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*Article*

# Assessment of toxic heavy metals and trace elements in poultry feeds, consumer chickens and eggs in Bangladesh

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**Abstract:** Considering the potential health hazardous effects, the distribution and deposition of heavy metals and trace elements including Mn, Fe, Cu, Zn, Cd, Cr, and Pb in different tissues (skin, brain, liver, meat, and bone) of broiler and layer chickens, their feeds, litter, and eggs were analyzed using atomic absorption spectrophotometer (AAS) after nitric/perchloric acid digestion. The samples for this study were taken from four poultry industries located at Sreepur upazila of Gazipur district of Bangladesh. The observed levels of heavy metals and trace element contents in different studied samples were to be in the range of 0.143±0.001 to 7.324±0.004 mg/kg for Mn, below the detection limit (BDL) to 324.43±0.003 mg/kg for Fe, 0.451±0.002 to 4.329±0.005 mg/kg for Cu, 0.014±0.001 to 7.413±0.003 mg/kg for Zn, 0.082±0.002 to 7.806±0.002 mg/kg for Cr, 0.112±0.002 to 1.581±0.002 mg/kg for Cd, and BDL to 34.775±0.004 mg/kg for Pb. Although some elements were found at a lower concentration in feed samples, they were found at a higher level in various parts of the examined chickens, eggs, and litter which may be resulted from other sources such as drinking water, soil, and the surrounding environment of the poultry industries. The brain contained a maximum amount of Cd, Cr, and Pb in comparison to other studied tissues of poultry. Most of the chicken body parts showed an excess level of Cr than the recommended guidelines. The Cu and Zn contents were usual in studied egg samples, whereas Mn, Fe, Cd, Cr, and Pb were found higher than their tolerance limits which are highly alarming for public health and demand regular governance and monitoring.

**Keywords:** heavy metals; trace elements; poultry; feeds; chicken; Bangladesh

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## 1. Introduction

A balanced and healthy diet is indispensable for humans as it provides the energy and nutrients vital for survival and keep the body fit, healthy, and function properly. An ideal diet should comprise of carbohydrates, proteins, fats, vitamins, and minerals. As a source of animal protein with high biological amino acids, low levels of collagen, unsaturated lipids which are primarily found in the skin, vitamins, and mineral contents, poultry meats are considered as valuable and highly nutritious foodstuffs. Due to the rapid growth of population with time, the high demand for protein in the diet is a matter of great concern worldwide, especially in developing countries like Bangladesh as compared to the developed countries. Chicken is a common protein source around the world because of its superior nutritional value and relatively low price compared with red meats, beef, and mutton. In addition, the consumption of poultry meat in substitution of red meat for protein sources may reduce the risk of coronary disease (Hu, 2005). Poultry meat constitutes an important source of non-vegetable protein in Bangladesh, where its poultry industries contributed 28.0% of the total meat production in 2014-2015. Approximately 82 parent stock farms and hatcheries were in operation in 2011 where 55-60 lac day old broiler and 5 lac day old layer chicks per week were produced (DLS, 2013). The chicken egg, the cheapest source of animal protein, has been consumed widely, readily digested, and can provide a significant portion of the nutrients required daily for the proper growth and maintenance of body tissues (Basha *et al*., 2013). Chicken egg contains some metals and minerals naturally where minerals have important biological functions and it is desirable to take them in sufficient amounts for maintaining the biological processes accurately. As the body is not able to synthesize them, the human diet must supply regular amounts of minerals whereas deficiencies may result in developmental abnormalities, reduced productivity, and loss of resistance to diseases (Demirulus, 2013). So poultry meats and eggs play crucial roles in meeting the national demand of nutritional requirement of protein and minerals at low cost for large numbers of individuals. As the demand for poultry meat is increasing in Bangladesh, poultry farm possessors collect one day chicks and rear them with a special type of feed which makes their growth faster and ensures their supply to market within 30 to 40 days for broiler chickens. To meet the increased and uninterrupted demand of poultry feed supply, 70 large-scale and 300 small-scale feed mills are being established and functioning throughout Bangladesh where 65000 MT of broiler feeds and 25000 MT of layer feeds are manufactured every month (Annual report, 2015). Poultry feed contains a small percentage of animal protein which is essential for a balanced diet of chicken to maintain the dietary nutritional demand of protein for their growth and egg production (Islam *et al*., 2007). To meet up these protein demands, the poultry is being contaminated with heavy metals which inadvertently enter into the food chain and pose risks to humans and animals, therefore raising a worldwide concern to food safety issues (Islam *et al*. 2007). The major source of metals in poultry arises from contaminated poultry feeds, drinking water, and the environment (Hossain *et al*. 2014b). An average of 220 metric tons of hides is processed daily in the tannery industries located in Hazaribagh of Bangladesh where 600–1000 kg of solid wastes from each ton of processed hides are released (Zahid *et al*., 2004). Protein is the principal constituent of this waste and the waste is converted to protein concentrate to be used as poultry feed, fish feed, and in the production of organic fertilizers. Among these constituents of animal protein, processed tannery skin cut waste, as well as shaving dust, are highly economical and affordable (ATSDR, 2012). To manufacture one kg of feed, only half the amount of skin-cut meat bone of tannery wastes is required in comparison to the imported protein (Mazumder *et al*., 2013). Tannery waste obtains a large amount of toxic metals when the hides and skins are treated with different chemicals and salts in various stages of the tanning process (Hossain *et al*. 2007; Mazumder *et al*. 2013). About 40 heavy metals and various acids are applied during the processing of raw hides (UNIDO, 2005). Besides, nearly 80% of tanneries of Bangladesh practice chrome tanning procedures whereas chromium has been found in percentage level, other heavy metals are at parts per million (ppm) levels (Basha *et al*., 2013). Hence, the use of the hazardous tannery wastes in poultry feed production as protein source without appropriate treatment would contaminate the feed and the toxic elements can be deposited in different body parts of poultry and their eggs which subsequently be transported and bio-accumulated to the human body and pose serious health hazards (Hossain *et al*., 2007; Mariam *et al*., 2004). The excess level of toxic metals in the chicken litter is also a great concern as poultry litter can be used for crops as fertilizer (Hossain *et al*., 2014a).

