ACCUMULATION OF CHROMIUM FROM FEED TO DIFFERENT EDIBLE ORGANS OF BROILER

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Roll No: 0117/04

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A thesis submitted in the partial fulfillment of the requirements for the Degree of Master of Science in Food Chemistry and Quality Assurance

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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Dedication

DEDICATED TO MY BELOVED PARENTS

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TABLE OF CONTENTS

CHAPTER-I: INTRODUCTION

CHAPTER-II: REVIEW OF LITERATURE

CHAPTER-III: MATERIALS AND METHODS

List of Tables

List of Figures

List of Abbreviations

Abbreviation	**Elaboration**
ATSDR	Agency for Toxic Substances and Disease Registry
°C	Degree Centigrade
FAO	Food and Agriculture Organization
Fig	Figure
gm	Gram
IDF	International Dairy Federation
IPCS	International Program on Chemical safety
PPM	Parts Per Million
%	Percentage
SCAN	Scientific Committee on Animal Nutrition
SD	Standard Deviation
MPL	Maximum Permissible Limit
µg	Microgram
µm	Micrometer
Mg	Milligram
Mg/kg	Milligram per Kilogram
Mg/L	Milligram per Liter
µg /dl	Microgram per Deciliter

Abstract

Ensuring food safety is a worldwide concern whereas heavy metal contamination into food chain is an emerging crisis throughout the world at this present age. This study was conducted to evaluate the status of chromium (Cr) concentration in broiler feeds and water with the risk assessment of chromium (Cr) in broiler meat, liver and brain. Five broiler rearing farms were selected for this study where feed used for rearing the broiler were different in the farms. A total fifteen broiler chickens was collected along with the water samples from those broiler farms. Atomic Absorption Spectrophotometer (AAS) was used to determine the chromium (Cr) concentration in the feed, water, broiler meat, liver and brain samples. The mean concentration of chromium in feed, meat, liver, brain and water sample were found 0.00-3.51 mg/kg, 0.00-4.702 mg/kg, 0.00-2.7 mg/kg, 0.00-3.55 mg/kg and 0.00 mg/kg respectively. The highest concentration of chromium in broiler feed, meat, liver and brain samples was 0.857 ± 0.076 mg/kg, 3.510 ± 0.095 mg/kg, 2.700 ± 1.084 mg/kg and 3.550 ± 1.281 mg/kg respectively. However, study findings revealed that two feed samples were contaminated with chromium, but water samples were not contaminated. Chromium was found only in meat, liver and brain sample of those farm where chromium containing feed were used for rearing the broiler. Brain samples had the highest accumulation than those of liver and meat samples. Chromium concentration in broiler brain and liver were found much higher than permissible levels determined by FAO and WHO. Meat samples almost safe to eat as it had the accumulation rate within the permissible limit. The correlation coefficient between feed, meat, liver, and brain was strongly positive. Therefore it is suggested that precarious steps should be taken to detect the chromium in poultry feed to evaluate the health risks and to protect the human from food that might cause severe harmful effect on the health.

Keywords: Heavy metal, Chromium, Broiler Feed, Meat, Liver, Brain

Chapter I

Introduction

1.1 General Feature

Poultry is one of the major protein sources, cheap and available for people in Bangladesh. It is a promising sector. It requires large scale feed production activities. There are various sources of raw materials for poultry feed production. In many ways these sources can be associated with anthropogenic heavy metals, in particular Cr, pollution. It is better to monitor any probable propagation of heavy metals like Cr into the food chain through the final feeds rather than the various raw materials for feeds. There was news in the Daily Star on July 24, 2010, titled “Toxic poultry feed poses health risk" that expressed concern over the possible presence of heavy metals, particularly Cr in the poultry in Bangladesh. Although, some of these metals are essential for normal physiology of the systems, they can become lethal when consumed in food above a threshold (Jaishankar *et al*., 2014). Therefore it is important to quantify Cr in the poultry feed and its percentage transporting into chicken and eggs (Mazumder *et al.,* 2013).

In Bangladesh, several large and many small mills are converting the solid wastes into protein concentrates without following any appropriate standard operating procedure. This protein concentrate is mixed with other ingredients to prepare poultry feeds. Each large mill produces 200–250 tons of protein concentrate per day (Hossain *et al*., 1998).

Human exposure to chromium occurs from both natural and anthropogenic sources. The oxidation state of Cr determines chemical and toxicological behaviors of Cr. Cr is considered as one of the most noxious heavy metals, which is listed as one of 129 priority pollutants by the USEPA. Cr can exist in many oxidation states but its most stable forms are Cr (III) and Cr (VI). Cr (VI) is highly toxic and responsible for health hazards like mutation, cancer and cell damage (Agency for Toxic Substances and Disease Registry, 2000).

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 Centre, 1999).

The increasing interest currently developed by researchers globally on the challenges of heavy metal contamination of meat product is borne out of the fact that heavy metals have been known to cause serious tissues and organ toxicity at trace amounts. In addition, some heavy metals are non-biodegradable, and can easily accumulate in tissues of organisms thereby posing serious health issues along the food chain (Tchounwou *et al.,* 2013).

Contamination of chicken meat with heavy metals may cause severe health hazard because of their toxicity, bio-accumulation and bio-magnifications. Serious illness such as kidney disease, damage to the nervous system, diminished intellectual capacity, heart disease, gastrointestinal diseases, bone fracture, cancer and even death can also happen due to the absorption of heavy metals through food (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 (Scientific Committee on Animal Nutrition, 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).

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.

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).

Among the pollutants generated by various industrial, domestic and commercial sewerage effluents, heavy metals and various pathogenic bacteria are directly related to the health problems. Health safety is very important aspect of food quality. Hazardous substances enter the food chain and are the main sources of contamination for humans. Heavy metals are most dangerous contaminants. All mineral elements, whether it is theorized as essential or potentially toxic, may have an adverse effect if included in the diet at excessively high concentration Chicken meat could accumulate heavy metals if the chicken feed is contaminated through the various raw materials used for its production (Okoye *et al.*, 2011). High concentration of Cr was found in different parts of chicken in Lahore, Pakistan (Mahmud *et al.*, 2011) and in Tamil Nadu, India (Sudha, 2010).

Heavy metal contamination is one of the major consequences of environmental pollution arising from anthropogenic activities such over-dependence on agro-chemicals, uncontrolled use chemical raw materials and fossil fuel combustion (Jaishankar *et al.*, 2014).

In Bangladesh, every year 5.9×10^4 tons of hides and skins (bovine , sheep, lamb, goat, and kid) are used are raw material for leather production (FAO, 2013), leading to huge amount of soid wastes being generated by the tanneries cause environmental pollution, which ultimately has detrimental effect on human healthwhen exposed it through various agencies (Sundar *et al.*, 2011). Due to generation of solid, liquid and gaseous pollutants, Department of enviromnet (DoE), Bangladesh has categorized tanneries as a 'Red' category industry. Many research groups have already investigated different ways to manage the solid wastes originating from the tanneries (Cabeza *et al.*, 1998; Ravindran and Sekaran, 2010; Shanmugam and Horan, 2009).

Transfer of chromium into poultry may occur through these feeds, which then enters into the human food chain. It is very important to know the quantity of chromium present in the feed and the amount of chromium transmitted to the body parts of chicken which are consumed as human food.

1.2 Objectives

1.2.1 General Objective

This research aims at determining the concentrations and accumulation rate of Chromium in meat and edible organs of Broiler consumed in Chattogram, Bangladesh.

1.2.2 Specific Objectives

- To identify the presence of heavy metal in water, poultry feed and different body parts of broiler chicken in Chattogram metropolitan area farm
- To quantify the accumulation of Chromium from feed to different body parts of poultry like flesh, liver and brain
- To evaluate the public health risk associated with heavy metal contamination in broiler meat

Chapter II

Review of literature

2.1 Heavy metal

The term heavy metals have been widely used as a group name for metals and metalloids that have been associated with contamination and potential toxicity or eco-toxicity. Different terminologies have been used in the definition of heavy metals.

In terms of density; they are referred to as metals with density (specific gravity) greater than 5 g/cm^3 (Lozet and Mathieu, 1991; Morris, 1992).

In terms of atomic weight; they are metals with high atomic weight (relative atomic mass) (Porteous, 1996; Oxford Dictionary of Sci., 1999).

In terms of atomic number; they are metals with atomic number greater than 20 (Hale and Margham, 1988) or metals with atomic number between 21 (scandium) and 92 (uranium) (Lyman, 1995).

Definition in terms of toxicity; elements commonly used in industry and generically toxic to animals and to aerobic and anaerobic processes, but not everyone is denser or entirely metallic. Includes: As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Zn (Scott and Smith, 1981).

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 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; fertilizers; paints; treated woods; aging water supply infrastructure; and micro plastics floating in the world's oceans (Halliwell *et al*,. 2000).

2.4 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 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 in organisms as they are hard to metabolize (Pezzarossa *et al.*, 2011).

2.5 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.

2.5.1 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 (Agency for Toxic Substances and Disease Registry, 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) (Agency for Toxic Substances and Disease Registry, 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).

2.5.2 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 (Agency for Toxic Substances and Disease Registry, 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) (Agency for Toxic Substances and Disease Registry, 2000)

2.5.3 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 *et al.*, 1997).

2.6 Heavy metals in water

The contamination of fresh waters with a wide range of pollutants has become a matter of great concern over the last few decades (Al-Weher, 2008). The aquatic systems receive a large amount of heavy metals from natural occurring deposits and natural processes and anthropogenic activities (Wogu and Okaka, 2011). Anthropogenic sources arising from human activities such as industrial, municipal effluents, as well as non-point source run off are the main sources of metals in rivers (Sonayei *et al.*, 2009).

