

**ASSESSMENT OF SELECTED HEAVY METALS IN PARTS OF
SLAUGHTERED CATTLE AND GOATS CENTRAL ABATTOIR FROM
AKINYELE, IBADAN**

BY

OLADIPO, TUNBOSUN ADEMOLA

MATRIC NUMBER 123544

D.V.M.

(UNIVERSITY OF IBADAN)

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CERTIFICATION

I certify that this research work was carried out by Oladipo Tunbosun Ademola of the department of Environmental Health Sciences, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan.

Supervisor

Dr. Oladapo Titus Okareh

B.Sc, M.P.H (Ib), Ph.D (Ib)

Department of Environmental Health Sciences, Faculty of Public Health, College of
Medicine, University of Ibadan, Nigeria

DEDICATION

This research work is dedicated to Almighty God. Also, to my late brother Dr. Oladipo Olawale David; may his gentle soul rest in peace, Amen.

UNIVERSITY OF IBADAN

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ABSTRACT

Meat and offal obtained from cattle and goats sold in open markets serve as sources of protein, but may be susceptible to heavy metal contamination due to illicit disposal and emission in the environment. Consumers' knowledge on the possible exposure to heavy metal contamination in meat and its associated health risks is low. Therefore, this study aimed at assessing heavy metal levels in these products.

A cross-sectional study design with observational and laboratory analysis components was adopted. Lead, cadmium and chromium residues in blood, muscle, liver and kidney of cattle and goats slaughtered in central abattoir, Akinyele, Ibadan were determined. The abattoir was selected because it is the largest abattoir supplying live animals and meat to residents of Ibadan. A total of 40 animals (20 each of white Fulani cattle and red Sokoto goats) were purposively selected over a period of 8 weeks in conformity with FAO quality control guidelines. Values were compared with Joint FAO/WHO guidelines. These animals were of different sexes and ages (determined by the use of their dentition). Samples of blood was collected from jugular vein at slaughter using 5ml syringe and 21 gauge needle, external abdominal muscles, liver apical lobes and kidney cortices were collected. Samples were analysed using Atomic Absorption Spectrophotometer (AAS) for lead, chromium and cadmium. Statistical analysis was done using descriptive statistics and t-test at 5% level of significance.

Age of 14 male and 6 female cattle was 49.5 ± 14.7 months while the age of 12 male and 8 female goats was 28.8 ± 8 months. Cadmium was found in 115 samples (71.9%), lead in 26 samples (16.3%) and chromium in 17 samples (10.6%). Chromium value in blood of cattle ($0.6 \pm 0.0 \mu\text{g/ml}$) was significantly lower compared to goats' ($2.4 \pm 2.6 \mu\text{g/ml}$). In muscle tissue, liver and kidney, chromium values for cattle and goats were 0.6 ± 0.0 , 1.3 ± 0.9 and $2.3 \pm 3.0 \text{ mg/kg}$ and 0.0 , 0.6 ± 0.0 and 0.0 mg/kg respectively. Chromium residues were higher in liver and kidney of cattle and goats' blood than permissible limits of 1 mg/kg . Cadmium value in blood of cattle ($5.7 \pm 2.1 \mu\text{g/ml}$) was significantly lower compared to goats' ($6.8 \pm 3.0 \mu\text{g/ml}$). In muscle tissue, liver and kidney of cattle and goats cadmium values were 3.8 ± 2.1 , 5.2 ± 2.3 and $5.7 \pm 2.3 \text{ mg/kg}$ and 5.4 ± 1.9 , 5.5 ± 2.9 and $4.8 \pm 1.9 \text{ mg/kg}$ respectively. These values were higher than permissible limit of $0.5\text{-}1.0 \text{ mg/kg}$. Lead residual values in blood of cattle and goats were 0.5 ± 0.6 and $0.0 \mu\text{g/ml}$, while in muscle, liver and kidney of cattle and goats were 0.1 ± 0.2 , 0.0 and 0.0 mg/kg and 0.0 , 0.0 and 0.0 mg/kg respectively. These values were within the permissible limits ($0.1\text{-}0.5 \text{ mg/kg}$). Cattle were found to have higher levels of bioaccumulation for lead, cadmium and chromium residues when compared to those of goats.

From central abattoir, Akinyele, Ibadan, cattle and goat meat contained high levels of cadmium and chromium which could pose health risk to consumers. Therefore, public health awareness on the risk associated with cadmium and chromium ingestion and measures to reduce them in meat and meat products should be advocated.

Keyword: Cadmium, Lead, Chromium contamination

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TABLE OF CONTENTS

CONTENTS	PAGES
Title Page	i
Certification	ii
Dedication	iii
Acknowledgements	iv
Abstract	v
Table of contents	vii
List of tables	xii
List of figures	xiii
List of plates	xiv
CHAPTER ONE	
1.0 Introduction	1
1.1 Problem statements	3
1.2 Justification	4
1.3 Broad objective	4
1.4 Specific objectives	4
CHAPTER TWO	
2.0 Literature review	5
2.1 Heavy metals	5
2.1.1 Classification of heavy metals	5
2.1.2 Heavy metals of public health importance studied	6
2.2 Cadmium	7
2.2.1 Health effects of cadmium	11

2.2.2	Study on the Cadmium-induced cancer	14
2.3	Chromium	15
2.3.1	Forms of chromium	15
2.3.2	Chromium Toxicity	16
2.3.3	Essentiality of chromium	16
2.3.4	Absorption of chromium	17
2.3.5	Distribution of chromium	19
2.3.6	Excretion of chromium	19
2.3.7	Carcinogenicity and Genotoxicity	20
2.4	Lead	21
2.4.1	Lead toxicity	22
2.4.2	Sources of exposure of lead	23
2.4.3	Industrial processes of lead	23
2.4.4	Food and smoking relationship with lead residues	24
2.4.5	Drinking water as source of lead of lead pollution	25
2.4.6	Domestic sources of lead contamination	25
2.4.7	Health effects of lead contamination	25
2.4.8	Effects of lead residues in children and pregnant women	26
2.4.9	Effects following acute lead exposure	26
2.4.10	Effects following chronic lead exposure	27
2.4.11	Organ systems mostly affected by lead exposure	27
2.4.12	Recent lead toxicity scenario in Nigeria	29
2.5	Heavy metal pollution	32
2.6	Heavy metals remediation	34

2.6.1	Technologies for remediation of polluted soils	34
2.6.2	Technologies for remediation of polluted water bodies	37
2.7	Determination of heavy metal(s)	38
2.8	Ruminant animals	38
2.8.1	Classes of Ruminant Animals	39
2.8.2	Economic importance of keeping ruminant animals	39
2.9	Digestion in the mouth and oesophagus	40
2.10	Digestion in the stomach	40
2.11	Digestion in small and large intestine	41
2.12	Types of cattle	43
2.13	Breed of cattle used for this research	44
2.14	Breed of goat used for this research	46
2.15	Instrument used for heavy metals analyses	48
CHAPTER THREE		
3.0	Materials and Methods	49
3.1	Study Location	49
3.2	Study Design	49
3.3	Community Entry	49
3.4	Study Duration	49
3.5	Sample Collection	50
3.6	Study Materials	51
3.7	Methods	51
3.7.1	Age estimation using dentition for cattle	51
3.7.2	Use of teeth eruption, leveling and wearing for age estimation	52

3.8	Lead, chromium and cadmium digestion process	52
3.9	Heavy metals analysis of samples	53
3.10	Heavy metals analyses of lead, chromium and cadmium	53
3.11	Data management	57

CHAPTER FOUR

4.0	Results	58
4.1	Age (in months) and sex characteristics of cattle sampled	58
4.2	Age (in months) and sex characteristics of goats sampled	60
4.3	Age categorization of cattle and goat samples	61
4.4	Number of cattle samples containing the selected heavy metals	64
4.5	Number of goat samples containing the selected heavy metals	66
4.6	Total proportion of cattle and goat having heavy metals	68
4.7	Heavy metal residue distribution in different tissue types of cattle	70
4.8	Heavy metal residue distribution in different tissue types of goats	74
4.9	Concentration of heavy metals in cattle residue as measured against FAO/WHO daily intake guideline limits	77
4.10	Concentration of heavy metal residue in goats as measured against FAO/WHO daily intake guideline limits	82

CHAPTER FIVE

5.0	Discussion	87
5.1	Age of animals sampled	87
5.2	Sex of animals sampled	87
5.3	Heavy metals proportion in cattle	88

5.4	Heavy metals proportion in goats	88
5.5	Total heavy metals proportion in both cattle and goats	89
5.6	Distribution of cadmium residue in cattle and goats	89
5.7	Distribution of chromium residue in cattle and goats	90
5.8	Distribution of lead residue in cattle and goats	90
CHAPTER SIX		
6.0	Conclusions and recommendations	92
6.1	Conclusions	92
6.2	Recommendations	94
6.3	Further research	95
REFERENCES		97

LIST OF TABLES

TABLES	PAGES
4.1 Age (in months) and sex characteristics of cattle sampled	59
4.2 Age (in months) and sex characteristics of goats sampled	61
4.3 Age categorization of cattle and goats sampled	63
4.4 Number of cattle samples with selected heavy metals	65
4.5 Number of goat samples with selected heavy metals	67
4.6 Total proportion of cattle and goats samples with heavy metals	69
4.7 Heavy metal residue distribution in different tissue types of cattle	72
4.8 Heavy metal residue distribution in different tissue types of goats	75
4.9 Concentration of heavy metals in cattle residue as measured against FAO/WHO daily intake guideline limits	78
4.10 Concentration of heavy metal residue in goats as measured against FAO/WHO daily intake guideline limits	83

LIST OF FIGURES

FIGURES	PAGES
4.1 Heavy metals residue distribution in parts of sampled cattle	73
4.2 Heavy metals residue distribution in parts of sampled goats	76
4.3 Concentration of cadmium in cattle residue as measured against FAO/WHO daily intake guideline limits	79
4.4 Concentration of chromium residue in cattle as measured against FAO/WHO daily intake guideline limits	80
4.5 Concentration of Lead residue in cattle as measured against FAO/WHO daily intake guideline limits	81
4.6 Concentration of cadmium in goats residue as measured against FAO/WHO daily intake guideline limits	84
4.7 Concentration of chromium residue in goats as measured against FAO/WHO daily intake guideline limits	85
4.8 Concentration of lead residue in goats as measured against FAO/WHO daily intake guideline limits	86

LIST OF PLATES

2.1	Picture of a boy and his ram at Bagega river after both had taken their bath	31
2.2	Digestive system of a ruminant	43
2.3	White Fulani breed of cattle	45
2.4	Maradi or Red Sokoto breed of goat	47
3.1	Collection of Samples from the abattoir	54
3.2a	Singeing of goat at Akinyele abattoir, Ibadan	55
3.2b	Singeing of cattle at Akinyele abattoir, Ibadan	55
3.3	Atomic absorption spectrophotometer used for heavy metal analyses	56

CHAPTER ONE

1.0 INTRODUCTION

Nigeria is one of the four leading livestock producers in Sub-Saharan Africa. The population of livestock (ruminant) in Nigeria was estimated to be about 14 million cattle, 13 million sheep and 23 million goats (RIM, 1990). However, these figures have since increased to 15.2 million cattle, 28 million goats and 23 million sheep (FAO, 2001).

Livestock plays a very important role in Nigerian agriculture contributing about 12.7% of the total agricultural gross domestic product (GDP) (CBN, 1999). Most of the ruminant livestock in Nigeria are raised in commercial quantities in the Northern part of the country but the enterprise is not known to be associated with the Southern Nigeria due to the prevalence of hemoparasites like trypanosomiasis, babesiosis, anaplasmosis, ehrlichiosis etc.

Cattle, goats, sheep etc are classified together as ruminant animals. Goat belongs to the family: Bovidae, subfamily, Caprinae and genus, Capra, while cattle belong to the Sub family Bovinae and genus Bos. A ruminant is a mammal that digests plant-based food by initially softening it within its first stomach known as the rumen, then regurgitates the semi-digested mass known as cud and chews it again. This process of re-chewing the cud to further break down plant matter and stimulate digestion is called rumination.

Livestock production has been a source of supply of animal protein worldwide. Meat from slaughtered cattle at various abattoirs constitutes the largest source of animal protein for the Nigerian populace (Idahor *et al.*, 2009). Heavy metal contamination

of grazing floors and water bodies constitute a serious threat to the animals due to their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Askloy, 2006). These cattle and goats are grazed on a free range system during which they eat grasses in the surroundings and also drink water from any nearby streams and stagnant water which could have been contaminated with heavy metals.

Presently, due to increased industrial and mining activities around livestock farms in most regions of the country, there have been a lot of reported cases of poisoning associated with heavy metal contamination of food and food products. For instance, in year 2010, Zamfara lead (Pb) poisoning case was reported in Nigeria due to lead ore mining along the water course where livestock feed and drink and children go for swimming, led to high fatalities that attracted international interventions and remediation. These activities have led to metal dispersal in the environment and consequently, impaired health of the population by the ingestion of edible meat contaminated by harmful elements (Zukowska and Biziuk, 2008).

Life-threatening health problems have been known to develop as a result of excessive uptake of dietary heavy metals. This could include depletion of some essential nutrients in the body thereby causing a decrease in immunological defences, intrauterine growth retardation, impaired psycho-social behaviours, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal tract cancer (Arora *et al.*, 2008). In the report of Swarup *et al.*, 2005 the authors assessed the lead (Pb) burden in the blood of goats reared around a primary lead-zinc smelter and they concluded from the study that goats reared around a primary lead-zinc smelter had higher blood lead levels in a dose-dependent manner.

Liu (2003) also reported high levels of lead (Pb) and cadmium (Cd) in some tissues including blood in sheep and horses, correlating the values with those observed in soil, water, forage and feed in the vicinity of non-ferrous metal smelters and suggested that the disease of sheep and horses in that region was caused by lead (Pb) poisoning combined with cadmium (Cd), as a result of heavy metal pollution by industrial activity.

Cadmium, lead and chromium are classified as some of the most dangerous heavy metals to health and environment (Michael, 2010). For the purpose of this research work, investigation of bio-accumulation of selected heavy metals that are of public health importance in the blood and tissue of cattle and goats readily used as food was studied.

1.1 **Problem statements**

The problem statements of this study include the following:

- i. There has been an increased in illicit the usage of agro-chemical products and indiscriminate disposal of toxic substances on the grazing floors and water bodies where animals graze.
- ii. Most consumers of meat and meat products are unaware of the presence of heavy metals in meat and its associated health risks

1.2 **Justification**

The justifications for carrying out this study include:

1. Heavy metal residues in animals slaughtered for daily human consumption are implicated in serious health challenges such as nephrotoxicity, hepatotoxicity, neurotoxicity, gastrointestinal disorders etc
2. This study will help to reveal the level of selected heavy metals and also establish the need to educate the consumers of meat on the adverse health issues heavy metals in meat and meat products in animals slaughtered for daily human consumption could predispose them to.

1.3 **Broad objective**

The broad objective of this study was to assess levels of cadmium, chromium and lead in blood, muscle, liver and kidney of cattle and goats slaughtered in central abattoir Akinyele, Ibadan.

1.4 **Specific objectives**

The specific objectives of this study were to:

1. Establish the presence of cadmium (Cd), chromium (Cr) and lead (Pb) residues in animals slaughtered in the abattoir, and determine the percentage distribution in the different tissue types.
2. Evaluate the patterns of distribution of heavy metal residues in animal body.
3. Compare the research findings with permissible limits as set by regulatory international organisations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy metals

Heavy metals are a group of chemical elements with relatively high density and are highly toxic. Heavy metals have mass density greater than 4.5g/cm^3 and tend to release electrons in chemical reactions and form simple cations. Some heavy metals have important physiological roles but high doses of these essential heavy metals or exposure to other heavy metals that lack physiological roles in organisms may create stress through interference with the function of enzymes or with the information-coding molecules (DNA or RNA) in cells (Robert and Nishanta,2008). Robert and Nishanta, 2008 also stated that some organisms are less susceptible to these effects than others and this heavy metal tolerance has become an important factor of core biological concepts such as adaptation and evolution.

