**Chapter I**

**Introduction**

Heavy metal contamination is a major international concern due to food safety issues and human health risk through the food chain. They are transferred into the environment through anthropogenic activities such as mining, industrial processing, waste water irrigation, agricultural activities, transport and fuel combustion, iron and steel production, coal and oil combustion, waste incineration, non-ferrous manufacturing and cement kilns (Dietz *et al.,* 1998).

Heavy metals find their way into living organisms from dietary and non dietary exposure where they accumulate and persist for long time. They enter into the food material and from there they ultimately make their passage into the tissue (Baykov *et al.,* 1996). Lead, chromium, cadmium, mercury, arsenic are among the main toxic metals which accumulate in food chains and have a cumulative effect (Cunningham and Saigo, 1997). Accumulation of heavy metal depends on the organ of interest and on the metal characteristics. Heavy metals often have physiologically toxic effects and are stored in living tissues. The uptake of heavy metals by living organisms is related to the bioavailability of such elements, represented by the characteristics of the metal, the nutritional facts and the age of the organisms.

Egg is a highly nutritious food among the human food stuff cycle and can be an effective delivery system for health regulating nutrients, vitamins, proteins fats etc (Hashish *et al.,* 2012). Eggs are readily digested and can provide significant portion of nutrients for the proper growth and maintenance of body tissue. Global environmental pollution with trace elements are leading to increase the investigations concerning metal contamination of food-stuffs including eggs, which represent an important part of humans’ diet, especially children (IDF, 1991).

Industrializations Progress throughout the world has been accompanied by the extraction and distribution of mineral substances from their natural deposits. Many minerals, especially trace elements, have undergone chemical changes through technical processes as finally pass, dispersed and in solutions into water and air and consequently into food chain. Poultry could take up heavy metal compounds from different sources; metal residues may concentrate in their meat, and eggs (Nisianakis *et al.,* 2009). Some trace elements are common in the diet and necessary for good human health. Iron (Fe) and copper (Cu) are essential trace elements which required by humans nevertheless, all metals are toxic at higher concentrations (Chronopoulos *et al.,* 1997; Lane and Morel, 2009).

Other heavy metals as arsenic (As), Cadmium (Cd), lead (Pb), chromium(Cr) are toxic and their accumulation over time inside chicken can cause serious illness. Certain elements that normally toxic are beneficial for certain organisms or under certain conditions (Lane *et al.,* 2005). Heavy metals may cause acute or chronic toxicity of human. Intake of heavy metals through the food chain has been widely reported through the world (Muchuweti *et al.,* 2006). Heavy metal toxicity can result in damaged or reduced mental and central nervous system functions, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs (International Occupational Safety and Health Information, 1999).

However poultry egg contain elevated levels of heavy metal that originate from food and water feed which are mainly influenced with the surrounding environment. Assessment of the heavy metal of egg is very important for many reasons that are related to health and nutritional value of eggs, the consequences of egg metals on its embryonic development and the use of eggs as bioindicators for environmental metal pollution (Surai and Sparks ,2001; Sparks 2006; Pappas *et al.,*2006).Therefore assessment of heavy metal in poultry egg is important for nutritional, toxicological and environmental aspects (Abdulkhalid *et al.,* 2012).

Poultry meat is another important source of protein and fat which used as major component of diet. Chemical composition of meat depends on both the kind and degree of the feeding animal. The need for mineral compounds depends on the age, physiological state and feed intake as well as on living conditions (Baykov *et al.,* 1996). Contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006). Although contamination of animal feed by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is a clear need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health (SCAN, 2003).

The risk associated with the exposure to heavy metals present in food product had aroused widespread concern in human health. Improvements in the food production and processing technology had increased the chances of contamination of food with various environmental pollutants, especially heavy metals. Ingestion of these contaminants by animals causes deposition of residues in meat. The risk of heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations (Santhi et al., 2008; Mahaffey, 1977).

Chromium is an essential element for human beings, further it acts in the organism as maintaining normal glucose tolerance (Upreti RK *et al.,* 2004). Trivalent chromium Determination of heavy metals in hen eggs found in most food and nutrient supplements, is an essential nutrient with very low toxicity. Hexavalant chromium compound have been shown to be potent occupational carcinogens (Zmudzki J and Szkoda J, 1996 ) .

Lead found everywhere in the environment and at low levels in almost all living organisms (Doganoc, 1996). The general population is exposed to Pb from air and food. During the last century, Pb emissions from petrol to ambient air caused considerable pollution (Järup, 2003). Lead ingested by chicken is deposited in bones, soft tissues and eggs, so contaminated egg yolk represents a potential public health hazard especially to children repeatedly consuming eggs (Trampel *et al.,* 2003), moreover children have high gastrointestinal uptake and permeable blood-brain barrier (Järup, 2003). As Pb can be passed from hen to the egg, repeated consumption of contaminated eggs from a family owned flocks provide continuing dietary source of Pb (Hui, 2002 and Trampel *et al.,* 2003). Lead is a neurotoxicant and of major public health concern which causes both acute and chronic intoxication (Gossel and Bricker, 1990), moreover it causes encephalopathy in children (Carl, 1991).

The rate of urbanization and industrialization is increasing day by day in several cities of Bangladesh. Besides many problems associated such developments, major one is the contamination and is principally associated with lead and chromium contamination. The accurate determination of trace metals in eggs is still an analytical challenge, due to their low concentration level and difficulties that arise from matrix characteristics. So, assessment of heavy metal in poultry meat has great importance for human health.

**1.1 Objectives**

* To identify the presence of heavy metal in poultry egg and meat
* To determine the level of selected heavy metal (lead and chromium) in poultry egg and meat
* To evaluate the public health risk associated with heavy metal contamination in poultry egg and meat

**Chapter II**

**Review of literature**

**2.1 Heavy metal**

Heavy metals are generally defined as [metals](https://en.wikipedia.org/wiki/Metal) with relatively high [densities](https://en.wikipedia.org/wiki/Density), [atomic weights](https://en.wikipedia.org/wiki/Atomic_weight), or [atomic numbers](https://en.wikipedia.org/wiki/Atomic_number). It is a subset of elements that exhibit metallic properties such as hard, opaque, shiny, and having good electrical as well as thermal conductivity. It comprises the transition metals (zinc, mercury etc.), some metalloids (arsenic, boron etc.), lanthanides (lanthanum, cerium etc.), and actinides (actinium, protactinium etc.). Heavy metal is also defined as those having a specific density of more than 5 g/cm3 **(**Suciu *et al.,* 2008). They can also be chemical elements with the density greater than 4 g/cm3  found in all kinds of soils, rocks and water in terrestrial and freshwater ecosystem (Adelekan and Abegunde, 2011).

**2.2 Types of heavy metal**

There are two main classes of heavy metal:

* Essential metals and
* Non-essential metals

Essential metals are Co, Cr, Cu, Zn, Fe, Ni and non-essential metals Pb, Hg, As, Cd. A deficiency in essential elements results in improper biological function, essential elements may become toxic when present in excess. Non-essential metals also have toxic effects, when enter into the body (Uluozlu *et al.,* 2009).

**2.3 Properties of heavy metal**

Heavy metal shows different physical and chemical properties those are:

**Table 2.1: Properties of heavy metal**

|  |  |
| --- | --- |
| **Physical properties** | |
| Density | Usually higher |
| Hardness | Most are quite hard |
| Thermal expantivity | Mostly lower |
| Melting point | Low to very high |
| Tensile strength | Mostly higher |
| **Chemical properties** | |
| Periodic table location | Nearly all found in group 3 through 16 |
| Abundance in Earth's crust | Less abundant |
| Main occurrence (or source) | Lithophilesor [chalcophiles](https://en.wikipedia.org/wiki/Chalcophile) ([Au](https://en.wikipedia.org/wiki/Gold) is a [siderophile](https://en.wikipedia.org/wiki/Siderophile_element)) |
| Reactivity | Less reactive |
| [Sulfides](https://en.wikipedia.org/wiki/Sulfide) | Extremely insoluble |
| [Hydroxides](https://en.wikipedia.org/wiki/Hydroxide) | Generally insoluble |
| [Salts](https://en.wikipedia.org/wiki/Salt_(chemistry)) | Mostly form colored solutions in water |
| [Complexes](https://en.wikipedia.org/wiki/Coordination_complex) | Mostly colored |

**2.4 Contamination sources of heavy metal**

Heavy metals are found naturally in the earth, and become concentrated as a result of human caused activities. Metals occur naturally in our environment, but rarely at toxic levels especially in the earth’s crust where they contribute to the balance of the planet. Common sources are from mining and industrial wastes; vehicle emissions; [lead-acid batteries](https://en.wikipedia.org/wiki/Lead%E2%80%93acid_battery); fertilizers; paints; treated woods; aging water supply infrastructure; and [micro plastics](https://en.wikipedia.org/wiki/Microplastics) floating in the world's oceans (Howell *et al.,*2012).

**2.5 Entry routes**

Heavy metals enter plant, animal and human tissues via air inhalation, diet and manual handling. Motor vehicle emissions are a major source of airborne contaminants including arsenic, cadmium, cobalt, nickel, lead, antimony, vanadium, zinc, platinum, palladium and rhodium. Water sources (groundwater, lakes, streams and rivers) can be polluted by heavy metals leaching from industrial and consumer waste; [acid rain](https://en.wikipedia.org/wiki/Acid_rain) can exacerbate this process by releasing heavy metals trapped in soils. Plants are exposed to heavy metals through the uptake of water; animals eat these plants; ingestion of plant- and animal-based foods is the largest sources of heavy metals in humans. Absorption through skin contact, for example from contact with soil, is another potential source of heavy metal contamination. Toxic heavy metals can [bioaccumulation](https://en.wikipedia.org/wiki/Bioaccumulation) in organisms as they are hard to [metabolize](https://en.wikipedia.org/wiki/Metabolism) (Pezzarossa et al., 2011).

**2.6 Lead**

Lead is the most common of the heavy elements. Several stable isotopes exist in nature, 208 pb being the most abundant. The average molecular weight of lead is 207.2. Lead is a soft metal that resists corrosion and has a low melting point (327°C). Lead is an element of risk for the environment and human health and has harmful effects that may exceed those of other inorganic toxicants. Industries are the major sources of lead pollution that contribute toxic metal lead to the environment (Hernandez *et al.*, 1987).

Lead (Pb) interferes the hemato-biochemical pathways with cumulative accumulation of lead to the body tissues *(Mazliah et al., 1989).* The whole food chain becomes contaminated due to the extensive contamination of all parts of environment. This pollution gives rise to the progressive accumulation of lead compounds in the aquatic environment (Hernandez *et al.,* 1987). Solid and liquid (sludge) wastes account for more than 50% of the lead discharged into the environment, usually into landfills, but lead has been dispersed more widely in the general environment through atmospheric emissions particularly from car exhausts. With the introduction of unleaded fuel, lead emissions from this source declined.

