), Cu ( $5.59\text{--}24.93\text{ mgkg}^{-1}$ ), and Zn ( $28.19\text{--}73.12\text{ mgkg}^{-1}$ ). Most of the metals were within permissible limits except Cr exceeded safety limit in several samples, indicating contamination from tannery waste. Egg samples contained significantly elevated concentrations: As ( $6.41\text{--}21.77\text{ mgkg}^{-1}$ ), Pb ( $1.20\text{--}6.33\text{ mgkg}^{-1}$ ), Cd ( $0.08\text{--}1.67\text{ mgkg}^{-1}$ ), Cr ( $8.76\text{--}15.08\text{ mgkg}^{-1}$ ), and Ni ( $0.44\text{--}4.62\text{ mgkg}^{-1}$ ); all above international limits. Essential elements were also high: Cu ( $3.48\text{--}11.73\text{ mgkg}^{-1}$ ), Zn ( $70.64\text{--}208.23\text{ mgkg}^{-1}$ ), Co ( $0.01\text{--}0.31\text{ mgkg}^{-1}$ ), and Be ( $0.002\text{--}0.013\text{ mgkg}^{-1}$ ). ADI values for As ( $0.035\text{--}0.121\text{ mg/person/day}$ ) exceeded PMTDI in most samples, and HI values surpassed 1.0 in all sheds, signifying cumulative human health risks.

**Conclusion:** Layer eggs from Savar are heavily contaminated with toxic metals, especially As and Cr, posing significant health risks to consumers.

**Keywords:** Heavy metals, Poultry feed, Eggs, Hazard quotient, Hazard index, Food safety, Bangladesh

## INTRODUCTION

In Bangladesh, poultry farming is a vital source of protein due to its affordability and nutritional value (Mottalib et al., 2018). An egg provides calories, protein, and fat. A 50 gram weighing chicken egg has around 297 KJ of energy, 6g of protein, 5g of fat (1.5g saturated, 2.0g monounsaturated, and 0.8g polyunsaturated), and 190mg of cholesterol (FAO, 2007). However, environmental heavy metals can contaminate layer chicken eggs, a staple food that poses serious concerns to public health (Alam et al., 2002; Islam et al., 2013). Heavy metal pollution in ecosystems has been accelerated by Bangladesh's rapid industrialization, expansion of agriculture, and inadequate implementation of regulations; contaminants enter poultry systems through a variety of channels (Hossain et al., 2008; Alam et al., 2002).

Heavy metals infiltrate the poultry food chain primarily via contaminated feed and water. Agricultural soils irrigated with polluted river water accumulate metals like Cr from tannery waste, which transfers to crops used in poultry feed (Islam et al., 2018; Mohiuddin et al., 2011). For instance, Cr concentrations in sediments near industrial zones exceed permissible limits, posing bioaccumulation risks (Islam et al., 2016). Arsenic enters eggs

through two dominant routes: (1) roxarsone, an arsenic-based poultry feed additive historically used to promote growth, and (2) arsenic-contaminated groundwater used in farming (Real et al., 2017; Hasan et al., 2022). Elevated arsenic concentrations found in eggs near agricultural zones using As-tainted water (Shahriar et al., 2025). Cadmium and Pb, originating from industrial discharge and vehicular emissions, adsorb onto soil particles and enter feed crops (Islam et al., 2015).

Total Arsenic (As), Cobalt (Co), Lead (Pb), Cadmium(Cd), Copper(Cu), Chromium(Cr), Zinc(Zn), Manganese (Mn), Beryllium (Be) and Nickel(Ni) have their potential for human exposure and increased health risk. Arsenic, Chromium, Lead, Arsenic, Zinc and Cadmium are among the main toxic and abundant metals which accumulate in food chain (Dermirezen and Uruç, 2006) and easily absorbed from atmospheric air and from the digestive tract (Krejpcio and Trojanowska, 2000). Beryllium (Be) contamination in poultry feed may arise from environmental exposure, including weathering of rocks, industrial effluents, and runoff from contaminated sites, which introduce beryllium into water and soil used for crop and feed production (EPA, 1980; EPA, 1983). Cobalt is an essential trace element but can be critical for physiological functions in poultry and can become problematic at high concentrations (Hashemi et al., 2023).

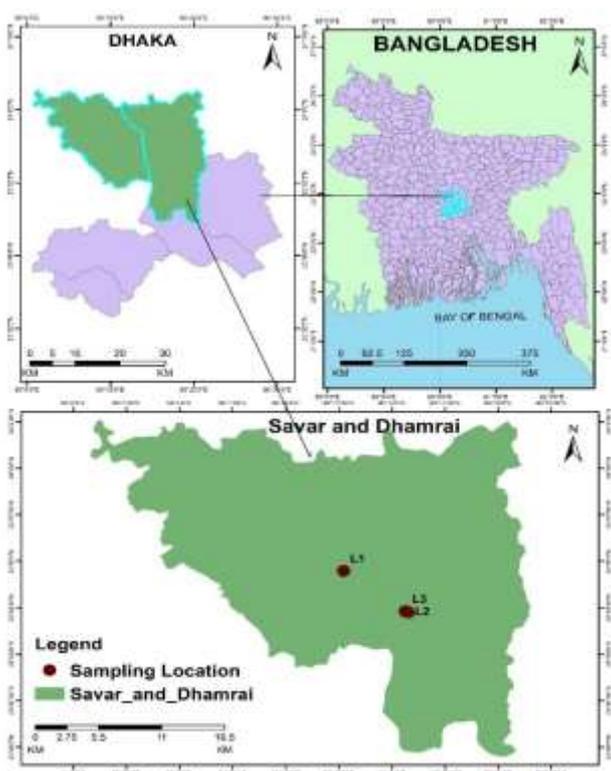
This study aims to evaluate the concentrations of arsenic, lead, chromium, cadmium, cobalt, beryllium, zinc, copper, and nickel in layer chicken eggs collected from selected regions of Bangladesh. Additionally, the study assesses associated human health risks using Average daily intake (ADI), hazard quotient (HQ) and hazard index (HI) models to determine potential risks for different consumer groups.