2.1.1 Classification of heavy metals

The International Agency for Research on Cancer classified heavy metals into different groups which include groups 1, 2A, 2B, 3 and 4 (IARC Monograph, 2006).

Group 1 include heavy metals classified as carcinogenic (ability to cause cancer) to humans and there are 113 agents found in this group. This group can be further explained based on the sufficient evidence in humans or in animals and strong mechanistic data in humans as its ability to be carcinogenic.

Group 2A comprises those probably carcinogenic to humans and 66 agents are found in this group. These have been known to have limited evidence in humans and sufficient evidence in animals for their carcinogenicity.

Group 2B include those possibly carcinogenic to humans and 285 agents are found. In this group, limited evidences are found in humans and less than sufficient evidence found in animals.

Group 3 are those not classifiable as to its carcinogenicity to humans and here 505 agents are found. There is inadequate evidence in humans and inadequate or limited evidence in animals.

Group 4 are those not probably carcinogenic to humans (1 agent found). In this, there is no evidence of carcinogenicity in humans and in animals.

For the purpose of this research work, cadmium, lead and chromium were assessed. Cadmium and chromium belong to group 1 and lead belongs to group 2B on IARC classification. Cadmium is known to be highly carcinogenic to humans. In non-smoker populations, food is the major source of cadmium exposure.

2.1.2 Heavy metals of public health importance studied

There have been some heavy metals classified of importance to public health and which pose serious health risk to the entire universe. Studying the levels of these heavy metals in our environment help in policy formulation on issue of disposal, emission, remediation etc. hence this work studied some of these heavy metals.

2.2 Cadmium (Cd)

Cadmium is the most abundant, naturally occurring element; it was discovered in early 19th century (Bernard, 2008). Cadmium is a soft, malleable, ductile, bluish-white bivalent metal and is highly carcinogenic for living beings. It is found in nature in mineral form and is extracted from cadmium ore known as “greenockite” (ATSDR, 2000; NTP, 2004). Cadmium compounds are extremely toxic for plants, animals and human beings. The most important sources of cadmium contamination are smelters. Other sources include burning fossil fuels such as coal and incineration of metropolitan waste such as plastics and nickel-cadmium batteries (Sahmoun *et al.*, 2005). Cadmium may also flee into the air, from iron and steel production process. When cadmium is released into the atmosphere by smelting or mining or some other processes, its particles are carried to long distances. Cadmium can also deposit on the earth by rainfall and its solubility in water is enhanced by increasing acidity of water (Ros and Slooff, 1987). Cadmium can easily move through water movement system in the upper layers of soil, absorbed by plants and become accumulated in leafy vegetables, root crops, cereals and grains, which enables it enter into the food chain (ATSDR, 1999a).

Cadmium concentrations in drinking water supplies are up to 1 part per billion (ppb) are considered the permissible limit (ATSDR, 1999a). Groundwater rarely contains high levels of cadmium until or unless it is contaminated by industrial wastewater, wastes from mining or seepage from hazardous waste sites. Soft or acidic water has higher tendency to dissolve cadmium from water lines and even its low levels by deposit in body tissues (ATSDR, 2008).

Environment exposed to cadmium can lead to contamination of the food and water (ATSDR, 1989b). Concentrations of cadmium present in food items vary widely depending on the place of their production, as the industrial areas are much responsible for contamination (Bokori and Fekete, 1995). The dietary intake of cadmium has been found in the range of 10-35 μ g (Galal-Gorchev, 1991). It has been found that cadmium has no single physiological function within the human body therefore attention has been diverted to its bio-hazardous potential. Once cadmium is absorbed, it accumulates in the body throughout life (Bernard, 2008).

Even at low concentrations, cadmium can adversely affect a number of metabolic processes in the animal body (Bernard, 2004; Nordberg *et al.*, 2007). Cadmium intoxication can lead to kidney, bone and pulmonary damages (Godt *et al.*, 2006). Data indicates that cadmium toxicity affects various organs such as the liver, lungs, testes and the hematopoietic system in animals (Kocak and Akc, 2006). Also, literature indicate that excessive intake of cadmium in cattle can lead to loss of appetite, anaemia, poor growth, abortions and teratogenic effects. Excessive intake of cadmium alters the metabolism of zinc and copper in animals (Powell *et al.*, 1964; Miller *et al.*, 1967; Neathery and Miller, 1976; Doyle, 1977; Bremner and Campbell, 1978; Wright *et al.*, 1997).

In addition, there are also some evidences which, indicates that mitochondrial dysfunction could be as a result of cytotoxicity of cadmium (Byczkowski and Sorenson, 1984; Wallace and Starkov, 2000; Sokolova, 2004). Acute toxicity in humans with cadmium can occur at the level of 1,500 to 8,900 mg or 20 to 30 mg kg^{-1} which results in human fatalities (ATSDR, 1989b). Besides human fatalities, high doses of cadmium are known to cause gastric disturbance that finally results in

vomiting, abdominal pain and diarrhoea (ATSDR, 1989b). Acute toxicity symptoms generally include abdominal and muscular cramps, headache, overtiredness, shock and ultimately death (USAF, 1990). Cadmium is also absorbed in significant quantities from cigarette smoke which ultimately causes toxic effects on both human and animal health. The deleterious effects are especially on kidneys, liver and vascular system but the most undesirable effects have been seen on reproductive tissues and developing embryos (Thompson and Bannigan, 2008).

The earliest signs of kidney damage to workers who are exposed to cadmium during mining, smelting or roasting processes in industries include an increase in urinary levels of β 2-microglobulin and retinal binding protein. But these signs are absent in the general population (Elinder *et al.*, 1985; Chia *et al.* 1992; Jarup and Elinder, 1994; Chen *et al.* 2006a, 2006b). High doses of cadmium cause a suppression of immune response in goats (Haneef *et al.*, 1995). That is why a threshold limit has been given for the animals. For elemental cadmium, the oral LD50 values range from 63-88 mg kg⁻¹ for cadmium chloride, 72 mg kg⁻¹ for cadmium oxide and 590-1125 mg kg⁻¹ for cadmium stearate (USAF, 1990).

Studies on cadmium toxicity in animals as well as in humans are well documented (Satoh *et al.*, 2002; Thompson and Bannigan, 2008). Krajnc *et al.* (1983) found that contamination in animals occurs through forage, feed and water while in human being cadmium contamination can occur by the utilization of dairy products like meat and milk. He also studied the relationship between cadmium concentration in organs of cattle and cadmium contents in soil and found that contamination in cattle organs was due to the feeding on forages growing on contaminated soils. This has necessitated most countries of the world to give great attention to the production of

safe and healthy meat for human use (Sabir *et al.*, 2003; Staniskien and Garalevicien, 2004).

Many researchers have studied the metal toxicity in the meat and other organs of cattle (Zasadowski *et al.*, 1999; Alonso *et al.*, 2000). In this regard, an experiment was performed on the liver and kidneys of cattle to find the heavy metal accumulation in these organs. The results suggested that cadmium gradually and progressively accumulated in animal tissues, especially in the kidneys (Zasadowski *et al.*, 1999; Jukna *et al.*, 2006). A study also showed that kidneys of cattle older than 5 years are unfit for human consumption because of accumulation of cadmium in high amounts (Doganoc, 1996). It was also observed that organically raised cows had lower levels of cadmium in the kidneys, liver and mammary tissue than conventionally raised cows which may be due to feeding of organically raised animal on the roughage containing less amount of cadmium (Olsson *et al.*, 2002; Caggiano *et al.*, 2005).

There is some controversy as regards the results of the above review as a study conducted on cattle indicated that cadmium concentrations in Galician cattle rarely exceeded acceptable maximum concentrations that have been adopted by many countries. It is important to note that the regulatory values of cadmium differ markedly from country to country. Similarly, another study was performed to determine the level of cadmium in the muscle, liver and kidneys of cattle from Isfahan, Iran. The results of the experiment indicated that the cadmium concentration in muscles was (0.002 $\mu\text{mol/kg}$ fresh weight), Liver (0.044 $\mu\text{mol/kg}$

fresh weight), kidney (0.122 $\mu\text{mol/kg}$ fresh weight) and these concentrations were within maximum acceptable limit as established by the European Commission (EC).

As the anthropogenic sources are responsible for pollution, an experiment was also conducted on the cattle of industrialized area and rural areas of Asturia (northern Spain). Results indicated that cadmium concentrations were significantly higher in the liver and kidney tissues of cattle of the industrial area than the cattle of the rural area (Miranda *et al.*, 2005). Another study showed that the concentration of cadmium was high in the muscle and liver of cattle reared in the vicinity of a metallurgic industry. So cadmium contamination is ecologically important (Korenekova *et al.*, 2002).

Studies conducted at Morocco by Sedki *et al.* (2003) indicated that bovine grazing on the municipal wastewater-spreading field of Marrakech City (Morocco) had high level of cadmium in liver and kidneys. Another study showed that the levels of cadmium were higher than $1.0 \mu\text{g g}^{-1}$ in the liver and kidneys of cattle (Korsrud *et al.*, 1985). Milk is an essential diet for children as well as for adults. If lactating cows are exposed to high quantities of toxic metals, such as cadmium and lead, these metals disturb different metabolic activities as well as health of children. Therefore, Jeng *et al.* (1994) studied 107 milk samples collected from different dairy farms for cadmium and lead contamination and found that cadmium was not at toxic levels. Another study endorses the above findings that the concentration of cadmium was found to be under permissible levels in cow and buffalo milk of Madras city (India) which ranged from $4.0\text{-}25.2 \text{ ng ml}^{-1}$ (Ayyadurai *et al.*, 1998).

2.2.1 Health effects of cadmium

Inhalation of cadmium fumes or particles can be life threatening and although acute pulmonary effects and deaths are uncommon but sporadic cases still occur (Seidal *et al.*, 1993; Barbee and Prince, 1999). Cadmium exposure may cause kidney damage. The first sign of the renal lesion is usually a tubular dysfunction evidenced by an increased excretion of low molecular weight proteins [such as β 2-microglobulin and α 1-microglobulin (protein HC)] or enzymes [such as N-Acetyl- β -D-glucosaminidase (NAG)] (WHO 1992, Jarup *et. al*, 1998). It has been suggested that the tubular damage is reversible (Hotz *et. al*, 1999) but there is overwhelming evidence that the cadmium induced tubular damage is indeed irreversible (Jarup *et. al*, 1998). WHO (1992), estimated that a urinary excretion of 10 nmol/mmol creatinine (corresponding to *circa* 200 mg Cd/kg kidney cortex) would constitute a 'critical limit' below which kidney damage would not occur. However, WHO calculated that *circa* 10% of individuals with this kidney concentration would be affected by tubular damage. Several reports have since shown that kidney damage and/or bone defects are likely to occur at lower kidney cadmium levels. European studies have shown signs of cadmium induced kidney damage in the general population at urinary cadmium levels around 2–3 μ g Cd/g creatinine (Buchet *et. al.* 1990, Jarup *et. al.* 2000).

The initial tubular damage may progress to more severe kidney damage and already in 1950, it was reported that some cadmium exposed workers had developed decreased glomerular filtration rate (GFR). This was confirmed in later studies of occupationally exposed workers (Bernard *et al.* ;1992, Jarup *et al.* 1995). An excess

risk of kidney stones, possibly related to an increased excretion of calcium in urine following the tubular damage, has been shown in several studies (Jarup *et. al.*, 1998).

Recently, an association between cadmium exposure and chronic renal failure [end stage renal disease (ESRD)] was shown (Hellström *et al.* 2001). Using a registry of patients who had been treated for uraemia, the investigators found a double risk of ESRD in persons living close to (<2 km) industrial cadmium emitting plants as well as in occupationally exposed workers. Long-term high cadmium exposure may cause skeletal damage, first reported from Japan, where the itai-itai (ouch-ouch) disease (a combination of osteomalacia and osteoporosis) was discovered in the 1950s. The exposure was caused by cadmium-contaminated water used for irrigation of local rice fields. A few studies outside Japan have reported similar findings (Jarup *et. al.*, 1998). During recent years, new data have emerged suggesting that also relatively low cadmium exposure may give rise to skeletal damage evidenced by low bone mineral density (osteoporosis) and fractures.

Animal experiments have suggested that cadmium may be a risk factor in cardiovascular disease but studies of humans have not been able to confirm this (Jarup *et. al.*, 1998). However, a Japanese study showed an excess risk of cardiovascular mortality in cadmium-exposed persons with signs of tubular kidney damage compared to individuals without kidney damage (Nishijo *et. al.* 1995).

2.2.2 Study on the Cadmium-induced cancer

The IARC has classified cadmium as a human carcinogen (group I) on the basis of sufficient evidence in both humans and experimental animals (IARC 1993). IARC, however, noted that the assessment was based on few studies of lung cancer in occupationally exposed populations often with imperfect exposure data and without the capability to consider possible confounding effect by smoking and other associated exposures (such as nickel and arsenic). Cadmium has been associated with prostate cancer but both positive and negative studies have been published. Early data indicated an association between cadmium exposure and kidney cancer (Kolonel, 1976).

Later studies have not been able to clearly confirm this but a large multi-centre study showed a (borderline) significant over-all excess risk of renal cell cancer, although a negative dose-response relationship did not support a causal relation (Mandel *et. al.* 1995). Furthermore, a population-based multi-centre study of renal cell carcinoma found an excess risk in occupationally exposed persons (Pesch *et. al.* 2000). [In summary, the evidence for cadmium as a human carcinogen is rather weak, in particular after oral exposure. Therefore, a classification of cadmium as 'probably carcinogenic to humans' (IARC group 2A) would be more appropriate. This conclusion also complies with the EC classification of some cadmium compounds (Carcinogen Category 2; Annex 1 to the directive 67/548/EEC)].

2.3 Chromium

Chromium is the hardest of all known metal element in the periodic table. It is odourless, tasteless and highly corrosion-resistant. It is required in small amounts, although its mechanisms of action in the body and the amount needed for optimal health is well defined.

2.3.1 Forms of chromium

Chromium is found in two forms:

1. **Trivalent (Cr^{3+}):** This is biologically active and found in food. It is required in trace amounts for sugar and lipid metabolism, which has been widely discussed because of the mechanism of action.
2. **Hexavalent (Cr^{6+}):** It is a toxic form that results from industrial pollution. It can be carcinogenic and highly harmful to human and animal health.

Several in vitro studies indicated that high concentrations of chromium (III) in the cell can lead to DNA damage. Acute oral toxicity ranges between mg/kg 1.9 + 3.3mg/kg (Katz and Salem,1992). The proposed beneficial effects of chromium (III) and its use as dietary supplements yielded some controversial results but recent reviews suggest that moderate uptake of chromium (III) through dietary supplements pose no risk (Katz and Salem, 1992). World Health organization (WHO) recommended maximum allowable concentration in drinking water for chromium (VI) is 0.05milligrams per liter (WHO Guidelines).

The lethal dose 50 (LD₅₀) for chromium (VI) ranges between 50 and 150mg/kg (Katz and Salem,1992). In the body, chromium (VI) is reduced by several mechanisms to chromium (III) in the blood before it enters the cells. The chromium (III) is excreted from the body. The acute toxicity of chromium (VI) is due to its strong oxidation properties. After it reaches the bloodstream, it damages the kidneys, liver and blood cells through oxidation reactions (Dayan and Paine, 2001).

