Biochemical responses of Sprague dawley rats and New Zealand rabbits following long-term dietary exposure to heavy metal contaminated fish

CHAPTER ONE

1.0. INTRODUCTION

1.1 Background to the Study

The environment is man's surroundings, which include all the circumstances, influences and events that he encounters in his life time. The natural environment encompasses all living and non living things occurring naturally on earth (Dale, 2007). Without health, development and a protected environment, health has little value. Development can only be achieved through the contributions of healthy people. Continued population growth, irrational use of resources, and increasing generation of wastes create unsustainable demands on the environment (Oldroyd, 2006). Sound management of the environment brings major health benefits while inadequate or no management results in large adverse effects on health and sustainable interaction between people and their environment.

There are limits to the extent to which the soil and fresh water resources can be exploited and ecosystems are used as a receptacle for the wastes generated by human society (Adams and Lambert, 2006). There are also global limits to the exploitation of non-renewable resources and to the capacity of the planetary system to absorb wastes. These global limits have

become apparent as in the depletion of stratospheric ozone layer, which has implications for health and agricultural production and in the possibility of climatic disruption as a result of the release of greenhouse gases (Adams and Lambert, 2006).

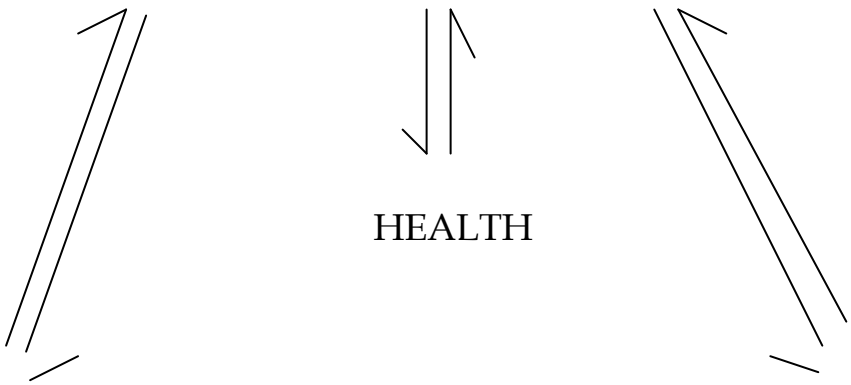
In developing countries, growth in industrial production has been accompanied by an increased incidence of environmental related diseases and physical hazards (Adams and David, 2006). Many of the workforces suffer exposure to occupational hazards. Priority given to human health raises an ethical dilemma if "Health for all" conflicts with protecting the environment. Respect for nature and the control of environmental degradation is a 2nd order principle which must be observed lest it conflicts with the 1st order principle of meeting and ensuring human survival needs (Seaton *et al*., 2005).

The report of the World Council on environment and development defined sustainable development as development meeting the needs of all the present population without compromising the ability of future generation to meet their own needs. This could prove contradictory if meeting present needs implies the irreversible depletion of natural resources and the degradation of ecosystem. But the intelligent application of what is known, combined with caution and a continuous commitment to improving understanding of links between the environment, development and health can change these trends. Focusing on health provides many insights into how a better balance can be achieved between the environment and development (Dale, 2007).

Health is no longer the responsibility only of doctors, nurses, midwives and other health professionals but it is also the responsibility of planners, architects, teachers, employers and all others who influence the physical or social environment. Each adult has the duty as a citizen to ensure that health risks within human environments are minimized and government resources wisely used.

Human health ultimately depends on society's capacity to manage the interaction between human activities and the physical and biological environment in ways that safeguard and promote health but do not threaten the integrity of the natural systems on which the physical and biological environment depends (Fig. 1.1), (Press-Ustinov, 2011). This includes maintaining a stable climate and continued availability of environmental resources (soil, fresh water, clean air). It also includes continued functioning of the natural systems that receive the wastes produced by human societies, domestic, industrial and agricultural, without exposing people to pathogens and toxic substances (Oldroyd, 2006).

The scale and nature of human activities including agricultural, industrial and energy production, the use and management of water and wastes, urbanization, the distribution of income and assets within and between countries, the quality of health and other public services, and the extent of protection of the living, working and natural environment.



Physical environment
(Soils and other chemical composition, air and water resources, climate including temperature, humidity, radiation, precipitation and seasonal changes)

Biological Environment
(type and distribution of habitats and their flora and fauna, including pathogens reservoirs and vectors.

Figure 1.1 Interactions between human activities and the physical and biological environment, (Hogan, 2010).