## METHODOLOGY

### Study Area:

The present study involved sample collection from several key poultry-rearing regions of Savar and Dhamrai, Bangladesh. The geographical coordinates of Savar is located near longitude  $90.2435^{\circ}$  E and latitude  $23.8462^{\circ}$  N and for Dhamrai it is  $90.2167^{\circ}$  E longitude and  $23.9083^{\circ}$  N latitude. These regions represent important hubs for poultry farming in Dhaka, which is practiced year-round to supply both domestic consumption and commercial markets. Due to logistical and financial constraints, sampling was limited to two representative commercial farms. The selected farms were chosen based on production scale and accessibility. Although the sample size is limited, the study provides preliminary baseline data for the region. Similar sample sizes used in previous regional studies (Chowdhury et al., 2011).

Map 1: Study Area and Sampling Locations of collected feeds and egg samples



**Sample Collection and preparation:**

The study was conducted on two commercial poultry farms selected based on accessibility, production scale, and operational consistency. A total of five feed samples and eighteen egg samples were collected during the study period (Table 1). Feed samples were collected directly from manufacturers, farms, and distributors in sealed, food-grade sampling bags to prevent contamination. For ethical and privacy considerations, and to avoid potential commercial conflicts, the specific brand names associated with these feed samples are not disclosed.

While the sample size may appear limited, the sampling strategy was designed to provide preliminary baseline information on heavy metal contamination in the selected production systems. Similar exploratory studies in comparable contexts have employed similar sampling scales due to logistical and financial constraints. However, it is acknowledged that the sample size may limit broader generalization.

Egg samples were collected from two types of Layer chickens: white Layers and brown Layers (Table 2). The eggs were obtained from the same poultry farms where the respective Layer chickens were reared. To ensure their integrity during transport, the eggs were placed in standard egg cases and transported carefully to the laboratory to prevent breakage. The number of farms and samples was constrained by access, laboratory capacity, and resource availability. The selected farms represent typical commercial layer operations in industrially influenced peri-urban areas of Savar and Dhamrai, allowing an initial assessment of contamination trends rather than national representativeness.

**Table 1: List of collected branded and non-branded feed samples**

Feed Sample Name	Company No.	Sample ID	Location
Layer Starter ( 0-19 weeks)	C1	LsC1	Dhulivita, Dhamrai
Layer Layer 1 ( 19-45 weeks)	C2	LL1C2	Jamsing, Savar,Dhaka
Layer Layer 1 ( 19-45 weeks)	C3	LL1C3	Jamsing, Savar,Dhaka
Layer Layer 1 ( 19-45 weeks)	C4	LL1C4	Jamsing, Savar,Dhaka
Layer Layer 1 ( 19-45 weeks)	C5	LL1C5	Dhulivita,Dhamrai

**Table 2: Details of Collected Egg samples from Savar Layer chicken rearing area**

Sample Type	Farm ID	Location
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 1)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 1)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 1)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 2)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 2)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 2)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 3)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 3)
Edible part(egg white and yolk)	Farm 1	Jamsing farm (shed 3)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 1)



Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 1)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 1)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 2)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 2)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 2)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 3)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 3)
Edible part(egg white and yolk)	Farm 2	Dhulivita farm (shed 3)

Upon arrival at the laboratory, the whole eggs were individually wrapped in aluminum foil and the fresh weight of each edible sample was recorded using a digital balance. The samples were then subjected to oven drying at 60–70°C until a constant weight was achieved, as recommended for moisture removal in biological samples (AOAC, 2000). Following drying, the eggs were homogenized by grinding to achieve uniform size. The homogenized samples were then stored in clean, dry, and properly labeled polyethylene bags to avoid contamination and moisture absorption prior to further analysis.

### Sample Digestion for Heavy Metal Analysis

To determine the concentration of heavy metals in the samples, acid digestion was employed due to the high organic content of the material. This method enables the effective breakdown of organic matrices, ensuring complete release of metal ions into solution for accurate analysis.

Approximately 1.00 g of oven-dried sample was accurately weighed and transferred into a 100 mL borosilicate glass beaker. To this, 15 mL of concentrated nitric acid (HNO<sub>3</sub>) and 10 mL of concentrated perchloric acid (HClO<sub>4</sub>) were carefully added. The selection of the HNO<sub>3</sub>–HClO<sub>4</sub> digestion mixture over conventional nitric acid extraction was based on its superior oxidative strength and efficiency in decomposing complex organic materials (Hossain et al., 2007). The beaker containing the sample-acid mixture was placed on a hot plate and heated gradually to a temperature between 150°C and 200°C. The digestion continued until the solution became clear or nearly clear, indicating the substantial decomposition of the organic matter.

Following digestion, the mixture was allowed to cool to room temperature. The cooled digest was then filtered using Whatman No. 42 filter paper to remove any particulate residue. The filtrate was quantitatively transferred into a 100 mL volumetric flask, and the volume was brought up to the mark with distilled water to ensure consistent sample dilution.

Finally, the prepared sample solutions were transferred into acid-washed polyethylene bottles and stored at room temperature until further analysis.

### Analysis of Heavy Metal Concentrations

The concentrations of selected heavy metals such as chromium (Cr), lead (Pb), nickel (Ni), copper (Cu), and zinc (Zn), arsenic (As), cobalt (Co) and beryllium (Be) in the digested extracts were quantitatively determined using Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICPMS). Specifically, a Varian AA240 instrument was employed for AAS analysis, while a Varian NixION 2000 system was used for ICP-MS measurements. These techniques are widely recognized for their sensitivity and reliability in trace metal analysis (Skoog et al., 2014).

### Health Risk Assessment

An assessment of the health risk posed in human beings by the consumption of contaminated chicken and fish

body parts was made by comparing the concentration of the contaminants recorded from the analysis with national and international safe limits. Different parameters like Average Daily Intake (ADI), Hazard quotient (HQ) and Hazard index (HI) were estimated to assess the health risk.