2.3.2 Chromium Toxicity

The toxicity of chromium depends on its oxidation state. Hexavalent chromium is more toxic than the trivalent form. Cr (VI) compounds penetrate biological membranes much more readily than do Cr (III) compounds. After crossing cellular membranes, Cr (VI) may be reduced to Cr (III) via a number of hypothesised reactions. Some of the reactive intermediates are – pentavalent and tetravalent chromium species and oxygen radicals are thought to be involved in the reduction process. These intermediates may interact with essential constituents of the cells (including genetic material) which they can damage through oxidation and complexation with the resulting Cr (III) species. As well as the inherently greater toxicity of hexavalent chromium compared with trivalent chromium, the former is the more readily absorbed by both the inhalation and oral routes.

2.3.3 Essentiality of chromium

Studies on humans and experimental animals have shown that trivalent chromium has an essential role in the maintenance of normal glucose and fat metabolism. The biologically active form of an organic Cr (III) complex is believed to function by facilitating the interaction of insulin with its cellular receptor sites. Studies have shown that chromium supplementation in deficient and marginally deficient subjects

resulted in improved glucose, protein and lipid metabolism. A number of authors consider that many people do not have an adequate intake of chromium (Hunt and Stoeker, 1996). The signs of chromium deficiency, which are often alleviated by increased dietary chromium, are similar to those of maturity-onset diabetes and cardiovascular diseases.

Supplementation of the diets of subjects showing symptoms of chromium deficiency with up 1mg/kg of chromium per day has usually shown benefits (Anderson, 1989, 1997).

2.3.4 Absorption of chromium

The presence of chromium in the urine and serum of men occupationally exposed to airborne, soluble Cr (III) or Cr (VI) compounds shows that chromium can be absorbed via the inhalation route.

Human and animal studies suggest that once deposited in the lungs, Cr (VI) compounds are generally transferred to the systemic circulation more readily than Cr (III) compounds. For any inhaled aerosol, the main determinants of the fractional deposition and the fractional transfer to the systemic circulation are the particle size and *in vitro* solubility. For chromium, there is the additional consideration that Cr (VI) compounds are more able to cross biological membranes than are Cr (III) compounds.

Both human and animal studies have shown that there is a difference in the efficiency of absorption of ingested Cr (VI) and Cr (III) compounds. For all the species examined, the absorption of hexavalent chromium is generally greater. The results usually quoted from human studies are that the fractions of ingested Cr (VI) and Cr (III) transferred across the gut are about 2% and 0.5% respectively. However,

in one study of a group of elderly subjects, the absorption of Cr (III) in the diet was estimated to be about 2.5% (Anderson *et al*, 1983). In another study of volunteers who received Cr (VI) as chromate in drinking water, a wide range of uptake values was found but most were in the range 3–6% (Kerger *et al*, 1997); most of the ingested Cr (VI) was thought to be reduced to Cr (III) organic complexes in the gastrointestinal tract before absorption. There is evidence that the extent of absorption is dependent on the dietary intake (being higher at low levels of chromium intake), that absorption is greater in immature animals than in adult animals and that absorption is usually greater in fasting animals than when taken with food (ATSDR, 1993).

However, if the chromium ion binds to certain ligands (some of which may be present in some foodstuffs), absorption may be increased by up to a factor of 5 (WHO, 1988). It seems likely that the relatively poor transfer of Cr (VI) compounds across the gut is a consequence of the reduction of some or most of the Cr (VI) to Cr (III) in the stomach. Systemic toxicity has been observed in humans following dermal exposure to chromium compounds, indicating significant transfer across the skin. A number of animal and human studies on the dermal penetration of chromium have been reported. The rate of transfer to the systemic circulation depends upon a number of variables, such as the solubility of the compound, its concentration, the solvent used and the oxidation state of the chromium (ATSDR, 1993).

Transfer rates were generally higher with organic solvents than with aqueous solutions and increased with increasing concentrations. Rates were usually but not always higher for Cr (VI) than for Cr (III) compounds. In a study with human

volunteers, transfer rates across the forearm skin using 0.01 M, 0.1 M and 0.2 M solutions of sodium chromate were about 1, 6 and 10 $\mu\text{g Cr (VI) cm}^{-2} \text{ h}^{-1}$ respectively (Baranowska-Dutkiewicz, 1981), indicating that with increased concentration, the transfer rate is faster.

2.3.5 Distribution of chromium

Some of the chromium that enters the systemic circulation will reach all organs and tissues but there appears to be little long-term accumulation. It was found that the concentrations were highest at birth and tended to decrease with age. The decrease was most pronounced during the first and second decades and (except for the lung) was followed by a more gradual decrease for the remainder of life. It is not known whether this decrease is a consequence of some physiological mechanism or of a dietary deficiency; an alternative explanation could be that as all the samples were taken over a relatively short time interval, the observed decrease in tissue concentration with age was a consequence of a lower historical dietary chromium intake for older subjects. Schroeder *et al.*(1962) found that the concentration of chromium in the lung began to increase again during middle and old age but did not regain its initial high value. In several rodent studies, higher tissue levels of chromium were found after administration of Cr (VI) than after administration of Cr (III). This presumably reflects the greater tendency of Cr (VI) to cross biological membranes and bind to intracellular proteins in the various tissues.

2.3.6 Excretion of chromium

Absorbed chromium is excreted primarily in the urine as Cr (III). The half-life for urinary excretion of chromium orally administered as potassium chromate in drinking water was estimated to be 35–40 hours in humans. Hair and nails are minor pathways of excretion.

2.3.7 Carcinogenicity and Genotoxicity

The IARC states that “there is sufficient evidence in humans for the carcinogenicity of chromium (VI) compounds as encountered in the chromate production, chromate pigment production and chromium plating industries”. Cr (VI) is described by the USEPA “as a known human carcinogen by the inhalation route of exposure”. Both organisations place Cr (VI) in their highest cancer category: Group 1 and Group A respectively (IARC, 1990; USEPA, 2001b). There is also agreement on Cr (III) not classifiable (or not classified) as to its human carcinogenicity: Group 3 in the IARC scheme and Group D under the USEPA guidelines (IARC, 1990; USEPA, 2001a). IARC has similarly assigned metallic chromium to Group 3 (IARC, 1990, 1999).

Epidemiological studies carried out in a number of countries have shown an association between exposure to chromium and lung cancer. Among the industries investigated are chromate production, chromate pigment production and use, chromium plating, stainless steel welding, ferrochromium alloy production and leather tanning. Studies of chromate production workers (who are exposed to both hexavalent and trivalent chromium compounds) and of chromate pigment workers (who are exposed mainly to hexavalent chromium compounds) have consistently shown excess risks for lung cancer. Studies in the chromium plating industry where exposure is mainly to Cr (VI), generally support the conclusion that Cr (VI) is carcinogenic. Studies of stainless steel welders exposed to Cr (VI) and other chemicals and of ferrochromium workers who are exposed mainly to Cr (0) and Cr (III) but also to some Cr (VI) have been inconclusive. Studies of leather workers who are exposed mainly to Cr (III) have been negative.

2.4 Lead

The general population is exposed to lead from air and food in roughly equal proportions. Earlier, lead in foodstuff originated from pots used for cooking, storage and lead acetate was previously used to sweeten port wine. During the last century, lead emissions into ambient air have further polluted our environment; over 50% of lead emissions originating from petrol. Over the last few decades, however, lead emissions in developed countries have decreased markedly due to the introduction of unleaded petrol. Subsequently, blood lead levels in the general population have decreased.

Lead in blood is bound to erythrocytes and elimination is slow and principally *via* urine. Lead accumulates in the skeleton and is only slowly released from this body compartment. Half-life of lead in blood is about 1 month and in the skeleton 20–30 years (WHO, 1996). In adults, inorganic lead does not penetrate the blood–brain barrier whereas this barrier is less developed in children and the tendency or penetration is high. The high gastrointestinal uptake and the permeable blood–brain barrier make children especially susceptible to lead exposure and subsequent brain damage. Organic lead compounds penetrate body and cell membranes. Tetramethyl lead and tetraethyl lead penetrate the skin easily. These compounds may also cross the blood–brain barrier in adults and thus adults may suffer from lead encephalopathy related to acute poisoning by organic lead compounds.

The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Lead encephalopathy is characterized by sleeplessness and restlessness. Children may be affected by

behavioral disturbances, learning and concentration difficulties. In severe cases of lead encephalopathy, the affected person may suffer from acute psychosis, confusion and reduced consciousness. People who have been exposed to lead for a long time may suffer from memory deterioration, prolonged reaction time and reduced ability to understand. Individuals with average blood lead levels under $3\mu\text{mol/l}$ may show signs of peripheral nerve symptoms with reduced nerve conduction velocity and reduced dermal sensibility. If the neuropathy is severe the lesion may be permanent. The classical picture includes a dark blue lead sulphide line at the gingival margin. In less serious cases, the most obvious sign of lead poisoning is disturbance of haemoglobin synthesis and long-term lead exposure may lead to anaemia.

2.4.1 Lead toxicity

Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. It is a cumulative toxicant that affects multiple body systems including the neurological, haematological, gastrointestinal, cardiovascular and renal systems. Children are particularly vulnerable to the neurotoxic effects of lead and even relatively low levels of exposure can cause serious and in some cases, irreversible neurological damage (Fewtrell *et. al.* 2003, IPCS 1995). Lead exposure is estimated to account for 0.6% of the global burden of disease with the highest burden in developing regions (WHO 2009).

Recent reductions in the use of lead in petrol (gasoline), paint, plumbing and soldering works have resulted in substantial reductions in lead levels in the blood (Fewtrell *et. al.* 2003). However, significant sources of exposure to lead still remain, particularly in developing countries. Further efforts are required to continue to

reduce the use and releases of lead and to reduce environmental and occupational exposures particularly for children and women of child-bearing age.

2.4.2 Sources of exposure to lead

Lead is found at low levels in Earth's crust, mainly as lead sulfide (IARC 2006). However, the widespread occurrence of lead in the environment is largely the result of human activity such as mining, smelting, refining and informal recycling of lead; use of leaded petrol (gasoline); production of lead-acid batteries and paints; jewelry making, soldering, ceramics and leaded glass manufacture in informal and cottage (home-based) industries; electronic waste UNEP (2008) and use in water pipes and solder. Other sources of lead in the environment include natural activities such as volcanic activity, geochemical weathering, sea spray emissions and remobilization of historic sources such as lead in soil, sediment and water from mining areas.

As lead is an element, once it is released into the environment, it persists (IPCS 1995). Because of lead's persistence and potential for global atmospheric transport, atmospheric emissions affect even the most remote regions of the world WHO (2007).

2.4.3 Industrial processes of lead

Lead is used mainly in the production of lead-acid batteries, plumbing materials and alloys. Other uses are in cable sheathing, paints, glazes and ammunition. Human occupational exposure can also take place during the application and removal of protective lead-containing paints during the grinding, welding and cutting of materials painted with lead-containing paints, such as in shipbuilding, construction, demolition industries and fabrication of heavy lead glass and crystal and in crystal

carving. Mining, smelting and informal processing and recycling of electric and electronic waste can also be significant sources of exposure.

Lead has been used widely in the form of tetraethyl and tetramethyl lead as antiknock and lubricating agents in petrol although the majority of lead is emitted from vehicles in the form of inorganic particles. This use has been phased out in most countries which has resulted in a significant reduction of human exposure and mean blood lead levels. In the few parts of the world where leaded petrol is still in use however, it continues to be a major source of exposure. Old industrial hotspots that have not been cleaned up can also represent a hazard even years after contamination has stopped, particularly to children who might ingest contaminated soil or dust as a result of their hand-to-mouth behaviour.

2.4.4 Food and smoking relationship with lead residues

For the non-smoking general population, the largest contribution to the daily intake of lead is derived from the ingestion of food, dirt and dust. The amount of lead in food plants depends on soil concentrations and is highest around mines and smelters. Cereals can contain high levels of lead and spices may be contaminated with lead. The use of lead-soldered food and beverage cans (which is now diminishing) may considerably increase the lead content of the food or beverage especially in the case of acidic foods or drinks. As alcoholic drinks tend to be acidic the use of any lead-containing products in their manufacture, distribution or storage will raise lead levels. Migration of lead into food from lead-glazed ceramic or pottery dinnerware is also a source of exposure. Smoking tobacco increases lead intake (IPCS, 1995).

2.4.5 Drinking-water as source of lead pollution

Lead present in tap water is rarely the result of its dissolution from natural sources but is mainly due to household plumbing systems containing lead pipes, solders and fittings. Water that has been in contact with lead in this way for an extended period (e.g. overnight) will have a higher concentration. Thus, lead concentrations can vary over the day and flushing of the taps before use is a control mechanism. Soft acidic water dissolves the most lead WHO (2003).

2.4.6 Domestic sources of lead contamination

Contaminated dust may be the main source of exposure for infants in countries that no longer use leaded petrol. The weathering, peeling or chipping of lead-based paints mainly found in older houses plays a role in children's exposure especially as some young children eat the fragments or lick dust-laden fingers. Lead-containing dust may be brought into the home on the clothes of those who work in industries where such dust is generated. Some toys either are made from lead or contain lead (e.g. some plastics or paints) WHO (2008). Some traditional medicines and makeup (e.g. kohl) contain lead IPCS (1995).

2.4.7 Health effects of lead contamination

It has been estimated that lead exposure was responsible (in 2004), for 143 000 deaths and 0.6% of the global burden of disease (expressed in disability-adjusted life years, or DALYs); taking into account mild mental retardation and cardiovascular outcomes resulting from exposure to lead WHO (2009). Lead in the body is distributed to the brain, liver, kidneys and bones. It is stored in the teeth and bones where it accumulates over time. Human exposure can be assessed directly through measurement of lead in blood, teeth or bones (bone and tooth lead reflect cumulative exposure).

2.4.8 Effects of lead residues in children and pregnant women

Young children absorb 4–5 times as much lead as adults (apart from pregnant women). Infants, young children (especially those less than 5 years of age) and pregnant women are most susceptible to the adverse effects of lead. The potential for adverse effects of lead exposure is greater for children than for adults, because in children

- (1) The intake of lead per unit body weight is higher
- (2) More dust may be ingested
- (3) Lead absorption in the gastrointestinal tract is higher
- (4) The blood–brain barrier is not yet fully developed and
- (5) Neurological effects occur at lower levels than in adults WHO (2007).

The most critical effect of lead in young children is that on the developing nervous system. Subtle effects on intelligence quotient (IQ) are observed with blood lead levels as low as 5 µg/dl (50 µg/l) and the effects gradually increase with increasing levels of lead in blood (Fewtrell *et. al.* 2003). A 2010 review of the latest scientific evidence indicating effects at lower levels did not provide any indication of a threshold for the key adverse effects of lead. Lead exposure has also been linked epidemiologically to attention deficit disorder and aggression.

Exposure of pregnant women to high levels of lead can cause miscarriage, stillbirth, premature birth and low birth weight as well as minor malformations (IPCS 1995).

2.4.9 Effects following acute lead exposure

Lead is classically a chronic or cumulative toxin hence acute adverse effects are usually observed only following short-term exposures to high concentrations. Acute exposures to lead may cause gastrointestinal disturbances (anorexia, nausea, vomiting, abdominal pain), hepatic and renal damage, hypertension and neurological

effects (malaise, drowsiness, encephalopathy) that may lead to convulsions and death.

