Addis Ababa University

College of Natural and Computational Sciences

Center for Food Science and Nutrition

Proximate Composition and Levels of Essential/Toxic Metals in Chicken Meat Raised Around Industry Areas in Ethiopia

By

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Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been duly acknowledged.

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Signature **Date**

____________ 23/08/2019

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Lists of Acronyms

Name	Abbreviation
Al	Aluminum
ANOA	Analysis Of Variance
AOAC	Association of Analytical Chemists
As	Arsenic
ANZFA	Australia- New Zealand Food Authority
B	Boron
Ba	Barrium
bw	body weigh
B-Pb	Blood lead
Ca	Calcium
$CdCl_2$	Cadmium Chloride
CdO	Cadmium Oxide
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
DM	Dry Matter
DOM	Dissolved Organic Matter
EDTA	Ethylene Diamine Tetra-Acetic Acid
EPA	Environmental Protection Agency
EEPA	Ethiopian Environmental Protection Agency
FAO	Food and agriculture organization
Fe	Iron
g/L	gram per liter
GTF	Glucose Tolerance Factor
HCA	Heterocyclic Amine
Hg	Mercury
HNO_3	Nitric acid

H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulfuric acid
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
Li	Lithium
mg/L	milligram per liter
mg/Kg	Milligram per kilogram
Mn	Manganese
MoH	Ministry of Health
ND	Not Detected
Ni	Nickel
Pb	Lead
PbO	Lead oxide
$PbSO_4$	Lead sulfate
P	Phosphorus
PDCAAS	Protein Digestibility-Corrected Amino Acid Scores
Ppm	Parts per million
PTWI	Provisional Tolerable Weekly Intake
PUFA	Polyunsaturated Fatty Acid
RGM	Reactive Gaseous Mercury
Se	Selenium
SOD	Superoxide Dismutase
Sn	Tin
SPSS	Statistical Package for Social Sciences
TPN	Total Parental Nutrition
WHO	World Health Organization
SPSS	Statistical Package for Social Science
US	United States
Zn	Zinc
µg/kgbw	Microgram per kilogram body weight

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Abstract

The general trend of poultry production and consumption has been steadily increasing globally in recent because of its benefit to consumers' health from chicken meat and eggs The objective of this study was to determine proximate composition and levels of some selected essential metals (Zn, Cu, Mn, Cr, Co, & Ni) and toxic heavy metals (As, Pb, Hg, and Cd,) of chickens raised around Akaki, Bishofitu and Modjo industry areas, Ethiopia. Purposefully 5 hens from local markets of each industry area which were cultivated in a traditional way were purchased and used for the study purpose. Parts of chest and thigh were used for proximate analysis and parts of liver, thigh and chest were used for essential and toxic metals analysis purposes. Proximate analysis was done using AOAC (2000) using official methods. Wet digestion technique to extract heavy metals from sample was used and metals analysis was carried out using inductively coupled plasma optical emission spectroscopy. Results of proximate composition of chest and thigh samples collected from industry areas are summarized as, Protein 22.49, 21.97; Moisture 72.23, 70.50 Fat 1.14, 1.24, ash 1.11, 1.13 respectively. These results were found being comparable with literatures reviewed. The results of mean concentrations of metals from studied parts of industry areas of liver, thigh and chest samples (mg/Kg) ranged ; Zn 24.04-134.53 , Cu 2.90-9.24 Mn 1.40-8.64 , Cr 4.44 - 4.75, Co ND - 0.02, Ni 3.23 - 4.48 As 0.11- 0.33, Cd 0.01 - 0.98, Pb 0.02 - 0.16, Hg 0.05 - 0.09. The results of the mean concentrations of those essential and toxic metals were compared against permissible limits of each trace metal set by international standards; Cu from all studied sample parts; Zn from liver of Akaki, Cd from liver samples of Akaki and Bishofitu. Mean values of Mn, Cr and Ni from all sample parts were found to be higher than their respective permissible limits. Whereas all other trace metals other than Co, whose permissible limit is not mentioned in World health organization standard were found to have mean concentrations below than their permissible limits. Though essential metals are important for human life in various ways excessive and prolonged exposures of those trace metals may impose health risks to the community. Respiratory problems, increased risk of lung cancer, neurological deficits, developmental deficits in children and increased blood pressure are health risks due to Ni exposures. Neurological disorders similar to Parkinson's disease may result due to excessive Mn exposure. Similarly inhibition leading to Wilson's diseases after long time Cu exposure and, skin rashes, stomach ulcer, kidney, liver damages, lung cancer and ultimate death due to prolonged higher Cr exposures. The result also indicated that chickens of liver samples from Akaki and Bishofitu industry areas were found with much higher Cd concentrations (1.08±0.36mg/kg, and 1.45±0.68mg/kg respectively) reflecting the environment is polluted with Cd. Higher level Cd exposures might cause health impacts such as hypertension, elevated blood pressure, renal dysfunction, acute and chronic pulmonary dysfunctions to the community.

Taking the health risks due to environmental pollutions around industry areas into account, chicken meat that contains good nutritional values like proteins, fat, and essential minerals such as zinc, can also be used to alleviate poverty for the country.

Key words: *Chicken meat, proximate composition and Essential/Toxic metals, Environmental pollution.*

Chapter One

1. Introduction

1.1 Background

The general trend of poultry production and consumption has been steadily increasing globally in recent years that poultry meat accounts for about 33% of the global meat consumption of which 87% comes from chicken. Similarly, the consumer demand for chicken meat has been growing progressively over the last decade because of its benefit to consumers' health from chicken meat and eggs, which provide food containing high-quality protein with desirable amino acid profiles (Melesse, 2014). The proportional contribution of poultry to the total animal protein production of the world by the year 2020 is believed to increase to 40%, the major increase being in the developing world (Asresie & Mitiku, 2015). Approximately 800 million chickens are currently found on the African continent, of which 80% are kept under traditional scavenging production systems (Melesse, 2014). Previous studies reveal that the total poultry population in Ethiopia is estimated to be more than 51.35 million (Sebho, 2016), where the majorities (99%) of these chickens are maintained in a traditional way (Leta & Endalew, 2010).

The scavenging system plays an important role in supplying local populations with additional income and high-quality food in the form of meat and eggs (Goromela, *et al*., 2006). Religions and cultural considerations are also amongst the reasons for keeping chickens by resource poor farmers in Africa including Ethiopia (Asresie & Mitiku, 2015). In addition, the local chicken sector constitutes a significant contribution to human livelihood and contributes significantly to food security of poor households (Leta & Endalew, 2010)

Meat and meat products are important for human diet because they provide a great part of nutrients, including the necessary trace elements. As the flesh of animals used for food, it is a relevant dietary source of high biological value proteins, essential amino acids, chemical elements (e.g. iron, zinc) and vitamins (e.g. B12, D) (Zahrana, & Hendyb, 2015) which are either not present in plant derived food or have a poor bioavailability (Gerber, 2007). It also contains a range of fats, including essential omega-3 polyunsaturated fats (Williams, 2007). Among meat sources, chicken meat contains higher protein as well as lower fat and cholesterol contents than red meat, and consequently is considered superior for human health (Jung., *et al* .2015).

Essential elements have several important roles in human bodies. In fact, although the trace elements are essential components of biological activities, the excessive levels of these elements can be toxic for the body health and may lead to many fatal diseases, such as cancers (Al-Fartusie and Mohssan, 2017) ,i.e metals such as iron, copper, manganese, cobalt, zinc are essential for human body but chronic metabolic disturbances may occur due to the deficiency or excess of these metals . It is important to keep the level of these metals in their proper ranges for maintaining proper metabolic functions in human body (Golub, *et al.*, 2004).

Toxic metal is defined as that metal, which is neither essential nor has beneficial effect, on the contrary, it displays severe toxicological symptoms at low levels and is defined as a metal with a specific weigh more than 5 g/cm^3 (WHO, 2007). Non-essential elements such as lead, cadmium, mercury and arsenic are considered to be toxic and their presence in the body can cause profound biochemical and neurological changes in the body (Lukáčová *et al.*, 2014).

With increasing industrialization, more and more metals are entering into the environment. These metals stay permanently because they cannot be degraded in the environment. Eventually these heavy metals enter into the food material and from there they ultimately make their passage into the tissue (Onyeka and David, 2015). Population exposure to toxic metals is of greatest concern due to their non biodegradable nature (Chowdhury, *et al.*, 2011). The risk associated with the exposure to heavy metals present in food product has aroused widespread concern in human health. Contamination of food with various environmental pollutants, especially heavy metals and ingestion of these contaminants by animals causes deposition of residues in meat (Badis,, Rachid, & Esma,2014). The presence of heavy metals in chicken meat may result from natural occurrence in the soil, from where they are taken up by the plants that feed the chicken, or due to the use of contaminated fish powder as a source of animal protein feed, or from the remnants of vehicle exhausts, which are hit by air to the source of fodder and water to drink used in poultry (Abdolgader, *et al.* 2013). Hence though poultry meat provides nutritional benefits it may also be a source of toxic heavy metals and therefore should be clean and safe from all hazardous agents like cadmium, lead, mercury, and arsenic (Chowdhury, *et al.*, 2011).

1.2 Statement of the Problem

More than one in seven people today still do not have access to sufficient protein and energy from their diet, and even more suffer from some form of micronutrient malnourishment (Godfray, *et al.*, 2010). Poverty and food insecurity are among the most pressing social issues in the sub-Sahara continent at the beginning of the 21st century (Lemke, *et al.*, 2003). Food in security at the household level in Ethiopia dates back a long period, it has remained as a challenging goal even today (Bogale, & Shimelis, 2009). Poultry, where chickens and their products are an important source of food and income considerably contributes the cash income of the rural families in Ethiopia (Alem, *et al.*, 2014). Because it has fast generation interval and high productivity rate will be an interesting tool to respond rapidly to poverty gaps if included in rural development strategies (Reta, 2009). Scavenging poultry provide their owners with economic and nutritional benefits with little or without any inputs (Moreki, *et al.*, 2010).

Food safety is a term broadly applied to food quality that may adversely affect the human health (Lee,*et al*.,, 2001). Environmental pollutants always have hazardous impact on living organisms that affects life-style of living entities throughout the globe (Katole, *et al.*, 2013). Due to the focus only on development program on economic growth and industrialization, limited resources for environmental management and weak pollution legislation in most developing countries like Ethiopia has worsened heavy metal pollution. In a study, levels in (mg/kg) of Cd (0.345 ±0.18) in lettuce, Cr (24.11±2.4) in lettuce and Zn (130.1±6.4) in spinach exceeding recommended limits were recorded in Ethiopia (Yabe, *et al.*, 2010). Arsenics, chromium, iron, and lead from a study done to evaluate the levels of heavy metals in leafy vegetables grown in Addis Ababa were found to surpassed maximum permitted metals concentrations (Itanna, 2002).

Even though it's reported by different publications regarding the levels of essential/toxic metals on different food items in Ethiopia there was no study on this area on chicken meat and its proximate composition of chicken meat in our country, the present study attempted to investigate and put an effort on this hidden issue. That is leading us to have insufficient knowledge in order to draw firm conclusions on how industrialization affect the composition of essential/toxic heavy metals in chicken meat. The study also tried to determine the proximate composition of chicken meat.

1.3 Significance of the Study

This study was aimed to assess and determine the proximate composition, and level of essential/ toxic heavy metals in chicken meat raised around selected industry areas in Ethiopia. The nutritional composition of Ethiopian chickens may be used as a means for poverty alleviation. Besides to say food is quality it is not merely means it contains high nutritional components, rather it should be free from food contaminants too. Therefore knowing the level of the composition of those toxic trace heavy metals in chicken meat will help to take proper actions if the result showed beyond the permissive intake level.

In general this work will have the following uses

- ✓ Provide a baseline data for parties concerned regarding proximate, essential/toxic metals composition level in chicken meat
- ✓ Regulatory agencies like Ethiopian environmental protection agency (EEPA) may use the data for planning
- ✓ Health professional and Ministry of Health (MoH) may use this data concerning disease like cancer and other chronic illnesses
- ✓ Poultry farm individuals and agencies may use the information regarding the source of poultry feed and location of poultry farming selection
- ✓ The work will be a reference material for researchers, students and for policy makers who are concerned on proximate composition, essential/ toxic trace heavy metals and its health importance and consequences.

1.4 Objectives

1.4.1 General Objective

- The main objective of this study was to determine proximate composition and levels of essential/toxic heavy metals found in chicken meat raised around three selected industry areas; namely Akaki, Bishofitu and Modjo by the year 2018/19 GC

1.4.2 Specific Objectives

The specific objectives of this study were to:

- To determine the proximate composition (protein, moisture, fat and ash) of chicken meat raised around industry areas of Akaki, Bishofitu and Modjo
- To determine, compare and contrast the level of essential (zinc, manganese, copper, chromium, cobalt and nickel) and toxic (arsenic, cadmium, lead and mercury) heavy metals composition among chicken grown around selected industry areas
- To compare pooled mean composition of each essential and toxic trace heavy metals among the selected industry areas.

Chapter Two

2. Literature Review

2.1 Chicken Meat

Meat has exerted a crucial role in human evolution and is an important component of a healthy, especially the brain and intellectual development and well balanced diet due to its nutritional richness (Pereira & Vicente, 2013). It is very rich and convenient source of nutrients including also a large extent of microelements (Lukáčová *et al.* 2014). As the flesh of animals used for food, meat is a relevant dietary source of high biological value proteins, essential amino acids, chemical elements (e.g. Fe, Zn) and vitamins (e.g. B12, D) (Zahrana, & Hendyb, 2015) which are either not present in plant derived food or have a poor bioavailability (Gerber, 2007). It also contains a range of fats, including essential omega-3 polyunsaturated fats (Williams, 2007).

Meat is frequently associated with a ''negative'' health image due to its ''high'' fat content and in the case of red meat is seen as a cancer-promoting food. This was due to epidemiological early case control and cohort studies suggested a positive correlation between fat intake and incidences of breast, colon and prostate cancer. However, more recent cohort, large case control and pooled analysis of case control studies failed to detect an association between fat intake and colon cancer. (Biesalski, 2005). Instead meat as a protein-rich and carbohydrate-low product contributes to a low glycemic index, which is assumed to be beneficial with respect to obesity, diabetes development and cancer (Gerber, 2007). However it should be considered that carcinogens and promoters, e.g. heterocyclic amines (HCAs) are formed when meat is fried or cooked and may contribute more or less to the individual cancer risk, especially in colorectal, breast and prostate cancer (Biesalski, 2005).

The chemical composition of meat depends on both the kind and degree of the source animal (Badis, *et al.*, 2014). Chicken meat is an important and good source of essential amino acids, vitamins, and minerals/essential metals for human consumption (Olusola, *et al.*, 2012). It is also distinguished for its low energy concentration and, consequently, it has high nutrient density. Poultry meat, as well as other meats, is a good source of high biological value protein (20-22%). Poultry meat is distinguished for its low energy concentration and, consequently, it has high nutrient density. Besides, it provides Fe and Zn of high bioavailability in lower quantities than red meats, but important amounts compared with food of vegetable origin. Poultry meat has significant content of vitamins from group B such as thiamin, riboflavin, niacin and vitamin B6, although vitamin B12 content is less than in other meats. The quantity of vitamin E, pantothenic acid, folic and biotin of poultry meat is considerably low. Fat content in poultry meat is relatively low (Barroeta, 2007). Particularly the free rearing (scavenging) chickens have superior qualities than farm base ones, i.e., the free rearing eggs are higher in vitamins, minerals, and lower in cholesterol, while free range poultry is found to be leaner and tougher due to the chickens more active lifestyle and natural diet(Brower, *et al.*, 2013).

Though meat is an important source food, it may potentially accumulate toxic minerals and represents one of the sources of heavy metals for humans (Zahrana, & Hendyb, 2015). The risk associated with the exposure to heavy metals present in food product has aroused widespread concern in human health. Ingestion of these contaminants by animals including chicken causes deposition of residues in meat (Badis, *et al.*, 2014).

2.2 Nutritional Value of Chicken Meat

2.2.1 Meat Protein Content and Protein Value

Literatures showed that the average protein content of meat usually ranges from 20 - 25% (Williams, 2007). Poultry meats is considered as a good source of animal protein with high biological value as it contains all the essential amino acids, many vitamins and minerals which are required for human nutrition (Goyer & Clarkson,1996). It is also important to note that this protein has high digestibility scores as determined by the Protein Digestibility-Corrected Amino Acid Scores (PDCAAS). The higher PDCAAS 1.00 have been attributed to egg white and casein proteins. Meat scored 0.92, while pinto beans, lentils, peas and chickpeas which are broadly considered important protein sources in vegetarian diets scored values from 0.5 to 0.71(Williams, 2007) and wheat gluten had been classified with a 0.25 score (Pereira & Vicente, 2013). This can be explained to some extent with the fact that plant proteins are mostly embedded into polysaccharide matrices (cell walls) where they cannot be reached by the proteolytical enzymes (Gerber, 2007). Cereals like rice and wheat are especially poor in lysine while legumes have low contents of methionine (Pereira & Vicente, 2013).