Discharge of heavy metals into rivers or any other aquatic environment can change both aquatic species diversity and ecosystems due to their toxicity and accumulative behaviour (Al-Weher, 2008). Heavy metals dissolved in water also endanger the lives of the public who use it for drinking and also irrigation. When used for irrigation heavy metals have the danger of being incorporated in food chain and therefore ingested by the public (Wogu and Okaka, 2011). Heavy metals accumulate in the soils at toxic levels as a result of long term application of untreated waste water and therefore soils irrigated by wastewater accumulate heavy metals in their soil surface (Sonayei *et al.*, 2009). When the capacity of the soil to retain heavy metals is reduced due to repeated application of waste water, the metals leach into ground water or soil solution available for uptake (Sonayei *et al.*, 2009). Table 2.1 shows metal limits in water set by national and international organizations.

Table 2.1 Heavy metal limits (μg/l) in water set by different national and international organizations

	Cd	Cr	Mn	Pb	Zn
USEPA	5	100	50	10	500
EU	5	50	50	10	Nm
WHO	3	50	400	10	NGL
Iranian	10	50	500	50	Nm
Australian	2	50	500	10	3000
Indian	10	50	100	100	5000
New Zealand	4	50	400	10	1500

Nm- not mentioned

NGL – No guideline, because it occurs in drinking water at concentrations well below those at which toxic effects may occur

Source: Mebrahtu and Zerabruk (2011)

2.7 Heavy Metal- Chromium

Chromium is a naturally occurring element found in rocks, soil, plants, animals, and in volcanic dust and gases. Chromium is present in the soil as (Cr III) or chromate (Cr VI) ions Chromium (III) is an essential nutrient in the diet, but it is required in a very small amount (Cataldo *et al*., 1981).

2.7.1 Forms of Chromium

Chromium is the 21st most abundant mineral in the crust of the earth. Although chromium (relative atomic mass 51.996 g) may theoretically occur in all oxidation states from –2 to +6, it is most often found in 0, +2, +3 and +6. Elemental chromium (0) is not naturally present in the earth crust and is biologically inert.

Almost all naturally occurring Cr is trivalent while hexavalent Cr is mostly of industrial origin. Most Cr compounds are halides, oxides or sulphides.

2.7.2 Divalent chromium (Cr^{2+})

It is a strong reductant; the form is readily oxidised when in contact with air, producing Cr^{3+}.

2.7.3 Hexavalent chromium (Cr^{6+})

It is the second most stable form and a strong oxidising agent, especially in acidic media. Hexavalent chromium is bound to oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) with a strong oxidative capacity. This form of Cr crosses biological membranes easily, reacting with protein components and nucleic acids inside the cell while being deoxygenated to Cr^{3+}. The reaction with genetic matter makes Cr^{6+} carcinogenic.

2.7.4 Trivalent chromium (Cr^{3+})

It is the most stable oxidation state in which Cr is found in living organisms. It does not have the capacity to cross cell membranes easily (Mertz, 1992) and has a low reactivity, which is the most significant biological feature distinguishing it from Cr^{6+}. Trivalent Cr forms a number of coordination complexes, hexadentate ligands being the basic form *e.g*:

Inorganic forms

1. Chromium chloride ($CrCl_3$)
2. Chromium oxide (Cr_2O_3)

Organic forms

1. Chromium polynicotinate ($Cr(C_6H_4NO_2)_3$)
2. Chromium- methionine ($Cr(C_5H_{10}NO_2S)_3$)
3. Chromium picolinate : ($Cr(C_6H_4NO_2)_3$) contains Cr and three picolinic acid (pyridine compound with a structure similar to nicotinic acid) molecules.

2.7.5 Chromium in Poultry

A meat is a food product from animals. It is a major source of proteins along with certain minerals, vitamins and trace metals, which are essential for healthy growth and development. Poultry is one such nutritious animal product. Among them, meat from chicken has remained a major source of animal protein in the daily diet of humans (Salawu *et al.,* 2014. Chicken is an important source of protein in Bangladesh and widely consumed in all over Bangladesh. The risk associated with the exposure to Chromium (Cr) present in food product had aroused widespread concern in human health. Investigated all the poultry feeds contained considerable amount of Cr. Over the last several years poultry has become a very popular and promising sector in Bangladesh. A large section of Bangladesh people consume poultry meat and egg to fulfill their protein demand (Shahidul *et al.,* 2007). The increasing interest currently developed by researchers globally on the challenges of heavy metal contamination of meat product is borne out of the fact that heavy metals have been known to cause serious tissues and organ toxicity at trace amounts.

The poultry farming has now turned into one of the most important divisions of agriculture in the world and improved rapidly as a dynamic industry in Asia (Mahesar *et al.,* 2010). Nowadays, poultry feed is produced from various raw materials such as fish by-products that can transfer heavy metals to poultry feed in undesirable levels following collecting them from contaminated waters (Shah *et al.,* 2010). The chicken is used as the most consumable food item in Iran and many countries of the world due to low cost, easy availability and valuable nutritional constitutes such as protein, vitamins and essential minerals (Fallah *et al.,* 2011; Saei-Dehkordi and Fallah, 2011; Shah *et al.,* 2010).

Almost all of the tanning industries in Bangladesh tan hides using chrome tanning method which is associated with vigorous chromium contamination both in liquid and solid waste form disposal. About 40 heavy metals and various acids are used during the processing of raw hides (UNIDO, 2005).

Generally basic chromium sulphate salt is used in conventional chrome tanning of which about 60% to 70% of chromium compound is consumed by hides and skins. Serious illness such as kidney disease, damage to the nervous system, diminished intellectual capacity, heart disease, gastrointestinal diseases, bone fracture, cancer and even death can also happen due to the absorption of heavy metals through food (Adeel *et al.,* 2012).

About 90% tanning industries use basic Chromium Sulfate (BCS) during tanning, (Aravindhan *et al.,* 2004); this chromium sulfate binds with the collagen protein to make stabilize it against degradation. On an average, only 60% of the entire chromium used is taken up by the pelt, while the remaining 40% chromium expelled through solid and liquid wastes, especially as spent chrome liquor (Fabiani *et al.,* 1997).

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)

Solid wastes generated in tannery industries mainly consist of skin trimmings, keratin wastes, fleshing wastes, chrome shaving wastes, and buffing wastes (Kanagaraj *et al.*, 2006). Chrome shaving dusts, wet blue scraps, and buffing dusts contain huge amount of chromium. It is reported that chrome shaving dusts contain chromium in the range of 10.68 ± 1.98 mg/g (Swarnalatha *et al.*, 2008). Disposing of this high chromium content solid wastes as land fill or dumping these may cause leaching of chromium to ground water and thus cause its entry into the human food chain.

As far as the feed ingredients and the compound feed for swine and poultry are an integral part of the consumer’s food chain, they need to be assessed as potential sources of heavy metal contamination.

2.7.6 Health effects

- Chromium (VI) compounds are toxins and known human carcinogens, whereas Chromium (III) is an essential nutrient.
- 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.
- Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation. (Suparna *et al.,* 2011)

2.7.7 Mechanisms of Toxicity and Carcinogenicity

Major factors governing the toxicity of chromium compounds are oxidation state and solubility. Cr (VI) compounds, which are powerful oxidizing agents and thus tend to be irritating and corrosive, appear to be much more toxic systemically than Cr (III) compounds, given similar amount and solubility (Connett et al., 1983). Although the mechanisms of biological interaction are uncertain, the variation in toxicity may be related to the ease with which Cr (VI) can pass through cell membranes and its subsequent intracellular reduction to reactive intermediates. Since Cr (III) is poorly absorbed by any route, the toxicity of chromium is mainly attributable to the Cr (VI) form. It can be absorbed by the lung and gastrointestinal tract, and even to a certain extent by intact skin. The reduction of Cr (VI) is considered as being a detoxification process when it occurs at a distance from the target site for toxic or genotoxic effect while reduction of Cr (VI) may serve to activate chromium toxicity if it takes place in or near the cell nucleus of target organs (Dayan et al., 2001). If Cr (VI) is reduced to Cr (III) extracellularly, this form of the metal is not readily transported into cells and so toxicity is not observed. The balance that exists between extracellular Cr (VI) and intracellular Cr (III) is what ultimately dictates the amount and rate at which Cr (VI) can enter cells and impart its toxic effects (Cohen *et al.*, 1993).

Cr (VI) enters many types of cells and under physiological conditions can be reduced by hydrogen peroxide (H_2O_2), glutathione (GSH) reductase, ascorbic acid, and GSH to produce reactive intermediates, including Cr (V), Cr (IV), thiylradicals, hydroxyl radicals, and ultimately, Cr (III). Any of these species could attack DNA, proteins, and membrane lipids, thereby disrupting cellular integrity and functions (Mattia *et al.*, 2004).

Studies with animal models have also reported many harmful effects of Cr (VI) on mammals. Subcutaneous administration of Cr (VI) to rats caused severe progressive proteinuria, urea nitrogen and creatinine, as well as elevation in serum alanine aminotransferase activity and hepatic lipid peroxide formation (Kim et al., 1991). Similar studies reported by Gumbleton and Nicholls found that Cr (VI) induced renal damage in rats when administered by single sub-cutaneous injections. Bagchi et al. demonstrated that rats received Cr (VI) orally in water induced hepatic mitochondrial and microsomal lipid peroxidation, as well as enhanced excretion of urinary lipid metabolites including malondialdehyde (*Bagchi et al.*, 2004).

Adverse health effects induced by Cr (VI) have also been reported in humans. Epidemiological investigations have reported respiratory cancers in workers occupationally exposed to Cr (VI)-containing compounds. DNA strand breaks in peripheral lymphocytes and lipid peroxidation products in urine observed in chromium-exposed workers also support the evidence of Cr (VI)-induced toxicity to humans (Chu *et al.*, 2002). Oxidative damage is considered to be the underlying cause of these genotoxic effects including chromosomal abnormalities (Goulart *et al.*, 2005), and DNA strand breaks. Nevertheless, recent studies indicate a biological relevance of non-oxidative mechanisms in Cr (VI) carcinogenesis (Zhitkovich *et al.*, 2001).