The annual consumption of lead is in the order of 3 million tons, of which 40% is used in the production of electrical accumulators and batteries, 20% is used in gasoline as alkyl additives, 12% in building construction, 6% in cable coatings, 5% in ammunition, and 17% in other usages. It is estimated that approximately 2 million tons are mined yearly. Probably 10% of this total is lost in treatment of the ore to produce the concentrate, and a further 10% is lost in making pig lead. The amount of lead discharged into the environment is equal to the amount weathered from igneous rocks. In global lead level terms, the power storage battery industry may have a relatively low impact on the environment because about 80% of all batteries are recycled.

**2.6.1 Sources of lead**

Lead is the most prevalent heavy metal contaminant. As a component of [tetraethyl lead](https://en.wikipedia.org/wiki/Tetraethyl_lead), (CH3CH2)4 pb, it was used extensively in [gasoline](https://en.wikipedia.org/wiki/Gasoline) during the 1930s–1970s. Lead levels in the aquatic environments of industrialized societies have been estimated to be two to three times those of pre-industrial levels. Lead (from [lead azide](https://en.wikipedia.org/wiki/Lead_azide) or [lead styphnate](https://en.wikipedia.org/wiki/Lead_styphnate) used in firearms) gradually accumulates at firearms training grounds, contaminating the local environment and exposing range employees to a risk of [lead poisoning](https://en.wikipedia.org/wiki/Lead_poisoning). The sources of lead include: old lead-pigment paints, batteries, industrial smelting and alloying, some types of solders, ayruvedic herbs, some toys and products from China, glazes on (foreign) ceramics, leaded (antiknock compound) fuels, bullets and fishing sinkers, artist paints with lead pigments, and leaded joints in some municipal water systems (Carson et al.,1986).The widespread industrial production of perfumes, oils and fats, cement-making, quarrying (especially limestone), and brick-making, as well as, agricultural discharges, sewage effluents, high ways or motor boat traffic , consider main sources of lead in the environment (Humphreys, 1991; Mahaffey, 1977).

**2.6.2 Exposure**

Food can be contaminated by naturally occurring lead in the soil as well as by lead from sources such as atmospheric fall out or water used for cooking. The total intakes and uptakes of lead from all sources are 29.5 and 12.5 mg/d, respectively, for children and 63.7 and 6.7 mg/d, respectively, for adults in urban areas (WHO, 1987). The relative contribution of water to average intake is estimated to be 9.8% and 11.3% for children and adults, respectively. The total intake of lead from three of the four major sources air, food, and dust appears to have dropped significantly since the mid-1980s as a result of regulatory and voluntary actions to control lead from air (via gasoline) and food (via cans) (Mushak and Crocetti , 1989).

**Most Lead contamination occurs via oral ingestion** of contaminated food or water or by children mouthing or eating lead containing substances. The degree of absorption of oral lead depends upon stomach contents (empty stomach increases uptake) and upon the body’s mineral status. **Deficiency of zinc, calcium or iron may increase lead uptake**. Transdermal exposure is slight. Inhalation has decreased significantly with almost universal use of non leaded automobile fuel.

**2.6.3 Health Effects**

Lead can be absorbed by the body through inhalation, ingestion, dermal contact (mainly as a result of occupational exposure), or transfer via the placenta. In adults, approximately 10% of ingested lead is absorbed into the body. Young children absorb from 40% to 53% of lead ingested from food. Once lead is absorbed, it enters either a “rapid turnover” biological pool with distribution to the soft tissues (blood, liver, lung, spleen, kidney, and bone marrow) or a “slow turnover” pool with distribution mainly to the skeleton. Of the total body lead, approximately 80–95% in adults and about 73% in children accumulate in the skeleton. The biological half-life of lead is approximately 16–40 days in blood and about 17–27 years in bones (Rabinowitz *et al.,* 1976).

Lead accumulates extensively in bone and inhibits formation of heme and hemoglobin in erythroid precursor cells. Bone Lead is released to soft tissues with bone remodeling that can be accelerated with growth, menopausal hormonal changes and osteoporosis (ATSDR, 2007).

Lead has physiological and pathological effects on body tissues that may be manifested from relatively low lead levels up to acutely toxic levels. **In children, developmental disorders and behavior problems may occur at relatively low levels: loss of IQ, hearing loss, poor growth.** In order of occurrence with increasing lead concentration, the following can occur: impaired vitamin D metabolism, initial effects on erythrocyte and erythroid precursor cell enzymologys, increased erythrocyte protoporphyrin, headache, decreased nerve conduction velocity, metallic taste, loss of appetite, constipation, poor hemoglobin synthesis, colic, frank anemia, tremors, nephrotoxic effects with impaired renal excretion of uric acid, neuropathy and encephalopathy (Piomelli *et al.,* 1990).

**At** relatively low **levels, lead can participate in synergistic toxicity with other toxic elements** (e.g. cadmium, mercury). Excessive retention of lead can be assessed by urinalysis after provocation with Ca-EDTA (IV) or oral DMSA. Whole blood analysis can be expected to reflect only recent exposures and does not correlate well with total body burden of lead (Piomelli *et al.,* 1990).

Young children are particularly affected and can suffer mental retardation and semipermanent brain damage. One of the most insidious effects of inorganic lead is its ability to replace calcium in bones and remain there to form a semipermanent reservoir for long-term release well after the initial absorption. The usual indicator of the degree of inorganic lead poisoning in humans is the content of this element in whole blood. Different authorities suggest safety levels in the range of 0.2–0.8 ppm. The figure 0.2 ppm seems to reflect a worldwide minimum. The disturbing fact is that the natural levels in human blood are already very close to what is considered a reasonable toxicological limit, not leaving us with any margin for exposure to lead (ATSDR, 1990).

Lead is a cumulative general poison, with fetuses, infants, children up to six years of age, and pregnant women (because of their fetuses) being most susceptible to adverse health effects. Lead can severely affect the central nervous system. Overt signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, hallucinations, and loss of memory with encephalopathy occurring at blood lead levels of 100–120 μg/dL in adults and 80–100 μg/dL in children. Signs of chronic lead toxicity, including tiredness, sleeplessness, irritability, headaches, joint pain, and gastrointestinal symptoms, may appear in adults with blood lead levels of 50–80 μg dL-1(ATSDR, 1990)

After one or two years of exposure, muscle weakness, gastrointestinal symptoms, lower scores on psychometric tests, disturbances in mood, and symptoms of peripheral neuropathy were observed in occupationally exposed populations at blood lead levels of 40–60 μg/dL (Baker *et al.,* 1984).

At levels of 30–50 μg/dL, there were significant reductions in nerve conduction velocity. Renal disease has long been associated with lead poisoning; however, chronic nephropathy in adults and children has not been detected below blood lead levels of 40 μg/dL. Finally, it has been demonstrated that interactions between calcium and lead were responsible for a significant portion of the variance in the scores on general intelligence ratings, and that calcium had a significant effect on the deleterious effect of lead (Lester *et al.,* 1986).

Several lines of evidence demonstrate that both the central and peripheral nervous systems are principal targets for lead toxicity. These include subencephalopathic neurological and behavioral effects in adults and electrophysiological evidence of both central and peripheral effects on the nervous system in children with blood lead levels well below 30 μg/dL.The carcinogenicity of lead in humans has been investigated in several epidemiological studies of occupationally exposed workers (IARC, 1982).

**2.7 Chromium**

Chromium (Cr) is one of the world’s most strategic and critical materials having a wide range of uses in the metals and chemical industries. Cr alloys enhance metal resistance to impact, corrosion, and oxidation. Cr is used in stainless steel and non iron alloy production for plating metals, development of pigments, leather processing, and production of catalysts, surface treatments, and in refractories. Human exposure to chromium occurs from both natural and anthropogenic sources. Chromium is present in the Earth's crust, with the main natural source of exposure being continental dust present in the environment (Barnhart 1997; Fishbein 1981; Pellerin and Booker 2000). Chromium is released into the environment in larger amounts as a result of human activities, which account for 60–70% of the total emissions of atmospheric chromium (Alimonti et al. 2000; Barceloux 1999; Seigneur and Constantinous 1995). Cr occurs in various oxidation states, of which chromium (VI) is a suspected carcinogen and a potential soil, surface water, and groundwater contaminant. Chromium is used mainly in metal alloys such as metal-ceramics, stainless steel, and is used as chrome plating. It has high value in the industrial world because it can be polished to a mirror-like finish, and provides a durable, highly rust resistant coating, for heavy applications. Cr (VI) may also occur in the natural environment, but human-caused Cr (VI) contamination has recently been the focus of much scientific discussion, regulatory concern, and legal posturing.

A common source of chromium exposure is from food. Total chromium levels in most foods typically range from <10 to 1,300 μg/kg, with the highest concentrations being found in meat, fish, fruits, and vegetables (WHO 2003). The general population is exposed to chromium by inhaling air, drinking water, or eating food or food supplements that contain chromium. However, the primary source of exposure for the general population and non-occupationally exposed workers to chromium comes from food sources, although drinking water can contribute significantly when the levels are >25 μg/L (WHO 2003).

Dermal exposure to chromium may also occur during the use of consumer products that contain chromium, such as fertilizer, wood treated with copper dichromate or chromated copper arsenate and leather tanned with chromic sulfate. In addition, people who reside in the vicinity of chromium waste disposal sites and chromium manufacturing and processing plants have a greater probability of elevated chromium exposure (Pellerin and Booker 2000).

Exposure to chromium for occupational groups can be two orders of magnitude higher than the exposure to the general population (Hemminki and Vainio 1984). Occupational exposure to chromium occurs mainly from chromate production, stainless steel production and welding, chrome plating, production of ferrochrome alloys, chrome pigment production and user industries, and from working in tanning industries (Pellerin and Booker, 2000 and Stern, 1982)

**2.7.1 Sources of chromium** (WHO 1990)

**2.7.1.1 Environmental sources**

Environmental sources of chromium include

* Airborne emissions from chemical plants and incineration facilities,
* Cement dust,
* Contaminated landfill,
* Effluents from chemical plants,
* Asbestos lining erosion,
* Road dust from catalytic converter erosion and asbestos brakes,
* Tobacco smoke, and
* Topsoil and rocks.

**2.7.1.2 Occupational sources**

Occupational sources of chromium include

* Anti-algae agents
* Antifreeze
* Cement
* Chrome alloy production
* Chrome electroplating (soluble Cr [VI])
* Copier servicing
* Glassmaking
* Leather tanning (soluble Cr [III])
* Paints/pigments (insoluble Cr [VI])
* Photoengraving
* Porcelain and ceramics manufacturing
* Production of high-fidelity magnetic audio tapes
* Tattooing
* Textile manufacturing
* Welding of alloys or steel and
* Wood preservatives i.e. Acid Copper Chromate.

## 2.7.2 Transport of chromium into the Environment

Chromium enters the environment through both natural processes and human activities. Increases in Chromium III are due to leather, textile, and steel manufacturing; Chromium VI enters the environment through some of the same channels such as leather and textile manufacturing, but also due to industrial applications such as electro painting and chemical manufacturing. Groundwater contamination may occur due to seepage from chromate mines or improper disposal of mining tools and supplies, and improper disposal of industrial manufacturing equipment

## 2.7.2.1 Air Contamination

According to the Toxics Release Inventory, in 1997, the estimated releases of chromium were 706,204 pounds to the air from 3,391 large processing facilities which accounted for about 2.2% of total environmental releases.