Non-essential toxic elements such as lead (Pb) and cadmium (Cd) can be harmful even at low concentrations. They can cause profound biochemical and neurological changes in the body when ingested over a long time (Chowdhury *et al*., 2011b). Because of their oxidative and carcinogenic potential, these are considered critical contaminants with proven hazardous nature that should be omitted in food for human consumption. Scientists observed that metals can act as carcinogens through oxidative mechanisms, generating free radicals and reactive oxygen species (ROS), which attack and damage DNA as well as many important enzymatic proteins (Bal, 2002). Heavy metals may also interrupt metabolic functions in the biological system through accumulation in the vital organs where they cause dislocation of the important minerals from their original place and thereby hamper their biological function. Pb exposure is associated with pathological changes in organs and the central nervous system, hence may cause seizures, mental retardation, and behavioral disorders. Lead ingested by chicken is deposited in bones, soft tissues, and eggs, so consumption of contaminated poultry items and eggs causes a potential public health hazard especially to children (Trampel *et al*., 2003). Absorption of ingested Cd may be toxic to virtually every system in the body which may induce kidney dysfunction, skeletal damage, prostate cancer, mutations, and reproduction deficiencies (Abou-Arab, 2001; Uluozlu *et al*., 2008). Some essential trace elements including iron (Fe), copper (Cu), zinc (Zn), chromium (Cr), and manganese (Mn) are essential for living beings where they play a variety of biochemical and physiological functions to maintain good health throughout life. These vital minerals usually work by binding with organic molecules where they function mainly as a catalyst to induce or enhance enzymatic activities (Regan, 1993). In addition to their essentiality for human nutrition, these can also produce toxic effects when ingested and reaching a certain level (Rahman *et al*., 2014). Considering the probable serious health effects of essential and non-essential elements on human health, this study was designed to estimate the level of trace elements such as Mn, Fe, Cu, Zn, Cr, and heavy metals including Cd and Pb in different branded poultry feeds, different parts of the chicken (skin, bone, meat, liver, and brain), poultry eggs, and poultry litter.

## 2. Methods

### 2.1. Study area

The study area was Sreepur Upazila of Gazipur district in Bangladesh. The total farm including broiler and layer is near about 114763 in Bangladesh. Among them, around 56% is located in the Dhaka district. In the Dhaka district approximately 50% of poultry is produced in the Sreepur Upazila (Bangladesh national portal, 2015). The sampled area is 465.24 sq km, located in between 24°01' and 24°21' north latitudes and in between 90°18' and 90°33' east longitudes.

### 2.2. Sample collection and preservation

Four different types of samples were collected from four poultry industries (labelled as Ind-Kz, Ind-Pln, Ind-Ajn, and Ind-RRP). The studied samples were feed consumed by the chickens, broiler chickens from Ind-Kz and Ind-RRP, layer chickens from Ind-Pln and Ind-Ajn, eggs of the sampled layer chickens, and litter of the sampled chickens. Table 1 presents the selected chicken breed, age, locality along with the Global positioning system (GPS) location.

#### 2.2.1. Feed sample

Around 50 gm of the layer-layer (Ind-Ajn), grower (Ind-RRP), layer starter (Ind-Kz), and pre-layer (Ind-Pln) feed samples which were consumed by the chickens from the respective farms were collected directly by opening the feed sacks using a sterile hook in sterile plastic bags, labeled and transferred to the Agrochemical and Environmental Research Division (AERD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka and stored in a dark cool and dry place.

#### 2.2.2. Chicken sample

Three layers of two ISA brown and one ISA white chicken from Ind-Pln and Ind-Ajn, and three broilers of two ISA brown and one ISA white chicken from Ind-Kz and Ind-RRP were selected. After returning from the farm area to the AERD laboratory, the chickens were slaughtered with a sterile stainless steel knife, and the targeted 5 body parts including brain, skin, liver, meat, and bone were collected. Then the samples were properly labeled and stored in separate sterile plastic bags below -20ºC up to sample preparation in the laboratory.

#### 2.2.3. Egg and litter sample

Eggs of layer chickens from Ind-Ajn and Ind-Pln feed industries were selected. To collect eggs and litter samples, the selected chickens were separated previously in a cage for one week. The eggs were washed, water-soaked, cleaned, labeled, and stored in sterile plastic bags in the AERD laboratory. About 20gm of litter from the separated chicken cage were collected from each of the farms in sterile plastic bags, airtight, labeled, and stored in a cooler box at about 4ºC.

### 2.3. Laboratory preparation of samples

The selected body parts of the chicken were cleaned and washed with distilled water to remove clotting blood, debris, and fat portions. Then they were air-dried and chopped into small chunks with a sterile stainless steel knife and thawed with a pre-cleaned glass pestle. Egg white, yolk, and eggshell were separated carefully in a sterile pyrex petri dish. After that, the studied samples (chicken body parts, eggs, litter, and feed) were placed in a continuous oven (OP100, LTE Scientific Ltd, Greenfield, Oldham, Great Britain) at 85ºC for 48 hours to obtain constant weight. After oven-dry, samples were ground in a glass mortar, kept in an airtight polythene bag, labeled, and stored in a cool and dry place.

### 2.4. Acid digestion of the studied samples

All the powdered chicken tissues, eggs, feeds, and litter samples were prepared by the wet digestion method. 1g of each sample was weighed in the crucible by Analytical balance (CRYSTAL 200). 10 ml of 65% nitric acid (Merck, Germany) and 70% perchloric acid (Merck, Germany) mixture in a ratio of 3:1 were added to each sample. The samples were then heated to 150°C for 15-20 minutes on a hotplate until near dryness. It was then cooled down for 5-10 minutes and diluted with 50 mL of distilled water followed by filtration with Whatman filter paper No. 1 and stored in LDPE 50 ml plastic bottles. Figure 1 summarizes the processes of preparing of different samples before AAS analysis.

### 2.5. Analysis of samples

The extract of the samples was analyzed for the studied metals by Atomic Absorption Flame Spectrophotometer (AA-6300, Shimadzu, Japan). Each standard solution was measured three times and the mean was plotted. A blank solution of distilled water was used to check the accuracy of the standard solutions and it was run after every 10 samples. For sample analysis, an aliquot of the digested samples was injected into the air acetylene flame for Pb, Cd, Zn, Cr, Cu, Mn, and Fe using a Shimadzu auto sampler ASC-6300. The instrument setting and operational conditions were maintained according to the manufacturers' specifications. The operating conditions for AAS are summarized in Table 2.

### 2.6. Statistical analysis

All analysis was performed in triplicate. Results were expressed as mean±SD (Standard Deviation). The whole data were subjected to a statistical analysis using the statistical software, Statistical Package for Social Sciences (SPSS) version 16. The standard graphs were drawn with MS Excel 2007.

## 3. Results and Discussion

In this study, seven different elements (Fe, Cr, Cu, Mn, Zn, Cd, and Pb) were measured in selected poultry feeds, different body parts (liver, meat, brain, bone, and skin) of studied broiler and layer chickens, their litter, and egg samples.