The physical environment has a major influence on human health not only through temperature, precipitation and composition of air and water but also through its interaction with the type and distribution of the flora and fauna (the biological environment). The biological environment is a major influence on the food supply and on the reservoirs and transmission mechanisms of many diseases (Press-Ustinov, 2011). Environmental factors that impair health include:

a. Pathogenic agents and their vectors

b. Physical and chemical agents present in the environment (Fig 1.1) that are independent of human activities and can impair health either by their presence (e.g. naturally occurring ultraviolet light) or by their relative deficiencies e.g. iodine, selenium.

c. Noxious physical and chemical agents added to the environment by human activities (e.g. nitrogen oxides, polycyclic aromatic hydrocarbons, particulates arising from fossil fuel combustion, gaseous, liquid and solid wastes produced by industry and radioactive wastes. The effect of these agents can be magnified or diminished by human intervention or activity (Hogan, 2010).

Environmental Health is a branch of public health that is concerned with all aspects of the natural and built environment that may affect human health (Davies, 2013). It addresses the physical, chemical and biological factors external to a person, and all the related factors impacting behavior. It encompasses the assessment and control of those environmental factors that can potentially affect health. It is targeted towards preventing disease and creating health supportive environments. This definition excludes behaviors not related to the environment and behaviors related to social and cultural environment and genetics (WHO, 2014). The Healthy People 2020 Environmental Health objectives focus on six themes, each of which highlights an element of environmental health:

1. Outdoor air quality
2. Surface and ground water quality
3. Toxic substances and hazardous wastes.
4. Homes and communities.
5. Infrastructure and surveillance.
6. Global environmental health (WHO, 2014).

1.2. STATEMENT OF PROBLEM

Contaminated food and water remain a major public health problem. Some biological contaminants of food can be eliminated or considerably reduced by improvement in personal hygiene, safe piped borne water, good quality sanitation, effective health programmes and the application of technologies, such as pasteurization and irradiation. These will help in the elimination or reduction of several food borne diseases such as typhoid fever, cholera and shigellosis in many developing countries, (USEPA, 2003).

Shellfish grown in contaminated water have been recognized as a source of hepatitis A. An epidemic of shellfish-borne hepatitis A in China affected some 282,000 persons (with at least 32 fatalities) and was linked to the consumption of contaminated clams (Silver, 2006). The consumption of fish is by far the most significant source of ingestion-related mercury exposure in humans and animals. Consumption of whale and dolphin meat as the practice in Japan is a source of high levels of mercury poisoning. The big pollution diseases of Japan were a group of man-made diseases all caused by environmental pollution due to improper handling of industrial wastes by Japanese companies (Hamilton, 2013). Ending poverty begins with clean water. Poor health, hunger and lack of education are symptoms of the true problem-no clean water. Together, they lead to a cycle of poverty (UN, 2006; WHO, 2002).

In the developed world, an extensive food safety infrastructure (legislation, enforcement mechanism, surveillance and monitoring systems of responsible industries) ensures that the food supply is largely safe from the health viewpoint, (WHO, 2002)

A number of chemical substances may occur in the food supply owing to contamination, environmental or otherwise and their effects on health may be serious. For instance, lead-soldered food cans may contain food with higher amounts of lead than in raw commodities and unsoldered cans. There are also concerns about the health effects of polychlorinated biphenyls (PCBs), which are used in various industrial applications, (Silver, 2006).

The continued release of persistent toxic elements such as cadmium and mercury into the environment will inevitably lead to rising levels of these substances in various food stuffs and eventually to levels that may be harmful to human health. Fresh water has become a scarce commodity due to pollution of water. Increasing population and its necessities have led to the deterioration of surface and subsurface water. Water crisis is not the result of natural factors rather it is been caused by human factors. Much of ill health which affects humanity, especially in the developing countries can be traced to lack of safe and wholesome water

supply (Hogan, 2010). The quality of water is the resultant of all the processes and reactions that act on the water from the moment it is condensed in the atmosphere to the time it is discharged by a well or spring. It varies from place to place and with the depth of the water table (Hogan, 2010).

In Nigeria, industrialization and urbanization have major impact on the water environment. Both surface and sub surface water sources are getting polluted due to developmental activities. In many cities of the world including Nigeria, domestic and industrial waste water goes directly into major water bodies without treatment (Kusemiju *et al., 2001*). Hazardous chemicals used in the home and industry sometimes find their way into the aquatic environment, causing damage to the ecosystem and contaminating drinking water supplies e.g. the city of Bucharest, Romania (population of two million) has no wastewater treatment plant. All wastewater is dumped in the river Danube (Newton, 2008). Hazardous chemical wastes from industrial sources are often dumped into poorly prepared and managed landfill sites with little or no separation of toxic wastes. This frequently results in contamination of drinking water, soil and air.