2.4.10 Effects following chronic lead exposure

Chronic lead exposure commonly causes haematological effects such as anaemia or neurological disturbances, including headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors and paralysis WHO (2010). There is some evidence that long-term occupational exposure to lead may contribute to the development of cancer. The International Agency for Research on Cancer (IARC) has classified inorganic lead compounds as *probably carcinogenic to humans*

2.4.11 Organ systems mostly affected by lead exposure

The major health effects of lead are manifest in three organ systems: the haematological system, the central nervous system (CNS) and the renal system. Major health effects attributed to lead in environmentally exposed populations are as follows:

1. Haematological system

Inhibition of ALA-D (δ-aminolevulinic acid dehydratase) and haem synthetase and corresponding accumulation of ALA and FEP (free erythrocyte protoporphyrin) at a concentration that is higher than the threshold level. At higher levels of exposure, reduced haem synthesis and anaemia. The haematological effects of lead have been recognized for many years and the biochemical bases for such changes are now reasonably well established. Two of the most sensitive effects on this system arise at the first and final steps of the haem synthesis pathway. The enzyme catalyzing the first step, δ-aminolevulinic acid dehydratase (ALA-D) is uniquely sensitive to lead and erythrocyte ALA-D is often reported to be inhibited in the general population.

This partial inhibition is not considered to be a deleterious effect because the enzyme exhibits a large reserve capacity.

In addition, erythrocyte ALA-D in mammals has no function as such because mature erythrocytes do not participate in haem synthesis. The inhibition of ALA-D in other haem-forming tissues will, if exposure is sufficiently high, result in an increased concentration of the substrate ALA in the body and urine. Lead also interferes with the last stage of haem synthesis (the incorporation of iron into protoporphyrin), catalyzed by haem synthetase (ferrochelatase). This step takes place in the inner matrix of the erythroid cell mitochondria in the bone marrow. It is now thought that lead does not interfere with the enzyme but rather inhibits the trans-mitochondrial transfer of iron. This effect results in the accumulation of protoporphyrin in the mitochondria, its incorporation into the globin molecule in place of haem and the presence of elevated levels of free erythrocyte protoporphyrin (FEP) in the peripheral blood.

2. Nervous system

Central nervous system impairment at moderate exposure in children, reflected by inattention, cognitive difficulties, fine motor dysfunction and altered EEG patterns. Under heavy exposure, encephalopathy may arise. Effect on the peripheral nervous system (PNS) is indicated by reduced nerve conduction velocity.

3. Renal system

The effect on the renal system is functional impairment of the tubular region characterized by mild aminoaciduria, glucosuria and hyperphosphaturia.

Morphological effects include mitochondrial damage and intranuclear inclusion bodies. Long-term heavy exposure may result in irreversible nephropathy.

2.4.12 Recent lead toxicity scenario in Nigeria

The Centers for Disease Control and Prevention (CDC) defines childhood lead poisoning as a whole-blood lead concentration equal to or greater than 10 μ g/dl - 0.010mg/dl micrograms/dL. Acute lead poisoning, 0.01mg/dl though uncommon shows up more quickly and can be fatal when a relatively large amount of lead is taken into the body over a short period of time. Children constitute the vast majority of such cases. Symptoms can include severe abdominal pain, diarrhea, nausea and vomiting, weakness of the limbs, seizures and coma.

In March 2010, an unusually high number of deaths, primarily among children under age 5 in Bukkuyum and Anka Local Government Areas (LGAs) of Zamfara State were reported by Médecins sans Frontières (MSF-Holland) to state health authorities. Further study of blood samples taken by MSF revealed that the increased mortality was the result of acute lead poisoning determined to be caused by massive environmental contamination from artisanal mining and processing of gold found in lead-rich ore (Ruddiger, 2010). The grinding of the ore into fine particles resulted in extensive dispersal of lead dust in the villages concerned, including within family compounds. Ingestion and inhalation of the fine lead particles was determined to be the major reason for high blood lead levels in victims' bodies. Blood lead levels (BLLs) were "unprecedented" for human beings according to the CDC.

Following the identification of the mass acute lead poisoning situation in Zamfara State, an immediate, two-pronged response approach was developed consisting of a medical component and an environmental component.

It has been reported that since 2010 widespread incidence of acute lead poisoning in Zamfara state has killed at least 400 children. Considered the worst outbreak of lead poisoning in modern history, more than 3500 affected children require urgent, life saving treatment. Notable is also the high rates of infertility and miscarriages among women of child-bearing age. In Bagega, the largest and the most contaminated village, environmental remediation and the implementation of safer mining practices to prevent recontamination are urgently needed and must be put in place before comprehensive treatment can be provided for children.



Plate 2.1 Picture of a boy and his ram at the Bagega River after both had taken their bath (Ruddiger, 2010).

2.5 Heavy metal pollution

Heavy metal pollution is the single biggest environmental issue facing the mining industry worldwide. It affects most countries and all mining sectors, including coal, precious and base metal, tin, tungsten, molybdenum, uranium, and rare earth element (REE) mines. It also affects industrial mineral deposits (e.g. mineral sands, diamonds) and quarries (Taylor, 2012). Heavy metal pollution has a combined worldwide economic impact estimated to be in excess of US \$10 billion and impacts can continue for more than 2,000 years (Taylor, 2012).

Social and environmental impacts can affect agricultural productivity, food security, water resources (i.e. drinking, irrigation, livestock, fishing, recreation, and tourism), human health, aquatic ecosystems and cause loss of habitat and also community displacement and outrage. The economic and business impacts of heavy metal pollution include accumulation of liabilities for governments and mining companies, expensive remediation programs, increasing compensation payments and impacts on company reputation and future project approvals (Taylor, 2012)

Heavy metals make significant contributions to environmental pollution as a result of anthropogenic activities such as mining, energy and fuel production, power transmission, intensive agricultural practices, sludge and industrial effluent dumping and military operations (Orcutt and Nilsen, 2000; Cseh, 2002)

Pollution with heavy metal is a global threat to the environment as they are widely present in the earth's crust, in air, water and food (Matthew *et al.*, 2002). Heavy metal pollution of soil is usually related to human activities. These activities include

dumping of wastes, unintentional spillages, use of agricultural pesticides and related chemicals, movement of contaminants into fertile land as vapors, by mobilization of soil, or as dust, or dispersal of sewage mire. Sites near mining activities or heavy metals industry are often highly contaminated with toxic metals. In addition, application of fertilizers increases cadmium content in top soils (Lado *et al.*, 2008). Such polluted soils are hardly usable for agricultural purposes because the pollution can be transferred to the food chain. Heavy metals bound in the soil are leachable and they can also spread further via ground water. All these sources contribute towards contamination of the environment. To avoid the spread of contaminants, it is possible to use phytoremediation techniques which can immobilize or decrease the pollution (Salt *et al.*, 1998).

Plants are able to immobilize metals in soil by forming insoluble compounds as a result of interactions of contaminants with plant exudates in rhizosphere or by absorption on root system (Kidd *et al.*, 2009). Some plant species are also able to accumulate heavy metals in their plant tissues so the contaminant is removed from such sites with harvested plant (McGrath and Zhao, 2003).

Heavy metals in feed and food symbolize a severe risk and their long-term exposure can lead to toxicological effects. Excessive exposure to elements such as cadmium, lead, chromium, etc is toxic for plants, animals and human beings (Llobet *et al.*, 2003). These metals have direct effect on animal health and indirect effect on human health (Hooda *et al.* 1997; SCAN, 2003). Heavy metal pollution in rural areas is due to disposal of industrial effluents and sewage sludge which cause problem for grazing animals because they dispose heavy metals on pastures grasses or forages (Smith *et al.*, 1991).

2.6 Heavy metals remediation

In order to reduce the challenges posed by heavy metal contamination, there is need to decontaminate water bodies and soil in our environment of any heavy metal introduced into them as these two remain the major pollution sites. Presently, different approaches have been developed to remediate or reclaim the heavy metal-polluted soils and waters including the landfill or dumping sites. These may be broadly classified into physicochemical and biological approaches.

2.6.1 Technologies for remediation of polluted soils

Below are the technologies involved in the remediation of polluted soil.

A. Physicochemical remediation of heavy metal polluted soils

The physicochemical approach includes excavation and burial of the soil at a hazardous waste site, fixation or inactivation (chemical processing of the soil to immobilize the metals), leaching by using acid solutions or proprietary leachants to desorb and leach the metals from soil followed by the return of clean soil residue to the site (Salt *et al.*, 1995), precipitation or flocculation followed by sedimentation, ion exchange, reverse osmosis and microfiltration (Raskin *et al.*, 1996).

B. Biological remediation of heavy metal polluted soils

Biological approaches of remediation include:

- (1) Use of microorganisms to detoxify the metals by valence transformation, extracellular chemical precipitation, or volatilisation (some microorganism can

enzymatically reduce a variety of metals in metabolic processes that are not related to metal assimilation).

(2) Use of special type of plants to decontaminate soil or water by inactivating metals in the rhizosphere or translocating them to the aerial parts. This approach is called phytoremediation, which is considered a new and highly promising technology for the reclamation of polluted sites and cheaper than physicochemical approaches (Garbisu and Alkorta, 2001; McGrath *et al.*, 2001; Raskin *et al.*, 1997).

Phytoremediation, also referred to as botanical bioremediation (Chaney *et al.*, 1997), involves the use of green plants to decontaminate soils, water and air. It is an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt *et al.*, 1998). However, the ability to accumulate heavy metals varies significantly among species and among cultivars within species, as different mechanisms of ion uptake are operative in each species based on their genetic, morphological, physiological and anatomical characteristics. There are different categories of phytoremediation which include phyto-extraction, phyto-filtration, phyto-stabilization, phyto-volatilisation and phyto-degradation depending on the mechanisms of remediation.

Phyto-extraction involves the use of plants to remove contaminants from soils. The metal ions accumulated in the aerial parts that can be removed to dispose or burnt to recover metals.

Phyto-filtration involves the plant roots or seedling for removal of metals from aqueous wastes.

In phyto-stabilization, the plant roots absorb the pollutants from the soil and keep them in the rhizosphere, rendering them harmless by preventing them from leaching.

Phyto-volatilization involves the use of plants to volatilise pollutants from their foliage such as selenium and mercury.

Phyto-degradation is the use of plants and associated microorganisms to degrade organic pollutants (Garbisu and Alkorta, 2001). Some plants may have one function whereas others can have two or more functions of phytoremediation.

1. Plant species for phytoremediation of heavy metal-polluted soil

To identify plant populations with the ability to accumulate heavy metals, 300 accessions of 30 plant species were tested by Ebbs *et al.*, (1997) in hydroponics for 4 weeks, having moderate levels of cadmium (Cd), copper (Cu) and zinc (Zn). The results indicate that many *Brassica* spp. such as *B. juncea* L., *B. juncea* L. Czern, *B. napus* L. and *B. rapa* L. exhibited moderately enhanced Zn and Cd accumulation. They were also found to be most effective in removing Zn from the contaminated soils. To date, more than 400 plant species have been identified as metal hyper-accumulators, representing less than 0.2% of all angiosperms (Brooks, 1998; Baker *et al.*, 2000). The plant species that have been identified for remediation of soil include either high biomass plants such as the willow (Landberg and Greger, 1996) or those that have low biomass but high hyper-accumulating characteristics such as *Thlaspi* and *Arabidopsis* species.

The hyper-accumulators that have been most extensively studied by scientific community include *Thlaspi* sp., *Arabidopsis* sp., *Sedum alfredii* sp. (both genera

belong to the family of Brassicaceae and Alyssum). *Thlaspi* sp. are known to hyper-accumulate more than one metal, i.e., *T. caerulescens* for cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn), *T. goesingense* for Ni and Zn, *T. ochroleucum* for Ni and Zn, and *T. rotundifolium* for Ni, Pb and Zn (Prasad and Freitas, 2003). Among the genus *Thlaspi*, the hyper-accumulator plant *Thlaspi caerulescens* received much attention and has been extensively studied as potential candidates for Cd and Zn contaminated soils.

1. Enhancement of phytoremediation by chemical and biological approaches

In order to cope with heavy metal contaminated soils, various phytoremediation approaches (phyto-stabilization, phyto-immobilisation and phyto-extraction) can be applied. However, the choice will depend on many factors, such as plant tolerance to pollutants, soil physicochemical properties, agronomic characteristics of the plant species, climatic conditions (rainfall, temperature) and additional technologies available for the recovery of metals from the harvested plant biomass. The solubility of heavy metals in polluted soils can be increased by using organic and inorganic agents thus enhancing the phyto-extraction capabilities of many plant species.

1.6.2 Technologies for remediation of polluted water bodies

Technologies involved in the remediation of polluted water bodies are as given below:

Rhizo-filtration is the removal of pollutants from contaminated waters by accumulation into plant biomass. Several aquatic species have been identified and tested for the phytoremediation of heavy metals from polluted waters. These include sharp dock (*Polygonum amphibium* L.), duck weed (*Lemna minor* L.), water

hyacinth (*Eichhornia crassipes*), water lettuce (*P. stratiotes*), water dropwort [*Oenathe javanica* (BL) DC], calamus (*Lepironia articulate*), pennywort (*Hydrocotyle umbellata* L.) (Prasad and Freitas, 2003). The roots of Indian mustard are found to be effective in the removal of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn), and sunflower can remove lead (Pb), Uranium (U), caesium (Cs-137) and strontium (Sr-90) from hydroponic solutions (Zaranyika and Ndapwadza, 1995; Wang *et al.*, 2002; Prasad and Freitas, 2003).

2.7 Determination of heavy metal(s)

The presence and dose of heavy metals in the biological systems can be determined by using neutron activation analysis (N. A .A), atomic spectroscopy forms which could be- atomic emission spectroscopy (A.E.S) and atomic absorption spectroscopy (A.A.S), fluorimetric analysis, extended x-ray absorption fine structure (E.X.A.F.S), infra-red and ramen spectrophotometry.

2.8 Ruminant animals

Ruminant animals are mammals that belong to the order *Artiodactyla*. They are animals with a complex stomach unlike the non-ruminants that have simple stomach. They eat and digest forages or plant based feed by swallowing it first and allowing it to get moistened in the rumen which is the first compartment of the complex stomach. The swallowed food is later regurgitated by the animal and re-chewed to break down the plant materials for digestion (Buckett, 1973). This process is called rumination or chewing the cud. The cud is a semi-solid and semi-degraded digesta usually in a bolus form which is regurgitated from the

reticulorumen of the animal. Examples of ruminant animals are cattle, sheep, goats, camel, water buffaloes, giraffes, antelopes etc.

2.8.1 Classes of Ruminant Animals

Ruminant animals are categorized into two main classes based on their body size namely the large ruminant animals and small ruminant animals. Examples of large ruminants are cattle, water buffalo, giraffe, camel etc while small ruminants are sheep, goat, antelope etc. Ruminants have an advantage of the ability to eat and utilize low quality fibrous food that cannot be eaten by human or non-ruminants (Okorie,1978).

2.8.2 Economic importance of keeping ruminant animals

Ruminant animals and their products have tremendous nutritional and economic values to man as stated below:

1. Meat and milk of cattle, sheep, goats and other ruminants are good sources of animal protein to man which are of better quality than plant protein.
2. They serve as sources of raw materials used in industries e.g. leather goods. Goat hair is also used for making carpets, bag and ropes. Wool from sheep is a raw material for the production of clothing for human wear.
3. They serve as a means of foreign exchange earnings. For instance, some countries in Europe such as Denmark and Botswana in the southern region of Africa export beef to earn foreign exchange. Others export dairy products from milk to earn foreign exchange.
4. They serve as source of income to subsistence farmers. In Nigeria, cattle, sheep and goats are kept at subsistence level by farmers.

5. These animals are able to survive on fallow lands and others that are not good for arable crop farming thereby maximizing the use of the available land resource.
6. They are also used as gifts or bride price which serves as family wealth.
7. They are sources of gainful employment. (Payne and Wilson,1999).

2.9 Digestion in the mouth and oesophagus

The whole process of digestion starts from the mouth of the animal. The combination of the tongue and the lips help ruminant animals to pick and roll the plant material into the mouth. The teeth help in the mastication or chewing of the plant material. There are two types of dentition in ruminants. The first is called deciduous dentition which is found in young animals with formula I 0/4; C 0/0; P 3/3. The second is called the permanent dentition with formula I 0/4; C 0/3; P 3/3; M 3/3 where I = Incisors, C= Canine, P= Premolar and M= Molar.