### Average Daily Intake (ADI)

The ADI of a heavy metal was calculated as a product of average daily consumption per person, percentage of dry weight of chicken and egg samples and average heavy metal concentration per dry weight of samples as shown in the following equation (Kacholi and Sahu, 2018):

$$ADI = A_{V\text{consumption}} \times \% DW_{\text{Egg}} \times C_{\text{heavy metal}}$$

Where ADI is Average Daily Intake (ADI) of heavy metal per person per day (mg/person/day),  $A_{V\text{consumption}}$  is average daily consumption of studied samples per person per day (g/day),  $\% DW_{\text{Studied samples}}$  is percentage of dry weight of studied samples ( $\% DW = [(100 - \% \text{ moisture})/100]$ ), and  $C_{\text{heavy metal}}$  is average heavy metal concentration of dry weight samples (mg/kg). Estimating heavy metal exposure level is indispensable in determining organism health risk (Othman, 2001). The degree of toxicity of heavy metal to human being depends upon their daily intake (Singh *et al.*, 2010). In the present study, the Average Daily Intake (ADI) of nine metals were calculated by taking average consumption of whole edible part of eggs as 22 g for an adult individual of 60 kg body weight according to “Household Income and Expenditure Survey in Bangladesh” (HIES, 2011), respectively, by considering the mean concentration of each metal in edible chicken, egg samples, corresponding dry weight of samples and average body weight of 60 kg of a person which may not reflect variability among children or high-consumption groups.

### Hazard Quotient (HQ)

Hazard quotient is a proportion of the probable exposure to an element/chemical and level at which no negative impacts are expected. When the quotient is  $<1$ , no potential health effects are expected from exposure, but when it is  $>1$ , it signifies that there are potential health risks due to exposure (Bermudez *et al.*, 2011). The HQ is calculated as a fraction of determined dose to the reference dose as shown in the following equation:

$$HQ = ADI/R_fD$$

Where ADI is the average food intake per day (mg/person/day) and  $R_fD$  is the oral reference dose of the metal (mg/kg/day).  $R_fD$  is an approximates of daily tolerable exposure to which a person is expected to have without any significant risk of harmful effects during a lifespan.  $R_fD$  for As – 0.0003 (US EPA, 2007); Pb – 0.0035, Cu – 0.04, Ni – 0.02, Cr – 0.003, Cd- 0.0005, Zn – 0.3 (WHO, 2013); Co – 0.03 (Finley *et al.*, 2012) and Be – 0.0002 (US EPA, 1998) mg/kg/day.

### Hazard Index (HI)

An exposure to more than one pollutant results in additive effects. Thus, hazard index (HI) is a vital index that assesses overall likely impacts that can be posed by exposure to more than one contaminant. When the HI is  $>1$ , this suggests that there are significant health effects from consuming pollutants contained in a foodstuff. The HI is calculated as an arithmetic sum of the hazard quotients for each pollutant as shown in the following equation (Kacholi and Sahu, 2018).

$$HI = \sum_{i=1}^n HQ$$

### Statistical Analysis

The experimental data were subjected to statistical analysis using Analysis of Variance (ANOVA) to determine the significance of differences among treatment means. Although the study is observational, inferential statistics were used to explore variability among sample groups. ANOVA was applied to explore relative differences among sheds and farms rather than to infer population-level significance, given the limited sample size. Among these farms sample significant differences were observed, Duncan’s Multiple Range Test (DMRT) was employed

to separate the means at the 5% probability level ( $p \leq 0.05$ ). Statistical computations were performed using IBM SPSS Statistics software (version 20). This analytical approach follows the methodology described by Gomez and Gomez (1984), which is widely accepted in agricultural and biological research for evaluating treatment effects. Alphabetic superscripts were assigned to mean values to indicate statistically significant differences, with means sharing the same letter considered not significantly different at the 0.05 level of probability. The statistical analysis was intended to identify potential differences rather than establish causal relationships.

## RESULTS AND DISCUSSION

### Heavy Metals Concentration in Collected Feed Samples

Nickel (Ni), cadmium (Cd) and lead (Pb) are not essential elements for poultry. Main sources of their contaminations are anthropogenic such as chemical industries, incineration of waste, fertilizers, industrial aerosols, mining and metallurgy, battery, combustion of coal and from raw materials of feed such as grains, oilseed meals (Mukherjee et al., 2022; Angon et al., 2024; Islam et al., 2024). As shown in Table 3, the mean concentrations of Ni, Cd and Pb were ranged within 3.95 – 5.66, 0.11 – 0.33 and 0.44 – 2.83  $\text{mgkg}^{-1}$ , respectively. The highly significant concentrations of Ni, Cd and Pb recorded in LL1C3 (5.66  $\text{mgkg}^{-1}$ ), LL1C2 (0.33  $\text{mgkg}^{-1}$ ) and LsC1 (2.83  $\text{mgkg}^{-1}$ ) respectively at  $p \leq 0.05$ . However, none of them crossed their safety limit of 50  $\text{mgkg}^{-1}$  (NRC, 2005) for Ni, 0.5  $\text{mgkg}^{-1}$  (EU, 2002) and 5  $\text{mgkg}^{-1}$  (EU, 2002) presented in Figure 1. According to Korish and Attia (2020), nickel (Ni), cadmium (Cd) and lead (Pb) concentrations in Layer feed (starter to finisher) found within 1.51 – 2.63, 0.055 – 0.061 and 3.02 – 4.14  $\text{mgkg}^{-1}$ , respectively, in Saudi Arabia.

The mean Concentration of chromium (Cr) observed in collected feed samples was within 0.00 – 13.48  $\text{mgkg}^{-1}$ , where almost all samples except LL1C4 and LL1C5 exceeded the maximum permissible limit endorsed by FSA (2024). The highly significant ( $p \leq 0.05$ ) concentration of Cr recorded in LsC1 (Layer starter of Company 1) which was 13.48  $\text{mgkg}^{-1}$  presented in Table 3 and Figure 2. Chromium contamination in poultry feed mainly originates from tannery wastes, contaminated crops irrigated with wastewater, exhaust of toxic smoke of vehicles and industrial emissions depositing Cr onto soils and feed ingredients (Ahmed et al., 2017; Ahmad et al., 2021). In Saudi Arabia, a study observed the amount of chromium in Layer feed samples within the range of 1.51 – 2.63  $\text{mgkg}^{-1}$  (Korish and Attia., 2020). According to Okeke et al. (2015), chromium concentrations in Layer feed were 0.042 – 0.212  $\text{mgkg}^{-1}$ .

