ASSESSMENT OF ETHYLENEDIAMINETETRAACETIC ACID AND ACTIVATED CHARCOAL AS CHELATORS OF SELECTED HEAVY METALS IN BROILER CHICKEN MEAT IN DELTA STATE, NIGERIA

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DEDICATION

This work is dedicated to God almighty, the giver of life, the source of all we have and are, the fountain of all wisdom anddiscernment for His protection, mercy, blessings and grace upon my life.

Also to the 21st century livestock farmers who in the midst of the odds, contribute to the socio-economic and health of humanity through the supply of animal proteins to satisfy the ever growing nutritional requirement of a teeming populace.

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ABSTRACT

Pollution of water, soil and air by petrochemical effluents, oil spills and gas flare containing heavy metals is a common occurrence in oil producing areas such as Delta State, Nigeria. The Drinking Water (DW) are thus inadvertently polluted with heavy metals with resultant systemic poisoning of life. Ethylenediaminetetraacetic acid (EDTA) and Activated Charcoal (AC) have the potential to reduce Heavy Metal Concentration (HMC) in DW. However, report on theiruse as chelators when incorporated in the DW of chickens during production is scanty. Hence, the effectiveness of EDTA and AC as chelators in DW of chickens during management was evaluated.

Twenty-one Local Government Areas (LGA) in Delta State, were purposively selected based on the intensity of crude oil exploration and grouped into seven zones: Urhobo, Isoko, Ijaw, Itsekiri, Ukwani, Aniocha and Ika. Samples of DW, eggs and meat were collected from each zone and analysed forHMC (ppm) using standard procedures, and results were compared with World Health Organization (WHO) standards. Water Relatively High in: Vanadium (WRHV), Cadmium (WRHC) and Iron (WRHI) were each treated with or without either 50 mg/l EDTA or AC. One-day old Arbor Acres broiler chicks (n=288) were randomly allotted to the treatments with four replicates in a completely randomised design and fed for 42 days. At day 35, two chickens were selected and housed in cages. The total excreta were collected, dried and stored. At day 42, two chickens were randomly selected per replicate and slaughtered. Deboned thighs of slaughtered chickens and excreta were assayedfor HMC using standard procedures. Data were analysed using descriptive statistics andANOVA at $\alpha_{0.05}$.

Vanadium, cadmium and Iron concentrations in the DW ranged from 0.02±0.0001 (Aniocha) to 29.29±0.0042 (Itsekiri), 7.86±0.2903 (Aniocha) to 15.68±0.3900 (Urhobo), 497.80±0.0043 (Aniocha) to 2002.20±12.0031 (Isoko), respectively. In the eggs, vanadium, cadmium and Iron concentrations ranged from 0.01±0.001 (Ika) to 0.08±0.001 (Itsekiri), 10.96±0.020 (Aniocha) to 21.03±0.032 (Urhobo) and 3060.40±4.001 (Ukwani) to 4594.40±7.001 (Isoko), respectively. In the meat, vanadium content ranged from non-detection (Ika) to 0.48±0.01 (Ijaw), cadmium ranged from non-detection (Aniocha) to 1.49±0.01 (Urhobo), while iron ranged from 1.41±0.01 (Ika) to 1.75±0.02 (Isoko). The HMC levels were higher than WHO tolerable levels of 0.34 (vanadium), 0.01 (cadmium) and 0.30 (iron). The EDTA reduced vanadium (25.0%), cadmium (50.0%) and iron (11.0%) concentrations in chicken meat. Similarly, AC reduced vanadium from 0.011±0.010 to 0.010±0.001 (10.0%), cadmium from 0.052±0.031 to 0.030±0.001 (42.0%) and iron from 0.209±0.070 to 0.057±0.021 (73.0%) in chicken. Voided vanadium of broiler on WRHV+EDTA (10.62±0.20) was significantly higher than WRHV+AC (0.67±0.012) and WRHV (0.007±0.001). Voided cadmium ranged from 0.001±0.001 (WRHC+EDTA) to 1.100±0.100 (WRHC+AC), while iron increased from 193.66±3.001 (WRHI+EDTA) to 622.20±8.020 (WRHI+AC). Chelation by activated charcoal increased concentration of vanadium, cadmium and iron by 99.0%, 99.0% and 17%, respectively in excreta.

Ethylenediaminetetraacetic acid was more effective in chelating cadmium while, activated charcoal was potent in chelating cadmium and iron in the drinking water and subsequent lowered concentration in chicken meat. Therefore,a combination of these chelators in drinking water of chicken is recommended.

Keywords: Activated charcoal, Chelation, Heavy metals, Oil spillage, Polluted water,
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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The crude oil exploration industries in Nigeria, particularlythe oil producing areas of theNiger Delta region have made enormous contribution to the economic growth and development of the nation, but uncontrollable oil exploration and poor waste management practices have caused the Niger Delta to become one amongst the most severely damaged ecological systems of the world (Tolulope, 2004). Oil spillages caused by the activities of the crude oil exploration companies concentrated mostly in the region of the Niger Delta, have resulted in extensive destruction and degradation of drinking water sources, farmlands, fishing sites, fishing grounds pollution, mangrove forestand declination in fish population and other sea foods such as periwinkles, crabs, molluscs and other animals such as birds(Ajayi*et al*., 2009).Contamination of fresh water, wastage of agricultural land, loss of forestland, destruction of fishing grounds and declination in the population of fish which used to be one of the majorsourcesof income, sources of food and a means of livelihood for the Niger Delta people, is inevitable (Tolulope, 2004).

The crude oil exploration impact on the composition and concentration of heavy metals in the environment in oil exploration areas is much higher in the Niger Delta hence, the higher concentration of heavy metal contents in soil, crops, air and ground water, where exploration activities are being carried out (Mueller *et al.*, 1992). In Nigeria, the flaring of gas is a continuous practice. It is estimated that the Niger Delta harbours about 123 gas flaring sites, producing heat of about 45.8 billion kilowatts which is emitted into the atmosphere being released from about 1.8 billion cubic feet of gas generated daily (Energetic Solution Conference, 2004). The ultimate resultant effect is the temperature

increase that have rendered large expanse of land uninhabitable (Agboola and Olurin, 2003). Gas flaring has been reported to have a direct impact on agricultural productivityand a direct relationship on the declining agricultural productivity (Salau, 1993; Adeyemi, 2002).

A very close relationship exists between gas flaring, oil spillage and environmental pollution, and United Nation Development Programme (2006) estimated that about 75% of thegas Nigeria produced is flared into the atmosphere, much more than any other oil or gas producing country in the world. There have been well documented incidences of fire outbreak due to crude oil exploration activities, which have resulted in a wide range of fatalities on animals, humans and property losses. Explosion of gas pipes, fire incidences and oil spillage are common occurrences in the Niger Delta resulting in heavy metal toxicity on animals and humans causing health challenges such as; respiratory diseases, kidney failure, neurological disorders and subsequent death (Ndubisi and Asia, 2007).

Gas emission is a common source of particulate black matter, Nox and the subsequent cumulative environmental impact that leads to accumulation of contaminants on land and in shallow underground water, subsequent greenhouse effect and total global warming. The utilization of total gas and associated products, reduction of gas flaring or the total end to gas emission between 2004 and 2008 were among the Federal Government policies that the crude oil companies in the Niger Delta were to comply with. Still, about 84.60% of the total volume of gas generated was flared and only about 14.86% was utilized locally (Ukoli, 2005). Despite the Federal government policy to end gas emission by the year 2008, gas flaring in the region is still a lingering problem (Ndubisi and Asia, 2007).

Acid rain in the Niger Delta has become another serious problem due to gas emission resulting in loss of biodiversity, pollution and destruction of economic crops and wastage of viable forest areas. Rainwater adsorbs impurities from the atmosphere during precipitation thus, the salts or acid concentration in the rain water seems to be

relatively higher in the Niger Delta and it recedes further away from the region (Uyigue and Agho, 2007). Pollution of water is directly proportional to the level of pollution of the surrounding environment. Rivers, streams and other water sources accumulate impurities and other contaminants through surface runoff, or direct soil percolationwhichare transferred further into water reservoirs or lakes and shallow aquifers which are sources of drinking water for both industrial and local uses (Adieze *et al*., 2003). Almost all the pollutants and chemicals generated through the activities of manwould subsequently end up in the drinking water and water supply sources such as; streams, lakes, rivers and underground water systemsthus, polluting the water sources.Water is a universal solvent and a natural endowment that is required by all living organisms (Opukria and Ibaba, 2008).

Heavy metal impact on water pollution are devastating to humans and animals alike, killing animals, birds and fish. Furthermore, water pollutionproblems pose a serious threat today and in the future to society generally (Grossman, 2006). Pollution of drinking water sources in the Niger Delta is a recurrent phenomenon with the drinking water obtainedfrom shallow water sources and uncontained aquifers (Abu *et al*., 1996; Adieze *et al*., 2003). Contamination of the food chain and water sources due to heavy metal has generated serious concern by many researchers thus, Hart *et al*. (2005) estimated some heavy metal concentrations such as; iron, lead, copper and zinc in a study on various crops which included cassava, yam, okra, pumpkin, water leaf and cocoyam in some crude oil producing locations in Rivers state, Nigeria and found them to contain heavy metals which include iron and lead, with high corresponding concentrations of zinc and copper all in higher levels in the food crops, produced in areas with intense industrial settlements and operations in Rivers State, Nigeria when compared with areas with no crude oil industrial activities. Ndoiokwere *et al*. (2000) also reported three to seven times intense concentrations of several metals in the soil, in a study conducted on levels of heavy metal in the soil near Warri refinery in Delta State, Nigeria.

Degradation of the ecological system in the oil-rich Niger Delta has resulted in an inevitable wanton destruction and a continuous deleterious impact on the overall animal welfare, food safety, public health, social and economic implications on the people. For over five decades crude oil refining companies produced a wide range of air pollutants, water and solid hazardous wastes. Most contaminants regularly introduced into the environment include the products of distillation process, refining and intense industrial activities (Worgu, 2000). Crude oil pollution and its deleterious impact on human health, animal lives and the environment have no limit, as every aspect of the animal lives, reproduction, growth and development is affected (Akporhuarho *et al*., 2011).

In Nigeria, heavy metals contamination of the environment is a serious problem threatening the health of human, animals and safety of the environment (Deko-Fehinti *et al*., 2012), and the resultant accumulation of heavy metals with varying half-lives in the body system in one organ or another resulting in organ deterioration, direct inhibition of enzyme activities and the indirect alteration of equilibrium of the essential metallic ionsin the body (Teresa *et al*., 1997). Human and animal exposure to heavy metal contamination through direct and indirect channels such as; feed, foods and water consumption, exposure to industrial operations and automobile fumes have been reported (Ghaedi *et al*., 2005; Demirezen *et al*., 2006). Some of the heavy metals which pose serious health hazardto both secondary and primary consumers include; vanadium, cadmium, iron, mercury and nickel (Demirezen *et al*., 2006). Some naturally occurring heavy metals appear less hazardous to the environment due to their presence in very minute quantities (Sanayei *et al*., 2009). However, the impact of these heavy metals changed negative, when their concentration in the surrounding exceed tolerable limits.

Metals and metalloids in the body system are known to be highly toxic to animals even at minute concentrations, including those that are considered essential minerals when present in excess (Hossen *et al*., 2001). Present in the body of all living organisms are the metallic elements which perform veryvital function, as constituents of mechanisms that control the body such as hormones and enzyme activators, building and structural parts of nerves and muscles.Some of the heavy metals which are known to be toxic

are;cadmium (Cd), vanadium (V), lead (Pb), mercury (Hg), arsenic and nickel are very toxic even at minute concentration and can cause health damage due to their biomagnification attributeand when ingested for a long period of time (Leistevuo *et al*., 2001; Young, 2005).

The oil industry sub-sector although, is the largest in the Niger Delta and is at least the six of the eight industrial sub-sectors that is most environmental polluting in Nigeria, it is also among the eight industrial sectors that is most polluting in the world (Ghaedi *et al.,* 2005). Currently, the Niger Delta is posed with the challenge of environmental pollution problems including heavy metals contamination, with no hope or solution to this problem in sight. There are no effluents treatment plants, petrochemical waste processing or recycling plants yet, thus industrial products and used materials which constitute high toxic levels of heavy metals are routinely introduced into the environment (Shohda *et al.*, 2001).

Inspite of all these negative environmental and health implications of crude oil contaminations, the product remains a very valuable and highly sought commodity in Nigeria, because of its economic benefit. It is constantly explored and being exploited primarily in the Niger Delta, because it is found in abundance. However, livestock production is of great importance to human due to its nutritional and health benefit, and the economic contributions to the nation, as a source of eggs, milk and meat to satisfy the protein needs of the teeming populace and as a means of fulfilling the socio-economic wellbeing of the people (Rodenburg *et al*., 2003).

Poultry meat is a good animal protein source and very high biological value as it contains all the essential amino acids, minerals and vitamins required for normal human nutrition (Institute of Medicine, 2003).Poultry eggs which represent an important part ofthe human's diet particularly children because the avian egg is very rich in nutrients and contains all the proteins, lipids, minerals and vitamins (Sparks, 2006). Avian eggs have been used for environmental pollution assessment since they can accumulate heavy metals in the drinking water, diets and the surrounding (Burger *et al*., 2009;

Soong *et al*., 1991). Issues concerning food safety and public health have generated research associated with the consumption of foods polluted with heavy metals (DMello, 2003).

The National Bureau of Statistics as of 1991, from the National Census stated about 25% of Nigerian population lives and work in the Niger Delta (Twumasi and Merem, 2001; Uyigue and Agho, 2007). The Niger Delta with approximately 30 million people as of 2005, with a steady growing population increase annually and accounts for more than 23% of the total population of Nigeria (Uyigue and Agho, 2007). A larger number of the peopleliving in the Niger Delta region survive on products and services provided by the ecosystem; shelter, industry, agriculture, employment, food, fishing, drinking water, wood, medicine and aesthetics. All aspects of the local biodiversity and the entire ecosystem has been negatively impacted by the activities of oil exploration and exploitation with every single impact exhibiting a rippling adverse effect (Twumasi and Merem, 2006; Uyigue and Agho, 2007).

Delta State which is one of the nine oil producing states in the Niger Delta is greatly endowed with abundant natural, agricultural and mineral resources, resulting in increased industrial and economic activities in the state. Petrochemical industries and refining companies account for about 70-75% of the industrial operations in the state (Ogjurwederhie *et al*., 2006). According to the Federal Office of Bureau of Statistics 1995, about 50% of the labour forcein Delta State, is actively involved in different forms of Agricultural activities with poultry production, yam, cassava, maize, plantain, cocoyam and vegetables as the predominantly grown food crops within the state. These heavy metals and other waste products pollute the soil and underground water systems in the state where crude oil exploration operations are being performed.

Petrochemical effluents discharges and formation water are highly essential in the dissolved total solids and injected chemicals into the oil wells to inhibit corrosion of equipment or enhanceseparation of oil and water. Such mixtures of water and chemicals could have detrimental impact on animals, plants and the quality of the environment

(Amatya *et al*., 2002). The negative impact of petrochemical exploration activities can be severe and ranged from eenvironmental degradation, soil pollution, contamination of surface and underground water.

The existence of oil in the region has resulted in increasing industrialization and thus, increased oil exploration activities in the Niger Delta region of Nigeria and this has stimulated consciousness of the degree of environmental contamination and possible dangers resulting from their activities. Environmental studies have revealed wide-spread contamination of water by different chemicals used in the chemical industry and agro-allied industry during manufacturing process (Porth, 2005). There is dearth of information in literature on heavy metals in the environment and the possible implications of the degree of their concentrations in livestock, the entire food chainand drinking water channels in the Niger Delta.

All poultry species must receive sufficient amount of clean water daily for its biochemical balance and provide optimum amount of water needed for normal biochemical and physiological processes in the body of an animal (Akporhuarho *et al*., 2011). Sources of drinking water for poultry production are very important particularly with intensive system. Crude oil exploration leads to the pollution of our water ways, thus exposureto itcauses potential hazard to both terrestrial and aquatic species (Shore and Douben, 1994). However, studies have revealed the devastating consequences of crude oil contamination on various parameters for farm animals. Thus, Nwokolo *et al*. (1994) reported poor hatchability in chickens, Berepubo *et al*. (1994) reported shrinkage in the size of testes with possible sub-fertility among male rabbits when supplied graded levels of dietary crude oil. Also, hyperemia vascular dilation and progressive necrosis in the liver as well as reduced spermatogenesis in rabbit was reported by Ovuru and Oruwan (2005).

In some regions like the Niger Delta, the main sources of drinking water supply to urban cities and communities in the rural areas is from bore holes, wells and streams. Though, there is lack of systematic water quality check or comprehensive water quality assessment programmes, the indications are, there is increasingdrinking water pollution

problem in the Niger Delta (Akporhuarho *et al*., 2011). Oil spillage, industrial wastes and petrochemical effluentsamong others are the major causes of this contamination. Literature is replete of sufficient data on heavy metal pollution of drinking water due to oil spillage and exploration activities in the Niger Delta for poultry production.

Water of good quality is importantfor biochemical processes in human and animal life and also for agriculture, commercial and domestic uses. The daily need for water for industrial and domestic utilization is increasing while all the water resources available at our disposal are rapidly becoming unfit for use due to uncontrollable waste disposal. Quality of water for drinking has a very important role on the health of human and animals. Assessment of the major health risk factors due to consumptionof unsafe wateraccounted for over two million deaths, which stand for a leading environmental risk factor on a global scale (Porth, 2005).The distribution of the burden of diseases and mortality geographically related to poor water quality represented substantial differences amonglow, middle and high income countries. Generally, over 99% of deaths attributed to unsafe drinking water happen in the developing countries, where the most vulnerable age group are children (Porth, 2005).

Toxic effects of these heavy metals to poultry among others include, low feed intake, low hatchability, low digestibility, retarded growth, weight loss, liver and kidney damage e.t.c. (Hassan *et al*., 1998). Shih *et al*. (2007) stated the toxic impacts of these metals to humans include central nervous systems effects, liver and kidney damage, anxiety, constipation, depression, cancer, respiratory distress, DNA alteration and death in extreme cases. Thus, an examination of the concentrations of heavy metals depositions in chicken products that form part of the daily protein requirements of the populace necessitated this research.

In view of the aforementioned facts, it becomes pertinent to introduce measures to prevent or reduce water contamination due to exposure to oil spillage and heavy metals. Provision of proper water treatment facility for all polluting sources is a difficult task and also very expensive hence, there is direneed for innovative technologies which are

low cost, require minimal maintenance and energy efficient (Wilde and Benemann, 1993). Research focus is now to develop or introduce suitable and adaptableinnovations which can prevent or reduce heavy metal concentrations in drinking water and ultimately to prevent or minimize their deposition in poultry products. This can be done either by limiting the heavy metal afflux to the receiving bodies; sources of drinking water, sewers, rivers, streams and lake, etc. by direct adsorption from contaminated media or masking their impact in the body system of animals that have been exposed to heavy metals (Davila *et al*., 1992). Different treatment technologies have been introduced for heavy metals removal from wastewater and water, but the most widely adopted methods for the removal of heavy metals from wastewater involves chemical precipitation, ion exchange, membrane filtration, reverse osmosis and evaporation. This type of treatment technique adopted byan industry depends upon the nature, effluent composition, rate of flow and the quality control necessary to be achieved. The effectiveness of the adopted technique can be optimized by applying one or the appropriate combination of two or more techniques. But most of these techniques suffer certain setbacks which include the disposal of residual metal sludge, high capital and operational cost,or are not adaptableby low-levelusers (Kobya *et al*., 2005).

Effective removal of heavy metals from drinking water and the environment is still a challenge. Several attempts to purifying or decolourizing type carbon from plant materials like palm kernel shell, groundnut husks, coal, coconut shells, plantain peels, walnut shells, have been made without much success. Several, inadequate ineffectiveness have been reported against conventional techniques with concentrations which range from 1 - 100 mg/l, and beside its high operational and capital cost (Kapoor and Viraraghavan, 1995). Activated charcoal can be used as adsorbent for Cd, Pb and other heavy metals particularly when they are in association with particulate organic matter in water (Cowan *et al*., 1991). The process of adsorption for removing heavy metals from water seems to be a promising one, which is suitable even at very low metal ion concentration such as 1mg/l (Chong and Volesky, 1995).

Crude oil spillage, waste dumpingand gas flaring are a common occurrence in Delta State and limited data exist on theirimpact on the quality of surface and underground water consumed by humans and livestock. Adequate information will be generated on the toxic consequences of oil spillage, heavy metals and the levels of hydrocarbons and other toxic residues on poultry products. The parameters to be evaluated are valuable tools and indicators for assessing exposure and toxicity status or effects of the animals under investigation. Limited data exist on water contaminationdue to crude oil on poultry. Exposure to heavy metal pollutants through drinking water or ingestion of contaminated meat, offal and eggs, pose serious health risks to humans and animals. Researches in this regard are necessary to determine the level of toxicity by heavy metals in water consumed by broilers and layers produced within crude oil exploration areas in Delta State. Investigations were focused on haematology, weight of organs, histology of tissues and serum biochemical parameters which are valuable tools and indicators for assessing the effects and toxicity status of animals under investigation.

This study was to assess the heavy metal concentration in the environment through underground water to chickens in selected areas of Delta State, Nigeria where poultry farms are now being established. This research endeavoured to determine the levels of selected heavy metals; cadmium, iron, nickel, vanadium and mercury, as environmental and ground water pollutants and assess the bioaccumulation of cadmium, vanadium, mercury, nickel and iron residues, with some of the major heavy metals in the tissue muscles and eggs of some poultry farms and to compare the results from those sample analysis with the World Health Organization (WHO) recommended standards. However, in Nigeria, there is high consumption of eggs and chicken more frequently in urban than in rural areas, but to this date, no studies on heavy metal toxicity have been undertaken in this area where poultry eggsand chickens and their products consumption is on the increase and the possible effects of heavy metals in water on broilers and layers in poultry farms in crude oil exploration areas of Delta State. The result of this study could help in taking precautionary steps in evaluating heavy metal contamination

in ground water and to provide information for the authorities on the health impact on the chicken and egg consuming population.

1.2 STATEMENT OF THE PROBLEMS

The effect of crude oil exploration and pollution on water consumed by livestock and humans require attention, because of the issues surrounding bioaccumulation and bioconcentration of heavy metals in poultry and poultry products.

Oil exploration has been on-ogoing in the region and has adversely affected the people in habiting that region and livestock produced. The Niger Delta consists of diverse ecosystem of mangrove, swamp, fresh water, rain forest but with the activities of oil exploration and pollution, the area is now characterised by contaminated streams, rivers and other sources of drinking water, forest destruction and biodiversity loss.

1.3 RESEARCH QUESTIONS / HYPOTHESIS

Ho: There is no relationship between crude oil exploration and heavy metals concentration in the drinking water available in crude oil producing areas of Delta State, Nigeria.

H_A: There is a relationship between crude oil exploration and heavy metals concentration in the drinking water available in crude oil producing areas of Delta State, Nigeria.

Ho: Thereis no negative impact of heavy metals in drinking water on poultry production

H_A: There is a negative impact of heavy metals in drinking water on poultry production

Ho: Ethylenediaminetetraacetic acid (EDTA) and Activated Charcoal (AC) as mitigating agents are not effective against heavy metals toxicity in broiler chickens,

H_A: Ethylenediaminetetraacetic acid (EDTA) and Activated Charcoal (AC) as mitigating agents are effective against heavy metal toxicity in broiler chickens,

1.4 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to assess the effects of heavy metals in drinking water on poultry and to evaluate the effectiveness of selected metal-binding agents in the drinking water of poultry during production and also to assess the impacts of crude oil exploration on heavy metals concentrations and their effects on poultry production. In addressing this during the study, the specific objectives are:

a) To determinethe concentrations of heavy metals in drinking water available in poultry sites located in crude oil exploration areas of Delta State,
b) To assess the toxic effects of heavy metals present in drinking water for poultry on broiler chickens and their subsequent concentrations in the meat,
c) To assess the toxic effects of heavy metals present in drinking water for poultry on layer chickens and their subsequent concentrations in the eggs,
d) To evaluate the effectiveness of Ethylenediaminetetraacetic acid (EDTA) and Activated Charcoal (AC) in reducing heavy metals toxicity in broiler chickens.

1.5 JUSTIFICATION OF THE STUDY

Extensive crude oil spillage, seepage and industrial wastes release heavy metals which lead to water pollution, present serious challenge to animal and human health, and the quality of the environment. Soil, air and groundwater contamination caused by oil spills, gas flare, petrochemical effluents are some of the most extensive environmentally devastating pollution problems which arecategorized as potential challenge to human, animals and ecosystem health, with particularly more damage to

the areas near active oil exploration, which has adversely impacted the livestock and people living in the Niger Delta.

One of the major sources of Nitrogen oxide (Nox) and particulate matter in the environment is gas flaring which leads to contaminants accumulation on land, water aquifers, ground water, greenhouse effect and overall global warming. Another problem caused by gas flaring in the Niger Delta is acid rain which has resulted in biodiversity loss. The acid concentration of rain water seemsto be higher in the Niger Delta and reducesfurther away.In the Niger Delta, contamination of water sources is prevalent and where most of the supply water is obtained from uncontained and shallow aquifers.The effects of water pollution by heavy metals through crude oil exploration activities are devastating to humans, animals, birds and fish. The impacts of these water pollutions present a dangerous threat to the livestock sector, both now and in the future. Its deleterious impacts on human, animal lives and the environment has no bound as all phases of life including reproduction, growth and development is affected.

However, poultry production is of great importance to humans due to its nutritional contribution to the teeming population as a veritable source of eggs and meat to meet the protein requirements of the populace. Global crude oil ecosystemdestruction and pollution by heavy metals has stimulated interest in metals contamination of drinking water and foodstuffs. Metals contamination of water and foods are major public concerns in the world over in recent time due to growing demand for safe and healthy foods, which has prompted research interest regarding the risk associated with the ingestion of water, eggs and meat contaminated by heavy metals. Thus, it becomes pertinent to evaluate the impacts of heavy metals in drinking water, in crude oil exploration areas on poultry and introduce methods by which its damaging effects can be eliminated or reduced in poultry products.

1.6 SIGNIFICANCE OF THE STUDY

The study is designed to ascertain the level of heavy metals deposition in drinking water, and to seek for possible measures through the heavy metal chelators to

ameliorate the negative impact of heavy metals concentration to life, and to provide information that could serve as the basis for more studies on the use of heavy metal chelators in drinking water for livestock during production and management. Also, data from this study could serve as a bed rock for the general public, health experts, nutritionists, livestock farmers, policy makers and other government agencies to take appropriate measures to protect the environment, sustain the means of livelihood of the people, and safe-guard the health of the public through proper crude oil exploration monitoring, waste management and the adoption of effective technology for water sanitation and food safety.

1.7 SCOPE OF THE STUDY

Contamination of water, air and soil by the activities of crude oil exploration and petrochemical effluents, gas flare and oil spill consisting of heavy metals is a common phenomenon in the oil rich Niger Delta, Particularly Delta State, Nigeria. The sources of water for drinking are subsequently polluted with heavy metals with resultant systemic poisoning of life. Previous studies done in this regard, were simulation studies which did not present a true picture of the impart of the contamination due to heavy metals with the resultant threat to animal life and the safety of food produced in the area. Thus, assessment of samples of drinking water, chicken and eggs produced in Delta State, with the introduction of ethylenediaminetetraacetic acid and activated charcoal as heavy metals chelators in the drinking water of chickens to reduce the concentration of heavy metals in the water for drinking and eventual heavy metal residues in the meat of chickens.

CHAPTER TWO

LITERATURE REVIEW

2.1. ENVIRONMENTAL POLLUTION

For years, the world's load of disease due to the effect of pollution from the environment has raised concern on public health. WHO (2008) reported that extended exposure to pollution from the environment was responsible for about 25% of the disease conditions faced by mankind presently. Humanity is at present seriously affected by environmental pollution. This, in the past years has been on exponential increase and attained far-reaching levels on plants and animals (Twumasi and Merem, 2001). Although, there are numerous sources of environmental pollutions, Da-silva *et al*. (1997) penciled crude oil as the most prominent and the most occurring.

Environmental pollution from crude oil and related products has been identified to be responsible for toxic consequences and alterations of biochemical system in the lives of animals both marine and terrestrial (Ovuru *et al*., 2004). The danger in crude oil pollution on animal lives is enhanced by the existence of high levels of aromatic and polycyclic aromatic hydrocarbons which alter normal biochemical processes in animal life. It has been reported that polycyclic aromatic hydrocarbons enhances mono-oxygenase activities and alters the biochemical components of blood in animals that ingest it (Akporhuarho *et al*., 2011). Water ways, as part of the environment, are also polluted due to the exploration of crude oil. Therefore, terrestrial and aquatic species are at risk to the effect of crude oil exposure (Shore and Douben, 1994). The deleterious effects of pollution due to crude oil on animals' lives are limitless since they affect every area of its development with growth and reproduction inclusive. Human and animal health has been seriously challenged by the heavy metals present in environmental pollution resulting from crude oil exploration.

Since 1971, Nigeria registered with Organization of Petroleum Exporting Countries (OPEC) to be a member and from the perspective of energy and industrial prominence; crude oil constitutes a real source of her economic development. As Africa's basic oil producer, it holds the largest reserve for both natural gas and oil as well as its second largest reserve for oil. In the 1980s, only 10% of Nigeria's foreign exchange earnings and 15% government revenue was not from oil (Odeyemi and Ogunseitan, 1985). About 62% and 100% of Nigeria's energy and fuels for transportation respectively are derived from oil and natural gas which are the main sources of fuel (NEPDG, 2001). This awareness resulted in widespread exploration for greater number of oil reserves and has become more prominent for some decades now. These operations of crude oil exploration have consequently led to widespread environmental pollution (Okoh, 2006). The petroleum industry is grouped into crude oil and natural gasproduction, exploration, marketing and distribution, refining and transportation.

Pollution from crude oil could be as a result of pipes and storage tank spills, oil well blowouts, breakages, pipeline overflow, effluents, seepage, wastes, and deballasting operations (Obire and Wemedo, 1996). Also, soil and water pollution from inflow over vegetation, land and water surface caused by some accidental outburst or discharges of petroleum products from tankers pose critical ecological challenges (Twumasi and Merem, 2001). A greater proportion of the environment is contaminated by the widespread utilization of petroleum products and the addition of crude oil waste into the environment as a waste discarding policy also contaminates as well (Flowers *et al.*, 1984). The intentional or chance discharge of crude oil in waste disposal results in grave environmental pollution consequences (Tousand and Latshew, 1994). Spence *et al.* (2005) stated that even minute discharge of petroleum products into aquifers can result in accumulated proportions of dissolved hydrocarbons beyond regulatory standard. The intentional release of effluent or oil field waste water as a source of environmental contaminant was reported by Twumasi and Merem (2001). Onuoha (2008) reported that for better distribution of petroleum products within the country oil and gas pipelines extending to about 7,000 Km has been in use.