Turan, (2008) reported the presence of heavy metals in some commercial fish species captured from the Black sea and Mediterranean coast of Turkey. Carvalho in 2009 reported the presence of mercury in *Tilapia* fish . It was also reported that aquatic organisms are considered excellent biomarkers of mercury occurrence in the environment. Latif *et al.,* (2009) reported high levels of arsenic, iron and chromium in foodstuffs, soils and sediments in Bangladesh while Gowd and Pradip (2008) reported the distribution of heavy metals in surface water of Ranipet industrial area in India. The report revealed that the surface water in the area is highly contaminated showing very high concentration of some of the heavy/toxic metals like cadmium, chromium, copper and lead.

The Lagos Lagoon is one of the meandering networks of lagoons and creeks found along the coastline of southern Nigeria (Lawson, 2011). It has continued to be under intensifying pressure from pollution such as sawdust and petrochemical materials, untreated sewage, detergents and industrial effluents, petroleum products and fecal pollution (Lawal-Are and Kusemiju, 2006; Soyinka and Kusemiju, 2004; Lawal-Are, 2004; Kusemiju *et al.*, 2001). The Lagos State Government is worried about the effects of uncontrolled waste water discharges and other related forms of pollution on the ecosystems in the state. Water pollution from waste water, sewage and the use of pesticides, herbicides and fertilizers in the state are major problems. Wastewater not properly managed can cause a lot of pollution in the state. Factors responsible for failing waste water management includes high mitigation costs, low public awareness, inadequate funding and lack of political will as well as weak polices and institutional frameworks. Typically, polluters are unwilling to assume responsibility and reluctant to remedy such a situation because it requires substantial effort and money and because they do not feel affected by problems they create elsewhere. Due to this it is necessary to show that the problem of waste water management affects us all. Reports from the Lagos State Environmental Protection Agency (LASEPA) showed that 57% of effluents collected from industries in Lagos State were within the acceptable state Effluent Limitation Standard while 43% were unacceptable. Industrial monitoring showed that only 25% of industries in Lagos State have pollution abatement in place (LASEPA, 2009). Discharges from factories in Lagos State are being emptied into open drains such as gutters or water bodies such as rivers or the Lagoons. What then is the fate of these water bodies and the aquatic life present within? With this aforementioned, it is important to investigate the pollution load of selected Lagos State water bodies and the aquatic life present within. It

is also important to investigate the biochemical effects of the consumption of fishes from some of the polluted water bodies on Nigerians, using animal model (rabbits and rats).

1.3 AIM OF STUDY

The aim of this study is to conduct a biochemical study on the effects of feeding laboratory rodents with heavy metal contaminated Black jaw Tilapia (*Sarotherodon melanotheron)* obtained from Carter Bridge and Makoko markets in Lagos, Nigeria.

1.4 OBJECTIVES OF THE STUDY

The specific objectives of the study are as follows:

i. To determine physiochemical parameters and heavy metal concentrations (lead, cadmium, chromium, copper) in water samples obtained from Makoko, Maroko, Majidun, Molatori, Ibeshe and Carter bridge water sites.

ii. To determine the proximate composition of *Sarotherodon melanotheron* and heavy metal concentrations (lead, cadmium, chromium, copper) in Tilapia fish *(Sarotherodon melanotheron)* samples obtained from the Carter Bridge and Makoko markets.

iii. To investigate the effects of the consumption of heavy metal contaminated Tilapia fish *(Sarotherodon melanotheron)* by rats and rabbits, on their blood chemistry, hematological, hormonal, antioxidant and histological parameters.

iv. To evaluate the use of zinc as a potential reversal agent for the amelioration of the heavy metal toxic effects on the biochemical, hormonal, hematological and antioxidants parameters of the rats and rabbits.

1.5 SCOPE OF THE STUDY

This study seeks to evaluate the potential health risks associated with the consumption of Tilapia fish *(Sarotherodon melanotheron)* obtained from selected markets close to the Lagos Lagoon on residents of Lagos State, using laboratory animals as models.