Carcinogenicity appears to be associated with the inhalation of the less soluble/insoluble Cr (VI) compounds. The toxicology of Cr (VI) does not reside with the elemental form. It varies greatly among a wide variety of very different Cr (VI) compounds (Katz *et al.*, 1993). Epidemiological evidence strongly points to Cr (VI) as the agent in carcinogenesis. Solubility and other characteristics of chromium, such as size, crystal modification, surface charge, and the ability to be phagocytized might be important in determining cancer risk.

Studies in our laboratory have indicated that chromium (VI) is cytotoxic and able to induce DNA damaging effects such as chromosomal abnormalities, DNA strand breaks, DNA fragmentation and oxidative stress in Sprague-Dawley rats and human liver carcinoma cells (Patlolla *et al.,* 2009). Recently, our laboratory has also demonstrated that chromium (VI) induces biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *carassius auratus* (Velma *et al*., 2009).

Various hypotheses have been proposed to explain the carcinogenicity of chromium and its salts, however some inherent difficulties exist when discussing metal carcinogenesis. A metal cannot be classified as carcinogenic per se since its different compounds may have different potencies. Because of the multiple chemical exposure in industrial establishments, it is difficult from an epidemiological standpoint to relate the carcinogenic effect to a single compound. Thus, the carcinogenic risk must often be related to a process or to a group of metal compounds rather than to a single substance. Differences in carcinogenic potential are related not only to different chemical forms of the same metal but also to the particle size of the inhaled aerosol and to physical characteristics of the particle such as surface charge and crystal modification (Norseth, 1983).

2.8 Bioaccumulation of Heavy Metals

Bio-accumulation is a process whereby organisms store up metals acquired from their surrounding food medium into tissues by chelation process. The accumulation of a metal in an organism is the product of equilibrium between the concentration of the metal in the organism's environment and its rate of ingestion and excretion.

When organic forms of mercury are formed, they escape from water sediments onto the surface layers of the water. Aquatic organisms absorb them and the organisms are eventually eaten up by fish e.g. predator or tuna fish. The organic mercury contents pass into the gills and intestines and are stored up there for a long period of time. The fish is caught and eaten up by humans who get poisoned through it. In 1946, it was found in Japan that the run-off waste waters from a mine containing lead, cadmium, zinc were drained into one river called Jinzu. This river was used as a source for drinking, rice irrigation and fishing. The toxic heavy metals thus discharged into the river accumulated in the agricultural and aquatic biomass and passed up the food chain to human consumers. In 1960, chemical analysis carried out on the body tissues of a patient who died of metal poison in the area revealed that 530 mg Pb, 3,800 mg Cd, 7000 mg Zn were contained per kilogram of the bones of the patient (Aderomoti, 1996).

Within the past four to five decades, evidence has been obtained to support the conclusion that heavy metal content of certain foods, particularly fish, is often directly related to wastes discharged into streams, lakes and oceans. One of the first indications was provided in the early 1950’s by Japanese scientists who reported the death of 43 people who had consumed fishes caught in Minamata bay. The fishes were found to contain abnormally high levels of mercury which had been discharged into the bay by a chemical manufacturing plant (Aderomoti, 1996).

2.8.1. Factors affecting Bio-accumulation of heavy metals

Factors which influence bio-accumulation include:

- Persistence
- Surface area or total size of organism
- Some biological factors

2.8.1.1 Persistence

For any element or compound to accumulate in any organism, the exposure must continue over an extended period of time, particularly if the exposure is through food chain. This implies that the given element or compound must be resistant to available break down processes. In this case, it persists in the organism for as long as it lives.

2.8.1.2 Size of organism

In an aquatic environment, adsorption processes appear to be a significant step in bio-accumulation. Rice and Sikka (1973) and also Munson *et al*. (1976) cited in Aderemoti (1996) independently found that the size of an organism is a factor in determining bio-accumulation. Tinsley (1979) cited in Aderemoti (1996) observed that filamentous algae that grow in long strings have large surface area and show greater tendency to accumulate compounds from aquatic environment. Also, most organisms contain significant fat deposits which help accumulation of heavy metals in their tissues.

2.8.1.3 Biological factors

Different organisms have different metabolic rates and different food amounts and requirements to sustain these rates. Hence rates of food intake may also be a significant factor. Organisms that require high amounts of food intake may tend to accumulate high levels of a particular compound provided that they do not compensate by more active excretion.

2.8.2. Mechanism for Bio-Accumulation

Two mechanisms have been proposed for bioaccumulation. They are:

- Direct absorption
- Food chain

2.8.2.1 Direct absorption

In an aquatic environment, all organisms are exposed to the same concentrations, of the substances present in the environment. They all have equal opportunity to accumulate by absorption the substances from the environment into their tissues. Thus, for aquatic environment, direct uptake i.e. absorption appears more important than the food chain.

2.8.2.2 Food chain

In the terrestrial environment, the food chain process mechanism holds sway. The ultimate sink for metals and their compounds is the soil. The tendency for an element or its compound to accumulate must involve food chain process. A metal or its compounds in the soil is absorbed by root hairs of the plants, then to the stem and branches and finally to the fruits they bear. Man, animals and birds partake of the fruits. Thus, the metal has passed on food chain to higher animals. Similarly, grasses grown on soil infested by metal pollution absorbs these metals through their root hairs to their leaves. Grazing animals eat the grass and the metal is passed on to them. Man kills the animal for food and thus the metal is passed by food chain to him.

2.9 Analytical technique used for heavy metal analysis

Several techniques for the determination of heavy metal elements are currently in use. These include flame atomic absorption spectroscopy, inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Sonayei *et al.,* 2009), inductively coupled plasma –mass spectroscopy (ICP-MS) (Nassef *et al*., 2006), X-ray fluorescence and neutron activation analysis (Magdaleno *et al*., 2011). The AAS was used in this study because of its simplicity, reliability and sensitivity (Sarkar, 2005). A lot of studies on heavy metals in water, soil plants and animals have used atomic absorption spectroscopy as the method of analysis for the heavy metals. A study by Begum *et al*. (2009) in the analysis of lead, iron, zinc, nickel and copper in soil and plants employed the method for analysis. Similarly, Fong *et al*. (2008) in the analysis of copper, Cd, manganese, lead and zinc in urban roadside soils used atomic absorption spectroscopy in their analysis. Other researchers who have employed atomic absorption spectroscopy in their analysis of heavy metals include, Mamtaz and Chowdhury (2006) who studied iron, copper, manganese and zinc levels in urban solid waste, Awokunmi *et al*. (2010) in their study of Cd, cobalt, chromium, copper, lead, manganese, nickel and zinc levels in soils from a dumpsite, Mico *et al*., (2006) employed atomic absorption spectroscopy method in analysis of Cd, cobalt, chromium, copper, iron, manganese, nickel, lead and zinc in the agricultural soils of Segura River Valley in Spain. Al weher (2008), analysed levels of Cd, copper and zinc in three species of fish using atomic absorption spectroscopy method. Similarly Wieczorek *et al*. (2005) employed the same method of analysis in determining the levels of lead in cereal grain and soils adjacent to roadways.

2.9.1 Atomic spectroscopy

This technique is applicable to most gas phase elements over a wide range of concentrations and involves detecting, measuring and analyzing radiation that is either absorbed or emitted from the atoms or ions of the element of interest (McMahon, 2007). It involves three techniques: Absorption, emission and fluorescence. In all the above, the sample is decomposed by intense heat into hot gases consisting of free atoms and ions of the element of interest (McMahon, 2007). As atoms are the simplest and purest form of matter and cannot rotate or vibrate as a molecule does when subjected to high energy radiation, electrons within the atom undergo transitions. The high energy radiation is commonly produced by

a. Flame in flame atomic absorption spectroscopy (FAAS)
b. Electrothermal furnace in flameless graphite furnace atomic absorption spectroscopy (GFAAS)
c. Plasma in inductively coupled plasma-optical emission spectroscopy (ICP OES)
d. X-ray in X-ray fluorescence spectroscopy (XRF) ((Lajunen and Paavo, 2007))

The above four belong to one of three major types of atomic spectroscopy namely absorption, emission and fluorescence ((Lajunen and Paavo, 2007)).

2.9.2 Principle of AAS

An atom is made up of positively charged nucleus surrounded by a number of negatively charged particles necessary to provide neutrality. These atoms occupy discrete energy levels but it is possible for an electron to be moved from one level to another by introduction of energy. Such transitions will only occur if the available energy is equal to the difference between the two levels. Energy levels and the energies associated with electron transitions are unique for each element. When light (energy) of a characteristic wavelength enters an analytical system, outer shell electrons of corresponding atoms within the light path will be excited as energy is absorbed. The amount of light transmitted through the system from a source to the detector will be less. The loss of light is proportional to the number of atoms. The measurement of the radiation transmitted (using Beer-Lambert’s law) in such a transition form the basis of AAS. Beer- Lambert’s law relates absorbance, a to the concentration of metallic atoms in the atom cell, c as follows

$$\mathrm{Log}T^{-1} = a\ b\ c$$

Where

a is the absorptivity in grams per litre-centimetre

b is the atom width in centimeters

c is the concentration of atoms

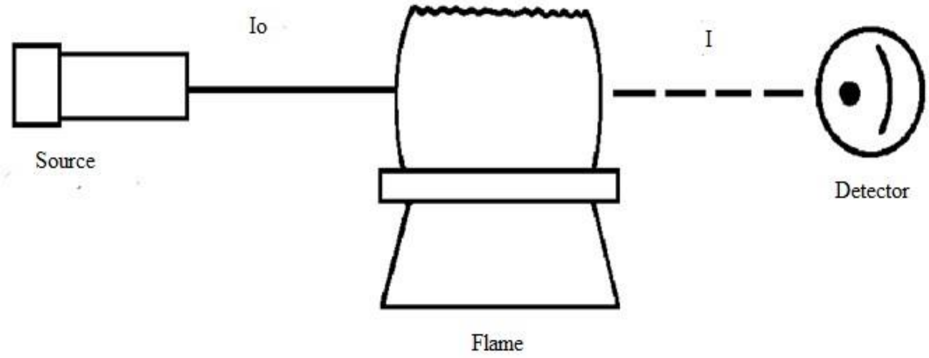


Figure 2.1: Diagram to illustrate instrumentation of AAS

The AAS involves the measurement of the drop in light intensity of initial radiation I_o to final radiation I depending on the concentration of the metal. Modern instruments automatically convert logarithmic values into absorbance (Nollet, 2011).