2.2 CRUDE OIL SPILLS

In Africa, oil spillage has been identified as a major source of contamination (Snape *et al*., 2001) with nearly every part of the environment being polluted due to widespread use of petroleum products (Flowers *et al.*, 1984). The Forcados estuary in Delta state were seriously polluted in July 1979 affecting both the aquatic and surrounding swamp forest when 570,000 barrels of oil was spilled by Forcados tank 6 (Ukoli, 2005)**.** Well No.5 in Funiwa Oil Field exploded with barrels of oil totalling about 421,000 emptied into the coastal environment with extensive destruction of 334.4 hectares of mangrove forest within six miles offshore (Ukoli, 2005)**.** On the 10th of May 1980 there was yet another oil spill of about 30,000 bbl in Oyakama (Ukoli, 2005). Ebocha-Brass pipeline in Oshika village in Rivers state in August 1983 experienced about 5,000 barrels of oil spill with an earlier spill of about 500 barrels in September 1979 which covered the lake and swamp forest with its attendant negative impact such as mortality in fish, shrimp and crabs (Ukoli, 2005).

The presence of oil sediments in the lake about eight months post spill resulted in increased death rate in embryonic shrimp as well as lowered reproduction (Gabriel, 2004). From Ogada-Brass pipeline close to Etiama Nembe in February 1995, barrel of oil totalling about 24,000 was spilled which flooded the fresh water swamp forest and extended to the brackish water of the mangrove swamp. A mean of 221 oil spills covering a total of about 7,350 bbl annually since 1989 was reported by Shell Petroleum Development Company (SPDC) in its areas of activities (SPDC, 1995). About 1,820,410.5 (77%) barrels of oil were not recovered out of the 2,369,470 barrels spilled into the environment from 1976-1996 in a sum of 4647 oil spill occurrences. A greater proportion of these spills in Niger Delta took placed offshore, swamp, and land environment (Uyigue and Agho, 2007). For about twenty years between 1976 to 1996 NNPC approximated about 2,300 cubic meters of oil to have been unintentionally discharged into the environment in 300 occurrences (Twumasi and Merem, 2001).

From the day crude oil was discovered and exploration started, oil spillage has been a worldwide occurrence. The uncovering of crude oil by Shell British Petroleum took place at Oloibiri in present day Bayelsa state situated in Niger Delta region in 1956 (Anifowose, 2008), by 1958 production was commercialized. Globally, the Niger Delta region is one out of the five most critically damaged ecosystems by petroleum due to unsustainable exploration despite decades-long of operations (FME *et al*., 2006). This has negatively affected the environment and the inhabitants of the region. The rain forest, fresh water swamps, and mangrove swamps are the different ecosystems present in the Niger Delta and the region is largest wetland in Africa. However, the rivers and streams have been contaminated, forest destroyed, and biodiversity lost in the region due to crude oil exploration. As it were, the region could best be described as an ecological wasteland. According to FME *et al*. (2006), a sum of 2,105,393 barrels of oil was accidentally discharged to the environment between 1976 and 1990 in 2,796 oil spillages. Between 1976 and 2001, in 6,817 spillages, about 70% of the 3 million barrels of oil discharged were unrecovered (United Nations Development Programme, 2006). SPDC (1995) reported in its Western Operations, that only 14.2% recovery from 5,187.14 barrels of oil spilled which occurred in 115 spillages. Mobil, in January 1998, unintentionally discharged 40,000 barrels of oil in Eket and in January 1980 the largest offshore spillage in Nigeria took place with an estimated barrel of crude oil totalling 200,000 was discharged into the Atlantic Ocean with the consequent destruction of mangrove forest measuring about 340 hectares (Nwilo and Badejo, 2005). Shell Petroleum Development Company (1996) reported that the four decades of crude oil exploration in Nigeria has witnessed about 6000 oil spills with a mean occurrence of 150 spills per year. Between 1996 and 1997, of the 2,369,407.04 barrels of oil spilled approximately 76.8% was lost to the environment.

Oil spillage is one of the most extreme causes of environmental contaminations in Nigeria with its negative effect on the ecosystem increasing the susceptibility of the already weak environment with each significant occurrence (Ekpo and Thomas, 2007). There is a complex and widespread network of pipelines in the Niger Delta region;

although, oil companies attribute a greater proportion of spillages to vandalism, most spillages took place *via* failures from storage facility and pipelines which could be as a result of ground erosion, material defect, and corrosion of pipeline. About 88% of crude oil spillages is caused by failure of equipment, however, Nwilo and Badejo (2004) reported unintentional and intentional discharge of oil by tankers at sea, flow stations, blowouts, and vandalism as the main causes of spillage in the Niger Delta. In the last five decades, the ecosystems of the Niger Delta have received approximately tons of crude oil totalling 1.5 million in the form of spillages and this was 50 times more than the approximated quantity discharged in 1989 during Exxon Valdez spillage in Alaska (CEESP, 2006). .

The second largest Delta in the world is the Niger Delta, which has an estimated 450km of coastline terminating at Imo River entrance and is located at the Atlantic coastline of Southern Nigeria (Awosika, 1995). The Niger Delta which is about 20,000sq/km as estimated is said to be the largest wetland in Africa and the third largest globally, respectively (Chinweze and Abiola-Oloke, 2009). In the region, estuaries and creeks covering about 2,370 sq/km stagnant swamps are estimated at 8600 sq/Km while the Delta mangrove swamp which is also Africa's largest mangrove is approximately 1900 sq/km (Awosika, 1995). Diverse terrestrial and aquatic fauna and flora are present in the ecosystem of the region which is classified as the tropical rainforest; mangrove swamp zone, coastal inland, lowland rainforest zone, and freshwater zone are the four ecological zones present in the region of the Niger Delta; a region that is the domain of one of the ten most important marine and wetlands ecosystems in the world (Uyigue and agho, 2009). Niger Delta is an important region for the preservation of Africa's western coast due to the exceptional biodiversity in the ecological zones therein (Nenibarini, 2004).

Despite the adverse effect of exploration on the environment, crude oil remains a very significant commodity in which the Nigeria's economy is largely dependent on. Consequently, exploration and exploitation are done unabatedly in the region due to its

abundance. This therefore increases the incidence of its spillage and consequent pollution of the environment.

2.3 HEAVY METALS

The biological status of living things during contamination and the constituents and quantity of petroleum products accumulated in the environment affects the level of toxicity. Plants and animals are instantly affected negatively when in highly polluted environment (NDWC, 1995). The toxic effects in an organism due to petrochemicals and hydrocarbons are manifested subsequently as a result of bioaccumulation of the offending xenobiotic. All living things contain metal based elements and these are significant for their role in structure, activation of enzyme or redox systems, and constituent of control mechanism such as in muscles and nerves. Essential minerals such as zinc (Zn), calcium (Ca) and copper (Cu), are beneficial in modulating vital biological processes when available within limits (Halder, 2009). Some metals including cadmium (Cd), lead (Pb) etc., that are not vital, even in trace quantity are poisonous (Ikeda *et al*., 2005). Deficiency of essential minerals such as copper and zinc can lead to destruction of biological role while their excess intakes cause sexual maturation of younger children or animals, skin and eye injuries (Jadhav *et al*., 2007).

The origin of heavy metal compounds uptake by poultry varies and these may accumulate in the eggs (Erdogan *et al*., 2005). The biomagnification, poisonous character and bioaccumulation of heavy metals along the food chain are accountable for the critical health danger attributed to pollution (Jones *et al*., 2007). Bioaccumulation is related to the uninterrupted movement of compounds into the circulatory fluids by bioconcentration, through body surface, or they are transmitted through food ingested in the gastrointestinal tract (GIT) across into the circulatory fluid by a process called biomagnifications (Cunnigham and Saigo, 1997). Metal based elements of high density or metals that are stable with density more than 5.00 to 6.00 g/cm^3 are referred to as heavy metals, these, when in quantity more than should occur naturally could be poisonous to the habitats of living organisms (Keepax *et al*., 2011).

The heavy metals are classified majorly into two groups; the essential (Ni, Co, Cu, Zn, Cr, Fe), and non essential (As, Cd, Hg, Pb). These can further be grouped into potentially toxic (arsenic, lead, aluminium, cadmium, mercury and -antimony), semi-essential (nickel, cobalt, vanadium) and essential (iron, manganese, selenium, zinc, copper etc) (Szentmihalyi and Then, 2007). Poisoning from heavy metals originate from food chain, contaminated water pipes, or increase environmental air concentrations close to emission sources. Bioaccumulation, over time increment in the quantity of chemical compound in living thing in comparison to its environmental concentration; makes heavy metals hazardous. The rate of metabolism or excretion of chemical compounds is slower than their uptake and accumulation in living organisms. Through a number of chemical and geochemical processes, heavy metals which are natural componentsof the earth crust can gain access to water and food cycles (Ibitoye *et al*., 2011). Water supply can be polluted with heavy metals through the degradation of soil by acidic rain with the breakout of heavy metals into water bodies like streams, lakes, and ground water, and by consumer and industrial waste.

2.4 SOURCES OF HEAVY METALS

Domestic sewage, waste disposal, exploration and mining industries, and combustion of fossil fuels, are the metallic sources in the environment (Baykov *et al*., 1996). The metal constituents in the environment can also be attributed to forestry and farming. The concentration of metals in the environment is on the increase with increase industrialization and because they are not degradable in the environment remains permanent. Consequently, through the food and food chain they eventually end in the tissue (Baykov *et al*., 1996). The major poisonous metals with cumulative effect due to accumulation in the food chain are arsenic, mercury, cadmium, lead (Cunningham and Saigo, 1997), while Mercury, cadmium, and lead do not have natural occurrence in living things and are not known for any physiological or biochemical functions in human (Lenntech, 2004). Therefore, even very small quantities of dietary uptake of these metals could be hazardous due to bioaccumulation. Roberts (1999) reported

mutagenic or carcinogenic effect as the major attribute of high amount of heavy metals from industrial effluents.

Eye and skin contact, air inhaled, water and ingestion through food are the main avenues a greater amount of elements can enter the body. Only small quantity of a few beneficial heavy metals such as strontium, vanadium, molybdenum, manganese, cobalt, copper, and zinc are needed in the body. These useful heavy metals become poisonous with higher concentration of intake which requires only very small quantity to be in excess. Besides, well above twenty heavy metals which are not essential for well-being are utilized and these are destructive to the cells through the production of harmful free radicals resulting in cancer and other disease conditions.

Twenty three of the 35 metals which are of universal interest due to residential or occupational exposure are the heavy metals and they include : vanadium, uranium, tin, thallium, tellurium, silver, platinum, mercury, manganese, iron, gold, gallium, cobalt, chromium, cerium, bismuth, arsenic, antimony, zinc, nickel, lead, copper, and cadmium (Robert, 1999), selenium, manganese, cobalt, zinc, nickel, and copper are trace elements with structural usefulness in molecules as well as function as enzymatic cofactors in metabolic processes but are poisonous when in excess amount (Chang and Cockerham, 1994). In some decades back, land food or food additives were observed to have been polluted by metals and this raised concerns on food and water contamination leading to the setting of optimum standard for metals by WHO (2004).

The total environmental concentration of toxins from heavy metals has experienced serious increase due to industrialization. Heavy metal compounds have for several purposes been burnt and manipulated, manufactured, refined, and mined actively by industries and commercial processes. Resultantly, food, water, air, and soil are in today's world loaded with increased amount of heavy metals, About 50% of the not less than 20 metals grouped as poisonous are released to the environment in amounts that are risky to the health of both animals and humans (Bakali *et al*., 1995). A greater number of these heavy metals released to the environment through the rivers, lakes,

and streams cannot be recycled and are poisonous to the well-being of humans and aquatic animals. Potable water source of humans and livestock are contaminated mainly by some of these metal elements. These compounds move and contaminate ground water, which could be a hindrance to the growth of plant while posing greater danger to health of human and the quality of the environment (Ibitoye *et al*., 2011).

2.5 GROUND WATER

Industrial, agricultural, and domestic operations in diverse locations of the universe have destroyed the quality of groundwater that was part of the healthiest wellspring of potable water. This has been the cause for water borne disease conditions in developing countries like Nigeria, thus, the quality assessment of groundwater for ingestion by animals is necessary for their existence. The quality of groundwater can be polluted naturally *via* the interaction of water and rocks such as erosion of minerals within deposits, products of volcanic eruption, and percolating of ore sediments, and or anthropogenic means by the operations of humans like domestic or industrial out-flowing, and discarding of solid waste (Marcovecchio *et al.,* 2007). Anthropogenic contamination of groundwater is worse compared to the natural cause since the water is left unbefitting for utilization than its original condition (Abimbola *et al*., 2005).One of the vital sources of potable water for humans and animals is groundwater. It is a crucial reservation for good water quality with about 90% of its resources being fresh water (Armon *et al.,* 1994). Water contamination by heavy metals has been of growing concerns to the scientific world and general public since they are poisonous to humans and other living things (Cunnigham and Saigo, 2004). This concern has been heightened by the strong toxicity of heavy metals even at small quantities (Marcovecchio *et al.,* 2007), the exhibition of their presence in different forms such as in dissolved phases, particulate, and colloidal in water (Adepoju - Bello *et al.,* 2009), and their presence in water happening naturally or of anthropogenic origin (Marcovecchio *et al.,* 2007).

2.6 DRINKING WATER

Potable water, just like unpolluted air, is a birth right for humans. However, potable water is scarce in a greater proportion of African and Asian countries, and in some comparatively developed nations like India. Above one billion people out of the population of 7 billion in the universe do not have access to potable water, and approximately 2.5 billion lack access to adequate water supply (TWAS, 2002). Moreover, an annual mean of greater than 6 million children are killed by different waterborne disease conditions which translates to 20,000 children per day (TWAS, 2002). The contamination of the bodies of water like groundwater, oceans, rivers, and lakes is referred to as water pollution. This takes place by the direct or indirect discarding of pollutants into water bodies without sufficient expelling of hazardous compounds by means of treatment. The contamination of water has adverse effect on living organisms inhabiting the water bodies and a larger population depending on it for survival but most times it becomes destructive to populations and individual species as well as to the native ecosystems (Anne *et al*., 2007).

Almost 70% of water on earth is salt water. A small fraction of 3% of water on earth is fresh water with a good portion of it located in the Antarctic and Greenland polar ice in frozen form. The usable fresh water for human and animal utilization is sourced from subsurface aquifers, lakes, and rivers and these sources represent only 1% of and all the water in the world. The dependence of 7 billion people on this supply at a time when a greater proportion of the earth's population is challenged with water deficits is worrisome. Presently, 2.8 billion people in 31 nations with the inclusion of Peru, Nigeria, Ethiopia, Kenya, India, and China are faced with chronic water challenges. The population of the world will increase to about 8 billion in less than 30 years. Nevertheless, the quantity of water will stay constant (Bishnoi and Arora, 2007).

The problem is crystal clear and compelling as pristine water flowing down from a mountain stream: we must seek equitable and new ways of conserving, consuming and recycling water (CEESP-IUCN, 2006). Apart from water deficit, both the economic

and health state of consumers are affected by the differently contaminated water (Anonymous, 1992). Salt, nitrates, heavy metals, viruses, and bacteria as contaminants have entered available water provisions as a result of excessive use of scarce resources of water, insufficient treatment more so the discarding of animal and human wastes, and industrial discharges (Singh and Mosley, 2003). Presently, polluted groundwater from harmful waste locations including other types of industrial contamination as well as systems of city waste are mostly the likely sources of pollution (Henry and Miles, 2001). Metals polluted water is an avenue for the health dangers of humans and animals. Among the numerous environmental contaminants, there exist a direct connection with each other; human diseases and heavy metals. The grouping of trace metals into essential and toxic groups is difficult but then, the toxicity of essential metal is sure when intakes are adequately high (Chang and Cockerham, 1994).

There are diverse possible interactions between micronutrients and toxic metals in the body. These include, secondary mechanisms such as oxidative stress, metabolism and sequestration of toxic metals, binding to target proteins, transport of metals in the body, absorption and excretion of toxic metals. Consequently, micronutrient deficient diet significantly affects the non-essential metals like arsenic, mercury, lead, and cadmium in terms of toxicity (Hurrell, 2001). There seem to be a link with the sulphur in protein with a greater proportion of heavy metals (Rossi and Santaroni, 1976). Rivers, lakes, and streams have heavy metals constituents not exceeding 0.1 ppm though combined quantities of up to 80 ppm can be obtained in locations close to diverse heavy metal sediments (Bachman *et al*., 2002).

2.7 FOOD SOURCES

Almost all the elements get into the body mainly through diets. One of the essential nutrients that is produced by both plants and animals is protein. The most significant avenue to pile up greater proportion of both essential and toxic chemical elements is through food. More of the elements like mercury found in living things of lower trophic stratus can be accumulated at the upper part of the food chain, a phenomenon known as biomagnification, through efficient transfer to higher levels organisms (Silva

et al., 2005). The discharge of pollutants into water bodies like lakes, rivers, sea, or even irrigation passages has polluted water and soil with important pollution contaminate plants, seeds, and plant products with poisonous elements (SCAN, 2003). Ingestion of polluted vegetation can significantly cause animal exposure to heavy metals. There are a number of other means of animal exposure to pollutants but crucially among these are those exposed through respiration and that is mainly for gases and particulate matters; skin contact for pollutants that can break through the skin barrier; and from different sources of food.

Globally, there is rising concern on human and animal health danger due to heavy metals exposure in food and related products. This is heightened by technological advancements in the production and processing of food which has increased the possibility of food pollution with diverse contaminants from the environment, particularly, heavy metals. Residue deposition in meat is a function of consumption of these pollutants by animals. Sabir *et al*. (2003) reported increased metal deposition in mutton and beef due to animal grazing. Also, Gonzalez-Waller *et al*. (2006) reported excess concentration of poisonous metals like cadmium and lead in products of meat. A greater proportion of organic vegetables and fruits available in urban markets are produced from farms in the rural areas. Illegal cancer-causing insecticides and growth promoters which may also contain toxic heavy metals may have been applied on some of these crops which are inaccurately labelled as organic and passed through a number of middlemen to get to the desired markets. The poisonous nature of heavy metals, their bioaccumulation and biomagnification in the food chain are threatening to health of animals (Demirezen and Uruc, 2006).

The environmental dominance of these contaminants has made it difficult to prevent pollution of animal feed by poisonous metals, however, there is need to reduce contamination with a view to minimizing both the indirect and direct impact on the health of animal and human respectively (SCAN, 2003). The ingestion of all minerals in excess quantities can lead to diseases due to poisoning in animals. The difference

between beneficial concentration for ingestion in feed or water and the quantity with negative effect on animal health is dependent on the type of metal. However, there exist some minerals with no defined role in the system of animals and are poisonous. Although, the environment is dominated with many of such heavy metals including but not limited to zinc, manganese, copper, and iron utilized as trace minerals and additives in poultry nutrition are essential for the well-being of animals, also toxic are metals like mercury, arsenic, lead, cadmium, etc. and harmful to health (Jadhav *et al.,* 2007). There is need for adequate supply of essential minerals for optimal performance while reducingpossible minimum the ingestion of others for the safety of both the animals and human consumers of animal products such as meat and eggs.

2.8 MEAT

Meat is a foodstuff made up of basically protein, fat and certain significant essential chemical elements necessary for growth as well as sustenance of satisfactory health.Metals found in foodstuffs such as meat and eggs are mostly sources from the feed fed to the animals. Tannery among other agricultural and industrial effluents could have polluted groundwater or other water sources used in watering crops used as feed ingredients. However, chickens may be exposed to heavy metals polluted water for consumption and which has brought about extensive concerns on the health of animal product consumers due to the danger linked with access to heavy metals through foodstuffs and drinking water. Meat provides an easier and abundant avenue for nutrients including greater proportion of micro elements.

Comparatively small concentrations of animals exposed to heavy metals have been reported to have poisonous impact (Kostial, 1986) with the obstruction of the metabolism of trace element (Lo'pez-Alonso *et al*., 2002). The poisonous nature of heavy metals even at comparatively small quantities calls for greater concern on the pollution of foodstuffs such as meat on the basis of food wholesomeness (Flowers *et al*., 1986). Flowers *et al*. (1986) reported pollution of meat products by heavy metals during processing. In Nigeria, chicken, chevon, mutton, beef, and presently, animal

organs like the liver and kidney are extensively utilized as main protein sources by the populace. Heavy metals in contaminated water and feed are the major sources in the meat of chicken and turkey. Miranda *et al*. (2005) reported meat with heavy metal pollution due to keeping of livestock close to contaminated environment and intake of polluted animal feed. Vehicular emission and also unclean slaughter houses can pollute meat as well. Heavy metals are included as a part of the living tissues where they exhibit their physiological impact (Baykov *et al*., 1996). John and Jeanne (1994) found in different tissues of goats increased accumulation of arsenic, cadmium, lead and mercury. Similarly, heavy metals were found in organs of calf but with increased accumulation of trace metals discovered mostly in small intestines, kidneys and livers (Horky *et al*., 1998).

2.9 POULTRY

The need for animal protein by the world's growing population is on the increase and birds are bred to meet this need. In the last 30 years, intensive system of poultry production has been used by developing nations as an effort to meet the need for protein of animal origin. This is in view of the rapid and higher supplyof acceptable and healthy animal protein for the increasing populace by intensive system of rearing birds (Adema *et al*., 1991).

One of the most significant and nutritious foods in the daily diets of humans is egg. Additionally, there are several reasons for the inclusion of eggs in food products (Ibitoye *et al*., 2011). Growing concern in the pollution of food materials is due to the contamination of the world environment with heavy metal with egg not just being inclusive but a significant part of the daily diets of humans and children in particular (Erdogan *et al*., 2005). Readily obtainable data on the status of trace metals in eggs is limited notwithstanding the large amount of attention generated by trace metals constituents of eggs from environmental scientists, avian nutritionists and breeders (Jones, 2007).

At the moment poultry rearing is centered all over the nation in areas around the big cities. Diverse negative health impact from poultry products can result from excess mineral element in poultry feed. The addition of untreated industrial and other wastes to the environment as it stands now is dangerous. There is need to limit the heavy metal constituents of poultry diets to safe levels for the well being of the consumers. The Maximum Tolerable Levels (MTL) of heavy metals in poultry diet and egg has been reported by Ghosh *et al*. (2012).About 9.5% total livestock growth as well as 19% of the total meat production is a function of the poultry industry and broiler chicken production (Farooq, 2011). Therefore, diets for poultry birds should meet their nutritional needs including requirements for minerals and some heavy metals; iron is a basic constituent of haemoglobin and cytochromes, zinc is required for DNA structure motifs whereas zinc, selenium, manganese and copper are needed for adequate enzymatic functions. Zinc as well as selenium are significant for immune system strengthening and feathering (Henry and Miles, 2001). Growth promotion and anticoccidiostat's attribute of arsenic was reported by AAFCO (1999). In other to meet their requirements, mineral supplements are indescrimately introduced into the diets of poultry. Most often, increased quantity of heavy metals such as nickel, mercury, lead, cadmium, and chromium, etc except for selenium, zinc, manganese, copper have been found in broiler diet by man-made causes (Hossain *et al.*, 2007).

Poultry birds ingest heavy metals from several sources leading to the accumulation of metal in eggs (Nisianakis *et al.*, 2009). Essential trace elements needed by human beings include copper (Cu) and Iron (Fe) yet, all metals at increased quantities are poisonous (Lenntech, 2004).

2.10 EGGS

Poultry eggs are susceptible to contaminants whene exposed to the environment and that is an expression of the exposure of the birds laying them (Burger, 1993). The piling up of heavy metals in eggs from ingested water and feed and the environment make them indicators of contaminations in the environment (Burger *et al*., 2009). Egg

has for a long time been a significant nutritional foodstuff for human. In the foodstuff cycle of human, it is one of the good providers of nutriments and useful as an efficient health delivery system, providing and modulating nutrients such as fat, proteins, vitamins, etc. Eggs are healthy to consume and are economical for inclusion in different products of victuals as a result of their numerous biological functions (Tyokumbur and Daramola, 2014). Hen egg provide good amount of nutrients that are so effective for the benefit of the healt h of human. Nevertheless, they may have increased content of heavy metals introduced mostly through water and feed. Some mineral constituents present in eggs are becoming important for both the health and nutritional worth of eggs. Eggs contain important concentrations of beneficial protein to the body.

The ingestion of poisonous elements even at minute quantities for a period of time can be hazardous (Young, 2005). Therefore, eggs consumption resulting in exposure to metals may become threat to human health, especially for a population with high egg consumption. Inspite of the nutritional constituents of eggs, certain potential threats to health are associated with their consumption, these include exposure to contaminants in the environment and in some cases individual intolerance. Eggs may have increased constituents of heavy metals obtained mostly from water and feed, as affected by the surrounding environment. The metals on early phase of embryo development can be used as biological biomarker indicators for metal contamination of the environment and exposure of the mother or hen laying the eggs. Chickens, eggs, milk and dairy product make up most of the wellspring of food around the globe. Therefore, screening of heavy metals concentrations in milk, dairy products, eggs, and chickens are very significant for toxicological, nutritional and environmental purposes (NDWC, 1995). Egg which is known for its high protein content is also a source of selenium, phosphorus, choline, folate (vitamin B_9), and retinol (vitamin A) and rich in iodine, biotin (vitamin B_7), cobalamins (B_{12}), riboflavin (B_2), calciferols (vitamin D). The bioavailability of nutrients like lutein is improved by the matrix of lipids present in the egg yolk. The richest combination of essential amino acids is supplied by eggs which

is crucial for young adults, adolescents and children due to their increase need for growth and muscle building (Layman and Rodriguez, 2009; Daniel and Edward, 1995).

There is fast increase in the expectation of consumers on egg quality with the consumption of egg in the world tripling in the last four decades. Eggs are affordable for consumption to the populace belonging to the middle class in most Asian countries like India and China. Generally, the level of egg consumption differs with countries. Yearly egg consumption is mostly a function of the wealth of a country and it ranges between 300 g/capita in African nations to 19100 g/capita in Japan. Among the sub-Saharan African nations about 9 out of 43 have a mean intake that is more than 2000g whereas a greater number of Americans and Asians consume a minimum of twice that quantity (Zaheer, 2015).

Iron and manganese, beside zinc are some of the significant major micro elements that have been standardized in poultry nutrition. Depending on dosage, the form of the elements, as well as a number of other factors with those physiological in nature inclusive are amassed in varying amounts in eggs and shells (Jones, 2007). In humans, acute or chronic poisoning of heavy metals may result. Globally, ingestion of heavy metals via water and the food chain has been documented (Muchuweti *et al*., 2006). Destruction or reduction of the normal functioning of the central nervous system, reduction in mental and energy levels, and destruction of the constituents of liver, kidneys, lungs, and other important body organs can result from poisoning from heavy metals (IOSHI, 1999).

The essential roles and poisoning of metals such as Zn, Cd, Cu and Pb in eggs to consumers have made the quantification crucial to a great extent. Resultantly, table eggs evaluation for heavy metals is crucial because of the threat to the liver and kidney of consumers due to their carcinogenic effect (Karavoltsos *et al*., 2002). Naturally, chicken eggs have in them a certain number of metals and minerals. The necessity in

adequate consumption of minerals is due to their crucial biological functions (Demirulus, 2013). Micronutrients also referred to as essential metals include Zn, Mn, Fe, Cu, Co, and Cr are poisonous when ingested beyond the needed quantities while, the non-essential metals which include cadmium (Cd), arsenic (As), lead (Pb) and mercury (Hg) are poisonous even at trace quantities.

2.11 HEAVY METALS HAZARDS IN EGGS

Hazardous materials, referred to as Persistent, Toxic and Bioaccumulative substances (PTBs), are chemicals not degraded when exposed to the environment. These substances critically pile up in fatty tissues and are slowly metabolized; which subsequently result in increasing their accumulation through the food chain (Salar-Amoli and Ali-Esfahani, 2015). A number of factors that are involved earlier than the time of laying and following oviposition determine egg quality. The environmental quality, safety of feed, and the health status of the laying bird are factors that inherently affect quality. The shelf-life and eggs' internal quality are specifically affected by transportation, handling, processing, grading and packing systems and the conditions of the environment. The likely danger that may take place due to the application or availability of diverse chemicals at various locations in the egg supply chain necessitates eggs and egg products assessment. Heavy metals pollution may get into the egg supply chain through veterinary, water and feed administration at the early stages of production and later through additives included in food, chemicals used for cleaning, and chemicals from packaging materials at the point of production and sale of egg products. Salar-Amoli and Ali-Esfahani (2015) reported some of the dangerous substances in food to include packaging materials, indirect additives, inks, colour additives, heavy metals, fungicides, fertilizers, toxins, hormones, antibiotics, and pesticides.

2.12 HEAVY METALS AND THE EMBRYO

The association between advancing infant and the environment has been established in the most recent of 100 years by several researches. Developing foetus is specifically

affected adversely by heavy metals as these are transferred exactly to the foetus from the dam through the tissues of developing foetus without placental filtration. Bioaccumulation is a major negative attribute of heavy metals. Detoxification of females prior to conception is therefore needful for the procreation of healthy offspring. In this wise, identification and removal of toxicity must first be done. This should be followed by the detoxification of the excretory organs such as the lungs, kidneys and liver (Salar-Amoli and Ali-Esfahani, 2015). Birth abnormality could result with the exposure of foetus to certain agents of the environment. The most significant impact in the foetal life is in the quantity of the teratogenic agent, the time interval of consumption, the phase of growth of the placenta, the solubility of lipid and the material molecular weight and importantly, the foetus phase of life in which the physical matter was taken up.

The environment may have either positive or adverse impact during gestation and after birth on infant. Teratogenic and mutagenic agents are numerous and birth abnormalities are exhibited differently ranging from death of infant, to neurobehavioral disorder including mental deceleration and several physical dysmorphologies with decelerated growth inclusive. Ingested feed and water, medications and environment, may result in exposure to metals. Birth abnormalities are known to be specifically caused by heavy metals like chromium, arsenic, cadmium, mercury and lead. Foetus accessed by such agent *in-utero* may lead to critical side impact in health due to the ability of the material to move across the barrier of the placenta easily and this may be without any effects on the dam.

Nutrition from the mother is the most all significant connection between the mother and the advancing foetus. Despite the nourishment of the dam and the foetus some pollutants emanating from the environment could still lead to abnormalities in the act of giving birth. Feed holding bacteria and feed polluted with mercury are some of the causes of the negative impact of pollution on animal reproductivity and subsequently, on the health and welfare of the offspring. The relationship between ingestion and

mortality of the foetus is directly proportional. Exposure close to the final period of gestation enhanced the impact of teratogenics (Bakalli *et al.,* 1995). There is environmental and scientific interest due to the careless and uncontrolled waste disposal and release of out-flowing, petroleum products, crude oil spills, and waste water with heavy loads of heavy metals in the environment which is aggravated by population and technological advancement (Ersteniuk, 2004). The spread and quality of heavy metals in the water bodies, land, and atmosphere has been made higher recently by human operations. In watery environments, biota, sediments, and water serve as reservoirs for metals (Erdogan *et al*., 2005). However, based on yearly and seasonal variations the amount of heavy metals present may differ. The lipid and biological tissue constituents of the biota, the chemical impacts of the metal, and preference of the metals to bind to particular materials determines the degree of pile up of heavy metals in the living organism of a region. Recently, the heavy metals concentration and spread in the water bodies, land, and atmosphere are influenced by a number of operations. The extensive and diffused pollution of the environment by heavy metals has generated increased interest in their potential danger to humans, animals and plants.