The nutritional quality of a protein depends on the proportion of essential amino acids present; high quality dietary proteins containing the necessary amounts of essential amino acid to cover the needs of the human body where as low quality dietary proteins show an imbalanced ratio of essential amino acids; the most lacking is called the limiting amino acid (Gerber, 2007). Protein from meat provides all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) and has no limiting amino acids (Pereira & Vicente, 2013). The amino acid glutamic acid/glutamine is present in meat in the highest amounts (16.5%), followed by arginine, alanine and aspartic acid (Williams, 2007). Therefore, the requirement for dietary protein consists of two components; (a) a requirement for the nutritionally essential amino acids, and (b) the need to meet the requirement for non-specific nitrogen in order to supply the nitrogen necessary for synthesis of the nutritionally not essential amino acids and other physiologically important nitrogen containing compounds (nucleic acids, creatine, porphyrins) (Gerber, 2007).

2.2.2 Fat in Meat

Fat is the richest dietary energy source and storage; supplies essential nutrients such as essential fatty acids as well as precursors of compounds that regulate a number of physiological functions (e.g. prostaglandins) and helps to absorb fat-soluble vitamins (A, D, E and K). Fat also acts as a protection of the organs and as source of fatty acids which again act as structural element of cell membranes. Fat also provides palatability and flavor to food. In the right proportions it is therefore an essential component of any balanced diet (Gerber, 2007). Due to their antithrombotic effects, the presence of omega 3 fatty acids in meat could counteract in part the pro thrombotic effect of omega 6 fatty acids (arachnidonic acid) which increases the risk of thrombosis (Pereira & Vicente, 2013).

The most ubiquitous fatty acids in meat are oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acid. Linoleic acid (C18:2*n*-6) is the predominant Poly Unsaturated Fatty Acids (PUFA) (0.5 – 7 %), followed by alpha-linolenic acid (C18:3*n*-3). *Trans*-fatty acids comprise below 0.5 % of total fatty acids across all types of meat from monogastric animals; in ruminant meats they represent around 2 – 4 % (Gerber, 2007, Biesalski, 2005). Poultry meat is an important provider of the essential PUFAs, especially the omega (n)-3 fatty acids. Scavenging chickens are particularly good source because of their varied diet. The fat content of cooked chicken varies depending on whether it is cooked with the skin on or off, the portion of the bird, and the bird's diet and breed. Breast meat contains less than 3 g fat/100 g and skin having the highest proportion. About half of the fat from chicken meat is made up of the desirable monounsaturated fats, and only one-third of the less healthy saturated fats. Chicken meat is therefore seen as a healthy meat. Chicken meat does not contain the trans fats that contribute to coronary heart disease (Ravindran, 2013).

2.2.3 Mineral Contents of Chicken Meat

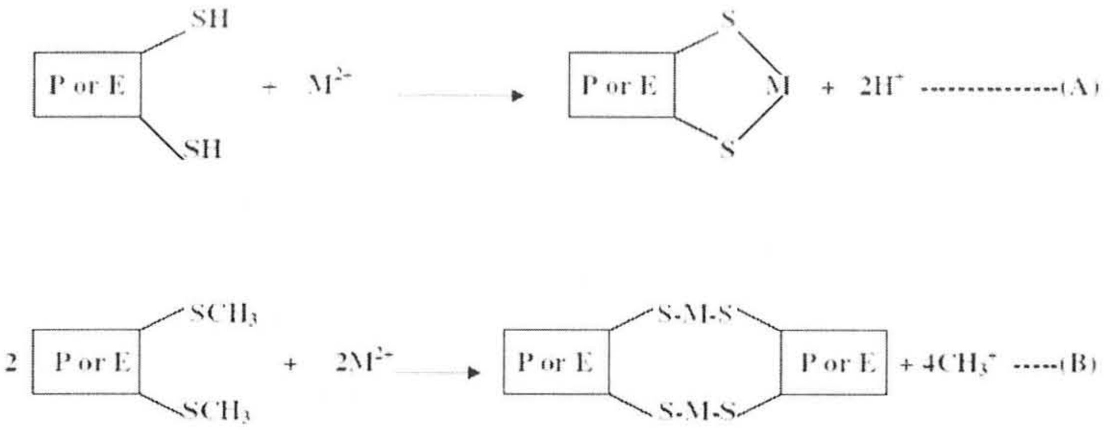
Meat is a major contributor of many of the essential minerals (e.g. Fe, Zn) (Lukáčová *et al.* 2014) required in the human diet. Many of these biogenic minerals are more readily available to humans from animal tissues than from any other food source (Zarkadas, *et al.*, 1987). Poultry meat provides Fe and Zn of high bioavailability in lower quantities than red meats, and it also has significant content of vitamins from group B such as thiamin, riboflavin, niacin and vitamin B6, although vitamin B12 content is less than in other meats (Barroeta, 2007). Meat is the food richest and represents as a primary source of heme iron, the iron form having the highest bioavailability (Lombardi-Boccia, *et al.*, 2005). Especially as many authors pointed out, those free rearing chickens has increased substantially as a result of the greater demand for the so-called natural products, which avoids the use of dietary animal by-products and antibiotics for poultry growth. Many consumers believe these products have superior sensory qualities and report that they "taste better" (Wei, *et al.*, 2016).

2.3. General Features of Heavy Metals

Heavy metals are by definition metals having densities higher than $\mu 5$ g mL^{-1}, for example, Fe, Cu, Pb, Cd, Hg, Ni, Zn, and Mn. Heavy metals can be classified into four major groups on their health importance: essential (cobalt, chromium III, copper, Iron ,manganese, molybdenum, selenium, zinc), metals with possible beneficial roles (boron,nickel,silicon,vanadium), non-essential (aluminum, antimony, barium, beryllium), toxic (Cd, Pb, Hg) heavy metals (Al-Fartusie & Mohssan, 2017). But classification of the trace elements into essential, non-essential, and toxic groups can be inaccurate and misleading due to the fact that all the essential elements become toxic at sufficiently high intakes; it is also probable that other elements will be added to the essential list as experimental techniques are further refined and applied. Consequently, it can be predicted that some of the trace elements present in living tissues that are now regarded merely as contaminants, rejecting the contact of the organism with its environment, will be found to perform some vital function; as nickel, tin, silicon, and vanadium have emerged as essential nutrients only in relatively recent years (WHO, 2004).

Essential metals (elements) have several important roles in human bodies, some are essential for enzymes reactions where they attract and facilitate conversion of substrate molecules to specific end products (Golub *et al.*, 20014). Others donate or accept electrons in redox reactions that are of primary importance in the generation and utilization of metabolic energy. Some of them have structural roles and responsible for the stability of important biological molecules. Furthermore, some trace elements have important actions throughout biological processes, for example, iron (Fe) which can bind, transport, and release oxygen in the body. In fact, although the trace elements are essential components of biological activities, the excessive levels of these elements can be toxic for the body health and may lead to many fatal diseases, such as cancers (Al-Fartusie & Mohssan, 2017). Iron, iodine, copper, zinc, manganese, cobalt, selenium, chromium, nickel, tin, and vanadium are essential metals (WHO. 2004).

A toxic metal is a metal, which is neither essential nor has beneficial effect, on the contrary, it displays severe toxicological symptoms at low levels. Toxic elements can be very harmful even at low concentration when ingested over a long time period due to their ability to accumulate in human and animal body (Badis, *et al.*, 2014). With increasing industrialization, more and more metals are entering into the environment. Those heavy metals stay permanently because they cannot be degraded in the environment (Katole, *et al.*, 2013). Eventually those heavy metals enter into the food material and ultimately they make their passage into the tissue (Islam, *et al* 2015). The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, they are converted in to their stable oxidation states and combine with the body's bio-molecules such as proteins and enzymes to form very stable biotoxic compounds, which become difficult to be dissociated, due to their bio-stabilities, during extraction from the body by medical detoxification therapy. The equations below show their reactions during bond formation with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH3) (Ireri, 2014).

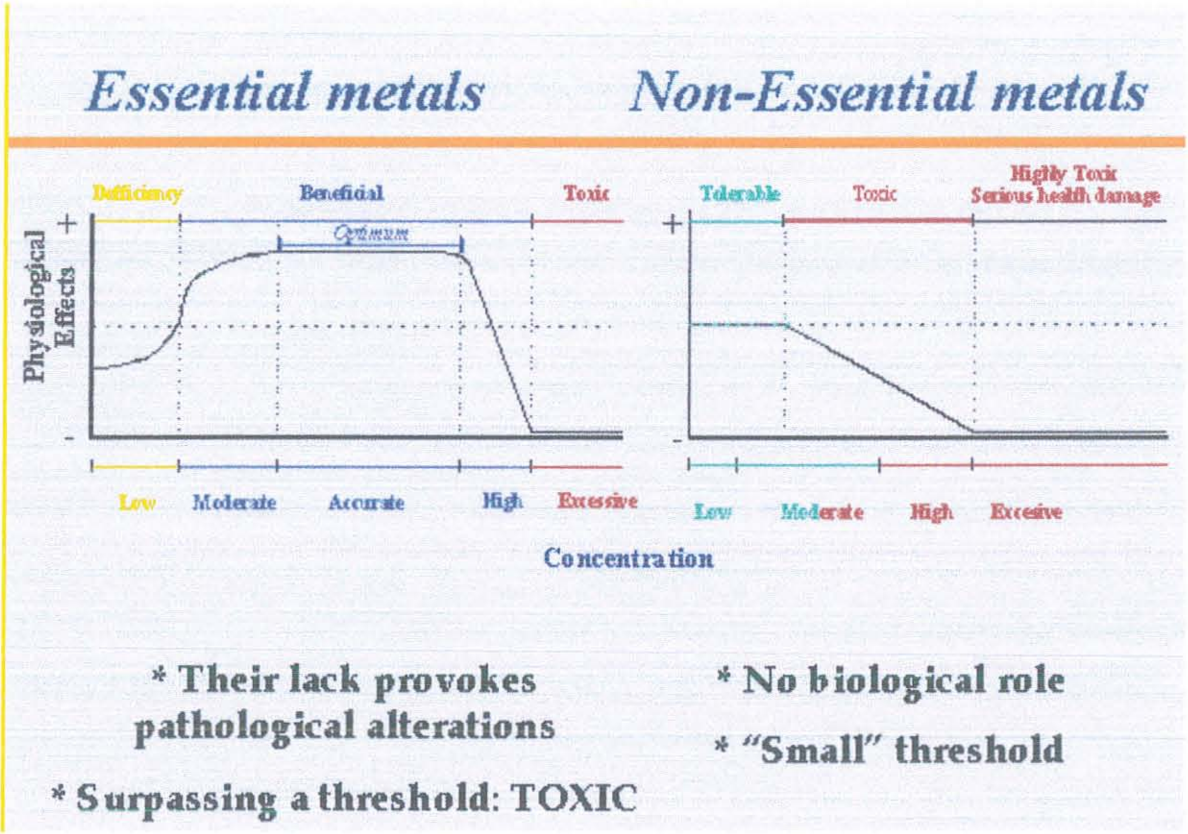


The hydrogen atoms or the metal groups in the above case are replaced by the poisoning metal and the enzyme is thus inhibited from functioning, whereas the protein–metal compound acts as a substrate and reacts with a metabolic enzyme (Ireri, 2014).

Where: A = Intramolecular bonding; B = Intermolecular bonding; P =

Protein; E = Enzyme; M = Metal

Figure 1 Illustrating the mechanism of action of metals on biological systems (Ireri, 2014)



* Their lack provokes pathological alterations

* Surpassing a threshold: TOXIC

* No biological role

* "Small" threshold

Figure 2. Conceptual response of metals concentrations to living things (Jadia & Fulekar, 2009)

Increased environmental exposure to several of the more toxic trace elements—notably lead, cadmium, mercury and arsenic as a consequence of the increasing industrialization and motorization of urban communities and the rapidly rising consumption of fossil fuels is also a matter of public health concern (Bordajandi, *et al.*, 2010). Population is generally being exposed to metal toxicity. ''soil–plant–human'' and/or ''plant–animal–human'' and/or ''soil–water–animal'' could be the potential food chain pathways of metal accumulation in human populations.

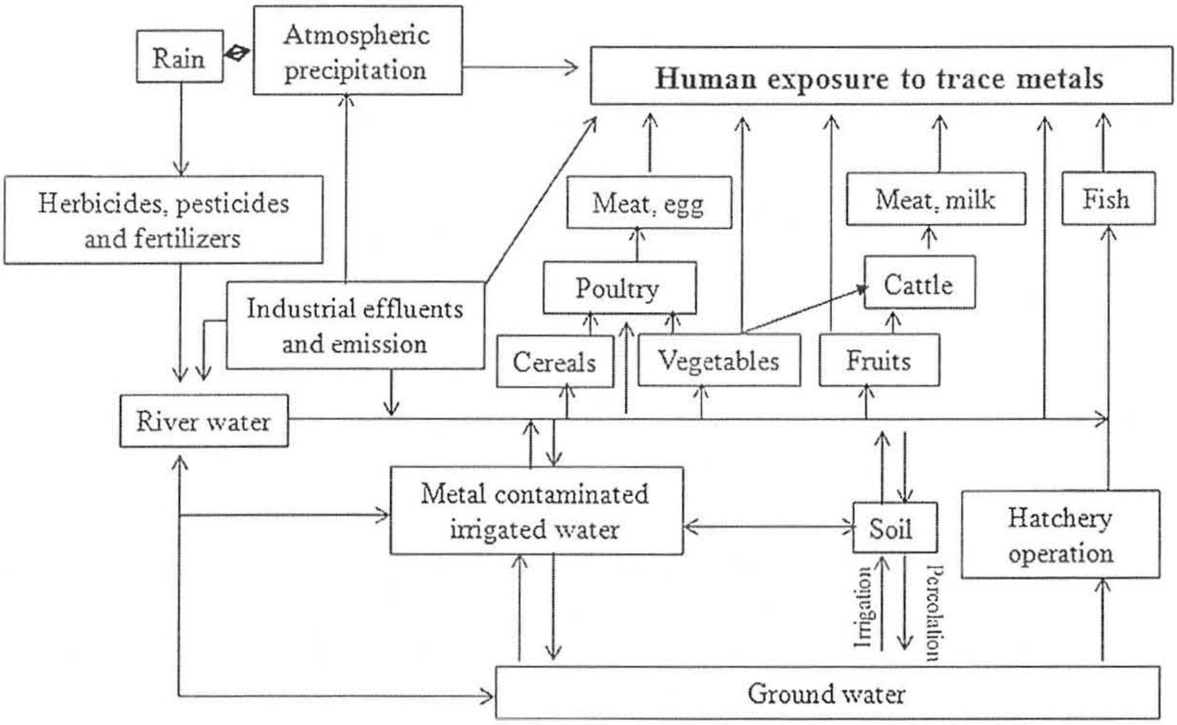


Figure 3. Possible food chain pathways through which humans may be exposed to trace metals (Islam, et al, 2015).

The different composition of air pollutants, the dose and time of exposure and the fact that humans are usually exposed to pollutant mixtures than to single substances, can lead to diverse impacts on human health (Katole, *et al.* , 2013). Human health effects can range from nausea and difficulty in breathing or skin irritation, to cancer. They also include birth defects, serious developmental delays in children, and reduced activity of the immune system, leading to a number of diseases. Moreover, there exist several susceptibility factors such as age, nutritional status and predisposing conditions (Goyer & Clarkson, 1996). Health effects can be distinguished to acute, chronic not including cancer and cancerous. Epidemiological and animal model data indicate that primarily affected systems are the cardiovascular and the respiratory system. However, the function of several other organs can be also influenced (Onyeka & David, 2015).

2.4 Sources, Pathways and Health Hazards of Some Selected Essential and Toxic Trace Heavy Metals

2.4.1 Zinc (Zn)

Zn is an essential trace element for animals and human diet, being involved in protein synthesis and as a constituent of many metalloenzymes (Akan, *et al.*, 2010, WHO, 1973). It is extraordinarily useful in biological systems and involved in many biochemical processes that support life and required for a host of physiological functions including normal immune function, sexual function, neurosensory function such as cognition and vision. Numerous proteins, enzymes and transcription factors depend on zinc for their function. Zinc is an essential component of hundreds of proteins and metalloenzymes including alkaline phosphatase, lactate dehyrogenase, carbonic anhydrase, carboxypeptides, and DNA and RNA polymerases found in most body tissues. It plays specific and important catalytic, co-catalytic and structural roles in enzyme molecules and in many other proteins and biomembranes (Nriagu, 2007).

Too little Zn can cause problems; however, too much Zn is harmful to human health. The permissible limit of Zn is 150ppm set by Australia-New Zealand Food Authority (ANZFA, 2001) (Akan, *et al.*, 2010). Zinc deficiency is a significant health problem among the elderly. Conditioning factors include physiological stress, disease, or exogenous substances such as alcohol, drugs, toxins and food constituents such as fibers and phytate (Sandstead, *et al.*, 1982).

Zinc is used in a variety of industrial processes. It enters aquatic environments through mining, industrial, and domestic effluents (Widianarko, *et al.*, 2001). Animal studies suggest that the availability of Zn for intestinal absorption is less from plant sources than from animal foods (WHO, 1973). All cereals and most vegetables contain phytate which can bind Zn, particularly in the presence of calcium, and reduce the biological availability of Zn. The complexing action of phytate appears to be an important etiological factor in the genesis of Zn deficiency in regions where the main staple foods are undermilled, unleavened cereals (WHO, 1973). Other plant constituents that may bind Zn and reduce its availability include certain hemicelluloses and amino acid carbohydrate complexes (Goyer & Clarkson, 1996). On the other hand, it is possible that Zn may complex with certain amino acids or peptides in the intestinal milieu to form chelates that are more readily absorbed. The various factors that influence the availability of this nutrient deserve further investigation, as also the mechanism of its absorption by man (WHO, 1973).