2.9.3 Instrumentation of AAS

Any atomic absorption spectrometer consists basically of a light source which emits the sharp line spectrum of elements to be determined, a method to produce atomic vapour of the sample to be analyzed, a monochromator for the spectral dispersion of the source radiation, a detector connected to an amplified read out system and a computer.

2.9.3.1 Light source

A continous source of radiation is required. A series of sources which can give sharp emission lines for a specific element are used. A hollow cathode glow discharge lamp is used. A hollow cathode lamp has two electrodes; one is cup shaped and made of a specific element. The metal used for the cathode is the same as the metal to be analyzed. The lamp is filled with noble gas at low pressure. It will produce a glow discharge from the hollow cathode. Metal atoms are evaporated by sputtering. The atoms accept energy of excitation and emit radiation with the lines of the metal. Hollow cathode lamps made out of several elements are available (Khopkar, 1998). The figure 2.3 below shows one of the light source used by AAS machine; a hollow cathode lamp

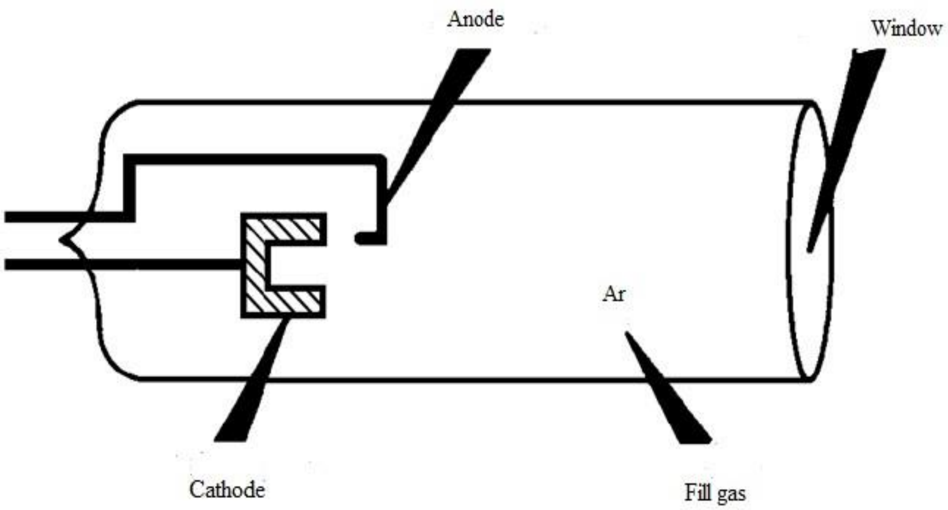


Figure 2.2: Hollow cathode lamp

2.9.3.2 Atomization

Several types of atomizers are used for atomization. These include flame, electrothermal, cold vapour technique for mercury and hydride generation (Nielsen, 2010). The flame atomizers consist of a nebulizer and a burner. The nebulizer is designed to convert the solution into a fine mist or aerosol. In flame atomization, atomization is carried out by flame. Heat energy is utilized to convert the metallic element to atomic dissociated vapour. The temperature should be controlled very carefully to convert it to atomic vapour. At too high or too low temperatures, atoms will be ionized and they will not be absorbed.

In atomization, fuel and oxidant gases are fed into a mixing chamber which passes through baffles to the burner head. A flame is produced and the sample is aspirated through the air into the mixing chamber. Only droplets of a small size pass through the baffles to the burner head. A narrow burner is therefore preferred and careful readjustment of the gas (Khopkar, 1998; Nielsen, 2010).

In electrothermal atomization, electrothermal atomizers are used. Electrothermal atomizers are typically cylindrical graphite tubes connected to an electric power supply. The sample is introduced into the tube through a small hole using a microlitre syringe. The system is flushed with an inert gas to prevent the tube from burning and exclude air from the sample compartment. The tube is heated electrically to evaporate the solvent, the sample is then ashed and further heated to quickly vapourized and atomize the sample (Nielsen, 2010). Cold vapour technique of atomization works for mercury only. In this technique, mercury compounds in a sample are reduced to elemental mercury by the action of stannous chloride, a strong reducing agent. The elemental mercury is then carried in a stream of air or argon into an absorption cell where absorption takes place (Nielsen, 2010). In hydride generation technique of atomization, volatile hydrides of elements are formed by reacting samples with sodium borohydride. The hydrides are carried into an absorption cell and heated to decompose them into free atoms. The atomic absorption measurements are then carried out (Nielsen, 2010).

2.9.3.3 Monochromator

A monochromator produces monochromatic light by removing unwanted wavelengths from the source light beam. It isolates a single atomic resonance line from the spectrum of lines emitted by hollow cathode lamp. Essentially it is an adjustable filter that selects a specific, narrow region of spectrum for transmission to the detector and excludes all wavelengths outside this region.

i. A monochromator comprises an entrance slit, a dispersion device and an exit slit.
ii. The entrance slit selects a defined beam of light from the source
iii. The dispersion device causes the different wavelength of light in the source beam to be dispersed at different angles
iv. The exit slit enables selection of a particular wavelength to produce the required monochromatic light

Figure 2.3 below shows a model of a monochromator used by an AAS machine

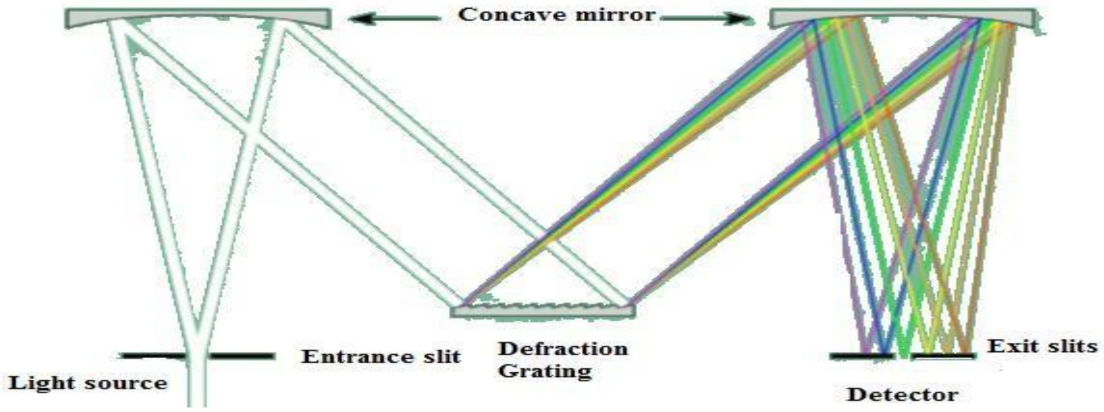


Figure 2.3: A model of a monochromator

There are two types of monochromators; prism and grating systems. Prisms and grating systems separate various wavelengths of light in different fashions. Prisms refract light at the interface of two surfaces with differing refraction indexes creating angular dispersion. Prisms have limitations, their resolution is significantly lower than a grating system and their separation technique is non-linear which creates mechanical problems with focusing a specific wavelength on the exit slit.

Diffraction gratings are materials with a large number of parallel and closely spaced slits or ridges. Diffraction causes constructive interference at unique points for each wavelength. The separated wavelengths are collimated with a concave mirror towards the exit slit. The tilt angle of the grating device determines the band of wavelength exiting the monochromator and reaching the detector.

2.9.3.4 The detector

Two detectors are used in atomic absorption spectrometers; photomultiplier tubes and solid state detectors (Nielsen, 2010). Detectors convert the radiant energy reaching it into an electrical signal. The signal is processed to produce either an analogue or digital read out. Modern instruments are interfaced with computers for data collection, manipulation and storage. The photomultiplier tubes are the most common types of detectors used.

Chapter III

Materials and Methods

3.1 Study area

Five broiler farms belonging to Chattogram 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.

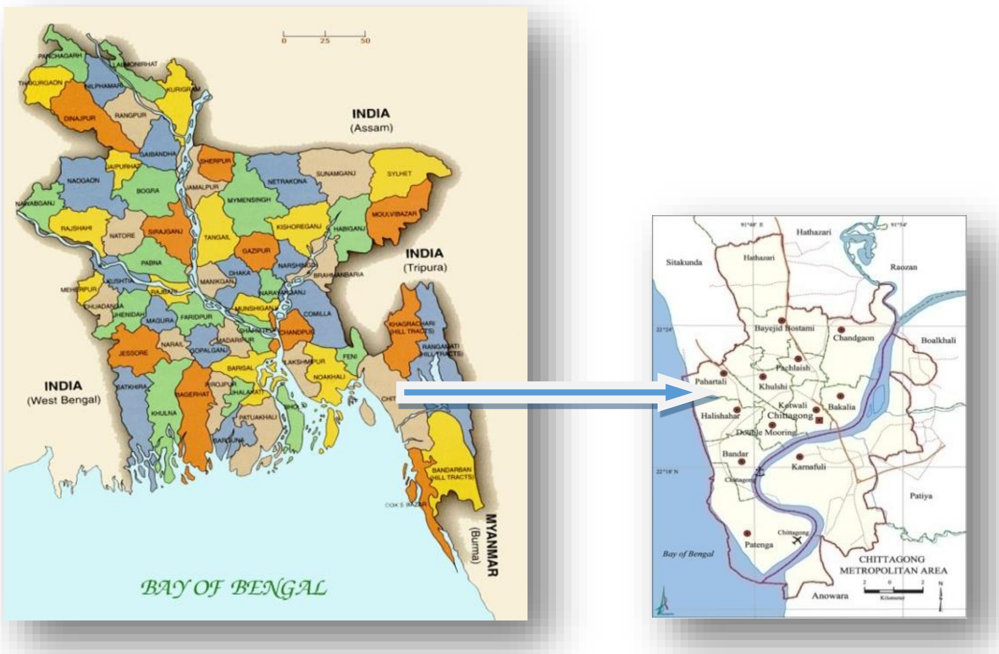


Fig.3.1 Study sites locating in the map of Bangladesh

3.2 Study period

The study was carried out during the period of July to December, 2018.