The unknown wellsprings of metal exposure may be unveiled by a complete account on nutrition and management system. Nutritional supplements may contain metal pollutants, or the pollutants leached from metal storage of feed and sources of drinking water such as lead decanters or containers. Toxicity may result eventually in animals exposed deliberately to colloidal metals for supposed health management advantage. Certain number of inappropriate use of antibiotics may be made complex by metal toxicity, for instance, in Quebec and subsequently in Minnesota, “beer drinkers’ cardiomyopathy” was discovered in alcoholics at a time, cobalt was added briefly in the 1970s in beer on tap for the stabilization of the head (Soghonian and Sinert, 2008). More recently, manganese poisoning has been implicated in a Parkinsonian syndrome among Latvian injection drug users of methcathinone.

There are two conditions where heavy metals can be poisonous to animals: the nonperformance of any metabolic roles and then, sudden exposure which can without any difficulty disrupt the usual bodily cellular processes. For example, gastrointestinal and anaemic symptoms may result due to sudden exposure to chromium and lead (IOCCC, 1996). Chromium toxicity, similar to copper's, also results in liver and kidney destruction with convulsions,respiratory and eye irritation.

2.13 BIOAVAILABILITY AND DISTRIBUTION OF CHEMICAL POLLUTANTS

Biota takes up chemical elements through a process that is flexible and complicated. These chemical elements are taken up by means of consumption and breathing in of air carried fragments and vaporized metals by animals and plants from water, sediments, and soil by coming in close association with their outside surfaces. Chemical speciation, pH, and solubility of inorganic medium are some of the chemical and physicochemical factors in which the integration of the bio-available particle is dependent on. In soils, the occurrence of metalloids and metals are in solid and aqueous state, that is soil solution phases. These elements can be expressed in form of free ions or suspended colloidal fragments, diverse complexes linked to organic or inorganic ligands, when in solution. The elements can be absorbed or adsorbed on inorganic and organic soil constituents that are present in the form of minerals or precipitated with other minerals when in solid phase. Generally, as soon they enter the food chain, ions present in solution are to a greater extent readily obtainable for uptake by plant and animal. Nevertheless, some biological and physicochemical requirements like the discharge of unique chelating agents, desorption, reduction and oxidation, changes in pH among others could influence the availability of metal ions in the solid phase (Silva *et al*., 2005).

Accumulation, mucociliary and alveolar clearance, solubilization and chemical binding are processes influencing metal absorption and inhalation of metal compound in particle form (Silva *et al.*, 2005). The metals which are accumulated in the

nasopharygeal, tracheobronchial, or pulmonary sections of the body after entering the body may be conveyed to the gastrointestinal tract by mucociliary action. Phagocyting of metals by macrophages can also take place. The competition for proteins that would carry a particular metal is responsible for certain complex involvement for absorption in the gastrointestinal tract like the observation made for Pb in the presence of Fe, Zn, and Ca (Bressler *et al*., 2004). Almost all metalloids and metals are absent as free radicals in biological fluids and tissues.

The rate of elemental excretion, load in the body and level of present uptake are determinants of the status of the element in the blood. These elements are normally tied to plasma proteins or red cells in the blood. Silva *et al*. (2005) reported that cadmium and lead are very close to being fully tied to red blood cells. The elements readily obtainable for movement in and out of tissues are those tied to carrier proteins. The diffusibility of the carried elements determines their movement into the interstitial fluid and intracellular sections from the blood. The concentration gradient in the various sections is connected exactly to the spread of those metals available in the ionized and free form. A plasma protein called albumin, is very good at fastening numerous metals, but will require for certain metals, proteins like ceruloplasmin or transferrin with a unique movement function. A defensive mechanism of great significance against metal poisoning is part of the roles of these fastening. Therefore, the availability and comparative quantities of biological ligands and also the capability of the metal complexes formed to act as substrates for the different organic solute transporters mostly controls the ability of toxic elements (Bressler *et al*., 2004).

2.14 HEAVY METALS BIOCHEMICAL TOXICITY

The pile up of more than the necessary quantity of heavy metals like nickel, cadmium, aluminium, arsenic, mercury or lead,in the body results in toxicity. This affects certain elements like selenium, iron, and copper functions as antioxidant, a requirement by blood cells, and an integral portion of a number of enzymes, respectively. Increased proportion of non-essential and essential metals can result in their toxicity in the body

leading to cell membrane demolition, modification of molecular and enzymatic functions. DNA structure is also susceptible to alteration by these metals (Bruins *et al*., 2000). The uptake of heavy metals beyond the tolerance limits results in destructive impacts in the body and this is referred to as the heavy metals biotoxic effects. Inspite of the exhibition of particular signs of toxicity by single metals such as cadmium, aluminium, arsenic, copper, zinc, lead and mercury have been shown to have general toxicity signs like: pneumonia, depression, vomiting and convulsion (McCluggage, 1991).

Mutagenic, teratogenic, carcinogenic, neurotoxic, or chronic and acute are the different expressions of the heavy metals impacts on the body. The existence of these metals when within the tolerance level of the body is not toxic while it is a natural process for their merging with protein in the body, however may result in pathogenicity if the bio-recommended limit is exceeded (Jaffer, 1988). Domestic and industrial contaminations have destructive impacts to a greater extent on the agricultural sector and natural vegetation. Severe health difficulties results from the amassing of these heavy metals in tissues storage locations for a long duration. Toxicity due to heavy metals from drinking contaminated water and ingestion of feed in which heavy metals are present at concentrations exceeding the upper safe limit results in severe damages to the gastro intestinal health condition of animals exposed.

Transition metals easily create complexes that are stable and covalent in nature and normally relate as component of macromolecules like hormones, enzymes, protein, etc based on their chemical attributes such as state of oxidation (Schoof, 2003). This propensity guarantees the formation of complexes *in-vivo* between these metals and specific biological sets, like carboxylic (-COOH), disulphide (-SS), hydroxyl (-OH), amino (-NH), and sulphydryl (-SH) sets of ascorbate, citrate, phospholipids, proteins, peptides, amino acids, and other components of the tissue. Also, these sets are present in crucial biological molecules with the exhibition of transport, structural or catalytic, uses (Silva *et al*., 2005). The affinity to organically bind is an attribute of each of the

transition metals. Biological molecules with abundant –SH groups seem to have equilibrium constants that are generally high and specific reactivity is indicated towards such by metals like Hg, As, and Pb (Silva *et al*., 2005). Poisonous metals can be bound in biological matrixes of proteins (biological molecules) like amino acids - histidine, cysteine, glutathione (GSH), and others, ceruloplasmin, hemosiderin, melanotransferrin, lactoferrin, transferrin, ferritin, and metallothioneins. The destructive actions of a considerable number of metal ions at molecular level are a function of the reactivity for an extensive range of biological ligands which also ascertain the typical toxicity of the metal absorbed. The reactive elements in food which respond to stimuli, mainly as natural complexes or related to fibers always have a decreased solubility in the intestinal lumen and also often not well absorbed.

Decreased quantities or lack of agents such as oxalates, phytate and fiber in diet would enhance the absorption of minerals (Hazell, 1985). Also, metal absorption and poisoning are crucially affected by the impact of other micronutrients. In the body, there are a number of ways in which micronutrients and toxic metals can relate. These include, in secondary mechanisms like oxidative stress, transport of metals in the body, metabolism binding to target proteins, sequestration of toxic metals, and absorption and toxic metals excretion.Consequently, micronutrient deficient feed significantly affects the nonessential metals like arsenic, mercury, lead, and cadmium in terms of toxicity (Hurrell, 2001). Therefore, adequate supply of essential minerals for optimal effect is necessary with the reduction in the intake of other minerals for whole safety for animals and human consumers of meat and eggs.

There are numerous haematological studies on the poisonous constituents of petroleum products (Cairney *et al.,* 2002). Berepubo *et al.* (1994) reported lower growth with comparatively brief exposure to petroleum products in weaner rabbits. Similar report by Tinsley, (1979) was observed in juvenile pink salmon (*Oncorhyncus gorbuscha*). Breathing difficulties, irritability, restlessness, drowsiness, coughing, cramps, swelling of the stomach, diarrhoea and vomiting can result from the ingestion of products like

kerosene or petrol. Coma or death may result from the consumption of more than an ounce. The rate of transit, pH, simultaneous administration of other substances, dose, solubility, particle size, presence of transport systems and a number of factors like sex, nutritional status, age and species are the determining factors in the inorganic salts absorption in the gastrointestinal tract. The absorption differ with metals; from <10% for Pb and Cd, to virtually whole absorption of 90-100% for thallium, germanium, and arsenic which are water soluble inorganic salts of trivalent ions (Silva *et al*., 2005).

2.15 BIOCHEMICAL MECHANISM OF HEAVY METAL TOXICITY

The interference of heavy metals with the usual biochemistry of the body in the usual metabolic processes is responsible for the impacts of toxicity. In the stomach with its acidic condition after ingestion heavy metals are converted to oxidation states that are stable (Ag^{+}, Hg^{2+}, As^{3+},As^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+}) which fuse with the biomolecules of the body like enzymes and proteins to create bonds that are strong and chemically stable. Ogwuegbu and Ijioma (2003) reported the equation for the reactions for the formation of bond with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine ($-SCH_3$) as follows.

Where: (A) = Intramolecular bonding;

(B) = Intermolecular bonding;

P = Protein;

E = Enzyme;

M = Metal

In the case above, the toxic metal displaces the metal groups or hydrogen atoms thereby preventing the enzyme from functioning, while the protein–metal compound serves as a substrate and reacts with a metabolic enzyme. In a scheme presented below (equation C), enzymes (E) react with substrates (S) in either the lock-and-key pattern or the induced-fit pattern.

In both cases, a substrate fits into an enzyme in a highly specific fashion, due to enzyme chirality, to form an enzyme–substrate complex (E-S*) as follows (Holum, 1983).

E + S E-S E – S* E – P E + P

(E = Enzyme; S = Substrate; P = Product; * = Activated Complex)

While at the E-S, E–S* and E-P states, except an enzyme is free it cannot have space for any other substrate.

Sometimes, there is coexistence of the enzymes for a complete series in one multi-enzyme complex made up of three or four enzymes. The output from one enzyme reacts with a second enzyme in a chain process, with the last enzyme yielding the final product as follows:

The final product (F) then goes back to react with the first enzyme thus preventing another reaction since it was not initial substrate for the process. Therefore, the enzyme E1 is unable to accept any other substrate until F is removed and that can only be done by its utilization by the body. The inability of the body to make use of the output produced from the heavy metal – protein substrate will result in an indefinite blockage of the enzyme with the inhibition of the initiation of any other bio-reaction of its function. Thus, significantly different bio-dysfunctions will result as the metal stays securely surrounded by the tissue (Holum, 1983).

Additionally, some other metal ions of the same size can easily replace a metal ion in the metallo-enzyme of the body. Therefore, cadmium toxicity can occur with the replacement of Zn^{2+} with Cd^{2+} in certain enzymes that dehydrogenates. Mutilation of the structure of a protein molecule to a bio-inactive form and the destruction of enzymes could result during inhibition. For instance, bioactivities of enzymes can be inhibited by the destruction of enzymatic -SH groups caused by toxic As^{3+} which forms in insecticides, fungicides, and herbicide as presented below (Ogwuegbu and Ijioma, 2003). The most stable oxidation states of these metals in their ionic species are the forms in which they are most poisonous. For instance, As^{3+}, Ag^{+}, Hg^{2+},Pb^{2+}, and Cd^{2+}. In their most stable oxidation states, bodily extraction by medical detoxification therapy is difficult since they make up truly stable bio-toxic compounds with bio-molecules in the body, making separation hard as a result of their biostability.

Mercury toxicity as usual with other heavy metals comes from pollution from the environment and from some unforeseen origins. For mercury, it is the nature of the environmental origin that is specifically raising concern in the context of nutrition. The agricultural industry with its attendant use of large volumes of fungicide for the dressing of seeds, production plants for electrical apparatus, and chloro-alkali plants are the major industrial avenues through which mercury enters the environment. The quantity of mercury in natural water is at times ten times less present in sewage outflow from industries. Adsorption of mercury on sediments in water results in the production of methyl mercury (CH_3Hg) and dimethyl mercury $(CH_3)_2Hg$ by sulphate reducing bacteria in sediments; these products volatilise and get discharged into the water and absorbed by fish. The created neutral complex of CH_3HgCl from CH_3Hg^+ ion in the saline biological fluids goes through the biological membranes and gets spread in the fish's tissues. The chloride is replaced by peptide sulphydryl groups in the tissues. The affinity of mercury for sulphur ligands will result in the slow elimination of the methyl mercury causing bioaccumulation and subsequently poisoning of bigger fish on feeding on the smaller ones. Biomethylation of mercury takes place in all sediments and fish but with higher concentrations in water bodies with sediments polluted by mercury from waste outflows (Jones and Lopez, 2006). In the 1950s in Minimata, Japan, the worst incidence of environmental mercury toxicity took place with the concentration of methyl mercury in fish to levels tending to 100 ppm. Through the poisoned fishes thousands and hundreds of people were poisoned and died, respectively. The ability of methyl mercury to translocate the blood-brain barrier made it to adversely affect the brain. Motor imbalance and delay in mental development in infants was attributed to methyl mercury as it can be passed from mother to child.

2.16 HEAVY METAL CONTAMINATION

About 50% of the not less than 20 metals grouped as poisonous are discharged into the environment in amounts that are dangerous to the health of human (Jones and Lopez,

2006). A greater proportion of these chemicals released into the environment through lakes, rivers, and streams cannot be recycled and are poisonous to aquatic creatures and the health of humans. Potable water sources for animals and humans are polluted mainly by some of these chemicals. Contamination, deterioration of the growth of plant, and the posing of risk to animals, health of humans, and the ecosystem are the impact of contamination by these chemicals which got into the groundwater (Jones and Lopez, 2006).

Heavy metals pollution is a reality that is both harmful to health and deadly. They are subtle, quiet, stalking killers. Heavy metals access the body through skin contact, inhaled air, water, and feed where they are amassed gradually with time in the brain, central nervous system, bones, pancreas, liver and kidneys and the eventual unnoticed and undiagnosed degradation of health. They can and do cause cancer without ever being implicated in the diagnosis. They can and do cause delay in mental development and heart disease. All animals and humans are polluted with heavy metals, without ever knowing it (Jones and Lopez, 2006). Aging, and also severe diseases and death are attributed to ions of nonessential heavy metals. Increased aging by otherwise truly healthy persons are caused by the ions of heavy metal cross linking with the body's normal molecules. These ions are at times known as "free radicals." Diseases like nerve damage, degeneration of organs, carpal tunnel syndrome, skin ailments, hardening of the arteries, etc. have been known to have been caused by the cross linking.

2.17 SYMPTOMS OF HEAVY METALS POISONING

Symptoms of poisoning resulting from heavy metal pollution include; in children mental deterioration and in adults dementia, headaches, memory loss, panic attacks, depression, emotional instability, personality changes, insomnia, liver (hepatic) diseases, kidney (renal) diseases, central nervous system (CNS) disorders, vision disturbances including peripheral neuropathy, lack of coordination (ataxia), excess sweating, and excess salivation. Death may result from cardiovascular diseases (CVD) or encephalopathy - diseases of the brain. Acidic blood pH may result from poisoning

from heavy metals. The extracted calcium from the bones by the body is used to buffer the acidity. Arteries hardening due to the accumulation of calcium and not heavy metals in the soft tissues of the arteries ensue. Calcium removal from soft tissue is difficult, as well as poisonous heavy metals removal (Jones and Lopez, 2006).

With time, these poisonous elements are piled up in the tissues like fat and bone in the body which are gradually degraded and destroyed. In the end, several physical and cognitive disorders, like fatigue, memory loss, anxiety and depression are triggered by the increasing poisonous load. Nerves and tissues can be destroyed by the destructive ability of poisonous elements even at comparatively low levels. Neurodegenerative states of aging like Alzheimer's and Parkinson's disease as well as early neurodevelopment disorders, like Attention Deficit Hyperactivity Disorder are caused by the role of elemental toxicity. Chronic exposure to poisonous elements have been linked to increased risk of cancer, gastrointestinal dysfunction, weakened immune function, respiratory illness, impaired kidney function, and heart disease (Jones and Lopez, 2006).

2.18 VANADIUM

Vanadium occurs naturally in the air, soil and water. It exists in crude petroleum deposits, iron ores, rocks and earth’s crust. Dissolved amounts of vanadium in groundwater are widespread with naturally presented vanadium found in almost all geologic settings.The most usual of the various oxidation states in which vanadium exist are +3, +4, and +5. The most poisonous form is pentavalent vanadium which is also the most chemically stable (WHO, 1988). The major vanadium source for exposure to the populace is through feed with approximately few tens of micrograms per capita per day as intake (WHO, 1996). The amounts of vanadium present in water for drinking do not broadly go beyond few micrograms per liter as such make lesser contribution. Notwithstanding, rocks with abundant content of vanadium influences the content in water supplies in such locations to increased quantities of more than 100 µg/l (Wright and Belitz, 2010). After vanadium is absorbed, it is quickly circulated by

blood to different tissues with the highest quantities initially located in lungs, kidneys and liver, while the long-term storage locations are the muscles and bones. The translocation of Glucose transporter type 4 (GLUT4) to the cell membrane is increased by vanadium due to its ability to improve insulin signal. Stimulation of insulin-independent phosphorylation mechanism, preservation of pancreatic cells, and improvement in insulin sensitivity by vanadium has been reported in animal studies (Sakurai, 2002). Nausea and greenish discoloration of the tongue are rarely caused by vanadium. Large doses are nephrotoxic. WHO (1996) reported the predomination of vanadium's pentanvalent and tetravalent in extracellular and intracellular fluids, respectively.

Different biological impacts are exerted by the different oxidative states of vanadium. A good number of which is based on the reactive oxygen species produced during the reduction of one electron, V^{+5} to V^{+4}, followed by the destruction of DNA, retardation of enzymes, signal transduction alteration and gene expression (Beyersmann and Hartwig, 2008). In poultry feed, the highest limit of tolerance for vanadium is 10 ppm. Romoser *et al*. (1960) found vanadium quantities of as much as 6,000 ppm in certain deposits of rock phosphate, in a commercial tricalcium phosphate. Commercial phosphate supplies 26 to 796 ppm of vanadium for individual samples but mostly between 50 and 200 ppm. Vanadium is also present in the form of peroxide and that can imitate insulin response (Fantus *et al.*, 1989).

2.18.1 VANADIUM TOXICOLOGY

The extent of toxicity of vanadium compounds is influenced by a number of factors including the route of exposure, oxidation status, and chemical form. Negative impacts in lungs, kidneys and spleen of rodents, elevated pulse rate in rats, developmental and reproductive toxicity in mice and rats have been reported for orally offering vanadium compounds like vanadylsulphate, sodium orthovanadate, and sodium and ammonium metavanadate (EFSA, 2004). Draghici *et al,* (2010) reported degradation of internal quality of egg resulting in impairment of magnum movement during the formation of

egg. Ingestion of feed with more than 10 ppm vanadium retarded the growth of birds fed for more than 21 to 28 days. As small as 6ppm of vanadium supplied by dicalcium phosphate reduced the quality of albumin in eggs from layers (Sell *et al*., 1982). The application of vanadium from calcium orthovanadate at 25 ppm through feed for 20 weeks reduced hatchability in birds (Kubena *et al*., 1980) and the weight as well as specific gravity of egg require 40 ppm to be influenced (Ousterhout and Berg, 1981). Also, 50 ppm vanadium in the form of calcium orthovanadate was offered to hens for five consecutive 28-day periods with no mortality (Kubena and Phillips, 1982). Haugh units reduced massively within 3 days of offering feed with ammonium metavanadate as source of 20 ppm vanadium (Toussant and Latshaw, 1994).

Increasing vanadium in feed reduced the proportion of inner thin albumen but with increased proportion of the outer thin albumen. The genotoxic potential of vanadium compounds has been investigated. These studies reported specific significance for the examination of risk and also for the absence of sufficient studies on carcinogenicity (EFSA, 2004). Generally, both tetravalent and pentavalent vanadium were distinctly genotoxic in *in-vitro* test systems, expressed by the modification of chromosome segregation, destruction of chromosome, and the breaking of DNA induction strand (EFSA, 2004). These impacts are due to indirect mechanisms like the production of reactive oxygen species *via* a Fenton-like reaction and not a direct interaction with DNA (EFSA, 2004). Leopardi *et al*. (2005) and Villani *et al*. (2007) examined the genotoxic danger of tetravalent and pentavalent vanadium in drinking water of mouse These studies revealed that pentavalent vanadium (vanadate) alone had the ability to elicit a certain number of genotoxicity *in vivo* which was limited to those given high dose repeatedly through drinking water. Hens exposed to the toxic impacts of 40 ppm vanadium can be protected with dietary inclusion of 0.4 to 0.5% ascorbic acid or 20% cotton seed meal (Ousterhout and Berg, 1981). Antioxidants like carotene (500ppm), vitamin E (200 IU/kg) or ascorbic acid (100 ppm) to reduce the adverse impact on the interior quality of egg caused by offering 10 ppm of vanadium to hens (Miles *et al*., 1997).

2.19 CADMIUM

The potential poisonous impacts of cadmium are as a result of its increased discharge into the environment from cigarette smoke, disposal of waste, and industrial processes. The utilization of zinc sulfate or improperly processed ores of zinc as the supply for supplemental zinc is probably the main avenue of pollution in the feed industry. The utilization of urban sewage sludge for soil fertilization for crops or pastures, disposed products of cadmium chloride, corrosion of metal-plated iron, mining and smelting activities are other sources of cadmium toxicity (NRC, 1980).

Cadmium is basically a toxic metal with cumulative impact due to its biological half-life which is very prolonged so much that an exposure in the distant past could be directly poisonous on residual metal (Ademachi *et al*., 1991). Plants around industrial locations may tolerate cadmium but with significant effect on animals specifically on their visceral organs, like kidneys and liver. The ingestion of cadmium altered the signals of oxidative stress as well as superoxide dismutase (SOD) operations, catalase, blood glutathione and MDA which reliably indicate the imbalance between oxidative and antioxidative capability of blood (Baykov *et al*., 1996).

The concentration of cadmium is increased with movement across the food chain reaching about 50 to 60 times its concentration on getting to the level of the carnivores (Daniel and Edward, 1995). Injury to lungs, liver damage, hypertension and dysfunction of the kidneys are the poisonous impacts of cadmium (John and Jeanne, 1994). Enzymes for detoxification in the kidney and liver were seriously modified by teratogenic dose of cadmium chloride (Draghici *et al*., 2010). Cadmium pollution also emanates from cigarettes, as cadmium is more effectively absorbed in the lungs than in the stomach from food. Induction of tubular dysfunction in kidney due to the production of urinary beta 2-microglobulin is attributed to cadmium. Adult poultry birds were used by Kostial *et al*. (1991) and Tinsley (1979) to investigate the effects of cadmium on antioxidant defense system and Lipid Peroxidation (LPO) of red blood

cells and they reported that sub-acute cadmium toxicity elevated the level of erythrocytic catalase (CAT) and superoxide dismutase (SOD).

Birds exposed to cadmium had decreased glutathione (GSH) level in the blood but with elevated lipid peroxidation. Cadmium is capable of inducing oxidative stress in adult poultry bird by elevating lipid peroxidation and the modifications of activities of antioxidant enzymes. The activation of the body's antioxidant defense system caused elevated operations of erythrocytic catalase and SOD due to cadmium exposure. Antioxidants lower reactions caused by oxidative radicals and consistently defend metabolic processes in the red blood cells that is against the advancement of oxidation stress and hypoxia. Cell could be shielded against cadmium toxicity by the use of GSH (Singhal *et al*., 1987).

Cadmium is a by-product from the production of zinc since it forms a small part of zinc ores. Although cadmium is a toxic metal, one enzyme, a carbonic anhydrase with cadmium as reactive centre has been discovered (Jarup, 1998; Zhou *et al.*, 2007). Incineration of municipal solid waste, production of nonferrous metals, production of cement and connected operations, production of iron and steel, natural sources, phosphate fertilizers, and the combustion of fossil fuel are the basic sources of exposures of animals and humans to cadmium in the environment. Dysfunction in the kidney with its characteristic tubular proteinuria is an adverse impact of the long term exposure of humans to cadmium. Blocked lung disease results from high exposure. The disruption of the oxidative and anti-oxidative balance of adult poultry birds due to cadmium exposure generates oxidative stress. Oxidative stress can be induced in adult poultry birds by the ingestion of 100 mg/L of cadmium sulphate in drinking water (Kant *et al*., 2011). Reduced cadmium toxicity and residues in broiler tissues were reported after offering zinc, with improved renal and hepatic functions as well as the hematological indices (Kostial *et al*. 1991). In poultry, the highest tolerable limit for cadmium is 0.50 ppm.

The absorption of poisonous quantities of cadmium can be prevented in animals by inducing the formation of the gastrointestinal tract protein-metallothionein as a basic defensive means. Cadmium is shed and excreted in faeces by protein and epithelial tissues that already separated it. Tousand and Letshew (1994) reported that not up to 4% of the opening1 ppm radio-cadmium offered Japanese quail in feed for 7 days followed by a 50-day basal feed, was left. Likely, in association with the protein-metallothionein, about 12%, 25%, and 25% were located in the gastro-intestine, kidneys, and liver, respectively. Adding iron to feed could to some degree mitigate anemia due to cadmium poisoning (Hamilton and Valberg, 1974). Kidney destruction in broilers due to cadmium poisoning can best be prevented by adding selenium and ascorbic acid in feed (Rambeck and Kollmer, 1990). Exposure to cadmium has been implicated in prostate and lung cancer. Nevertheless, the carcinogenicity of cadmium is still a subject of debate to researchers. Muscular weakness, dyspnea, abdominal cramps, vomiting and nausea are some of the signs of poisoning depending on the degree of exposure. Pulmonary edema and death may result from severe exposure to cadmium. Sub-chronic exposure by breathing in cadmium and its compounds may lead to renal and pulmonary (alveolitis, bronchiolitis, and emphysema) impacts (Young, 2005).

Cadmium pneumonitis, arising from the inhalation of fumes and dusts causes cancer of the lungs. Death of lung tissue lining due to watery fluids amassing beyond limits, cough with bloody foamy sputum and chest pain are the signs of cadmium pneumonitis. Defects such as myocardic dysfunctions, increased blood pressure, osteoporosis and spontaneous fractures, and osteomalacia are also connected to cadmium toxicity.The use of poultry manure from birds offered feed with average cadmium content of 0.12-1 mg for plants or soil fertilization with other matter may have adverse environmental impacts.

2.20 IRON

The most plenteous trace element present in the body is iron (Fe) with its deficiency (anemia) being universally, the most usual micronutrient deficiency. Cytochrome enzymes, ferritin and hemosiderin, myoglobin, and haemoglobin contain iron. Oxygen is transported to different tissues by haemoglobin. Deterioration of the immune function is connected to the deficiency of iron. Researches using animals and humans have shown bactericidal activity, myeloperoxidase, natural killer cell, reduced neutrophil, and deterioration of cell mediated immunity, when iron is limited. Concentrations of 8mg/day, 11mg/day, 15 mg/day and 30mg/day of iron are the Recommended Dietary Allowance (RDA) for men and post menopausal women, adolescents, premenopausal women and pregnant women, respectively. Heme iron present in sea food, chicken, red meat and other animal products and non-heme iron in dried fruits, nuts, whole grains and dark green leafy vegetables are the two forms found in food. Absorption for the two forms of dietary iron in the gastrointestinal tract is 15-20% and 1-8% for the heme and non-heme iron, respectively. However, this is reduced by tannins and phytates but elevated by ascorbic acid.

Hookworm infestation, inadequate absorption, insufficient intake, increased physiological need, and other infections could result in iron limitation. Hypochromic, microcytic anaemia linked to, dysphagia occurring once in a while, pica, poor cognitive development, and easy fatigability result from iron deficiency. Repeated parenteral administration, excessive absorption in the gastro-intestine and elevated intake in diet could lead to excess iron in the system. Iron overload is linked with hemochromatosis and hemosiderosis. Appraising the amount of iron stored in the bone marrow, the total capacity to bind iron and serum ferritin could give an insight into the iron status of the body. Bone marrow stores is first reduced followed by serum ferritin when the iron balance is negative while the absorption of iron and copper are depleted by the absorption of zinc beyond limit (Lenntech, 2004). With elevated deficiency, serum iron declines, microcytosis and hypochromasia appear on the peripheral smear.

Elevated risk of myocardial necrosis is a function of increased amount of iron in the tissues (Hazell, 1985).

2.21 NICKEL

Nickel (Ni) exposure leads to prostate, larynx, nose, and lung cancer. The biomagnification of heavy metals along the food chain is due to their natural propensity for tissue bioaccumulation. The concentration of nickel beyond limit is poisonous except that only small quantity is required in the human body for erythrocytes production. Skin irritation, destruction of liver and heart, and decline in body weight are signs of long term exposure to nickel.

Nickel (Ni) is silvery-white, hard, malleable, ductile metal that is extensively spread in the environment, and the 24th naturally plenty element found on the earth's crust (Kasprzak *et al*., 2003). It is utilized in the production of various consumer and industrial products like special alloys, coinage, magnets and stainless steel. Nickel is also used as a green tint in glass and for plating. Nickel occurs naturally in plants, animals, drinking water, soil, air, wetlands, rivers, and oceans. Seriously elevated concentration of it is found in fats and chocolate whereas foodstuffnaturally contains little quantities. The higher oxidation states of nickel have reduced stability and this makes nickel a biochemically significant element (Atkins, 1978). Nickel, an essential element needed for the correct functioning of chicks and rats 'liver, and morphological sustenance but with the risk of respiratory cancer when the exposure is unabated. Nickel pollution has dangerous impacts on animals and humans, specifically pulmonary disorders. Lipid per oxidation in target organs is caused by nickel poisoning (Nielson, 1991).

2.22 MERCURY

Mercury is a natural occurring metal that can also be discharged through industrial contamination into the environment. Mercury can be converted into methylmercury by bacteria present in water. For poultry, the highest tolerable limit is 2 ppm. Less

quantity of organic methylated mercury can be tolerated in poultry than the inorganic form. Nevertheless, there are chances for elevated toxicity resulting from the biomethylation of inorganic mercury in the animal or environment. Death, coma, reproductive difficulties, loss of sensation in toes and finger, lack of coordination, loss of hearing, slurred speech, and loss of memory are caused by the destructive nature of this metal to the central nervous system due to increase concentration.

Mercury causes the pollution of the environment and subsequently penetrates the water system via soil, lakes, streams, and rivers. The inorganic mercury is the grade present in drinking water and can be changed by bacterial action in water and soil to methylmercury, which is the organic form. It enters the food chain ultimately in this form. The highest permissible limit for mercury present in water for drinking available by the EPA is 2 ppb. Tap water generally has mercury content within this limit but this safe limit is evidently exceeded by water sourced from wells. Universally, in aquatic ecosystems, increased quantities of heavy metals carried by water are toxic to the environment. Some penetrate groundwater while others are amassed in plants or sea food thereby becomes a means of toxicity to animals and humans. Sea animals and birds' deformation plus certain abnormal conditions in animals and humans are due to heavy metals particularly mercury.