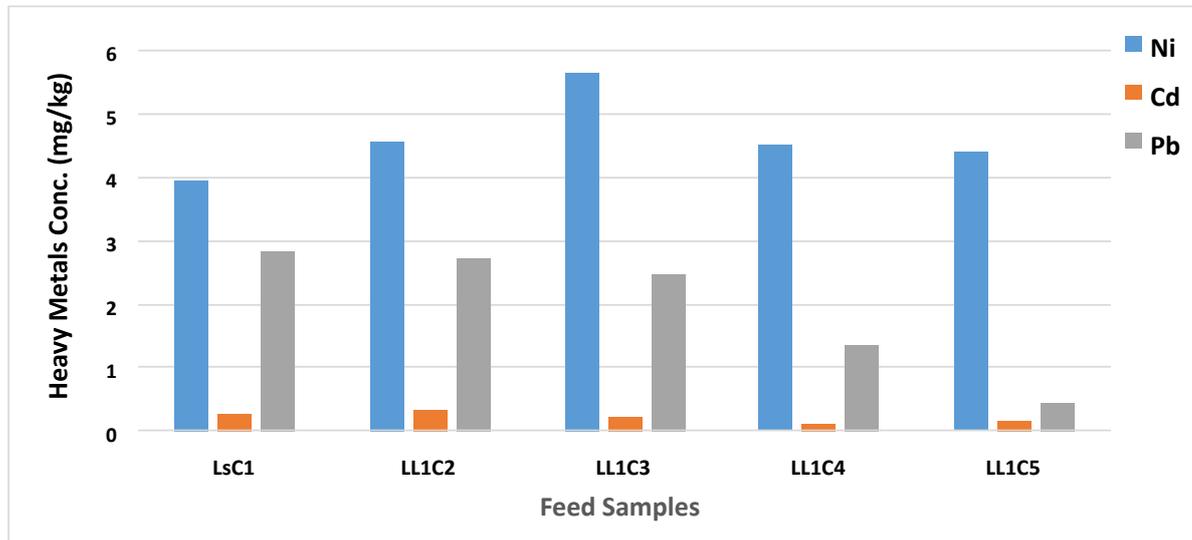
**Table 3: Heavy Metals Concentrations in Collected Poultry Feeds Samples from Different Branded and Non-Branded Company**

Heavy Metals Concentrations in Collected Poultry Feeds ( $\text{mgkg}^{-1}$ )							
Feed Samples	Sample ID	Ni	Cr	Cu	Cd	Pb	Zn
Layer Starter( 0-19 weeks)	LsC1	3.95 a	13.48 c	24.93 a	0.26 ab	2.83 b	64.57 b
Layer Layer 1 ( 19-45 weeks)	LL1C2	4.57 a	12.13 b	5.59 a	0.33 b	2.72 b	28.19 a
Layer Layer 1 ( 19-45 weeks)	LL1C3	5.66 a	11.29 b	21.39 a	0.22 ab	2.49 b	73.12 b
Layer Layer 1 ( 19-45 weeks)	LL1C4	4.51 a	0.00 a	5.83 a	0.11 a	1.37 ab	29.63 a
Layer Layer 1 ( 19-45 weeks)	LL1C5	4.41 a	0.00 a	10.77 a	0.16 ab	0.44 a	43.34 a
<b>Feed MPL</b>		<b>50<sup>1</sup></b>	<b>0.5<sup>2</sup></b>	<b>25<sup>3</sup></b>	<b>0.5<sup>4</sup></b>	<b>5<sup>4</sup></b>	<b>120<sup>3</sup></b>

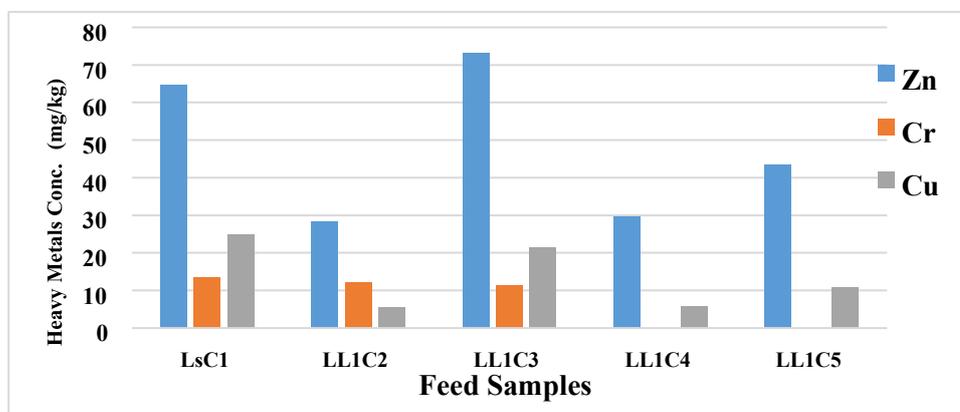
<sup>\*1</sup> National Research Council (NRC, 2005) <sup>\*2</sup> Food Standard Agency (FSA,2024) <sup>\*3</sup> European Regulations 2016/1095 <sup>\*4</sup> EU Directive 2002/32/EC; Ls – Layer Starter, LL1 – Layer Layer 1, C1 – C5 (Company ID)

**N.B.** Mean values followed by the same letter (s) in a column do not differ significantly from each other at 5% level by DMRT

**Figure 1: Comparative levels of Ni, Cd, and Pb (mgkg<sup>-1</sup>) in poultry feed samples (LsC1–LL1C5). The observed variability among feed companies indicates differences in contamination sources and provides a basis for evaluating potential bioaccumulation in poultry and the consequent risk to food safety and public health.**



**Figure 2: Comparative levels of Zn, Cr, and Cu (mgkg<sup>-1</sup>) in poultry feed samples (LsC1–LL1C5). The observed variability among feed companies indicates differences in contamination sources and provides a basis for evaluating potential bioaccumulation in poultry and the consequent risk to food safety and public health.**



Copper (Cu) and zinc (Zn) are used in feeds as the essential elements of chicken growth and bone development (Mamun as al., 2024; Huang et al., 2021). The mean concentrations of copper (Cu) and zinc (Zn) found within 5.59 – 24.93 and 28.19 – 73.12 mgkg<sup>-1</sup>, respectively, as shown in Table 3 and Figure 2.