### 3.1. The concentration of heavy metals and trace elements in feed samples

The average concentration (±SD) of Mn, Fe, Cu, Zn, Cd, Cr, and Pb in the analyzed feed samples collected from different companies in Sreepur Upazila are presented in Table 3. The obtained results showed that the range was 1.773±0.002-3.021±0.003 mg/kg, 16.477±0.001-108.392±0.002 mg/kg, 0.554±0.003-1.317±0.002 mg/kg, 0.014±0.001- 2.223±0.002 mg/kg, 0.312±0.001-1.839±0.001 mg/kg, 0.112±0.002-0.471±0.003 mg/kg, and BDL-9.178±0.005 mg/kg for Mn, Fe, Cu, Zn, Cr, Cd, and Pb respectively. The maximum level of Mn was detected in Layer Layer samples of Ind-Ajn feed industry and the lowest was found in the Layer Starter feed of Ind-Kz farm industry. All the studied feed samples contained Mn at a concentration lower than the recommended value . The concentration detected for all the feed samples was found lower than those obtained by many previous reports (Alkhalaf*et al*., 2010; Rahman *et al*., 2014; Okoye*et al*., 2011b; Korish and Attia, 2020) and higher than the studies investigated (Imran *et al*., 2014; Suleiman *et al*., 2015). Among the four different studied samples, layer feeds of Ind-Ajn farm was also identified with the highest amount of Fe content (108.392±0.002 mg/kg) which was slightly higher than the mean maximum value (102.0175 mg/kg) obtained by Islam *et al*., 2017 and 91.86±8.98 mg/kg reported by Imran *et al*., 2014, Rahman *et al*., 2014 and Korish *et al*., 2020 recorded the Fe level in the poultry feed samples in the ranges from 1026.67±8.15-3053.33±18.24 mg/kg and 99.4-142.9 mg/kg, respectively. The obtained Fe level in all the feed samples of the present study was within the maximum acceptable concentration of 500 mg/kg for Fe in feed which was proposed by the National Research Council (NRC), 2005. The Cu content in the feed from different Industries is found to reduce from 1.317±0.002 to 0.554±0.003 mg/kg where pre-layer feed samples from Ind-Pln showed the maximum Cu concentration and layer starter from Ind-Kz farm had the minimum level. The mean concentration of Cu in the feed from another study of Gazipur was found at 1.75 mg/kg which was slightly higher than the present studied samples (Mottalib *et al*., 2016). The Cu concentration in the feed samples was found very lower than that obtained by different authors (Rahman *et al*., 2014; Imran *et al*., 2014; Korish and Attia, 2020; Okoye *et al*., 2011b). Alkhalaf *et al*., 2010 noted that in almost all the studied samples, the level of Cu was higher than the present findings except layer feed containing 0.56±0.18 mg/kg of Cu which is lower than the Cu level of Ind-Ajn layer-layer feed of 1.307±0.002 mg/kg. From Table 3, it appeared that the highest concentration of Zn in feed was found in grower and the lowest was found in layer starter. Zn was found lower than that obtained by the investigated feed samples of many previous studies (Islam *et al*., 2017; Rahman *et al*., 2014; Imran *et al*., 2014; Korish and Attia, 2020). European Union (EU), 2003 has set the maximum acceptable concentration of 100 mg/kg for Cu and 500 mg/kg for Zn in feed which is far higher than our present analysis. From the analyzed feed samples of four different Industries, the range (0.312±0.001-1.839±0.001 mg/kg) for Cr was observed lower than that obtained by prior reports (Imran *et al*., 2014; Mottalib *et al*., 2016; Alkhalaf *et al*., 2010; Jothi *et al*; 2016) and the highest concentration of Cr was identified in Layer Layer and the lowest was in Grower. Cr concentration was found to be much lower than the permissible limit set by NRC, 2005. The mean(±SD) concentration of Cr in feed samples from Gazipur Sadar, Gazipur and Sreepur, Gazipur were 1.71(±0.24) µg/g, 1.64(±0.61)µg/g, respectively (Islam *et al*., 2016).

The Cd concentration in studied feed samples of Ind-RRP and Ind-Pln was 0.471±0.003 mg/kg and 0.463±0.002 mg/kg respectively which showed quite similar value obtained by Imran *et al*., 2014 (0.44±0.31 mg/kg) and from another study on poultry feeds collected from Gazipur which was done by Mottalib *et al*., 2016 (0.45 mg/kg). It was also observed that the highest level of Cd was found in Grower and the lowest was found in Layer Starter feeds in this study. However, Cd concentration found in the present examination is lower than (3.8-33.6 mg/kg) and (3.33±0.05-16.67±0.58 mg/kg) reported by Mahesar *et al*., 2010 and Rahman *et al*., 2014 respectively but higher than the feed samples of both broiler (0.03 mg/kg) and deshi poultry feeds (BDL) analyzed by Bari *et al*., 2015. The mean concentration of Cd (0.176±0.25mg/kg) from the Grower feed of Okoye *et al*., 2011 showed a quite similar value to the current examined layer feeds Cd level (0.172±0.001 mg/kg). The highest Cd level in feeds in Hubei Province, China was 1.200 mg/kg which was quite higher than the study done on Sreepur Upazila (Tao *et al*., 2020). The Cd concentration in feed samples was higher than the reported starter, grower, and layer feed samples, obtained by Korish *et al*., 2020. All the feed samples contained the Cd value within the maximum acceptable concentration of 1 mg/kg proposed by EU. A grower from Ind-RRP and pre-layer feed from Ind-Pln did not contain any Pb content which was quite similar to the study reported (Rahman *et al*., 2014) whereas both the starter feed from Ind-Kz farm and the layer feed from Ind-Ajn was found with a level of 5.492±0.003 mg/kg and 9.178±0.005 mg/kg, respectively. The Pb concentration in broiler and deshi poultry feeds was 10.32 mg/kg and 10.27 mg/kg which is higher than the detected Pb content of the present study (Bari *et al*., 2015). The Pb content in collected poultry feed from Gazipur was 14.36 mg/kg which is also higher than the present work (Mottalib *et al*., 2016). The lead concentration from Ind-Kz and Ind-Ajn feed samples was higher than that obtained by other studies (Okoye *et al*., 2011b; Alkhalaf *et al*., 2010) as well as the limit (5 mg/kg for Pb in feed) given by EU.

### 3.2. The concentration of heavy metals and trace elements in chicken samples

The concentrations of Mn, Fe, Cu, Zn, Cd, Cr, and Pb in the studied samples were determined and the mean±SD values for the analyzed samples are listed in Table 4 for chicken body parts and litter samples in comparison to those from other areas.