3.3 Study design

A cross-sectional study was carried out on different feed, meat, liver, brain and water samples which were collected from 5 types of selected broiler farms in the study area in order to determine the chromium.

3.4 Sample collection

Live poultry samples, water used for drinking and feed used for feeding were collected from broiler farms of Chattogram city area. After slaughtering the broiler, different edible parts such as breast meat, liver and brain were separated. Then meat, liver and brain samples were collected in polyethylene bags and stored at freezing temperature for analysis.

3.5 Number of samples

A total number of five types of market available feed, five water samples used for rearing broiler chicken as well as 15 broiler samples were randomly collected from respective five broiler farms.

3.6 Diagnostic evaluation

3.6.1 Processing of samples

3.6.1.1 Microwave digestion of meat, liver and brain sample

Fresh meat, liver and brain samples were digested in an acid solution with an Analytik Jena microwave digestion system. Firstly, 750 mg of the respective sample was weighed and transferred into the digestive vessel. Then 10 ml freshly prepared 65% Nitric acid(HNO_3) was added and closed the vessel. Thereafter, digestive vessel were placed in the microwave oven at $200℃$ for 20 min and then cooled to room temperature about 20 min.

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

3.6.1.2 Microwave digestion of feed sample

Feed samples were digested in an acid solution with an Analytik Jena microwave digestion system. Firstly, 300 mg of the sample was weighed and transferred into the digestive vessel. Then 10 ml freshly prepared 65% Nitric acid(HNO_3) was added. After shaking the mixture carefully, then closed the digestive vessel. Thereafter, digestive vessel were placed in the microwave oven at $230℃$ for 10 min and then cooled to room temperature about 40 min.

Filtrate sample was taken into felcon tube from standard volumetric flask and then samples were analyzed through Atomic Absorption spectrometer.

3.6.1.3 Digestion of water sample

100 ml water sample was taken in a conical flask. Then 65% Nitric acid (HNO_3) was added drop by drop until P^H of the water reached to 2. Thereafter, the water sample was transferred to volumetric flask and heated at 80-90℃ until the water became clear. Finally, made upto 100ml with distilled water.

3.6.2 Standard preparation

Chromium metal standard solution was prepared for the calibration of instrument for element being determined on the same day due to avoiding possible deterioration of standard with time. All samples were prepared from chemicals of analytical grade with distilled water. Firstly, 1g Chromium was dissolved in water and made up to 1 liter in volumetric flask with distilled water, thus stock solution of 1000 mg/l of Cr was prepared (Cantle, J.E. 1982). Then 100 ml of 0.1, 0.25, 0.5, 0.75, 1.0 and 2.0 mg/l of working standards of Cr metal was prepared from these stock using micropipettes in 5ml of 2N nitric acid. Reagent blank was prepared in the same manners of sample preparation without sample to avoid reagents contamination.

3.7 Detection and estimation

The atomic absorption instrument was set up and flame condition and absorbance were optimized for the analyte. Then blanks (deionized water), standards, sample blank and samples were aspirated into the flame in AAS (Model- iCE 3300, Thermo Scientific, Designed in UK, Made by China). The calibration curves obtained for concentration vs. absorbance. Data were statistically analyzed using fitting of straight line by least square method. A blank reading was also taken and necessary corrections were made during the calculation of concentration of various elements. All laboratory works were performed at Chittagong Veterinary and Animal Sciences University and Bangladesh Scientific and Industrial Research, BCSIR (Chattogram), Bangladesh.

3.8 Statistical analysis

All laboratory data were stored in Microsoft Excel 2007 and then exported into STATA^{IC} 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. The level of significance was set ≤ 0.05.

Chapter IV

Results

Heavy metal concentration estimated in fresh wet weight basis. The absorption wavelengths for the heavy metals were 357.9 nm for Cr. 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 feed, the highest mean concentration of chromium level was found 3.510 mg/kg. The highest chromium level shows the farm D. Chromium concentration in feed sample was found 0.00 in farm A, Farm C and Farm E respectively (Table-4.1).

Table-4.1: Concentration of chromium) in different feed sample

Sample Id	Mean ± SD	Minimum	Maximum	Maximum Permissible Limit
Farm A	0.000 ± 0.000	0.00	0.00	0.00
Farm B	3.157 ± 0.050	3.11	3.21	0.00
Farm C	0.000 ± 0.000	0.00	0.00	0.00
Farm D	3.510 ± 0.095	3.42	3.61	0.00
Farm E	0.000 ± 0.000	0.00	0.00	0.00

In case of meat, the highest mean concentration of chromium level was found 0.857 mg/kg. The highest chromium level shows the farm D. Chromium concentration in meat was found 0.00 in farm A, Farm C and Farm E respectively (Table-4.2).

Table-4.2: Concentration of chromium in different meat sample

Sample Id	Mean ± SD	Minimum	Maximum	Maximum Permissible Limit
Farm A	0.000 ± 0.000	0.00	0.00	1.00
Farm B	0.847 ± 0.095	0.74	0.92	1.00
Farm C	0.000 ± 0.000	0.00	0.00	1.00
Farm D	0.857 ± 0.076	0.0.79	0.94	1.00
Farm E	0.000 ± 0.000	0.00	0.00	1.00

In case of liver, the highest mean concentration of chromium level was found 2.7003 mg/kg. The highest chromium level shows the farm D. Chromium concentration in liver was found 0.00 in farm A, Farm C and Farm E respectively. (Table-4.3).

Table-4.3: Concentration of chromium in different liver sample

Sample Id	Mean ± SD	Minimum	Maximum
Farm A	0.000 ± 0.000	0.00	0.00
Farm B	2.017 ± 0.447	1.71	2.53
Farm C	0.000 ± 0.000	0.00	0.00
Farm D	2.700 ± 1.084	1.53	3.67
Farm E	0.000 ± 0.000	0.00	0.00

In case of brain, the highest mean concentration of chromium level was found 3.550 mg/kg. The highest chromium level shows the farm D. Chromium concentration in brain was found 0.00 in farm A, Farm C and Farm E respectively (Table-4.4).

Table-4.4: Concentration of chromium in different brain sample

Sample Id	Mean ± SD	Minimum	Maximum
Farm A	0.000 ± 0.000	0.00	0.00
Farm B	2.497 ± 0.966	1.88	3.61
Farm C	0.000 ± 0.000	0.00	0.00
Farm D	3.550 ± 1.281	2.10	4.53
Farm E	0.000 ± 0.000	0.00	0.00

In present study it was revealed that in case of water concentration of chromium was found 0.00 mg/l in all farm (Table-4.5).

Table-4.5: Concentration of chromium in different water sample

Sample Id	Mean ± SD	Maximum Permissible Limit
Farm A	0.000 ± 0.000	0.10
Farm B	0.000 ± 0.000	0.10
Farm C	0.000 ± 0.000	0.10
Farm D	0.000 ± 0.000	0.10
Farm E	0.000 ± 0.000	0.10

In case of all farms, correlation coefficient of feed and meat was strongly positive. Correlation coefficients of chromium in feed and meat at different farms are given below:

Table-4.6: Correlation coefficient of chromium in feed and meat at different farms

Sample Name	Farm A	Farm B	Farm C	Farm D	Farm E
Feed	0.000	0.995	0.000	0.772	0.000
Meat					

In this study, in case of farm A, farm C farm D and farm E correlation coefficient of chromium in feed and liver was strongly positive but farm B was strongly negative. The correlation coefficients of chromium in feed and liver at different farms are given below:

Table-4.7: Correlation coefficient of chromium in feed and liver at different farms

Sample Name	Farm A	Farm B	Farm C	Farm D	Farm E
Feed	0.000	-0.731	0.000	0.438	0.000
Liver					

In present study, it was revealed that in case of all farms correlation coefficient of feed and brain was strongly positive. The correlation coefficients of Chromium (Cr) in feed and brain at different farms are given below:

Table-4.8: Correlation coefficient of chromium in feed and brain at different farms

Sample Name	Farm A	Farm B	Farm C	Farm D	Farm E
Feed	0.000	0.941	0.000	0.287	0.000
Brain					

Chapter V

Discussion

Poultry is one of the major source of protein especially broiler in the developing country like Bangladesh. Now-a-days, it is an alarming issue that chromium is found highly accumulated in broiler meat and other edible organs. This may be occurred due to the chromium in commercial poultry feed, water and surrounding environment from which chromium is transmitted and accumulated during farming of broiler. Hence it is important to assess the chromium in poultry feed, water and surrounding environment to check the accumulation in broiler meat and other edible organs which might have serious harmful and carcinogenic effects. The current study was conducted to investigate the presence of chromium level in broiler feeds and meats from selected farms of Chattogram City, Bangladesh.

Cr (III) is a micro-nutrient and required in a very small amount for normal growth and development of animal and human but Cr (VI) is carcinogenic according to many research. The present study shows the total amount of Cr in different parts of chicken where Cr (III) and Cr (VI) might be present. 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).

In the regulations of EU (2003), there is no maximum allowed concentrations of chromium for feed ingredients and compound feed. However, chromium was detected in two commercial feed meal samples at high level. The maximum allowed limits for chromium concentrations in food lies between 0.1 and 0.5 mg/kg for human (Alkhalaf *et al.*, 2010).