All the samples were within safe limit of 25 mgkg<sup>-1</sup> and 120 mgkg<sup>-1</sup> set by FSA (2024) and EU (2016) for Cu and Zn, respectively. The significantly ( $p \leq 0.05$ ) highest concentrations in copper recorded in LsC1 (24.93 mgkg<sup>-1</sup>) and in zinc it was found in LL1C3 (73.12 mgkg<sup>-1</sup>). A similar study conducted in Saudi Arabia where the researchers found the Cu and Zn concentrations in Layer feeds were 11.14 – 16.61 and 49.9 – 60.3 mgkg<sup>-1</sup>, respectively (Korish and Attia., 2020).

### Heavy Metal Concentrations in collected Layer Egg Samples

The average concentrations of arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) in samples of Layer chicken eggs ranged within 6.41 – 21.77, 1.20 – 6.33, 0.08 – 1.67, 8.76 – 15.08 and 0.44 – 4.62 mgkg<sup>-1</sup>,

respectively (Table 4). The highest value of As found in Shed 3 of Farm 2 (21.77 mgkg<sup>-1</sup>), was much higher (p≤0.05) than values from other sites and was more than 200 times higher than maximum permissible level (MPL) of 0.1 mgkg<sup>-1</sup> set by JECFA (2005), depicted in Figure 3. The amount of lead (Pb) featured in Figure 4, where in Shed 2 of Farm 1 (6.33 mgkg<sup>-1</sup>) and Shed 3 of Farm 2 (2.05 mgkg<sup>-1</sup>) had significantly highest concentrations of Pb that were much higher (p≤0.05) than those at other locations.

**Table 4: Concentrations (mgkg<sup>-1</sup>) of different heavy metals in Layer chicken egg samples collected from different sampling location**

Heavy metal concentrations in Layer Chicken Egg (mgkg <sup>-1</sup> )							
Location	Farm	sample	As	Pb	Cd	Cr	Ni
Jamsing, Savar, Dhaka	Farm 1	Shed 1	16.20 bc	2.13 a	0.33 a	15.08 a	1.15 a
		Shed 2	18.15 bc	6.33 a	1.67 b	13.59 a	4.62 a
		Shed 3	11.79 ab	3.78 a	0.54 a	8.76 a	3.46 a
Dhulivita, Dhamrai, Dhaka	Farm 2	Shed 1	6.41 a	3.57 a	0.26 a	12.81 a	2.19 a
		Shed 2	18.15 bc	1.20 a	0.08 a	15.04 a	0.44 a
		Shed 3	21.77 c	2.05 a	0.15 a	13.98 a	2.64 a
<b>MPL</b>			<b>0.1<sup>a</sup></b>	<b>0.1<sup>b</sup></b>	<b>0.05<sup>c</sup></b>	<b>0.1<sup>d</sup></b>	<b>0.5<sup>a</sup></b>
Heavy metal concentrations in Layer Chicken Egg (mgkg <sup>-1</sup> )							
Location	Farm	sample	Cu	Zn	Co	Be	
Jamsing, Savar, Dhaka	Farm 1	Shed 1	6.05 a	91.74 a	0.09 a	0.008 ab	
		Shed 2	11.73 b	208.23 b	0.28 a	0.013 b	
		Shed 3	3.90 a	129.9 ab	0.31 a	0.012 b	
Dhulivita, Dhamrai, Dhaka	Farm 2	Shed 1	4.46 a	105.38 a	0.25 a	0.007 ab	
		Shed 2	3.48 a	70.64 a	0.01 a	0.002 a	
		Shed 3	4.62 a	101.24 a	0.11 a	0.006 ab	
<b>MPL</b>			<b>1.0<sup>e</sup></b>	<b>20<sup>e</sup></b>	<b>0.05<sup>f</sup></b>	-	

\*\* a JECFA (2005); b Codex Alimentarius (2009), EFSA (2023); c – FAO/WHO (2015); d - FSANZ. (2008); e – FAO/WHO (2002); f - Codex Alimentarius (2019) \*ND-Not done

**N.B. Mean values followed by the same letter (s) in a column do not differ significantly from each other at 5% level by DMRT**

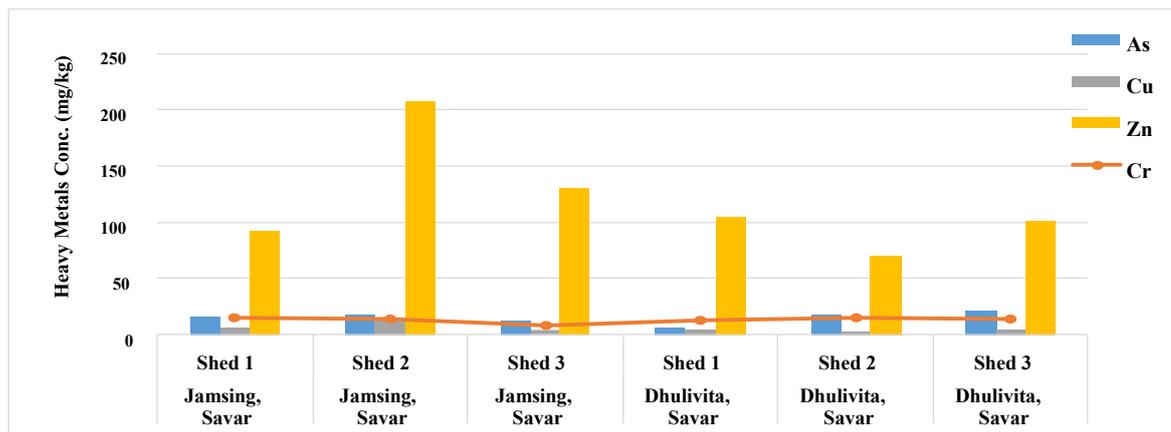
All samples for As were above the MPL of 0.1 mgkg<sup>-1</sup> (Codex, 2009; EFSA, 2023). The highest level of cadmium was in Shed 2 of Farm 1 (1.67 mgkg<sup>-1</sup>), which was more than 30 times higher than the MPL of 0.05 mgkg<sup>-1</sup> endorsed by FAO/WHO (2015). The Food Standards Australia New Zealand (FSANZ, 2008) MPL of 0.1 mgkg<sup>-1</sup> was far lower than the chromium levels at all sites (8.76–15.08 mgkg<sup>-1</sup>). Nickel levels ranged from 0.44 to 4.62 mgkg<sup>-1</sup>, and Shed 2 of Farm 1 had a level of 4.62 mgkg<sup>-1</sup> that was much higher (p≤0.05) than the levels at other sites. The JECFA (2005) MPL of 0.5 mgkg<sup>-1</sup> was exceeded by all nickel values. According to a published study, the average amounts of heavy metals in chicken eggs are as follows: arsenic, lead, cadmium concentrations were

under detection limit, chromium (7.96 – 8.62 mgkg<sup>-1</sup>), and nickel found under detection limit (Korish and Attia, 2020).