Pathological conditions in man that appear to be the consequence of inadequate Zn nutrition includes growth failure and sexual infantilism in teenage individuals, idiopathic impaired taste (hypogeusia), impaired smell (hyposmia) and impaired wound healing. Zn -responsive growth failure and sexual infantilism in both sexes. Other features that appear to condition the deficiency include blood loss due to hookworm and schistosomiasis, geophagia , and perspiratory losses due to high ambient temperature (Militaru & Sscppo, 2012).

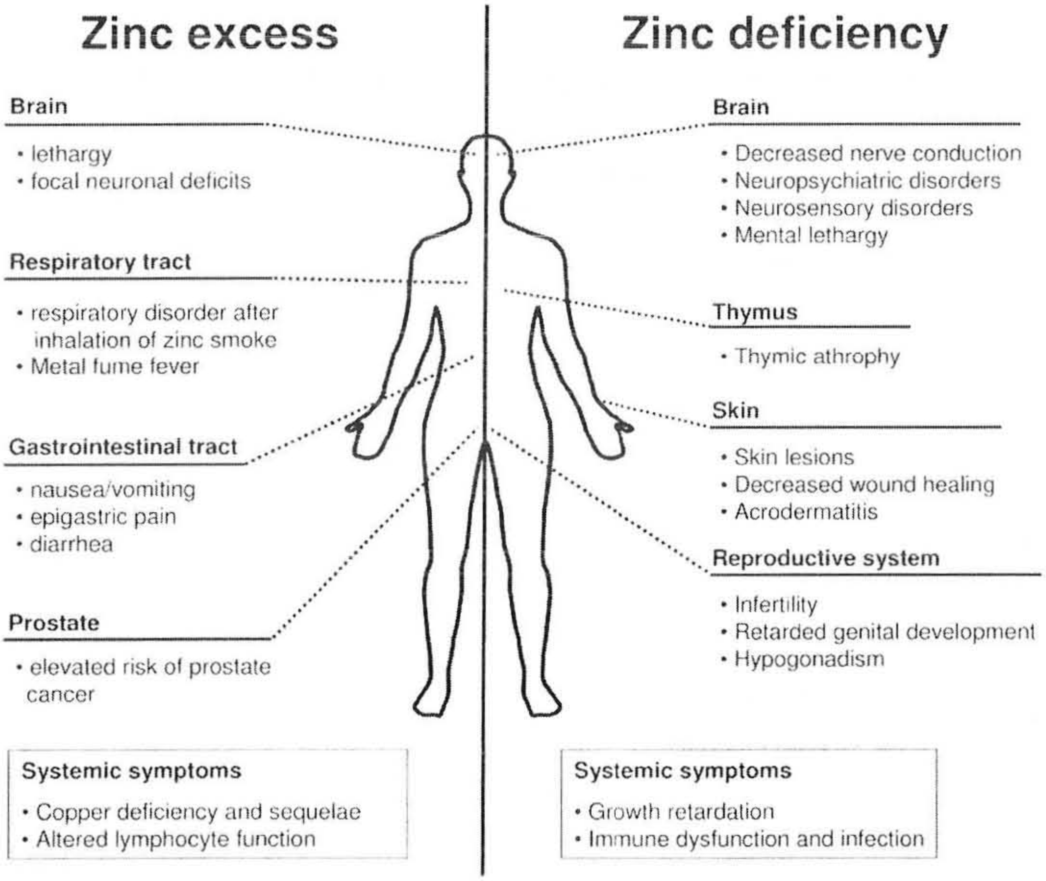


Figure 4. Pathological conditions following Zn toxicity/deficiency (Plum *et al.*, 2010).

2.4.2 Copper (Cu)

Cu is an essential micronutrient required by all life forms involved in a variety of biological processes like embryonic development, mitochondrial respiration, synthesis and regulation of hemoglobin levels as well as hepatocyte and neuronal functions (Krupanidhi, Sreekumar, & Sanjeevi, 2008), bone formation, skeletal mineralization, and maintaining the integrity of connective tissues (Abduljaleel, 2016). Being a transition metal, Cu gets biologically converted between different redox states namely oxidized Cu (II) and reduced Cu (I) (Krupanidhi, *et al.*, 2008). Copper, ubiquitous in the diets of humans, is an important cofactor for angiogenesis. Copper stimulates proliferation and migration of human endothelial cells. Patients with Wilson's disease, a rare autosomal recessive disorder, have a metabolic defect of copper transport that results in life-threatening accumulation of copper in multiple organs, notably liver and the brain (Santore, *et al.*, 2010).

Cu is widely distributed in nature. Food, beverages, and drinking water are potential sources of excess exposure. It is one of the major contaminants released from metal-finishing, electroplating and electrical industries (Krupanidhi, *et al.*, 2008). Copper ions are mainly found in wastewaters of industries such as metal cleaning, plating baths, refineries, paper and pulp, fertilizers, tanneries, and wood preservatives. High concentration of copper causes enzyme inhibition leading to Wilson's diseases. It was estimated that the annual industrial discharges of copper into fresh water environments was 1.4×10^{10} g/year, and the amount of copper in industrial wastes and sewage sludge that have been dumped into the ocean was 1.7×10^{10} g/year worldwide (Farouq & Yousef, 2015). The increase in copper concentrations in the environment results from industrial and domestic waste discharge, disposal of mining washings, refineries, and the use of copper as a base material for antifouling paint (Ulusoy, Gürkan, & Akcay, 2011). Rich amounts of copper found in the soil are taken up by plants using very elaborate transportation machinery. Plants, thus serve as a direct source of elemental copper for higher organisms. Additionally, human breast milk has the highest concentration of Cu (0.25 to 6.0 mg/l). A few plants uniquely accumulate copper. They are: *Aeolanthusbiformifolius, Athyrium yokoscense, Azolla filiculoides,Bacopa monnieri, Brassica juncea* L., *CallisneriaAmericana, Eichhornia crassipes, Haumaniustrumrobertii, Helianthus annuus, Larrea tridentate, Lemnaminor, Pistia stratiotes* and *Thlaspi caerulescens* (Krupanidhi, *et al.*, 2008). Cu exposures in industry are to particulates in miners or to metal fumes in smelting operations, welding, and related activities (Bakalli, *et al.*, 1995).

Daily intake of copper in adults varies between 0.9 and 2.2 mg. Intake in children has been estimated to be 0.6 to 0.8 mg/day (0.07 to 0.1 mg/kg body weight per day). The EPA's maximum contaminant level for Cu in drinking water is 1.3 mg/L where as the provisional WHO guideline for Cu in drinking water is 2 mg/L (Liao, *et al.*, 2007).

Gastrointestinal absorption of copper is normally regulated by homeostatic mechanisms. It is transported in serum bound initially to albumin and later more firmly to ceruloplasmin and transcuprein (WHO, 2007). The normal serum level of Cu is 120 to 145µg/L. The bile is the normal excretory pathway and plays a primary role in Cu homeostasis (Goyer & Clarkson, 1996). Most Cu is stored in liver and bone marrow, where it may be bound to metallothionein. Cu as Cu (II) entering into hepatocytes is initially reduced and complexed by glutathione prior to binding with metallothionein. Alternatively, copper entering the cell may be exported by a copper ATPase translocase. Cu is not an effective inducer of metallothionein relative to Zn or Cd. Nevertheless, Cu bound to metallothionein is thought to be a normal storage form of Cu, particularly in infancy and childhood (Bakalli, *et al.*, 1995). Isolated hepatic cells are protected from Cu toxicity by prior induction of metallothionein with Zn. Cu-metallothionein accumulates in lysosomes, facilitating the biliary excretion of Cu (Goyer & Clarkson, 1996). The newborn is dependent on stored Cu, which may not be adequate in premature infants. The amount of Cu in milk is not enough to maintain adequate Cu levels in the liver, lungs, and spleen of the newborn. Tissue levels gradually decline up to about 10 years of age, remaining relatively constant thereafter (Goyer & Clarkson, 1996). Brain levels, on the other hand, tend to almost double from infancy to adulthood. The ratio of newborn to adult liver copper levels shows considerable species difference: human, (15:4); rat,(6:4), and rabbit, (1:6). Since urinary Cu levels may be increased by soft water, concentrations of approximately 60 g/L under these conditions are not uncommon (Liao, *et al.*, 2007).

2.4.3 Chromium (Cr)

Cr is an essential element helping the body to use sugar, protein and fat. Excessive amounts of Cr may cause adverse health effects (El-Salam, *et al.*, 2013). 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 and has no beneficial role in the human body. Particularly Cr (III) plays an important role in the body function (metabolic functions, cofactor of insulin etc.) in trace amount but it turns to be toxic when it exceeds the tolerance limit (Mottalib, *et al.*, 2016).

The interest in chromium as a nutritional enhancement to glucose metabolism can be traced back to the 1950s, when it was suggested that brewer's yeast contained a glucose tolerance factor (GTF) that prevented diabetes in experimental animals. This factor was eventually suggested to be a biologically active form of trivalent chromium that could substantially lower plasma glucose levels in diabetic mice (Cefalu, & Hu, 2004). Trivalent chromium is essential for proper insulin function and is required for normal protein, fat and carbohydrate metabolism, and is acknowledged as dietary supplement (Ireri, 2014). Cr III is also required in a very small amount for normal growth and development of animal and human (Iwegbue, *et al*,. 2008).

Chromium, one of the most common elements in the earth's crust and seawater, exists several oxidation states, principally as metallic (Cr^0), divalent, Trivalent ($^{+3}$), four-valent, five- valent and hexavalent ($+^6$) chromium (Cefalu, & Hu, 2004). It can be solid, liquid, and in the form of gas (Jaishankar, *et al*., 2014). The stable forms of Cr are the trivalent Cr (III) and the hexavalent Cr (VI) (Ireri, 2014). The latter is largely synthesized by the oxidation of the more common and naturally occurring and is highly toxic, which usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) (Cefalu, & Hu, 2004). Chromium is present in rocks, soil, animals and plants. Chromium compounds are very much persistent in water sediments (Jaishankar, *et al*., 2014). The wide use of Cr compounds by modern industries has resulted in the discharge of large quantities into the environment via emission, waste water or solid waste disposal. It is used on a large scale in many different industries, including metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production, and pulp and paper production (Ireri, 2014). Occupational sources of chromium include protective metal coatings, metal alloys, magnetic tapes, paint pigments, rubber, cement, paper, wood preservatives, leather tanning and metal plating (Jaishankar, *et al*., 2014). Incineration of all sorts (municipal, sludge, hazardous waste) releases chromium, and it is necessary to understand the species that are released and what interchanges occur in the heated plume and subsequently in the atmosphere (Gochfeld & Witmer, 1991).

The general population is exposed to Cr by eating food, drinking water and inhaling air that contains the chemical (Ireri, 2014). Consumption of refined foods, including simple sugars, exacerbates the problem of insufficient dietary chromium because these foods are not only low in dietary chromium but also increase its loss from the body. Chromium losses are also increased during pregnancy and as a result of strenuous exercise, infection, physical trauma, and other forms of stress. Reduced chromium levels are reported in the elderly and in patients with diabetes (Cefalu & Hu, 2004).

The body has several systems of reducing Cr (VI) to Cr (III). Once absorbed into the bloodstream, Cr (VI) is rapidly taken up by erythrocytes after absorption and reduced to Cr (III) inside the red blood cells. In contrast, Cr (III) does not readily cross red blood cell membranes, but binds directly to transferrin, an iron-transporting protein in the plasma. This Cr (VI) detoxification leads to increased levels of Cr (III). The estimated safe and adequate daily dietary intake of Cr is 50 to 200 μg for adult and adolescents (Ireri, 2014).

Chromium (VI) compounds, such as calcium chromate, zinc chromates, strontium chromate and lead chromates, are highly toxic and carcinogenic in nature. The uptake of hexavalent chromium compounds through the airways and digestive tract is faster than that of trivalent chromium compounds. When broken skin comes in contact with any type of chromium compounds, a deeply penetrating hole will be formed, consequently that can result in the formation of ulcers, which will persist for months and heal very slowly. Ulcers on the nasal septum are very common in case of chromate worker (Jaishankar, *et al*., 2014).

There is no clinically defined state of chromium deficiency, but diabetes has been shown to develop because of low chromium levels in experimental animals and in humans sustained by prolonged total parenteral nutrition (TPN). These results suggest that there may be a more general relationship between chromium levels and glucose and/or lipid metabolism. It has also been suggested that low chromium concentrations and the associated impairments in insulin, glucose, and lipid metabolism may also result in increased cardiovascular risk (Cefalu & Hu, 2004).

The problems that are associated with Cr involve skin rashes, stomach ulcer, kidney, liver damages, lung cancer and ultimate death. Long term exposure can cause kidney and liver damage and damage to circulatory nerve tissue. At an elevated concentration it is toxic for both plant and animals. Hexavalent Cr causes marked irritation of the respiratory tract. Studies on experimental animals have reported hexavalent Cr to cause various forms of genetic damage in short-term mutagenicity tests, including damage to DNA, and misincorporation of nucleotides in DNA transcription (Ireri, 2014).

2.4.4 Manganese (Mn)

Manganese is a trace mineral that is present in tiny amounts in the body and is one of the most important nutrients in human health (Al-Fartusie & Mohssan, 2017). Mn exists in 11 oxidation states, from _3 to +7. Cycling between Mn^{2+} and Mn^{3+} may be potentially deleterious to biological systems because it can involve the generation of free radicals (Liao, *et al.*, 2007).

The trace element manganese is essential for normal development and body function across the life span of all mammals (Crossgrove, & Zheng, 2004). It is a cofactor for a number of enzymatic reactions, particularly those involved in phosphorylation, cholesterol, and fatty acids synthesis (Liao, *et al.*, 2007). Manganese binds to and/or regulates many enzymes throughout the body. For example, Mn is a required co-factor for arginase, which is responsible for urea production in the liver, superoxide dismutase (SOD), which is critical to prevent against cellular oxidative stress, and pyruvate carboxylase, an essential enzyme in gluconeogenesis. In brain, about 80% of Mn is associated with the astrocyte-specific enzyme glutamine synthetase, where Mn plays a regulatory role, although it is not a required co-factor. Interruption of Mn homeostasis has also been associated with a variety of disease states in humans (Crossgrove, & Zheng, 2004). Mn helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation, normal brain and nerve function. In addition, Mn is a key component of enzyme systems, including oxygen-handling enzymes. It is a component of the antioxidant SOD, which helps fight free radicals (Al-Fartusie & Mohssan, 2017).

Manganese is present in all living organisms. The principal source of intake is food. Vegetables, the germinal portions of grains, fruits, nuts, tea, and some spices are rich in manganese (Liao, *et al.*, 2007). The low intakes are from diets high in meat, milk, sugar, and refined cereals, which are characteristically low in manganese. The high Mn diets are high in whole cereals, nuts, legume seeds, and green leafy vegetables, all of which are high-manganese foods. Tea is exceptionally rich in Mn, one cup of tea contributing as much as 1.3 mg of manganese. In the developing countries, where unrefined cereals are the staple foods, dietary intakes of Mn are invariably high (WHO, 1973. Manganese is available in a wide variety of forms, including Mn salts (sulfate and gluconate) and Mn chelates (aspartate, picolinate, fumarate, malate, succinate, citrate, and amino acid chelate). Manganese supplements can be taken as tablets or capsules, usually along with other vitamins and minerals in the form of a multivitamin (Al-Fartusie & Mohssan, 2017).

Homeostatic mechanisms involving the liver and biliary excretion, gastrointestinal mechanisms for excreting excess manganese, and perhaps the adrenal cortex, plus the tendency for extremely large doses of manganese salts to cause gastrointestinal irritation, account for the lack of systemic toxicity following oral administration or dermal application (Crossgrove, & Zheng, 2004).

There are few reports of Mn deficiency in general human populations with self-selected diets, which contain 2– 4mg Mn daily. In rats, long-term dietary Mn deficiency (<1 ppm vs control at 66 ppm) correlates with an increased serum level of calcium and phosphorous and a decreased bone calcium, suggesting an interference of bone metabolism (Crossgrove, & Zheng, 2004). And it is assumed that functions of manganese, the clinical and biochemical signs of manganese deficiency would be expected to be similar in man (WHO, 1973). The low levels of manganese in the body can causes hypercholesterolemia, impaired glucose tolerance, dermatitis, changes in hair color, skeletal abnormalities, infertility, deafness, and impaired synthesis of vitamin K-dependent clotting factors (Crossgrove& Zheng, 2004). Low blood Mn in humans has been noted in bone modeling and remodeling diseases, including osteoporosis, Perth's disease, and also in adults and children with epilepsy. It is suspected that the presence of neurological symptoms in epileptics may correlate with low brain Mn, which may result from a low blood Mn. It can also cause nervous disorders (ataxia of the newborn) (WHO, 1973). On the other hand abnormal concentrations of Mn in the brain, especially in the basal ganglia, are associated with neurological disorders similar to Parkinson's disease (Al-Fartusie & Mohssan, 2017). Chronic Mn poisoning occurs in miners following prolonged working with Mn ores. In this case Mn enters the body mainly as oxide dust via the lungs and also via the gastrointestinal tract from the contaminated environment.' The lungs apparently serve as a depot from which the Mn is continuously absorbed (WHO, 1973). Victims of chronic manganese poisoning tend to recover slowly, even when removed from the excessive exposure. Metal sequestering agents have not produced remarkable recovery; L-dopa, which is used in the treatment of Parkinson's disease, has been more consistently effective in the treatment of chronic manganese poisoning than in Parkinson's disease (Cotzias *et al.*, 1971).