Cr (III) and Cr(VI) are released to the environment primarily from stationary point sources (facilities that are identified individually by name and location) resulting from human activities. The estimates of atmospheric chromium emissions in 1976 and 1980 in the Los Angeles, CA and Houston, TX areas indicate that emissions from stationary fuel combustion are about 46-47% of the total, and emissions from the metal industry range from 26 to 45% of the total (ATSDR 2000).

Coal and oil combustion contribute an estimated 1,723 metric tons of chromium per year in atmospheric emissions; however, only 0.2% of this chromium is Cr(VI). In contrast, chrome-plating sources are estimated to contribute 700 metric tons of chromium per year to atmospheric pollution, 100% of which is believed to be Cr (VI) (ATSDR 2000).

Cr (III) in the air does not undergo any reaction. Cr (VI) in the air eventually reacts with dust particles or other pollutants to form Cr (III). However, the exact nature of such atmospheric reactions has not been studied extensively (EPA 1998).

## 2.7.2.2 Water Contamination

According to the Toxics Release Inventory, in 1997, the estimated releases of chromium was 111,384 pounds to water from 3,391 large processing facilities which accounted for about 0.3% of total environmental releases (ATSDR 2000).

Electroplating, leather tanning, and textile industries release relatively large amounts of chromium in surface waters. Leaching from topsoil and rocks is the most important natural source of chromium entry into bodies of water. Solid wastes from chromate-processing facilities, when disposed of improperly in landfills, can be sources of contamination for groundwater, where the chromium residence time might be several years.

A survey conducted from 1974 to 1975 provides estimates of chromium concentrations in U.S. drinking water. The survey reported the concentration of chromium in tap water in U.S. households was from 0.4 to 8.0 micrograms per liter (µg/L) (ATSDR 2000)

## 2.7.2.3 Soil Contamination

According to the Toxics Release Inventory, in 1997 the estimated releases of chromium was 30,862,235 pounds to soil from 3,391 large processing facilities accounted for about94.1% of total environmental releases (ATSDR 2000).  
Total chromium has been identified in 939 soil and 472 sediment samples collected from 1,036 National Priority Lists (NPL) hazardous waste sites (HazDat 2000).

Chromium waste slag containing potentially hazardous levels of Cr (VI) compounds was used as fill material at more than 160 residential, industrial, and recreational sites. Persons living or working in the vicinity of the sites may have been exposed through inhalation, ingestion, or skin contact with contaminated soils and dusts (Fagliano, Savrin et al. 1997).

**2.7.3 Impacts on human health (**EPA 1998)

Cr (III) is an essential dietary nutrient. It is required to potentiate insulin and for normal glucose metabolism. Cr (III) deficiency has been associated with

* cardiovascular disease,
* decreased lean body mass,
* decreased sperm count,
* elevated percent body fat,
* fasting hyperglycemia,
* glucosuria,
* impaired fertility,
* impaired glucose tolerance
* Maturity-onset diabetes.

Chromium VI is the most dangerous form of chromium and may cause health problems including: allergic reactions, skin rash, nose irritations and nosebleed, ulcers, weakened immune system, genetic material alteration, kidney and liver damage, and may even go as far as death of the individual. Hexavalent chromium is considered to be lethal for a dose higher than 3 g for adult humans. The first symptoms are vomiting and persisting diarrhea. After a week, hemorrhagic diathesis and epitasis are commonly observed. Convulsions occur during the final stages of the illness. Repeated occupational inhalation of hexavalent chromium compounds causes perforations of the nasal septum and skin ulceration “chrome holes.” The sense of smell and acute irritative dermatitis or allergic eczematous dermatitis have frequently been reported in case of chronic exposure to chromic acid vapors as well as an increased incidence of cancer in the respiratory organs. Bronchial asthma due to chromate dust or chromic acid fumes has been experienced by a number of workers. Environmental contamination with chromium seems trivial compared to mercury or cadmium. Nevertheless, severe toxic effects on plants have been reported at Cr (VI) concentrations of approximately 0.5 mg L-1 (Naja *et al.,* 2009).

**2.8 Concentration of lead and chromium in poultry egg and meat**

The concentration of lead and chromium has been reported in Pakistan and samples show the lead content increases 2-3fold than permissible limit (lead 0.5 mg/kg) and chromium content has been undetectable (ul Islam et al., 2014). These results can be used as primary tools to further ensure egg quality control and also can work as dataset for the close monitoring of food contamination by governments and environmentalist.

Another investigation has been reported the highest concentration of lead (Pb) in bone (0.096±0.048mg/kg), followed by brain (0.085±0.044mg/kg), kidney (0.077±0.007mg/kg), liver (0.053±0.011mg/kg) and muscles (0.040±0.0211mg/kg). The natural accumulation of Pb significantly (P<0.01) present in egg shell (0.251±0.032ml/kg) compared with to the yolk (0.089±0.010ml/kg) and albumen (0.066±0.003ml/kg). A significant level of (Pb) also detected in poultry litter (0.580±0.067 mg/kg). Lead (Pb) levels in chickens meat and eggs in this study at present showed accepted level which is at present not in alarming stage but repeated consumption of lead (Pb) contaminated chicken meats and eggs may bring a serious public health hazard (Hossain *et al.,* 2014)

The concentration of Cr in animal meat, organ meat and meat products are in the ranges 0.02-5.36 mg/kg, 0.06-1.57 mg kg-1 and 2.89-4.33 mg kg-1 respectively and the amounts are within the permissible limit in eggs. The investigation shows that the concentration of Cr in the meat products and a very limited number of meats of Chittagong city is significantly higher than those studied in abroad. The highest concentration of Pb found in animal meat of local cow and local hen of Chittagong that are 24.9 and 41.94 mg kg-1 respectively, whereas the highest concentration of Pb found in animal meat of Indian cow and local goat of Comilla that are 43.37 and 1.36 mg kg-1 respectively. The organ meat of cow and buffalo of Chittagong contain the Pb in the ranges of 0.67 to 16.3 mg kg-1 and 0.51 to 7.47 mg kg-1 respectively. Beef roll, chicken wing, and beef pizza contain higher amount of Pb among the meat products, their values are 4.62, 3.94 and 2.45 mg kg-1 respectively. The amount of Pb in egg is recorded very negligible except the egg of local hen 12.1 mg kg-1 (Chowdhury *et al.,* 2011).

Another investigation showed the concentration of Pb 0.59 mg kg-1 in eggs and it is greater than the averages of 0.048 and 0.489 mg kg-1 reported in eggs in china and India, respectively. The concentration of chromium recorded higher than reference value (Khan and Naeem, 2006).

The mean concentration of of heavy metals in the liver, heart and muscle of chicken samples are 3.87 ± 3.94, 3.77 ± 2.77 and 2.27 ± 1.07 mg/kg (for Cr), 3.79 ± 3.64, 2.65 ± 1.88 and 1.65 ± 1.09 mg/kg (for Pb) respectively, indicting higher heavy metals levels in liver and heart samples (Sadeghi *et al.,* 2015).

Furthermore, mean concentrations of Pb 0.00694 ppm in chicken meat consumed by the population in Tenerife Island, Spain (González-Weller *et al.,* 2006)

The aforementioned concentration estimates of different heavy metals in poultry egg and meat have been produced by the sporadic and unstructured studies and no study has previouslybeen conducted in Chittagong region. Hence, to address the gaps of those studies thepresent cross-sectional study has been performed in Greater Chittagong.

**2.9 Risk factors for the presence of lead and chromium in poultry egg and meat**

The main sources of heavy metal contamination are growing and represented, especially, by pesticides, fertilizers, industrial processes and exhaust gases from automobiles (Albu, 2010).

The whole food chain becomes contaminated due to the extensive contamination of all parts of environment by trace elements (Bhaskaran and Gupta, 2006). Heavy metals from manmade pollution sources are continuously released into aquatic and terrestrial ecosystems and therefore, the concern about the effect of anthropogenic pollution on the ecosystems is growing.

In Bangladesh, chicken meat is a cheapest source of protein to the population and is widely consumed. The main source of metals in chickens arises from contaminated poultry feeds and water. The determination of heavy metals in the body tissues of chickens received serious attention (Iwegbue *et al,* 2008)

In view of the fact that poultry feed has been reported to be affected due to the use of heavy metals contained feed additives in poultry feed production system (Islam *et al.,* 2007). Many studies are conducted to assess the heavy metals from poultry feed and results showed the mixing of high concentration of heavy metals (Abdullah *et al.,* 2010). When these metals are added in feed more than the required level, these can accumulate in body tissues of broiler and in human being on its consumption and can be released in litter to cause environmental impacts (Mcbride and Dpiers, 2001).

Heavy metals can enter in food chain on consumption of broiler. There are many studies carried out to detect the heavy metals accumulation in chicken meat. Khan and Meijer (2005)assessed the risk of polluted and excessive amount of various ingredients used in animal feed. These toxic elements present in feed pose serious health hazards to consumers and secondary consumers due to biomagnifications. Deposition of heavy metals in body of broiler result from their excessive use in poultry feed. Concentration of toxic metals are also higher in litter depending on composition of poultry feed.

At present poultry farming is being concentrated around the large urban centers all over the country. All mineral elements present in poultry feed at higher amounts can pose several adverse health issues to poultry products. Currently, industries wastes as well as other wastes are being added to environment untreated. Therefore, it is essential that heavy metals must be restricted in the diet at the adequate levels in poultry products to ensure consumer health. A few research has been carried out at National research council (NRC) for both, dietary maximum tolerable levels of these elements in poultry feed as well as maximum tolerable levels (MTL) concentrations of these metals in dietary poultry eggs (NRC,1994).

In Nigeria, chicken meat, gizzard and turkey meat are a major source of protein to the population and are widely consumed. The main source of metals in water source and processing chicken and turkey meat arises from contamination of poultry feeds, and drinking (Iwegbue *et al,* 2008).

In general, lead accumulates in the plants and animals, while its concentration is magnified in the food chain (Halliwell et al., 2000).

Due to the fact that high demand for poultry meat has in recent years influenced their production significantly and alongside is the increased production and extensive modifications of poultry feeds to meet these demands. However, in view of the fact that poultry feeds, whether it is natural or locally sourced or the improved modifications from special manufacturing processes have been reported to be affected by the content of heavy metals in poultry feeds (Islam et al., 2007).

Commercial poultry farmers now use inorganic materials in poultry feed for augmentation of health and production. Grains, such as maize, soybeans and wheat, which are the major components of feed, could also have picked up or bioaccumulated Pb, Cr and Cd directly from contaminated soil, depending on the location in which they were grown and the chemical nature of the soil there. Excessive and improper use of inorganic or organic fertilizer could lead to the accumulation of metals in the soil (particularly Cd, Cr and Pb), which would be biomagnified by plants and eventually end up in animal feed, Close proximity of farms and crop plantations to industry could also result in crop contamination by metals. Increased concentrations of lead, chromium and other metals have been reported in Pakistan in soils and vegetation around a lead battery and particularly at Kasur Leather factories (Khan and Naeem, 2006).