**Manganese (Mn):** Mn is an essential trace mineral that is required for many biological processes such as carbohydrate metabolism (Costa, 2000). In the present work, it was revealed that the average concentration of Mn in five different chicken body parts from different farms of Sreepur Upazila showed the descending order of brain>skin>meat>bone>liver. In those selected samples of both layer and broiler chickens, the level of Mn was found in the range of 0.869±0.001-1.886±0.003 mg/kg, 0.812±0.002-5.159±0.003 mg/kg, 0.143±0.001-1.684±0.003 mg/kg, 0.163±0.003-1.548±0.002 mg/kg, and 0.530±0.002-1.395±0.001 mg/kg, in skin, brain, liver, meat, and bone respectively. It was noticed that the concentration of Mn was detected higher in almost all the samples collected from layer chickens than the broiler samples. The level of Mn in the liver (3.58±0.03-3.88±0.04 mg/kg), meat (2.57±0.03-3.33±0.04 mg/kg), and bone (3.15±0.03-3.16±0.02 mg/kg) of poultry was found higher in a previously reported study than the current studied data (Rahman *et al*., 2014). Alturiqi *et al*., 2012 found a very high level of Mn (21.48-34.42 mg/kg) in chicken meat samples whereas a lower amount of Mn was observed in the report presented by Uluozlu *et al*., 2008. Uluozlu *et al*., 2008 also recorded a low Mn level (0.14 ± 0.011 mg/kg) in poultry skin but a high level of Mn (2.51±0.178 mg/kg) in the liver compared to our present data. In liver samples of poultry, Ghimpețeanu *et al*., 2014 detected the range (0.74 to 2.92 mg/kg) of Mn which is higher than the concentration obtained in layer chicken liver but lower than broiler samples.

Most of the chicken samples exceeded the permitted concentration of Mn (0.5 mg/kg) which indicates that the chickens are not safe for consumption.

The range of Mn content was 5.290±0.003 mg/kg to 7.324±0.004 mg/kg in investigated litter samples while the highest concentration was found in broiler chicken and the lowest was from the layer chicken. Korish *et al*., 2020 showed that both broiler and layer litter samples had high levels of Mn content ranges (263.2-297.7 ppm) and (122.3-141.1 ppm) than this current study.

**Iron (Fe):** Fe is an essential mineral for the growth and development of living organisms. It is well established that sufficient Fe intake in the diet is highly essential for decreasing the incidence of anemia (Ghaedi *et al*., 2006). Fe helps the red blood cells to transport oxygen from the lungs to the rest of the body. In this present research work, the level of Fe was analyzed in different body parts of poultry and the concentration was found following the order of bone>brain>skin>liver>meat. The concentration of Fe was detected in the range of 14.560±0.002-51.320±0.004 mg/kg, 22.317±0.003-324.437±0.003 mg/kg, and BDL-24.978±0.004 mg/kg in the liver, bone, and meat, respectively which were very lower than a study of Rahman *et al*., 2014. The liver contained a low level of Fe than the literature values reported (Uluozlu *et al*., 2008; Korish and Attia, 2020) but had a quite similar value of (14.11 to 54.65 mg/kg) reported by Ghimpețeanu *et al*., 2014. In the current work, the analyzed skin samples of both broiler and layer were identified in the range of 13.528±0.002 to 128.898±0.003 mg/kg. The highest concentration was detected in layer chicken of Ind-Ajn farm which was quite higher than the level of Fe identified in the skin of poultry samples in Uluozlu *et al*., 2008. The obtained results for chicken muscles in the current study were far lower than those obtained by different authors (Iwegbue *et al*., 2008; Alturiqi and Albedair, 2012) but higher than the report conducted by Uluozlu *et al*., 2008. In brain tissue samples, the level of Fe was detected from 39.383±0.003 to 288.690±0.004 mg/kg. Furthermore, comparing with the maximum acceptable concentration of 150 mg/kg for Fe in chicken, both layer and broiler chicken tissues were found Fe at permissible concentration with few exceptions (Ashraf *et al*., 2016).

In litter samples, the Fe was found in the ranges from 100.581±0.003 to 185.241±0.001 mg/kg where layer from Ind-Ajn showed high Fe value and lowest was in the broiler chicken of Ind-Kz farm. Korish *et al*., 2020 found the Fe content in the range of (536.4-581.9 ppm) and (617.4-719.1 ppm) in broiler and layer litter samples respectively which was much higher than this study.

**Copper (Cu):** Copper is an essential micronutrient required for the proper functioning of different oxidative and reductive enzymes where Cu acts as a cofactor. Although Cu is an essential nutritional element, a high intake of this trace element can cause several health complications such as liver and kidney damage (Chowdhury *et al*., 2011a). The current results of this study revealed that the deposition of Cu in brain samples was higher than the other body parts of poultry and the range was between 1.197±0.001 and 4.329±0.005 mg/kg where the minimum level of Cu was identified in bone samples in the ranges from 0.522±0.002 to 1.183±0.002 mg/kg. Mottalib *et al*., 2016 found the average Cu concentration in chicken bone 18.74 mg/kg which was extremely higher than the Cu concentration found in chicken bone samples of the present work. From the analyzed data, it was observed that most of the parts of broiler chicken had a higher level of Cu than layer chicken samples. In the analysis of skin and liver samples, the Cu concentration was obtained in the range of 0.683±0.003-1.636±0.002 mg/kg and 1.047±0.003-1.626±0.001 mg/kg, respectively which was far lower than the skin and liver of poultry samples analyzed by Uluozlu *et al*., 2008. The meat samples contained Cu in the range of 0.965±0.002 to 1.568±0.001 mg/kg which was lower than the previous results (Alturiqi and Albedair, 2012; Islam *et al*., 2015; Rahman *et al*., 2014). Mottalib *et al*., 2018 identified Cu in the liver of both broiler and layer with a mean value of 4.092 mg/kg and 3.039 mg/kg, respectively which was far higher than the liver samples analyzed in both broiler and layer chicken of our study. Both the broiler and layer poultry samples contained an excess level of Cu than the assigned limit of 1 mg/kg.

The range of Cu in litter samples was 0.856±0.003-1.713±0.004 mg/kg and 1.048±0.002-1.511±0.006 mg/kg for broiler and layer, respectively. Korish *et al*., 2020 showed that both broiler and layer litter had a very high level of Cu content (23.3-28.5 ppm) and (27.8-36.6 ppm) respectively in comparison to this present studied data.

**Zinc (Zn):** Concentrations of Zn varied in different parts of studied poultry tissues and the maximum amount of Zn accumulation was recorded in bone (2.775±0.003-3.908±0.002 mg/kg) and the lowest amount was observed in meat (1.255±0.001-2.284±0.002 mg/kg). The obtained results from poultry meat samples were lower than that obtained by Iwegbue *et al*., 2008 (6.12-33.21 mg/kg), Alturiqi *et al*., 2012 (27.93-36.93 mg/kg), and lower than Rahman *et al*., 2014 (26.67±0.25-28.67±0.34 mg/kg. All the values in the studied samples were below the permissible limit (150 mg/kg) set by the Australia New Zealand Food Authority (ANZFA) (Okoye *et al*., 2011a). The mean concentration of Zn in different studied poultry samples was far lower than the maximum acceptable concentration of Zn indicating the safe consumption of the poultry items considering the level of Zn. The highest level of Zn in skin and brain samples among the studied samples was noticed in broiler samples collected from Ind-Kz farm industries. The examined poultry skin and liver showed the Zn concentration in the range of 0.474±0.001 to 7.41±0.003 mg/kg and 1.319±0.002 to 3.117±0.002 mg/kg respectively whereas Uluozlu *et al*., 2008 detected the level of Zn in the skin at 7.1 ± 0.6 mg/kg and in liver at 22.5 ± 2.1 mg/kg. Ghimpețeanu *et al*., 2014 found the Zn concentration in poultry liver in the range of 4.37 to 17.86 mg/kg.