The result of this study indicates that in poultry feed samples, the mean concentration of Cr was 0 to 3.51 mg/kg. Cr content in Farm D (3.61 mg/kg) was higher and in Farm A, Farm C and Farm E (0 mg/kg) was not found in poultry feed samples. Chromium was detected in two feed samples.

Jothi *et al.*, (2016) reported in a study that the mean concentration of Cr in commercial poultry feed was in a range Cr 10.63 to 218.10 mg/kg which is higher than our findings at this study.

WHO and EU permissible limit of chromium in drinking water is 0.05 mg L^{-1}. Chromium concentration in the water samples at this study was not found.

It is worthwhile to point out that, Chromium, particularly Cr (III) plays an important role in the body function (metabolic functions, co-factor of insulin production etc.) in trace amount but it turns to be toxic when it exceeds the tolerance limit (Alam *et al.*, 2011). The permissible limit of chromium in meat is 1 mg kg^{-1}. Iwegbue *et al.*, (2008), found that the concentration of Cr in chicken meat ranged between 0.01- 3.43 mg/kg which is above the permissible limit. Mean Chromium level in meat found at this study was 0 to 0.857 mg/kg· The maximum concentration of Cr was observed in Farm D sample (0.94 mg kg^{-1}) and concentration of Cr was observed zero in the Farm A, Farm C and Farm E meat sample. A similar observation was also reported by Uluozulu *et al.*, (2009) whereby they reported Cr in low concentrations in the muscles than in other internal tissues like the liver or gizzard. Islam *et al.*, (2016) found higher concentration in muscle (0.61 to 1.31 mg/kg) that was higher than permissible levels determined by FAO and WHO (Choi, 2011). According to the International Program on Chemical Safety (IPCS, 1998), chromium concentrations in chicken are in the range of 10-60 µg/kg. This study found chromium in some of the meat sample of broiler but lower than the permissible limit.

Nesheim *et al.*, (1979) noted that the liver is the site where metabolism occurs while the muscle has no known metabolic function.

Okoye *et al.*, (2015) reported that the concentration of chromium (Cr) in liver samples of broiler was 0.045 mg kg^{-1}. This study reveals that the mean concentration of chromium (Cr) in all liver samples were 0 to 2.7 mg/kg which were higher than the permissible limit. The maximum concentration of Cr was observed in Farm D sample and the minimum concentration of Cr was observed in the Farm A, Farm C and Farm E liver sample. Sadeghi *et al.*, (2015), also reported the mean concentration of chromium in liver was 3.87 mg/kg.

This study revealed that the mean Chromium concentration in brain of the broiler at was 0 to 3.550 mg/kg· The maximum concentration of Cr was observed in Farm D sample and the minimum concentration of Cr was observed in the Farm A, Farm C and Farm E brain sample. However, this finding is lower than the previously reported Cr concentration by Rajib *et al.*,2016 that was 4.5 mg/kg in the 29 days old chicken.

Regarding Cr accumulation in chicken this study found that highest amount of Cr was found in Brain and minimum amount was found in breast meat. 40% of commercial feed samples has Cr concentration higher than the maximum permitted concentrations, 40% of meat samples, 40% of liver and brain sample of broiler contaminated with higher than the permitted Cr concentration recommended by EU (2003) and all the water samples are free from Cr. In addition, the accumulation of Cr in different parts followed the order Brain>Liver > Breast.

Chapter VI

Conclusions

In the developing countries like Bangladesh broiler is one of the major source of protein which is essential for the synthesis of body tissue and growth of the body. It provides human with companionship, food and fiber in the form of eggs, meat and feathers. Alarming news is that several study revealed broiler meat and other edible organs are possessed with significant amount of Chromium which has highly toxic and carcinogenic effect on the human body for the long time consumption over the permissible limit. The result of this study indicates that broilers raised with commercial feed have significant concentrations of chromium over the permissible FAO/WHO levels. High concentration of chromium in broiler feeds resulted in their bioaccumulation in muscle tissues of broiler. There is significant correlation among feed, meat, liver and brain of the broiler. The higher the chromium in feed, the higher the accumulation in the different edible organs of the broiler. It has found that higher concentration of chromium is bio accumulated in the brain of the broiler other than the liver which is higher than the permissible limit and accumulation of chromium in broiler meat is lower than the permissible limit. Hence, broiler meat is almost safe to eat. But consumption of liver and brain of the broiler having bio accumulated chromium over the long time is harmful for human body stated by FAO, WHO and other standard organizations. To maintain the safety of food chain and to minimize the heavy metals contamination, it is mandatory for the feed producers to always observe and maintain standards for heavy metals in chicken feeds. Feed companies should carry out heavy metal assessment in their feed products to keep them at a safe limit. Government should take initiative to check the feed companies either they produce feed maintaining standard or not, ultimately ensuring the safe food and sound human health.

Chapter VII

Recommendations and Future perspectives

Ensuring food safety is current world's concern. The world is changing, so do the people. They are more conscious about food as well as their health. They try to keep their health free from diseases ensuring the food chain safe from any type of contamination. Heavy metal contamination in food is one of the major everybody's concern at this present age. Heavy metals are bio accumulated into the food while producing in the field either from the soil, environment or feed used for growing the poultry. Several study suggested to avoid boiler due to accumulation of heavy metals. It is quite impossible to avoid broiler only due to accumulation of several heavy metals from feed but they are the good and available source of protein. In this cases authorities and feed producers should take steps to avoid any type of heavy metal contamination of feed for using tannery waste as a source of protein in the feed meal. They should produce feed maintaining permissible limit of heavy metals set by the FAO, WHO and many other standard organizations. Broiler growers or the farmers also have some responsibilities such as testing the feed before selecting the feed for feeding the broiler to avoid those feed having higher concentration of heavy metals or which exceed the permissible limit, thereby ensuring safe feed for the broiler which is the major sources of heavy metal bioaccumulation into the meat, egg and others edible organs. Besides, farmers should test the water before using for drinking the broiler.

This study had some limitations. I had tested only Chromium (Cr) in the feed, water, meat, liver and brain. But along with chromium several heavy metals like Lead (Pb), Cobalt (Co), Arsenic(As), Cadmium (Cd), Nickel (Ni) etc. may come into the feed from the protein sources used for manufacturing poultry feed and they are also harmful for human when exposed with food for the long time above the permissible limit. They can also lead to carcinogenicity in the human health. Hence, it is also important to detect and determine the concentration of these heavy metals not only in the feed and water but also in all the edible organs as well as in the eggs of broiler and layers respectively. Besides all the poultry products should also checked for the detection and determination of heavy metals before entrance into the market.

References

Adeel SS, Wajid A, Hussain S, Malik F, Sami Z, Haq IU, Hameed A, Channa RA. 2012. Recovery of chromium from the tannery wastewater by use of Bacillus subtilis in Gujranwala, Pakistan. Journal of Pharmacy and Biological Sciences. 2(2):36-45.

Al-Weher SM. 2008. Levels of heavy metal Cd, Cu and Zn in three fish species collected from the Northern Jordan Valley, Jordan. Jordan Journal of Biological Sciences. 1(1):41-46.

Aravindhan R, Madhan B, Rao JR, Nair BU, Ramasami T. 2004. Bioaccumulation of chromium from tannery wastewater: an approach for chrome recovery and reuse. Environmental science & technology. 38(1):300-306.

ATSDR. 1999. HazDat (Hazardous Substances Database). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Bagchi D, Vuchetich PJ, Bagchi M, Hassoun EA, Tran MX, Tang L, Stohs SJ, 1997. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. Free Radical Biology and Medicine. 22(3):471-478.

Barnhart J. 1997. Chromium chemistry and implications for environmental fate and toxicity. Soil and Sediment Contamination. 6(6): 561-568.

Baykov BD, Stoyanov MP, Gugova ML. 1996. Cadmium and lead bioaccumulation in male chickens for high food concentrations. Toxicological & Environmental Chemistry. 54(1-4): 155-159.

Begum A, Ramaiah M, Khan I, Veena K. 2009. Heavy metal pollution and chemical profile of Cauvery River water. Journal of Chemistry. 6(1): 47-52.

Cabeza LF, Taylor MM, DiMaio GL, Brown EM, Marmer WN, Carrio R, Celma PJ, Cot J. 1998. Processing of leather waste: pilot scale studies on chrome shavings. Isolation of potentially valuable protein products and chromium. Waste management. 18(3): 211-218.

Choi YY. 2011. International/national standards for heavy metals in food. Government Laboratory (Australia).

Chowdhury MZA, Siddique ZA, Hossain SA, Kazi AI, Ahsan AA, Ahmed S, Zaman MM. 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-Sereli A, Massas I. 1997. Variations in plant and soil lead and cadmium content in urban parks in Athens, Greece. Science of the Total Environment. 196(1): 91-98.

Chu CC. 2002. Inhibitory effect of tannic acid and its related compounds on DNA damage in human lymphocytes exposed to H2O2 and food mutagens. Master's Thesis. 1-65.

Cohen MD, Kargacin B, Klein CB, Costa M. 1993. Mechanisms of chromium carcinogenicity and toxicity. Critical reviews in toxicology. 23(3): 255-281.

Connett PH, Wetterhahn KE. 1983. Metabolism of the carcinogen chromate by cellular constituents. In Inorganic Elements in Biochemistry. 93-124.

Cunningham WP. Saigo BW. 1997. Environmental Science a Global Concern. 4th Ed. WMC Brown Publisher, New York. pp: 389.

Das S, Grewal A, Banerjee M. 2011. A brief review: Heavy metal and their analysis. International journal of Pharmaceutical Sciences Review and research. 11(1): 13-18.

Dayan AD, Paine AJ. 2001. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000. Human & experimental toxicology. 20(9): 439-451.

Demirezen D, Uruç K. 2006. Comparative study of trace elements in certain fish, meat and meat products. Meat science. 74(2): 255-260.