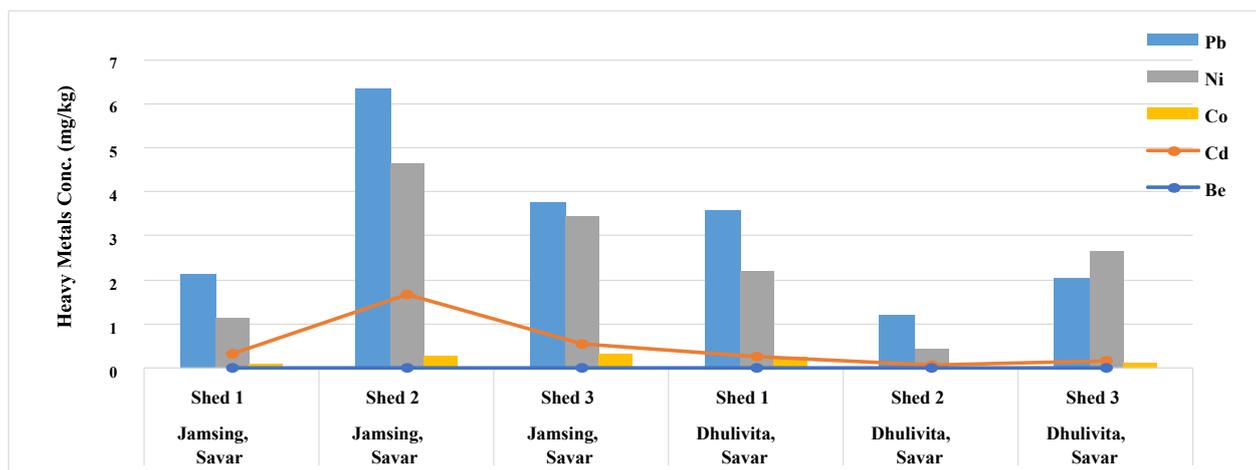
The mean concentrations of copper (Cu), zinc (Zn), cobalt (Co), and beryllium (Be) in the egg samples presented in Table 4. The amount of copper in the samples varied from 3.48 to 11.73 mgkg<sup>-1</sup>. Shed 2 of Farm 1 (11.73 mgkg<sup>-1</sup>) had a lot more copper than the other sites (p≤0.05). The FAO/WHO (2002) maximum permissible level (MPL) for copper is 1.0 mgkg<sup>-1</sup>; however, all the values were 3.5 to 11.7 times higher. The MPL for zinc was 20 mgkg<sup>-1</sup>, but the levels were between 70.64 and 208.23 mgkg<sup>-1</sup>, which is 3.5 to 10.4 times higher and they depicted in Figure 3.

The current assessment represents a deterministic approach and does not incorporate probabilistic modeling or sensitivity analysis. Consequently, the calculated risk values should be interpreted as indicative estimates rather than precise individual exposure predictions. The present study quantified total arsenic concentrations without distinguishing between inorganic and organic arsenic species. This distinction is toxicologically important, as inorganic arsenic is considerably more toxic and carcinogenic than organic forms. The absence of arsenic speciation analysis represents a limitation in accurately characterizing carcinogenic risk. Therefore, the health risk interpretation for arsenic should be viewed cautiously. Future investigations should incorporate speciation analysis to improve risk accuracy.

**Figure 3: Concentrations of As, Cu, Zn, and Cr (mgkg<sup>-1</sup>) in layer chicken egg samples collected from different sheds in Jamsing and Dhulivita, Savar. The figure compares spatial variations in heavy metal levels to identify potential location-based contamination and evaluate their implications for dietary exposure and food safety risk.**



**Figure 4: Concentrations of Pb, Ni, Co, Cd and Be (mgkg<sup>-1</sup>) in layer chicken egg samples collected from different sheds in Jamsing and Dhulivita, Savar. The figure compares spatial variations in heavy metal levels to identify potential location-based contamination and evaluate their implications for dietary exposure and food safety risk.**



The graphical illustration of the mean concentrations of Co and Be shown in Figure 4. Cobalt levels were found to be between 0.01 and 0.31 mgkg<sup>-1</sup>, and all Savar sheds had levels that were up to six times higher than the Codex Alimentarius (2019) MPL of 0.05 mgkg<sup>-1</sup>. There was a small amount of beryllium (0.002 to 0.013 mgkg<sup>-1</sup>) in Layer eggs, but there is no MPL for this element. The high levels of heavy metals in eggs collected from Savar farms are probably due to contamination of the water and feed sources. These higher amounts of arsenic, lead, cadmium, chromium, nickel, copper, zinc, and cobalt could be detrimental to human health.