**Chromium (Cr):** Regarding Cr accumulation in chicken organs, the average concentration of the analyzed Cr was found to follow the order of brain>skin>liver>meat>bone. The brain had the maximum level of Cr ranges from 1.594±0.003 to 7.806±0.002 mg/kg and the analyzed bone samples had a minimum level (0.365±0.004-1.151±0.002 mg/kg). From Table 4, it was noticed that most of the parts of Ind-Kz broiler chicken have a higher level of Cr than other poultry samples analyzed. Poultry meat samples contained Cr levels in the range of 0.378±0.002 to 2.382±0.001 mg/kg. The mean±SD concentration of Cr in broiler meat samples from Gazipur Sadar and Sreepur of Gazipur district was 1.139(±0.26) µg/g, and 1.31(±0.20) µg/g, respectively (Islam *et al*., 2016). The range of Cr in studied skin, liver, and the meat was 0.875±0.002-6.568±0.001 mg/kg, 0.991±0.003-2.175±0.002 mg/kg, and 0.378±0.002-2.382±0.001 mg/kg, respectively which was far higher than the study reported (Uluozlu *et al*., 2008). Islam *et al*., 2015 obtained a similar amount of Cr (1.4 mg/kg) which was found in the present analyzed layer meat samples of the Ind-Pln (1.417±0.004 mg/kg) and Ind-Ajn (1.498±0.004 mg/kg) farms. Mottalib *et al*., 2020 found the average Cr concentration in meat samples of both the broiler and layer chickens at 3.976 mg/kg and 2.401 mg/kg, respectively, higher than the current analyzed report. The concentration of Cr in most of the analyzed edible tissues was much higher than the maximum permissible level of 1 mg/kg might pose risk to the consumers (Choi, 2011).
The Cr content in litter samples was ranged from 0.083±0.002 to 2.731±0.002 mg/kg. Korish *et al*., 2020 obtained a very high level of Cr in both broiler (1.74-17.1 ppm) and layer (10.9-15.2 ppm) litter.

**Cadmium (Cd):** Exposure of non-essential Cd has been implicated in several human pathologies. The Cd concentration in investigated poultry body parts showed that Cd was mostly accumulated in the brain than the other studied edible parts of chicken (skin, liver, meat, and bone). Rahman *et al*., 2014 found the Cd concentration in the bone, liver, and meat at the range of 2.67±0.04-2.67±0.04 mg/kg, 4.25±0.04-4.33±0.03 mg/kg, and 3.32±0.03-3.33±0.05 mg/kg, respectively which were quite higher than the present studied levels (Table 4). The Cd level in meat samples was detected 0.249±0.003 to 0.404±0003 mg/kg which was higher than 0.030 mg/kg (Islam *et al*., 2015) but lower than many other studies contained (1.36-1.68 mg/kg) (Alturiqi and Albedair, 2012), 6.09±0.49 mg/kg (Uluozlu *et al*., 2008), and the maximum Cd content (1.27 mg/kg) detected by Iwegbue *et al*., 2008. Broiler from Ind-Kz farm showed a similar amount of Cd (0.243 mg/kg) in broiler samples obtained from Mottalib *et al*., 2018. The layer breast muscle samples showed the Cd value (0.166 mg/kg) which is lower than both the layer muscle samples (Ind-Pln and Ind-Ajn) while the case was different for liver samples (0.480 mg/kg) which was higher than the poultry layer liver samples (Mottalib *et al*., 2018). The detected Cd level in studied liver samples was lower than the range (2.24±0.20 mg/kg) detected by Uluozlu *et al*., 2008. The permissible limit for Cd in chicken is 0.5ppm . Both layer and broiler chicken tissues were found within the proposed limit except brain tissues of both layer and broiler chicken.
In litter, the Cd ranges from 0.172±0.001 to 0.499±0.003 mg/kg whereas the maximum Cd was found in broiler sample collected from Ind-RRP farm. A study done by Korish and Attia, 2020 showed that broiler litter had a low level of Cd (0.137-0.172 ppm) than this current study.

**Lead (Pb):** Lead is a nonessential toxic metal that is of direct concern to human and livestock health as it may accumulate in the body, particularly in the kidney, liver, and to a lesser extent in the muscle and causes adverse health effects such as neurotoxicity and nephrotoxicity (García-Lestón *et al*., 2010;Li *et al*., 2005). From the study, it was observed that all of the analyzed samples from Ind-RRP farm did not contain any Pb residue which is similar to the study reported by Rahman *et al*., 2014. The detected Pb content is mostly accumulated in brain tissues at a level of 34.775±0.004 mg/kg, 15.4826±0.003 mg/kg, and 11.232±.005 mg/kg in Ind-Kz, Ind-Pln, and Ind-Ajn poultry samples, respectively. The detected Pb content in meat samples was lower than (7.61-10.49 mg/kg) (Alturiqi and Albedair, 2012), but higher than (0.17 mg/kg) (Islam *et al*., 2015). The maximum limit of Pb content for meat and offal is 0.1 mg/kg and 0.5 mg/kg, respectively . Although most poultry samples did not show any Pb, the detected samples with Pb were higher than the tolerable limit.