The uptake of Pb from eggs in children is approximately 40%.The rate of egg consumption depends on socioeconomic status, age group and place of residence either urban or rural. The rate of egg consumption among lower class individuals and those residing in rural areas is very low. Boiling and frying are the predominant ways of egg preparation among lower-class individuals and their average intake is 6 eggs/week (egg per capita: 3.7 kg; i.e., the total weight of eggs consumed in a year calculated by multiplying the average weight of eggs taken per day by 365). The upper class, the rich and the affluent consume more eggs prepared in several ways, in addition to the normal boiled and fried eggs. These individuals eat more egg containing cakes, homemade biscuits, drinks (eggovin) and salad than poor individuals; such behaviours could amount to an average intake of 3 eggs per day (egg per capita: 18.6 kg). On average, a middle-class individual (i.e., healthy urban inhabitant) consumes an average of 2 eggs per day, for an egg per capita of 12.4 kg (Khan and Naeem, 2006).

**2.12 Public health risk due to lead and chromium**

Public health risk has been reported due to higher concentration of heavy metal exposed through poultry egg and meat. The health risk of human due to lead and chromium are given below:

**Table 2.2 Public health risk due to lead and chromium**

|  |  |  |
| --- | --- | --- |
| **Metal name** | **Permissible limit (mg/kg)** | **Effects** |
| **Lead (Pb)** | 0.5a (Egg white)  0.5a (egg yolk)  0.5b(meat) | * Decreased performance in nervous system * weakness in ﬁngers,wrists, or ankles * small increases in blood pressure; anemia * damage the brain and kidneys and ultimately cause death. * In pregnant women, high levels of exposure to lead may cause miscarriage. * High level exposure in men can damage the organs responsible for sperm production. |
| **Chromium (Cr)** | 0.002c (Egg white)  0.002c (egg yolk)  1.0d (meat) | * Breathing high levels can cause irritation to the lining of the nose; nose ulcers; runny nose; and breathing problems, such as asthma, cough, shortness of breath, or wheezing. * Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted. * Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation |

Source: (aZmudzki and Szkoda 1996; bFAO/WHO, 1987 ; cRoychowdhury *et al*., 2003; dEU,2002)

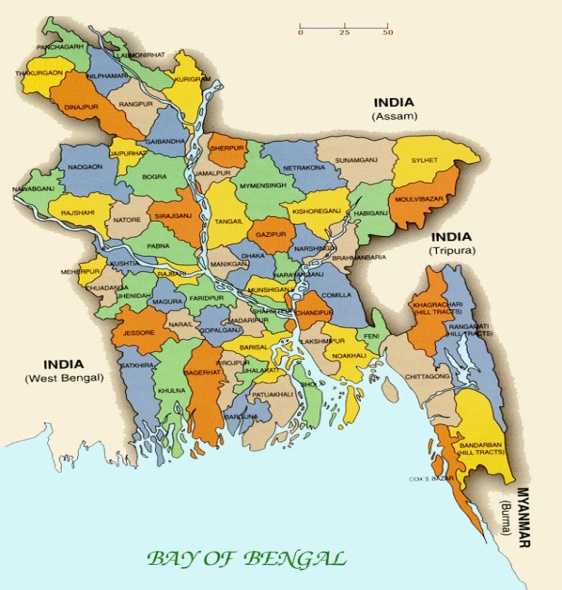
No investigation has earlier been carried out to assess the health risk due to exposure of heavy metals through poultry egg and meat in Greater Chittagong. The present study has therefore been conducted to assess health risk in the study areas.

**Chapter III**

**Materials and Methods**

**3.1 Study area**

Different broiler and layer farms are belonging to Chittagong city area were selected for the current study. The selection of study area depends on the purpose of the research, convenient of collection and analyze of the sample

**Fig.3.1 Study sites locating in the map of Bangladesh**

**3.2 Study period**

The study was carried out during the period of July to December, 2016

**3.3 Study design**

A cross-sectional study was carried out on different egg and meat samples from selected layer and broiler farms in the study area in order to determine the heavy metals.

**3.4 Sample collection**

Poultry egg and meat sample were collected from different layer and broiler farms of Chittagong city area. Egg samples were transferred into plastic bags and kept at refrigerated temperature to the laboratory. The meat samples were collected in polyethylene bags and stored at freezing temperature for analysis. Yolk and white portion of egg were analyzed separately.

**3.5 Number of samples**

A total number of 25 eggs and 25 meat samples were randomly collected from five layer and five broiler farms.

**3.6 Detection and Estimation**

The amount of (Pb, and Cr) heavy metal was measured by Atomic Absorption Spectrophotometer (AAS) Model: ZEE nit 700P, Germany (Shahriar *et al.,* 2014). All laboratory works were performed at Chittagong Veterinary and Animal Sciences University, Bangladesh.

**3.7 Diagnostic evaluation**

**3.7.1 Processing of samples**

**Egg sample**

The collected samples were decomposed by wet digestion method for the determination of various heavy metals. Each egg was cut in the air cell end using pointed forceps and dissecting scissors for each egg separately. The egg white portion was separated from egg yolk.

A known quantity, 5g of egg samples was introduced into separate crucible and kept in oven at 1050C for 2 hours to remove moisture content. Then 5ml nitric acid was added with each samples and kept for 24 hours. Thereafter samples were heated in digestion chamber at 2000C for 15-20 minutes and cool for 5-10 minutes.

5 milliliters aqua regia solution were added and again heated the samples until dried. Finally the samples were kept for cooling and added 100 milliliter deionized water in volumetric flask for dilution. The digested samples were filtered through Whatman No.1 filter paper.

Then 12 ml filtrate sample was taken into felcon tube and processed sample analyzed through Atomic Absorption spectrometer.

**Meat sample**

A 5 gm of meat samples was taken into crucibles and placed into oven at 1050C for 2 hours to remove moisture content. Then 5ml nitric acid was added with each samples and kept for 24 hours. Thereafter samples were heated in digestion chamber at 2000C for 15-20 minutes and cool for 5-10 minutes.

After digestion 5 milliliters aqua regia solution were added and again heated the samples until dried. Finally the samples were kept for cooling and added 100 milliliter deionized water in volumetric flask for dilution. The digested samples were filtered through Whatman No.1 filter paper.

12 ml filtrate sample was taken into felcon tube from standard volumetric flask and processed sample analyzed through Atomic Absorption spectrometer.

**3.8 Atomic Absorption Spectrometer (ZEEnit 700) (About Atomic Absorption Spectrometer)**

Atomic absorption spectrophotometer (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed.

Approximately 12 ml of processed samples (water or milk) were taken in a cup suitable to fit into the sample tray of the Atomic Absorption Spectrophotometer (ZEEnit 700). On starting the machine around 2-3 ml processed sample were automatically drawn for a single metal detection (lead/nickel/chromium). The sample solution was aspirated by a pneumatic analytical nebulizer and transformed into an aerosol, which was then introduced into a spray chamber, where the sample was mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (less than 10 μm) entered into the flame. This conditioning process was responsible that only about 5% of the aspirated sample solution reached the flame.

On top of the spray chamber was a burner head that produced a flame that was laterally long (usually 5–10 cm) and only a few mm deep. The radiation beam passed through this flame at its longest axis, and the flame gas flow-rates was adjusted to generate the highest concentration of free atoms. The burner height was also adjusted, so that the radiation beam passed through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

**3.8.1 Principles**

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the [Beer-Lambert Law](https://en.wikipedia.org/wiki/Beer-Lambert_Law).

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given [wavelength](https://en.wikipedia.org/wiki/Wavelength)). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The [radiation flux](https://en.wikipedia.org/wiki/Radiation_flux) without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

**3.8.2 Instrumentation**

In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electro thermal ([graphite](https://en.wikipedia.org/wiki/Graphite) tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a [monochromatic](https://en.wikipedia.org/wiki/Monochromator) in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

The processes in a flame include four stages:

**Dissolution (drying)**

The solvent was evaporated and produced dry samples.

**Vaporization (Transfer to the gaseous phase)**

The solid particles converted into gaseous molecules

**Atomization**

The molecules were dissociated into free atoms.

**Ionization**

In this stage atoms converted to gaseous ions

In flame Atomic Absorption Spectrometer a steady-state signal was generated during the time when the sample was aspirated and read the results on the screen of the machine. Concentration of metals was expressed in average mg/L.

**3.9 Statistical analysis**

All laboratory data were stored in Microsoft Excel 2007 and then exported into STATAIC 13.0 (Stata Corporation, College Station, TX, USA) for statistical analysis. Descriptive analysis was performed by using percentages, mean and standard deviation for different variables. Finally oneway ANOVA was used to compare the level of heavy metal residues in egg and meat of different farms of Chittagong. The level of significance was set ≤ 0.05.

**Chapter IV**

**Result and Discussion**

Heavy metal concentration estimated in fresh dry weight basis. The absorption wavelengths for the heavy metals were 357.87 nm for Cr and 217.0 nm for Pb. The metal content calculated by using formula:

Concentration (mg/kg or ppm dry wt)

= Concentration of the element through AAS (ppm) ×Volume made up/Sample Weight

In case of egg white highest mean concentration of lead and chromium level was found 3.213 mg/kg and 7.614 mg/kg. The highest lead and chromium level shows the farm A and farm D.The lowest mean concentration of lead and chromium in egg white was found 1.226 in farm C and 5.29 in farm A (Table-4.1 and Table-4.2)

**Table-4.1: Concentration of lead (Pb) in egg white**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farm** | **Mean ± SD** | **Minimum** | **Maximum** |
| **A** | 3.213 ± 2.872 | 0.504 | 7.04 |
| **B** | 2.308 ± 2.209 | 0 | 4.93 |
| **C** | 1.226 ± 2.412 | 0 | 5.52 |
| **D** | 1.613 ± 1.979 | 0 | 4.69 |
| **E** | 0.813 ± 1.662 | 0 | 3.78 |

**Table-4. 2: Concentration of Chromium (Cr) in egg White**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farms** | **Mean ± SD** | **Minimum** | **Maximum** |
| **A** | 5.29 ± 2.825 | 0.86 | 8.46 |
| **B** | 5.699 ± 4.768 | 0.548 | 13.35 |
| **C** | 6.240 ± 4.893 | 0 | 11.88 |
| **D** | 7.614 ± 5.690 | 0.47 | 14.16 |
| **E** | 6.014 ± 4.195 | 0 | 10.69 |

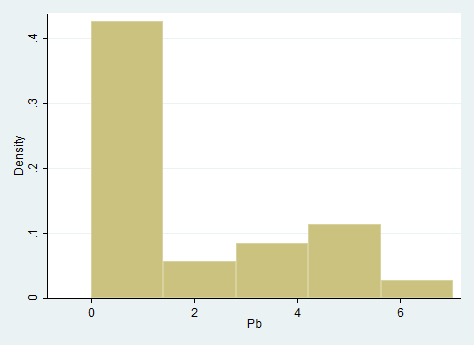
The concentration of lead was found in egg white at farm B is lower than farm A. The result indicate that farm C contain lower lead concentration than farm D. Otherwise in egg white lower chromium concentration was found in farm A than farm B. The result also showed that farm D contains highest chromium level than farm C and farm E. The mean concentration of lead (Pb) and chromium (Cr) in egg white at different farm are graphically represented below:



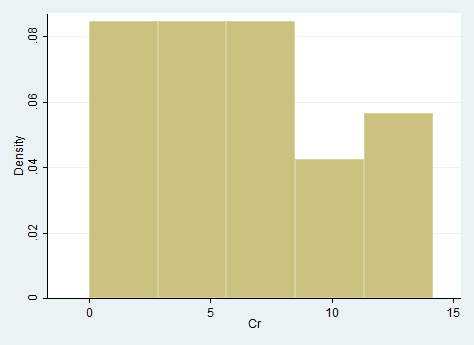
**Fig. 4.1 Mean concentration of lead (Pb) in egg white**



**Fig. 4.2 Mean concentration of Chromium (Cr) in egg white**



**Fig.4.3.Concentration of lead in egg white at different farm**



**Fig.4.4 Concentration of chromium in egg white at different farm**

The comparison between reference value with mean value of different samples in different farm for egg white showed that lead was significantly different in farm A and farm E (p<0.05) and lead was not significantly different in farm B, farm C and farm D (p>0.05).Otherwise chromium concentration was significantly different (p<0.05) in all farms.