2.4.5 Nickel (Ni)

Small amounts of nickel are needed by the human body to produce red blood cells, however, in excessive amounts, can become mildly toxic. Short-term overexposure to nickel is not known to cause any health problems, but long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation (ME, *et al.*, 2014)

Nickel is normally present in human tissues and, under conditions of high exposure; these levels may increase significantly. In the general population, contributions to the body burden from inhalation of nickel in the air and from drinking water are generally less important than dietary intake and ingestion is considered to be the most important route of exposure (Cempel & Nikel, 2006).

Food is a major source of exposure for most people. The Environmental Protection Agency (EPA) estimates that an average adult consumes 100 to 300 µg of nickel per day. Drinking water contains very small amounts of nickel (Liao, *et al.*, 2007)

The range between required and toxic levels of nickel is extremely wide. Human intoxication with nickel due to dietary contamination has not been reported, as far as is known. High levels of nickel fed to calves have been shown to result in a reduction in nitrogen retention and impaired growth (ME., *et al.*, 2014).

Although a number of cellular effects of nickel have been documented, a deficiency state in humans has not been described (Cempel & Nikel, 2006). Short-term overexposure to nickel is not known to cause any health problems, but long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation (ME., *et al.*, 2014). Nickel is a respiratory tract carcinogen in workers in the nickel refining industry. Other serious consequences of long-term exposure to nickel are not apparent, but severe acute and sometimes fatal toxicity may follow exposure to nickel carbonyl (Liao, *et al.*, 2007).

2.4.6 Cobalt (Co)

Cobalt is an essential trace element for the human body, where it is a key constituent of cobalamin (the scientific name of vitamin B_{12}) (Al-Fartusie& Mohssan, 2017). Cobalt is used to treat anemia for pregnant women because it stimulates the production of red blood cells (Abduljaleel, 2016). It also has a substantial role in the formation of amino acids and neurotransmitters (Al-Fartusie & Mohssan, 2017). Cobalt is unique among the trace elements in that it is physiologically active in man only when supplied in one particular form-cyanocobalamin or vitamin B_{12}. The problem of cobalt in human nutrition is therefore primarily a question of the dietary sources and supplies of vitamin B_{12} and of the absorption of this vitamin, rather than of cobalt itself (ME,*et al.*, 2014).

Cobalt occurs naturally in soil, rock, air, water, plants, and animals (Abduljaleel, 2016). Co is one of the three magnetic minerals (in addition to Fe and nickel) that is often used in magnet alloys. It has considerable industrial applications. It is used in paints and dyes, where it has been used since the middle ages in the production of a blue colored glass (smalt). The radioactive counterpart Co-60 is a powerful gamma ray source that used in medical applications, such as radiotherapy trace and cancer fighter. It is also used for sterilization of medical supplies and medical waste (Al-Fartusie & Mohssan, 2017).

It has been found that the cobalt deficiency is associated with disturbances in vitamin B_{12} synthesis. It might cause anemia and hypothyroidism, as well as increase the risk of developmental abnormalities and failure in infants. The excess level of this metal in the human body might cause hypothyroidism and overproduction of erythrocytes, fibrosis in lungs and asthma (Al-Fartusie & Mohssan, 2017).

2.4.7 Cadmium (Cd)

Cadmium is a non-essential toxic, very reactive heavy metal that has no biological role, found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources (Abdolgader, *et al*,. 2013). It is one of the most toxic elements in the world, and can accumulate in the body with a half-life 10 to 30 yrs and it can disrupt a number of biological processes in human organs such as in the kidney and the lungs (Al-Rmalli, *et al*,. 2012, Sobhan, 2017). Cadmium may accumulate in the human body and eventually may induce kidney dysfunction, skeletal damage, reproduction deficiencies, prostate cancer, mutations, and foetal (embroyonic) death (Abdolgader, *et al*., 2013). Decreased rate of glomerular filtration, significant proteinuria, and increased frequency of kidney stone formation are the chronic effects of oral exposure to this metal. Low-level postnatal Cd exposure may cause neurotoxic effects in children (Sobhan, 2017). It is most commonly found as inorganic compounds in the 2^+ oxidation state and is mainly present as ($CdCl_2^0$) and ($CdCl^+$) complexes in seawater (Bosch, *et al*., 2016). In the atmosphere, Cd occurs attached to particles; especially those in the submicron category (approximately 0.5–1μm) also identified a second, smaller maximum particle size of approximately 0.1μm. The main Cd species upon emission are oxides, chlorides, sulfides and the elemental form. Oxides (CdO) are emitted by most of the anthropogenic sources (Goyer & Clarkson, 1996).

Elemental Cd is released during high-temperature processes such as organic fossil fuel combustion and waste incineration. Cd-Sulfides (CdS) are prominent in the emissions from non-ferrous metal production and coal combustion. Refuse incineration is a source of Cd- chloride ($CdCl_2$) (WHO, 2007).

Sources of Cd that will be released into the atmosphere are natural and anthropogenic means. Volcanoes, windborne particles and biogenic emissions are considered the main natural sources of Cd in the atmosphere. The anthropogenic sources of Cd include non-ferrous metal production, stationary fossil fuel combustion, waste incineration, iron and steel production and cement production (WHO, 2007). For persons in the general population, the major source of Cd is food (Goyer & Clarkson, 1996). Refined foods, water foods, water pipes, coffee, tea, coal burning and cigarettes are all the most important source of Cd (Anjulo & Mersso, 2015). Plants readily take up Cd from soil contaminated by fallout from the air, Cd-containing water used for irrigation and Cd-containing fertilizers. Another source of concern about potential sources of Cd toxicity is the use of commercial sludge to fertilize agricultural fields. Commercial sludge may contain up to 1500 mg of Cd per kilogram of dry material (Anjulo & Mersso, 2015).

In the general population, exposure from inhalation is low, but house dust is potentially an important and persistent source of Cd exposure in areas with contaminated soils, especially where driveways have been covered with residues from non-ferrous metal production (e.g. zinc ashes or sintels as oven sludge) (Anjulo & Mersso, 2015). Cigarette smoking represents an additional source of Cd, which may exceed that from food (WHO, 2007). Workplace exposure to Cd is particularly hazardous in the presence of Cd fumes or airborne Cd like electrolytic refining of Pb and Zn and other industries that employ thermal processes e.g. Fe production, fossil fuel combustion, cement manufacture, manufacture of paint pigments, Cd-nickel batteries, and electroplanting all of which release airborne Cd, the metal being a constituent of the natural raw material (Goyer & Clarkson, 1996). For non-smokers, food constitutes the principal environmental source of Cd. Absorption is enhanced by dietary deficiencies of calcium (Ca) and Fe and by diets low in protein. Low dietary Ca stimulates synthesis of Ca-binding protein, which enhances Cd absorption (Goyer & Clarkson, 1996). Pulmonary absorption of inhaled Cd ranges from 10% to 50% (WHO, 2007) where as gastrointestinal absorption accounts 5-8% (Goyer & Clarkson, 1996). Cd in the tissues is mainly bound to metallothionein (Goyer & Clarkson, 1996). The synthesis of this protein probably represents the body's defense mechanism against the toxic Cd ion. Liver and kidney tissues are the two main sites of Cd storage. The newborn infant is virtually free of Cd but, over a lifetime, these organs accumulate considerable amounts of Cd (about 40–80% of the body burden). Blood Cd levels in adults without excessive exposure is usually less than 1µg/dL. Newborns have a low body content of Cd, usually less than 1mg total body burden. The placenta synthesizes metallothionein and may serve as a barrier to maternal Cd, but the fetus may be exposed with increased maternal exposure (WHO, 2007).

Human breast milk and cow's milk are low in Cd, with less than 1µg/kg of milk. About 50 to 75 percent of the body burden of Cd is in the liver and kidneys; its halflife in the body is not known exactly, but it may be as long as 30 years. With continued retention, there is progressive accumulation in the soft tissues, particularly in the kidneys, through ages 50 to 60, when the Cd burden in soft tissues begins to decline slowly (WHO, 2007). An average man accumulates as about 30 mg Cd in his body by the age of 50 years (Anjulo & Mersso, 2015).

Cadmium elimination from blood is effective with an open two-compartment model, as having a fast-decay half-time of 15–120 days and a slow-decay half-time of 7.4–16 years. Cd is eliminated in urine and faeces: daily faecal and urinary excretion is estimated to constitute 0.007% and 0.009% of the body burden, respectively (Goyer & Clarkson, 1996).

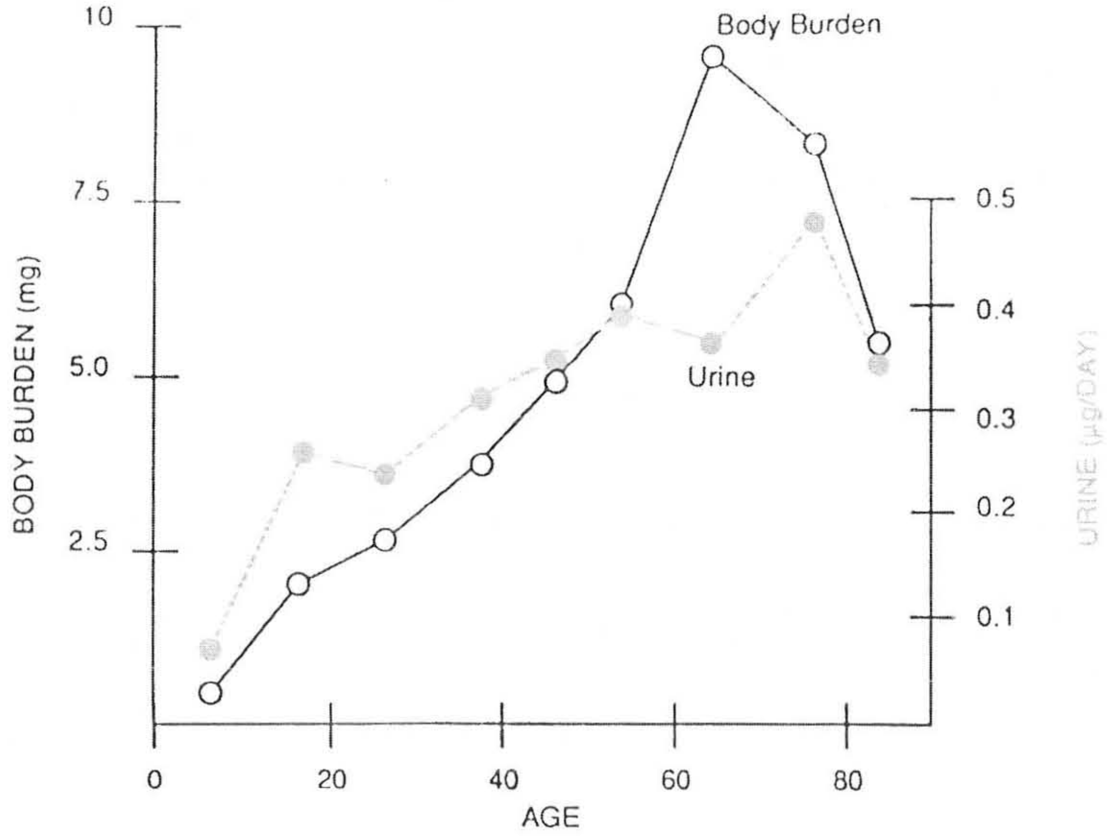


Figure 5 Illustration of the accumulation of Cd with age (Goyer & Clarkson, 1996)

Important health endpoints include kidney and bone (by affecting Ca and phosphorus (P) metabolism) damage and cancer. Painful bone disorders, including osteomalacia, osteoporosis and spontaneous bone fracture, have been observed in humans chronically exposed to Cd (WHO, 2007). The kidney is the critical organ with regard to long-term occupational and environmental exposure to Cd, and all health-based recommendations relate to the early disturbance of renal function. There are also evidences that Cd exposure can result to induce hypertension, cause blood pressure elevation and increased cardiovascular diseases (Goyer & Clarkson, 1996).

Cd toxicity may result in both acute and chronic pulmonary dysfunctions. Acute toxicity can result in acute chemical pneumonitis, and pulmonary oedema. If the chemical dose is high it might be resulted in lethal situations (Goyer & Clarkson, 1996). Chronic toxicity may cause chronic obstructive pulmonary disease and emphysema and chronic renal tubular disease (Anjulo &

Mersso, 2015). Cadmium accumulated in the kidney and liver over long time and interacts with numbers of minerals mainly Zn, Fe, Cu and selenium (Se) due to chemical similarities and competition for binding stage (Akan, *et al.*, 2010). Others symptoms can also be included like loss of appetite, poor growth, retarded testicular development, parakeratosis, etc (Daniel, 2015).

2.4.8 Arsenic (AS)

As is an environmental toxicant (Zahrana & Hendyb, 2015) metal that occurs at ultratrace levels, for which its specific biochemical function has not totally been well defined. However circumstantial evidence suggests that dietary deprivation in some animal models results in a suboptimal biological function that is prevented or reversed by an intake of physiological amounts of the element. It has been suggested that this metal could play an essential role in humans because decreases in serum arsenic concentration have been correlated with injuries of the central nervous system, vascular disease and cancer (Delgado-Andrade, *et al.*, 2003).

There are two types of As; organic As consisting of As combined with oxygen, and hyrogen and inorganic oxygen,chloride and sulfur. The most toxic forms of As are the inorganic arsenic (III) and (V) compounds; the inorganic As trioxide is well known as a rat poison, which was also sometimes used for homicide. Methylated forms of As have a low acute toxicity; arsenobetaine which is the principal As form in fish and crustaceans is considered nontoxic (WHO, 1973). Arsenic has a complex chemistry and can be present in several organic (trivalent and pentavalent arsenic) and inorganic (elemental, trivalent and pentavalent arsenic) forms which vary in their degree of toxicity. Inorganic As is seen as the most toxic form as it is stable and soluble and therefore absorbed by the digestive tract, abdominal cavity and muscles in the human body. Whilst organic arsenic does not accumulate in the human body due to rapid excretion. Inorganic As is often found in high levels in drinking water whereas organic arsenic is primarily found in fish and meat. Seafood can contain several times the amount of As than other foods and is therefore the main source of dietary intake in humans Early symptoms of As exposure in humans include abdominal pain, vomiting, diarrhoea, muscle weakness and skin flushing whereas chronic As toxicity has led to skin defects and cancer (Bosch, *et al.*, 2016).

Arsenic is widely distributed in nature due to environmental sources such as volcanic activity and weathering minerals (WHO, 1973) and anthropogenic pollution which is largely due to smelting activities, glass manufacturing, manufacture and use of arsenic pesticides, herbicides, fungicides and wood preservatives, veterinary or human medicinal drugs (Bosch, *et al.*, 2016). Because it occurs naturally in the environment it is possible to be exposed to it through air, water and soil contact (ME, *et al.*, 2014), and can occur as a large number of organic or inorganic chemical forms in food (species). As relatively present in Crustacea and other shellfish but the concentration may increase by industrial pollution and by contamination from using As as insecticides and as additives to animal feeds. Because of its availability in foods and beverages, and in environment even naturally, maximum limits of As in foods and liquids have been legally imposed in many countries (WHO, 1973). Especially in the marine environment As is often found in high concentrations of organic forms, up to 50 mg/kg of As on a wet weight basis in some seafood including seaweed, fish, shellfish and crustaceans. In fresh water and in the terrestrial environments arsenic is normally found in much lower levels (typically 0-20 ug/kg) in crop plants and in livestock. Higher levels may be found in rice, mushrooms and sometimes in poultry which is fed fish meal containing arsenic (WHO, 1973).

Several studies have shown that inorganic arsenic can cause lung, bladder, liver, and kidney, prostate and skin cancer. Emerging science also shows that inorganic arsenic may harm pregnant women and their fetuses. As has been shown to cross the placenta to the fetus and has been found in breast milk. Chronic exposure to arsenic has been shown to affect child development, lowering their IQ scores. The most toxic forms of As are the inorganic As (III) and (V) compounds; the inorganic arsenic trioxide is well known as a rat poison, which was also sometimes used for homicide. Methylated forms of arsenic have a low acute toxicity; arsenobetaine which is the principal arsenic form in fish and crustaceans is considered nontoxic (WHO, 1973). There is very little information available on the effects of organic As compounds in humans (ME, *et al.*, 2014). Chronic As toxicity is mostly manifested in weight loss, capricious appetite, conjunctivitis, mucosal and erythematic lesion including mouth ulceration, anemia, liver and kidney damage, hyper pigmentation, skin damage and black foot disease. Acute toxic effects include abdominal cramping, hyperesthesia in extremities, abdominal patellar reflexes and abdominal electrocardiogram (Katole, *et al.*, 2013).

2.4.9 Lead (Pb)

Lead is one of the oldest known and most widely studied occupational and environmental toxins (Gidlow, 2004). As far as is known, lead has no beneficial or desirable nutritional effects. Low dietary intakes of calcium, iron, and protein may increase its absorption in experimental animals (WHO, 1973). Lead is a toxic heavy metal with widespread industrial use. Chronic exposure at relatively low levels can result to damage to kidneys and liver and to the immune, reproductive, cardiovascular, nervous and gastrointestinal systems (Okoye &Ugwu, 2010).

Lead is released into the atmosphere from natural emissions such as wind resuspension and from sea salt, volcanoes, forest fires and biogenic sources. The other anthropogenic emission sources of lead on a global scale include the combustion of fossil fuels from, for example, traffic, non-ferrous metal production and iron and steel production. Some contributions are also made by cement production and waste disposal (WHO, 2007). The other sources of lead exposure include mainly industrial processes, food and smoking, drinking water and domestic sources (Jaishankar, *et al.*, 2014).