2.22.1 METABOLISM OF MERCURY

The volatility of mercury than other metals that are reactive to sulfhydryl makes it elemental form more greatly absorbable. Mercury contaminates water naturally by drawing from the earth's crust and by man's operations through industrial contamination. Methylated mercury by bacteria and algae in aquatic environment goes up the food chain and gets highly concentrated in huge aquatic organisms that are predators like the tuna, salmon, shark, and swordfish. Elemental mercury (Hg) and methyl mercury (MeHg) are the two highly absorbed forms of mercury. Mercury, if consumed, is improperly absorbed but through the efficient absorption of its vapour via the lungs it rapidly gets to the brain by means of the blood brain barrier. Mercury has

preference for lipid membranes and myelin because it is lipophilic. Mercury is oxidized after cellular penetration by the catalase to greatly reactive Hg^{2+}. Fish adsorb methyl mercury (MeHg) as well as di-methyl mercury easily in the intestine. Demethylation and oxidation of methyl mercury to Hg^{2+} can also be done. Protein residues of cysteine and glutathione bond covalently with Hg^{2+} and $MeHg^{+}$ the moment cellular incorporation of the later takes place. The low rate of Hg excretion is responsible for the greater quantity of the incorporated quantities being held leading to continual piling up in the liver, neurological tissue, and kidneys.

Mercury directly takes away Glucose stimulating hormone (GSH) from the cell and also hinders the operations of GSH reductase and GSH synthetase involved in the metabolism of GSH. The operations of enzymes like GSH peroxidase, superoxide dismutase, and catalase which put out free radicals are also hindered by mercury. Sulfhydryl reactive metals enhance lipid peroxidation, reduce GSH, hinder antioxidative processes, and interrupt the anatomy and physiology of several proteins by directly forming bond with free sulfhydryl group. Complete sulfhydryl groups are significant for biological operations of almost all proteins with Na/K ATPase inclusive. Astrocytes swelling and damaging can be caused by the hindrance of Na/K ATPase as induced by metal (Jones, 2007). Existing microtubules can be prevented from undergoing polymerization by mercury. Lesions in animals' brains tend to have same similarity with those found in Alzheimer's disease patients (Ghosh *et al*., 2012).

2.22.2 EFFECT OF MERCURY ON FERTILITY AND FOETAL DEVELOPMENT

Infertility and developmental impacts of mercury on foetus and infants are less and without significant impacts on mature animals. The low weight of the body with more feed ingested per time per kilogram of body weight, higher rate of absorption in the intestine, reduce excretion from the kidney, and reduce blood-brain barrier in foetus and newborns make them more sensitive to mercury than adults even at low exposure concentration (Halder *et al*., 2009). The basic means of transferring mercury was by

creating bond with albumin. In breast milk and foetus, the presence of notable concentrations of methyl mercury in breast milk has also been reported (Schumann, 1990). The concentrations of mercury in breast milk under normal physiological state should be between 1.7ug/l and 3.5ug/l. This seems a sufficient screening level for health risk. The concentration of mercury accumulated in the dam is less than that present in breast milk and foetus (Schumann, 1990). Samples from milk were between 0.2 and 57 ug/l (Dexler and Schaller, 1998).

The most dependable pointer of exposure to foetal mercury is the first stool (meconium) and frequently has notable concentration when levels in the blood of the dam and foetus are low. Developmental dangers can be examined without difficulties using maternal tests since mercury from hair and maternal blood correlated significantly with the concentrations in meconium and nursing infants (Cernichiari *et al*., 1995). There could be notable levels in foetus despite low concentrations in the blood of the mother. The foetal pituitary gland which has impacts on development of the reproductive systems, immune, and endocrine is where the highest concentration of mercury are normally found. Mercury disrupts the endocrine system in humans and animals, with the affinity of piling up in the pituitary gland, thyroid and hypothalamus gland (Lindqvist, 1996; Ukoli, 2005). Mercury hinders with the production processes of enzymes (Markovich and James, 1999), transfer of glucose and interferes with numerous hormonal functions (Gerhard *et al*., 1998) atexposure to very low concentration. The pituitary gland dictates for the generality of the roles of the endocrine system of the body and releases hormones that influence most processes of the body, with the reproductive and immune systems inclusive (Colborn, 1992). Temperature of the body and a large number of metabolic processes are regulated by the hypothalamus. Acetylcholine, a neurotransmitter that provides the brain with sharpness and good memory, is disrupted by mercury, aluminium, and lead. There are four major sources of mercury poisoning: acquired by baby from the mother, vaccinations, dental amalgams or silver fillings, contraception pills and contact lens solution.

2.22.3 EFFECT OF MERCURY ON FERTILITY

Destruction is permanently done to the brain of developing foetus and baby by mercury which had access through the membranes of the placenta in pregnant adults. A special relationship is shared between the dam and the foetus with particular regard to mercury circulation. Cord blood has much more elevated concentrations of methylmercury than the maternal blood. There is much less concentration of mercury present in the maternal brain tissue than that in the foetal brain tissue. Mercury exposure disrupts the hormone and immune system and its roles with adverse effect on fertility. The occurrences in female fertility cycle are greatly connected to posterior pituitary operation, which can disrupt fertility and other roles not related to pregnancy. Amalgam affects the role of estrogen, a notable hormone of the female fertility. Blood serum phosphorus is a rule of thumb for endocrine balance. The level of Phosphorus lower than 3.5 mg in the body points towards an endocrine disturbance. The most effective substances in balancing the phosphorus level are the sex hormones.

Both females and males are producers of both testosterone and estrogen. The production of testosterone is more in male while estrogen production is more in female, but with a balance in the sexes for the two hormones. Serum phosphorus is balanced using small quantities of both hormones in both sexes. The pituitary, hypothalamus, and ovaries, through feedback mechanism that is really complex, influence reproductive cycles. Follicle stimulating hormones (FSH) always have a feedback relationship that is negative with estradiol. At lower concentration of estrogen there is increased secretion of leutinizing hormone (LH) and vice versa. This ebb and flow dictates the hormonal role resulting in ovulation and the mid cycle sudden transient increase in the concentrations of both leutinizing hormone and follicle stimulating hormone and the decline in leutinizing hormone and follicle stimulating hormone at the luteal phase connecting to a feedback relationship with progesterone.
Increased concentration of ovulation progesterone is the result of progesterone released by the ovary prior to ovulation. The elevated progesterone and estrogen concentrations

connected with the luteal phase jointly subdue follicle stimulating hormone and leutinizing hormone during the corpus luteum phase. Destruction of cellular membranes in the anterior pituitary by mercury hinders the secretion of follicle stimulating hormone from the pituitary.

2.22.4 EFFECT OF MERCURY ON THE PLACENTA

Maternal and foetal circulatory systems are apart from each other by a placental membrane that is really light weighted. This membrane guarantees the blood of the mother and foetus are not mingled. The placental membrane was previously referred to as the placental barrier. The defense of the foetus from likely harm such as poisoning, from the maternal blood was the assumed role of the placental membrane. Nevertheless, the tragic birth abnormalities and deformities associated with the Thalidomide disaster in 1961 showed the possibility of toxic exchange between maternal and foetal blood. The decline in oxygen carrying ability of the blood due to mercury exposure may not affect blood flow in the foetus, but hypoxic state due to lower oxygen content in the blood may result. The balance of greater number of essential nutrients in the body may be affected by mercury. The offspring relies on maternal sources for all of its nourishment from fertilization to birth. The critical parts in influencing the result of foetal development are the state of maternal nutrition; quality of the placental anatomy and physiology; the offspring's genetic composition and the presence of maternal and offspring communication means, be it mechanical, chemical, or physical during gestation.

Adequate result from the development of foetus can be affected in the above areas by mercury. Foetotoxicity of cadmium and mercury affects transmembrane movement of nutrients like amino acids, to the foetus from the placenta. Delay in growth, abnormal formations from birth, or death of the foetus may result from hinderance to the movement of nutrient. The prevention of the movement of the needed nutrients to the embryo/foetus is an expression of the toxic impacts of cadmium and mercury in the placenta. Although numerous substances can be blocked by placental membrane,

however, its fat molecules makeup will allow fat soluble substances like methylmercury and mercury vapour to penetrate it.

There is absence of scientific information on the mechanisms of mercury toxicity as it links to the reproductive cycle of humans and animals. A greater number of reports on mercury are on methyl mercury or inorganic mercury. Not much has been done on the menace in which chronic exposures at low level to toxic metals constitute. Most acute exposures investigated were based on the injection of large concentration of toxic metal at once. The movement of mercury is without a preventive barrier but not so with cadmium while lead is slightly prevented. Bodily and cellular penetration of mercury vapour is far more easily done compared to its other forms.

There is a directly proportional relationship in the placental and infant hair's concentrations of mercury and bodily mercury load in infant. Lower concentrations of total mercury, methylmercury, iron, and cadmium were found in the blood of the dam than in cord blood, whereas zinc and copper had higher concentrations. The concentrations of total mercury, methylmercury, manganese, cadmium, and lead had correlated positively between the blood of the mother and umbilical cord. The significant correlations between a number of metals pairs, especially in the umbilical cord and its blood is an indication of the heavy metals influence on infants compared to the dams. The transplacental movement of mercury to the embryo/foetus can be reduced by the availability of selenium in the placenta. The merging of mercury with proteins in the plasma or its penetration of the erythrocytes may allow it access into other body organs but not easily allowed into the foetus or brain (El-Shenawy and Hassan, 2008). The metabolism of mercury takes place mainly in the liver and this causes serious liver destruction due to the piling up of mercury. Ultrastructural and histopathological injuries in the liver indicated by cell necrosis and periportal fatty deterioration are caused by $HgCl_2$. Cell lesions caused by $HgCl_2$ notably affected DNA which played notable roles in the cell operations (Schurz *et al*.,2000).

Health officials worldwide are faced with water contamination with mercury as one of their serious challenges. Mercury is an extensive industrial and environmental pollutant that causes serious tissues modifications (Sener *et al*., 2007), several neurological aberrations (Auger *et al*., 2005) and generates peripheral neuropathy (Chuu *et al.*, 2007) in animals and humans. The rate of industrialization and urbanization has been on the rise. Apart from the numerous challenges related with such social alterations, pollution is of paramount interest for national health, feed, food and water safety. The pollution of food chain needs to be given serious consideration that is instant, of all the other types of environmental contamination that are dangerous to animals and humans due to its increasing threat (Draghici *et al*., 1991). Therefore, bodily detoxification of heavy metals has positive anti-aging impact and in the right direction even in persons who seem adequately healthy. Vitamins and mineral supplements with affinity for heavy metal ions are antioxidants since they get fastened to free radicals and take same away from the body. The identification of targets and associated biomarkers of impacts is a function of the knowledge of mechanisms of action. Health impacts caused by exposure to poisonous metals differ greatly; from irritant and acute or chronic systemic toxic impacts to teratogenic, mutagenic and carcinogenic impacts (Silva *et al.*, 2005).

2.23 REDUCING THE TOXICITY OF HEAVY METALS

The environmental dominance of toxic metals has made it difficult to prevent pollution of animal feed, drinking water, and food products by these metals, however, there is need to reduce contamination with a view to minimizing both the indirect and direct impact on human health and animal safety, respectively (SCAN, 2003). Taking away these contaminants sufficiently from the environment is still a challenge. Raw stuff like oil palm empty fruit, walnut shells, plantain peel, palm kernel shell, coconut shells, groundnut husks, and charcoal has been on for years. Every effort on metal detoxification requires as a prior condition a foundation of healthy mineral.The body can be detoxified using substituting minerals as electrolytes like magnesium, calcium, potassium, and sodium to aid the movement of toxic waste through the extracellular

chamber toward the lymphatic and venous vessels. Metals get fastened only to sites meant for metal ions and the deficiency of minerals makes room for empty binding sites to be fastened to by toxic metals.

Humans require high quality water while acceptable water quality is of high importance for commercial, domestic, industrial, and agricultural uses. The activities also result in water contamination. These operations on daily basis empty into freshwater bodies billions of gallons of wastes. The unsuitable disposal of waste has gradually rendered available water resources unfit for use even in the face of increasing demand for water. The provision of suitable facility for treatment for all sources of contamination is not just difficult but also expensive, hence the urgent need for innovative technologies that are cost effective, needing low sustenance and efficient in terms of energy use. Adsorption is as a result of surface energy, the same as surface tension. Adsorption occur when a gas or liquid solute piles up on solid or a liquid adsorbent surface, creating a molecular or atomic film, the adsorbate. This occurs in a greater number of systems like chemical, biological, physical and natural and is extensively utilized for water purification, synthetic resins and activated charcoal. Adsorption is normally expressed via isotherms-roles which link the quantity of adsorbate on the adsorbent, with its pressure or concentration of liquid.

Toxic heavy metals are removed from the body by chelation. Chelating molecules, agents, or compounds, have the affinity of two or more of the atoms binding with metal ions. This results in a much stronger bond than the metal ion bond that existed in the body. The metal ion is pulled and carried away by the chelator from the molecules of the body. Another crucial characteristic of a chelator is that it holds the metal ion while the liver expels it into the digestive tract or the kidneys to the urine. Recycling of the metal ion back into the blood stream from the gastro-intestine must be prevented (Kingmann *et al.*, 2005).

Capturing of toxic heavy metals within the body can be done. Blood borne ions are captured or released by the liver or kidneys. These ions released into the intestine by

the liver are usually captured again and recycled back into the blood. Certain number of these ions stay in faeces and urine and can be measured. Disposed skin cells, sweat, hair, urine and faeces are avenues through which ions of toxic heavy metals can be expelled from the body. Chelating vitamins, minerals and supplements, oral chelators, and intravenous chelators are the means for the chelation of toxic heavy metals from the body (Kingmann *et al.*, 2005).

The use of inexpensive matters like egg shell, chitosan, mango leaves, sawdust, coconut shell, activated carbon, and other adsorbents with elevated capacity for adsorption have been researched on. The taking away of heavy metals from waste stream has been carried out by researchers using adsorption process. The exploration of inexpensive agro-based adsorbents should be carried out and the feasibility for their heavy metals removal abilities studied.

2.24 CHARCOAL OR ACTIVATED CARBON

Activated carbon is widely used adsorptive substance with a surface area that is large and an adsorptive capacity that is efficeint. It is a widely used toxin adsorbent and usually used for digestive toxicities of different kinds. Heavy metals detoxifications include the displacement of metals from tissues into the blood by mineral antagonists balanced with chelators that bind the metals in the blood and channel same to the kidneys for excretion by keeping from been re-deposited elsewhere. Normal detoxification methods like kidney cleansers, liver flush, fasting, etc. are not effective with heavy metals.

2.25 Na-EDTA

Na-EDTA or EDTA (EDTA Disodium) creates chelates with metals that are polyvalent in nature, particularly calcium, thereby elevating their excretion in the urine. Chelation of calcium from the arteries and the reduction in arterial hardening is usually accomplished with the use of EDTA which has been shown to be effective also in chelating aluminum and other metals. The redistribution of heavy metals that are

toxic to other parts of the body in place of complete removal is a demerit that is neither inevitable nor impossible.

2.26 PROTEIN COMPLEXES AS BINDING AGENTS

Endogenous detoxification of metal and shuttle agents, like glutathione, metallothioneine, ceruloplasmin and others are furnished with by proteins as precursors. Independent detoxification impacts of increased value can be found in amino acids that are branched chain in cow and goat whey. Supplements of amino acids, particularly the ones with high concentrations of branched chain amino acids, are valuable.Whey proteins are powerful, natural and healthy chelators which discharge toxic heavy metals from the system. A number of amino acids present in meat proteins are satisfactory chelators. Foods like sea food, chicken, fish, red meat are natural chelators of heavy metals that are toxic. Soya protein is deficient in the required branched chain amino acids (Kingmann *et al.*, 2005).

2.27 METALLOTHIONEINS

Metallothioneins (MTs) belong to the family of cysteine-rich, low molecular weight, metal-chelating proteins that are produced naturally within the cells of the body. The levels of Selenium, copper, and zinc concentrations are controlled by MTs which also detoxify or bind to silver, platinum, mercury, and cadmium. Reactive oxygen species (ROS) or free radical ions like heavy metals are kept from causing injury to the lipids of the cell membrane, DNA of the mitochondrion and the destruction of the cell's ATP (energy) production structure by MTs. This is otherwise referred to as oxidative stress. Cellular DNA destruction by free radical results in cancer. The availability of dietary amino acids; cysteine, histidine, and minerals (selenium, copper, and zinc) determine the generation of MTs. Inadequate metallothioneins and the piling up of heavy metals results from the insufficient supply of these minerals and amino acids. Piling up of heavy metal in adults and infants' autism is caused by insufficient defensive metallothioneins due to zinc and amino acids deficiency which is typical of vegetarian diet. Although poor maternal nutrition may have already caused widespread destruction to the baby's brain, diet for elevated metallothioneins and reduction in

heavy metal pollution in newborn can still be useful. L-glutathione which is a Tripeptide is a product of amino acid which is considered as the most powerful antioxidant in the body. It is degraded in the digestive system into its component amino acids of glycine, glutamic acid, and cystine as such not administered orally.

About 60-80 % of the central nervous system constitutes lipids made from fatty acids, which needs to be steadily refilled. Susceptibility of the nervous system to fat soluble metals like metallic mercury which steadily escapes as odourless and invisible vapour from dental amalgam sets in with the advent of deficiency. Inadequate fluid intake may cause toxic metals to be deposited in the kidneys. The swelling of the basal membranes will adversely affect efficient filtration of toxins. Restoration of intra and extracellular fluid balance can be accomplished by the addition of small quantity of electrolyte solution that is balance in water (Klinghardt, 2002). Antioxidants like vitamins A, C and E, CoEnzyme Q-10, zinc, selenium and alpha lipoic acid, which can get fastened to toxic heavy metals and naturally remove them from the body. Colloidal minerals and mineral supplements are healthy metals that displace toxic heavy metals and gradually remove them from the body. Toxic heavy metals are responsible for the depletion of trace minerals which can be replaced by the ingestion of colloidal mineral supplements.

2.28 DESFERAL

Desferal also referred to as desferrioxamine or deferoxamine, is a subcutaneous detoxification agent for the discharge of aluminum and excess iron from the body. Blood borne free iron is fastened for elimination in the urine by desferal. Excess iron can be destructive to different organs of the body, including the liver and has been implicated in coronary artery disease.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY ONE

3.1.1 LOCATION OF STUDY

Twenty one Local Government Areas (LGA) of Delta state, were purposively selected due to the intensity of exploration activities of crude oil extraction and refining companies and were grouped into seven zones: Urhobo, Isoko, Ijaw, Itsekiri, Ukwani, Aniocha and Ika. Samples of drinking water were obtained from one farm site in each of the LGAs and analysed for Heavy Metal Concentration (HMC) (ppm) using standard procedures. Three intensively managed poultry farms in each of the zones were surveyed and used for the study**.**

URHOBO ZONE:

VETO FARM

This farm is located in Jesse town in Okpe L.G.A. of Delta state. It is an integrated farm which specializes on poultry, fish, and palm oil production. Veto farm operates a battery cage system with open water troughs as drinkers. Jesse town is characterized by a lot of vegetation and swampy ground, associated with a lot of economic activities. Jesse is one of those communities with a long history of oil pipes vandalism and crude oil spillage both on land and water. Jesse river often shows a shiny thin film on the water surface and the bank side of the water surface which served as evidence of the presence of crude oil pollution.

AGROFLOURISH FARM

This farm is situated in Ubogo town in Udu L.G.A. of Delta State, It is also an integrated farm which specializes mainly on poultry, fish, and feed milling. This farm operates a battery cage system with automatic water drinking system. Also, earthen and

concrete ponds used for catfish production. Ubogo town is characterized by a lot of economic activities which are enhanced by the existence of oil exploration companies. It is surrounded by other oil producing communities and gas flaring sites. It also consists of thick vegetation and swampy ground, streams with brackish water. Ubogo town is located between Otor-Udu, Emadadja and Egini towns.

CHRIS FARM:

This farm is located in Orogun town in Ughelli North L.G.A. of Delta State. It engages in poultry, fish and pig production. This farm operates a poultry battery cage with open trough drinking system. Orogun town has oil wells and a network of oil pipelines. Orogun town is characterized by thick vegetation and swampy areas of land.

ITSEKIRI ZONE:

EJIRO FARM

This farm is established in Ubeji town in Warri North L.G.A. of Delta State. Ejiro farm engages in poultry and crops production. Ubeji community has oil wells and a network of pipelines which supply crude oil to the Warri NNPC refinery.

KPENU FARM

Kpenu farm is located in Omadino town in Warri North L.G.A. of Delta State. Kpenu farm engages in poultry and crops production. Omadino is characterized by a lot of oil exploration activities and is associated with an age long practice of petrochemical pipelines vandalism causing spillage. Omadino community is surrounded by river (Omadino River) and swampy land. It also has thick forest vegetation and wetland.

OLOTU FARM

This farm is situated in Koko town in Warri South L.G.A. of Delta State. Olotu farm is situated on a marshy land, and is located close to a petrochemical production and processing plant. It specializes in poultry and fish farming.

ISOKO ZONE:

OVIORIE FARM

Oviorie farm is located in Igbide town after Olomoro town in Isoko South LGA of Delta State. Oviorie farm engages in poultry, fish and oil palm tree production. Igbide town is characterized by a lot of oil wells and gas flaring sites and a network of oil pipes. The town is surrounded by thick vegetation, farm land and a river (Igbide River).

GODSPOWER FARM

This farm is situated in Oleh community and it engages in poultry production only. Oleh has a Shell Petroleum Development Company's camp where a lot of oil exploration activities are taking place and it is characterized by a cross session of oil pipelines and gas flaring site.

LEO ODEGOLO FARM

This farm is located in Ozoro town in Isoko North L.G.A. of Delta State. Leo farm engages in poultry and fish production. Ozoro town is characterized by heavy socio - economic activities, it has thick forest vegetation, swamps and farm land with pockets of swampy areas.

IJAW ZONE:

JOHNSON FARM

Johnson farm is located in Ogulaha town in Burutu L.G.A. of Delta State. Johnson Farm engages in poultry and catfish production. Ogulaha town has a SHELL company tank farm, a lot of oil wells and a network of oil pipelines. Ogulaha town is surrounded by the Atlantic Ocean.

LIZZY FARM

This farm is located in Ogbe-Ijoh in Warri South-West L.G.A of Delta State. Lizzy farm is a poultry farm with a laying capacity of about 1,500 birds. The community is also surrounded by river.

EBI POULTRY FARM

This farm is situated in Burutu town in Burutu L.G.A. of Delta State. Ebi farm is a poultry farm with a laying capacity of about 900 birds in battery cages.

UKWANI ZONE

AZUNZE FARM

This farm is found in Kwale town in Ndokwa west L.G.A. of Delta State. Azunze farm is a poultry farm with laying birds in battery cages and broilers in the deep litter system. Azunze farm is situated at the outskirt of Kwale town. Kwale town is characterized by oil exploration activities which enhance the socio – economic state of the town. It is surrounded by trees and large expanse of farm land.

KONUN FARM

Konum farm is situated in Amai in Ukwani L.G.A. of Delta State. It is an integrated farm with poultry and catfish sections. It also has accommodation and suite, restaurant and conference centre in the same premises. It is situated in the suburb of Amai town.

ELIKE FARM

Elike farm is located in Ubiaruku in Ukwani L.G.A. of Delta State. It engages in poultry production with a Garden for the production of exotic fruits and vegetables.

IKA ZONE:

PETEROSA FARM

This farm is located in Agbor South L.G.A. of Delta State. It engages in poultry production only with a laying capacity of about 6,000 birds in battery cage system and about 1,000 broilers in deep litter.

ABIO FARM

This farm is situated in Owa-Oyibo in Ika North L.G.A. of Delta State. It engages in poultry production with the laying birds of about 1,900 on deep litter system. Agbor North is across the river Niger.

PHED FARM AND HATCHERY

This farm is located in Agbor South L.G.A. of Delta State. It engages in the production and sales oftable eggs andDayold chicks. PHED farm is situated at the outskirt of Agbor along Isele-ukwu Benin High way.

ANIOCHA/OSHIMILI ZONE:

OBIORA FARM

This farm is situated in Ajasha village after Ibusa town in Aniocha south L.G.A. of Delta State. It engages in poultry and fish production.

NWANKWO POULTRY FARM

This farm is found in Isele-ukwu, in Oshimili South in Delta State. Nwankwo farms engage in poultry production.

FAITH FARM

Faith farmis located in Ogbolu town in Oshimili South L.G.A. of Delta State. It engages in poultry production and it is situated along Illah-Asaba road, in Delta State.

3.1.2 COLLECTION OF SAMPLES

Samples of drinking water were collected from each farm in each L.G.A., a sample per farm, across seven zones in Delta State. About 500 ml sample of drinking water was obtained per farm stored in a clean glass container and coveredproperly. The samples were transferred immediately to the laboratory for heavy metals determination.

3.1.3 PRESERVATION OF SAMPLES

Samples of water were treated with approximately 3ml of HNO_3 and deionised water per 250ml in a ratio of 1:3 (USEPA, 2002).

3.1.4 DIGESTION OF WATER SAMPLES

Water samples were digested and according to the method described by Draghici *et al*. (2010).

3.1.5 CHEMICAL ANALYSIS

Digested water samples were analysed according to AOAC (1995), while metal concentrations were analysed using the Atomic Absorption Spectrophotometer (AAS).

3.1.6 PARAMETERS ANALYSED

Chemical analysis of water samples used for heavy metals - Nickel (Ni), Mercury (Hg), Vanadium (V) Iron (Fe) and Cadmium (Cd). Water samples were analysed according to AOAC (1995). The heavy metals concentrations were analysed by the process of Spectrophotometry (AAS).

3.1.7 STATISTICAL DESIGN

Statistical design was a Completely Randomised Design

3.1.8 STATISTICAL ANALYSIS

Analysis of data was carried out using ANOVA, while separation of means was done using Fisher's Least Significant Difference (LSD) on SAS (2009).

3.2 STUDY TWO

3.2.1 LOCATION OF STUDY

Twenty one Local Government Areas (LGAs) in Delta State were purposively selected due to the intensity and activities of crude oil exploration and grouped into seven zones: Urhobo, Isoko, Ijaw, Itsekiri, Ukwani, Aniocha and Ika. Broiler chicken samples were obtained from one farm in each LGA and analysed for Heavy Metal Concentration (HMC) (ppm) using standard procedures. Three intensively managed poultry farms in each zone were surveyed and used for on-farm trials**.**

3.2.2 COLLECTION OF SAMPLES AND SAMPLE SIZE

Broiler chickens totalling 105 were randomly selected and used for the study, 3 farms in each zone, across 7 zones in Delta State.

Sampling: Samples of chicken blood were obtained from three birds per farm, through the large veins in the chicken neck using 5mls clinical syringe into EDTA bottles and test tubes.

3.2.3 PARAMETERS ASSESSED

Haematological parameters include: Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Count (MCHC) and leukocyte count.

Serum indices include: albumin, globulin, total protein, Alkaline Phosphatase (ALP), uric acid, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and total cholesterol.

Histology: Kidney and liver sections from each bird were obtained, prepared into slides for histological evaluation and microscopic examination (Pandey and Chauchan, 2007)

3.2.3.1 ASSESSMENT OF MEAT SAMPLES

Evaluation of meat samples for heavy metal residues according to AOAC (1995) and AAS methods.

The samples analyzed in this study were the breast cut from slaughtered Broiler chickens. All samples were obtained from poultry farms in Delta State. One hundred and five samples were collected, broiler chickens weighing about 2 to 3.5 kg live weights. The collected chicken samples stored inside a flask containing ice flakes were transferred to the laboratory. The samples were oven dried at 65°C until no further weight changes was observed. Samples were crushed and pulverized with the aid of ceramic mortar and pestle. About 10 to 20g of ground meat samples were put in polyethylene vials and stored in envelopes before they were finally stored in air-tight containers. Preparation of standards was done by adding 5 to l1% aliquot of newly made standard solution (1000 ppm) of each element on Whatman's filter paper. Atomic absorption spectroscopy was used as the characterization technique for the conducted study.

3.2.4 DETERMINATION OF HEAVY METALS IN MEAT

Preparation and determination of heavy metals in Chicken meats samples were carried out according to the procedure described by Keepax *et al*. (2011)

3.2.5 STATISTICAL ANALYSIS

Analysis of the data was carried out using ANOVA, while separation of means was done using Fisher's Least Significant Difference (LSD) on SAS (2009).

3.3 STUDY THREE

3.3.1 LOCATION OF STUDY

Twenty-one Local Government Areas (LGA) of Delta state, were selected purposively due to the intensity and activities of the exploration of crude oil and were grouped into seven zones: Urhobo, Isoko, Ijaw, Itsekiri, Ukwani, Aniocha and Ika.

Layer chicken were obtained from a farm from each LGA and analysed for Heavy Metal Concentration (HMC) (ppm) using standard procedures. Three intensively managed poultry farms in each zone were surveyed and used for on-farm trials**.**

Samples of eggs were obtained from a farm from each LGA and analysed for Heavy Metal Concentration (HMC) (ppm) using standard procedures.Three intensively managed poultry farms in each of the zones were surveyed and used for the on-farm trials**.**

3.3.2 SAMPLE SIZE

A total of 105 laying chickens were used for this study, 3 farms in each zone, across seven zones in the State.

3.3.3 EGG SAMPLES COLLECTION

In March, 2015 fresh egg samples from laying hens (5 per cent hen day egg production) per farm were randomly collected from 21 different commercial poultry farms across the seven zones. A total of 1890 eggs were collected for the study, 3 farms from each zone in Delta State. Among them 5 per cent of the total egg samples collection per farm was randomly selected for analysis. Fresh egg samples in well labelled transparent clean egg crates were immediately transferred to the laboratory for samples preparation, digestion and analysis of heavy metals; Nickel (Ni), Mercury (Hg), Vanadium (V), Iron (Fe) and Cadmium (Cd) concentrations.

3.3.4. HEAVY METAL DETERMINATION IN EGGS

The preparation, digestion and determination of heavy metals in egg samples were carried out according to the procedure described by Keepax *et al*. (2011).

3.3.5 BLOOD SAMPLE COLLECTION

A total of 105 randomly selected layer hens were used for the study, 3 farms in each zone, across 7 zones in Delta State.

Blood Sampling: About 5 ml of blood samples were obtained with the aid of a 5ml clinical syringe fitted with a 24-gauge sterilized needle through the jugular veins of three birds in each farm. About (2.5 ml) of the blood was released into a pre–labelled blood collection bottle containing ethylenediaminetetraacetic acid (EDTA) as anti-coagulant and shaken gently in order to prevent clotting of the blood. The rest of the blood was transferred into blood collection test tubes without EDTA to form clot. The serum was decanted into clean, labelled blood collecting tubes for serum analysis. Haemolysed blood samples were discarded. The tubes containing blood were taken for serum biochemical and haematological analysis in the laboratory, using routine clinical laboratory procedures.

3.3.6 PARAMETERS ASSESSED

Haemoglobin (Hb): The haemoglobin content was determined with a Digital Photo Colorimeter Model 321E (Digital Photo Instruments Germany) at a wavelength of 540nm after blood had been mixed with Drabkins solution in a ratio of 2:200 and expressed in g/dl.