**Table -4.3: Variation of lead concentration in egg white among different farm**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farm** | **Mean value of lead (Pb) in egg white** | **Reference Value of Pb in Egg white** | **t-test P value** |
| **A** | 3.213 | 0.5 | .05 |
| **B** | 2.308 | 0.5 | .15 |
| **C** | 1..266 | 0.5 | .24 |
| **D** | 1.613 | 0.5 | 0.14 |
| **E** | 0.813 | 0.5 | .004 |

**Table -4.4: Variation of Chromium concentration in egg white among different farm**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farm** | **Mean value of Chromium in Egg white** | **Reference Value of Chromium in Egg white** | **t-test P value** |
| **A** | 5.29 | 0.02 | 0.023 |
| **B** | 5.699 | 0.002 | 0.04 |
| **C** | 6.24 | 0.002 | 0.03 |
| **D** | 7.61 | 0.002 | 0.04 |
| **E** | 6.01 | 0.002 | 0.04 |

In case of all farms correlation coefficient of Pb and Cr was strongly negative except farm E. The correlation coefficients of egg white in different farms are given below:

**Table-4.5: Correlation coefficient of Pb and Cr in egg white at different farms**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Metal name** | **Farm A** | **Farm B** | **Farm C** | **Farm D** | **Farm E** |
| **Pb** | -0.7 | -0.3 | -0.7 | -0.6 | 0.02 |
| **Cr** |

In present study it was revealed that in case of egg yolk highest mean concentration of lead was found in farm E at 4.702mg/kg and highest chromium concentration was found in farm B at 6.808mg/kg. The lowest mean concentration of lead and chromium in egg yolk were found 1.050 at farm A and 3.326 mg/kg at farm E respectively (Table-4.5 and Table- 4.6).

**Table-4.6: Concentration of lead (Pb) in egg yolk**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farm** | **Mean ± SD** | **Minimum** | **Maximum** |
| **A** | 1.050 ± 1.830 | 0 | 4.300 |
| **B** | 2.343 ± 4.671 | 0 | 10.680 |
| **C** | 1.582 ± 1.313 | 0 | 3.300 |
| **D** | 3.828 ± 5.395 | 0 | 12.320 |
| **E** | 4.702 ± 4.551 | 0 | 11.260 |

**Table-4.7: Concentration of chromium (Cr) in egg yolk**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farms** | **Mean ± SD** | **Minimum** | **Maximum** |
| **A** | 5.358 ± 2.292 | 2.27 | 7.870 |
| **B** | 6.808 ± 3.187 | 4.13 | 12.240 |
| **C** | 3.922 ± 2.055 | 0.704 | 6.080 |
| **D** | 6.070 ± 5.583 | 1.040 | 12.280 |
| **E** | 3.326 ± 2.410 | 0 | 6.300 |

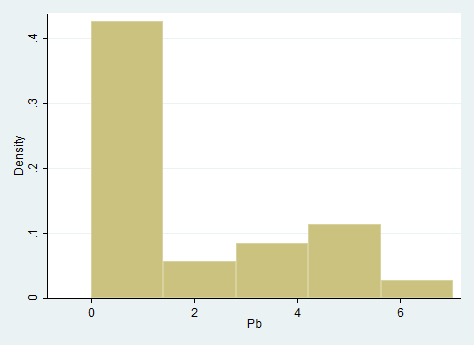
The result clearly indicates that the concentration of lead in egg yolk was found at farm B is higher than farm A. The concentration of lead was found in farm C is lower than farm D and farm E. Otherwise the concentration of chromium was found in farm A is lower than farm B and farm C is lower than farm D. The concentration of chromium was found in farm D is higher than farm E. The mean concentration of lead and chromium in egg yolk at different farm are graphically represented below:



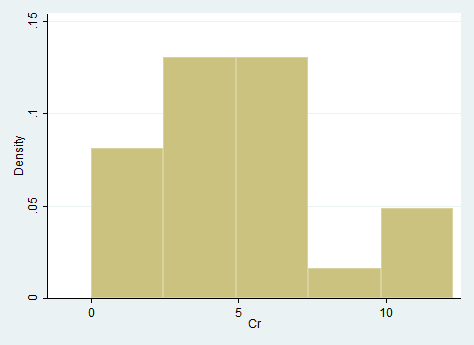
**Fig.4.5 Mean plot of lead (Pb) in egg yolk**

****

**Fig.4.6 Mean plot of chromium (Cr) in egg yolk**

****

**Fig.4.8 Concentration of lead in egg yolk at different farm**

****

**Fig.4.7 Concentration of chromium in egg yolk at different farm**

The comparison between reference value with mean value of different samples in different farm for egg yolk showed that lead was not significantly different (p>0.05) in all samples but chromium was not significantly different (p<0.05) in case of all farms except farm D (p<0.05).

**Table -4.8: Variation of lead concentration in egg yolk among different farm**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of farm** | **Pb Mean value of lead (Pb) in Egg (Yolk)** | **Reference Value of lead (Pb) in Egg yolk** | **t-test P value** |
| **A** | 1.05 | 0.5 | 0.26 |
| **B** | 2.343 | 0.5 | 0.23 |
| **C** | 1.582 | 0.5 | 0.12 |
| **D** | 3.82 | 0.5 | 0.23 |
| **E** | 4.70 | 0.5 | 0.12 |

**Table: 4.9: Variation of Chromium (Cr) concentration in egg yolk among different farm**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of farm** | **Mean value of chromium (Cr) in Egg (Yolk)** | **Reference Value of Chromium (Cr) in Egg yolk** | **t-test**  **P value** |
| **A** | 5.39 | 0.002 | .01 |
| **B** | 6.808 | 0.002 | .01 |
| **C** | 3.922 | 0.002 | .02 |
| **D** | 6.07 | 0.002 | 0.08 |
| **E** | 3.32 | 0.002 | .04 |

In this study, in case of farm A, farm C and farm D correlation coefficient of Pb and Cr was strongly positive but farm B and farm E was strongly negative.

**Table: 4.10: Correlation coefficient of Pb and Cr in egg yolk among different farm**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Metal Name** | **Farm A** | **Farm B** | **Farm C** | **Farm D** | **Farm E** |
| **Pb** | 0.3 | -0.5 | 0.9 | 0.9 | -0.1 |
| **Cr** |

The comparison of lead concentration in egg white and egg yolk with reference value showed that Pb content higher than permissible limit. It also indicates that lead concentration was higher in egg yolk than egg white at farm B, farm C, farm D and farm E. Otherwise it was found that chromium concentration higher than tolerable limit. The result showed that chromium concentration was higher in egg white than egg yolk at farm C, farm E and farm E (fig.4.8 and fig. 4.9)

Pb mg/kg

Name of farms

**Fig. 4.8 Comparison of lead concentration of egg white and egg yolk with reference value**

Cr mg/kg

Name of farms

**Fig. 4.9 Comparison of chromium concentration of egg white and egg yolk with reference value**

It appears that in case of poultry meat the highest mean concentration of lead was found 0.372 at farm D and lowest mean concentration was found 0.228 at farm B. Otherwise the result showed that highest chromium concentration was found 0.112 at farm C and lowest chromium concentration was found 0.048 at farm B (Table- 4.10 and 4.11).

**Table-4.11: Concentration of Lead (Pb) in poultry meat**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farm** | **Mean ± SD** | **Minimum** | **Maximum** |
| **A** | 0.242± 0.33 | 0 | 0.72 |
| **B** | 0.228±0.257 | 0 | 0.66 |
| **C** | 0.370±0.363 | 0 | 0.85 |
| **D** | 0.372±0.312 | 0.04 | 0.78 |
| **E** | 0.290±0.309 | 0.02 | 0.7 |

**Table-4.12: Concentration of Chromium (Cr) in poultry meat**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of farm** | **Mean ± SD** | **Minimum** | **Maximum** |
| **A** | 0.06±0.079 | 0 | 0.17 |
| **B** | 0.048±0.067 | 0 | 0.16 |
| **C** | 0.112±0.165 | 0 | 0.39 |
| **D** | 0.06±0.058 | 0.01 | 0.16 |
| **E** | 0.054±0.061 | 0 | 0.15 |

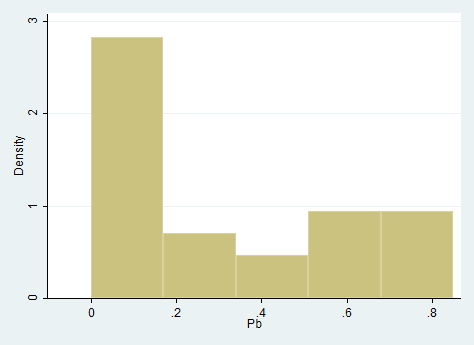
It can be illustrated that lead content of meat was higher in farm A than farm B. It also indicates that lead content in farm E was lower than farm D and farm E. Otherwise chromium content of was higher in farm B than farm A. The mean plot showed that chromium content was lower in farm E than farm C and farm D.