The general population could be significantly exposed owing to poorly glazed ceramic ware, the use of lead solder in the food canning industry, high levels of lead in drinking water, the use of lead compounds in paint and cosmetics and by deposition on crops and dust from industrial and motor vehicle sources (Gidlow, 2004). Children can be exposed to lead by drinking contaminated water, eating lead-based paint chips, chewing on objects painted with lead-based paint or swallowing house dust or soil containing lead (Chowdhury, *et al.*, 2011). Acute exposure to lead is also more likely to occur in workplace, particularly in manufacturing processes that include the use of lead such as battery manufacture and in petrol filling stations. Also printing ink, paint, and fertilizer contain lead (Jnr, 2011). Lead is one of the elements that is found in varying amounts in dental enamel. In a recent study on the etiology of dental caries it was the only element in food that appeared to have a strong direct correlation with the prevalence of caries (WHO, 1973).

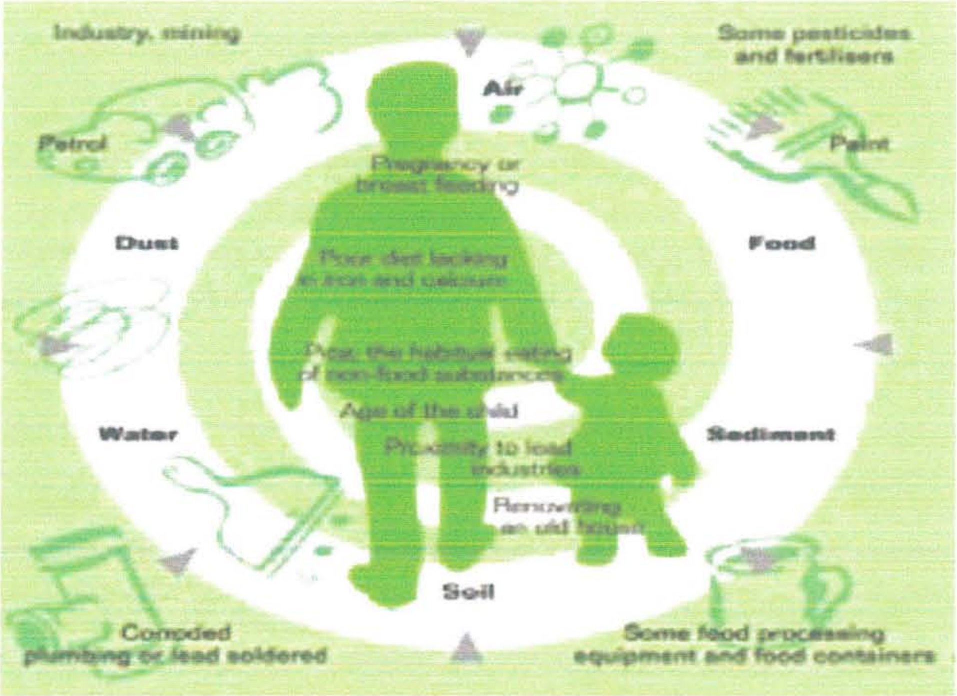


Figure 6 Sources of lead exposure (Jnr, 2011).

The toxicity of Pb is dependent on its chemical form where the organolead compounds are more toxic than the inorganic Pb form. Lead is mostly found in its dissolved form in the ocean, of which a large proportion (50–70%) is organic compounds. The bioavailability of Pb in the environment as organic compounds can be significantly increased by the presence of dissolved organic matter (DOM). The more methyl or ethyl carbon groups linked to the Pb molecule, the higher its toxic effect (Sobhan Ardakani, 2017). Lead is rapidly absorbed into the bloodstream of human body through inhalation, ingestion, or by skin contact. Through the bloodstream, lead is distributed among three main compartments: Blood, soft tissue that includes kidney, bone marrow, liver, brain, and mineralized tissue that includes bones and teeth (Al-Fartusie & Mohssan, 2017).

Lead affects almost every organ system in the human body (Chowdhury, *et al.*, 2011). The central nervous system is particularly vulnerable in infants and children under age six. The effects are the same whether it is breathed or swallowed. Large amounts of lead exposure may lead to blood anemia, severe stomachache, muscle weakness, and brain damage. Lower levels of exposure, may affect a child's mental and physical growth leading to learning disabilities and seizures (Chowdhury, *et al.*, 2011, Benouadah, *et al*,. 2015). To a great extent, it can cause damage to the brain, kidneys, nervous system, reproductive system, and can cause high blood pressure. In general the nervous system is the most sensitive to lead poisoning (Al-Fartusie & Mohssan, 2017).

2.4.10 Mercury (Hg)

Mercury is a toxic heavy metal and a persistent environmental pollutant (Badis, *et al.*, 2014). It is a potent toxicant metal that is liquid at ambient temperature and pressure and can be present in several different inorganic and organic chemical forms and compounds in the environment. It is deemed by the US EPA to be a top three priority pollutant of concern (Bosch, *et al.*, 2016, Goodrich, 2011). Inorganic Hg exposure is mainly to the elemental (Hg^0) or mercuric (Hg^{2+}) forms while exposure to organic Hg is primarily via the methylated ($MeHg^+$) form (Goodrich, 2011). Metallic mercury (Hg^o) is used in thermometers, dental amalgams, and some batteries. In its pure form, metallic mercury is a liquid (Martin & Griswold, 2009).

Industrial wastes and sewage water from the chloroalkali industry (Anjulo & Mersso, 2015), evaporation from the sea surface, and geothermal activity (WHO, 2007), degassing of the earth's crust are a major source of mercury pollution (Liao, *et al.*, 2007). Other source is human activity and occupational involvement like plastic production (vinyl chloride), metal production, cement production, combustion of coal and other fuels, waste disposal and cremation (WHO, 2007), chlor-alkali industry, where it is used as a cathode in the electrolysis of brine; in making a variety of scientific instruments and electrical control devices; in dentistry, as amalgam tooth filling; and in the extraction of gold (Liao, *et al.*, 2007).

Mercury is known to be toxic to many biological systems. It can cause neurological disorders, irritability, memory loss, insomnia, and gastro- intestinal disorders. Mercury may penetrate in the food materials from food processing or environmental contamination (Islam, *et al.*, 2015). The adverse health effects of chronic, low dose exposure to methylmercury are less well characterized, though evidence suggests several outcomes (e.g., neurological, cardiovascular) may be affected by low dose exposure in both prenatally exposed children and adults. Methylmercury impairs cognitive development (learning, memory, attention) among children exposed in utero. (Goodrich, 2011).

2.5 Practice and Utilization of Poultry in Ethiopia

Animal production in general and chickens in particular play important socioeconomic roles in developing countries. Provision of animal protein, generation of extra cash incomes and religious/cultural considerations are amongst the major reasons for keeping village chickens by communities (Moges, *et al.*, 2010). The proportional contribution of poultry to the total animal protein production of the world by the year 2020 is believed to increase to 40%, the major increase being in the developing world (Asresie & Mitiku, 2015). Nearly all rural and peri-urban families in developing countries keep a small flock of free range chickens that accounts for more than 60% of the total national chicken population in most African countries (Moges, *et al.*, 2010) particularly in Ethiopia the majorities (99%) of these chickens are maintained in a traditional way (Leta & Endalew, 2010). Previous studies reveal that the total poultry population in Ethiopia is estimated to be more than 51.35 million which represents a significant component of the rural household livelihood (Sebho, 2016), as a source of cash income and nutrition. In addition, the local chicken sector constitutes a significant contribution to human livelihood and contributes significantly to food security of poor households (Leta & Endalew, 2010). Religions and cultural considerations are also amongst the reasons for keeping chickens by resource poor farmers in Africa and in Ethiopia in particular (Asresie & Mitiku, 2015).

2.6 Heavy Metals Pollution in Ethiopia

Environmental pollution associated with heavy metals has been of global concern over many decades. Metals for which there are no nutritional requirements may react with biological systems to cause adverse effects. Excessive doses of nutritionally essential metals can also cause adverse effect (Rahlenbeck, *et al.*, 1999, Goyer & Clarkson, 1996). Heavy metals like Pb, Cd, Hg, As,Cr Fe, Cu and Zn get deposited in the vital organs through the food chain. Their high concentration may have deleterious effect causing disorders on vital organs like kidney, liver, lung and may cause for cancer (WHO, 2007). Environmental risk factors account for a quarter of the total burden of disease and 2.97 million human deaths every year in Africa (Yabe, *et al.*,, 2010).

Due to the focus only on development program on economic growth and industrialization, limited resources for environmental management and weak pollution legislation in most developing countries like Ethiopia has worsened heavy metal pollution. As a result, industrial expansion and increased extraction of natural resources have resulted in widespread heavy metal pollution (Katole, *et al.*, 2013). There are some data regarding the status of essential and non-essential metals in Ethiopia. Regarding essential metals high concentrations Cr from water sample of modjo (Rehrahie, 2018), and in milk at Borena Zone ((Belete, *et al*,. 2014) were reported. Other essential metals like Mn, Cu and Zn from water samples in Tigrai region were found being below their respective permissible limits (Mebrahtu, & Zerabruk, 2011), but higher accumulations of Mn in leaves of the tea plant (Camellia sinensis L.) and soil of Wushwush farms, (Yemane, *et al.*, 2008) were reported. On the other hand levels of different values of toxic heavy metals are reported at different cases. Higher concentrations of Pb in Ethiopian wines (Woldemariam, & Chandravanshi, 2011) and milk samples from Holetta and Bishoftu (Rehrahie, 2018) but lower concentrations in leaves of the tea plant (Camellia sinensis L.) and soil of Wushwush farms, Ethiopia (Yemane, *et al.*,, 2008) were reported. Similarly cadmium was not detected in Ethiopia wines in a study by Woldemariam, & Chandravanshi, 2011. On other report cadmium concentrations in milk studied at eastern shoa were found being within permissible limits (Rehrahie, 2018 similarly the levels of As in milk samples in western and eastern Shoa was found being within permissible limits (Rehrahie, 2008)

Chapter Three

3. Materials and Methods

3.1 Study Area

Ethiopia has selected some areas of the country to be industry zones. Of those industry zones of the country Akaki, Bishofitu and Modjo are among the oldest industry areas in the country. On each industry zone varieties of factories, like plastic, steel, leather etc are planted thereby producing trace and heavy metals effluents to the surrounding environment. Example Bishofitu comprising 14 kebeles, being situated 47kms at altitude of 1800-2100m south of Addis Ababa accommodates a total of 108 functioning industries (i.e. 26 metal and metal works, 10 chemical producing, 10 plastic, 3 furniture, 5 textile and 54 agro- processing). In the same way Modjo being located within Oromia region with a distance of 73km south of Addis Ababa at altitude of 1600.-1800m comprises a total of 40 industries (i.e. 12 tannery, 18 agro-processing, 3 chemical producing and 7 textile) with its 2 kebele administration systems. Akaki; one of the ten sub cities of Addis Ababa comprising 243 wood and metal works, 39 textile, 65 chemicals producing, 115 agro processing industries.

3.2 Sample Collection and Preparation

3.2.1 Sample Collection

Purposively 5 hens of local breed from each of the three selected industry areas which were laying/have laid eggs and grown in a traditional way by local residents were purchased from local markets. Sellers were orally asked regarding their residence to check that their residence is not far from areas of interest. Besides sellers were asked if hens started laying eggs/ have laid at least once in life. Purchased hens were slaughtered at home and samples from chicken thigh and chest was used for proximate analysis and samples from liver, thigh and chest was used for heavy metals analysis purpose. Ceramic knife instead of metal knife was applied to avoid metals contamination of the samples with heavy metals. All samples were stored at polyethylene bag under refrigeration until it goes to laboratory areas for preparation and treatment (Shaheen, *et al.*, 2016). Then samples were transported from storage to laboratory areas using Ice box.

3.2.2 Inclusion and exclusion criterion

3.2.2.1 Inclusion Criteria

Hens which were grown by local residents in a traditional way who have started/had laid eggs were taken as an inclusion criteria

3.2.2.2Exclusion Criteria

Hens cultivated in farm base, hens sold by sellers by bringing from far areas, hens that have not started laying eggs were exclusion criterion

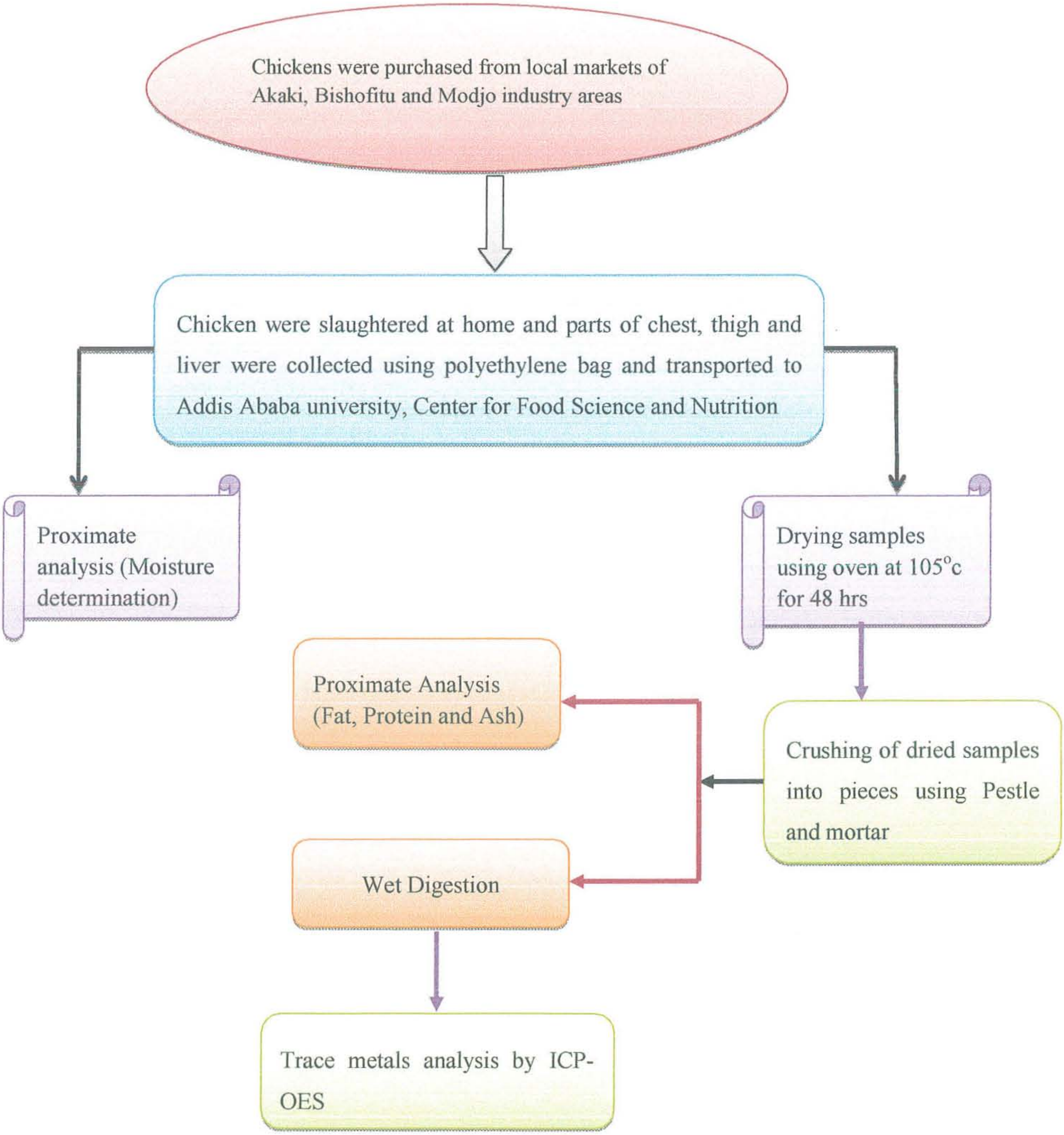


Figure 7 Flow chart of sample collection and preparation

3.3 Sample Treatment and Preparation

The collected samples of liver, thigh and chest underwent through the following preparation processes until the sample reached for analysis. The samples were cut into small pieces using plastic knife. Samples were dried with an oven of around $105C^0$ for 48 hrs (Zahrana, & Hendyb, 2015). After drying the samples were ground into a fine powder using a ceramic pestle and mortar, sieved and then stored in polyethylene bags in the dark until used for proximate analysis according to AOAC 2000 official methods and acid digestion for heavy metals analysis.

3.4 Proximate Analysis

Fresh sample was used for moisture determination where as dried and finely grounded samples were used for protein, fat and ash analysis. Proximate compositions of chest and thigh chicken parts were determined accordingly to AOAC 2000 official methods.

3.4.1 Moisture Content

Moisture content was determined according to AOAC (2000) using the official method. A crucible was dried in an oven at 105°C for 1 hour and placed in desiccators to cool. The weight of crucible was recorded as W1. 5g sample was weighed in dry crucible and recorded. The crucible with a sample were dried at 105°C for 48 hour and after cooling to room temperature in desiccators it was again weighed and recorded as W3. Moisture content was measured by weighing differences before and after oven drying.

3.4.2 Crude Protein Content

Crude protein proximate analysis was done by Kjeldahl method (AOAC, 20000 and the following procedures were carried out.