EPA U, 1998. Toxicological Review of Trivalent Chromium (CAS No. 16065-83-1). National Center for Environmental Assessment, Office of Research and Development, Washington, DC.

EU. 2003. Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, Health and Consumer Protection Directorate-General, European Union, Belgium.

Fabiani C, Ruscio F, Spadoni M, Pizzichini M. 1997. Chromium (III) salts recovery process from tannery wastewaters. Desalination. 108(1-3): 183-191.

Fagliano JA, Savrin J, Udasin I, Gochfeld M. 1997. Community exposure and medical screening near chromium waste sites in New Jersey. Regulatory Toxicology and Pharmacology. 26(1): S13-S22.

Fallah AA, Saei-Dehkordi SS, Nematollahi A, Jafari T. 2011. Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (Oncorhynchus mykiss) using ICP-OES technique. Microchemical Journal. 98(2): 275-279.

Flores-Magdaleno H, Mancilla-Villa OR, Mejía-Saenz E, Olmedo-Bolantilde MDC, Bautista-Olivas AL. 2011. Heavy metals in agricultural soils and irrigation wastewater of Mixquiahuala, Hidalgo, Mexico. African Journal of Agricultural Research. 6(24): 5505-5511.

Fong FT, Chee PS, Mahmood AA, Tahir NM. 2008. Possible source and pattern distribution of heavy metals content in urban soil at Kuala Terengganu town center. Malaysian Journal of Analytical Sciences. 12(2): 458-467.

Goulart M, Batoreu MC, Rodrigues AS, Laires A, Rueff J. 2005. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutagenesis. 20(5): 311-315.

Halliwell D, Turoczy N, Stagnitti F. 2000. Lead concentrations in Eucalyptus sp. in a small coastal town. Bulletin of environmental contamination and toxicology. 65(5): 583-590.

Hemminki K, Vainio H. 1984. Human exposure to potentially carcinogenic compounds. IARC scientific publications. (59): 37-45.

Hossain SM, Barreto SL, Silva CG. 1998. Growth performance and carcass composition of broilers fed supplemental chromium from chromium yeast. Animal feed science and technology. 71(3-4): 217-228.

Iftikhar B, Arif S, Siddiqui S, Khattak R. 2014. Assessment of Toxic Metals in Dairy Milk and Animal Feed in Peshawar, Pakistan. Journal of British Biotechnology. 4(8): 883-893.

IPCS. 1998. Environmental Health Criteria for Chromium, Published by United Nations Environment Programme, the International Labor Organization, and the World Health Organization, Geneva.

Islam MM, Kabir SML, Sarker YA, Sikder MMH, Islam SKS, Akhter AHMT, Hossain MM. 2016. Risk assessment of chromium levels in broiler feeds and meats from selected farms of Bangladesh. Bangladesh Journal Veterinary Medicine. 14: 131-134.

Islam MS, Kazi MAI, Hossain MM, Ahsan MA, Hossain AM. 2007. Propagation of heavy metals in poultry feed production in Bangladesh. Bangladesh Journal of Scientific and Industrial Research. 42(4): 465-474.

Iwegbue CMA, Nwajei GE, Iyoha EH. 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.

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. 2014. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary toxicology. 7(2):60-72.

Jothi JS, Yeasmin N, Anka IZ, Hashem S. 2016. Chromium and lead contamination in commercial poultry feeds of Bangladesh. International Journal of Agricultural Research, Innovation and Technology. 6(2): 57-60.

Kanagaraj J, Velappan KC, Babu NK, Sadulla S. 2006. Solid wastes generation in the leather industry and its utilization for cleaner environment-A review.

Katz SA, Salem H. 1993. The toxicology of chromium with respect to its chemical speciation: a review. Journal of Applied Toxicology. 13(3): 217-224.

Kim E, Na KJ. 1991. Nephrotoxicity of sodium dichromate depending on the route of administration. Archives of toxicology. 65(7): 537-541.

Lajunen J, Peramaki P. 2009. Spectrochemical analysis by atomic absorption and emission. Burlington, Uk: Royal society of Chemists. 810.

Lozet J, Mathieu C. 1991. Dictionary of Soil Science, 2nd ed.

Lyman WJ. 1995. Transport and transformation processes, in Fundamentals of Aquatic Toxicology, G. M. Rand (Ed.), Taylor & Francis, Washington DC.

Mahesar SA, Sherazi STH, Niaz A, Bhanger MI, Rauf A. 2010. Simultaneous assessment of zinc, cadmium, lead and copper in poultry feeds by differential pulse anodic stripping voltammetry. Food and Chemical Toxicology. 48(8-9): 2357-2360.

Mamtaz R, Chowdhury M. 2006. Leaching characteristics of solid waste at an urban solid waste dumping site. Journal of Civil Engineering (IEB). 34(2): 71-79.

Mattia GD, Bravi MC, Laurenti O, Luca OD, Palmeri A, Sabatucci A, Mendico G, Ghiselli A. 2004. Impairment of cell and plasma redox state in subjects professionally exposed to chromium. American journal of industrial medicine. 46(2): 120-125.

Mazumder LT, Hasan S, Rahman ML. 2013. Hexavalent chromium in tannery solid waste based poultry feed in Bangladesh and its transfer to food chain. Journal of environmental science, Toxicology and Food Technology. 3: 44-51.

McMahon G. 2008. Analytical instrumentation: a guide to laboratory, portable and miniaturized instruments. John Wiley & Sons.

Mebrahtu G, Zerabruk S. 2011. Concentration and health implication of heavy metals in drinking water from urban areas of Tigray region, Northern Ethiopia. Momona Ethiopian Journal of Science. 3(1): 105-121.

Mendoza MP, Llopis CM, Sanchez J, Boix LR. 2006. Heavy metal content of agricultural soils in a Mediterranean semiarid area: the Segura River Valley (Alicante, Spain). Spanish Journal of Agricultural Research. (4): 363-372.

Muchuweti M, Birket JW, Chinyanga E, Zvauya R, Scrimshaw MD, Lester JN. 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implications for human health. Agriculture, Ecosystems & Environment. 112(1): 41-48.

Musante CL, Ellingwood MR, Stilwell DE. 1993. Cadmium contamination of deer livers in Connecticut. Bulletin of environmental contamination and toxicology. 51(6): 838-843.

Nassef M, EI-Tahawy MS, Hannigan R, Sayed KA, 2006. Determination of some heavy metals in the environment of Sadat industrial city.

Nesheim MC, Austic RE, Card LE. 1979. Poultry production (No. Ed. 12). Lea & Febiger.

Nielsen SS. 2010. Phenol-sulfuric acid method for total carbohydrates. In Food analysis laboratory manual (pp. 47-53). Springer, Boston, MA.

Nisianakis P, Giannenas I, Gavriil A, Kontopidis G, 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.

Norseth T. 1986. The carcinogenicity of chromium and its salts. British journal of industrial medicine, 43(10): 649.

Okoye COB, Ibeto CN, Ihedioha JN. 2011. Assessment of heavy metals in chicken feeds sold in south eastern, Nigeria. Advances in Applied Science Research. 2(3): 63-68.

Okoye PAC, Ajiwe VIE, Okeke OR, Ujah II, Asalu UB, Okeke DO. 2015. Estimation of Heavy Metal Levels in the Muscle, Gizzard, Liver and Kidney of Broiler, Layer and Local (Cockerel) Chickens Raised within Awka Metropolis and Its Environs, Anambra State, South Eastern Nigeria. Journal of Environmental Protection. 6(06): 609.

Patlolla AK, Barnes C, Yedjou C, Velma VR, Tchounwou PB. 2009. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. Environmental Toxicology: An International Journal. 24(1): 66-73.

Pellerin C, Booker SM. 2000. Reflections on hexavalent chromium: health hazards of an industrial heavyweight. Environmental health perspectives. 108(9): A402.

Pezzarossa B, Gorini F, Petruzzelli G. 2011. Heavy metal and selenium distribution and bioavailability in contaminated sites: a tool for phytoremediation. Dynamics and Bioavailability of Heavy Metals in the Roots Zone, CRC Press Taylor & Francis Group, Florida: 93-127.

Porteous A. 2013. Dictionary of environmental science and technology. John Wiley & Sons.

Rajib A, Islam AS, Ahmed MR, Tariqur M, Rahman M, Rahman A, Ismail ABM. 2016. Detection of Chromium (Cr) using X-ray Fluorescence Technique and Investigation of Cr Propagation from Poultry Feeds to Egg and Chicken Flesh.

Ravindran B, Sekaran G. 2010. Bacterial composting of animal fleshing generated from tannery industries. Journal of Waste management. 30(12):.2622-2630.

Sadeghi A, Hashemi M, Jamali-Behnam F, Zohani A, Esmaily H, Dehghan AA. 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.

Salawu MB, Ibrahim AG, Lamidi LO, Sodeeq AE. 2014. Consumption and consumer preference for poultry meat types in Ibadan Metropolis. Journal of Economics and Sustainable Development. 5(28): 20-25.

Sanayei Y, Ismail N, Talebi SM. 2009. Determination of heavy metals in Zayandeh Rood river, Isfahan-Iran. World Applied Sciences Journal. 6(9): 1209-1214.

Santhi D, Balakrishnan V, Kalaikannan A, Radhakrishnan KT. 2008. Presence of heavy metals in pork products in Chennai (India). American Journal of Food Technology. 3(3): 192-199.

Sarkar B. 2005. Heavy metals in the environment. New York, USA: Taylor and Francis. :33-41.

Scientific Committee on Animal Nutrition. 2003. Opinion of the Undesirable Substances in Feed.

Scott JS, Smith PG. 1983. Dictionary of waste and water treatment. Butterworths.

Shanmugam P, Horan NJ. 2009. Optimising the biogas production from leather fleshing waste by co-digestion with MSW. Bioresource Technology. 100(18): 4117-4120.