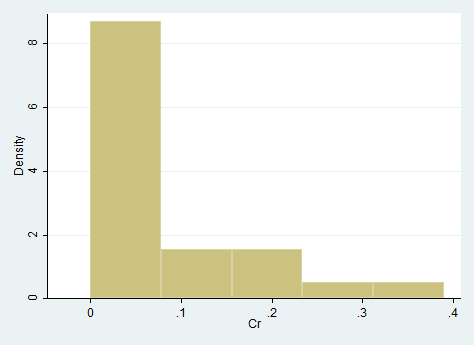
**Fig.4.10 Mean plot of lead (Pb) in poultry meat**



**Fig.4.11 Mean plot of Chromium (Cr) poultry in meat**



**Fig. 4.12 Concentration lead (Pb) in poultry meat at different farm**



**Fig. 4.13 Concentration chromium (Cr) in poultry meat at different farm**

The comparison between reference value with mean value of different samples in different farm for meat showed that lead was not significantly different (p>0.05) in all farms except farm B (p<0.05) but chromium was significantly different (p<0.05) in case of all farms (Table-4.13 and Table- 4.14)

**Table -4.13: Variation of lead concentration in poultry meat among different farms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farms** | **Pb mean value in Meat** | **Reference Value of Pb in meat (mg/kg)** | **t-test P value** |
| **A** | 0.242 | 0.5 | 0.156 |
| **B** | 0.228 | 0.5 | 0.046 |
| **C** | 0.370 | 0.5 | 0.447 |
| **D** | 0.372 | 0.5 | 0.387 |
| **E** | 0.290 | 0.5 | 0.167 |

**Table -4.14: Variation of chromium concentration in poultry meat among different farms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Farms** | **Cr mean value in Meat** | **Reference Value of Cr in Meat** | **t-test P value** |
| **A** | 0.06 | 1 | 0.001 |
| **B** | 0.048 | 1 | 0.001 |
| **C** | 0.112 | 1 | 0.003 |
| **D** | 0.06 | 1 | 0.001 |
| **E** | 0.054 | 1 | 0.001 |

In present study in case of farm A, farm B, farm C and farm D correlation coefficient was strongly negative except farm E (**Table-4.14)**

**Table-4.14: Correlation coefficient of Pb and Cr in meat among different farms**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Metal name** | **Farm A** | **Farm B** | **Farm C** | **Farm D** | **Farm E** |
| **Pb** | -0.6 | -0.2 | -0.2 | -0.4 | 0.01 |
| **Cr** |

The comparison of lead concentration between poultry meat and reference value indicates that lead content was lower than tolerable limit. Again it was found that chromium concentration also lower than acceptable range (Fig. 4.14 and Fig. 4.15).

Pb mg/kg

Name of farms

**Fig. 4.14 Comparison of lead concentration of poultry meat with reference value**

Cr mg/kg

Name of farms

**Fig. 4.15 Comparison of chromium concentration of poultry meat with reference value**

**Chapter V**

**Discussion**

Assessment of heavy metal in poultry egg and meat can become an important tool for food nutritionists. Lead is a ubiquitous harmful metal presence in food chain. It is one of the toxic heavy metals that have a commutative toxic effect in humans and animals. Lead has been shown to be associated with impaired neurobehavioral functioning in children. Impaired neurobehavioral development was considered to be the most critical effect (IPCS, 1995). The recommended daily allowance for lead is 0.3 mg per day and maximum permissible limit of lead in food stuff is 1-5 mg/ kg (IAEA, 1980). Excess lead is known to reduce the cognitive development and intellectual performance in children and to increase blood pressure and cardiovascular disease incidence in adults (Anonymous, 2005). The result of this study indicates that lead concentration of poultry meat 0.228-0.290 mg/kg. The high concentration of lead in the muscle indicates long term bioaccumulation. In this study the concentration of Pb exceeded the FAO/WHO standard of 0.5mg kg-1 for Pb. The high levels of Pb in poultry products emanate mainly from contamination of feeds and water sources. Mariam et al, (2004) reported mean levels of 3.15mg.kg-1 for lead in poultry. The levels found in this study were much lower than these values.

The permissible limit of lead in egg white and egg yolk is 0.5mg/kg (Zmudzki and Szkoda 1996).The result showed that lead concentration of egg white and egg yolk were 0.813-3.213 mg/kg and 1.050-4.702mg/kg. It was revealed that from this study more egg samples contain higher amount of lead but minimum sample contain lower amount. Meluzzi et al, (1996) reported 0.315 ppm Pb in egg white which is lower than our analyzed results. It is known that most eggs pollutants have tropism for yolks because they (including here heavy metals) are more soluble and addictive for a lipids rich environment, easily oxidable, favouring thus the chelating of highly reactive metallic ions within the organic catena of phospholipids (Bargellini et al.,2008 ). Thus, in literature, value of 0.12ppm Pb (Bargellini et al.,2008); 0,036ppm Pb (Polonis and Dmoch , 2007) and 0.397 ppm Pb (Meluzzi et al., 1996) were reported in yolk which were lower than our findings.

It might be due to the fact that migration of Pb across the membrane form albumen to yolk has been inhibited due to larger ionic radii of Pb+2.  
The recent review by [Kan and Meijer (2007)](http://smithandfranklin.com/current-issues/Concentrations-of-Heavy-Metals-and-Minerals-in-Poultry-Eggs-and-Meat-Produced-in-Khyber-Pakhtunkhwa-Pakistan/16/1/224/html#Kan--C.A.--and-G.A.L.-Meijer.-2007.-The-risk-of-c) gives a general insight on transfer of toxic  
metal from feed to eggs.

Chromium plays an important role in body function (metabolic function co-factor of insulin) in trace amount but it turn to be toxic when it exceeds the tolerance limit .The daily requirement of chromium for adult is estimated between 0.02 to 0.5 mg/day. Chromium (Cr) is an essential element which helps the body to use sugar, protein and fat at the same time it possess carcinogenic properties as well ([Institute of Medicine, 2002](http://smithandfranklin.com/current-issues/Concentrations-of-Heavy-Metals-and-Minerals-in-Poultry-Eggs-and-Meat-Produced-in-Khyber-Pakhtunkhwa-Pakistan/16/1/224/html#Institute-of-Medicine-.--2002.-Dietary-Reference-Intakes--for-Vitamin-A--Vitamin-K--Arsenic--Boron-C)).The trivalent form of chromium is considered as essential for normal carbohydrate and lipid metabolism (NRC, 1980). Cr (III) is ubiquitous in nature, occurring in air, water, soil, and biological materials. The major concern about chromium is the Cr (VI) form which has carcinogenic behavior in humans. The most important toxic effects after contact, inhalation, or ingestion of hexavalent chromium compounds include dermatitis, allergic and eczematous skin reactions, skin and mucous ulcerations, perforation of the nasal septum, allergic asthmatic reactions, bronchial carcinomas, gastro-enteritis, hepatocellular deficiency, and renal oligo-anuric deficiency (Baruthio, 1992).

The permissible limit of chromium in egg white and egg yolk is 0.002mg/ kg and in meat is 1mg/kg. The result indicate that chromium concentration in egg white, egg yolk and meat were 5.29-7.614mg/kg, 3.326-6.808mg/kg 0.048-0.112mg/kg. So the investigation showed that chromium concentration is higher in egg samples and lower in meat samples. Our measurements for Cr content in eggs were higher than those recorded by Hui.

Iwegbue et al (2006) found the concentration of Cr in chicken meat ranged between 0.01- 3.43mgkg-1 which is above the permissible limit. The findings of our study are comparable to the work done by [Akan et al. (2010)](http://smithandfranklin.com/current-issues/Concentrations-of-Heavy-Metals-and-Minerals-in-Poultry-Eggs-and-Meat-Produced-in-Khyber-Pakhtunkhwa-Pakistan/16/1/224/html#Akan--J.C.--F.I.-Abdulrahman--O.A.-Sodipo-and) who found higher concentration in muscle (0.29 ppm).

**Chapter VI**

**Conclusion**

Egg and meat is a good source of protein. Heavy metal contamination in food is important topic. Heavy metals are considered particularly dangerous to human health because, in the preparation of food, they do not decompose; on the contrary, their concentration tends to bioaccumulate. A long term consumption of heavy metal containing food above tolerance limit has a hazardous impact on human health. It is concluded that the present study showed that egg samples contain higher amount of lead and chromium which is alarming for public health but meat sample contain lower amount of lead and chromium below permissible limit which is safe to human health. Even if there were in small amounts, the presence of these heavy metals can generate worries due to their cumulative effect in the consumers’ organism. It is suggest that to reduce this heavy metal contamination poultry feed, water should be regular examined. It is also demand that regular checked poultry meat and egg before coming into market. Furthermore, garlic could be advised to antagonize Pb toxicity, as garlic contains chelating compounds capable of enhancing elimination of Pb. Garlic feeding can be exploited to safeguard human consumers by minimizing Pb concentrations in chicken meat which had been grown in a Pb polluted environment.

**Chapter VII**

**Recommendation and Future perspectives**

* To reduce heavy metal contamination poultry feed, water should be regular examined.
* Regular checked poultry meat and egg before coming into market.
* Garlic could be advised to antagonize Pb toxicity
* Assessment of heavy metal concentration in different body parts of poultry.
* Determination of heavy metal concentration in poultry products
* Comparison the level of heavy metal among different poultry species
* Determination of other heavy metal (As, Co, Hg, Cd, Ni) in poultry meat and egg

**References**

Abdul Khaliq, A., Swaileh, K., Jussein, R., Matani, M. (2012). Levels of metals (Cd, Pb, Cu and Fe) in cow’s milk, dairy products and hen’s eggs from the West Bank, Palestine. *International Journal of Food Research*, 19 (3), 1089 – 1094.

Abdullah NA, Osman K, Salaman KA (2010). Monitoring of aflatoxins and heavy metals in some poultry feeds. AJFS., 4(4): 192-199.

Adelekan, B., Abegunde, K., (2011). Heavy metals contamination of soil and groundwater at automobile mechanic villages in Ibadan, Nigeria. *International Journal of Physical Sciences 6*, 1045-1058.

Agency for Toxic Substances and Disease Registry (ATSDR) (1990). Toxicological profile for lead. Public Health Service/U.S. Environmental Protection Agency.

Agency for Toxic Substances and Disease Registry (2000). "Toxicological Profile for Chromium."

Akan, J.C., F.I. Abdulrahman, O.A. Sodipo and Y.A. Chiroma. (2010). Distribution of Heavy Metals in the Liver, Kidney and Meat of Beef, Mutton, Caprine and Chicken from Kasuwan Shanu Market in Maiduguri Metropolis, Borno State, Nigeria. Res. J. Appl. Sci. Engin. Technol. 2(8):743-748.

Albu A., (2010).Assessment of heavy metal and nitrate/nitrite residues in the plant, part of food chain and their risk for consumers, PhD Thesis, Ion Ionescude la Brad University of Agricultural Sciences and Veterinary Medicine, Iaşi, Romania.

Alimonti A, Petrucci F, Krachler M, et al. (2000). Reference values for chromium, nickel and vanadium in urine of youngsters from the urban area of Rome. J Environ Monit. 2(4):351–354.

Anonymous (2005). Commission regulation (EC)amending regulation E C N0: 466/2001 as regards heavy metals. Official J. L 16/43, 20/1/2005. 43-45.

Baker, E.L., Feldman, R.G., White, R.A., Harley, J.P., Niles, C.A., Dinse, G.E., and Berkey, C.S. (1984). Occupational lead neurotoxicity: A behavioral and electrophysiological evaluation. Study design and year one result. Br. J. Ind. Med., 41, 352–361.