1. Digestion: About 0.5 gm of fresh sample were added to a Tecator tube and 6 ml of acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulfuric acid) will be added and mixed again on to this mixture 3.5 ml of 30% hydrogen peroxide were added step by step. As soon as the violet reaction ceased, shaked and placed back to the rack. Three grams of catalyst mixture (ground 0.5 gm of selenium metal with 100 gm potassium sulfate) were added in to each tube and allowed standing and for about 10 minutes before digestion. When the temperature of the digester reached 370°C, the tubes in to the digester were lowered. The digestion was continued until appearance of clear solution is achieved (for about 4 hours). Then tubes in the rack were cooled in a fume hood: 25 ml of de-ionized water were added, and shaken to avoid precipitation of sulfate in the solution.

2. Distillation and titration: the digested and diluted sample solution were distilled using boric acid and the distillate were titrated using 0.1N hydrochloric acid until reddish color appeared,

The crude protein was determined as follow:

Nitrogen = [(VHCl (1) * NHCl)/Wo]*14*100 Eq1

% Protein = 6.25 * % Nitrogen Eq2

Where: V is volume of HCl in Litter consumed to the end point of titration, N is normality of HCl (0.1N is usually used), Wo is sample weight on dry matter basis and 14 is molecular weight of nitrogen. The % of nitrogen is converted to % of protein by using the following conversion factor based on the assumption that proteins contain 16% nitrogen. (% Protein = 6.25 * % Nitrogen)

3.4.3 Crude Fat Content

The crude fat was extracted according to AOAC (2000) Official method 4.5.01).

About 2 gm of dried meat sample were extracted with 50ml diethyl ether at least for 4 hours in a soxhlet extractor (SZC-D Fat Determinator, China). The solvent was then be evaporated and the extracted fat were dried in an oven and cooled in a desiccators. The crude fat is determined as follows

Crude Fat % Wt= [(W2-W1)/W] *100 Eq3

Where: W1 = Weight of extraction flask (gm)

W2 = Weight of extraction flask plus dried crude fat (gm)

W = Weight of sample (gm)

3.4.4 Ash Content

The ash contents were determined by the following ways:

Washed porcelain dishes with distilled and de ionized water were placed in a muffle furnace for 30 minutes at 550°C. The dishes will cool in desiccators (with granular silica gel) for about 30 minutes at room temperature and will be weighed and recorded as M1. About 2.5 gm of sample were weighed in a dish and recorded as M2. The dishes were then placed on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and samples become thoroughly charred. The dishes with the samples will be placed inside a muffle furnace at 550°C for 5 hours and then cooled in desiccators for 1 hour. When the color of the sample in a dish becomes clean and white let the dish containing the sample cool to room temperature, weigh it and record as M3.

Total Ash %=[(M3-M1)/(M2-M1)]*100 Eq4

3.5 Essential and Toxic Metals Analysis

3.5.1 Wet Digestion Technique

Wet digestion is preferred with the following procedures. A mixture of HNO_3, $HClO_4$ and hydrogen peroxide (H_2O_2) reagents were used. A 2.00gm dry sample were placed in a tectator tube and pre-digested in 10ml 68% concentrated HNO_3 at 135°C until the liquor became clear. Next, 10ml of HNO_3, 1 ml $HClO_4$ and 2 ml H_2O_2 were added and temperature was maintained at 135°C for 1 hr until the liquor became again clear. The digest was slowly evaporated to near dryness (avoid prolonged baking), cooled and dissolved in 1M HNO_3. The digests were subsequently filtered through Whatman filter paper (Daniel, 2015). Now sample is ready for heavy metals analysis.

Standard solutions: eight series of standard metal solution was by appropriate dilution of the metal stock solution with deionized water containing 0.5ml 3% nitric acid in 50ml graduated tube. Calibration curve for each metal using the prepared standard solution was prepared. Concentration determination was analyzed using ICP-OES Spectro Across FHS12, Germany, 2010, machine. Sample blank was run with the sample solution.

Metals concentrations was calculated as follows

Metals concentrations (mg/kg) = $(a - b)xv/w$

Where a= concentration (mg/kg) of sample solution
b= concentration (mg/kg) of blank solution
V= volumes used for extraction (25ml of 1M $NHNO_3$)
W= weight of dry sample used (2 mg)

3.5.2 Methods of Validation

Lower limit of detection (LOD), internal control method/approach to verify the accuracy of the studied data and calibration curves were tested to check the validity of the data

3.5.2.1 Lower Limit of Detection (LOD)

LOD is the lower linear range of calibration and recalculated after every regression.

Table 1. LOD of the studied heavy metals by the ICP-OES

Metal	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Zn
LOD	0.0011	0.00051	0.00087	0.0014	0.0013	0.0013	0.00017	0.0007	0.0007	0.0007

3.5.2 .2 Internal Control Method

An internal control method of the company where analysis was carried out (Horticoop, Ethiopia) was applied to check the accuracy of the data and is kept as follows

Table 2. Internal Control Measurements

Elementas	Control concentration (Mg/kg)/internal control data of heavy metals	Control Measured concentration of the study sample (Mg/kg)	Recovery %
As	2.00	2.059	102.95
Cd	2.00	1.939	96.95
Co	2.00	1.922	96.1
Cr	2.00	1.913	95.65
Cu	4.00	3.929	98.225
Hg	2.00	1.939	96.95
Mn	4.00	4.211	112.775
Ni	2.00	1.997	99.85
Pb	2.00	1.985	99.25
Zn	4.00	4.158	103.95

3.5.2.3 Accuracy

3.3.3.1 Calibration Curves

Calibration curve of the studied metals were made after grouping them into three standard groups. Concentrations of eight series each of the different metals were made by pouring from 1000ppm stock solutions.

Standard A

Cu 0.7 ml 2.8 ppm
Zn 0.7 ml 2.8ppm
Mn 1 ml 4 ppm

Standard B

Pb 0.35ml 1.4ppm
Ni 0.35 ml 1.4 ppm
Co 0.35 ml 1.4 ppm
Cr 0.35 ml 1.4 ppm
Cd 0.35 ml 1.4 ppm

Standard C

Hg 0.8 ml 3.2 ppm
As 0.8 ml 3.2 ppm

Table 3. Concentrations of Standard Group A metals

Standard series	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8
Volume(Ml)	1	2	3	10	20	30	40	50
Cu(Ppm)	0.2	0.4	0.8	1.2	1.6	2.0	2.4	2.8
Zn(Ppm)	0.2	0.4	0.8	1.2	1.6	2.0	2.4	2.8
Mn(Ppm)	0.08	0.16	0.24	0.8	1.6	2.4	3.2	4.0
Pb (Ppm)	0.028	0.056	0.084	0.28	0.56	0.84	1.12	1.4
Ni (Ppm)	0.028	0.056	0.084	0.28	0.56	0.84	1.12	1.4
Co(Ppm)	0.028	0.056	0.084	0.28	0.56	0.84	1.12	1.4
Cr (Ppm)	0.028	0.056	0.084	0.28	0.56	0.84	1.12	1.4
Cd(Ppm)	0.028	0.056	0.084	0.28	0.56	0.84	1.12	1.4
Hg(Ppm)	0.064	0.128	0.192	0.64	1.28	1.92	2.56	3.2
AS(Ppm)	0.064	0.128	0.192	0.64	1.28	1.92	2.56	3.2

NB. All Stadared stock solution were 1000ppm in 3% nitric acid which are imported from inorganic Ventures. By dilutions with 0.5M Nitric acid we prepare the calibration scheme As follows.

All standard Solutions were prepared in 50 ml graduated tube by filling to their final volume.

So the graph will be drawn against those intensity concentrations.

3.6. Statistical Analysis

For both heavy metals and proximate analysis data was evaluated statistically using the SPSS computer program. One way analysis of variance (ANOVA) was carried out on the data obtained in order to determine any significant difference in the studied metals in the various meat parts and any significant difference of proximate compositions of meat parts across studied industry areas. Results were expressed as mean ± standard error.

3.7 Limitations of the Study

- The sample size is small due to financial constraints needed to pay for heavy metals using ICP-OES. Hence it may cause the rise of question regarding the representativeness of the samples for the desired study.
- Failure to control the seller's bias regarding the location of the growth of hens with respect to the distance from industry area

Chapter Four

4. Results and Discussion

4.1 Proximate Composition

4.1.1 Proximate Composition of Thigh and Chest Sample Parts of Chicken from Akaki, Bishofitu and Modjo Industry Areas

As shown in table 4 below proximate composition of samples between plots of studied industry areas (namely Akaki, Bishofitu and Modjo) was done. The proximate composition of both thigh and chest body parts of chicken from each industrial area were evaluated. The mean proximate composition of thigh samples of chicken from Akaki, Bishofitu and Modjo industry areas can be summarized as; crude protein 22.31±0.21, 21.79±0.16, 21.81±0.31; moisture 70.08±0.44, 70.62±0.71, 70.80±0.47; fat 1.21±0.05, 1.26±0.02, 1.26±0.04; ash 1.17±0.04, 1.13±0.04, 1.10±0.05 respectively. Similarly the proximate composition of chest samples of chicken from Akaki, Bishofitu and Modjo industry area was evaluated and found as follows; protein 22.51±0.29, 22.57±0.21, 22.55±0.30; moisture 71.42±0.22, 73.09±0.25, 72.59±0.38; fat 1.11±0.03, 1.20±0.05, 1.12±0.06; ash 1.14±0.05, 1.04±0.04, 1.16±0.05 respectively ($P \geq 0.05$). On ANOVA there was no significant mean composition difference of moisture content, protein, fat and ash among thigh samples from industry areas of Akaki, Bishofitu and Modjo. Similarly there was no significant mean composition of chest samples from target industry areas except the value obtained that the mean moisture content of chest samples from Akaki industry area was significantly different from mean moisture content of chest samples from Bishofitu and Modjo industry areas (($P \geq 0.05$).

Table 4 Proximate Composition of Thigh and Chest Samples of Chicken From Akaki, Bishofitu and Modjo Industry Areas

Body part	Industry area	Proximate composition			
		Protein	Moisture	Fat	Ash
Thigh	Akaki	22.31 ± 0.21^{a}	70.08 ± 0.44^{a}	1.21 ± 0.05^{a}	1.17 ± 0.04^{a}
	Bishofitu	21.79 ± 0.16^{a}	70.62 ± 0.71^{a}	1.26 ± 0.02^{a}	1.13 ± 0.04^{a}
	Modjo	21.81 ± 0.31^{a}	70.80 ± 0.47^{a}	1.26 ± 0.04^{a}	1.10 ± 0.05^{a}
	Akaki	22.51 ± 0.29^{a}	71.42 ± 0.22^{b}	1.11 ± 0.03^{a}	1.14 ± 0.05^{a}
	Bishofitu	22.57 ± 0.21^{a}	73.09 ± 0.25^{a}	1.20 ± 0.05^{a}	1.04 ± 0.04^{a}
	Modjo	22.55 ± 0.30^{a}	72.59 ± 0.38^{a}	1.12 ± 0.06^{a}	1.16 ± 0.05^{a}

Within column, mean with different letters are statistically significant $p<0.05$

Values are kept as mean$\pm std$ error

4.1.2 Proximate Composition of Thigh and Chest Samples of Chicken from Industry Areas

As shown in table 5 below the mean proximate composition of thigh and chest samples from chicken raised around industry areas were recorded as; moisture content, 70.50±0.29, 72.37±0.20, crude protein, 21.97±0.14, 22.49±0.15, fat, 1.24±0.02, 1.14±0.03, ash, 1.13±0.02, 1.11±0.03 respectively.

Table 5 Proximate Composition of Thigh and Chest Samples of Chicken from Industry Areas

Body part	Proximate composition			
	Protein	Moisture	Fat	Ash
Thigh	21.97±0.14	70.50±0.29	1.24±0.02	1.13±0.02
Chest	22.49±0.15	72.37±0.20	1.14±0.03	1.11±0.03

Values are kept as mean$\pm std$ error

The crude protein content of studied meat samples (chest and thigh) from independent industry areas (as shown in table 5 and cumulative industry area (as shown in table 5) were lower when compared with a study at Nigeria in chicken muscle tissue being 27.01±0.03 (Daniel 2015). However a study from Korea by (Skalickd & Korenekovd, 2002), 21.93 in thigh and 24.32 in chest chicken samples was almost in line when compared with meat samples value found from Modjo and Bishofitu industry but lower from protein of chest samples from Akaki industry area. The results of protein composition found from studied sample body parts were almost in line with literatures; poultry meat (20-25%) (Gerber, 2007, Barroeta , 2007).

Moisture content of meat samples (chest and thigh) from study areas was lower when compared with a study done at Korea (74.79 from thigh and 73.01 from chest samples) (Skalickd & Korenekovd, 2002) but higher when compared with a study at Nigeria (67.03±0.04 from chicken muscle) (Skalickd & Korenekovd, 2002). Besides moisturecontent in food determines the keeping qualities of food, it also enhance the rate at which absorption takes place within the digestive system and influences the rate at which enzyme activities takes place on the food(Daniel, 2015).

The crude fat content of those studied samples is comparable with studies from Nigeria (1.11±0.11 from chicken meat) (Daniel, 2015) and Korea (1.41 from thigh of chicken and 0.72 from chest (Skalickd & Korenekovd, 2002). The mean fat content from studied samples was in line with a study from Nigeria (Daniel, 2015) but much more lower when compared with a study from Korea (Skalickd & Korenekovd, 2002)

4.2 Essential Metals Analysis

4.2.1 Essential Metals Concentrations from Liver, Thigh and Chest samples of Chicken Raised around Industry Areas

The mean concentrations of essential metals content from liver, thigh and chest samples of chicken meat raised around industry areas observed values ranged (mg/kg) Zn 24.04±0.61- 134.53±7.27; Cu 2.90±0.13 - 9.24±0.30 ; Mn 1.40±0.07 - 8.64±0.39; Cr 4.44±0.26 - 4.75±0.13; Co ND - 0.02±0.01; Ni 3.23±0.21 - 4.28±0.34

Table 6 Essential Metals Concentrations from three body parts Liver, Thigh and Chest samples of Chicken Raised Around Industry Areas (mg/Kg)

Body part	Essential metals concentrations (mg/Kg)					
	Zn	Cu	Mn	Cr	Co	Ni
Liver	134.53±7.27^{a}	9.24±0.30^{a}	8.64±0.39^{a}	4.53±0.15^{a}	0.02±0.01^{a}	3.23±0.21^{b}
Thigh	68.49±2.38^{b}	2.90±0.13^{b}	2.49±0.50^{b}	4.44±0.26^{a}	ND^{b}	4.28±0.34^{a}
Chest	24.04±0.61^{c}	4.23±2.48^{b}	1.40±0.07^{c}	4.75±0.13^{a}	ND^{b}	3.54±0.30^{b}

Within column, mean with different letters are statistically significant $p<0.05$

ND stands for Not Detected

Values are kept as mean$\pm std$ error

4.2.2 Concentration of Essential Metals from Three Body Parts of Chicken Samples of Akaki, Bishofitu and Modjo Industry Areas

The concentrations of essential heavy metals from liver, thigh and chest samples of chicken meat raised around industry areas of Akaki, Bishofitu, Modjo ranged(mg/Kg); Zn 24.94±0.63 - 155.51±4.24, 25.24±1.41 - 107.87±9.30, 21.94±0.79 - 140.22±17.57; Cu 1.62±0.28 - 9.79±0.36, 1.84±0.29 - 7.39±0.49, 2.26±0.16 - 10.53±0.34; Mn 1.64±0.18 - 7.87±0.63, 1.29±0.09 - 7.57±0.28, 1.27±0.03 - 10.49±0.76; Cr 4.26±0.14 - 4.75±0.70, 4.60±0.42 - 5.45±0.25, 3.93±0.23 - 4.73±0.07, Co ND - 0.03±0.02, ND, ND; Ni 2.14±0.11- 3.07±0.15, 3.07±0.30 - 5.45±0.92, 4.27±0.24 - 5.30±0.61 respectively

Table 7 Concentration of Essential Metals from body parts of Chicken samples of Akaki, Bishofitu and Modjo Industry Areas (mg/Kg)

Industry area	Body part	Essential metals concentration (mg/Kg)					
		Zn	Cu	Mn	Cr	Co	Ni
Akaki	Liver	155.51 ± 4.24^{a}	9.79 ± 0.36^{a}	7.87±0.63a	4.26 ± 0.14^{a}	0.03 ± 0.02^{a}	2.14 ± 0.11^{b}
	Thigh	77.90 ± 3.07^{b}	3.75 ± 0.18^{b}	1.69 ± 0.07^{b}	4.75 ± 0.70^{a}	ND^{a}	3.07 ± 0.15^{a}
	Chest	24.94 ± 0.63^{c}	1.62 ± 0.28^{c}	1.64 ± 0.18^{b}	4.45 ± 0.19^{a}	0.01 ± 0.00^{a}	2.27 ± 0.15^{b}
Bishofitu	Liver	107.87 ± 9.30^{a}	7.39 ± 0.49^{a}	7.57 ± 0.28^{a}	4.60 ± 0.42^{a}	0.03 ± 0.02^{a}	3.29 ± 0.42^{b}
	Thigh	70.85 ± 4.59^{b}	2.68 ± 0.15^{b}	4.15 ± 1.42^{b}	4.64 ± 0.26^{a}	ND^{a}	5.45 ± 0.92^{a}
	Chest	25.24 ± 1.41^{c}	1.84 ± 0.29^{b}	1.29 ± 0.09^{c}	5.45 ± 0.25^{a}	ND^{a}	3.07 ± 0.30^{b}
Modjo	Liver	140.22 ± 17.57^{a}	10.53 ± 0.34^{a}	10.49 ± 0.76^{a}	4.73 ± 0.07^{a}	ND^{a}	4.27 ± 0.24^{a}
	Thigh	56.70 ± 2.48^{b}	2.26 ± 0.16^{a}	1.63 ± 0.1^{b}	3.93 ± 0.23^{b}	ND^{a}	4.32 ± 0.11^{a}
	Chest	21.94 ± 0.79^{c}	9.22 ± 7.42^{a}	1.27 ± 0.03^{b}	4.34 ± 0.05^{a}	ND^{a}	5.30 ± 0.61^{a}

Within column, mean with different letters are statistically significant $p<0.05$

ND stands for Not Detected

Values are kept as mean$\pm std$ error

Zinc (Zn) content

As shown in table 6 the overall concentrations of Zn from chicken raised around industry areas samples taken from liver, thigh, chest were observed with values of 134.43±7.23, 68.49±2.38, and 24.04±0.61mg/kg respectively. On ANOVA the mean concentrations of Zn content from liver samples was significantly higher than the value from both thigh and chest samples. At the same time as indicated in table 7 mean Zn concentration of liver, thigh and chest samples of the three selected industry areas namely Akaki, Bishofitu and Modjo was compared among themselves; the highest Zn concentration value was observed from liver of Akaki industry area (155.51±4.24mg/kg) and the lowest concentration value was observed from chest of Modjo industry area (21.94±0.79mg/kg). Moreover the results obtained from studied sample body parts of selected industry areas were much higher when compared with the reported value of 3.11±0.025μg/g in liver and 1.1μg/g in meat samples respectively. A study done to assess heavy metals on poultry in tannery waste contaminated area in Bangladesh with an observed value of (19.95±0.01mg/kg) in liver and 4±0.00mg/kg in meat samples from Kohan market, Pakistan (El-Salam, *et al.*, 2013). The mean Zn concentrations found in the studied liver and meat (thigh and chest) samples were much higher when compared with a study by Akan, *et al.*, 2010 but lower from mean Zn concentrations of 190.00±29.00mg/kg, 42.00+12.20mg/kg in liver and meat samples respectively from a study in China (Zhuang,*et al.*, 2014). The studied body parts of chicken Zn content value obtained from liver, thigh and chest were below the permissible limit for Zn (150ppm) set by ANZFA (El-Salam, *et al.*, 2013).