1.6 SIGNIFICANCE OF THE STUDY:

- It is a well known fact that the Lagos Lagoon is polluted (Jimoh *et al.*, 2011; Lawson, 2011; Osibona and Kusemiju 2006). Little or nothing is known about the effects of consuming these aquatic products on the human body.
- The physiochemical parameters and heavy metal concentrations in water and fish samples would shed more light on the present and current state of the Lagos Lagoon.
- This will also throw more light on the state of fishes in these water bodies: either they are nutrients or poisons.
- The investigation of the effects of the consumption of fishes from these water sites by rats and rabbits will show if there is any effects on blood chemistry, hematological, hormonal, antioxidant and histological parameters.
- The potential amelioration and reversal of the harmful effects from the consumption of fish using zinc will also be investigated. The use of two animal species was determined by the aim of studying both physiological changes (rabbits) and histopathological and fertility changes (rats).

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Tilapia Fish

Tilapia is the common name for nearly a hundred species of cichlid fish from the tilapine cichlid tribe. Tilapia inhabits a variety of fresh water habitats, including shallow streams, ponds, rivers and lakes. Historically, they have been of major importance in artisan fishing in Africa and are of increasing importance in aquaculture (Yonas, 2006)

2.1.1 Etymology

The common name tilapia is based on the name of the cichlid genus Tilapia, which is itself a latinization of *thiape*, the Tswana word for fish. Tilapia goes by many names. The name St. Peter's fish comes from the story in the Gospel of Matthew about the Apostle Peter catching

a fish that carried a coin in its mouth, though the passage does not name the fish while the name also applies to *Zeus faber*, a marine fish not found in the area (Froese *et al,* 2012).

2.1.2 Fishing Farming

Tilapia is the fifth most important fish in fish farming, with production reaching 1,505,804 metric tonnes in 2000 (Herdson and Priede, 2011). Because of their large size, rapid growth and palatability, tilapiine cichlids are the focus of major farming efforts, specifically various species of *Oreochromis*, *Sarotherodon* and *Tilapia*, collectively known colloquially as Tilapias. Like other large fish, they are a good source of protein and popular among artisanal and commercial fisheries. Most such fisheries were originally found in Africa, but outdoor fish farms in tropical countries, such as Papua New Guinea, the Philippines and Indonesia are underway in freshwater lakes. In temperate zone localities, tilapiine farming operations require energy to warm the water to tropical temperature. One method uses wastes heat from factories and power stations (Espe *et al,* 2006). Commercially grown Tilapia is almost exclusively male. Cultivators use hormones, such as testosterone to reverse the sex of newly spawned females. Tilapia are prolific breeders because the presence of female Tilapia results in rapidly increasing populations of small fish, rather than a stable population of harvest – size animals. Other methods of Tilapia population control are polyculture, with predators farmed alongside Tilapia or hybridization with other species. Whole Tilapia fish can be processed into skinless, boneless fillets: the yield is from 30 – 37% percent, depending on fillet size and final trim. The use of Tilapia in the commercial food industry has led to the virtual extinction of genetically, pure bloodlines. Most wild Tilapia today is hybrids of several species (Espe *et al.,* 2006)

2.1.3. Nutrition: Tilapia is a low saturated fat, low calorie, low carbohydrate and low sodium protein source. It is a source of phosphorus, niacin, selenium, vitamin B_{12} and potassium.

Farm – raised Tilapia (the least expensive and most popular) has a high fat content, though low in saturated fats. The nutritional value of farm – raised Tilapia may be compromised by the amount of corn included in the feed. Short – chain omega – 6s in corn accumulate in the fish (Yonas, 2006).

2.1.4. Exotic Species

Tilapia is unable to survive in temperate climates because it requires warm water. The pure strain of the blue Tilapia, *Oreochromis aureus*, has the greatest cold tolerance and dies at 45^{o}F (7^{o}C) while all other species of Tilapia will die at range of 52 to 62^{o} F (11 to 17^{o}C). As a result, they cannot invade temperate habitats and disrupt native ecologies in temperate zones; however, they have spread widely beyond their point of introduction in many fresh and brackish tropical and subtropical habitats (Yonas, 2006).

2.1.5. Uses of fish other than supplying food.

Tilapia serves as a natural, biological control for most aquatic plant problems. Tilapia consumes floating aquatic plants, such as duckweed watermeal, most undesirable submerged plants and most forms of algae. In the United States and countries such as Thailand, they are becoming the plant control method of choice, reducing or eliminating the use of toxic chemicals and heavy metals – based algaecides. Tilapia rarely competes with other ponds fish for food. Instead, they consume plants and nutrients unused by other fish species and substantially reduce oxygen depleting detritus. Adding Tilapia often increase the population, size and health of other fishes (Yonas, 2006). In Kenya, Tilapia helps control mosquitoes which carry malaria parasites. They consume mosquito larvae, which reduces the numbers of adult females, the disease's vector. Tilapia also provides an abundant food source for aquatic predators.