The mouth has salivary glands that secrete saliva with pH of about 8.2. It stabilizes the pH of the mouth and reduces the acidity in the subsequent chamber called the rumen. The oesophagus is a tubular column through which food is swallowed and regurgitated for re-chewing. It has no sphincter valves and the muscles contract in both directions to allow movement of food (Chesworth, 1992).

2.10 Digestion in the stomach

The stomach forms the greater proportion of the ruminants' digestive system. It has four chambers. The first is the rumen, while the second is reticulum, omasum is the third and abomasum the fourth.

1. Digestion in the rumen and reticulum

The digesta moves through the oesophagus into the rumen. A thin wall separates the rumen and the reticulum and their contents always mix. Hence the two chambers are called the reticulorumen. It is in the rumen that fermentation of the plant materials takes place. The rumen harbours billions of microbes for this function. In the young and unweaned animal, there exists an oesophageal groove that enables the milk ingested to pass down into the abomasum which is the true stomach for digestion and subsequent absorption and utilization in the small intestine.

2. Digestion in the omasum

After fermentation, the digesta flows into the omasum chamber. The omasum is a spherical organ with muscular laminae and in this chamber, water and inorganic minerals are absorbed. The digesta is filtered to ensure that no harmful object enters into the omasum.

3. Digestion in the abomasum

This is the true stomach and the first glandular portion whereby digestion by enzymes takes place. Gastric juice containing hydrochloric acid, pepsin, renin and lipase are produced. In young unweaned animals, the abomasum is about 80% of the stomach while in the adult it is only 10%. The digesta stays in the abomasum for about 1-2 hours.

2.11 Digestion in small and large intestine

Further breakdown of the food digesta occurs at the upper part of the small intestine. Here, pancreatic juice and bile assist in the digestion process while

absorption of the end product takes place in the lower portion of the intestine through finger-like structure called villi (Ranjhan, 1980).

The large intestine is made up of the **colon** and **caecum**. In this part of the Gastro-Intestinal Tract (GIT), some of the food residues are deposited for further fermentation in the caecum which has a blind end containing some microbes. The absorption of water and other nutrients continues in the colon. The digesta moves until it reaches the **rectum** and **anus**. The undigested food material forms a solid mass in the colon and it is eventually expelled through the **anus** (Ranjhan, 1980).

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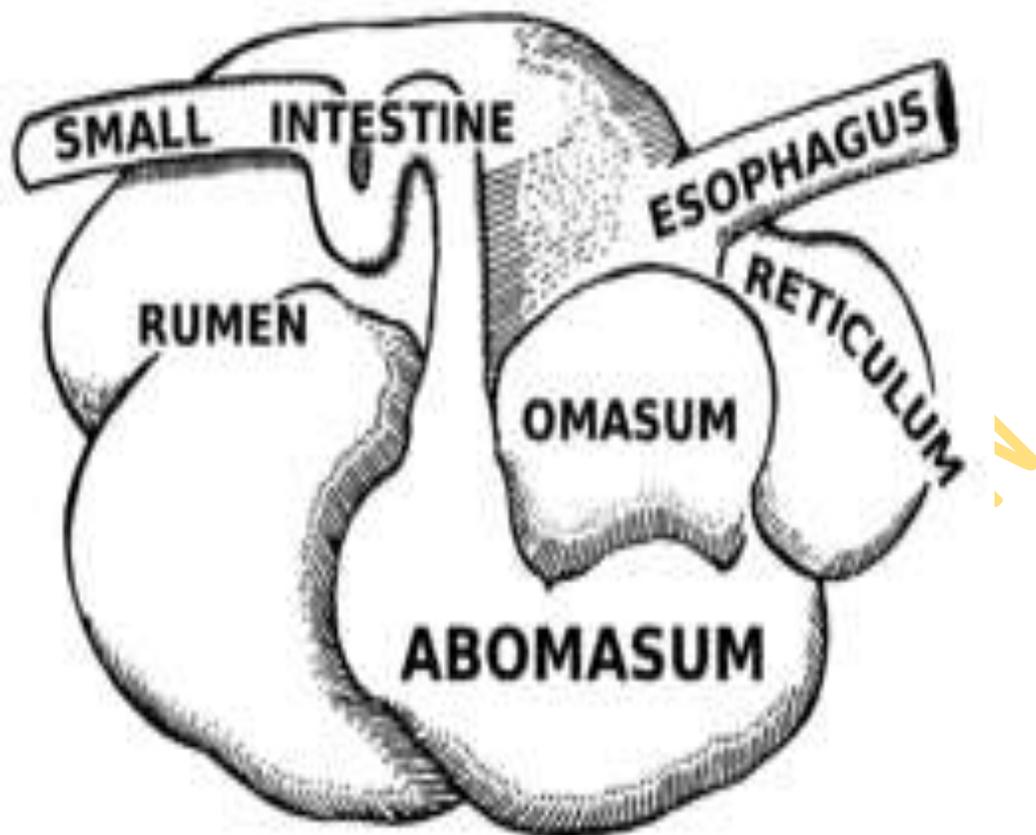


Plate 2.2 Digestive system of a ruminant (Chestworth, 1992)

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2.12 Types of Cattle

Cattle belong to the family *Bovidae* which is sub-divided into two subgroups: *Bos Taurus* and *Bos Indicus*. *Bos Taurus* cattle have no hump, while *Bos indicus* possesses humps. In addition, *Bos Taurus* cattle are the types of cattle found in the temperate regions of the world while *Bos Indicus* are found mostly in the tropics. Examples of *Bos Taurus* cattle includes breeds like the Holstein/Friesian, Ayrshire, Jersey, Hereford, Kuri, Ndama, Muturu and so many others while *Bos Indicus* are breeds like the White Fulani, Sokoto Gudali, Red Bororo, Keteku, Wadara (Shua Arab).

Cattle can also be classified based on the main purpose of production. Those specifically bred for milk productions are called dairy cattle (e.g. Holstein/Friesian) while those for meat are called beef cattle (e.g. Hereford). Some cattle have dual or even triple-purpose functions. They can be used for dairy, beef production and also used for draught. Examples include most of the local breeds in Africa e.g. White Fulani, Sokoto Gudali etc.

2.13 Breed of Cattle used for this research

White Fulani or Bunaji

This breed of cattle is the most prevalent in Nigeria especially in the Guinea and savannah zones of the country. They are large animals with medium to long up-curving horns. They have white coloured coat. The animal has well developed hump with skin folds. At maturity, the female White Fulani or Bunaji cattle weighs 330 to 350 kg while the male or bull weighs about 500 kg. It is a dual or triple purpose type of cattle as it may be fattened for beef, or kept for milk production or used as draught animal especially the bull.



Plate 2.3 White Fulani breed of cattle

2.14 Breed of goat used for this research

Maradi or Red Sokoto

This breed of goat is found mostly in the Sokoto area of Nigeria and part of the Niger republic. It is the most well defined breed of goat perhaps in Africa. It has red skin coat that is of good quality for leather production. Other varieties of the breed are the Kano brown or Boronu white. Both sexes carry horns with short ears that are horizontally positioned. At maturity, Maradi goats weigh between 20 and 30 kg.

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Plate 2.4 Maradi or Red Sokoto breed of Goat (FAO,2008)

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2.15 Instrument used for heavy metals analyses

Atomic Absorption (AA) occurs when a ground state atom absorbs energy in the form of light of a specific wavelength and is elevated to an excited state. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of analytes present in known standards can be used to determine unknown sample concentrations by measuring the amount of light they absorb. Performing atomic absorption spectroscopy requires a primary light source, an atom source, a monochromator to isolate the specific wavelength of light to be measured, a detector to measure the light accurately, electronics to process the data signal and a data display or reporting system to show the results. The light source normally used is a hollow cathode lamp (HCL) or an electrodeless discharge lamp (EDL).

In general, a different lamp is used for each element to be determined although in some cases, a few elements may be combined in a multi-element lamp. In the past, photomultiplier tubes were used as the detector. The source of energy for free-atom production is heat most commonly in the form of an air/acetylene or nitrous-oxide/acetylene flame. The sample is introduced as an aerosol into the flame by the sample introduction system consisting of a nebulizer and spray chamber. The burner head is aligned so that the light beam passes through the flame, where the light is absorbed.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study location

The study was carried out at the Central abattoir Akinyele, Ibadan, Oyo state, which falls within the rain forest agro-ecological zone and is found between latitude 7.5309⁰N and longitude 3.9110⁰E. This is the largest abattoir supplying both live animals and meat products for the general populace in Ibadan making the abattoir very important and it needs to be regulated in order to prevent meat borne disease and contamination to people living in Ibadan and its environs.

3.2 Study design

A cross-sectional study design with observational and laboratory analyses component were adopted.

3.3 Community entry

This was made possible through the Head Animal Health Technologist in the abattoir. He introduced me to overall head of the abattoir, the sub-heads of the cattle, sheep and goat sections. Also, the animal health workers were introduced and this helped in fast tracking the sample collection procedures.

3.4 Study duration

Samples were collected from the abattoir every Friday for eight weeks, while samples were sent to the laboratory for elemental analysis. The choice of Friday for the sample collection was important because, larger numbers of animals are being

slaughtered when compared to other days of the week. This is necessary for public health monitoring, surveillance and sampling.

3.5 Sample collection

A total of twenty (20) cattle and twenty (20) goats were purposively selected over a period of 8 weeks in conformity with FAO quality control guidelines. Samples of blood from the jugular vein at slaughter were collected using 5ml syringe with 21gauge needles. External abdominus muscles, liver apical lobes and kidney cortices were also collected from these animals. Samples were immediately taken to the Nigerian Institute of Science Laboratory, Samonda, Ibadan for digestion and elemental analysis in an ice pack. Below is the summary of the samples collected at the abattoir.

Number of animals used – 40 animals comprising 20 cattle and 20 goats.

Number of samples collected – 160 samples

Nature of samples collected – blood, muscle tissue, liver and kidney

Heavy metals analyzed – cadmium, chromium and lead.

Samples were collected every Friday morning (7am) for 8 weeks. Sample bottles used were coded as BD (bovine blood), BE (bovine muscle), BY (bovine kidney), BR (bovine liver) for cattle.

Goats' samples were coded as CD (caprine blood), CE (caprine muscle), CY (caprine kidney) and CR (caprine liver). This was done to prevent any error of bias in the study that may ensue during analysis.

3.6 Study materials

The materials used for the study were classified into the field and Laboratory materials. The field materials were the plain sample bottles, permanent markers for proper labeling and identification, disposable gloves, and nose masks. The laboratory materials were a sensitive weighing balance, glass petri-dishes, syringes, digestion flask, electric Bunsen burner, heating mantle, fume chamber, Nitric acid, cotton wool, funnels, digestion tubes and laboratory sample bottles. A Perkin Elmer Model 4100 Atomic absorption spectrophotometer was used for the study.

3.7 Methods

Methods involved in age estimation, digestion of samples and heavy metals analyses are explained below

3.7.1 Age estimation using dentition for cattle

In a well organized abattoir, cattle birth records should be kept and transferred with the cattle whenever presented for slaughter. In this way, the exact age of an animal will be known. When cattle age records are not available, dentition may be used to estimate cattle age. It is therefore important that veterinarians or other trained persons estimate their ages before slaughter or being marketed. At the Akinyele abattoir where the samples were collected, there were no records kept for age of the cattle presented for slaughter. However, permission was sought to carryout procedures for age estimation.

3.7.2 Use of teeth eruption, leveling and wearing for age estimation

The following part of the teeth was used to estimate the age of cattle:

1. Incisors

The patterns of eruption, leveling and wearing of incisor teeth are as follows:

- a. Eruption of pincher occurs between 18 and 24 months and leveled at 5 to 6 years, while noticeable wear occurs at 7 to 8 years.
- b. Eruption of first intermediate pair occurs between 24 and 30 months, leveled at 6 to 7 years, and the noticeable wear seen at 8 to 9 years.
- c. Eruption of second intermediate pair occurs between 36 and 48 months, leveled at 7 to 8 years and noticeable wear seen at 9 to 10 years.
- d. While the eruption of corner occurs between 48 months to 60 months, leveled at 9 years, then noticeable wear seen at 10 years.

The use of the dentition revealed that most cattle and goats slaughtered at the abattoir were between 18 and 48 months (categorized as young and matured animals), while old animals above 60 months were rarely encountered. It is important to note that patterns of age estimation for ruminants are the synonymous.

3.8 Lead, chromium and cadmium digestion process

Approximately 0.5kg each of collected samples of muscle, liver, kidneys and 5ml blood of goats and cattle were weighed and decomposed by ashing and wet digestion method for the determination of lead, chromium and cadmium residues. Known quantity, (5 g of each sample) were put into vycor crucible and pre-ashed for 1 to 2 hours until the sample was completely charred on a hot plate. After which the pre-ash sample was placed in a muffle furnace and ashed at 500 °C. After one hour, the

sample was removed from the furnace, and careful wetting of the ash with Nitric acid (HNO₃) was done. It is highly necessary not to use too much HNO₃, as the sample may splatter when returned to the furnace. The wetting procedure was repeated hourly until the ash turned white (usually about two hours of wetting). After the ashing procedure, quantitative transfer of the sample into 10 ml volumetric flask by carefully washing the crucible with 1 ml HNO₃, and then made up to 10ml by adding distilled water.

3.9 Heavy metals analysis of samples

The determination of heavy metals was made directly on each of the final solutions using Atomic Absorption Spectroscopy (A.A.S). For each heavy metal, there is a specific “hollow cathode lamp” and the machine set at a particular wavelength for the heavy metal to be analyzed. Formula for calculation of the heavy metal residue is

$$\frac{mg}{kg} = \text{instrument reading} \left(\frac{mg}{L} \right) \times \frac{\text{final volume (mL)}}{\text{weight (g)}}$$

3.10 Heavy metal analyses of lead, cadmium and chromium

Procedure was carried out using an Atomic Absorption Spectrophotometer (Perkin Elmer 4100, U.S.A). Air/acetylene is the primary source of heat and the burner was adjusted to set the flame height in order to allow the light source to pass through. For each heavy metal to be analyzed a particular hollow cathode lamp specific to the metal is inserted into the machine. This is followed by aspiration of the digested sample into the nebulizer, which aerosolizes the sample to spray the chamber. The atom becomes excited from the ground state and the photon is knocked off, goes through a monochromator and read on the detector. The absorbance was measured at 15mA of lamp and the peak height mode of the wave lengths used were 283.3nm, 228.8nm, 356.9nm for lead, cadmium and chromium respectively.



Plate 3.1 Collection of samples from the abattoir

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Plate 3.2a singeing of goat at Akinyele abattoir, Ibadan



Plate 3.2b singeing of cattle at Akinyele abattoir, Ibadan



Plate 3.3 Atomic absorption spectrophotometer used for heavy metal analyses

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3.11 Data management

Individual characteristics of the sampled cattle and goats were presented in frequency and percentages. Data obtained were summarized as mean \pm SD. Median, Minimum and maximum values. Descriptive statistics and t-test were done using SPSS 17.0 package.

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CHAPTER FOUR

4.0

RESULTS

This cross-sectional study was employed to assess some heavy metals of public health importance in selected parts of cattle and goats slaughtered at Akinyele abattoir, Ibadan, Oyo state. The results obtained are as presented in this chapter.

4.1 Age (in months) and sex characteristics of cattle sampled

Table 4.1 shows the summary of the age and sex characteristics of the sampled cattle. The age ranged from 30 to 84 months with the mean and standard deviation of 49.5 and 14.7 months respectively. Cattle of 36 months of age were mostly encountered.