Packed Cell Volume (PCV): The PCV was done with the aid of a Winthrobe's micro-haematocrit technique. Some quantity of uncoagulated blood was allowed to flow by capillary action into a 16mm capillary tube and sealed at one end with animprovised sealer. They were centrifuged at approximately three hundred rotations per minute (300 rpm) to separate the blood into its cell and plasma components. The lengths of plasma cells and red blood cells in the capillary tubes were measured with the aid of haematocrit-reader, and expressed as a percentage of the entire column.

Red Blood Cell Count (RBC): This was determined with the aid of Electronic counter model 2F. The RBC was expressed in million per micro millimeter cubed (mm^3) of blood.

White Blood Cell Counts (WBC): this parameter was obtained with the aid of standard diluting pipettes and counting in an improved Naubauer haemocytometer. The WBC counts was expressed in thousands per microlitres or per million.

MCV, MCH and MCHC: these parameters were computed with the formulae expressed by Lindqvist (1995).

Thus: MCV= $\frac{\text{Hb}}{\text{RBC}}$ x 100

expressed in picogram/cell MCHC=

$\frac{\text{Hb}}{\text{PCV}}$ x 100

expressed in gram per decilitre

Plasma Protein: The plasma protein was obtained through hand refractometer technique, and expressed in grams per litre (g/l) of blood.

Evaluation of differentials: The monocytes, lymphocytes, neutrophils, eosinophils and basophils was calculated using differential counter techniques with the formula;

$\frac{\text{Each differential counts}}{\text{Total Number of differential counts}} \text{ x } \frac{100}{1}\%$

Serum Parameters: The serum parameter that was determined include, total protein (TP), Albumin (Alb), cholesterol and Globulin.

Total protein (TP): Total protein was determined by the Biuret method and will be calculated thus; $TP = \frac{Samplexconc.ofstd}{Absorbentofstandard}$

Where standard = 0.290

Concentration = 5.99 g/dl

Albumin (Alb): Albumin was determined by the colorimetric technique with formula:

$$Albuming/dl = \frac{Samplexconc.ofstd}{Absorbentofstandard}$$

Where standard = 1.290

Concentration = 5g/dl

Cholesterol: Cholesterol was obtained by enzymatic colorimetric method with formula:

$$Cholesterol = \frac{Samplexconc.ofstandard}{Absorbentofstandard}$$

Where standard = 0.521290

Concentration = 206g/dl

Globulin: this serum index was determined by subtracting the Albumin from the total protein.

Thus;

Globulin = Total Protein – Albumin

3.3.7 ORGAN HISTOLOGY

Sections of the liver and kidney, from each bird were collected

Slide preparation and histological evaluation and microscopic examination (Pandey and Chauchan, 2007).

3.3.8 STATISTICAL ANALYSIS

Analysis of the data was carried out using ANOVA, while separation of means was done using Fisher's Least Significant Difference (LSD) on SAS (2009).

3.4 STUDY FOUR

3.4.1 STUDY LOCATION

The study was under-taken at the Poultry Section, of the Teaching and Research Farm, University of Ibadan, during a 49 day period (7 weeks) (March 6th – April 20th, 2017).

3.4.2 PEN PREPARATION

The poultry house was cleaned, washed and disinfected; thereafter it was partitioned into 44 pens of 2m×1m floor spacing, with wood and wire mesh, in open sided pen house. Wood shavings were evenly spread as litter materials on the floor and changed periodically as at when due. Feeders and drinkers were adequately provided for each pen.

3.4.3 EXPERIMENTAL ANIMALS

One-day old Arbor-Acres strain of broiler chicks (n = 288) were procured for the study. Brooding of the birds was done together for a period of 7-days under good illumination and poultry management condition. At the end of which, the average weight of the birdswas obtained and birds were distributed randomly into 9 groups of 32 birds per treatment, with four replicates of 8 birds each in a completely randomised design. Administration of water and feed was done *ad-libitum.*

3.4.4. EXPERIMENTAL TEST SAMPLES

Mitigating agents: ethylenediaminetetraacetic acid (EDTA) and activated charcoal (AC), were each introduced orally in drinking water at 50 mg/l for broiler chickens. EDTA and AC were procured from a reliable licenced laboratory chemical shop in Ibadan, Oyo State.

Drinking water samples were obtained from Koko, Udu and Igbide for Water Relatively High in: Vanadium (23.973 ppm) (WRHV); Cadmium (17.5 ppm) (WRHC) and Iron (1445 ppm) (WRHI), were used for the study.

3.4.5 EXPERIMENTAL LAYOUT

Treatment 1: Water Relatively High in Vanadium

Treatment 2: Water Relatively High in Vanadium + EDTA

Treatment 3: Water Relatively High in Vanadium + charcoal

Treatment 4: Water Relatively High in Cadmium

Treatment 5: Water Relatively High in Cadmium + EDTA,

Treatment 6: Water Relatively High in Cadmium + Charcoal,

Treatment 7: Water Relatively High in Iron,

Treatment 8: Water Relatively High in Iron + EDTA,

Treatment 9: Water Relatively High in Iron + charcoal,

3.4.6 ANIMAL HEALTH MANAGEMENT

A standard prophylactic poultry health programme was carefully carried out adhering to best practices. Routine monitoring of the health status of the birds was carefully carried out for any health challenges or signs of morbidity and mortality.

3.4.7 DATA COLLECTION

The growth performances of the birds, blood parameters, and histology of selected organs, heavy metals in chicken samples were assessed.

3.4.8 BLOOD SAMPLE COLLECTION

A 5 ml syringe was used to collect blood by insertion through the jugular veins from two birds per replicate, at the 3^{rd} and 6^{th} weeks. Half (2.5 ml) of the collected blood was released into a pre–labelled blood collection containing ethylenediaminetetraacetic acid (EDTA) as anti-coagulant and agitated gently in order to prevent clotting of the blood. The rest of the blood was transferred into blood collection test tubes without EDTA to clot. The serum was decanted into clean, labelled blood collecting tubes for biochemical analysis. The samples were transferred to the department's laboratory for haematological and serum biochemical analysis, using routine clinical laboratory procedures.

3.4.9 BLOOD PARAMETERS ASSESSED

Haemoglobin (Hb): The Haemoglobin content was determined with the aid of a Digital Photo Colorimeter, Model 321E (Digital Photo Instruments, Germany) at a wavelength of 540nm after blood had been mixed with Drabkins solution in a ratio of 2:200 and expressed in g/dl.

Packed Cell Volume (PCV): The PCV was done with the aid of a Winthrobe's micro-haematocrit technique. Some quantity of uncoagulated blood was allowed to flow by capillary action into a 16mm capillary tube and sealed at one end with an improvised sealer. They were centrifuged at approximately three hundred rotations per minute (300 rpm) to separate the blood into its cell and plasma components. The lengths of plasma cells and red blood cells in the capillary tubes were measured with the aid of haematocrit-reader, and expressed as a percentage of the entire column.

Red Blood Cell Count (RBC): This was determined with the aid of Electronic counter model 2F. The RBC was expressed in million per micro millimeter cubed (mm^3) of blood.

White Blood Cell Counts (WBC): This parameter was obtained with the aid of standard diluting pipettes and counting in an improved Naubauer haemocytometer. The WBC counts was expressed in thousands per microlitres or per million.

MCV, MCH and MCHC: These parameters were computed with the formulae expressed by Lindqvist (1995).

Thus:

$MCV = \frac{Hb}{RBC} x\ 100$ expressed in picogram/cell

$MCHC = \frac{Hb}{PCV}\ x\ 100$ expressed in gram per decilitre

Plasma Protein: The plasma protein was obtained through Hand Refractometer technique, and expressed in grams per litre (g/l) of blood.

Evaluation of differentials: The Monocytes, lymphocytes, Neutrophils, Eosinophils and Basophils will be calculated using differential counter techniques with the formula; $\frac{\text{Each differential counts}}{\text{Total Number of differential counts}} \text{X} \frac{100}{1}\%$

Serum Parameters: The serum parameters that were determined include, total protein (TP), Albumin (Alb), cholesterol and Globulin.

Total protein (TP): Total protein was determined by the Biuret method and will be calculated thus; $TP = \frac{Sample \text{ x } conc.of\ std}{Absorbent\ of\ standard}$

Where standard = 0.290

Concentration = 5.99 g/dl

Albumin (Alb): Albumin was determined by the colorimetric

$$Albumin\ g/dl = \frac{Sample \text{ x } conc.\ of\ std}{Absorbent\ of\ standard}$$

Where standard = 1.290

Concentration = 5g/dl

Cholesterol: Cholesterol was obtained by enzymatic colorimetric method with formula: $Cholesterol = \frac{Sample \text{ x } conc.of standard}{Absorbent\ of\ standard}$

Where standard = 0.521290

Concentration = 206g/dl

Globulin: this serum index was determined by subtracting from the total protein the Albumin.

Thus;

Globulin = Total Protein – Albumin

3.4.10 PERFORMANCE CHARACTERISTICS

The growth performance of birds was assessed by the initial body weight, weekly live weights, final body weight, live weight gain, daily water and feed intake, conversion efficiency of feed and percentage mortality of birds.

At day 35, two chickens were randomly selected per replicate housed in metabolic cages from which the total excreta were collected, dried and stored. On day fourty one of the trial all chickens were starved of feed overnight to minimise interference from intestinal contents and their final weight obtained. At day 42, two chickens were selected and randomly weighed, scalded and slaughtered before dissecting to obtain the visceral organs, for organs weights and Gut length. The characteristics of broiler chicken carcasses and primal parts and cuts were evaluated. Deboned thighs of slaughtered chickens and excretasamples collected from birds during the trial were analysed for residual heavy metals concentrations -Nickel (Ni), Mercury (Hg), Vanadium (Vn), Iron (Fe) and Cadmium (Cd), using standard procedures as described by AOAC (1995) and AAS methods (Haswell, 1991; Varma, 1984).

3.4.11 HISTOLOGY OF SELECTED ORGANS

Section of the liver and kidneys, from two birds per replicate were collected and prepared into slide by a method described by Ewuola (2009). Histopathological examination and microscopic evaluation was done by the procedure of Pandey and Chauchan (2007).

3.4.12 HEAVY METALS DETERMINATION

The analyzed samples used in the study were bore-hole water, Broiler chickens meat and excreta samples from broiler chickens.

Preparation and determination of heavy metals in Broiler chicken meat samples were carried out according to the procedure described by Keepax *et al*. (2011).

3.4.13 EXPERIMENTAL DESIGN

The design of the study was a 3x3x3 factorial arrangement in a complete randomised design; as heavy metal binding agents as either of EDTA or Ac and water with no binding agent, on Cd, V and Fe heavy metals in drinking water from three locations, using General Linear Model Procedure of SAS.

3.4.14. STATISTICAL ANALYSIS

Descriptive statistics and ANOVA were applied in the data analysis ($p<0.05$), while means of treatments were compared using orthogonal contrast.

Table 3.1. Experimental Diets gross composition (g/100g dm)

INGREDIENTS	STARTER	FINISHER
Maize	60.00	61.00
Soy bean meal	32.00	27.50
Fish meal	3.00	1.00
Wheat bran	2.00	7.00
DCP	1.00	1.50
Limestone	1.50	2.00
Salt	0.35	0.35
Vitamin Premix	0.25	0.25
DL Methionine	0.10	0.10
HCL Lysine	0.15	0.15
TOTAL	100.00	100.00
CP (%)	21.92	19.47
Calcium (%)	1.10	1.00
Available Phosphorus (%)	0.60	0.60
Lysine HCL (%)	1.20	1.10
Crude fibre (%)	3.90	4.00
Metabolizable Energy (Kcal/kg)	2976.80	2909.50

CHAPTER FOUR

RESULTS

4.1.1 HEAVY METAL CONCENTRATION (PPM) IN WATER SAMPLES IN URHOBO (ZONE ONE)

The Heavy metal concentrations in samples of drinking water in Urhobo zone one are shown in Table 4.1. Water samples in Urhobo zone vanadium level in water was highest at Orogun (0.105) and which was significantly ($P<0.05$) different when compared to those in Jesse (0.065) and Udu (0.031). Also, the level of V in water sample in Udu (0.031) significantly ($P<0.05$) was smaller than that of Jesse (0.065). Cadmium (Cd) concentration in water has a higher significant ($P<0.05$) values in samples from Udu (17.47) and Jesse (16.903) than those from Orogun (12.57), while the cadmium levels in Udu and Jesse were not different significantly ($P<0.05$). Similarly, the iron concentration in Orogun (1353.3) had a higher significant ($P<0.05$) value when compared to those in Udu (206.6) and Jesse (446.7) with Udu (206.6) having the least concentration. The levels of Nickel (Ni) in the samples of water had no significant ($P<0.05$) variation across the Locations. However, levels of Mercury (Hg) in Orogun (0.012) had a higher significant ($P<0.05$) value when compared with those of Udu (0.009) and Jesse (0.006).

Table 4.1. Heavy metal concentrations (ppm) in water samples in Urhobo zone, Delta State

	Locations				
Parameters	**Udu**	**Orogun**	**Jesse**	**SEM**	**Permissble Limits**
Vanadium	0.031[c]	0.105[a]	0.065[b]	0.011	0.09-0.34
Cadmium	17.467[a]	12.567[b]	16.903[a]	0.792	0.00-0.005
Iron	206.6[c]	1353.3[a]	446.7[b]	21.425	0.30
Nickel	0.010	0.011	0.007	0.002	0.00-1.12
Mercury	0.009[b]	0.012[a]	0.006[b]	0.003	1.00-2.68

[abc:]means within the rows with same superscripts are not different. significantly ($P<0.05$)

SEM; Mean Standard Error. *Permissible limits of heavy metals (WHO, 2004)

4.1.2 HEAVY METAL CONCENTRATION (PPM) IN WATER SAMPLES FROMISOKO ZONE

Table 4.2 presents the levels of Cadmium, Vanadium, Iron, Nickel and Mercury in drinking water samples in Isoko zone. Vanadium concentration had a higher significant ($P<0.05$) value water samples from Oleh (6.72) when compared with those from Ozoro (0.02) and Igbide (0.13). The cadmium concentration had a significant lower value ($P<0.05$) in the samples from Igbide (14.88) than those in Ozoro (16.12) and Oleh (16.45). However, the concentrations in samples from Ozoro (16.12) and Oleh (16.45) did not show any significant ($P<0.05$) variation between them. Iron concentration also displayed high valuesin Igbide (1445.0) and Oleh (1408.0), but varied significantly ($P<0.05$) from that of Ozoro (165.0). The concentration of Nickel in water samples was highest in Ozoro (0.021) and it varied significantly ($P<0.05$) from values obtained in samples in Igbide (0.011) and Oleh (0.007), with Oleh (0.007) having the least concentration.However, the concentrations of mercury in water samples from Ozoro (0.003) had the least concentration, while those of Igbide (0.016) and Oleh (0.015), did not show any significant ($P<0.05$) variation between them.

Table 4.2: Heavy metal concentration in water samples inIsokozone

	Locations				
Parameters	**Ozoro**	**Igbide**	**Oleh**	**SEM**	**Permissible Limits**
Vanadium	0.020[b]	0.130[b]	6.720[a]	0.946	0.09-0.34
Cadmium	16.120[a]	14.883[b]	16.453[a]	0.249	0.00 - 0.005
Iron	165.000[b]	1445.000[a]	1408.000[a]	203.189	0.30
Nickel	0.021[a]	0.011[b]	0.007[c]	0.002	0.00-1.12
Mercury	0.003[b]	0.016[a]	0.015[a]	0.003	1.00 - 2.68

[abc:]means within the same row with similar superscripts are not different significantly (P<0.05).
SEM: Standard Error of Mean. *Permissible limits (WHO, 2004)

4.1.3 HEAVY METAL CONCENTRATION (PPM) IN WATER FROM ITSEKIRI ZONE

Table 4.3 shows the heavy metals concentration in water in Itsekiri zone. The water samples from Koko contained (23.97) vanadium and was significantly ($P<0.05$) higher when compared with those from Ubeji (14.49) and Omadino (13.95), while the least vanadium concentration was observed in water samples from Ubeji (14.49). Cadmium level in Ubeji (14.907) had a higher significant ($P<0.05$) value which varied from that of Koko (13.60); Omadino (13.95). Also, the concentrations of iron in Ubeji (1413.0), Omadino (1263.0) and Koko (310.0) had varied significantly ($P<0.05$) across the locations, while thehighest concentration was observed in Ubeji (1413.0) and least in Koko (310.0). However, the levels of nickel in water samples was highest in Koko (0.023) and differed significantly ($P<0.05$) from the values observed in samples from Ubeji (0.011) and Omadino (0.009), but there were no differences between Ubeji (0.011) and Omadino (0.009). While, the mercury level in water samples in Omadino (0.058), Ubeji (0.026) and Koko (0.007) hadvaried significantly accross the locations, Omadino (0.058) was highest in concentration, with the least concentrations observed in Koko (0.007).

Table 4.3: Heavy metal ions concentration (ppm) in water from zone three (Itsekiri)

	Locations				
Parameters	**Koko**	**Ubeji**	**Omadino**	**SEM**	**Permissible Limits**
Vanadium	23.973[a]	14.490[c]	20.103[b]	3.712	0.09-0.34
Cadmium	13.597[b]	14.907[a]	13.947[b]	2.038	0.00–0.005
Iron	310.0[c]	1413.0[a]	1263.0[b]	81.795	0.30
Nickel	0.023[a]	0.011[b]	0.009[b]	0.006	0.00-1.12
Mercury	0.007[c]	0.026[b]	0.058[a]	0.007	1.00–2.68

[abc:]means within a row with different superscripts are different significantly (P<0.05).

SEM: Standard Error of Mean. *Permissible limits of heavy metals (ppm) in water (WHO, 2004)

4.1.4 HEAVY METAL CONCENTRATIONS (PPM) IN WATER INIJAW ZONE

The level of vanadium, cadmium, iron, nickel and mercury in water samples in Ijaw zone are shown in Table 4.4. The concentration of vanadium was least in Burutu (0.042) and varied significantly ($P<0.05$) when compared with those from Ogulaha (0.069) and Ogbe-ijoh (0.065), Ogulaha and Ogbe-ijoh had similar values. The cadmium concentration in water samples from Burutu (9.030) and Ogbe-ijoh (9.060) were similar($P<0.05$) and were significantly higher in Ogulaha ($P<0.05$) than 8.167 in Ogulaha (8.167). However, the iron concentration in Ogulaha (849.67), Ogbe-ijoh (340.00) and Burutu (670.00) were different significantly ($P<0.05$) across the locations, while the highest value was recorded in Ogulaha (849.67) and least in Ogbe-ijoh (340.00). Similarly, the levels of Nickel in Ogulaha (0.024) and Burutu (0.026) were similar ($P<0.05$) and both had higher in concentration of nickel than in Ogbe-ijoh (0.002). Water samples contained mercury concentrations which increased from 0.001 (Burutu) to 0.008 (Ogulaha), with Ogulaha having the highest ($P<0.05$) level while the least ($P<0.05$) mercury concentrations was noticed in Burutu.

Table 4.4: Heavy metal Concentrations (ppm) in water fromIjaw zone

	Locations				
Parameters	**Ogulaha**	**Ogbe-ijoh**	**Burutu**	**SEM**	**Permissible Limits**
Vanadium	0.069[a]	0.065[a]	0.042[b]	0.004	0.09-0.34
Cadmium	8.167[b]	9.060[a]	9.030[a]	0.147	0.00–0.005
Iron	849.667[a]	340.000[c]	670.000[b]	74.690	0.30
Nickel	0.024[a]	0.002[b]	0.026[a]	0.004	0.00-1.12
Mercury	0.008[a]	0.003[b]	0.001[c]	0.002	1.00-2.68

[abc:]means within the same row with the same superscripts are not different significantly (P<0.05). SEM: Standard Error of Mean. *Permissible limits of heavy metals (ppm) in water (WHO, 2004)

4.1.5 HEAVY METAL CONCENTRATIONS (PPM) IN WATER SAMPLES IN UKWANI ZONE

Table 4.5 presents the heavy metal concentrations in drinking water from Ukwani zone. Vanadium concentration was highest in Amai (0.132), which varied significantly ($P<0.05$) from the metal ion concentrations in water from Kwale (0.045) and Ubiaruku (0.012). The cadmium concentration in this zone ranged from 9.75 (Obiaruku) to 14.09 (Amai). Cadmium levels across all three locations showed significant ($P<0.05$) variations. Similarly, Iron concentrations in water from Ubiaruku (1308.00) was higher and had a significant ($P<0.05$) variation from the concentrations obtained in water from Kwale (150.00) and Amai (273.33).

However, the levels of nickel in water samples was higher and differsignificantly ($P<0.05$) from the levels observed for Ubiaruku (0.006) and Amai (0.005). Also, the levels of mercury in water sample in Kwale (0.012) was observed to be higher and varied significantly ($P<0.05$) when compared with those from Ubiaruku (0.009) and Amai (0.007).

Table 4.5: Heavy metal Concentrations (ppm) in water samples in Ukwani zone

	Locations				
Parameters	**Kwale**	**Ubiaruku**	**Amai**	**SEM**	**Permissible Limits**
Vanadium	0.045[b]	0.012[c]	0.132[a]	0.021	0.09-0.34
Cadmium	11.577[b]	9.733[c]	14.090[a]	0.632	0.00–0.005
Iron	150.00[b]	1308.00[a]	273.33[b]	185.365	0.30
Nickel	0.009[a]	0.006[b]	0.005[b]	0.002	0.00-1.12
Mercury	0.026[a]	0.009[b]	0.007[b]	0.006	1.00–2.68

[abc:]means within the rows with different superscripts are different significantly (P<0.05).

SEM: Standard Error of Mean. Permissible limits of heavy metals (ppm) in water (WHO, 2004).

4.1.6 HEAVY METAL CONCENTRATIONS (PPM) IN WATER SAMPLES IN ANIOCHA ZONE

The levels of heavy metal ions in the samples of water from Aniocha zone is shown in Table 4.6. The levels of vanadium in this zone did not show any significant ($P<0.05$) variations across the locations. The cadmium concentration in water samples was higher in Ogwachukwu (9.068) and showed significant ($P<0.05$) variation when compared with those observed in Isele-ukwu (8.167) and Asaba (6.355), with Asaba (6.355) having the least concentration of cadmium. Also, the concentrations of iron in Aniocha ranged from 324.3 (Ogwachukwu) to 799.3 (Isele-ukwu), with the least concentration observed in Ogwachukwu (324.3). However, the levels of nickel in water samples was higher in Isele-ukwu (0.012) and different significantly ($P<0.05$) from those of Asaba (0.004) and Ogwachukwu (0.002). Similarly, mercury in water samples from Iseluku (0.06) and Asaba (0.013), did not show any significant ($P<0.05$) variations between them, but were both higher than that of Ogwachukwu (0.003).

Table 4.6: Heavy metal concentrations (ppm) in water samples from Aniocha Zone

	Locations				
Parameters	**Isele-ukwu**	**Asaba**	**Ogwashukwu**	**SEM**	**Permissible Limits**
Vanadium	0.080[a]	0.08[a]	0.069[a]	0.032	0.09-0.34
Cadmium	8.167[b]	6.355[c]	9.068[a]	0.402	0.00–0.005
Iron	799.3[a]	369.7[b]	324.3[b]	110.028	0.30
Nickel	0.012[a]	0.004[b]	0.002[b]	0.003	0.00-1.12
Mercury	0.006[a]	0.013[a]	0.003[b]	0.003	1.00–2.68

[abc:]means within the rows with the same superscripts are not different significantly (P<0.05).

SEM: Standard Error of Mean. *Permissible limits of heavy metals (ppm) in water (WHO, 2004).

4.1.7 HEAVY METAL CONCENTRATIONS (PPM) IN WATER FROM IKA ZONE

Levels of vanadium, nickel, iron, cadmium and mercury in water samples in Ika zone are shown in Table 4.7. The concentration of vanadium ranged from 0.045 (Agbor North) to 0.067 (Agbor south), did not show any significant ($P<0.05$) variation across the location within this zone. Also, cadmium concentration in Agbor south-2 (11.24) had higher concentration which varied significantly ($P<0.05$) when compared to other values recorded from Agbor north (9.41) and Agbor south (9.70). However, the iron concentration in Ika zone ranged from 340.00 (Agbor north) to 849.67 (Agbor south) and all were different significantly ($P<0.05$) across the location. While, the nickel concentrations from Agbor south (0.018), Agbor north (0.011) and Agbor south-2 (0.001), exhibited significant ($P<0.05$) variation across the locations, the least concentration was observed in Agbor south-2 (0.001) and Agbor-south recorded the highest concentration (0.018). Mercury levels from Ika water samples had a range of 0.007 (Agbor south-2) to 0.058 (Agbor north), with Agbor-north having the highest concentration (0.058) and the least was in Agbor south-2 (0.007).

Table 4.7: Heavy metal concentrations (ppm) in water from Ika Zone

Parameters	Locations			SEM	PermissbleLimits
	Agbor North	Agbor South	Agbor South2		
Vanadium	0.045	0.067	0.050	0.005	0.09-034
Cadmium	9.407[b]	9.697[b]	11.237[a]	0.306	0.00-0.005
Iron	340.00[c]	849.667[a]	670.001[b]	74.690	0.30
Nickel	0.011[b]	0.018[a]	0.001[c]	0.003	0.00-1.12
Mercury	0.058[a]	0.011[b]	0.007[c]	0.008	1.00–2.68

[abc:]means within a row with the same superscripts are not different significantly (P<0.05).

SEM: Standard Error of Mean. Permissible limits of heavy metals (ppm) in water (WHO, 2004).

4.1.8 HEAVY METAL CONCENTRATIONS (PPM) IN WATER FROM SEVEN ZONES WITHIN DELTA STATE

The concentrations of vanadium (V), nickel (Ni), iron (Fe), mercury (Hg) and cadmium (Cd), in drinking water in seven zones within Delta State are shown in table 4.8. The concentrations of V in water samples in Isoko (22.29) and Itsekiri (29.19), showed higher significant ($P<0.05$) values when compared with those observed in Urhobo (0.067), Ijaw (0.059), Ukwani (0.055), Aniocha (0.023) and Ika (0.054) respectively. While, the concentrations of Vanadium in water samples from; Urhobo (0.067), Ijaw (0.059), Ukwani (0.055), Aniocha (0.023) and Ika (0.054) respectively, did not show any significant ($P<0.05$) variations among them. Cadmium concentrations in the samples of water from Urhobo (15.68) and Isoko (14.82) were higher and differ significantly ($P<0.05$) from those observed in all the other zones. While, the cadmium concentrations in drinking water in Itsekiri (12.32) was significantly different from those of Urhobo (15.68), Isoko (14.82), Ijaw (8.75) and Aniocha (7.86), it did not show any significant ($P<0.05$) difference from those recorded in Ukwani (11.80), together with Ika (10.11).

Also, drinking water in Isoko (2002.20) had significantly higher significant ($P<0.05$) iron concentration when compared with those from Ijaw (619.9), Ukwani (577.1), Aniocha (497.8) and Ika (619.9), but did not show any significant ($P<0.05$) differencewhen compared to those recorded in Urhobo (1334.6), Itsekiri (1162.0) together with Ika (1275.1). The nikel concentrations of drinking water in Isoko (0.013) and Ijaw (0.017) was a higher significantly ($P<0.05$) variation when they were compared with the concentration values recorded for water samples from Urhobo (0.010), Itsekiri (0.001), Ukwani (0.003), Aniocha (0.002) and Ika (0.009). Mercury concentrations in drinking water from Urhobo (0.001), Isoko (0.009), Ijaw (0.001), Ukwani (0.003) and Ika (0.002) were lowered significantly ($P<0.05$) than values observed in water from Itsekiri (0.030) and Aniocha (0.025) for Hg..

Table 4.8: Heavy metal concentrations (ppm) in water from seven zones in Delta State

	ZONES								
Parameter	**Urhobo**	**Isoko**	**Itsekiri**	**Ijaw**	**Ukwani**	**Aniocha**	**Ika**	**SEM**	**P. Limits**
Vanadium	0.067^{b}	22.290^{a}	29.189^{a}	0.059^{b}	0.055^{b}	0.023^{b}	0.054^{b}	2.261	0.09-0.34
Cadmium	15.682^{a}	14.819^{a}	12.317^{b}	8.752^{c}	11.800^{bc}	7.863^{c}	10.113^{bc}	0.828	0.00-0.005
Iron	1334.60^{ab}	2002.20^{a}	1162.00^{ab}	619.90^{b}	577.10^{b}	497.80^{b}	619.90^{b}	118.127	0.30
Nickel	0.010^{b}	0.013^{a}	0.001^{b}	0.017^{a}	0.003^{b}	0.002^{b}	0.009^{b}	0.002	0.00-1.12
Mercury	0.001^{b}	0.009^{b}	0.030^{a}	0.001^{b}	0.003^{b}	0.002^{b}	0.025^{a}	0.002	1.00-2.68

$^{abc:}$means along the same row with the same superscripts are not different significantly ($P<0.05$).. SEM: Standard Error of Mean.
*P: Permissible limits (WHO, 2004).

4.1.9 HAEMATOLOGICAL INDICES OF BROILER CHICKENS FROM SEVEN ZONES IN DELTA STATE

Table 4.9 shows the Haematological indices of birds from different zones with crude oil exploration activities. The Packed Cell Volume (PCV) of broiler chickens obtained in Ijaw 48.33% did not show any significant (P<0.05) variation from those in Ika 46.67%, but had a higher significant (P<0.05) value different from those recorded in Urhobo (44.78%), Isoko (37.22%), Itsekiri (35.11%), Ukwani (35.33%) and Aniocha (38.11%), while that of Ika (46.67%) showed no significant variation from that of Urhobo (44.27%).

The Red Blood Cell (RBC) values of broiler chickens in Ijaw (5.63ul) was higher significantly (P<0.05) than those values recorded in all other zones, but was not higher than that of Urhobo (5.33ul). No significant (P<0.05) variations was observed in values of red blood cells from Urhobo (5.33ul) and Ika (5.17ul). While, Ukwani (4.49 ul), Aniocha (4.27ul), Isoko (3.84ul) and Itsekiri (2.93ul) did not exhibit any significant (P<0.05) variations among them, but were different significantly (P<0.05) from those in Urhobo (5.33ul) and Ika (5.17ul). While, the red blood cell values of birds in Ukwani zone (4.49ul) vary significantly (P<0.05) from those in Isoko (3.84ul) together with Itsekiri (2.93ul), but they did not show any significant (P<0.05) variation from those of Aniocha (4.27ul). The platelets of birds in Urhobo (25.11), Ijaw (26.33) and Ika (27.56) were higher significantly (P<0.05) than those of Isoko (19.89), Itsekiri (18.22), Ukwani (14.67) and Aniocha (15.89), the platelets of broiler chickens in Ukwani (14.67), was lowered significantly (P<0.05) and differwhen compared to those of all other zones.

Higher significant (P<0.05) values were observed for Mean Cell Haemoglobin (MCH)in broiler chickens from Isoko (34.68) and Aniocha (33.46), when compared with values from all the other zones. The MCH of broiler chicken in Itsekiri (30.16) recorded higher significant (P<0.05) values when compared with those obtained from Urhobo (28.29) together with Ukwani (26.57). The MCH of chickens from Itsekiri (30.16) and Urhobo (28.29), did not vary significantly (P<0.05) comparing them with those obtained from

Ijaw (29.83) and Ika (29.08), while, MCH of broiler chickens in Ukwani (26.57) showed lowest significant ($P<0.05$) values among the zones.