### 3.3. The concentration of heavy metals and trace elements in eggs

The concentration of selected trace elements (Mn, Fe, Zn, Cu, and Cr) and heavy metals (Pb and Cd) in poultry eggs are depicted in Figure 2. The level of Mn was detected in the range of 0.936±0.004-1.425±0.002 mg/kg, 1.605±0.003-1.823±0.003 mg/kg, and 1.603±0.003-1.728±0.003 mg/kg in eggshell, egg yolk, and egg white, respectively where the studied egg white and yolk contained a higher amount of Mn than the reports recorded by Uluozlu *et al*., 2008) and Korish and Attia, 2020) but lower than Rahman *et al*., 2014. All the portions of eggs exceeded the maximum acceptable concentration of 0.5 mg/kg for Mn in eggs. In the case of Fe analysis in the studied egg contents, it was observed that egg yolk (24.737±0.002-43.067±0.001 mg/kg) had the highest amount of Fe followed by egg white (7.737±0.002-28.622±0.003 mg/kg) and eggshell (8.422±0.001-18.048±0.003 mg/kg). Chowdhury *et al*., 2011b and Uluozlu *et al*., 2008 also found that the Fe level was higher in the egg yolk portion than the egg white. Rahman *et al*., 2014 found a very high level of Fe content in the studied egg samples (egg white: 416.67±12.15-433.33±10.23 mg/kg; egg yolk: 106.67±21.25-180.45±14.21 mg/kg) than the present analyzed data. The tolerable limit is 15 mg/kg for Fe in eggs and most of the samples were found at a higher level than the acceptable concentration. The Cu concentration in egg shell (0.704±0.003 to 1.247±0.003 mg/kg), yolk (1.293±0.004 to 1.334±0.003 mg/kg), and egg white (1.268±0.002 to 1.276±0.005 mg/kg) were found within the maximum acceptable concentration of 10 mg/kg for Cu in eggs . The permissible Zn content in poultry eggs is reported 20 mg/kg (Zmudzki and Szkoda, 1996) and our current research found that the level of Zn in all the egg samples (eggshell: 0.231±0.003-0.614±0.002 mg/kg, egg yolk: 1.532±0.004-1.617±0.002 mg/kg, egg white: 0.607±0.002-0.723±0.003 mg/kg) was within the recommended limit. The obtained results for both Cu and Zn were higher than that obtained by Chowdhury *et al*., 2011b, Uluozlu *et al*., 2008 and lower than different studies (Rahman *et al*., 2014;Korish and Attia, 2020;Islam *et al*., 2015). The studied egg samples had exceeded the safe limit for Cr (1 mg/kg) . The Cr concentration in eggshell, egg yolk, and egg white were ranged from 0.995±0.002 to 1.157±0.001 mg/kg, 1.469±0.003 to 2.431±0.002 mg/kg, and 1.228±0.003 to 2.520±0.002 mg/kg, respectively which was higher than the reports that obtained by Uluozlu *et al*., 2008; Chowdhury *et al*., 2011b and Islam *et al*., 2015 but lower than Korish and Attia, 2020.

The Cd concentration was found in the maximum level in egg yolk samples where the layer chickens collected from Ind-Pln and Ind-Ajn contained the Cd value at 0.3858±0.003 mg/kg and 0.2736±0.002 mg/kg, respectively. The range of Cd in egg white and egg yolk was detected higher than that obtained by studied poultry farm eggs of Chowdhury *et al*., 2011b; Korish and Attia, 2020; Islam *et al*., 2015; Uluozlu *et al*., 2008 but far lower than that obtained by Rahman *et al*., 2014. Comparing with the maximum acceptable concentration of 0.05 mg/kg for Cd in eggs, all the samples were found at an exceeding level which might be a great concern for human health. The non-essential toxic Pb level was determined in egg contents (egg yolk: 0.285±0.001-7.037±0.002 mg/kg, egg white: 2.673±0.002-5.610±0.001 mg/kg, eggshell: BDL-3.478±0.001 mg/kg.). However, most samples exceeded the maximum acceptable concentration of 0.5 mg/kg of Pb in eggs (Zmudzki and Szkoda, 1996).

**Table 1. Studied chicken breed, bird age, locality and GPS location.**

| Feed Industry | Bird Type | Bird Age | Locality | GPS Location |
|---|---|---|---|---|
| Ind-Pln | Layer | 90 weeks | Sreepur | N- 24 11 45<br>E- 90 27 48 |
| Ind-AjnAjiron | Layer | 55 weeks | Soling More | N- 24 13 26<br>E- 90 22 555 |
| Ind-RRP | Broiler | 26 days | Goshinga | N- 2410 51<br>E- 90 32 17 |
| Ind-Kz | Broiler | 22 days | Kaowraid | N- 24 19 27<br>E- 90 30 57 |

**Table 2. The operating conditions of AAS used for metal analysis.**

| Elements | Wavelength | Lamp current | Slit width | Gas flow | BGC Mode |
|---|---|---|---|---|---|
| Mn | 279.5 nm | 12 mA | 0.7 nm | 2 L/min | BGC-D2 |
| Cd | 228.8 nm | 8 mA | 0.7 nm | 1.8 L/min | BGC-D2 |
| Cr | 357.9 nm | 10 mA | 0.7 nm | 2.8 L/min | BGC-D2 |
| Pb | 283.3 nm | 10 mA | 0.7 nm | 2 L/min | BGC-D2 |
| Cu | 324.8 nm | 6 mA | 0.7 nm | 1.8 L/min | BGC-D2 |
| Fe | 248.3 nm | 12 mA | 0.2 nm | 2.2 L/min | BGC-D2 |
| Zn | 213.9 nm | 8 mA | 0.7 nm | 2 L/min | BGC-D2 |

**Table 3. Mn, Fe, Cu, Zn, Cd, Cr, Pb concentration (mg/kg) on selected feed samples from selected companies.**

| Sample | Mn | Fe | Cu | Zn | Cd | Cr | Pb |
|---|---|---|---|---|---|---|---|
| **Starter** | 0.532 ±0.002 | 18.993 ±0.002 | 0.321 ±0.003 | 1.169 ±0.002 | 0.299 ±0.001 | 0.089 ±0.001 | 5.492±0.003 |
| **Grower** | 2.217 ±0.002 | 27.742 ±0.001 | 0.922 ±0.001 | 2.223 ±0.002 | 0.470 ±0.003 | 0.312 ±0.001 | BDL |
| **Pre-layer** | 2.579 ±0.004 | 18.952 ±0.003 | 1.317 ±0.002 | 1.478 ±0.003 | 0.463 ±0.002 | 0.765 ±0.003 | BDL |
| **Layer Layer** | 3.021 ±0.003 | 108.392 ±0.002 | 1.307 ±0.002 | 1.142 ±0.001 | 0.256 ±0.001 | 1.839 ±0.001 | 9.178±0.005 |

**Table 4. Mn, Fe, Cu, Zn, Cd, Cr, Pb concentration (mg/kg) on selected chicken tissue, and litter samples from different companies.**