The sex of the cattle sampled was as also presented in table 4.1. Out of twenty (20) cattle sampled, fourteen (14) were males (bulls) making 70% of the cattle sampled, while six (6) were females (cows).

Table 4.1 Age (in months) and sex characteristics of cattle sampled

Age	Frequency	Sex		Age summary (months)			
		Male	Female	Mean \pm S.D	Mode	Min	Max
30-39	7	7	0	49.5 \pm 14.7	36	30	84
40-49	4	3	1				
50-59	2	1	1				
60-69	5	3	2				
70-79	1	0	1				
80-89	1	0	1				
Total	20	14	6				
(%)	100	70	30				

4.2 Age (in months) and sex characteristics of goats sampled

Table 4.2 shows the summary of the age and sex characteristics of the sampled goats. The age ranged from 18 to 48 months with the mean and standard deviation of 28.8 and 8.7 months respectively. Goats of 24 months of age were mostly encountered.

The sex of the goats sampled was as also presented in table 4.2. Out of twenty (20) goats sampled, fourteen (12) were males (bucks) making 60% of the goats sampled, while eight (8) were females (does).

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Table 4.2 Age (in months) and sex characteristics of goats sampled

Age	Sex			Age summary (months)				
	Range	Frequency	Male	Female	Mean \pm S.D	Mode	Min	Max
10-19	4	4	4	0	28.8 \pm 8.7	24	18	48
20-29	6	5	5	1				
30-39	7	3	3	4				
40-49	3	0	0	3				
Total	20	12	12	8				
(%)	100	60	60	40				

4.3 Age categorization of cattle and goats

Table 4.3 shows age categorization of cattle and goats under study. The animals were categorized into young, grown and old. Animals between 0 and 24 months were considered as young and those between 25 and 60 months as grown and those 61 months and above as old. In this study cattle categorized grown and old were 17 (85%) and 3 (15%) respectively. While goats categorized as young, grown were 10 (50%) and 10 (50%) respectively. This indicated that young cattle were hardly found in the abattoir for slaughter, while old goats were rarely presented for slaughter as most goats were slaughtered before attaining such age except those proven and used as breeding stocks.

It is highly important to note that age has a positive association with the levels on heavy metals in the tissue of animals; the older the age the higher the level of heavy metals that would be bio-accumulated.

Table 4. 3: Age categorization of the cattle and goats sampled (in months)

Animal category	Animal type	
	Cattle	Goats
Young	0 (0%)	10 (50%)
Grown	17 (85%)	10 (50%)
Old	3 (15%)	0 (%)

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4.4 Number of cattle samples containing the selected heavy metals

Table 4.4 shows the number of cattle samples containing the selected heavy metals i.e cadmium, chromium and lead. Of eighty (80) samples analyzed for each heavy metal, sixty three (63) cattle had cadmium in their tissues, nine (9) had chromium and seventeen had lead residues amounting to 78.8%, 11.3% and 21.3% respectively.

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Table 4.4 **Number of cattle samples with selected heavy metals**

Animals	Cadmium	Chromium	Lead
Cattle	63	9	17
Percentage (%)	78.8	11.3	21.3
Total samples	80	80	80

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4.5 Number of goat samples containing the selected heavy metals

Table 4.5 shows the number of goat samples containing the selected heavy metals i.e cadmium, chromium and lead. Of eighty (80) samples analyzed for each heavy metal, fifty two (52) goats had cadmium in their tissues, eight (8) had chromium and eight (8) had lead residues amounting to 65.0%, 10.0% and 10.0% respectively.

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Table 4.5 **Number of goats' samples with selected heavy metals**

Animals	Cadmium	chromium	Lead
Goats	52	8	8
Percentage (%)	65.0	10.0	10.0
Total sample	80	80	80

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4.6 Total proportion of the cattle and goats having the heavy metals

The total number of cattle and goats having the heavy metals of interest are as presented in table 4.6. Of all the 160 animals, 115 (71.9%) animals had cadmium, 17 (10.6%) had chromium and 25 (15.6%) had lead residues. This shows that proportion of cadmium residue detected was higher significantly when compared to that of chromium and lead found the animals tissues under the study.

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Table 4.6 Total proportion of cattle and goats samples with heavy metals

Animals	Cadmium	chromium	Lead
Cattle	63	9	17
Goats	52	8	8
Total detected	115	17	25
Percentage (%)	71.9	10.6	15.6
Total sample	160	160	160

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4.7 Heavy metal residue distribution in different tissue types of cattle

Table 4.7 shows the heavy metal residue distribution in different tissue types of cattle slaughtered at the abattoir selected for study. Cadmium residues were found in 16 blood samples, 15 muscle samples, 16 kidney samples and 16 liver samples. The level in blood ranged from 1.32-8.90 $\mu\text{g/ml}$ with mean and standard deviation of 5.67 ± 2.08 $\mu\text{g/ml}$. While in muscle, kidney and liver, the values ranged from 0.01-6.11mg/kg, 2.41-8.89mg/kg and 3.33-14.44mg/kg respectively. Also, the mean and standard deviation values obtained from muscle, kidney and liver were 3.81 ± 2.09 mg/kg, 5.71 ± 2.31 mg/kg and 5.24 ± 2.30 mg/kg respectively.

Table 4.7 also shows chromium residue distribution in different tissue types of cattle slaughtered in the abattoir selected for study. Chromium residue was found in 1 blood sample, 2 muscle samples, 4 kidney samples and 2 liver samples. The level found in blood was 0.62 $\mu\text{g/ml}$ with mean 0.62 $\mu\text{g/ml}$. While in muscle, kidney and liver the values ranges from 0.62-0.62mg/kg, 0.62-6.79mg/kg and 0.62-1.94mg/kg respectively. Also, the mean and standard deviation values obtained from muscle, kidney and liver were 0.62 ± 0.00 mg/kg, 2.33 ± 2.99 mg/kg and 1.28 ± 0.93 mg/kg respectively.

Lead residue distribution in different tissue types of cattle slaughtered in the abattoir selected for study is also shown in table 4.7. Lead residue was found in 8 blood samples, 5 muscle samples, 2 kidney samples and 2 liver samples. The level in blood ranges from 0.01-1.21 $\mu\text{g/ml}$ with mean and standard deviation of 0.49 ± 0.56 $\mu\text{g/ml}$. While in muscle, kidney and liver the values ranges from 0.001-0.51mg/kg, 0.001-0.03mg/kg and 0.00mg/kg respectively. Also, the mean and standard

deviation values obtained from muscle, kidney and liver were $0.11 \pm 0.22 \text{mg/kg}$, $0.02 \pm 0.02 \text{mg/kg}$ and $0.01 \pm 0.01 \text{mg/kg}$ respectively.

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Table 4.7 Heavy metal residue distribution in different tissue types of cattle

Animal	Heavy metals residue (mg/kg)								
	Cadmium			Chromium			Lead		
	N	Mean+S.D	Range	N	Mean+S.D	Range	N	Mean+S.D	Range
Blood*	16	5.67±2.08	1.32-8.90	1	0.62	0.62	8	0.49±0.56	0.01-1.21
Muscle	15	3.81±2.09	0.01-6.11	2	0.62±0.00	0.62-0.62	5	0.11±0.22	.001-0.51
Kidney	16	5.71±2.31	2.41-8.89	4	2.33±2.99	0.62-6.79	2	0.02±0.02	.001-0.03
Liver	16	5.24±2.30	3.33±14.4	2	1.28±0.93	0.62-1.94	2	.001±.001	.001-.001

* Unit in blood presented as µg/ml

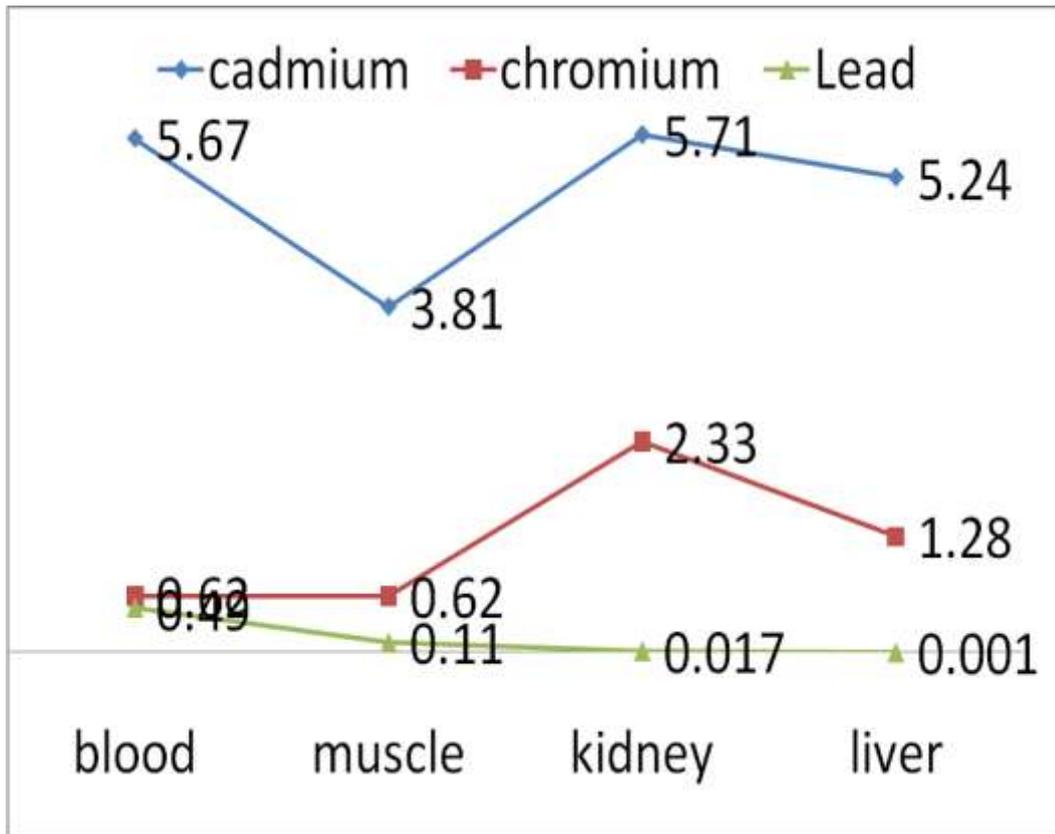


Fig 4.1 Heavy metals residue distribution in parts of sampled cattle

Table 4.8 Heavy metal residue distribution in different tissue types of goats

Table 4.8 shows the cadmium residue distribution in different tissue types of goats slaughtered at the abattoir selected for study. Cadmium residues were found in 13 blood samples, 12 muscle samples, 13 kidney samples and 14 liver samples. The level in blood ranged from 3.33-14.44 $\mu\text{g/ml}$ with mean and standard deviation of 6.75 ± 3.03 $\mu\text{g/ml}$. In muscle, kidney and liver, the values ranged from 3.33-8.89 mg/kg, 3.25-8.89 mg/kg and 0.61-11.67 mg/kg respectively. Also, the mean and standard deviation values obtained from muscle, kidney and liver were 5.43 ± 1.94 mg/kg, 4.77 ± 1.93 mg/kg and 5.51 ± 2.92 mg/kg respectively.

Table 4.8 also shows the chromium residue distribution in different tissue types of goats slaughtered at the abattoir selected for study. Chromium residues were found in 5 blood samples, none in muscle samples, 1 kidney sample and 2 liver samples. The level in blood ranged from 0.62-6.79 $\mu\text{g/ml}$ with mean and standard deviation of 2.37 ± 2.55 $\mu\text{g/ml}$. While the mean values in muscle, kidney and liver, the values were 0.00 mg/kg, 0.01 mg/kg and 0.62 mg/kg respectively.

Lead residue distribution in different tissue types of goats slaughtered at the abattoir selected for study is presented in table 4.8. Lead residues were found in 3 blood samples, 1 muscle sample, 1 kidney sample and 3 liver samples. The level in blood, muscle, kidney and liver in all the samples were not significantly different and at 1 place of decimal all were found to be 0.0 residual values.

Table 4.8 Heavy metal residue distribution in different tissue types of goats

Animal	Heavy metals residue (mg/kg)									
	Samples	Cadmium			Chromium			Lead		
		N	Mean+S.D	Range	N	Mean+S.D	Range	N	Mean+S.D	Range
Blood*	13	6.75±3.03	3.33-14.4	5	2.37±2.55	0.62-6.79	3	.007±.005	.001-.010	
Muscle	12	5.43±1.94	3.33-8.89	-	ND	-	1	.001	.001	
Kidney	13	4.77±1.93	3.25-8.89	1	0.01	0.01	1	.001	.001	
Liver	14	5.51±2.92	0.61-11.7	2	0.62±0.00	0.62-0.62	3	.005±.006	.001-.011	

* Unit in blood presented as µg/ml

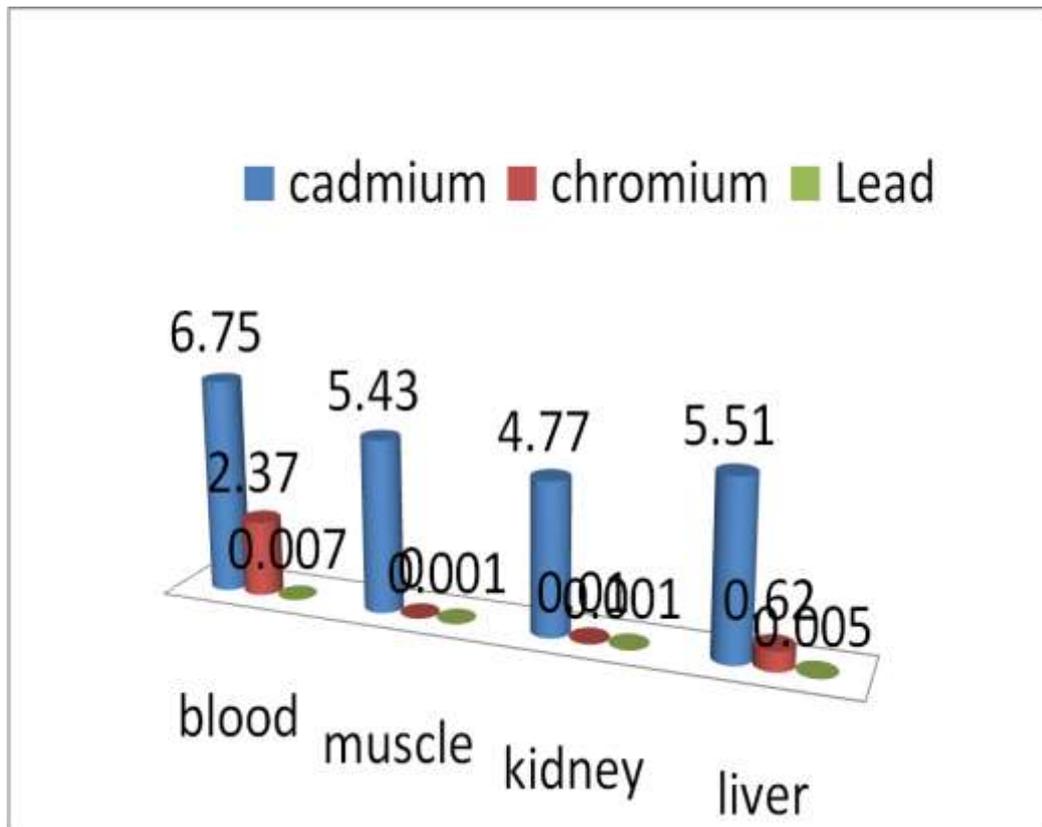


Fig 4.2 Heavy metals residue distribution in parts of sampled goats

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4.9 Concentration of heavy metals in cattle residue as measured against FAO/WHO daily intake guideline limits

Cadmium residues in blood, muscle, kidney and liver under study were 5.67 ± 2.08 , 3.81 ± 2.09 , 5.71 ± 2.31 and 5.24 ± 2.30 respectively. These values were found to be above the daily intake permissible limit (0.50-1.00mg/kg). Chromium residues were found to be within the permissible limit in blood and muscle, while the values in kidney (2.33 ± 2.99) and liver (1.28 ± 0.93) were above the permissible limit of 1mg/kg. The lead residues in the blood and all the tissues sampled were within the permissible limits (0.10-0.50mg/kg).