Barceloux DG. Chromium. ClinToxicol. (1999). 37(2):173–194.

Barnhart J. Chromium chemistry and implications for environmental fate and toxicity. J Soil Contam. (1997). 6(6):561–568.

Baykov, B.D., M.P. Stoyanov and M.L. Gugova, (1996). Cadmium and lead bioaccumulation in male chickens for high food concentrations. Toxicol. Environ. Chem., 54: 155-159.

Bhaskaran S, Gupta G (2006). Adsorption of trace elements from poultry litter by montmorillonite clay. J. Hazard Materl., 128: 80-83.

Carl, M. (1991). Heavy metals and other trace elements. Monograph on residues and contaminants in milk and milk products. Special Issue 9101, Chapter 6. *Internatiomal Dairy federation (IDF)*, Belgium.

Chowdhury, M. Z. A., Siddique, Z. A., Hossain, S. A., Kazi, A. I., Ahsan, A. A., Ahmed, S., &Zaman, M. M. (2011). Determination of essential and toxic metals in meats, meat products and eggs by spectrophotometric method.Journal of the Bangladesh Chemical Society, 24(2), 165-172.

Chronopoulos, J., Haidouti, C., Chronopoulou, A. and Massas, I. (1997). Variations in plant and soil lead and cadmium content in urban parks in Athens, Greece. Science Total Environment 196: 91–98.

Codex Alimentarius Commission. (2011). FAO/WHO, Joint Food Standards Programme, Codex Committee on Contaminants in Foods, Working document for information and use in discussions related to contaminants and toxins in the GSCTFF, List of Maximum Levels for Contaminants and Toxins in Foods, Part 1, March, CF/5 INF/1

Cunningham, W.P. and B.W. Saigo, (1997). Environmental Science a Global Concern. 4th Edn.,WMC Brown Publisher, New York, pp: 389.

Demirezen, D. and K. Uruç, (2006). Comparative study of trace elements in certain fish, meat and meat products. Meat. Sci., 74: 255-260.

Dietz, R., Pacyna, J., Thomas, D.J., Asmund, G., Gordeev, V., Johansen, P., Kimstach, V.,Lockhart, L., Pfirman, S.L., Rigét, F.F., Shaw, G., Wagemann, R. & White, M., Heavy metals (1998). AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP): Oslo, Norway, pp. 373–524.

Doganoc, D.Z.(1996). Distribution of lead, cadmium and zinc in tissues of hens and chickens from Slovenia. Bull Environ. Contam. Toxicol., 57:932-937.

EPA (1998). "Toxicological Review of Trivalent Chromium. CAS No. 16065-83-1. In support of Summary Information on the Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington, D.C."

EU, European Parliament of the Council. Brussels. Belgium. (2002). Setting Maximum Levels for Certain Contaminants in Foodstuffs. Report No.466/2002.

Fagliano, J. A., J. Savrin, et al. (1997). "Community exposure and medical screening near chromium waste sites in New Jersey." Regulatory Toxicology & Pharmacology 26(1 Pt 2): S13 22.

FAO/WHO, Joint Expert Committee on Food Additives, WHO Technical Report Series No. 505(1972); No.555 (1974c); No.647 (1980); No.683(1982); No 751 (1987) and No. 776 (1989).Evaluation of certain food additives andcontaminants, Geneva.

Fishbein L. (1981). Sources, transport and alterations of metal compounds: An overviewIArsenic, beryllium, cadmium, chromium and nickel. Environ Health Perspect. 40:43–64.

Gossel, T. A. and Bricker, J. D. (1990). Metals. In: Principles of Clinical Toxicology .2 nd Ed., Raven press, New York, 162-192.

Gonzalez-W aller, D., L. Karlsson, A. Caballero, F. Hernandez, A . Gutierrez, T. Gonzalez-Igalesias., M. Marino and A. Hardission. (2006). Lead and cadmium in meat and m eat products consumed by the population in Tenerife Islans,Spain. Food Addit. Contamin., 23: 757-763.

Hashish, S.M., Abdel – Samee, L.D., Abdel –Wahhab, M.A. (2012). Mineral and heavy metals content in Eggs of local hens at different Geographic Area in Egypt. Global Veterinaria, 8 (3), 298 – 304.

Halliwell D., Turoczy N., Stagnitti F., (2000). Lead concentrations in Eucalyptus sp. in a small coastal town, Bulletin of Environmental Contamination andToxicology, 65, 583-590.

HazDat (2000). "Hazardous substances database." Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA

Hemminki K, Vainio H. (1984). Human exposure to potentially carcinogenic compounds. IARC Scientific Publ No. 59:37–45.

Hernandez L, Rico MMC, MJ Gonzalez and Hernan MA (1987). Environmental contamination by lead and cadmium in plants from urban area of Madrid, Spain. Bulletin of Environmental Contamination and Toxicology 38: 203-208.

Hossain, M. A., Akanda, M. R., Belal, S. A., Hasan, M. M. I., Islam, S., Uddin, M. N., & Roy, A. C. (2014). Environmental Deposition of Lead (Pb) in Chickens and Litter Samples Collected from Selected Areas of Bangladesh. J. Vet. Adv, 4(9), 677-685.

Hui, C. A. (2002). Concentrations of chromium, manganese and lead in air and in avian eggs. Environ .Poll., 120:201-206.

Humphreys DJ (1991). Effects of exposure to excessive quantities of lead on animals. Br. Vet. J. 147(1):18-30.

IAEA, “Elemental analysis of Biological Materials” (Technical Report Series No. 197) IAEA Vienna, (1980).

International Dairy Federation “IDF”. (1991). Monograph on residue and contaminants in milk and milk products. In Heap, Carl, M. (Ed). Brussels (Belgium), chapter 6, p.112-119.

International Program on Chemical safety “IPCS”. (1995). Environmental health criteria for inorganic lead, (WHO, Food Additives Series 44, 2000).

Islam, M. S., M. Azizul Islam Kazi,a M. Moazzem Hossain, (2007) Propagation of Heavy Metals in Poultry Feed Production in Bangladesh. Bangladesh J. Sci. Ind. Res. 42(4), 465-474.

Iwegbue, C. M. A., Nwajei, G. E., &Iyoha, E. H. (2008). Heavy metal residues of chicken meat and gizzard and turkey meat consumed in southern Nigeria. *Bulgarian Journal of Veterinary Medicine*, 11(4), 275-280.

Järup, L. (2003). Hazards of heavy metal contamination. Br. Med. Bull., 68:167-82.

Kan, C.A., and G.A.L. Meijer. (2007). The risk of contamination of food with toxic substances present in animal feed. Anim. Feed Sci. Technol. 133: 84-108

Khan, K., &Naeem, M. (2006). Simultaneous determination of accumulated hazardous metals in hens egg by atomic absorption spectroscopy. *Journal of Applied Sciences*, 6, 198-201.

Lane, T.W., Saito, M.A., George, G.N., Pickering, I.J., Prince, R.C. and Morel, F.M. (2005). Biochemistry: A cadmium enzyme from a marine diatom. Nature 435: 42.

Lester, M.L., Horst, R.L., and Thatcher, R.W. (1986). Protective effects of zinc and calcium against heavy metal impairment of children’s cognitive function. Nutr. Behav., 3, 145–161.

Mahaffey, K. R (1977). Quantities of lead producing health effects in humans: sources and bioavailability. Environ. Health Perspect. 19: 285-295.

Mahaffey, K. R. (1977). Mineral concentrations in animal tissues: Certain aspects of FDA's regulatory role. *Journal of animal science,* 44(3), 509-515.

Mazliah JS, Barron E, Reznik I (1989). The effect of chronic lead intoxication in mature chickens. Avian Dis., 33: 566-570.

Mcbride M, Dpiers G (2001). Trace element contents of selected fertilizers and dairy manures as determined by ICP-MS. Soil Sci. Plant Anal., 32: 139-156.

Muchuweti, M., Birkett, J.W., Chinyanga, E., Zvauya, R., Scrimshaw, M.D. and Lister, J.N. (2006). Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implication for human health. Agriculture Ecosystem. Environment 112: 41–48.

Mushak, P. and Crocetti, A.F. (1989). Determination of numbers of lead-exposed American children as a function of lead source: Integrated summary of a report to the U.S. Congress on childhood lead poisoning. Environ. Res., 50, 210–229.

Naja, G., Mustin, C., Volesky, B., and Berthelin, J. Biosorption (2008).Study in a mining wastewater reservoir*. Intern. J. Environ. Pollut.*

Nisianakis, P., Giannenas, I., Gavriil, A., Kontopidis, G. and Kyriazakis, I. (2009).Variation in trace element contents among chicken, turkey, duck, goose, and pigeon eggs analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Biological Trace Element Research 128 (1): 62-71.

NRC. (1994). Nutrient Requirements of Poultry, ninth rev. ed. National Academy Press, Washington, DC.

Pappas, A. C., Karadas, F., Surai, P. F. N.A.R. Wood, N. A. R., Cassey, P., Bortolotti, G. R. and Speake, B. K. (2006). Interspecies variation in yolk selenium concentrations among eggs of free-living birds: The effect of phylogeny. Journal *o Trace Elements in Medicine and Biology* 20: 155-160.

Pellerin C, Booker SM. Reflections on hexavalent chromium. Health hazards of an industrial heavyweight. Environ Health Perspect. (2000). 108(9):A402–A407.

Pezzarossa, B; Gorini, F; Petruzelli, G (2011). "Heavy Metal and Selenium Distribution and Bioavailability in Contaminated Sites: A Tool for Phytoremediation". In Selim, HM. Dynamics and Bioavailabiliy of Heavy Metals in the Rootzone. CRC Press. pp. 93–128.[ISBN](https://en.wikipedia.org/wiki/International_Standard_Book_Number) [9781439826225](https://en.wikipedia.org/wiki/Special:BookSources/9781439826225).

Piomelli S. et al. (1990). “Management of Childhood Lead Poisoning”, J. Pediatr 105 p. 523-32.

Rabinowitz, M.B., Wetherill, G.W., and Kopple, J.D. Kinetic analysis of lead metabolism in healthy humans. J. Clin. Invest. 58, 260–270.

Roychowdhury T, Tokunaga H, Ando M. (2003). Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an arsenic affected area of West Bengal, India. Science of the Total Environment 308(1-3):15-35.

Sadeghi, A., Hashemi, M., Jamali-Behnam, F., Zohani, A., Esmaily, H., & Dehghan, A. A. (2015). Determination of Chromium, Lead and Cadmium Levels in Edible Organs of Marketed Chickens in Mashhad, Iran. *Journal of food quality and hazards control,* 2(4), 134-138.

Santhi, D., V. Balakrishnan, A. Kalaikannan and K.T. Radhakrishnan, (2008). Presence of heavy metals in pork products in Chennai (India). Am. J. Food Technol., 3(3): 192-199.

SCAN, (2003). Scientific Committee on Animal Nutrition: Opinion of the Undesirable Substances in Feed. Retrieved from: http://europa.eu.int /comm/ food/fs/sc/scan/out126\_bis\_en.pdf, (Accessed on: 25 April, 2003).