Copper (Cu) content

From table 6 the overall mean concentrations (mg/kg) of Cu from samples of studied areas from liver, thigh, chest was 9.24±0.30, 2.90±0.13, 4.23±2.48, respectively. Liver samples containing the highest concentrations. At the same time from individual selected industry areas as shown in table 9 the highest mean concentration was from liver of Modjo industry area (10.53±0.34mg/kg) and the lowest concentration was from chest sample of Akaki industry area (1.62±0.28mg/kg).

These Cu mean concentrations found from the studied sample parts of studied areas were higher when compared with a study at Enugu metropolis, Nigeria with mean concentrations of 0.24±0.06mg/kg, 0.08±0.024 mg/kg from meat and liver samples respectively (Onyeka & David, 2015) as well as the results from liver and meat samples were much higher when compared with Cu concentrations obtained by Akan, *et al.*, 2010. A study done at Kohat market, Pakistan showed mean Cu concentration of 20.863±0.03mg/kg from liver and 2.513±0.00mg/kg from meat samples of chicken (El-Salam, *et al.*, 2013). The permissible limit in chicken meat set by FAO/WHO as well as by the Commission of European communities is 1mg/kg (Zhuang,*et al.*, 2014). The sources of highest concentrations of Cu from chicken meat of studied industry areas could be from domestic sources and due to the availability of industries like tannery, metal works, and wood preservatives used for wood factories of the studied industry areas.

Manganese (Mn) Content

From table 6 the overall mean concentration of Mn from liver, thigh, chest sample parts of studied areas was 8.64±0.39, 2.49±0.50, and 1.40±0.07mg/kg respectively. And as shown from table 9 the mean concentrations of Mn from Akaki, Bishofitu and Modjo industry areas the highest Mn mean concentration was found from liver of Modjo industry area and the lowest was from chest sample of same industry area. The mean concentration of Mn found from studied industry areas sample parts were higher when compared with mean Mn concentrations of 0.52±0.03mg/kg , 0.48±0.22mg/kg from liver and muscle samples respectively of Mn a study at Uyo metropolis,Akwa Ibom state, Nigeria (Daniel, 2015). The results obtained from studied sample body parts chicken were much higher when compared with the WHO standard of 0.5mg/kg (Daniel, 2015).

Chromium (Cr) Content

From table 6 the mean concentration of Cr from liver, thigh, chest samples of studied areas was 4.53±0.15, 4.44±0.26, 4.75±0.13mg/kg respectively. From Cr concentrations of the selected industry study areas the highest Cr concentration was from chest of Bishofitu (5.45+0.25mg/kg) and the lowest was from thigh of Modjo industry area (3.93±0.23mg/kg). The results obtained from sample parts of studied areas were much higher when compared with Cr mean concentration of 0.02±0.01mg/kg, 0.24±0.02mg/kg from muscle and liver sample parts respectively a study held at Enugu metropolis, Nigeria (Onyeka & David, 2015). A study done on tannery waste contaminated area in Bangladeshi showed mean concentrations of 4.56±4.09mg/kg, 1.79±1.26mg/kg, 2.78±1.15mg/kg from liver, thigh and breast samples respectively (Mottalib, *et al.*, 2016). The permissible for Cr in food is generally 0.5ppm. The highest concentrations found from studied chicken meat parts could result from tannery, chemical, metal, wood preservatives effluents from industries of selected industry areas.

Cobalt (Co) Content

As shown in table 6 the mean concentrations of Co in liver, thigh, chest samples of the studied areas 0.02±0.01mg/kg, ND, ND respectively. Also as shown in table 9 the mean Co concentrations from the independent industry areas the highest concentrations were from liver samples of both Akaki and Bishofitu (0.03±0.02mg/kg for both cases) and the lowest concentrations was from from liver sample of Modjo and thigh and chest samples of all the three industry areas which were below detection limits. The results obtained in the studied samples were much lower when compared with Co mean concentrations of 0.20±0.00mg/kg and 0.20±0.00mg/kg from liver and meat samples respectively done at Kohan, Pakistan (El-Salam, *et al.*, 2013). The concentrations of cobalt whose permissible value is not mentioned in WHO in liver sample were almost in line with a study at Iraq (Abduljaleel, 2014).

Nickel (Ni) Content

As shown in tables 6 the mean concentrations of Ni in liver, thigh and chest samples of chicken rasised around industry areas was 3.23±0.21 mg/kg, 4.48±0.34 mg/kg, 3.54±0.30 mg/kg respectively. From the mean Ni concentrations from the selected industry areas of Akaki, Bishofitu and Modjo liver, thigh and chest samples, the highest concentration was from thigh sample of Bishofitu industry area. The studied Ni content values were much higher when compared with the permissible limits in foods (WHO 1966) which is 0.5mg/kg. The results found from body parts of the studied industry areas were also much higher when compared with a study done with cocoa producing area grown local hens in Nigeria whose Ni concentrations in muscle and liver with the value 1.13±0.01mg/kg and 1.13±0.22mg/kg respectively (Williams, *et al.*, 2017). Other study from Bangladesh were observed as 6.24±1.61mg/kg, 3.70±3.46mg/kg and 7.41±6.51 mg/kg in breast, thigh and liver being 6.24±1.61mg/kg, 3.70±3.46mg/kg and 7.41±6.51 mg/kg respectively (Mottalib, *et al.*, 2016). The reason for higher concentration of Ni in chicken meat might come from domestic discharges such as human food leftovers used as chicken food and water.

4.2.3 Pooled Mean Concentrations of Essential Metals

As indicated in table 8 below the pooled mean that contains mean of means of liver, thigh and chest samples of industry areas concentrations of essential heavy metals from Akaki, Bishofitu and Modjo industry areas; Zn 86.12±8.26, 67.99±6.13, 72.96±9.46; Cu 5.06±0.55, 3.97±0.41, 7.34±2.48; Co 0.01±0.00, 0.01±0.00, ND; Mn 3.74±0.49, 4.34±0.61, 4.46±0.69; Cr 4.48±0.24, 4.90±0.19, 4.34±0.09; Ni 2.49±0.10, 3.94±0.38, 4.63±0.23 respectively. On ANOVA of the essential trace heavy metals there existed significant pooled mean difference among selected industry areas only on pooled Cr and pooled Ni mean concentrations. Cr; the pooled mean concentration of Cr of Akaki and Bishofitu areas was significantly different from pooled Cr mean concentration of Mojo industry area, Ni; the pooled mean concentration of Ni of Bishofitu and Mojo industry areas is significantly different from pooled mean Ni concentrations of Akaki industry areas.

Table 8 Pooled Mean of Essential Metals Concentrations from Akaki, Bishofitu and Modjo industry Areas (mg/kg)

Industry area	Essential trace metal concentration					
	Zn	Cu	Co	Mn	Cr	Ni
Akaki	86.12±8.26^x	5.06±0.55^x	0.01±0.00^x	3.74±0.49^x	4.48±0.24^x	2.49±0.10^y
Bishofitu	67.99±6.13^x	3.97±0.41^x	0.01±0.00^x	4.34±0.61^x	4.90±0.19^x	3.94±0.38^x
Mojo	72.96±9.46^x	7.34±2.48^x	ND^x	4.46±0.69^x	4.34±0.09^y	4.63±0.23^x

Within column, mean with different letters are statistically significant $P<0.05$

ND stands for Not Detected

Values are kept as mean$\pm std$ error

4.3 Toxic Trace Heavy Metals analysis

4.3.1 Toxic Heavy Metals Concentrations from Liver, Thigh and Chest body parts of Chicken Raised Around three selected Industry Areas, Ethiopia

The mean concentrations of toxic heavy metals from liver, thigh and chest samples of studied industry areas ranged (mg/kg); As 0.11±0.00 - 0.33±0.12, Pb 0.02±0.0 - 0.16±0.07, Cd 0.01±0.00 - 0.98±0.26, Hg 0.05±0.02 - 0.09±0.02 respectively. as shown in table 11 liver sample parts containing relatively high toxic metals than thigh and chest sample parts.

Table 9 Toxic Heavy Metals Concentrations from Liver, Thigh and Chest samples of Chicken raised around selected Industry Areas, Ethiopia (mg/Kg)

Body part	Heavy metal concentrations (Kg/mg)			
	As	Pb	Cd	Hg
Liver	0.33 ± 0.12^{a}	0.16 ± 0.07^{a}	0.98 ± 0.26^{a}	0.09 ± 0.02^{a}
Thigh	0.12 ± 0.02^{a}	0.02 ± 0.0^{b}	0.01 ± 0.00^{b}	0.07 ± 0.01^{a}
Chest	0.11 ± 0.00^{a}	$0.02\pm.0^{b}$	0.01 ± 0.00^{b}	0.05 ± 0.02^{a}

Within column, mean with different letters are statistically significant $p<0.05$

ND stands for Not Detected

Values are kept as mean$\pm std$ error

4.3.2 Toxic Heavy Metals Concentrations from Liver, Thigh and Chest Samples of Chicken from Akaki, Bishofitu and Modjo Industry Areas

The mean concentration toxic heavy metals from samples of chest, thigh and liver of Akaki, Bishofitu and Modjo industry areas ranged (mg/kg); As 0.08±0.03 - 0.61±0.32, 0.05±0.03 - 0.29+0.13, ND - 0.23±0.06; Pb ND - 0.08±0.04, ND - 0.39±0.21, ND - 0.07±0.03; Cd ND - 1.08±0.36, ND - 1.45±0.68, ND - 0.42±0.16; Hg 0.09±0.02 - 0.12±0.02, 0.02±0.01 - 0.12±0.04, ND - 0.09±0.02 respectively.

Table 10 Concentrations of Toxic Heavy Metals from Liver, Thigh and Chest Samples of Chicken from Akaki, Bishofitu and Modjo Industry Areas (mg/Kg)

Industry area	Body part	Toxic metals concentration (mg/Kg)			
		As	Pb	Cd	Hg
Akaki	Liver	0.61 ± 0.32^{a}	0.08 ± 0.04^{a}	1.08 ± 0.36^{a}	0.08 ± 0.03^{a}
	Thigh	0.08 ± 0.03^{a}	ND^{b}	ND^{b}	0.12 ± 0.02^{a}
	Chest	0.23 ± 0.06^{a}	0.07 ± 0.03^{b}	ND^{b}	0.09 ± 0.02^{a}
Bishofitu	Liver	0.29 ± 0.13^{a}	0.39 ± 0.21^{a}	1.45 ± 0.68^{a}	0.12 ± 0.04^{a}
	Thigh	0.07 ± 0.02^{b}	ND^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}
	Chest	0.05 ± 0.03^{b}	ND^{b}	ND^{b}	0.03 ± 0.02^{b}
Modjo	Liver	0.09 ± 0.07^{b}	ND^{b}	0.42 ± 0.16^{a}	0.07 ± 0.01^{a}
	Thigh	0.23 ± 0.06^{a}	0.07 ± 0.03^{a}	ND^{b}	0.09 ± 0.02^{a}
	Chest	ND^{b}	0.06 ± 0.04^{a}	ND^{b}	ND^{b}

Within column, mean with different letters are statistically significant $p<0.05$

ND stands for Not Detected

Values are kept as mean$\pm std$ error

Concentration of Arsenic (As)

As shown in table 9 the mean concentrations of As from liver, thigh and chest from selected studied industry areas was 0.33±0.12, 0.12±0.02 and 0.11±0.00mg/kg respectively. The highest As concentration was observed from liver sample of the studied industry areas which was 0.33±0.12mg/kg. Whereas the lowest mean concentration was observed from chest samples. Of the 45 sample parts studied on 11 samples (3 from Akaki chest, 2 from Bishofitu chest, 2 from Bishofitu liver, 1 from Bishofitut thigh, and 3 from Modjo chest) were found to be below detection limit. A study conducted on chickens raised within Enugu metropolis, Nigeria is found to contain mean concentrations of As in liver and meat as 0.26±0.05 and 0.09±0.02 mg/kg respectively. (Onyeka & David, 2015). According ANZFA, 2001, the permissible limit for As in liver of chicken is 2ppm (Akan, *et al.*, 2010). This reveals that the mean concentrations of As both in chicken meats (chest or thigh) and liver samples from chickens raised around the industry areas was higher than the mean concentrations of As from respective sample body parts studied at Enugu metropolis area but the concentration of As from all industry area liver samples was lower than the permissible limits of As (Onyeka & David, 2015).

Concentration of Lead (Pb)

As shown in table 9 the mean concentrations of Pb from studied samples of industry areas of liver, thigh and chest samples were 0.16±0.07, 0.02±0.00, and 0.02±0.00mg/kg respectively. From the three industry areas the highest Pb concentration was observed from liver sample of Bishofitu industry area (0.39±0.21mg/kg). These values were lower when compared with the WHO standard for the permissible limit of Pb for chicken liver (0.5mg/g) and chicken meat of 0.1mg/g (Iwegbue, *et al.*, 2008) respectively. A study conducted on chickens raised within Enugu Metropolis, Nigeria the mean concentration from muscle and liver samples was found to be 0.08±0.00mg/g and 0.07±0.04mg/g respectively (Mansour, 2014). Results done in China showed mean Pb concentrations of 0.73+0.29mg/kg, 0.52+0.22mg/kg in poultry liver and meat samples respectively (Zhuang,*et al.*, 2014).

Concentration of Cadmium (Cd)

As shown in table 9 the mean concentration of Cd in liver, thigh and chest of the studied industry area samples were 0.98±0.126, 0.01±0.00, and 0.01±0.00 respectively; the highest mean concentration being from liver samples. Among the three studied industry areas the highest Cd concentration was from chicken liver of Bishofitu (1.45±0.68) and the lowest concentration was from chest samples of the three industry areas and thigh samples of both Akaki and Bishofitu industry areas which was below detection limit for both cases. The result obtained from liver samples of the studied industry areas was higher when compared with the permissible limits (0.5mg/kg in chicken liver and 0.1mg/kg in chicken meat (Zahrana, & Hendyb, 2015); however the concentrations obtained from meat samples were below the permissible limit, and the Cd concentration on studied meat samples is almost in line with Cd mean concentrations at Slovak (0.02mg/kg) (Skalickd & Korenekovd, 2002). A study from Kohant market Pakistan showed mean concentration of Cd on liver and meat samples being 1.213±0.00mg/kg and 1.15±0.00 respectively (El-Salam, *et al.*, 2013). The results obtained in thigh and chest from the studied sample parts were almost in line, but higher regarding Cd concentrations in liver when compared with a study at Libya (Abdolgader, *et al.* 2013). A study done in china showed mean Cd concentrations of 9.36±3.60mg/kg and 0.06±0.02mg/kg in chicken liver and meat samples respectively (Zhuang,*et al.*, 2014).

The highest Cd concentrations in liver samples of chicken might came both from natural emissions since the location of the selected industry areas is on rift valley region and from metal work industries of the selected areas. Though in Ethiopian culture chicken liver is not used for consumption and might not be a direct health threat for health of the community after consuming chicken meat; it is an indication that there is Cd pollution of the environment.