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Table 4.9: Concentration of heavy metals in cattle residue as measured against FAO/WHO daily intake guideline limits

Tissue type	Heavy metals in cattle (mg/kg)					
	Cadmium	FAO/WHO	Chromium	FAO/WHO	Lead	FAO/WHO
Blood*	5.67±2.08	0.50	0.62	1.00	0.49±0.56	0.50
Muscle	3.81±2.09	0.50	0.62±0.00	1.00	0.11±0.22	0.10
Kidney	5.71±2.31	1.00	2.33±2.99	1.00	0.017±0.022	0.50
Liver	5.24±2.30	0.50	1.28±0.93	1.00	0.001±0.00	0.50

* Unit in blood presented as µg/ml

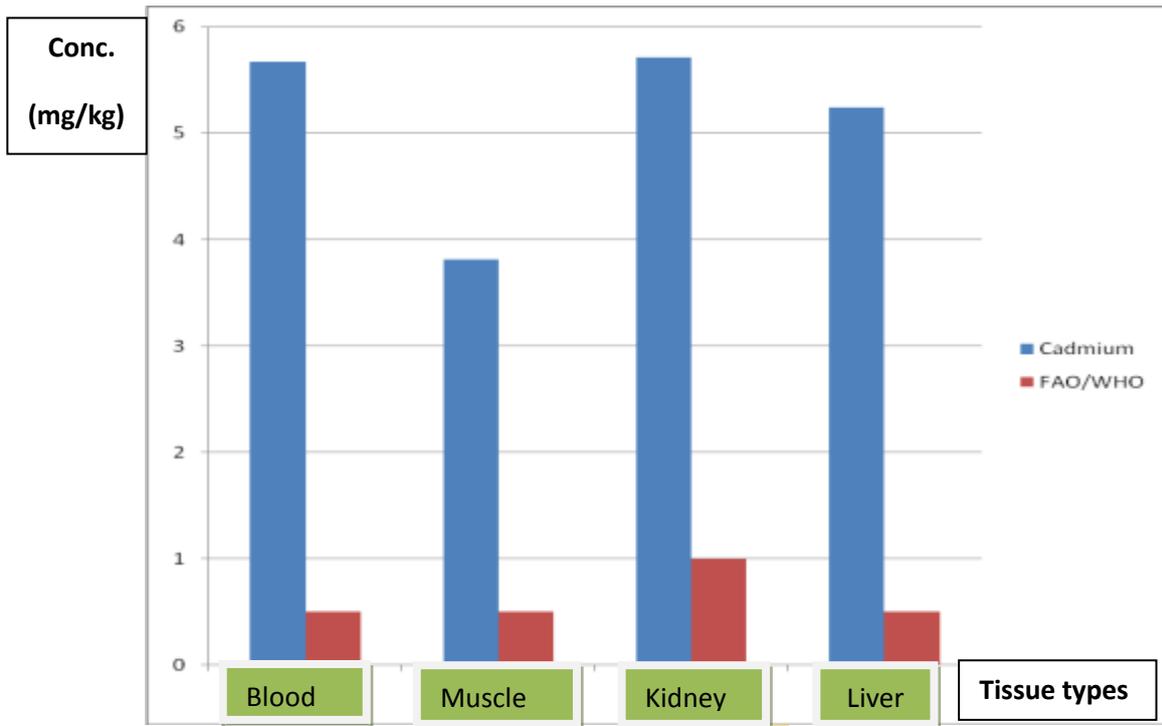


Fig 4.3: Concentration of cadmium in cattle residue as measured against FAO/WHO daily intake guideline limits

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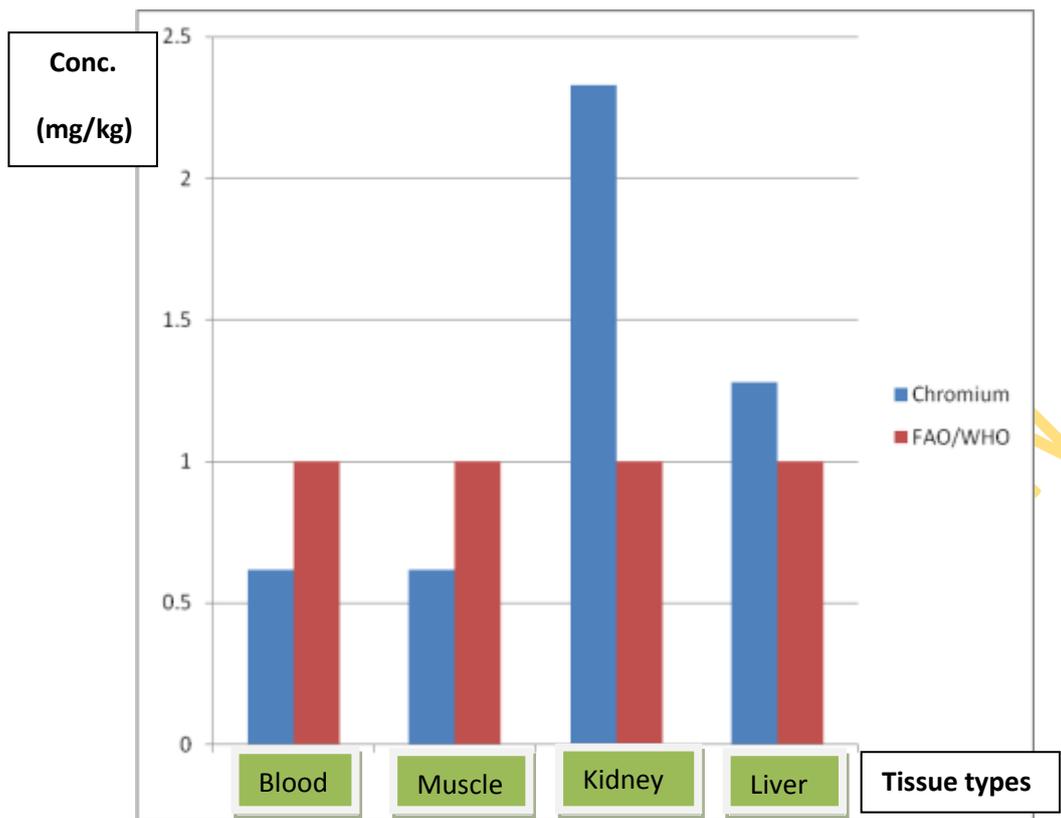


Fig 4.4: Concentration of chromium residue in cattle as measured against FAO/WHO daily intake guideline limits

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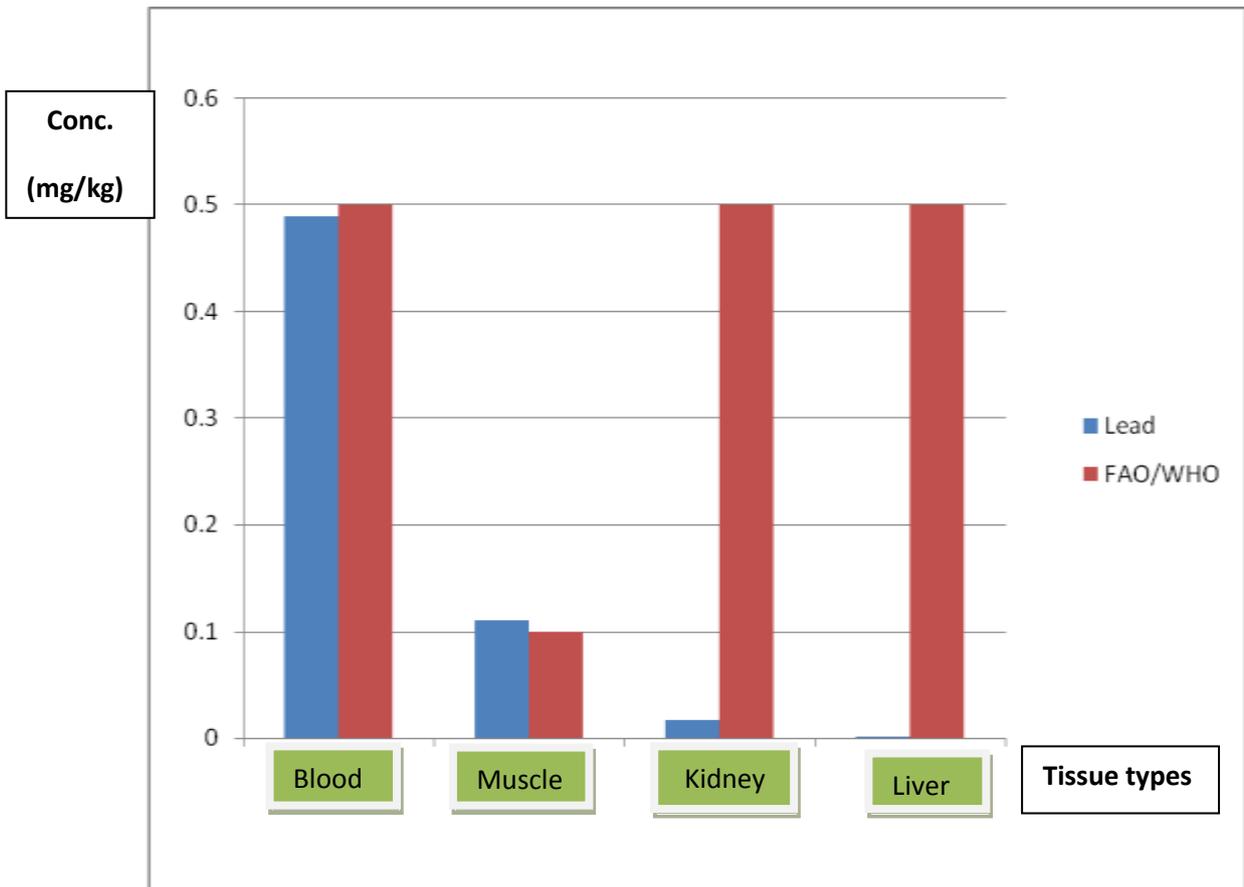


Fig 4.5 Concentration of Lead residue in cattle as measured against FAO/WHO daily intake guideline limits

4.10 Concentration of heavy metal residue in goats as measured against FAO/WHO daily intake guideline limits

Cadmium residues in blood, muscle, kidney and liver under study were 6.75 ± 3.03 , 5.43 ± 1.94 , 4.77 ± 1.93 and 5.51 ± 2.92 respectively. These values were found to be above the permissible limit (0.50-1.00mg/kg). Chromium residues were found to be within the permissible in muscle, kidney and liver, while the value in blood (2.37 ± 2.55) was above the limit of 1mg/kg. While the lead residues in the blood and all the tissues sampled were within the permissible limit (0.10-0.50mg/kg)

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Table 4.10: Concentration of heavy metal residue in goats as measured against FAO/WHO daily intake guideline limits

Tissue type	Heavy metals in goats (mg/kg)					
	Cadmium	FAO/WHO	Chromium	FAO/WHO	Lead	FAO/WHO
Blood	6.75±3.03	0.50	2.37±2.55	1.00	0.007±0.005	0.50
Muscle	5.43±1.94	0.50	Nil	1.00	0.001	0.10
Kidney	4.77±1.93	1.00	0.01	1.00	0.001	0.50
Liver	5.51±2.92	0.50	0.62±0.00	1.00	0.005±0.006	0.50

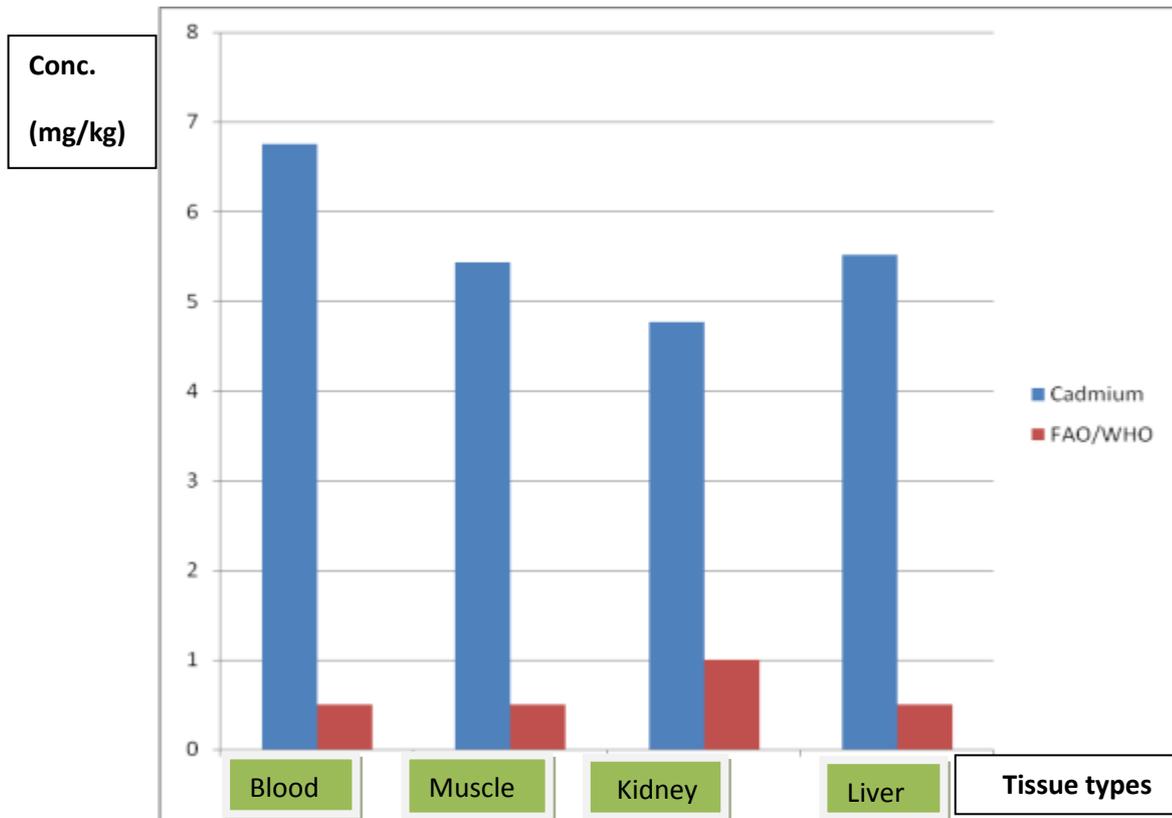


Fig4.6: Concentration of cadmium in goats residue as measured against FAO/WHO daily intake guideline limits