Seigneur C, Constantinous E. (1995). Chemical kinetic mechanism for atmospheric chromium. Environ SciTechnol29:222–231.

Sparks, N. H. C. (2006). The hen’s egg – Is its role in human nutrition changing? *World’s Poultry Science Journal* 62: 308-315.

Stern RM. (1982). Chromium compounds: Production and occupational exposure. In: Langard S, editor. Biological and environmental aspects of chromium. New York, NY: Elsevier Biomedical Press. pp. 5–47.

Suciu, I., Cosma, C., Todică, M., Bolboacă, S.D., Jäntschi, L., (2008). Analysis of soil

heavy metal pollution and pattern in central Transylvania. *International Journal of Molecular Sciences* 9, 434-453.

Surai, P. F. and Sparks N. H. C. (2001). Designer egg: from improvement of egg composition to functional food. Trends in Food Science and Technology 12: 7-16.

Trampel, D.W.; Imerman, P.M; Carson, T.L.; Kinker, J.A. and Ensley, S.M. (2003). Lead contamination of chicken eggs and tissues from a small farm flock *J. Vet. Diagn. Invest.,* 15 (5): 418-422.

ul Islam, M. S., Zafar, M., & Ahmed, M. (2014). Determination of heavy metals from table poultry eggs in Peshawar-Pakistan. *Journal of Pharmacognosy and Phytochemistry*, 3(3), 64-67.

Uluozlu O.D., Tuzen M., Mendil D., Soylak M. (2009). Assessment of trace element contents of chicken products from Turkey. *Journal of Hazardous Materials.* 163: 982-987.

Upreti RK, Shrivastava R and Chaturvedi UC. (2004). Gut microflora and Toxic Metals: Chromium as a model. Indian J. Med. Res; 119: 49-59.

World Health Organization (WHO) (1987). Report of the 30th Meeting of the Joint FAO/WHO. Expert Committee on Food Additives. Geneva and Rome, World Health Organization.

WHO (1996). World health organization: Health criteria and other supporting information. In: Guideline for drinking water quality Vol 2, 2nd edu., Geneva. 31-38

WHO (2003). Background document for development of WHO guidelines for drinking water quality. Geneva: World Health Organization, Chromium in drinking water.

Zmudzki J. and Szkoda J. (1996). Concentrations of trace elements in hen eggs in Poland. Bromatologia-iChemia-Toksykologiczna 29 (1): 55-

**Appendix I: Spreadsheet of heavy metal concentration in egg and meat samples**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of Farms** | **Name of Samples** | | **Sample ID** | | **Lead (mg/ kg)** | | | | | **Chromium (mg/ kg)** | | | |
| **Tested value** | | **Reference value** | | | **Tested value** | | **Reference value** | |
| **A** | **Egg white** | | 1 | | 0.734 | | 0.5a | | | 6.74 | | 0.002c | |
|  |  | | 2 | | 0.504 | | 0.5 a | | | 5.46 | | 0.002c | |
|  |  | | 3 | | 5.3 | | 0.5 a | | | 4.93 | | 0.002c | |
|  |  | | 4 | | 2.49 | | 0.5 a | | | 8.46 | | 0.002c | |
|  |  | | 5 | | 7.04 | | 0.5 a | | | 0.86 | | 0.002c | |
| **B** |  | | 6 | | 4.93 | | 0.5 a | | | 3.37 | | 0.002c | |
|  |  | | 7 | | 3.24 | | 0.5 a | | | 5.14 | | 0.002c | |
|  |  | | | 8 | 0 | | 0.5 a | | | 0.548 | | 0.002c | |
|  |  | | | 9 | 0 | | 0.5 a | | | | 13.35 | 0.002c | |
|  |  | | | 10 | 3.37 | | 0.5 a | | | | 6.09 | 0.002c | |
| **C** |  | | | 11 | 0.034 | | 0.5 a | | | | 2.37 | 0.002c | |
|  |  | | | 12 | 0 | | 0.5 a | | | | 11.88 | 0.002c | |
|  |  | | | 13 | 0 | | 0.5 a | | | | 8.65 | 0.002c | |
|  |  | | | 14 | 0.578 | | 0.5 a | | | | 8.3 | 0.002c | |
|  |  | | | 15 | 5.52 | | 0.5 a | | | | 0 | 0.002c | |
| **D** |  | | | 16 | 0.51 | | 0.5 a | | | | 14.16 | 0.002c | |
|  |  | | | 17 | 0 | | 0.5 a | | | | 3.78 | 0.002c | |
|  |  | | | 18 | 2.51 | | 0.5 a | | | | 7.57 | 0.002c | |
|  |  | | | 19 | 0.358 | | 0.5 a | | | | 12.09 | 0.002c | |
|  |  | | | 20 | 4.69 | | 0.5 a | | | | 0.47 | 0.002c | |
| **E** |  | | | 21 | 3.78 | | 0.5 a | | | | 5.95 | 0.002c | |
|  |  | | | 22 | 0.288 | | 0.5 a | | | | 9.1 | 0.002c | |
|  |  | | | 23 | 0 | | 0.5 a | | | | 4.33 | 0.002c | |
|  |  | | | 24 | 0 | | 0.5 a | | | | 0 | 0.002c | |
|  |  | | | 25 | 0 | | 0.5 a | | | | 10.69 | 0.002c | |
| **A** | **Egg yolk** | | | 1 | 0.29 | | 0.5 a | | | | 7.87 | 0.002c | |
|  |  | | | 2 | 0 | | 0.5 a | | | | 5.5 | 0.002c | |
|  |  | | | 3 | 4.3 | | 0.5 a | | | | 7.25 | 0.002c | |
|  |  | | | 4 | 0.66 | | 0.5 a | | | | 4.1 | 0.002c | |
|  |  | | | 5 | 0 | | 0.5 a | | | | 2.27 | 0.002c | |
| **B** |  | | | 6 | 0.784 | | 0.5 a | | | | 5.75 | 0.002c | |
|  |  | | | 7 | 0.252 | | 0.5 a | | | | 6.8 | 0.002c | |
|  |  | | | 8 | 0 | | 0.5 a | | | | 5.12 | 0.002c | |
|  |  | | | 9 | 0 | | 0.5 a | | | | 12.24 | 0.002c | |
|  |  | | | 10 | 10.68 | | 0.5 a | | | | 4.13 | 0.002c | |
| **C** |  | | | 11 | 1.49 | | 0.5 a | | | | 4.89 | 0.002c | |
|  |  | | | 12 | 2.41 | | 0.5 a | | | | 4.66 | 0.002c | |
|  |  | | | 13 | 3.3 | | 0.5 a | | | | 6.08 | 0.002c | |
|  |  | | | 14 | 0 | | 0.5 a | | | | 0.704 | 0.002c | |
|  |  | | | 15 | 0.714 | | 0.5 a | | | | 3.28 | 0.002c | |
| **D** |  | | | 16 | 12.32 | | 0.5 a | | | | 12.28 | 0.002c | |
|  |  | | | 17 | 6.12 | | 0.5 a | | | | 12.02 | 0.002c | |
|  |  | | | 18 | 0.704 | | 0.5 a | | | | 2.4 | 0.002c | |
|  |  | | | 19 | 0 | | 0.5 a | | | | 1.04 | 0.002c | |
|  |  | | | 20 | 0 | | 0.5 a | | | | 2.61 | 0.002c | |
| **E** |  | | | 21 | 6.62 | | 0.5 a | | | | 2.6 | 0.002c | |
|  |  | | | 22 | 0.95 | | 0.5 a | | | | 0 | 0.002c | |
|  |  | | | 23 | 11.26 | | 0.5 a | | | | 2.81 | 0.002c | |
|  |  | | | 24 | 4.68 | | 0.5 a | | | | 4.92 | 0.002c | |
|  |  | | | 25 | 0 | | 0.5 a | | | | 6.3 | 0.002c | |
| **A** | | **Meat** | | 1 | | 0.72 | | 0.5b | 0.01 | | | 1.0d |
|  | |  | | 2 | | 0.03 | | 0.5 b | 0.12 | | | 1.0d |
|  | |  | | 3 | | 0.46 | | 0.5 b | 0 | | | 1.0d |
|  | |  | | 4 | | 0 | | 0.5 b | 0.17 | | | 1.0d |
|  | |  | | 5 | | 0 | | 0.5 b | 0 | | | 1.0d |
| **B** | |  | | 6 | | 0.66 | | 0.5 b | 0.06 | | | 1.0d |
|  | |  | | 7 | | 0 | | 0.5 b | 0.16 | | | 1.0d |
|  | |  | | 8 | | 0.14 | | 0.5 b | 0.02 | | | 1.0d |
|  | |  | | 9 | | 0.09 | | 0.5 b | 0 | | | 1.0d |
|  | |  | | 10 | | 0.25 | | 0.5 b | 0 | | | 1.0d |
| **C** | |  | | 11 | | 0.6 | | 0.5 b | 0.02 | | | 1.0d |
|  | |  | | 12 | | 0.85 | | 0.5 b | 0.01 | | | 1.0d |
|  | |  | | 13 | | 0.04 | | 0.5 b | 0.14 | | | 1.0d |
|  | |  | | 14 | | 0.36 | | 0.5 b | 0.39 | | | 1.0d |
|  | |  | | 15 | | 0 | | 0.5 b | 0 | | | 1.0d |
| **D** | |  | | 16 | | 0.78 | | 0.5 b | 0.04 | | | 1.0d |
|  | |  | | 17 | | 0.23 | | 0.5 b | 0.06 | | | 1.0d |
|  | |  | | 18 | | 0.19 | | 0.5 b | 0.16 | | | 1.0d |
|  | |  | | 19 | | 0.62 | | 0.5 b | 0.01 | | | 1.0d |
|  | |  | | 20 | | 0.04 | | 0.5 b | 0.03 | | | 1.0d |
| **E** | |  | | 21 | | 0.54 | | 0.5 b | 0.05 | | | 1.0d |
|  | |  | | 22 | | 0.7 | | 0.5 b | 0.07 | | | 1.0d |
|  | |  | | 23 | | 0.06 | | 0.5 b | 0 | | | 1.0d |
|  | |  | | 24 | | 0.13 | | 0.5 b | 0 | | | 1.0d |
|  | |  | | 25 | | 0.02 | | 0.5 b | 0.15 | | | 1.0d |

Source: (aZmudzki and Szkoda 1996; bFAO/WHO, 1987 ; cRoychowdhury *et al*., 2003; dEU,2002)

**Appendix II: Picture Gallery**

****  ****

**** ****

**Brief Biography**

Mrs. Nilufa Yeasmin passed the Secondary School Certificate Examination in 2006 and then Higher Secondary Certificate Examination in 2008. Mrs. Nilufa Yeasmin obtained his B.Sc. (Hons.) in Food Science & Technology in 2013 from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Department of Food Processing & Engineering under Food Science & Technology Faculty; CVASU. She has immense interest to work in heavy metal detection in egg, meat, meat products, water, milk, vegetables etc.