Concentration of Mercury (Hg)

As shown in table 9 the mean concentration of Hg in liver, thigh and chest of studied industry areas were 0.09±0.02, 0.07±0.01 and 0.05±0.02mg/kg respectively. The highest mean concentration is from liver sample and the lowest was from chest. As indicated in table 10 the highest Hg concentration was from liver sample of Bishifitu (0.12±0.04mg/kg) where as the lowest was from chest sample of Modjo industry area. A study done on locally reared chicken of cocoa producing area in Cross River State, Nigeria, showed Hg concentration in meat and liver as 0.03±0.02 0.03±0.01mg/kg respectively (Williams, *et al.*, 2017).The established permissible limits set for Hg in consumable meats products is 0.50ppm (Onyeka & David, 2015).

4.3.2 Pooled Mean Concentrations of Toxic Heavy Metals

As shown below in table the pooled mean concentrations of toxic heavy metals from liver, thigh and chest samples of chaicken raised around industry areas of Akaki, Bishofitu and Modjo (mg/kg) As 0.32±0.13, 0.14±0.05, 0.11±0.03; Pb 0.03±0.01, 0.13±0.07, 0.04±0.02; Cd 0.37±0.14, 0.49±0.24, 0.14±0.06, Hg 0□1±0.02, 0.06±0.02, 0.05±0.01 respectively. Table 11 Pooled Mean Concentrations Of Toxic Heavy Metals (mg/Kg)

Table 11. Pooled Mean of Toxic Metals Concentrations from Akaki, Bishofitu and Modjo industry Areas (mg/kg

Industry area	Heavy metal of concentrations (mg/kg)			
	As	Pb	Cd	Hg
Akaki	0.32±0.13^{x}	0.03±0.01^{x}	0.37±0.14^{x}	0.11±0.02^{x}
Bishofitu	0.14±0.05^{x}	0.13±0.07^{x}	0.49±0.24^{x}	0.06±0.02^{y}
Mojo	0.11±0.0s3^{x}	0.04±0.02^{x}	0.14±0.06^{x}	0.05±0.01^{y}

Within column, mean with different letters are statistically significant $p<0.05$

Values are kept as mean±*std* error

As shown in table 11 pooled mean concentrations toxic trace heavy metals from Akaki, Bishofitu , Mojo; As; 0.32±0.13, 0.14±0.05, 0.11±0.03, 0.90±0.14, Pb; 0.03±0.01, 0.13±0.07, 0.04±0.02, Cd; 0.37±0.14, 0.49±0.24, 0.14±0.06, Hg; 0.11±0.02, 0.06±0.02, 0.05±0.01mg/kg respectively. On ANOVA of the toxic trace heavy metals there existed significant pooled mean difference among selected industry areas only on pooled Hg mean concentrations. Hg; the pooled mean concentration of Hg from Akaki industry area was significantly different from Hg pooled mean concentration of both Bishofitu and Modjo industry areas but there was no significant Hg pooled mean difference between Bishofitu and Mojo industry areas.

Chapter Five

Conclusions and Recommendations

5. 1 Conclusion

This study was conducted with the broad intention to point out proximate composition and levels of essential/toxic metals in chicken meat raised around industry areas of Ethiopia.

Proximate composition of the studied meat of chicken revealed that they are rich in protein contents; chest meat containing relatively high protein composition than of thigh meat. However there were no significant difference observed on the value of protein content of chest and thigh samples among chickens from the studied industry areas (Akaki, Bishofitu and Modjo). Moreover meat samples from all industry areas were also found containing low proportion of fat content though thigh samples from its respective chest sample containing relatively high amount of fat.

Regarding essential trace metals assessment the study showed chickens from all studied industry areas having rich amount of essential trace metals like Zn. Moreover and higher amounts of Cu, Mn, Ni, and Cr indicating that there is high amount of those metals effluents from the surrounding industries to the environment which may consequently cause health hazards to the community after long term exposures. All chest, thigh and liver sample parts of the three studied industry area chickens were found to contain much higher mean Cu, Mn, Cr and Ni concentrations when compared with the maximum permissible limits set by national and international standards. Thereby may impose health risks that can be associated with the excessive effects of those heavy metals. Respiratory problems, increased risk of lung cancer, neurological deficits, developmental deficits in children and increased blood pressure are health risks due to Ni exposures. Neurological disorders similar to Parkinson's disease may result due to excessive Mn exposure. Similarly inhibition leading to Wilson's diseases after long time Cu exposure and, skin rashes, stomach ulcer, kidney, liver damages, lung cancer and ultimate death due to prolonged higher Cr exposures. The result also indicated that chickens of liver samples from Akaki and Bishofitu industry areas were found with much higher Cd concentrations (1.08±0.36mg/kg, and 1.45±0.68mg/kg respectively) reflecting the environment is polluted with Cd. Higher level Cd exposures might cause health impacts such as hypertension, elevated blood pressure, renal dysfunction, acute and chronic pulmonary dysfunctions to the community.

5.2 Recommendations

The current study with its own different limitations has tried to investigate proximate composition and the levels of essential/toxic metals in chicken meat raised around industry areas in Ethiopia. Hence the following issues should also be considered in the future based on the outcomes of the current study.

Chicken from the three studied industry areas were found to contain high value of protein, low proportion of fat and rich amount of essential minerals like Zn. However there was a higher concentration of both essential (Cu, Mn, Cr, Ni) and toxic metal like Cd that may cause health hazards to the community. Therefore it is good to consider those health consequences.

Taking the effect of industrial pollution to the environment into account including chicken meat in regular meals could be considered by the community. Besides poultry food can be also one means to combat poverty in Ethiopia

Some toxic heavy metals were found to reside especially in liver being beyond their permissible limits; hence it is advisable to conduct series of studies.

It could be encouraged others to conduct similar researches including chickens raised in rural & as well as chickens raised in farm base to compare especially essential and toxic metals content differences

It could be advised others to conduct similar researches with large sample size to draw good representatives of the desired study since only few samples are utilized for this study due to financial constraints especially because of the expensive cost required to pay for ICP-OES to analyze heavy metals

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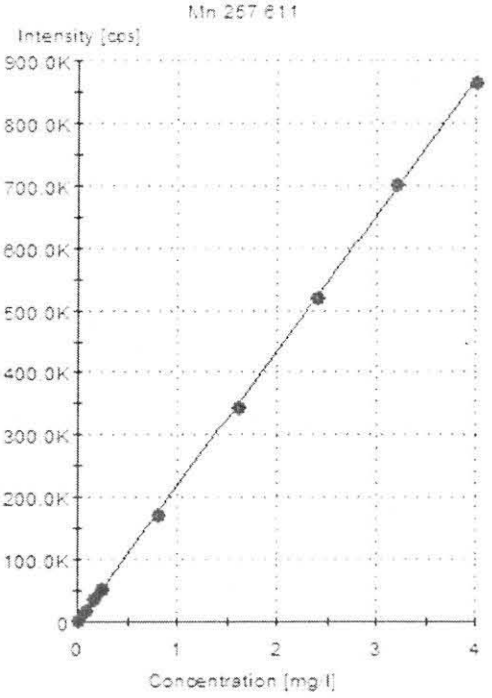
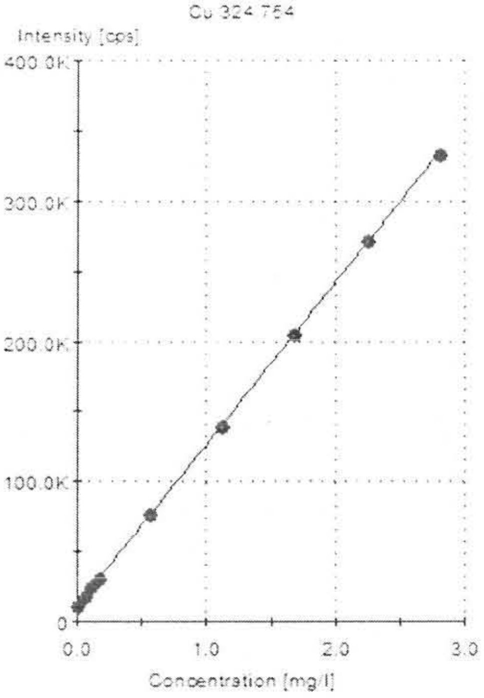
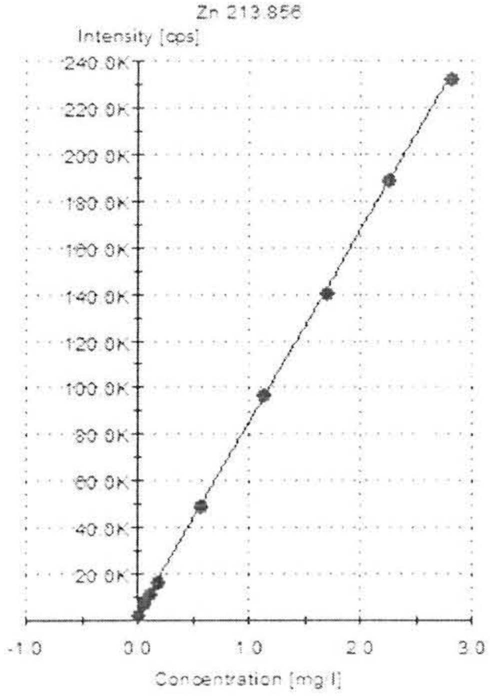
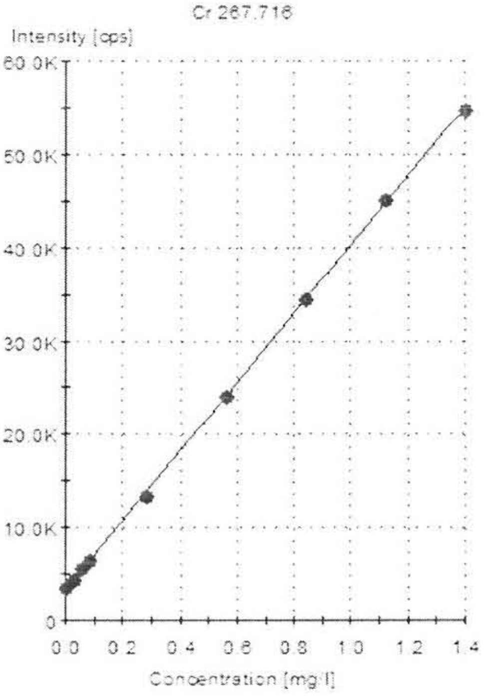
APPENDIX

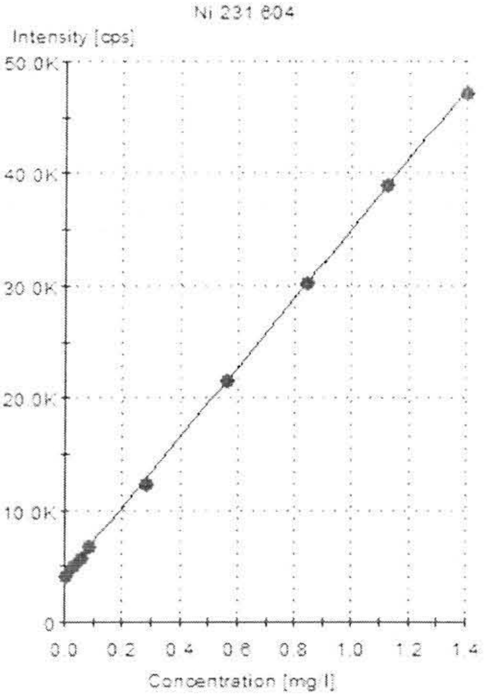
A. Calibration Curve of studied Heavy Metals

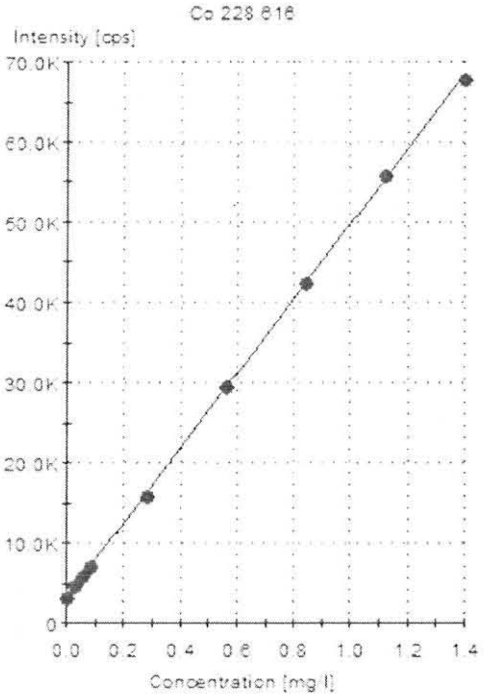


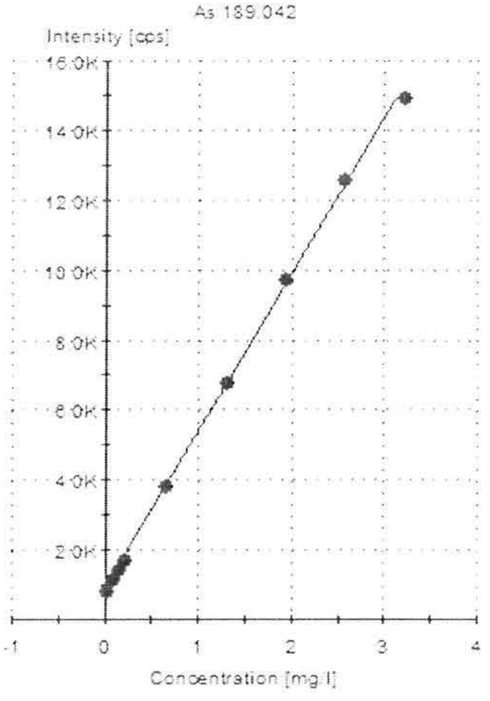


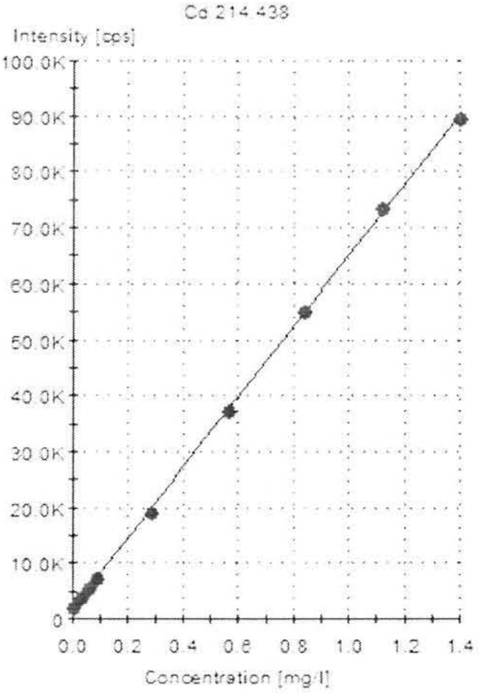


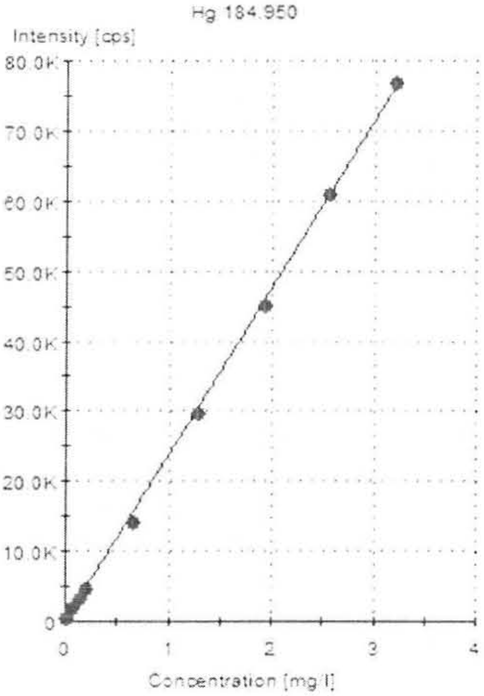



Ni 231.604
Intensity [cps]
50.0K
40.0K
30.0K
20.0K
10.0K
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Concentration [mg/l]

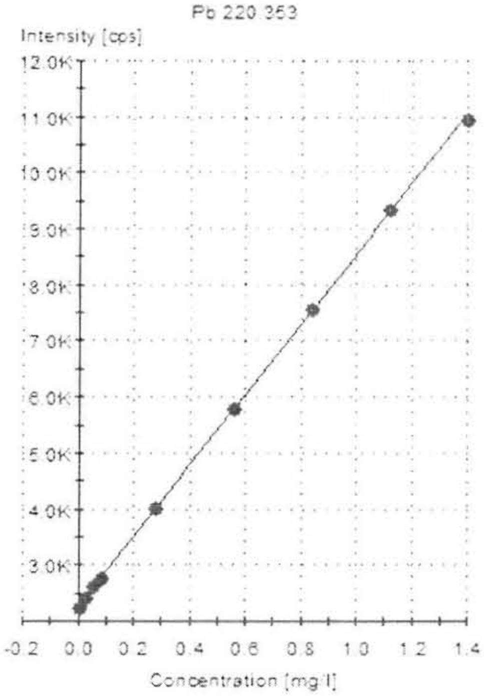

Co 228.616
Intensity [cps]
70.0K
60.0K
50.0K
40.0K
30.0K
20.0K
10.0K
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Concentration [mg/l]


As 189.042
Intensity [cps]
16.0K
14.0K
12.0K
10.0K
8.0K
6.0K
4.0K
2.0K
-1
0
1
2
3
4
Concentration [mg/l]


Cd 214.438
Intensity [cps]
100.0K
90.0K
80.0K
70.0K
60.0K
50.0K
40.0K
30.0K
20.0K
10.0K
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Concentration [mg/l]



Hg 184.950



Pb 220.353