The Mean Cell Haemoglobin Count (MCHC) obtained from broiler chickens in Ijaw (34.84) and Urhobo (34.79) vary significantly ($P<0.05$) with higher valueswhen compared to those in Isoko (34.18), Ukwani (33.73) and Ika (33.45), but they did not show any significant ($P<0.05$) variation when compared with those from Itsekiri (34.37) and Aniocha (34.32).

Table 4.9: Haematology of broiler chickens from different zones with crude oil exploration activities

Parameters	ZONES							SEM	Standards
	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika		
PCV (%)	44.78^{b}	37.22^{c}	48.33^{a}	35.11^{c}	35.33^{c}	38.11^{c}	46.67^{ab}	0.55	31.5-36.70
Hb (g/dl)	14.02	12.25	16.96	13.83	12.33	12.98	15.89	1.315	7.40-12.20
RBC (x10^{6}ul)	5.33^{ab}	3.84^{d}	5.63^{a}	2.93^{d}	4.49^{c}	4.27^{cd}	5.17^{b}	0.06	10.30-12.90
Platelets (x10^{3}mm^{3})	25.11^{a}	19.89^{b}	26.33^{a}	18.22^{bc}	14.67^{c}	15.89^{bc}	27.56^{a}	14.58	15.6-28.6
WBC (x10^{3}ul)	8.62	7.67	8.99	7.50	14.94	7.79	7.82	0.10	4.90-9.70
MCH (p%)	28.29^{c}	34.68^{a}	29.83^{bc}	30.16^{b}	26.57^{d}	33.46^{a}	29.08^{bc}	0.24	31.9-40.7
MCHC (%)	34.79^{a}	34.18^{b}	34.84^{a}	34.37^{ab}	33.73^{cd}	34.32^{ab}	33.45^{d}	46.77	25.9-33.9
MCV (fl)	84.49	95.31	89.72	85.35	81.66	90.98	87.26	0.75	102.0-129.0

$^{abcd.}$means within the rows with same superscripts are not significantly (P<0.05) different. SEM: standard Error of mean. Hb- Haemoglobin, MCV- mean cell volume, PCV- Packed cell volume, MCH- mean cell haemoglobin, MCHC- mean cell haemoglobin count, Standards (Kotula *et al.,* 1957).

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4.1.10 SERUM BIOCHEMICAL RESPONSE OF BROILER CHICKENS FROM THE SEVEN ZONES OF DELTA STATE

Serum biochemical indices of Broiler chickens from different zones with crude oil exploration activities are presented in Table 4.10.

The Albumin content of broiler chickens from Ika (4.74g/dl) had higher albumin content than the values observed in all other zones. While, those of Urhobo (4.06g/dl) and Ukwani (3.91g/dl) had significantly ($P<0.05$) higher albumin differences when compared to the values observed in Isoko (1.40g/dl), Ijaw (2.86g/dl) and Itsekiri (2.88 g/dl), but they did not show any significant ($P<0.05$) differencefrom the values of albumin obtained in Aniocha (3.50g/dl). The values of albumin content obtained from Ijaw (2.86g/dl) and Itsekiri (2.88g/dl) did not show any significant ($P<0.05$) differences between them, but they vary significantly ($P<0.05$) from those values obtained for Isoko (1.40g/dl), which had the lowest values when compared with all other zones.

The indirect bilirubin values in broiler chickens from Ika (0.87) showed higher significant ($P<0.05$) variation than the indirect bilirubin content observed across the zones, but they did not display any significant ($P<0.05$) differences when compared with values obtained from Ukwani (0.72) and Aniocha (0.72). While, the indirect bilirubin values obtained in Ukwani (0.72) and Aniocha (0.72) showed significant ($P<0.05$) differences when compared with those observed in Isoko (0.45), but did not show any significant ($P<0.05$) differences from the values obtained for Ijaw (0.60) and Itsekiri (0.60). Indirect bilirubin values from broiler chickens in Ijaw (0.60) and Itsekiri (0.60), did not exhibit any significant ($P<0.05$) differences from values obtained from Isoko (0.45) which also had the lowest value of Indirect bilirubin when compared with all other values across the zones.

The total protein observed in Urhobo (13.19g/dl) together with Isoko (13.47g/dl), showed significant ($P<0.05$) differences from the values obtained for broiler chickens in Itsekiri (8.10g/dl) and Aniocha (11.36g/dl), but had no significant ($P<0.05$) differences from the values observed in Ijaw (12.25g/dl), Ukwani (12.60g/dl) and Ika (11.92g/dl). Total

protein for broiler chickens in Aniocha (11.36g/dl) was different significantly (P<0.05) from those obtained for Itsekiri (8.10g/dl).

The uric acid values in Urhobo (3.07mg/dl), Ukwani (2.61mg/dl) and Aniocha (2.53mg/dl) showed significant (P<0.05) differences from values obtained for Ijaw (1.36mg/dl), which did not showed any significant (P<0.05) differences from those obtained in Isoko (2.18mg/dl), Itsekiri (2.04mg/dl) and Ika (2.41mg/dl). The uric acid of broiler chickens from Ijaw (1.36mg/dl) had the lowest value, but it was not different significantly (P<0.05) from values observed for Isoko (2.18mg/dl), Itsekiri (2.04mg/dl) and Ika (2.41mg/dl).

The Aspartate Amino Transferase (AST) value of broiler chickens in Ukwani (101.51 IU/l) had a higher significant (P<0.05) values than those observed in Urhobo (77.00IU/l) together with Aniocha (78.43 IU/l), but showed no significant (P<0.05) variation compared to those values obtained for broiler chickens in Isoko (84.53IU/l), Ijaw (80.25IU/l), Itsekiri (81.71IU/l) and IKa (96.85 IU/l). The AST of broiler chickens from Urhobo (77.00 IU/l) and Aniocha (78.43IU/l), had the lowest values and they did not show any significant (P<0.05) variation from those of Isoko (84.53IU/l), Ijaw (80.25IU/l), Itsekiri (81.71IU/l) and Aniocha (96.85 IU/l).

The Alanine Amino Transferase (ALT) of broiler chickens from Ukwani (52.99 IU/l) differ significantly (P<0.05) when compared with those from Urhobo (27.70 IU/l), Isoko (21.62 IU/l), Ijaw (29.50 IU/l), Itsekiri (28.73 IU/l), Aniocha (28.10 IU/l) and Ika (38.98 IU/l). While, the ALT value of broiler chickens in Ika (38.98 IU/l) was different significantly (P<0.05) from the values observed for broiler chickens from Isoko (21.62 IU/l), was not significantly (P<0.05) different from those obtained from Urhobo (27.70 IU/l), Ijaw (29.50 IU/l), Itsekiri (28.73 IU/l) and Aniocha (28.10 IU/l). Values observed for birds in Isoko (21.62 IU/l) had the lowest when compared with ALT values obtained from all other zones.

Table 4.10: Serum biochemical indices of broiler chickens in Delta State

ZONES / Parameters	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika	SEM	Standards
Albumin (g/dL)	4.06^{b}	1.40^{d}	2. 86^{c}	2.88^{c}	3.91^{b}	3.50^{bc}	4.74^{a}	0.15	2.10-3.45
Direct bilirubin	0.66	0.34	0.53	0.42	0.58	0.60	0.84	11.45	_____
Indirect bilirubin	0.87^{a}	0.45^{c}	0.60^{bc}	0.57^{bc}	0.72^{ab}	0.72^{ab}	0.88^{a}	0.03	_____
Total Protein (g/dl)	13.19^{a}	13.47^{a}	12.25^{ab}	8.10^{c}	12.60^{ab}	11.36^{b}	11.92^{ab}	0.26	5.20-6.90
Cholesterol (mg/dl)	98.12	101.74	128.17	98.58	96.64	113.97	99.61	14.51	52.0-148.0
Uric acid (mg/dl)	3.07^{a}	2.18^{ab}	1.36^{b}	2.04^{ab}	2.61^{a}	2.53^{a}	2.41^{ab}	0.12	2.47-8.08
AST (IU/l)	77.00	84.53^{ab}	80.25^{ab}	81.71^{ab}	101.51^{a}	78.44^{b}	96.85^{ab}	2.23	88.0-208.0
ALP (IU/l)	20.44	13.78	16.67	13.00	17.67	15.22	40.33	2.979	24.50-44.40
ALT (IU/l)	27.70^{bc}	21.62^{c}	29.50^{bc}	28.73^{bc}	52.99^{a}	28.10^{bc}	38.98^{b}	1.71	9.50-37.20

^{abc}: means within rows with the same superscripts are not different significantly ($p<0.05$). ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, AST- Aspartase Amino Transferase. SEM: standard Error of mean, Standards (Kotula *et al.,* 1957).

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4.1.11. HEAVY METAL CONCENTRATIONS (PPM) IN MEAT SAMPLES OF BROILER CHICKENS FROM SEVEN ZONES IN DELTA STATE

Table 4.11 presents the concentrationof heavy metalsin meat samples of broiler chickens from different zones with crude oil exploration.

Vanadium (V) concentration in broiler chickens from Ijaw(0.48) higher significant (P<0.05) values when compared with those obtained from Ukwani (0.07) together with those from Ika (0.00), but did not vary significantly (P<0.05) comparing it with those of Urhobo (0.23), Isoko (0.20), Itsekiri (0.18) and Aniocha (0.18), but the concentration of Vanadium (V) in broiler chickens from Urhobo (0.23), Isoko(0.20) Itsekiri (0.18) and Aniocha (0.18), did not show any significant (P<0.05) variations from those of Ukwani (0.07) together with Ika (0.001). The concentration of Cadmium (Cd) in broiler chickens from Urhobo (1.49) was higher and significantly varied (P<0.05) from the values obtained across the other zones. The values of cadmium obtained from Isoko (0.230), Ijaw (0.170), Itsekiri (0.190), Ukwani (0.120), Aniocha (0.002) and Ika (0.002), did not show any significant (P<0.05) differences among them.

The Concentration of Mercury (Hg) in broiler chickens from Urhobo 0.34 was higher and varied significantly (P<0.05) from those values obtained from Ika 0.004, but both did not show any significant (P<0.05) variations from those Hg concentrations across the other zones.

Table 4.11: Heavy metal concentrations (ppm) in meat samples of broiler chickens from different zones in Delta State

Parameters	Locations							SEM	P. Limits
	Urhobo	Isoko	Ijaw	Itsekiiri	Ukwani	Aniocha	Ika		
Vanadium	0.230^{ab}	0.200^{ab}	0.480^{a}	0.180^{ab}	0.070^{b}	0.180^{ab}	0.001^{b}	0.033	0.009-0.34
Cadmium	1.490^{a}	0.230^{b}	0.170^{b}	0.190^{b}	0.120	0.002^{b}	0.002^{b}	0.109	0.00-0.005
Iron	1.610	1.750	1.680	1.430	1.460	1.500	1.410	0.060	0.30
Nickel	0.003	0.002	0.002	0.001	0.001	0.001	0.002	0.0002	0.00-1.12
Mercury	0.34^{a}	0.23^{ab}	0.17^{ab}	0.11^{ab}	0.12^{ab}	0.12^{ab}	0.004^{b}	0.023	1.00-2.68

^{ab}: means within rows with the same superscripts are not different significantly (P<0.05). SEM: standard Error of mean.
*P: Permissible limits of heavy metals (ppm) in water (WHO, 2004).

4.1.12 HAEMATOLOGICAL INDICES OF LAYER CHICKENS FROM THE SEVEN ZONES IN DELTA STATE

Haematological indices of Layer chickens from different zones with crude oil exploration activities are presented in Table4.12. Layer chickens from Urhobo had the highest Packed Cell Volume (PCV) 45.56% and was different significantly (P<0.05), when compared with values recorded in layer chickens from all other zones. While, the PCV of layer chickens from Isoko (32.34%) and Aniocha (31.67%), did not show any significant (P<0.05) variation between them, but they were different significantly (P<0.05) when compared with those of Ijaw (39.22%), Itsekiri (38.11%), Ukwani (37.56%) and Ika (37.78%). The Haemoglobin (Hb) was higher and different significantly (P<0.05) in layer chickens in Ijaw (15.46g/dl), when compared with those from the other zones. While, the haemoglobin values for layer chickens in Urhobo (13.12g/dl), Itsekiri (12.6g/dl), Ukwani (12.54g/dl), Aniocha (12.77g/dl) and Ika (13.30g/dl), did not show any significant (P<0.05) differences among them, but had higher values and were significant (P<0.05) when compared with those that are from Isoko (10.22g/dl) which also had the lowest haemoglobin value.

The Platelets values of layer chickens obtained from Ijaw (95.11) and Ika (90.22) were significantly (P<0.05) higher than the values obtained from the other zones, with Urhobo having the least value. The white blood cell values of layer chickens in Urhobo (8.40 ul) showed higher significant (P<0.05) values than those from the other zones. White blood cell values of birds from Ijaw (7.79 ul) and Ika (9.72ul) zones, did not show any variation between them and they were not different significantly (P<0.05) when compared with those from Isoko (7.40 ul) and Ukwani (7.43 ul), but varied significantly (P<0.05) comparing with the values recorded from Itsekiri (6.84 ul) and Aniocha (6.37 ul), with Aniocha (6.37 ul) showing the least significant (P<0.05) value. While, the white blood cell values of layer chickens in Isoko (7.40 ul) and Ukwani (7.43 ul) had the highest significant (P<0.05) values than those in Aniocha (6.37 ul), which were not different significantly (P<0.05) when compared with values from layer chickens in Itsekiri zone (6.84 ul).

The Mean Cell Haemoglobin (MCH) presented higher significant ($P<0.05$) values in layer chickens in Urhobo (30.01pg), Ijaw (30.04pg) and Itsekiri (29.90pg) when compared with values from the other zones, and showed significant ($P<0.05$) differences from those values observed in layer chickens from Isoko (26.47pg) and Aniocha (27.96pg). The MCH values observed for layer chickens in Ukwani (28.76pg) and Ika (28.54pg) had no significant ($P<0.05$) variation from those in Urhobo (30.01pg), Ijaw (30.04), Itsekiri (29.90pg) and Ukwani (27.96pg), but showed significant ($P<0.05$) differences from those values observed in layer chickens from Isoko (26.47pg).

The Mean Cell Volume (MCV) obtained from layer chickens in Itsekiri (94.33fl) was highest and showed differences significantly ($P<0.05$) from the values recorded across other zones. The MCV from layer chickens in Urhobo (90.03fl), Ijaw (90.12fl) and Ukwani (89.76fl) showed significant ($P<0.05$) differences from those values observed in Isoko (79.41fl), Aniocha (80.78fl) and Ika (79.36fl). The values of mean cell volume obtained from laying birds in Isoko (79.41), Aniocha (80.78) and Ika (79.36) were not significantly ($P<0.05$) different from one anaother. The Red Blood Cell (RBC) values of layer chickens from Urhobo (5.10ul), and Ukwani (4.86ul) were significantly ($P<0.05$) higher across the zones, while no significant ($P<0.05$) variation was observed among those values obtained in Isoko (4.011), Ijaw (4.366), Itsekiri (4.457), Aniocha (4.150) and Ika (4.220).

Table 4.12: Haematological indices of Layer chickens from the seven zones within Delta State

	ZONES								
Parameters	**Urhobo**	**Isoko**	**Ijaw**	**Itsekiri**	**Ukwani**	**Aniocha**	**Ika**	**SEM**	**Standards**
PCV (%)	45.56^{a}	32.34^{c}	39.22^{b}	38.11^{b}	37.56^{b}	31.67^{c}	37.78^{b}	0.68	31.5-36.70
Hb (g/dL)	13.12^{b}	10.22^{c}	15.46^{a}	12.67^{b}	12.54^{b}	12.77^{b}	13.30^{b}	2.23	10.7-12.20
Platelets (x10^{3}/mm^{3})	21.56^{d}	84.56^{b}	95.11^{a}	69.33^{c}	85.56^{b}	65.56^{c}	90.22^{a}	16.19	———
WBC (x10^{3} ul)	8.4 0^{a}	7.40^{bc}	7.79^{b}	6.84^{cd}	7.43^{bc}	6.37^{d}	7.82^{b}	0.10	4.90-9.70
MCH (pg)	30.01^{a}	26.47^{c}	30.04^{a}	29.90^{a}	28.76^{ab}	27.96^{b}	28.54^{ab}	0.24	31.9-40.9
MCHC (%)	34.40^{a}	33.30^{a}	33.70^{a}	33.20^{a}	33.70^{a}	33.60^{a}	36.90^{a}	47.83	30.00-34.40
MCV (fl)	90.026^{b}	79.408^{c}	90.106^{b}	94.328^{a}	89.760^{b}	80.779^{c}	79.363^{c}	0.822	102.0-129.0
RBC (x10^{6}ul)	5.100^{a}	4.011^{b}	4.366^{b}	4.457^{b}	4.860^{a}	4.150^{b}	4.220^{b}	0.068	10.30-12.90

$^{abcd:}$means within the rows with the same superscripts are not significantly (P<0.05) different. Hb- Haemoglobin, PCV- Packed cell volume, MCHC- mean cell haemoglobin count, MCV- mean cell volume, MCH- mean cell haemoglobin, SEM: standard Error of mean, Standards (Kotula *et al.*, 1957).

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4.1.13 SERUM BIOCHEMICAL INDICES OF LAYER CHICKENS FROM DIFFERENT ZONES IN DELTA STATE

Serum biochemical indices of birds from different zones with crude oil exploration activities are presented in Table 4.13. Layer chickens from Urhobo had the highest albumin content (5.27g/dl) and was different significantly (P<0.05) from the values recorded across the zones. While, Albumin values from layer chickens from Ijaw 3.48g/dL and Ukwani (3.45g/dl) Zones, showed differences significantly (P<0.05) when compared to those from Isoko (0.87g/dl), Itsekiri (1.56g/dl), Aniocha (1.64g/dl) and Ika (1.16g/dl).

The direct bilirubin values of birds in Urhobo (0.80)and Ijaw (0.82), presented higher significant (P<0.05) values and were different from the values observed in layer chickens from other zones. Direct bilirubin value of laying birds from Ukwani (0.52) was significantly different from values observed in Isoko (0.13), Itsekiri (0.23), Aniocha (0.25) and Ika (0.17), while the values for Isoko (0.13), Itsekiri (0.23), Aniocha (0.25) and Ika (0.17), showed no significant variation from one another. Indirect Bilirubin values of birds in Urhobo (1.09) and Ijaw (1.09) showed significant (P<0.05) higher values when compared with the ones recorded in other zones. While, Isoko (0.17), Itsekiri (0.31) and Ika (0.22), had the lowest values, which were different significantly (P<0.05) from those in Ukwani (0.69), but those of Ukwani (0.69) and Aniocha (0.44)were not significantly (P<0.05) different from each other.

Total protein of laying birds in Urhobo (13.82g/dl) and Ijaw (14.38g/dl, presented higher significant (P<0.05) values than those values obtained for layer chickens in Itsekiri (10.72g/dl). But the values observed in Urhobo (13.82g/dl), Ijaw (14.38g/dl) and Itsekiri (10.72g/dl) showed no differences significantly (P<0.05) from those obtained in birds from Isoko (12.28g/dl), Ukwani (12.28g/dl), Aniocha (12.52g/dl) and Ika (12.94g/dl).

Aspartate Amino Transferase (AST) values of laying birds in Urhobo (122.72 IU/l) and Ukwani (123.71 IU/l) were higher significantly (P<0.05) than those from the other zones, but did not show any significant (P<0.05) variation when compared to those of Isoko

(83.58 IU/l), Aniocha (95.11 IU/l) and Ika (37.13 IU/l), while that of Ika (37.13 IU/l), was different significantly ($P<0.05$) comparing to those from other zones. Alkaline phosphatase (ALP) values of birds in Urhobo (35.11 IU/l)) and Ijaw (36.71 IU/l) were higher in values and showed significant ($P<0.05$) differences from the values observed in other zones. The layer chickens in Isoko (21.11 IU/l) and Ukwani (18.00 IU/l), recorded an ALP values which presented significant ($P<0.05$) variations from those values recorded in Itsekiri (12.67 IU/l), Aniocha (12.67 IU/l), and Ika (12.00 IU/l).

The Alanine Amino Transferase (ALT) of layer chickens in Urhobo (45.83 IU/l) and Ukwani (44.51 IU/l) were higher and not varied significantly ($P<0.05$) from those obtained from Itsekiri (38.72 IU/l) and Aniocha (34.48 IU/l), while those observed in Isoko (29.85 IU/l) and Ijaw (29.17 IU/l) presented higher significant ($P<0.05$) values compared to Ika (13.26IU/l), which did not show any significant ($P<0.05$) variation from those in Itsekiri (38.72 IU/l) and Aniocha 34.48 IU/L. The ALT values for laying birds in Isoko (29.85 IU/l), Ijaw (29.17 IU/l), Itsekiri (38.72 IU/l) and Aniocha (38.48 IU/l) presented no significant ($P<0.05$) variation among them, but presented higher significant ($P<0.05$) variation from those obtained in Ika (13.26 IU/l), which had the lowest value.

Table 4.13: Serum biochemical indices of laying hens from seven zones within Delta State

	ZONES								
Parameters	**Urhobo**	**Isoko**	**Ijaw**	**Itsekiri**	**Ukwani**	**Aniocha**	**Ika**	**SEM**	**Standards**
Albumin (g/dL)	5.27^{a}	0.87^{c}	3.48^{b}	1.56^{c}	3.45^{b}	1.64^{c}	1.16^{c}	0.26	2.10-3.45
Direct Bilirubin	0.802^{a}	0.131^{c}	0.817^{a}	0.23^{c}	0.52^{b}	0.25^{c}	0.17^{c}	0.04	———
Indirect Bilirubin	1.09^{a}	0.17^{c}	1.09^{a}	0.31^{c}	0.67^{b}	0.45^{bc}	0.22^{c}	0.06	———
Total protein (g/dl)	13.82^{a}	12.28^{ab}	14.38^{a}	10.72^{b}	12.281^{ab}	12.523^{ab}	12.943^{ab}	0.300	5.20-6.90
Cholesterol (mg/dl)	104.82^{a}	86.68^{a}	89.32^{a}	91.90^{a}	103.98^{a}	94.11^{a}	90.82^{a}	1.744	52.0-148.0
Uric acid (mg/dl)	4.98^{a}	4.84^{a}	4.29^{a}	4.53^{a}	3.96^{a}	4.24^{a}	9.54^{a}	0.81	2.47-8.08
ALP (IU/l)	35.11^{a}	21.11^{c}	36.00^{a}	12.67^{c}	18.00^{b}	12.667^{c}	12.000^{c}	5.046	24.50-44.40
AST (IU/l)	122.72^{a}	83.58^{b}	108.65^{ab}	106.58^{ab}	123.71^{a}	95.11^{b}	37.13^{c}	0.926	88.0-208.0
ALT (IU/l)	43.83^{a}	29.85^{b}	29.17^{b}	38.72^{ab}	44.51^{a}	34.48^{ab}	13.26^{c}	1.81	9.50-37.20

^{abc}: means within the rows with the same superscripts are not different significantly ($P<0.05$). AST - Aspartase amino transferase, ALT - Alanine amino transferase, ALP - Alkaline Phosphatase, SEM: standard Error of mean, Standards (Kotula *et al.*, 1957).

4.1.14 HEAVY METAL CONCENTRATIONS (PPM) IN EGGS FROM POULTRY SITES FROM DIFFERENT ZONES WITH CRUDE OIL EXPLORATION IN DELTA STATE

The levels of heavy metal residues present in eggs of laying chickens from different zones in crude oil exploration areas are shown Table 4.14. The levels of Vanadium (V) in the samples of eggs from Isoko 0.08 and Aniocha 0.07, showed significant ($P<0.05$) differences from the values observed in eggs in Urhobo 0.006 and Ika 0.021, but which had no significant ($P<0.05$) differences from those observed in Ijaw 0.056, Itsekiri 0.054 and Ukwani 0.053, respectively. Although, for the eggs samples from Urhobo 0.006, Ijaw 0.056, Itsekiri 0.054 and Ukwani 0.053 respectively, no significant ($P<0.05$) differences was observed among their values, but the concentration of vanadium in Urhobo 0.006 and Ika 0.021 respectively, had no significant ($P<0.05$) differences between them.

Iron (Fe) concentrations in eggs ranged from 3060.4 (Ukwani) to 4594.4 (Isoko).While, thehighest concentration of Fe in egg samples was recorded in Isoko 4594.4, it did not show any significant ($P<0.05$) difference when compared with those from Urhobo 3815.7, Ijaw 4174.2, Itsekiri 3632.8, Aniocha 3615.7 and Ika 4032.4 respectively. The level of Fe observed in Ukwani 3060.4 showed no different significant ($P<0.05$) values from those recorded in other zones.

While, the Nickel (Ni) concentration in egg samples from Urhobo 0.04 was different significantly ($P<0.05$) from the values obtained in other zones, but the Ni values in eggs from Isoko 0.02, Ijaw 0.02, Itsekiri 0.01, Ukwani 0.01, Aniocha 0.02 and Ika 0.02 were not different significantly ($P<0.05$) from one another.

Table 4.14: Concentrations (ppm)of heavy metals in table eggs from laying hens in Delta State

	ZONES								
Parameters	Urhobo	Isoko	Ijaw	Itsekiri	Ukwani	Aniocha	Ika	SEM	P. Limits
Vanadium	0.021^{c}	0.054^{ab}	0.056^{ab}	0.081^{a}	0.053^{ab}	0.070^{a}	0.006^{bc}	0.007	
Cadmium	21.032	12.066	12.328	14.728	12.628	10.963	13.434	1.156	0.00-0.005
Iron	3815.7^{ab}	4594.4^{a}	4174.2^{ab}	3632.8^{ab}	3060.4^{b}	3615.7^{ab}	4032.4^{ab}	132.367	0.30
Nickel	0.035^{a}	0.019^{b}	0.016^{b}	0.013^{b}	0.011^{b}	0.016^{b}	0.016^{b}	0.002	0.00-1.12
Mercury	0.029	0.007	0.031	0.030	0.002	0.011	0.002	0.004	1.00-2.68

abc: means within the same row with same superscripts are not significantly ($P<0.05$) different.
SEM: standard Error of mean,Permissible limits of heavy metal residues in water (WHO, 2004).

4.1.15 HISTOLOGY OF SELECTED ORGANS FROM LAYING HENS EXPOSED TO HEAVY METAL POLLUTED WATER

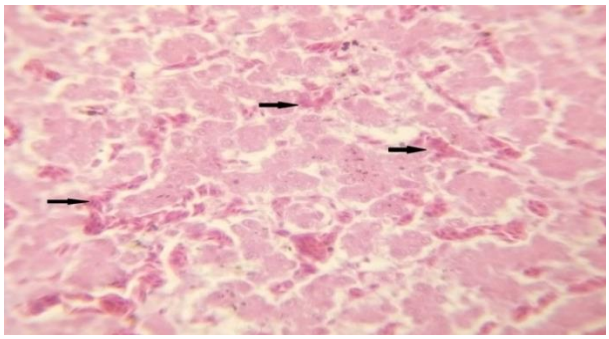
Histological lesion of selected organs (liver and kidney) of layer chickens from crude oil exploration areas of Delta state: Urhobo, Isoko, Ijaw, Itsekiri, Ukwani, Aniocha and Ika are shown in Table 4.15. Different degrees of damages done to livers and kidneys of layer chickens, exposed to water contaminated with heavy metals. The degree of damage caused to the organs by toxic effect of heavy metal residues, is rated in percentage of the total number of birds used for the study as: mild, moderate and severe. 100 % of birds showed moderate to mild hepatic necrosis of the liver. Mild hemorrhagic necrotic lesion with hyperplasia of the kuppler cells and mild periportal macrophage infiltration was observed in the detoxifying organ of birds the liver from Urhobo, Isoko, Ijaw, and Ukwani. In some of the livers observed for birds from Urhobo, Isoko, Ijaw, Itsekiri and Ukwani, there were moderate dissociation and thinning of hepatic cords, widespread congestion of central veins, numerous perivascular aggregate of macrophages and lymphocytes, with widespread marked vacuolar change of the hepatocytes, multiple foci of macrophages and lymphocytes aggregate.

The kidney of birds from Urhobo, Isoko, Ijaw, Itsekiri and Ukwani, showed mild to marked lesion of about 40% and 60% respectively. Some of the kidneys observed for birds in Urhobo, andIsoko, showed multiple foci of interstitial hemorrhagic lesion, marked congestion of the blood vessels, few foci of heterophils and lymphocytes aggregate in the interstiticum, multiple foci lobular necrosis. Dilated sinusoidal spaces and nephrosis were observed in the kidneys of birds from Urhobo, Isoko, Ijaw, Itsekiri and Ukwani.

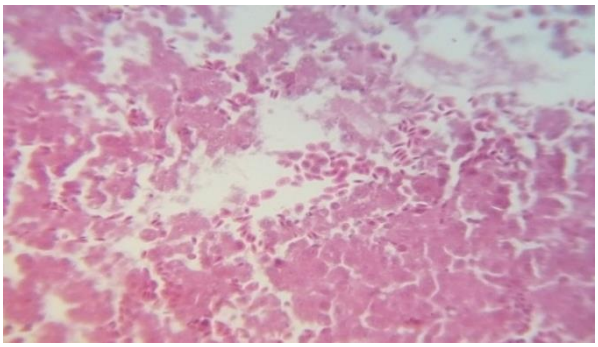
Table 4.15: Organ histology of laying birds from Delta State

	ZONES						
Parameters	**Urhobo**	**Isoko**	**Ijaw**	**Itsekiri**	**Ukwani**	**Aniocha**	**Ika**
LIVER							
Necrosis/Lesion							
Moderate/Mild	100(5/5)	60(3/5)	60(3/5)	20(1/5)	20(1/5)	NVL	NVL
Severe	0(0/5)	20(1/5)	0(0/5)	20(1/5)	0(0/5)	0(0/5)	0(0/5)
KIDNEY							
Necrosis/Lesion							
Moderate/Mild	40(2/5)	60(3/5)	0(0/5)	0(0/5)	NVL	NVL	NVL
Severe	0(0/5)	0(0/5)	0(0/5)	0(0/5)	0(0/5)	0(0/)	0(0/5)

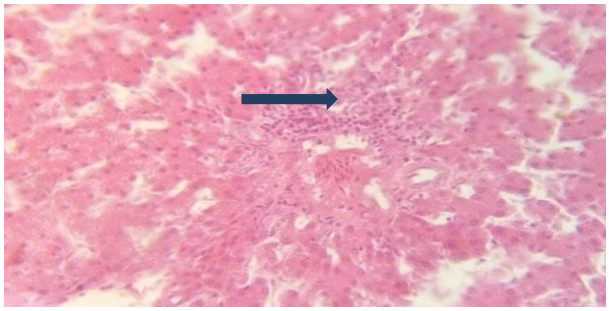
NVL: No visible lesion



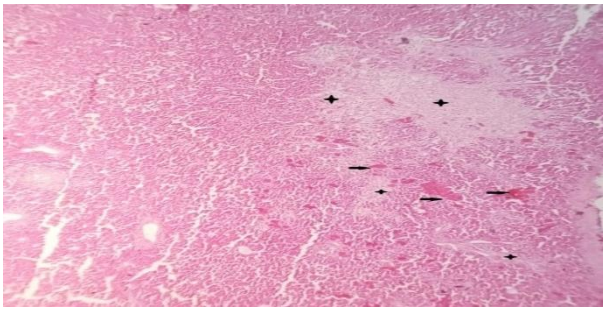
A: Liver with arrows highlighting hepatic sinusoids congested with erythrocytes



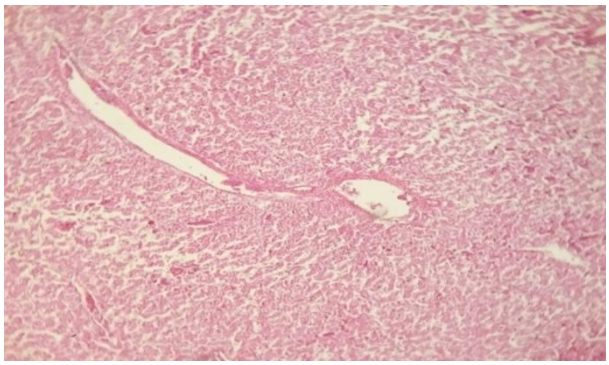
B: hepatic congestion



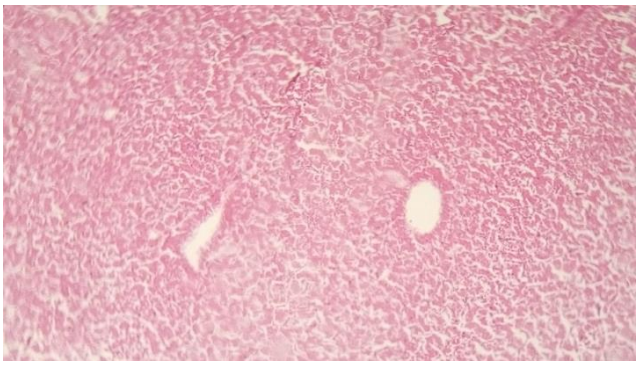
C: liver showing a focus of inflammation with lymphocytes aggregating (arrow).