| Metals | Samples | | Skin | Brain | Liver | Meat | Bone | Litter |
|---|---|---|---|---|---|---|---|---|
| **Mn** | Broiler | Ind-Kz | 1.361±0.003 | 0.812±0.002 | 0.143±0.001 | 0.163±0.003 | 0.746±0.002 | 6.799±0.003 |
| | | Ind-RRP | 1.347±0.003 | 2.056±0.002 | 0.516±0.001 | 0.983±0.002 | 0.530±0.002 | 7.324±0.004 |
| | Layer | Ind-Pln | 1.886±0.003 | 5.159±0.003 | 1.564±0.003 | 1.548±0.002 | 1.348±0.001 | 5.290±0.003 |
| | | Ind-Ajn | 0.869±0.001 | 4.279±0.002 | 1.684±0.003 | 1.516±0.002 | 1.395±0.001 | 5.460±0.001 |
| **Fe** | Broiler | Ind-Kz | 128.898±0.003 | 288.691±0.004 | 14.561±0.002 | BDL | 123.561±0.001 | 100.581±0.003 |
| | | Ind-RRP | 43.221±0.004 | 64.612±0.003 | 51.320±0.004 | 24.285±0.002 | 36.148±0.003 | 146.542±0.005 |
| | Layer | Ind-Pln | 13.528±0.002 | 53.654±0.005 | 26.390±0.002 | 16.955±0.002 | 22.317±0.003 | 122.866±0.003 |
| | | Ind-Ajn | 20.987±0.001 | 39.383±0.003 | 50.665±0.003 | 24.978±0.004 | 324.43±0.003 | 185.241±0.001 |
| **Cu** | Broiler | Ind-Kz | 1.636±0.002 | 1.197±0.001 | 1.626±0.001 | 1.568±0.001 | 0.522±0.002 | 0.856±0.003 |
| | | Ind-RRP | 1.091±0.004 | 2.214±0.003 | 1.047±0.003 | 0.965±0.002 | 0.451±0.002 | 1.713±0.004 |
| | Layer | Ind-Pln | 1.508±0.002 | 4.329±0.005 | 1.339±0.003 | 1.548±0.003 | 1.183±0.002 | 1.511±0.006 |
| | | Ind-Ajn | 0.683±0.003 | 3.322±0.002 | 1.274±0.002 | 1.287±0.003 | 0.733±0.001 | 1.048±0.002 |
| **Zn** | Broiler | Ind-Kz | 7.413±0.003 | 4.149±0.001 | 1.319±0.002 | 1.402±0.002 | 3.387±0.003 | 5.557±0.005 |
| | | Ind-RRP | 1.215±0.001 | 1.983±0.002 | 1.391±0.003 | 1.254±0.001 | 3.908±0.002 | 5.749±0.003 |
| | Layer | Ind-Pln | 1.354±0.002 | 2.755±0.004 | 2.196±0.001 | 1.821±0.003 | 3.147±0.004 | 3.345±0.002 |
| | | Ind-Ajn | 0.474±0.001 | 2.806±0.001 | 3.116±0.002 | 2.284±0.002 | 2.775±0.003 | 2.608±0.002 |
| **Cd** | Broiler | Ind-Kz | 1.087±0.002 | 1.247±0.005 | 0.423±0.004 | 0.249±0.003 | 0.212±0.001 | 0.242±0.001 |
| | | Ind-RRP | 0.433±0.002 | 1.023±0.002 | 0.489±0.001 | 0.393±0.001 | 0.242±0.003 | 0.499±0.003 |
| | Layer | Ind-Pln | 0.553±0.001 | 1.581±0.002 | 0.456±0.002 | 0.404±0003 | 0.456±0.002 | 0.485±0.004 |
| | | Ind-Ajn | 0.146±0.002 | 0.695±0.002 | 0.231±0.001 | 0.272±0.002 | 0.116±0.001 | 0.172±0.001 |
| **Cr** | Broiler | Ind-Kz | 6.568±0.001 | 7.806±0.002 | 2.175±0.002 | 2.381±0.001 | 1.003±0.003 | 0.082±0.002 |
| | | Ind-RRP | 1.719±0.002 | 1.594±0.003 | 0.991±0.003 | 0.378±0.002 | 0.365±0.004 | 0.817±0.002 |
| | Layer | Ind-Pln | 1.429±0.001 | 5.613±0.003 | 1.142±0.001 | 1.417±0.004 | 0.396±0.004 | 0.763±0.003 |
| | | Ind-Ajn | 0.875±0.002 | 4.471±0.002 | 1.631±0.004 | 1.498±0.004 | 1.150±0.002 | 2.731±0.002 |
| **Pb** | Broiler | Ind-Kz | BDL | 34.775±0.004 | 10.881±0.003 | 11.142±0.005 | BDL | BDL |
| | | Ind-RRP | BDL | BDL | BDL | BDL | BDL | BDL |
| | Layer | Ind-Pln | BDL | 15.4826±0.003 | BDL | BDL | BDL | BDL |
| | | Ind-Ajn | 2.628±0.004 | 11.232±.005 | 3.922±0.004 | 4.151±0.003 | 2.234±0.002 | 1.585±0.002 |