### Average Daily Intake (ADI) of Layer Chicken Egg

The Average Daily Intake (ADI) values of heavy metals in chicken eggs collected from two different farms and various sheds in Savar, Dhaka, demonstrated variability among the samples (Table 5). Total Arsenic (As) (Organic+Inorganic) levels ranged from 0.035 to 0.121 mg/person/day, notably surpassing the Provisional Maximum Tolerable Daily Intake (PMTDI) of 0.0168 mg/person/day for As in multiple samples, especially Shed 3 of Farm 2 (0.121 mg/person/day). The estimated daily intake was calculated using an average adult egg consumption rate of 22 g/day based on available national dietary data (HIES, 2011). However, actual consumption may vary depending on age, gender, and dietary habits. In the present study, the Average Daily Intake (ADI) of nine metals were calculated by taking average consumption of whole edible part of eggs as 22 g for an adult individual of 60 kg body weight according to “Household Income and Expenditure Survey in Bangladesh” (HIES, 2011), respectively, by considering the mean concentration of each metal in egg samples, corresponding dry weight of samples and average body weight of 60 kg of a person which may not reflect variability among children or high-consumption groups. Therefore, the risk estimates should be interpreted with caution. Elevated arsenic concentrations from egg consumption, which was over the PMTDI in some samples, poses potential health risks given arsenic's recognized toxicity and carcinogenicity. It should be noted that this study measured total arsenic rather than differentiating between inorganic and organic species. Since inorganic arsenic is considered more toxic and carcinogenic, the lack of speciation analysis may influence the accuracy of risk characterization. Future studies should incorporate arsenic speciation for more precise health risk assessment. Heavy metals present in poultry feed may accumulate in tissues and subsequently transfer to eggs through systemic circulation. Chronic exposure to elevated Cd and Pb levels has been associated with renal dysfunction, neurotoxicity, and oxidative stress.

Lead (Pb) concentrations ranged from 0.006 to 0.035 mg/person/day, all below the PMTDI of 0.210 mg/person/day. Cadmium (Cd) levels were low (0.0004 to 0.009 mg/person/day), safely within the PMTDI of 0.046 mg/person/day. Chromium (Cr) values spanned from 0.047 to 0.083 mg/person/day, beneath the PMTDI of 0.200 mg/person/day, and nickel (Ni) ranged from 0.002 to 0.026 mg/person/day, well within the PMTDI of 0.300 mg/person/day.

**Table 5: Average Daily Intake (mg/person/day) of different heavy metals by consumption of Layer chicken eggs collected from different sampling location**

ADI of Heavy Metals by Chicken Egg (mg/person/day)							
Location	Farm	sample	As	Pb	Cd	Cr	Ni
Jamsing, Savar, Dhaka	Farm 1	Shed 1	0.090 c	0.012 b	0.002 b	0.083 d	0.006 b
		Shed 2	0.100 c	0.035 d	0.009 d	0.075 bc	0.026 f
		Shed 3	0.063 b	0.020 c	0.003 c	0.047 a	0.019 e
Dhulivita, Savar, Dhaka	Farm 2	Shed 1	0.035 a	0.019 c	0.001 ab	0.069 b	0.012 c
		Shed 2	0.093 c	0.006 a	0.0004 a	0.077 cd	0.002 a
		Shed 3	0.121 d	0.011 b	0.001 ab	0.078 cd	0.015 d
<b>PMTDI</b>			<b>0.0168<sup>a</sup></b>	<b>0.210<sup>b</sup></b>	<b>0.046<sup>b</sup></b>	<b>0.200<sup>c</sup></b>	<b>0.300<sup>d</sup></b>

ADI of Heavy Metals by Chicken Egg (mg/person/day)						
Location	Farm	sample	Cu	Zn	Co	Be
Jamsing, Savar, Dhaka	Farm 1	Shed 1	0.033 c	0.507 b	0.0005 b	0.00004 c
		Shed 2	0.065 d	1.152 e	0.0015 d	0.00007 d
		Shed 3	0.021 ab	0.698 d	0.0017 e	0.00006 d
Dhulivita, Savar, Dhaka	Farm 2	Shed 1	0.024 ab	0.569 c	0.0014 c	0.00004 b
		Shed 2	0.018 a	0.364 a	0.0001 a	0.00001 a
		Shed 3	0.026 b	0.562 c	0.0006 b	0.00003 b
<b>PMTDI</b>			<b>2.00<sup>e</sup></b>	<b>20.00<sup>e</sup></b>	<b>0.096<sup>f</sup></b>	<b>0.12<sup>g</sup></b>

\*\* a- ANSES (2011), b – JECFA (2003), c – RDA (1989), d – FAO/WHO (2011), e – FAO/WHO (2011), f – EFSA (2012), g – ATSDR (2023)

**N.B. Mean values followed by the same letter (s) in a column do not differ significantly from each other at 5% level by DMRT**

Copper (Cu) levels fluctuated between 0.018 and 0.065 mg/person/day, zinc (Zn) ranged from 0.364 to 1.152 mg/person/day, cobalt (Co) values varied from 0.0001 to 0.0017 mg/person/day, and beryllium (Be) was present in trace amounts between 0.00001 to 0.00007 mg/person/day. All these measured concentrations were significantly below their respective PMTDI values, indicative of no immediate nutritional toxicity concerns. Lead, cadmium, chromium, and nickel levels, while detectable, did not surpass international safety thresholds, suggesting controlled exposure levels in these elements. The low levels of cobalt and beryllium further emphasize limited contamination by these metals, aligning with findings in similar poultry egg studies worldwide. Copper and zinc, essential trace elements, presented in ranges consistent with nutritional supply without risk of overexposure.

### Hazard Quotient (HQ) and Hazard Index (HI) of Layer Chicken Egg

The Hazard Quotient (HQ) values for individual heavy metals in Layer chicken eggs from Jamsing and Dhulivita ranged as follows: arsenic (As) 1.92–6.71, lead (Pb) 0.03–0.17, cadmium (Cd) 0.01–0.31, chromium (Cr) 0.26–0.46, nickel (Ni) 0.002–0.021, copper (Cu) 0.007–0.027, zinc (Zn) 0.020–0.064, cobalt (Co) 0.0000–0.0009, and beryllium (Be) 0.0009–0.0060 (Table 6). All the HQ value of arsenic (As) exceeded 1 and poses high risk from arsenic in each egg samples. None of the individual HQs exceeded 1 except arsenic (As), indicating low risk from single-metal exposure. Hazard prediction models suggest that continued consumption of eggs with elevated HI values (e.g., HI > 1.0) may increase the risk of neurological, renal, and developmental effects, particularly in vulnerable groups such as children and pregnant women (Aendo et al., 2019). In our study, all sheds exceeding HI > 1 clearly indicate the need for targeted interventions to reduce arsenic and chromium inputs, as these metals contributed most to the cumulative risk.