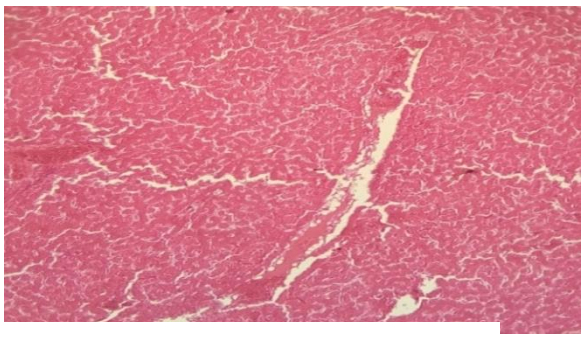
D: liver with areas of chronic inflammation and fibrosis (stars). Also shown is congestion of the hepatic sinusoids (arrows)



E: Normal liver



F: Normal liver



G: Normal liver

Figure 4.1: Comparative photomicrograghs of liverorgans of laying hens from different zones with crude oil exploration (magnification 400X).

(A) Urhobo

(B) Isoko

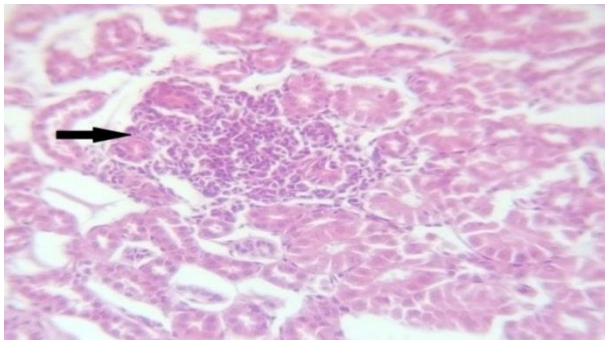
(C) Itsekeri

(D) Ijaw

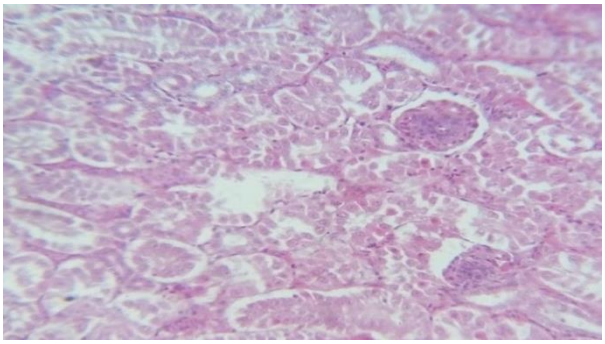
(E) Ukwani

(F) Aniocha/Oshimili

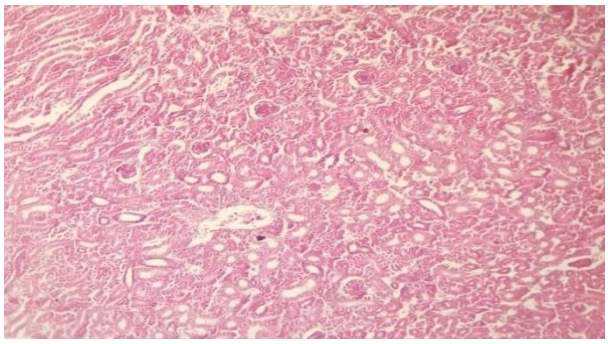
(G) Ika



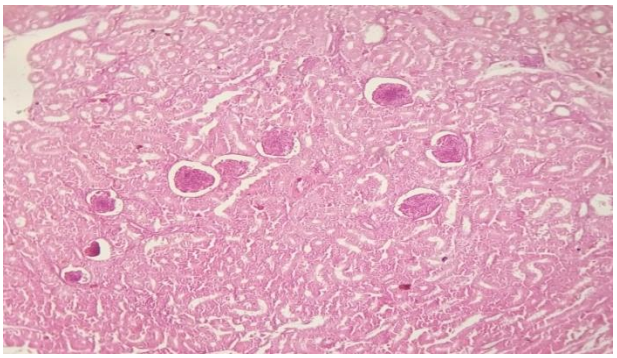
A: kidney with the arrow indicating a focus of tubulointerstitial inflammation with an aggregate of lymphocytes



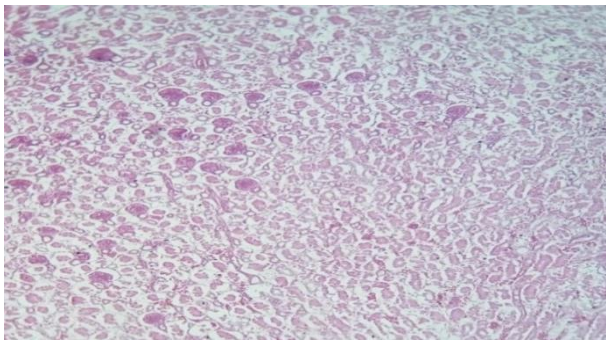
B: Normal kidney



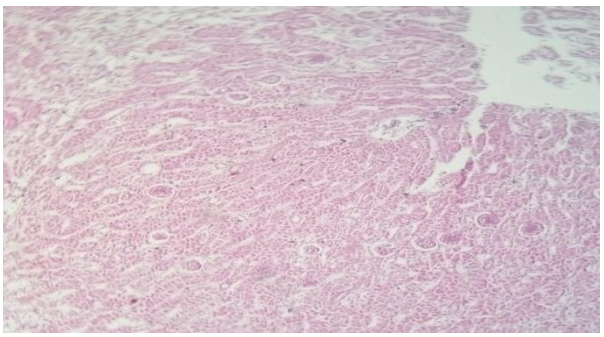
C: Normal kidney



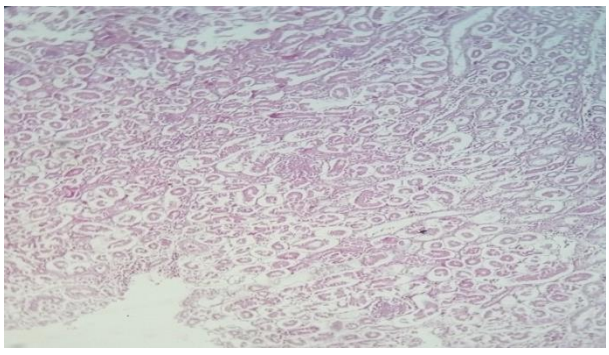
D: Normal kidney



E: Normal kidney



F: Normal kidney



G: Normalkidney

Figure 4.2: Comparative photomicrograghs of Kidney organs of layers from different zones with crude oil exploration (magnification 400X).

(A) Urhobo

(B) Isoko

(C) Itsekiri

(D) Ijaw

(E) Ukwani

(F) Aniocha/Oshimili

(G) Ika

4.1.16 EFFECT OF HEAVY METAL AND METAL CHELATING AGENTS ON GROWTH PERFORMANCE OF BROILER CHICKENS

Table 4.16, presented the effect of heavy metals and metal chelating agents on the initial weight, body weight gain, final live weight, daily feed intake and water intake of broiler chickens. The final body weight of broiler chickens in location 1 (vanadium-1282.84) showed significant ($p<0.05$) higher values than that of location 3 (Iron-1167.51). While, the final live weight of broiler chickens in location 2 (cadmium-1206.58) were not significantly ($p<0.05$) different from those of locations 1 (vanadium-1282.84) together with 3 (Iron-1167.51). However, the values observed for effect of metal binding agents on final body weight (g) of the chickens, did not show any significant response.

The total gain in weight (g) of broiler chickens in location 1 (vanadium 1162.35) was higher but differ significantly ($p<0.05$) from that of location 3 (iron, 1046.95), while the total weight gain of birds in location 2 (cadmium, 1086.05) did not show significant ($p<0.05$) difference from those of locations 1 (vanadium, 1162.35) and 3 (vanadium 1162.35) .

The daily water and feed intake of broiler chickens in this study across the locations, with or without binders did not show any significant ($p<0.05$) differences from one another.

Table 4.16: Growth performance (g/100g)of Broiler chickens supplied with drinking water from crude oil contaminated area and metal-binding agents

Treatment/ Parameters	Initial Wt	Final Body Wt/b	Body Wt G/b	Water Intake	Feed Intake g/d
1 (WRHV)	120.48	1282.84[a]	1162.35[a]	40.30	80.08
2 (WRHC)	120.53	1206.58[ab]	1086.05[ab]	40.2317	80.00
3 (WRHI)	120.57	1167.51[b]	1046.95[b]	40.3008	79.83
SEM	0.06	29.54	29.55	0.11	0.60
Binder					
0	120.55	1237.71	1117.16	40.19	80.17
EDTA	120.50	1204.91	1084.34	40.47	79.75
A.charcoal	120.53	1214.31	1093.81	40.17	80.00
SEM	0.58	29.54	29.55	0.11	0.60
P.Values					
Location	0.60	0.03	0.03	0.88	0.06
Binder	0.83	0.72	0.72	0.11	0.89
Binder x Location	0.72	0.37	0.37	0.68	0.28
Contrast					
Loc 1 vs Loc 2	1.00	0.01	0.01	0.48	0.01
Loc 2 vs Loc 3	0.33	0.09	0.09	0.01	0.01

[ab:] means within the same column with same superscripts are not significantly ($P<0.05$) different. WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron, SEM: standard Error of mean, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid. Wt G/b – weight Gain per bird, g/d – gram per day.

4.1.17 INTERACTION EFFECT OF BINDER AND HEAVY METALS ON GROWTH PERFORMANCE OF BROILER CHICKENS

Presented in Table 4.17, is the Interaction effects of binder together with location on the performance of broiler chickens. The interaction of binder and heavy metals in drinking water for broiler chickens showed a similar trend on growth performance. The Final Body Weight (FBW) as a result of interaction of WRHV with (0, binder), Ethylenediaminetetraacetic acid (EDTA) and Acticvated Charcoal (AC); 1301.25, 1239.46 and 1307.80 did not show any different significant ($p<0.05$) values. Also, the Gain in Body Weight (BWG) : 1180.75, 1118.91 and 1187.40 respectively, were similar ($p<0.05$).

Table 4.17: Effect of Interaction of heavy metals and metal binding agent on Growth Performance of broilers

Treatment	Parameters		
Location*Binder	**Initial Wt**	**Final Body Wt/b**	**Body Wt G/b**
1*AC	120.40	1307.80[a]	1187.40[a]
1*EDTA	120.55	1239.46[abc]	1118.91[abc]
1*0	120.50	1301.25[ab]	1180.75[ab]
2*AC	120.48	1191.49[abc]	1071.01[abc]
2 *EDTA	120.53	1157.62[bc]	1037.10[bc]
2*0	120.60	1270.64[abc]	1150.04[abc]
3*AC	120.63	1143.65[c]	1023.02[c]
3*EDTA	120.53	1217.65[abc]	1097.12[abc]
3*0	120.55	1141.25[c]	1020.70[c]
SEM	0.1005	51.1667	51.1753

[abc:] means within the same column with same superscripts are not significantly ($P<0.05$) different. SEM: standard Error of mean, Wt/b: weight/bird, WtG/b: weight gain/bird. 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid.

4.1.18 EFFECT OF METAL CHELATING AGENTS AND HEAVY METALS ON CARCASS CHARACTERISTICS OF BROILER CHICKENS

Heavy metals and metal binding agents, effects on broiler chicken carcass characteristics are shown in table 4.18. The presence of heavy metals in drinking water did not show any significant ($p<0.05$) impact on the weights of primal parts of chicken; head, neck, wing, shank, drum stick, thighs, breast cut, back and abdominal fat. The weight of primal parts ranged from: 30.29 (WRHC) to 32.08 (WRHV) head, 49.08 (WRHC) to 53.25 (WRHV) shank, 84.08 (WRHC) to 87.67 (WRHV) wing, 100.83 (WRHC) to 110.67 (WRHV) drum stick, 95.08 (WRHC) to 104.79 (WRHV) thighs and 187.21 (WRHC) to 211.71 (WRHV) breast cut. Similarly, addition of metal binding agents to contaminated drinking water did not make any significant ($p<0.05$) impact on head, neck, wing, shank, drum stick, thighs, breast cut, back and abdominal fat.

Table 4.18: Effect of Metal binding agents and Heavy metals on carcass characteristics of Broiler chickens

Treatments/Parameters	Head	Neck	Shank	Wing	Drum stick	Thighs	Breast	Back	Abdominal fat
Location									
1 (WRHV)	32.083	52.667	53.250	87.667	110.667	104.792	211.708	142.417	6.708
2 (WRHC)	30.292	49.500	49.083	84.083	100.833	95.083	187.208	125.542	5.917
3 (WRHI)	31.083	52.036	52.339	85.774	104.839	97.738	196.786	130.869	6.250
SEM	0.675	0.246	0.255	1.046	3.740	4.825	6.742	2.019	0.260
Binders									
0	31.375	52.000	51.000	84.292	104.375	100.292	197.667	135.708	7.583
EDTA	30.167	48.411	50.089	84.315	100.923	92.780	185.036	125.036	4.250
AC	31.917	53.792	53.583	88.917	111.041	104.542	213.000	138.083	7.042
SEM	0.681	0.264	0.265	1.146	3.802	5.538	6.721	2.110	0.360
P. Values									
Binders	0.439	0.125	0.339	0.483	0.199	0.106	0.078	0.152	0.040
Locations	0.428	0.443	0.205	0.716	0.219	0.192	0.129	0.053	0.841

0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid, SEM: standard Error of mean, WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron.

4.1.19 EFFECT OF HEAVY METALS AND METAL CHELATING AGENTS ON THE RELATIVE ORGAN WEIGHTS (g/g) OF BROILER CHICKENS

Heavy metals and metal chelating agents effect on the relative organ weights (g/g) of Broiler chickens is presented in table 4.19. Heavy metals present in water for drinking did not show any significant ($p<0.05$) effect on relative organ length and weights; intestinal length, gizzard, heart, kidneys, lungs, pancreas, spleen, bursa of fabricius and bile, there was a significant impact on the liver organ weight. The liver organ weight presented higher significant ($p<0.05$) values for birds in location 3 (iron, 2.50) than that of location 1 (vanadium, 2.03). While the liver weight of birds in location 2 (cadmium, 2.37) were not significantly ($p<0.05$) different fromthose in locations 1(vanadium, 2.03) and 3 (Iron, 2.50) respectively.

The values however, ranged from: 5.51 (WRHV) to 6.53 (WRHI) GIT, 1.72 (WRHV) to 2.05 (WRHI) Gizzard, 0.41 (WRHV) to 0.45 (WRHI) heart, 2.03 (WRHV) to 2.50 (WRHI) liver, 0.62 (WRHV) to 0.68 (WRHC) kidneys and 0.42 (WRHV) to 0.45 (WRHC) lungs, 0.26 (WRHV) to 0.30 (WRHI) pancreas, 0.41 (WRHV) to 045 (WRHC) bile.

Similarly, introduction of metal binding agents into water for drinking did not make any effect significantly ($p<0.05$) on the organs weight of broiler chickens, however, the values recorded ranged from: 5.80 (0) to 6.22 (AC) length of GIT, 1.76 (AC) to 1.99 (0) gizzard, 0.39 (AC) to 0.45 (EDTA) heart, 2.21 (AC) to 2.35 (0) liver, 0.61 (AC) to 0.68 (0, EDTA) kidneys, 0.41 (AC) to 0.46 (EDTA) lungs, 0.27 (AC) to 0.29 (0) pancreas, 0.024 (EDTA) to 0.15 (0) respectively.

Table 4.19: Effect of metals and metal chelating agents on the Relative organ weights (g/g) of Broiler chickens

Treatment	GIT	Gizzard	Heart	Liver	Kidneys	Lungs	Pancreas	Spleen	Bursa F.	Bile
Locations										
1 (WRHV)	5.509	1.725	0.407	2.026[b]	0.618	0.420	0.256	0.022	0.028	0.141
2 (WRHC)	6.123	1.906	0.415	2.371[ab]	0.680	0.445	0.276	0.028	0.028	0.149
3 (WRHI)	6.527	2.047	0.447	2.496[a]	0.664	0.438	0.301	0.021	0.027	0.145
SEM	0.362	0.116	0.029	0.152	0.053	0.037	0.027	0.022	0.028	0.023
Binder										
0	5.798	1.987	0.427	2.353	0.678	0.435	0.279	0.022	0.028	0.149
EDTA	6.146	1.933	0.451	2.328	0.677	0.459	0.288	0.022	0.028	0.024
A.charcoal	6.216	1.758	0.391	2.213	0.606	0.409	0.267	0.022	0.027	0.032
SEM	0.362	0.113	0.029	0.152	0.053	0.037	0.027	0.022	0.027	0.032
P.Values										
Binder	0.142	0.145	0.576	0.086	0.691	0.884	0.515	0.760	0.102	0.980
Location	0.683	0.335	0.334	0.786	0.544	0.628	0.871	0.856	0.320	0.180
Contrast										
Loc 1 vs loc 2	0.406	0.277	0.992	0.274	0.428	0.811	0.474	0.942	0.132	0.049
Loc 2 vs loc 3	0.491	0.793	0.274	0.846	0.352	0.806	0.881	0.866	0.945	0.224
EDTA vs A.charcoal	0.593	0.261	0.133	0.580	0.319	0.315	0.586	0.627	0.080	0.331

0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid, SEM: standard Error of mean. Bursa F: Bursa of Fabricius, WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron.

4.1.20 EFFECT OF HEAVY METALS AND METAL CHELATING AGENTS ON HAEMATOLOGY OF BROILER CHICKENS

Presented in Table 4.20, is the effect of metal binding agents and heavy metals on haematology of Broiler chickens. The existence of heavy metal residues in drinking water available for broiler chickens had a significant ($P<0.05$) affect on lymphocyte together with heterophils. The lymph value (65.08) in broiler chicken on WRHV was higher but significantly ($P<0.05$) different from that of WRHC (60.83) and both were not different significantly ($P<0.05$) from that on WRHI (62.00). While heterophil (dL) values for birds on WRHC (31.33) and WRHI (30.83) did not show any significant ($P<0.05$) differences between them, but they were higher significantly ($P<0.05$) than those in WRHV (27.00). The PCV, Hb, RBC, WBC, Platelet, Monocyte, Eosinophil and Basophils did not show any significant ($P<0.05$) impact due to heavy metals present in water available for drinking for broiler chickens. The values recorded for other haematological parameters ranged from: 26.92 (WRHV) to 28.00 (WRHC) PCV, 8.68 (WRHV) to 9.07 (WRHC) Hb, 2.91 (WRHV) to 3.23 (WRHI) RBC, 16154.17 (WRHI) to 26875.00 (WRHC) WBC, 176250.00 (WRHI) to 188083.33 (WRHV) Platelet, 2.33 (WRHI) to 3.42 (WRHC) Monocyte and 4.00 (WRHV) to 4.43 (WRHI) Eosinophil.

The platelet (dl) values in broilers chickens exposed to drinking water with EDTA (191750.00) and AC (199166.67) were higher and differ significantly ($P<0.05$) from that which has no metal-chelating agent, but had the lowest value (160583.33 dL) and was different significantly ($P<0.05$) from those with metal-binding agents. The values of platelets in blood cell of birds on EDTA and activated charcoal were not significantly differences between each other (EDTA, 191750.00 and AC, 199166.67).

Table 4.20: Effect of Metal binding agents and Heavy metals on haematology of Broiler chickens

Treatments	PCV	Hb	RBC	WBC	Platelet	Lymph	Hetero	Monoc	Eosino	Basop
Location										
1 (WRHV)	26.92	8.68	2.91	17375.00	188083.33	65.08^{a}	27.00^{b}	2.92	4.25	0.25
2 (WRHC)	28.08	9.07	3.22	26875.00	187166.67	60.83^{b}	31.33^{a}	3.42	4.00	0.25
3 (WRHI)	27.58	8.96	3.23	16154.17	176250.00	62.00^{ab}	30.83^{a}	2.33	4.43	0.25
SEM	0.61	0.25	0.18	6125.31	8833.94	1.30	1.26	0.38	0.46	0.14
Binders										
0	27.00	8.83	2.98	27441.67	160583.33^{b}	61.17	30.50	2.92	4.42	0.25
EDTA	28.33	9.16	3.25	17679.17	191750.00^{a}	63.50	29.00	2.58	4.51	0.33
AC	27.25	8.71	3.13	15283.33	199166.67^{a}	63.25	29.67	3.17	3.75	0.17
SEM	0.61	0.25	0.18	6125.31	8833.94	1.30	1.26	0.38	0.46	0.19
P. Values										
Binders	0.28	0.43	0.58	0.35	0.01	0.39	0.71	0.55	0.46	0.70
Location	0.42	0.53	0.37	0.41	0.58	0.08	0.04	0.15	0.81	1.00
Location 2	0.21	0.34	0.65	0.36	0.08	0.03	0.04	0.84	0.91	0.84
Contrast										
EDTA vs AC	0.78	0.73	0.55	0.15	0.15	0.25	0.20	0.43	0.32	0.28

Cntrl: control. 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediamine tetraacetic acid, SEM: standard Error of mean, Hb: Haemoglobin, RBC: Red Blood Cell, WBC: White Blood Cell, Hetero: Heterophil, Monoc: Monocyte, Eosino: Eosinophil, Basop: Basophil, WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron. .

4.1.21 EFFECT OF METAL CHELATING AGENTS AND HEAVY METALS ON SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS

Table 4.21 as shown below, is the effect of heavy metals together with metal binding agents on serum biochemistry of broiler chickens. Among the serum protein, AST (IU/L) and creatinine (IU/L) values, there were no significant ($P<0.05$) differences among the broilerchicken samples on heavy metal polluted water. Serum protein values ranged from 4.56 (WRHC) to 4.63 (WRHI), AST increased from 188.92 (WRHI) to 199.25 (WRHC) and creatinine from 0.53 (WRHI) to 0.55 (WRHC, WRHV) respectively.

For serum albumin of birds on WRHV (1.46) and WRHI (1.24 g/dL), there was no significant ($P<0.05$) variation between them, but showed significantly ($P<0.05$) higher values, when compared to values obtained from birds on WRHC (0.93 g/dL). The globulin value of birds on WRHC (3.59) displayed significant ($P<0.05$)highervalues than those of globulin obtained from birds on WRHV (3.15) but did not show any significant ($P<0.05$) variation from those on WRHI (3.39). The values of globulin of birds on WRHV (3.150) and WRHI (3.392), did not show any significant ($P<0.05$) variation between them.

The ratio of albumin to globulin in birds on WRHV (0.46) was significant ($P<0.05$) but higher than those on WRHC together with WRHI (0.23 and 0.33) respectively. While the values of birds on WRHC and WRHI (0.23 and 0.33) showed no significant variation between them. Aspartate amino transferase (ALT) values of birds on WRHI (33.25 IU/L) was significant ($P<0.05$) and higher than those on WRHV (26.50), while those of WRHC and WRHI (31.42 and 33.25 IU/L), no significant ($P<0.05$) differences existed between them. For the alkaline phosphatase (ALP) of birds on WRHV (424.75) the recorded values were reportedly highest in values and was different significantly ($P<0.05$) from those observed for birds on WRHC and WRHI (379.83 and 356.83) respectively, but there were no significant variation between birds on WRHC and WRHI (379.83 and 356.83).

The values of serum albumin (1.47) and A-G (0.45) of birds on 0-binder showed significantly ($P<0.05$) higher values than 1.07 EDTA, 1.10 AC (albumin) and 0.25 EDTA, 0.33 AC (A-G). Serum protein ranged from 4.43 (AC) to 4.79 (0-binder), AST increased from 190.58 (EDTA) to 195.75 (AC), ALT values ranged from 28.67 (EDTA)

to 31.50 (0-binder), ALP increased from 372.67 (AC) to 401.67 (0-binder), urea ranged from 10.20 (AC) to 10.45 (0-binder) and creatinine increased from 0.53 (EDTA) to 0.57 (0-binder).

Table 4.21: Effect of Metal chelating agents and Heavy metals on serum biochemical indices of Broiler chickens

Treatments	Parameters									
	Protein	**Album**	**Globuli**	**A-G**	**AST**	**ALT**	**ALP**	**Urea**	**Creat**	**Glucose**
Location										
1 (WRHV)	4.61	1.458^{a}	3.150^{b}	0.458^{a}	192.417	26.500^{b}	424.750^{a}	10.242^{ab}	0.550	268.417^{b}
2 (WRHC)	4.558	0.933^{b}	3.592^{a}	0.233^{b}	199.250	31.417^{ab}	379.833^{b}	9.992^{b}	0.550	299.333^{a}
3 (WRHI)	4.633	1.242^{a}	3.392^{ab}	0.333^{b}	188.917	33.250^{a}	356.833^{b}	10.633^{a}	0.525	289.917^{ab}
SEM	0.135	0.094	0.112	0.038	4.153	2.227	15.232	0.366	0.016	8.709
Binders										
0	4.792	1.467^{a}	3.292	0.450^{a}	194.250	31.500	401.667	10.450	0.567	272.750
EDTA	4.575	1.067^{b}	3.508	0.250^{b}	190.583	28.667	387.083	10.217	0.525	287.250
A.C	4.433	1.100^{b}	3.333	0.325^{b}	195.750	31.000	372.667	10.200	0.533	297.667
SEM	0.135	0.094	0.112	0.038	4.153	2.227	15.232	0.366	0.016	8.709
P. Values										
Binders	0.187	0.009	0.361	0.003	0.668	0.635	0.416	0.650	0.185	0.146
Locations	0.924	0.002	0.032	0.001	0.220	0.105	0.013	0.115	0.472	0.052
Location x Binder	0.700	0.047	0.333	0.070	0.986	0.737	0.548	0.103	0.903	0.594
Contrast										
EDTA vs AC	0.342	0.072	0.498	0.001	0.060	0.960	0.383	0.192	0.994	0.413

Cntrl: control, 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediamine tetraacetic acid, SEM: standard Error of mean. ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, AST- Aspartase Amino Transferase, A:G – Albumin to Globulin ratio, WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron.

4.1.22 EFFECT OF METAL CHELATING AGENTS ON HEAVY METAL RESIDUES (mg/g) IN MEAT SAMPLES OF BROILER CHICKENS

Effect of heavy metals and metal binding agents on heavy metals (mg/g) residues in meat samples of broiler chickens is shown in Table 4.22. Concentration of vanadium in meat samples of broiler chickens exposed to heavy metals in drinking water no significant ($P<0.05$) differences were present. The concentration of vanadium observed in broiler chickens ranged from 3.29 (WRHV) to 4.50 (WRHI). The level of cadmium in WRHC (0.84) in meat samples of broiler chickens exposed to heavy metals in drinking water was higher and vary significantly ($P<0.05$) from those in WRHV (0.001) together with WRHI (0.26) respectively. Significant ($P<0.05$) differences were noticed for iron concentration in samples of meat from broiler chickens. Iron concentration in broiler chickens on WRHC (628.10) was higher and vary significantly ($P<0.05$) from values of iron concentration of meat samples from broiler chickens on WRHV (151.57) and WRHI (259.78) and bothdid not show any significant ($P<0.05$) differences from each other.

The concentrations of residues of heavy metals in meat samples from broiler chickens were significantly ($P<0.05$) influenced by the presence of metal binding agents. The residual levels of vanadium was higher in 0-binder (10.62) and significantly ($P<0.05$) different when compared with those on EDTA (0.007) and AC (0.67), while the values of concentration of vanadium for the EDTA and AC did not show any significant ($P<0.05$) differences between them. For cadmium, the residual concentration was higher in 0-binder (1.10) and was significantly ($P<0.05$) different compared to those on EDTA (0.001) and AC (0.001), but both had no significant ($P<0.05$) differences between them.

The values recorded for iron residual concentration in meat samples of broiler chickens exposed to heavy metals and metal binding agent in water for drinking did show significant ($P<0.05$) values in meat samples of broilers chickens on EDTA (622.20) that was higher, when compared with those on 0-binder (233.66) and AC (193.59), and both had nosignificant ($P<0.05$) differences from each other.

Table 4.22: Effect of metal chelating agents on Heavy metal residues (mg/g) in meat samples of broiler chickens

Locations	Vanadium	Cadmium	Iron
1 (WRHV)	3.290	0.001 [b]	151.57 [b]
2 (WRHC)	3.508	0.839 [a]	628.10 [a]
3 (WRHI)	4.494	0.261 [b]	259.78 [b]
SEM	0.629	0.188	161.85
Binder			
0	10.619 [a]	1.099 [a]	233.66[b]
EDTA	0.007 [b]	0.001 [b]	622.20[a]
A.charcoal	0.666 [b]	0.001 [b]	193.59[b]
SEM	0.629	0.188	161.85
P.Values			
Location	0.367	0.013	0.112
Binder	0.001	0.000	0.132
Binder x Location	0.870	0.003	0.382
Contrast			
Location1 vs location 2	0.34	0.03	0.48
Location2 vs location 3	0.00	0.31	0.49
EDTA vs AC	0.44	1.00	0.06

[ab:] meanswithin the same column with the same superscripts are not different significantly (P<0.05), SEM: standard Error of mean, 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid, WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron.