Stern RM. 1982. Chromium compounds: production and occupational exposure. :5-47

Sudha PN. 2010. Are we eating chrome chicken. The Socioscan. 2(3-4): 69-71.

Sundar VJ, Raghavarao J, Muralidharan C, Mandal AB, 2011. Recovery and utilization of chromium-tanned proteinous wastes of leather making: A review. Critical reviews in environmental science and technology. 41(22): 2048-2075.

Swarnalatha S, Srinivasulu T, Srimurali M, Sekaran G. 2008. Safe disposal of toxic chrome buffing dust generated from leather industries. Journal of hazardous materials. 150(2): 290-299.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. In Molecular, clinical and environmental toxicology. :133-164.

Uluozlu OD, Tuzen M, Mendil D, Soylak M. 2009. Assessment of trace element contents of chicken products from Turkey. Journal of hazardous materials. 163(2-3): 982-987.

UNIDO. 2005. United Nations Industrial Development Organization Cost of Tanned Waste Treatment, 15th Session of the Leather and Leather Products Industry Panel, Leon, Mexico.

Velma V, Vutukuru SS, Tchounwou PB. 2009. Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. Reviews on environmental health. 24(2). 129-146.

WHO. 2003. Background document for development of WHO guidelines for drinking water quality. Geneva: World Health Organization, Chromium in drinking water.http://www.who.int/water_sanitation_health/dwq/chemicals/chromium/

Wieczorek J, Wieczorek Z, Bieniaszewski T. 2005. Cadmium and lead content in cereal grains and soil from cropland adjacent to roadways. Polish Journal of Environmental Studies. 14(4): 535-540.

Wogu MD, Okaka CE. 2011. Pollution studies on Nigerian rivers: heavy metals in surface water of warri river, Delta State. Journal of Biodiversity and Environmental Sciences. 1(3):7-12.

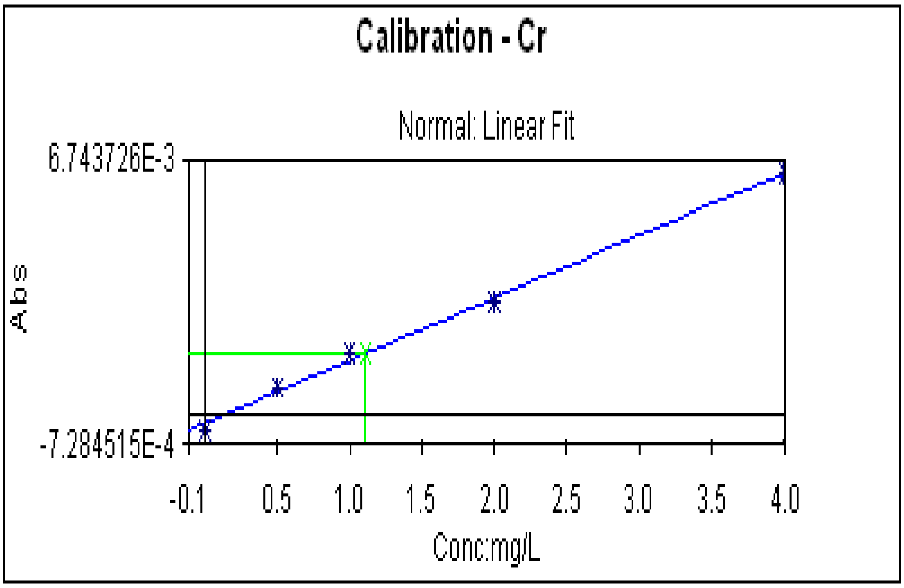
Yeasmin N, Ali MH, Begum A, Nazira Sharmin. 2018. Assessment of heavy metal concentration in poultry meat samples collected from chittagong city.

Zhitkovich A, Song Y, Quievryn G, Voitkun V. 2001. Non-oxidative mechanisms are responsible for the induction of mutagenesis by reduction of Cr (VI) with cysteine: role of ternary DNA adducts in Cr (III)-dependent mutagenesis. Biochemistry. 40(2): 549-560.

Appendix A: Spreadsheet of Chromium concentration in different samples

Sample Name	**Feed (mg / kg)**	**Meat (mg / kg)**	**Liver (mg / kg)**	**Brain (mg / kg)**	**Water (mg / kg)**
Farm A	0.00	0.00	0.00	0.00	0.00
Farm A	0.00	0.00	0.00	0.00	0.00
Farm A	0.00	0.00	0.00	0.00	0.00
Farm B	3.11	0.88	2.53	1.88	0.00
Farm B	3.15	0.92	1.71	2.00	0.00
Farm B	3.21	0.74	1.81	3.61	0.00
Farm C	0.00	0.00	0.00	0.00	0.00
Farm C	0.00	0.00	0.00	0.00	0.00
Farm C	0.00	0.00	0.00	0.00	0.00
Farm D	3.42	0.84	1.53	2.10	0.00
Farm D	3.5	0.79	3.67	4.53	0.00
Farm D	3.61	0.94	2.90	4.02	0.00
Farm E	0.00	0.00	0.00	0.00	0.00
Farm E	0.00	0.00	0.00	0.00	0.00
Farm E	0.00	0.00	0.00	0.00	0.00

Appendix B: Calibration curve of Chromium (Cr)



Appendix C: Sample Collection from broiler farm

Fig: Different steps of sample collection from broiler rearing farms

Appendix D: Sample preparation for Microwave Digestion

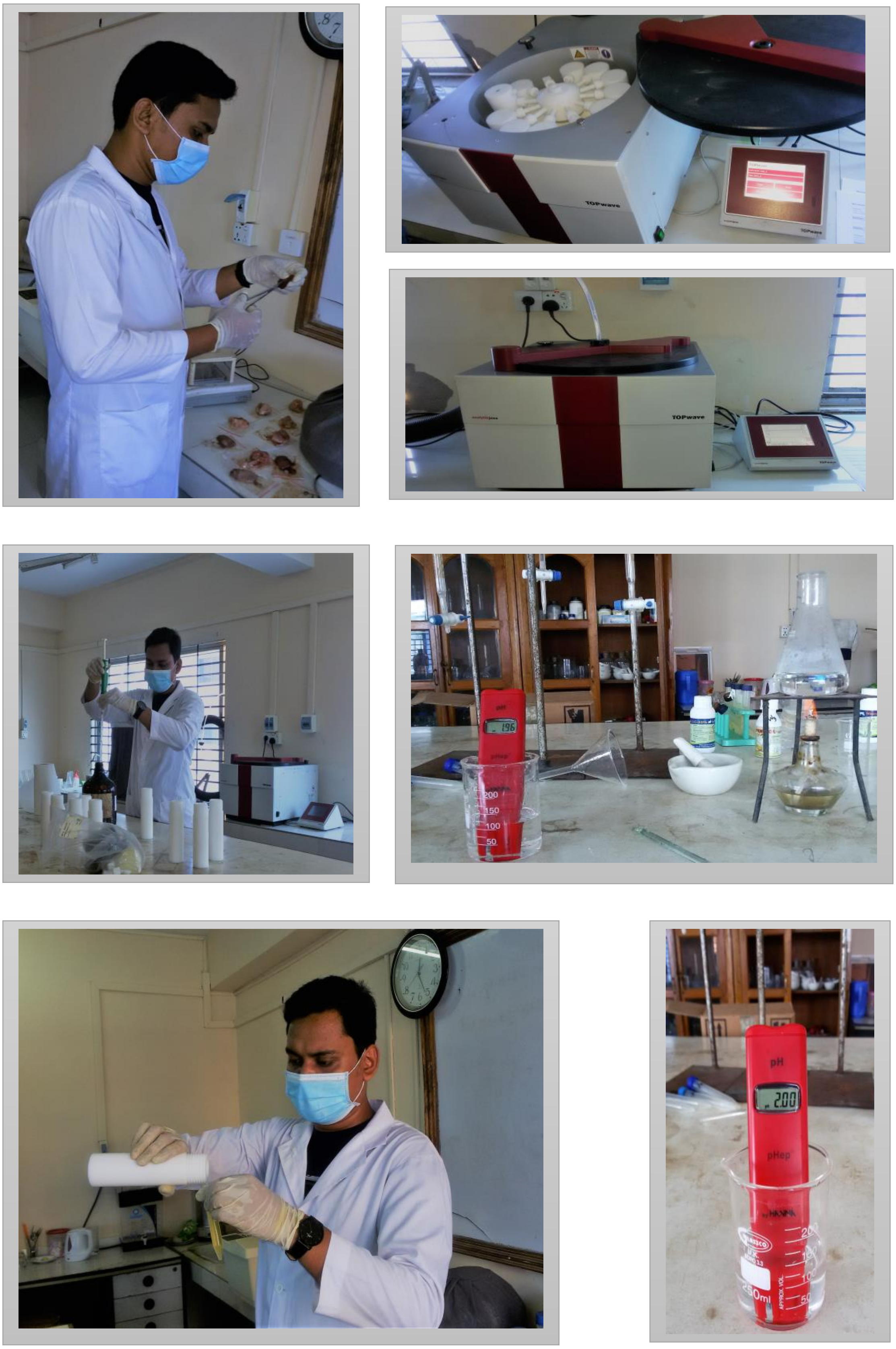


Fig: Different steps of sample preparation for analysis

Appendix E: Prepared sample and AAS instrument

Fig: Prepared sample for analysis

Fig: AAS instrument

Brief Biography

Md. Showkat Hossen completed B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh with CGPA 3.85 out of 4.00 and secures the second position in merit. Now, he is a candidate for the degree of MS in Food Chemistry and Quality Assurance under the Department of Food Chemistry and Quality Assurance, Chittagong Veterinary and Animal Sciences University (CVASU). He has immense interest to work in public health perspective like heavy metal detection in egg, meat, meat products, water, milk, vegetables and also in the area of Product development, Natural preservatives, Bio preservation of food, functional food product development and nutritional value analysis, relation of food properties and processing, quality control and quality assurance regarding food, taste and flavor.