Results are expressed as mean±SD

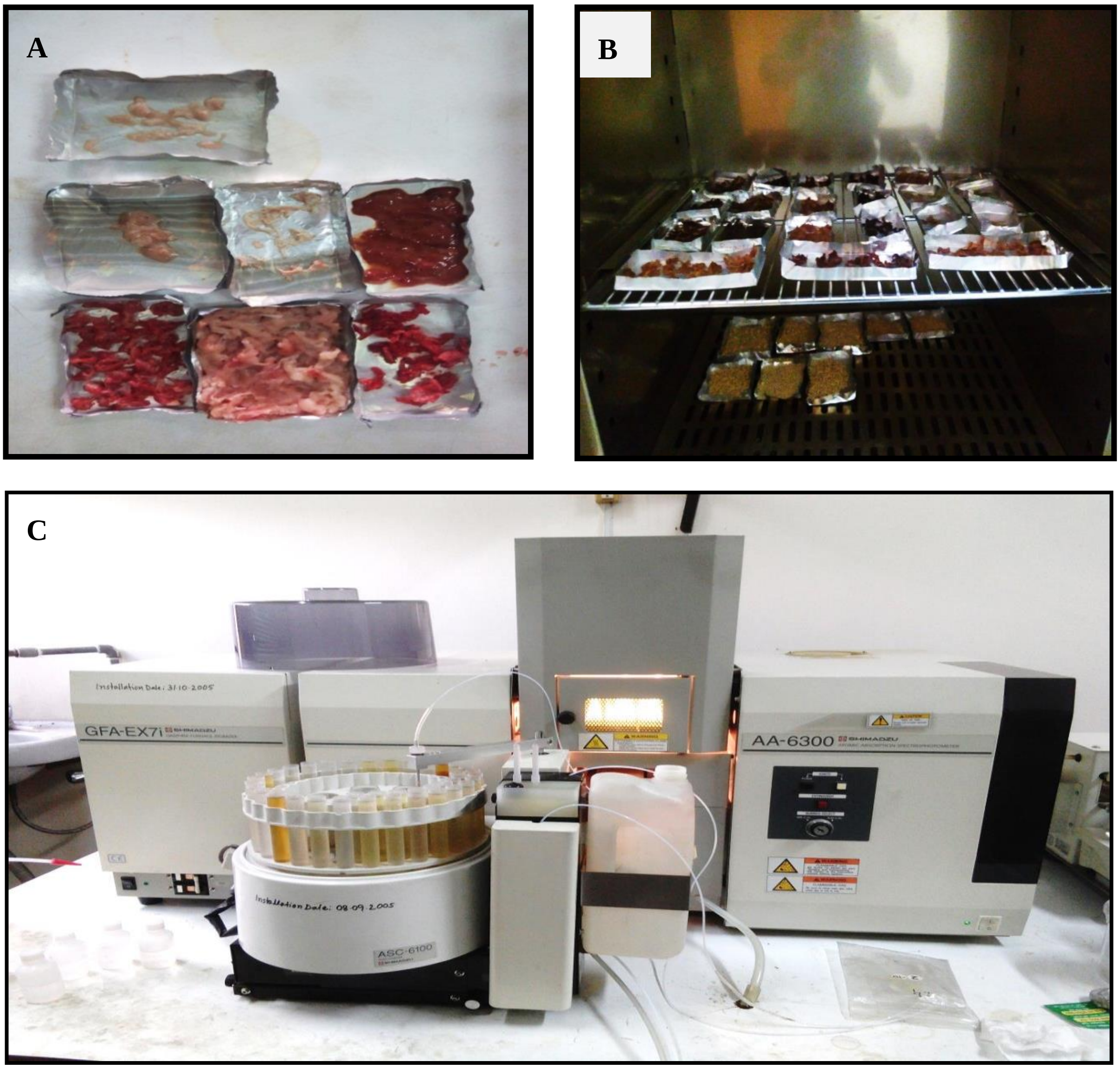


**Figure 1. (A) Sampling of chicken, (B) Oven drying of samples, (C) Analysis by AAS.**

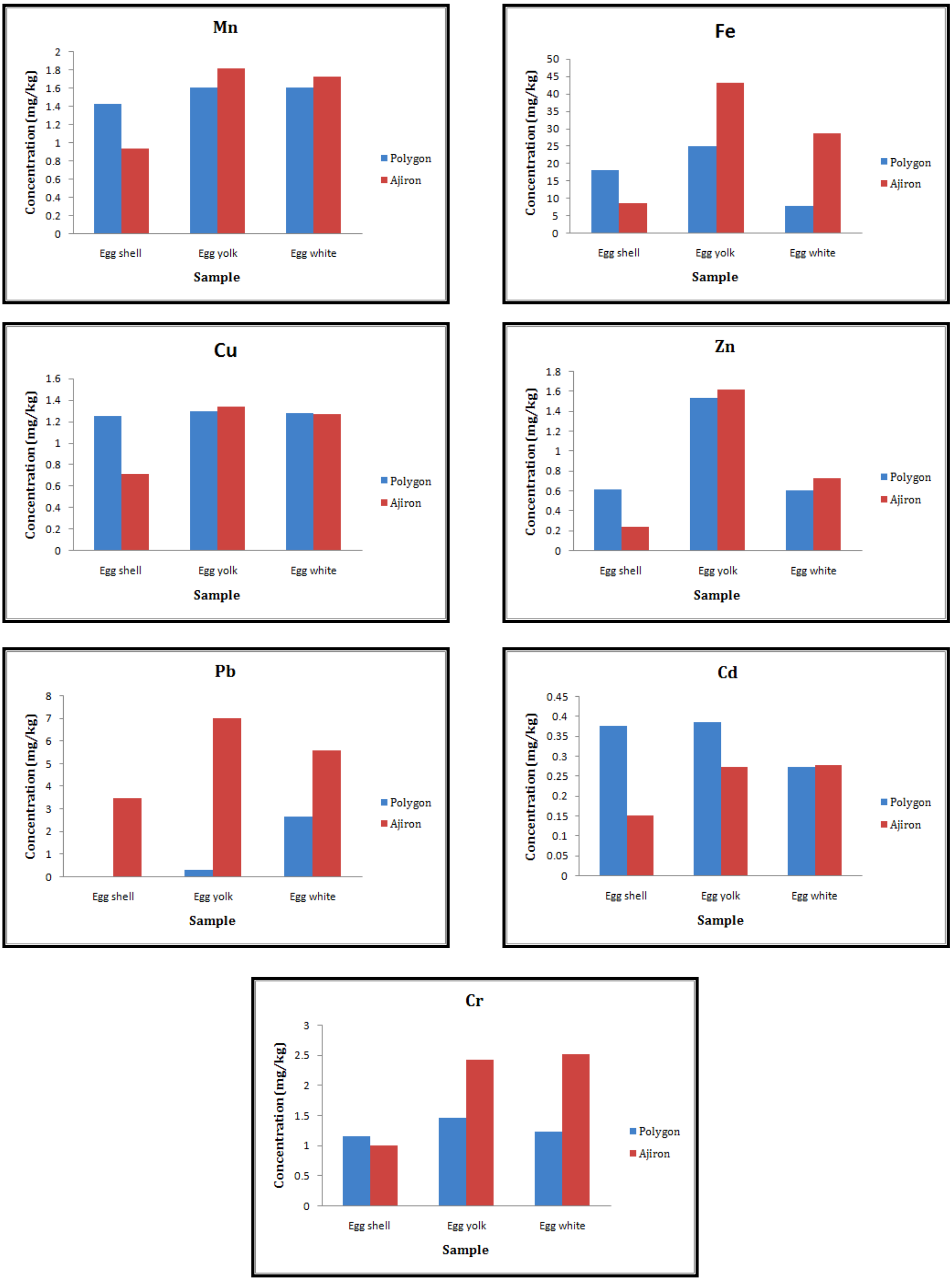


**Figure 2. Concentration of heavy metals and trace elements in selected poultry egg samples.**

## 4. Conclusions

In conclusion, the results of the study showed that the studied trace elements were within the recommended legal limits in collected feeds, but the Pb content in feed samples of Ind-Kz and Ind-Ajn was exceeded which may enter into the food chain through poultry consumption. This study in poultry samples revealed that a noticeable amount of metals such as Cd, Cr, and Pb were observed in brain tissue compared to other parts of the selected chickens and the values surpassed the recommended guidelines for human consumption. The Cr level was detected higher in almost all the chicken body parts which could be a great threat for public health accumulating heavy metals on tissues. Most of the studied metals (Mn, Fe, Cd, Cr, Pb) in egg samples crossed the safe permissible limit which may bring a serious health hazard whereas Cu and Zn contents were found lower than the suggested values. Most of the metals were found lower in the feed sample compared to the chicken tissues, eggs, and litter. This indicates that chicken feed is not the only source of metal contamination on chicken and eggs. Hence, this baseline data in the studied region suggests that contaminated poultry items should be avoided as much as possible. Proper monitoring is practiced for safe consumption to maintain standards for heavy metals and trace elements in chickens and eggs.

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**Conflict of interest**

None to declare.

**Authors' contribution**

M.A.Z. Chowdhury designed and supervised the experiment. Z. Fardous and M. Abir carried out the experiment. M.A.Z. Chowdhury and M. Nesha wrote the manuscript which was finely reviewed by H. Rahman and M. L. Bari and finalized in consultation with Z. Fardous and M. Abir. All authors have read and approved the final manuscript.

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