4.1.23 INTERACTION EFFECT OF METAL BINDING AGENTS AND HEAVY METALS ON METAL RESIDUES IN MEAT OF BROILER CHICKENS

The interaction effects of metal binding agents and heavy metals on the residual concentration in meat samples of broiler chickens are displayed in Table 4.23 The interaction effect of heavy metal and metal binding agent in the meat samples of broiler chickens showed a similar trend on metal residual concentration in the meat. The concentration of metal residues of vanadium, iron and cadmium in samples of meat from broiler chickens presented significant ($p<0.05$) differences. Values of residual concentration for vanadium presented significant ($p<0.05$) higher values in birds on (WRHV, 0-binder) 9.86 than those on (WRHV, EDTA) 0.002 and (WRHV, AC) 0.009 respectively. Also, similar trend was observed for cadmium as a result of interaction of (WRHC, 0-binder) 2.51, (WRHC, EDTA) 0.001 and (WRHC, AC) 0.001, which were also significantly ($p<0.05$)different. The iron residual concentration of meat samples presented no differences significantly ($p<0.05$) in broiler chickens exposed to (WRHI, 0-binder) 595.68 and (WRHI, EDTA) 512.45, and both showed significant ($p<0.05$) variation from those on (WRHI, AC) 207.33.

Table 4.23: Interaction effect of metal binding agents and Heavy metals on metals residues in meat of broiler chickens

Loc*Bind	Vanadium	Cadmium	Iron
1*AC	0.009 [b]	0.001 [b]	230.33[b]
1*EDTA	0.002 [b]	0.001 [b]	157.94[b]
1*0	9.860 [a]	0.002 [b]	66.017[b]
2*AC	0.011 [b]	0.001 [b]	142.69[b]
2 *EDTA	0.004 [b]	0.001 [b]	1196.22[a]
2*0	10.010 [a]	2.514 [a]	545.39[ab]
3*AC	1.481 [b]	0.001 [b]	207.33[b]
3*EDTA	0.016 [b]	0.001 [b]	512.45[ab]
3*0	11.985 [a]	0.781 [a]	595.68[a]
SEM	1.089	0.326	280.33

[abc:] means within the same column with different superscripts are significantly (P<0.05)different, SEM: standard Error of mean. 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid, Loc*Bind: Location*Binder.

4.1.24 EFFECT OF METAL CHELATING AGENTS ON HEAVY METAL (mg/g) RESIDUES IN FAECES OF BROILER CHICKENS

The effect of metal chelating agents on heavy metals (mg/g) residues in faeces of broiler chickens is shown in Table 4.24. Concentration of vanadium in faeces of broiler chickens exposed to heavy metals contaminated drinking water were different significantly ($P<0.05$). Vanadium residual concentration in voided faeces of broiler chickens were higher in birds on 0-binder (0.012) and AC (0.011) and different significantly ($P<0.05$) when in comparism with the concentration values of vanadium recorded due to the effect of EDTA (0.009) on vanadium. The cadmium and iron residual concentrations observed in broiler chicken faeces did not show any significant ($P<0.05$) variation between them. The ranges of values were from birds on WRHV (0.039) to WRHI (0.047) for cadmium, and from WRHV (0.129) to WRHC (0.25).

The effect of metal binding agents on heavy metals in drinking water for broiler chicken has significantly ($P<0.05$) influenced the metal residual level in the voided faecal samples. The vanadium residual concentrations were significantly ($P<0.05$) lower in faecal samples from broiler chickens on (WRHC, EDTA) 0.009 than those on (WRHV, 0-binder) 0.012 and (WRHI, AC) 0.012, which were not significantly ($P<0.05$) different from one another. Also, significant ($P<0.05$) differences in the residual concentration of voided heavy metals through faeces were noticed due to metal-binding effect on cadmium. The residual concentration of cadmium in faeces of broiler chickens on 0-binder (0.026) was lower significantly ($P<0.05$) compared to those on EDTA (0.052) and AC (0.054) respectively, which also did not show any differences significantly ($P<0.05$) between them. The concentration of iron residues in the excreta of broiler chickens exposed to heavy metal contaminated water and the addition of binding agents in the water showed no significant ($P<0.05$) differences from each other, and the values ranged from 0.158 (AC) to 0.209 (0-binder).

Table 4.24: Effect of metal chelating agents on Heavy metals (mg/g) residues in faeces of broiler chickens

Locations	Vanadium	Cadmium	Iron
1 (WRHV)	0.012 [a]	0.039	0.129
2 (WRHC)	0.009 [b]	0.045	0.250
3 (WRHI)	0.011 [a]	0.047	0.174
SEM	0.003	0.008	0.057
Binder			
0	0.012 [a]	0.026 [b]	0.209
EDTA	0.009 [b]	0.052 [a]	0.187
AC	0.012 [a]	0.054 [a]	0.158
SEM	0.000	0.008	0.057
P.Values			
Location	0.000	0.756	0.333
Binder	0.000	0.033	0.823
Binder*Location	0.000	0.376	0.230
Contrast			
Location1 vs location2	0.00	0.90	0.09
Location2 vs location3	0.00	0.03	0.10
EDTA vs AC	0.00	0.02	0.78

[ab:] means within the same column with different superscripts are significantly ($P<0.05$) different, 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid, SEM: standard Error of mean, WRH (V, C, I): Water Relatively High in Vanadium, Cadmium and Iron.

4.1.25 INTERACTION EFFECTS OF HEAVY METALS AND METAL CHELATING AGENTS ON RESIDUAL METAL CONCENTRATION IN FAECES OF BROILER CHICKENS

The interaction effects of metal chelating agents and heavy metals on the residual metal concentration in faeces of broiler chickens are shown in Table 4.25. The effect of binding agents and heavy metal in the meat samples of broiler chickens had a similar occurrence on the metal residual levels in the faeces. The residual levels of vanadium, iron and cadmium in the samples of faeces from broiler chickens presented significant ($p<0.05$) differences. The residual concentration of vanadium presented significant ($p<0.05$) higher values in birds on (WRHV, 0-binder) 0.016, followed by those on (WRHV, AC) 0.014 and least value was in faeces of birds on (WRHV, EDTA) 0.005 respectively. Also, similar trend was observed for cadmium as a result of interaction of (WRHC, 0-binder) 0.016, (WRHC, EDTA) 0.061 and (WRHC, AC) 0.058, did show significant ($p<0.05$) differences which ranges from (WRHC, 0-binder) 0.016 to (WRHC, EDTA) 0.061. The residual concentration of iron in faeces presented no significant ($p<0.05$) variation, while the values ranges from (WRHI, 0-binder) 0.060 to (WRHI, EDTA) 0.280.

Table 4.25: Interaction effects of heavy metals and metal chelating agents on residual metal concentration in faeces of broiler chickens

Location * Binder	Vanadium	Cadmium	Iron
1*AC	0.014 [b]	0.035 [ab]	0.149[ab]
1*EDTA	0.005 [f]	0.050 [ab]	0.068[b]
1*0	0.016 [a]	0.033 [ab]	0.171[ab]
2*AC	0.007 [e]	0.058 [a]	0.145[ab]
2 *EDTA	0.009 [d]	0.061 [a]	0.213[bc]
2*0	0.011 [c]	0.016 [b]	0.394[a]
3*AC	0.014 [b]	0.068 [a]	0.181[ab]
3*EDTA	0.012 [c]	0.044 [ab]	0.280[ab]
3*0	0.008 [de]	0.030 [ab]	0.062[b]
SEM	0.001	0.014	0.099

[a-f:] means within the same column with the same superscripts are not different significantly (P<0.05). 0: No metal binding agent, AC: Activated charcoal, EDTA: Ethylenediaminetetraacetic acid, SEM: standard Error of mean.

CHAPTER FIVE

DISCUSSION

5.1.1 HEAVY METAL CONCENTRATION IN DRINKING WATER

Water samples in Delta State contained varying concentrations (ppm) of heavy metals in drinking water. The levels ranged from 0.023 to 29.29 ppm vanadium, 7.87 to 15.68 ppm cadmium, 497.8 to 2002.2 ppm iron, 0.001 to 0.017 ppm nickel and 0.001 to 0.030 ppm mercury, in drinking water samples from poultry sites in Delta state. The results from these studies clearly showed that heavy metals, particularly for cadmium, vanadium and iron, had higher concentrations, which were above permissible ranged limits, when compared with the national and international safe limits of World Health Organisation (WHO, 2004; 2008). These reports were in line with that of Chaitali *et al*. (2013) who stated that well and bore-hole water samples from Central region of India contained high concentrations of cadmium, above the World Health Organisation maximum acceptable concentration (MAC). It was observed that the samples of drinking water examined for vanadium in some farm locations were above the tolerable limits, while iron and cadmium in Delta state, exceeded also the limits of permissible values, indicative of water pollution (Cd - 0.005, and Fe - 0.3 ppm) and poor water quality standard (WHO, 2008). It shows that the result obtained from this study is an indication of water contamination hazards encompassing high deposition of cadmium and vanadium from the operation of petrochemical industries and a poor treatment and management practices for drinking water in the areas around crude exploration activities, which have very important animal and human health implications. Similar reports were stated by Friberg *et al*. (1986) that cadmium concentrations approaching 5μg/l was contained in drinking water found in shallow wells in some areas in Sweden where the soil was treated with acids Mustafa *et al*. (1988) also found average concentrations between 1-26 μg/l in portable samples of water from ground wells. The results obtained in this research work were in consonance with the discovery made from the work of Danbatta (2006) who reported high levels of iron found in water from some bore-holes and wells dug by hands.

Thecurrent study has shown that Nickel and Mercury levels in drinking water fell below the permissible ranged limits of WHO (2008). Similarly, reports of Kingmann *et al.* (2005) showed that the concentration of mercury in rivers, streams and lakes was not above 0.1 ppm. While, Higgings and Dasher (1986) stated that various manufacturing processes or waste water from sewage plants can cause water contamination, and that heavy metals in samples of water for drinking goes together with chronic diseases and a negative health impact.

Thetoxic metals and wastes have deleterious impacts on animals but with no clear homeostatic mechanism (Draghici *et al*., 2010; Vieira *et al*., 2011). They are broadly categorised as very toxic and poisonous to animals. The effects on animal life due to heavy metal exposure, even at mild concentration, is diverse;adverse and include carcinogenic and neurotoxic actions (Jomova and Valko, 2011). Contamination of water due to mercury is a serious problem threatening the safety of animals and foods, which has been confronting public health everywhere (Singh *et al.*, 2011). Mercury is a potent diverse industrial and environmental contaminant, which causes severe alteration and malformation in tissues of animals (Lund *et al*., 1993; Mahboob *et al*., 2001; Sener *et al*., 2007).

Groundwater generally, is very well distributed around the globe and is about 98 percent of fresh water resources in the world (Buchanan, 1983; Bouwer, 2002). Groundwater has being for a period of time and it has been used or served as a potential water supply source, particularly, from hand dug wells, boreholes and springs. As a result of their increasing popularity as a veritable source of water supply for the teeming populace, it thus becomes pertinent to critically evaluate their quality and portability for fitness for livestock and human consumption.

5.1.2 HEAVY METALS IN EGGS

Heavy metals from egg samples within Delta state were higher than the range of permissible limits recommended by WHO (2008). This is am indication of the quality status of the eggs produced in the area and against the background of high levels of heavy metals concentration particularly: iron, vanadium and cadmium concentrations present in underground solvent, available in poultry sites. This result was backed-up from the reports of (Marettova *et al*., 2003), all of whom reported low levels of mercury deposited in the muscle, organs and tissues of blood

from chickens supplied feed fortified with Selenium and clay on mercury bioaccumulation in different studies. Abdou *et al.* (2016) also presented higher levels of cadmium in feed samples examined in industrial region than non industrial regions which presented lower levels of those heavy metals. In the same vein, USDA (2011) reported high levels of iron as 17.6 ppm in egg samples which was above the tolerable limits.

The outcome of this current research clearly presented cadmium concentrations in egg samples within Delta State which were significantly not different from one location to another across all the zones, but were higher above the recommended values by the WHO, (2008) as permissible values. Regarding the presence of cadmium in eggs of chickens exposed to cadmium contamination through drinking water, the World Health Organization (1972) had stated that the rate of accumulation of cadmium in the body is independent of the route of adsorption and the concentration of intake and that eventually toxic level would be attained, even if intake is at a minute concentration. This result also, is in line with the work of Daniel and Edward (1995), who stated that a small quality of cadmium concentration present in our environment may cause accumulation and can result into a toxicological hazard. Mercury and cadmium have been known to have no functions in the physiology or biochemistry of animals, and they are not naturally occurring in living organisms (Lenntech, 2004). Thus, exposure to these heavy metals, at very minute levels may be seriously harmful due to their bioaccumulative nature in living organisms. From this research, the mercury level in the assayed egg samples showed values below the permissible ranged limits of WHO (2008) standard.

The eggs of laying hens from poultry sites in Delta State, according to the current study showed iron residues. The values of Iron (Fe) concentrations (ppm) ranged from 3060.4 to 4594.4. The average concentration of Fe in eggs as stated by USDA (2011) is 17.6 ppm. High levels of iron residues in eggs from poultry sites showed an adverse effect of the high iron concentration on the gastrointestinal tract of the animals exposed to them. Several animal sources of iron, such as eggs, contain a form of iron called heme, which is readily more utilized by the body (USDA, 2011). From the intake of 100 g of eggs, the iron intake daily average is 2.652 mg/day/person. The consumption of this shows about 4.74% of Average daily intake (ADI) which is proscribed

by FAO/WHO respectively for iron (Codex, 2011). This has raised the importance of the impact of the concentrations of iron present in chicken eggs in the human diets, particularly children. These findings indicated that the concentration of iron in eggs are high and unsafe for consumption over long period, at moderate egg consumption, but even concentration extremely low may still pose adverse effect particularly when exposure to iron concentrations is for a prolonged time period (Zhou *et al*., 2007).

5.1.3 MEAT SAMPLES OF CHICKENS

Although, the results from previous studies on underground water clearly displayed in the water samples from various poultry sites, shows high levels of some heavy metals, particularly vanadium, cadmium and iron, above the permissible limits, while nickel and mercury were within the tolerable levels, when compared with the international organizations (WHO, 2004) standards. Yet, in the samples of meat examined, the values of some metals were within tolerable ranged limits of WHO, (2008). The low heavy metals residual levels found in the breast muscle parts of broiler chicken displayed the present status of and the quality of broiler chickens raised within the area which is in contrast with the background high levels of heavy metals particularly vanadium present in underground water, available in the poultry sites in Delta State. This report was in consonance with those of Marettova *et al*. (2003), who detected low mercury concentration in blood tissues, organs and chicken muscle when supplied mercury diet contaminated. Low levels of heavy metals detected in broiler chicken meat are indications of no adverse effects on broiler chickens and subsequently on the final consumer due to heavy metals in the short term, since broiler chickens are raised to slaughter within a short period of time.

The levels of Cadmium (Cd) in broiler chickens from Urhobo, Isoko, Ijaw, Itsekiri and Ukwani zones were higher than the International range of WHO (2004). One of the principal sources of cadmium in the environment is food (Baykov *et al*, 1996). According to this research, cadmium levels in chicken meat ranged from (0.002 – 1.49 ppm. kg^{-1}). Gonzalez-Weller *et al*, (2006) reported average concentrations of (1.90 μg. kg^{-1}) in beef, (1.22 μg.kg^{-1} Cd) in mutton and (1.68 μg. kg^{-1} Cd) in pork. Also, Mariam *et al*, (2004) reported cadmium concentration of: (0.37 mg.kg^{-1}, 0.31mgkg^{-1}, and 0.33mg.kg^{-1}) for lean meat of mutton, poultry and beef respectively. Cadmium concentrations detected in this research were in consonance and comparable with

those stated by Gonzalez-Weller *et al*, (2006) but were much lower when compared with the values stated by Mariam *et al*, (2004) and Iwegbue *et al*, (2008). The cadmium levels from this study were higher above the permissible limit of 0.05mgkg^{-1} recommended by the European commission. There were higher levels of iron in all the poultry farm locations. High level of iron in animal tissues can result into high risk of myocardial infarction (Kingmann *et al.*, 2005).

The range of nickel (0.001 – 0.003 mg/kg) in broiler chicken meat reported in this study is similar to those of Surtipanti *et al.* (2005) in meats, gizzard and liver from chicken raised in Indonesia. Nickel is known to cause respiratory challenges and it is also a carcinogen. The tolerable ranged limit of nickel in food is 0.5mgkg^{-1} as recommended by WHO (2008) and the USSR standard.

5.1.4 SERUM BIOCHEMICAL INDICES OF CHICKEN

The biochemical parameters of birds from poultry sites within Delta State revealed that AST (77.00 – 101.51 iu/l) was significantly reduced in birds, with values lower than the normal physiological range (88.00-208.00 Iu/l) for normal chickens. However, ALT values (27.70 – 52.99) were significantly higher than the normal range of a normal chicken (9.50 – 37.20 iu/l). From this study, it can be inferred that elevated serum enzymes could be as a result of the toxic impact of heavy metals in the water. This report is in agreement with those of ALT activity and cytoplasmic enzyme are indication of necrotic lesions of the internal organs especially the liver and degeneration of tissues in the kidney, while a reduction in the serum Superoxide Dismutase (SOD) and Catalase (CAT) concentrations is an indication of no cholestasis or congestion (Lysenko 2000; Borg *et al*. 2003; Cabanero *et al*., 2005). These authors in their reports stated that chickens subjected to $HgCl_2$ displayed significant increased in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities, whereas a significant reduction of the SOD and CAT activities in the body system.

Serum enzymes are normally present at low levels in the blood, but when the liver cells are damaged due to toxic effects of heavy metals, it would be expected that some of the enzymes would be released into the blood and increase in levels. This increase in ALT content in the blood indicates a positive hepatic damage since the enzymes are produced by the liver and in

other parts of the body (Crook, 2006). In the present research, changes in the normal levels of different biochemical serum parameters together with histological necrosis and degenerative alteration of the kidney and liver tissues were the major effects of heavy metal toxicicity observed in the chickens due to exposure to heavy metal contaminated water. In previous researches, some physiological alterations traceable to mercury toxicities, such as reduction; in the chicken body weight gain has been reported (Marettova *et al*., 2003). Similarly, necrotic alterations in the liver, increase in AST activities and qualitative vascular degeneration of kidney tissues has been observed in chickens, (Crook, 2006). Hence, the activity of ALT in serum may be used as an enzyme for marker to access the liver functional status as recommended by Sobutskii *et al*. (2007).

The findings from this research are in line with the works (Borg *et al*., 2003; Cabanero *et al*., 2005; Zraly *et al*., 2008). Also, Jagadeesan and Pillai (2007) stated significant improvement in serum ALT and AST levels of rats as a result of exposure to $HgCl_2$ for prolonged time period of 30 days.In a separate report, a meaningful increase in serum AST and ALT have also been reported due to rat exposure to mercury (Singh *et al*., 2007).

5.1.5 HISTOLOGY OF CHICKEN ORGANS

The liveris the main point of mercury metabolism and it accumulates in the liver leading to severe hepatic degeneration. Reports of various researches have shown that $HgCl_2$ induces ultrastructural and histopathological degeneration of the liver resulting in periportal fatty degradation and cell tissue necrosis. Schurz *et al*. (2000) reported that DNA was a major molecule in the activities of the cell and was a vital target for $HgCl_2$-induced cell injuries.

Accumulation of cadmium in the body negatively affecting various organs which include: the central nervous system, brain, bones, kidney, liver, lung and placenta (Castro-González and Méndez- Armenta, 2008). Other negative impacts that have been reported include immunological effects, reproductive, hepatic, development toxicityand haematological effects (Apostoli and Catalani, 2011).

Nickel can cause respiratory problems and is a carcinogen. The permissible limit of Ni in food according to WHO and USSR standard is 0.5mgkg-^{1}. In this currnt study, concentrations of nickel ranged between 0.011 – 0.035, which is lower than the recommended limit by the WHO (2004), and also those of Surtipanti *et al.* (2005) who stated 0.0421–0.0807 mg.kg^{-1}, 0.0353-0.1668mg.kg^{-1} and 0.0339–0.0744 mg.kg^{-1} for chicken gizzard, liver and muscle respectively. The reason for the low content of nickel observed in this study could be as a result of it low concentration in the environment where the study was carried out.

Toxicity due to mercury can emanate fromingestion, inhalation or absorption through the skin andcould be very toxic but corrosive once it is absorbed into blood stream. Also, it combines with proteins in the plasma or enters the red blood cells but does not readily transit to the brain or fetus and instead, may enter into other organs of the body (El-Shenawy and Hassan, 2008). Toxicity due to mercury causes tissue damage due to its toxic metabolites (Sharma *et al*., 2002). The toxic metabolite free radical is produced by cytochrome p450 which further reacts with oxygen to produce trichloromethyl peroxy radicals (Sharma *et al*., 2002). These radicals bind covalently with the macromolecule and cause peroxidative degradation of lipid membranes of the liver and kidney. Increased lipid peroxidation under contamination conditions can be due to increased oxidative stress in the cell resulting fromthe depletion of antioxidant scavenger systems. Associated with the changes in lipid peroxidation, the affected tissues showed decreased activities of key antioxidants CAT and SOD. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals in vivo. Previous studies have reported that the activity of SOD is low after mercury toxicities (Sobutskii *et al*., 2007) who measured biochemical indeces of blood after low doses of mercury exposures which come in agreement with the results of this study.

In the kidneys, there were congestion and haemorrhages similar among the birds in all treatments. There were multiple foci of tubular degeneration and necrosis with marked congestion of interstitial blood vessels which resulted to widespread necrosis of renal tubules.

The liver results showed that laying hens obtained from poutry sites in Urhobo, Isoko Itsekiri and Ijaw showed mild to moderate necrosis in their liver cells. This shows that the hepatic necrosis

with aggregates of heterophils at the portal tracts, while those from Ukwani, Anioch and Ika maintained normal functioning in their hepatic cords. Liver enlargement due to toxic effects in seeds has been reported in other animals including fish and rats (Bundit *et al*. 2014; Sobutskii *et al*., 2007). This study is in agreement with Bundit *et al*., (2014), who reported mild histopathological changes in the liver of Bocourti's catfish when fed with 50% and 75% of herbs in the diet of fish. The results of this study suggest that heavy metal when present in the environment will have toxic impact on lives over time.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.0 SUMMARY

From studies thus carried out to assess the existence and possible bioaccumulation of cadmium, mercury, nickel, vanadium and iron on water, table eggs and chickens in Delta State, the heavy metals of interest were all observed to be present in the samples. Although, some heavy metal concentrations were within their respective established permissible limits in drinking water, eggs and meat consumption.

The levels ranged from 0.023 to 29.29 ppm vanadium, 7.87 to 15.68 ppm cadmium, 497.8 to 2002.2 ppm iron, 0.001 to 0.030 ppm mercury and 0.001 to 0.017 ppm nickel, in drinking water samples. These results clearly showed that there were higher levels of heavy metals, particularly for iron, vanadium and cadmium above the World Health Organisation recommended permissible values. The present study showed that Nickel and Mercury were below the permissible range of WHO (2008).

The result from this research therefore, indicated that chickens reared in the oil endowed Delta State had higher cadmium and iron levels than the recommended tolerable ranged limits by WHO (2008). While, Vanadium was within tolerable limits, Nickel and Mercury, values of these heavy metals, in the samples of chicken meat were below the WHO (2008) recommended tolerable limits.

The detectable trace quantities of heavy metals discovered in the tissues and sample of broiler chicken meat is an evidence of their existence in the environment and their occurrence in drinking water and in the food chain. This is also a clear display of the present status and quality of chicken produced over time in the oil rich Delta State and against the backdrop of high

concentrations of heavy metals in the surrounding and underground water, resulting from lack or poor sanitation and improper water purification measures. And also the negligence on the part of the environmental agencies to monitor and ensures good waste management and proper removal of oil and its products from the environment in any case of outbreak or accident during crude oil transit.

Although, these heavy metals were present in chickens within tolerable limits, their contributions to the overall burden of metal in the body cannot henceforth be considered as negligible, therefore, the eggs and chickens may be assumed to be safe for consumption by humans in the short term. Caution and efforts however should be taken to eradicate or reduce the existence of Nickel and mercury from the surrounding and underground water to avoid their deposition in the eggs, chickens and other poultry products and thus prevent their ultimate bioaccumulation in the body and its subsequent toxic impact.

Level of heavy minerals in egg samples in Delta state, though, the values obtained for vanadium of 0.006 to 0.081 and nickel of 0.01 to 0.04 ppm in the eggs were within acceptable range, the cadmium of 10.96 to 21.03 and iron of 3060.4 to 4594.4, in the samples of eggs were higher than the permissible range. This is indicative of the status of the eggs produced and the health condition of the chickens reared in the area,

6.1 CONCLUSIONS

The concentrations of heavy metals V, Cd, Fe Ni and Hg in water, eggs and meat samples from poutry sites within different locations in Delta State were determined. The locations included: Orogun, Ubogo, Jesse, Ubeji, Koko, Omadino, Ogulaha, Burutu, Ogbeijoh, Igbide, Oleh, Ozoro, Kwale, Ubiaruku, Amai, Agbor, Asaba, Isele-ukwu e.t.c. The heavy minerals were determined using a model AA 701 of atomic absorption spectrophotometer and results compared with WHO standards and were found to be of higher values than the stipulated tolerable limits. These findings are worrisome and give cause for concern, especially heavy metal bioaccumulation in tissues of the body and present a severe health threat to animals and man, such as inflammation, cancer, vomiting, fever, joint calcification e.t.c.

Conclusively, eggs and chickens contamination are due to exposure to heavy metals, cadmium, vanadium, and Iron bioaccumulation in the meat and eggs. The toxic elements cadmium, vanadium and Iron are of utmost concern due to levels discovered in drinking water, chicken meat and eggs exceeded stipulated guideline values by International organization. Subsequent effect of this on the overall health of the local poultry and human population in the area with prolonged exposure to heavy metals and consumption of contaminated drinking water, eggs and chicken meats produced in polluted environment.

Heavy metal contaminated drinking water, poultry egg and chicken meats, in Delta State presented an important health issue, especially the occurrence of high amount of vanadium, Iron, mercury and cadmium in groundwater. This has posed a very daunting task for the body science and innovators especially now when global water supply is receding against the background of a global increasing population and when alternative water supply sources for human activities are not readily available.

At the present, the introduction of very sound and clear definition of organic or biologically based regulatory values demands urgency, as standard regulatory values would present a key strategy for efficient environmental pollution challenges, as a rule to adopting the best safety and protection of health of animals and humans with a minimum waste of tool resources. It is also concluded that Mitigating agents such as Ethylenediaminetetraacetic acid (EDTA) and Activated charcoal (AC) at the levels of 50 mg/l of drinking water for broiler chickens mitigate or reduced the concentration (43% EDTA and 73% AC) of heavy metals and ultimately reduced their induced toxic effects in broiler chickens with observed results on the concentrations of cadmium, vanadium and iron in chicken meat, faecal samples and histological characteristics.

The administration of drinking water high in heavy metal concentration to broiler chicken in the short term had no much negative effect on birds' mortality.Also administration of mitigating agents Ethylenediaminetetraacetic acid (EDTA) and Activated charcoal (AC) at 50 mg/l did not completely chelate the heavy metals present in the system of broiler chickens. While Ethylenediaminetetraacetic acid (EDTA) was more effective in chelating cadmium, Activated charcoal was more potent in chelating cadmium and iron in the drinking water and subsequently

lowered its concentration in the chicken meat. Therefore, a combination of these two chelators in drinking water of chickens is recommended.

6.2 RECOMMENDATIONS

The most essential element; Iron was high across all the zones. The results give cause for concern, particularly as heavy metals are bioaccumulative in the body system and portend a serious health threat to animals. The concerned authority should take necessary steps for reducing water, food and environmental contamination. Ethylenediaminetetraacetic (EDTA) acid and Activated charcoal (AC) as heavy metal-binding agents have great potency in the prevention of toxicity due to heavy metals, but there is need for further investigation into the mode of administration and appropriate dose of heavy metals binding agents for poultry. It is therefore, recommended that water sources and poultry products produced in the area of crude exploration should be routinely monitored to ascertain its suitability for drinking, consumption and for other purposes.

The use of metal chelators may have significant hidden health threat that may be associated with the indiscriminate and nonselective or non-specific metal binding agents, inclusion levels in drinking water, chelators' affinity to target or binding sites in the gastrointestinal tract (GIT), other organs or other essential or mineral materials in the body system of chickens. Therefore, heavy metal chelating agents should undergo rigorous tests with special attention to their efficacy or effectiveness and safety. The introduction of mitigating agents in broiler production and management as reported in this research is beneficial in the prevention and reduction of metal toxicosis, but the dose and duration of administration may play a significant role in this regard.

This study has merit as a means of testing the efficacy of new heavy metal binding agents and reappraising those of existing commercial products that can be used in poultry production. It is therefore recommended that an expanded version of this study be carried out preferably by a central body to assess the efficacy of heavy metal binding agents from either organic or synthetic sources, testing various inclusions levels on heavy metal contaminated drinking water for broiler chickens. There is also need to include recovery studies of broiler chickens exposed to heavy

metal ingestion or contamination, as this could be a loop area in the long term effect of heavy metals exposure in livestock and human conditions.

CONTRIBUTIONS TO KNOWLEDGE

Crude oil exploration contributes to the levels of heavy metals concentrations (Vanadium, Cadmium, Iron, Nickel and Mercury) causing health hazard and threat to the quality of poultry products in Delta State,

Water samples from poultry farms across sevens zones in Delta State contained varying levels of vanadium, cadmium and iron above permissible range limits,

Meat and egg samples contained vanadium, cadmium and iron at concentrations higher than tolerable limit,

Residues of nickel and mercury were also found in meat samples of broiler chcickens, but within acceptable range limits,

Ethylendiamine tetraacetic acid at 50mg/l in drinking water of broiler chickens, had ameliorative effect on cadmium, vanadium and iron toxicity in broiler chickens,

Activated charcoal at 50mg/l of drinking water for broiler chickens also had a reducing effect on cadmium, vanadium and iron residues in broiler chcickens,

Detoxification of vanadium, cadmium and iron, by Ethylendiaminetetraacetic acid or Activated charcoal in broiler chickens was effective against cadmium, and iron, since Ethylenediaminetetraacetic acid reduced cadmium up to 50.0 percent and activated charcoal also reduced cadium and iron residues in broiler chickens up to 43.0 percent and 73.0 percent,

EDTA and Activated charcoal as heavy metal-binding agents had potency in the reduction of toxicity due to heavy metals, but there is need for further investigation into the mode of administration and appropriate dose of heavy metals binding agents for chickens.

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Plate A: Massive oil spill at aaAmukpe near Sapele in Delta State, pouring crude oil into the neighbouring river for more than a month (pic. Israel Aloja, ERA/FoEN) NMPC, Amukpe Sapele.

Plate B: Abandoned oil spill farm site of 10 year old (Photo Dulue, Feb 7, 2008/IRIN). Amnesty International.com

Plate C: Impact of an oil spill (www.the guardian.com)

Plate D: Impact of Oil spill from the oil well head, near the village Oloibiri (www.guardian.com)

Plate E: Oil spill in the Niger Delta (www.Igbofocus.com)

Plates F: Cleansing of an oil spill site (www.theguiardian.com)

Plates G: Cleansing of an oil spill site (www.theguiardian.com)