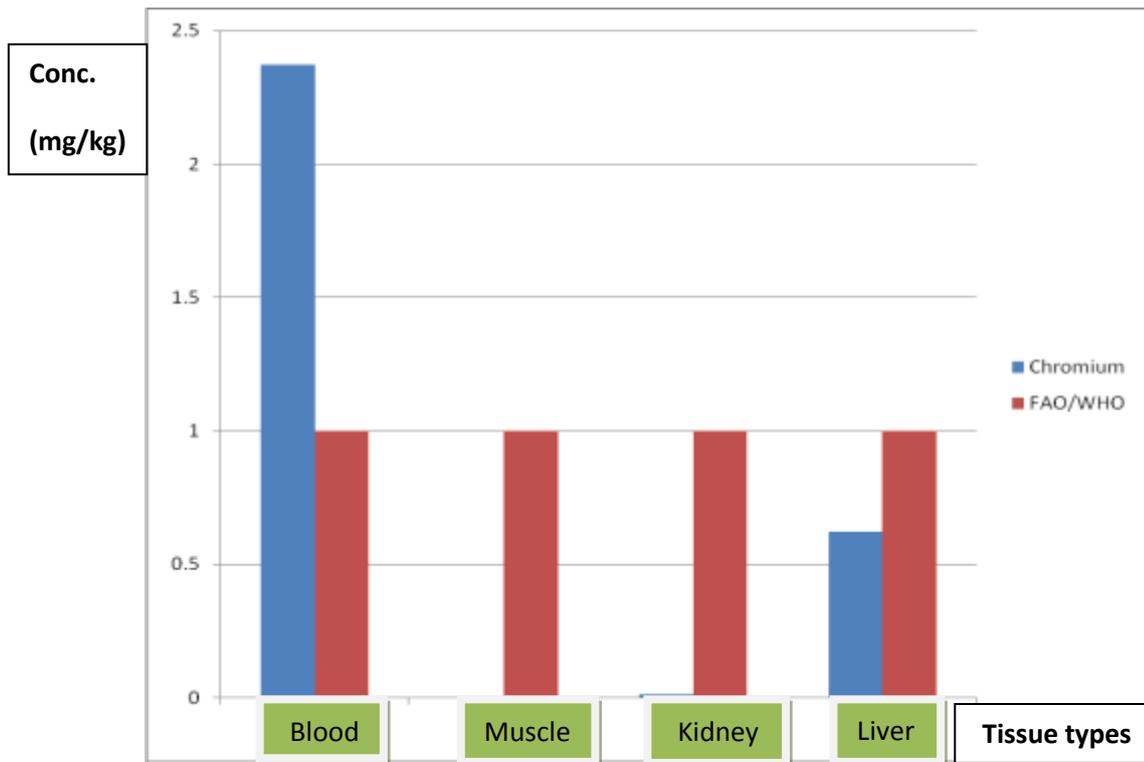


Fig 4.7: Concentration of chromium residue in goats as measured against FAO/WHO daily intake guideline limits

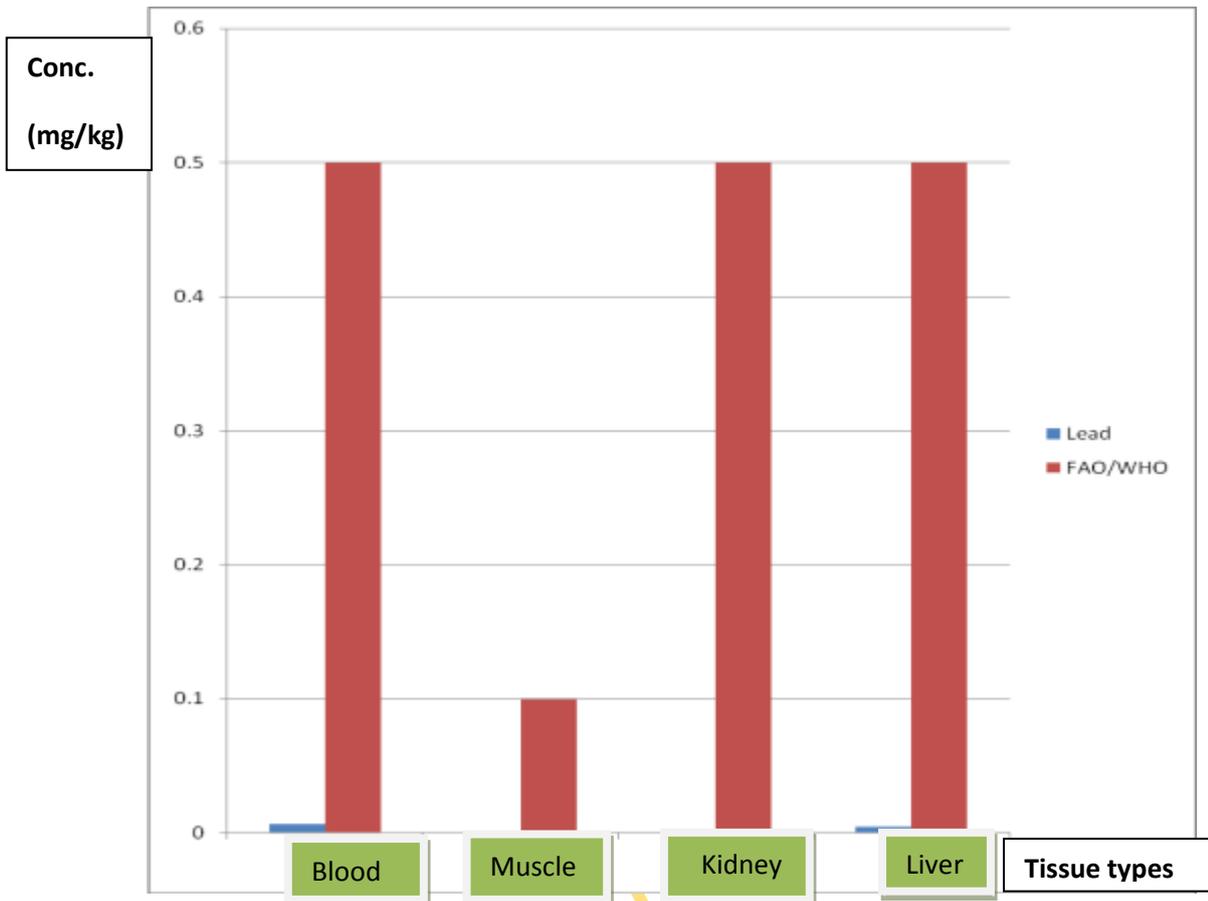


Fig 4.8: Concentration of lead residue in goats as measured against FAO/WHO daily intake guideline limits

CHAPTER FIVE

5.0 DISCUSSION

This chapter discusses the findings in line with the specific objectives of the study. These include establishment of presence of cadmium (Cd), chromium (Cr) and lead (Pb) residues in animals slaughtered in the abattoir; determination of percentage distribution of cadmium (Cd), chromium (Cr) and lead (Pb) in different parts of slaughtered animals; evaluation of pattern of distribution of heavy metal residues in animal body and comparison of the research findings with permissible limits as set by FAO/WHO standards.

5.1 Age of the animals sampled

It was found from this study that 85 percent of cattle slaughtered in the abattoir for daily consumption were categorized as grown i.e between 36 to 60 months, 15 percent were old, and no young cattle was encountered. This was because most cattle offered for slaughter were indigenous breeds which attain desirable weight for sale and slaughter at older age. While in goats, both young and grown animals were seen as this was largely due to the fact that most consumers of chevon (goat meat) have preference for the meat because of its tenderness and once it becomes old, this quality would be lost.

5.2 Sex of the animals sampled

In the course of sampling, 75 percent male cattle and 60 per cent male goats were encountered. This showed that majority of cattle and goats slaughtered in the abattoir were male. It is important to note that, female cattle and goats are being used for breeding purposes, while only the unproductive and old ones are culled for

slaughter in the abattoir. This is in line with the works of Riehn *et al.* 2010, reporting that sound economic management demand that, animals sold for slaughter should be mainly males and reproductively inactive females. Also, the meat quality of male cattle is better than female. Meat obtained from male is usually more tender and easy to chew.

5.3 Heavy metals proportion in cattle

The research work revealed that cadmium had the highest percentage of 78.8%, while chromium had the least prevalence 11.3%, in the cattle sampled. This result is in contrast with the work of Bala *et al.* (2012), who reported 100% prevalence for cadmium, chromium and lead in animals samples from Sokoto central abattoir. Bala *et al.* (2014), also reported 87.5%, 95.9%, 79.2% prevalence for cadmium, chromium and lead respectively in animal samples examined. The reduction in the lead (Pb) prevalence could be linked to increased awareness on health hazard it causes in man and animal population, as evident in Zamfara lead poisoning, which claimed many lives. It is important to note that, highest heavy metal levels seen in this research and that of Bala *et al.* was cadmium residues. This could be largely due to increased usage of cadmium in production processes in the northern and southwestern areas of Nigeria in the manufacture of batteries, smelter operation etc.

5.4 Heavy metals proportion in goats

Likewise, percentage prevalence of the goat samples containing cadmium, chromium and lead residues were 65.0%, 10.0% and 10.0% respectively. These percentages were generally lower when compared to that found in cattle samples.

This could be adduced to the fact that cattle presented for slaughter were older and also have more grazing time to bio-accumulate heavy metals.

5.5 Total heavy metals proportion in both cattle and goats

The total percentage proportion of cadmium, lead and chromium residues in both cattle and goats were 71.9% (115 out of 160), 15.6% (25 out of 160) and 10.6% (17 out of 160) respectively. These findings established the presence of heavy metals (cadmium, lead and chromium) in cattle and goats slaughtered for daily consumption at the central abattoir, Akinyele, Ibadan. This satisfied one of the specific objectives of this research work which was to establish the presence of heavy metal residues in animals offered for slaughter.

5.6 Distribution of cadmium residues in cattle and goats

The distribution of cadmium residue levels in the various tissues of cattle and goats followed almost the same pattern. Cadmium was detected in blood, muscle, kidney and liver, in almost the same number of samples and residual levels. The residual values seen were generally higher in the blood samples than in other tissues studied. This could be as a result of recent exposure to these heavy metals during transportation, confinement in lairage and singeing (burning of carcass) operations. It is also important to conclude from this finding that, the residual levels of cadmium were also high in muscle, liver and kidney tissues. This is affirmed in the study of García-Fernández *et. al.* (1996) who suggested that the kidneys are the main storage organs in animals subjected to chronic low-level cadmium exposure. In comparison, it was discovered that values in goats were higher than that of cattle except in kidney samples.

Considering the cadmium residues found in these tissues, it could be concluded that values found were above the permissible limit, which is in contrast with some works that found the levels of cadmium residues below the permissible limit.

5.7 Distribution of chromium residues in cattle and goats

Chromium residue levels in the tissues of cattle and goats sampled showed that, the total number and levels found were generally low especially when compared with that of cadmium residues. In cattle, blood and muscle tissues had the least mean values of 0.62mg/kg while kidney and liver had mean residual levels higher than the permissible limit (i.e 2.33 and 1.28mg/kg respectively). Fathy *et al.* (2011) also reported lower residual levels of chromium in muscle with 11.20 µg/kg (0.011mg/kg) fresh weight. From the result obtained from this research work, only 17 of 160 samples analyzed had chromium level.

In goats, chromium residue levels were lower than that of cattle except in blood which might also be due to recent exposure. . The level of chromium residues in goats were in agreement with work the of Bala *et. al.* (2012) who stated that the chromium level in kidney and liver were within the permissible limit. In addition, the chromium residue levels in muscle, kidney and liver were within the permissible limit.

5.8 Distribution of lead residue in cattle and goats

From this research work, it was discovered that the number of tissues having lead residue distribution in cattle and goats were seventeen (17) and eight (8) respectively. In cattle, blood sample had the highest mean residual values of 0.49µg/ml while the least value of 0.001mg/kg was found in the liver.

Also in goats, the mean lead residual level was highest in blood (0.007 μ g/ml) while the value in the liver was higher liver (0.005mg/kg) when compared to that of the kidneys and muscle (both having 0.001mg/kg). This is in line with the works of Koréneková *et al.* (2002) and Miranda *et al.*(2005) who reported that the liver accumulates lead more than any other tissues.

It is very striking to note that lead residues found in goat and cattle samples were within the permissible limits. This is important because lead residues in our environment has claimed so many lives, caused lots of teratology, mutagenesis, nervous impairment etc in the last decade. Nigeria has also suffered loss of human and animal population recently from lead toxicity. Therefore, reduction of lead residues as seen in this work could be largely due to public health awareness, reduction in the use of leaded gasoline and prevention of illicit disposal of lead containing substances.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

In this chapter, conclusions and recommendations based on what has been discovered in the course of the research would be made. This preamble is necessary so as to draw the attention of the public to some of the salient discoveries obtained from this work, which could be useful in public health safety.

6.1 Conclusions

The following can be concluded from the work:

- Sixty to seventy percent of cattle and goats slaughtered at central abattoir, Akinyele were male and only un-reproductive females were offered for slaughter.
- Most cattle slaughtered were usually between 36 and 60 months of age and age range for goats was between 24 and 36 months. This indicated that only young matured animals were slaughtered and old stocks were seldom encountered.
- Percentage distribution showed that cattle bio-accumulated heavy metals more when compared with goats. This agrees with the work of Bendeddouche *et. al.* (2014), which revealed higher levels of heavy metal bio-accumulation in cattle when compared with sheep and goats produced in Algeria.

- Likewise, there was a uniform pattern of heavy metals bioaccumulation in both species of animals sampled. Cadmium had the highest residual values, while Lead had the least residual values. Possibly this could be due to:
- Cadmium release into the atmosphere via natural and anthropogenic sources especially through cigarette smoke and singeing operations.
- The use of phosphate fertilizers which is a major source of cadmium input to agricultural soil.
- Blood cadmium levels are indicative of recent exposure rather than whole-body burdens. Cadmium mostly accumulates in the kidneys and liver.
- It is important to know that high level of public awareness on Lead (Pb) has drastically reduced its level in the environment and thereby lowering to the amount found in meat and offal to the barest minimum.
- The levels of heavy metals seen in different tissue parts, indicated high involvement of liver and kidney in the process of detoxification and elimination of the toxic compounds.
- Also, in comparison with the permissible limits, lead was found to be low in both animals; chromium was only higher in kidneys and liver of cattle and also in the blood of goats while others fell within limits. It is worthy of note that the prevalence of chromium was generally low in the study.
- Cadmium exceeded the permissible limits in all the samples of both animals, which was the striking finding. Hence, possible risk of public health

challenge in our population could be to a large extent from cadmium intake in meat and offal.

- It could be concluded that exposure rate of these animals to different heavy metals from exhaust from air, water bodies, soil etc was high. This is evident in the high levels of these metals in blood of the animals under study.

6.2 Recommendations

In view of the conclusions arrived at in this study and since the general population cannot do without consumption of meat and meat products, the following recommendations are proffered:

1. Proper disposal of solid wastes. Most of these waste materials have heavy metals in them and constitute serious health risk in the environment, leaching of metals into water bodies and surrounding soil usually occurs.
2. Pre-treatment of waste water and effluent before it is channeled into the environment. This would help in the reduction of toxic substances that could easily contaminate our environment.
3. Livestock farms should be sited far away from industrial sites, quarries, highways etc. This is highly necessary to prevent direct ingestion, inhalation and absorption of heavy metals that are in constant use in these areas.
4. Cadmium-containing equipment and wares usage should be minimized, if it cannot be totally avoided.
5. Use of fertilizers, agrochemicals, additives containing heavy metals, especially cadmium, should be avoided.

6. Consumption of too old stock/animals should be avoided, as these have higher levels of bioaccumulated heavy metals.
7. Consumption of younger stock should be advocated.
8. Goat is recommended for consumption over cattle as it has lesser levels of heavy metals bioaccumulation tendencies.
9. Government, through the ministries of health and agriculture should establish a department saddled with the responsibility of screening (animals meant for slaughter) for heavy metals.
10. There is need to embark on massive public health awareness on the need to reduce emission of cadmium into the soil, water bodies and air in our environment.

6.3 Further research

This research work has raised some important areas where further work can be done and these include the following

1. Research on the use of synthetic and/ or natural agents e.g. plant or animal extracts to chelate heavy metals within the body systems of live animals after dosing with known quantities.
2. Research on the use of different processing methods that could reduce heavy metals in meat and meat products after slaughter.
3. Research on the use of body fluids e.g. urine, discharges and blood as a monitoring tool for heavy metal detection.

4. Collaboration with other experts in the field of veterinary medicine, medicine, agriculture, engineering, biochemistry etc in developing biomarkers, that can be used to assay for levels of heavy metals in live animals.

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