**Table 6: Hazard Quotient (HQ) and Hazard Index (HI) of different heavy metals in Layer flesh against sampling location**

Hazard Quotient of Heavy Metals by Layer Chicken Egg												HI
Location	Farm	sample	As	Pb	Cd	Cr	Ni	Cu	Zn	Co	Be	
Jamsing, Savar, Dhaka	Farm 1	Shed 1	<b>4.98</b>	0.06	0.06	0.46	0.005	0.014	0.028	0.0003	0.0037	<b>5.61</b>
		Shed 2	<b>5.58</b>	0.17	0.31	0.42	0.021	0.027	0.064	0.0009	0.0060	<b>6.59</b>
		Shed 3	<b>3.52</b>	0.10	0.10	0.26	0.015	0.009	0.039	0.0009	0.0054	<b>4.04</b>



Dhulivita, Savar, Dhaka	Farm 2	Shed 1	<b>1.92</b>	0.09	0.05	0.38	0.010	0.010	0.032	0.0008	0.0032	<b>2.50</b>
		Shed 2	<b>5.19</b>	0.03	0.01	0.43	0.002	0.007	0.020	0.0000	0.0009	<b>5.69</b>
		Shed 3	<b>6.71</b>	0.05	0.03	0.43	0.012	0.011	0.031	0.0003	0.0028	<b>7.28</b>

The transfer of metals from feed to eggs was also evident, confirming biological magnification. Egg samples showed detectable residues of arsenic ( $6.41 - 21.77 \text{ mgkg}^{-1}$ ), chromium ( $8.76 - 15.08 \text{ mgkg}^{-1}$ ), nickel ( $0.44 - 4.62 \text{ mgkg}^{-1}$ ), copper ( $3.48 - 11.73 \text{ mgkg}^{-1}$ ), and zinc ( $70.64 - 208.23 \text{ mgkg}^{-1}$ ). The calculated HI for eggs ranged from 2.50 to 7.28, with Savar eggs reaching the highest value (7.28) due to elevated arsenic concentrations. These findings indicate that contaminants are metabolically transferred from feed to eggs, posing chronic exposure risks for regular consumers, particularly vulnerable groups such as children and pregnant women. The concentrations observed in this study were higher than those reported by Samad et al. (2023), potentially reflecting differences in feed composition, environmental contamination levels, or industrial proximity. According to Shahriar et al. (2025) only the concentrations of Pb and Cr exceeded the maximum permissible range established by WHO/FAO and according to the health risk assessment, the metals in the study (except for Pb) did not individually represent a concern by the target hazard quotient and estimated daily intake. Pb and Cr had threshold carcinogenic risks because their CR values ranged from  $10^{-4}$  to  $10^{-6}$  (Shahriar et al., 2025). Conversely, these lower concentrations compared to this study may be attributed to differences in analytical techniques or regional environmental burden. Such discrepancies highlight the importance of localized monitoring rather than direct numerical comparison alone.

Heavy metals present in poultry feed may accumulate in body tissues through gastrointestinal absorption and systemic circulation. These metals can subsequently be transferred into eggs via deposition in the yolk during follicular development. Chronic dietary exposure to cadmium and lead has been associated with nephrotoxicity, neurotoxicity, oxidative stress and developmental disorders. Even when individual metal concentrations remain within permissible limits, cumulative exposure from multiple dietary sources including rice, vegetables and fish commonly consumed in Bangladesh may increase long-term health risks. The findings of this study indicate the presence of measurable heavy metals in poultry feed and eggs from the selected farms. Although most concentrations were within internationally recommended limits, certain values suggest potential exposure concerns that warrant attention. Given the limited sample size and geographic scope, these results should be interpreted cautiously. Nevertheless, the study highlights the importance of routine monitoring and quality control in poultry production systems to safeguard public health. The findings also indicate potential risk in the studied farms and highlight the need for broader surveillance studies.

### Limitations of The Study

The results of this study should be interpreted within the context of some limitations. First, the sample size was relatively small and restricted to two farms, which may limit the representativeness of the findings at regional or national scales. Therefore, the results should be interpreted as preliminary evidence rather than definitive regional estimates. Second, sampling was conducted within a specific time frame and may not capture seasonal variation in contamination levels. Third, although the study provides valuable insight into feed-to-egg metal transfer, a larger multi-site investigation would be necessary to confirm broader exposure trends. Future studies incorporating more farms, larger sample sizes, and stratified sampling approaches are recommended to strengthen generalizability. The health risk assessed under standard exposure assumptions, which might not represent variability in the different groups of consumers. Furthermore, total arsenic was quantified without chemical speciation and this may affect the precision of the risk assessment. Therefore, the findings should be interpreted as preliminary evidence rather than definitive regional estimates. Future studies with larger and more geographically diverse sampling is recommended.

### CONCLUSION

This study provides baseline data on heavy metal contamination in layer chicken feed and eggs from selected farms in Bangladesh. Data observed in this study showed that the contamination of feed by chromium (over the MPL value) and maximum contamination of eggs by all the metals (As, Cr, Pb, Cd, Cu, Zn, Co, Ni and Be). The



feed analysis revealed chromium contamination from tannery waste, which bio-accumulates through the poultry food chain into eggs. Hazard assessment demonstrated that arsenic exceeded safe daily intake thresholds across most samples, with Hazard Index values surpassing 1.0 in six sheds of two farms, indicating cumulative health risks. Vulnerable populations might face particular neurological and developmental concerns from chronic exposure. Urgent interventions are needed to regulate industrial discharge sources, improve water quality management, and establish stricter feed quality standards to mitigate these public health risks. Although most concentrations were within recommended safety limits, measurable levels highlight the importance of routine monitoring. Given the limited sample size and geographic coverage, the findings should be interpreted cautiously. Expanded multi-site studies incorporating larger sample sizes and seasonal variation are recommended to strengthen future risk assessments.

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## Authors Contribution

Afrose Sultana Chamon contributed in conceptualization of the research, provided resources, developed methodology, investigation, helped in funding acquisition, writing-review and editing, validation and overall supervision of the research. M.N. Mondol supervised the research and administrated the project and helped in funding acquisition. Sayada Kowka Batul Jannat Tajnin contributed in formal analysis, investigation and visualization of data as well as wrote the original draft.

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## Conflicts of Interest

The authors declare that there is no conflict of interest.

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