2.1.6 Aquaria

Large Tilapia species are generally viewed as poor community aquarium fish because they eat plant, dig up the bottom and fight with other fish. However, they are often raised in aquarium as a food source due to their rapid growth and tolerance for high stocking densities and poor water quality. The smaller West Africa species and those species from the crater lakes of Cameroon, are more popular. In specialized cichlid aquaria, tilapias can be mixed successfully with non territorial cichlids, amored catfish, tinfoil barbs, garpike and other fishes. Some species including *Tilapia buttikoferi, Tilapia rendalli, Tilapia mariae, Tilapia joka* and the brackish-water *Sarotherodon melanotheron* have attractive patterns and are quite decorative (Espe *et al.*, 2006)

2.1.7. *Sarotherodon melanotheron*

Sarotherodon is a genus of tilapiine cichlids endemic to Africa and the Middle East. A few species from this genus have been introduced far outside their native range, and are important in aquaculture. They mainly inhabit fresh and brackish water, but a few can live in salt water. Members of this genus, as well as those of the genera Tilapia and Oreochromis share the common name Tilapia. There are currently 13 recognized species in this genus (Froese *et al.*, 2013).

The Nile Tilapia, *Oreochromis niloticus* is a relatively large cichlid fish which is native to Africa from Egypt South to East, Central and West Africa. It is also native to Israel and numerous introduced populations exist outside its natural range. It is commercially known as mango fish or Nilotica (Froese *et al.*, 2012).

2.1.8. *Ethmalosa fimbriata.*

Ethmalosa fimbriata, the Bonga shad is a fish that occurs along the coasts and in brackish water of coastal lagoons, rivers and lakes of western Africa. Bonga is caught by inshore small scale fisheries using seine fishing from a boat or by beach seine. It is an important food source in West and Central Africa (Momodou, 2012). It is usually smoked dried for 2-5 days depending on size and on the market. A hard smoked bonga can be kept for several months in ambient conditions (Froese *et al.,* 2011).

2.2. Environmental pollution

Environmental pollution is defined as energy or waste materials that are discharged into the environment where they can cause damage to human health (Karade, 2007; Martin and Grisworld, 2009). These polluting agents (waste materials, chemicals, toxicants) are known as environmental pollutants (Table 2.1). Environmental pollution is a worldwide problem and its potential to influence the health of human population is great. Over the last three decades, there has been increasing global concern over the public health impacts attributed to environmental pollution. There is no doubt that excessive levels of pollution are causing a lot of damage to human and animal health, plants and trees (WHO, 2010).

Table 2.1: Major Industrial Activities and Potential Sources of Pollution (Karade, 2007)

Industry	Air emission	Waste water	Solid waste
Coal mining	*	*	*
Meat production	*	*	*
Manufacture of dairy products		*	
Manufacture of soft drinks		*	
Tanneries and leather finishing		*	*
Manufacture of paper and products	*	*	*
Petroleum refineries	*	*	*
Manufacture of cement	*	*	*

Ion and steel industries	*	*	*
Electricity, light & power	*	*	*
Agricultural industry			*
Textile industry		*	

Note: An asterisk indicates a major source of pollution from the relevant industry.

Tehran is one of the victim cities in terms of environmental pollution. Indian was nominated among the most polluted cities in the world. The air we breathe is an essential ingredient for our well being and a healthy life. Unfortunately, polluted air is common throughout the world. The main pollutants found in the air we breathe include particulate matter, Polycyclic aromatic hydrocarbons (PAHs), lead, ozone, heavy metals, sulphur dioxide, benzene, carbon monoxide, nitrogen dioxide etc.

Forms of Pollution:

There are different types of pollution namely:

i. Air pollution
ii. Light pollution.
iii. Visual Pollution
iv. Water pollution

v. Noise pollution.

vi. Soil pollution.

vii. Radioactive pollution.

viii. Thermal pollution.

Water Pollution:

The water we drink is an essential ingredient for our well being and a healthy life. Unfortunately, polluted water and air are common throughout the world. Polluted water consists of industrial discharged effluents, sewage water, rain water pollution and so on (Hogan., 2010). Some water pollution effects are recognized immediately whereas others do not show up for months or years. Estimation indicates that more than 50 countries of the world with an area of 20 million hectares area are treated with polluted or partially treated polluted water (Hogan, 2010). This poor quality water causes hazards, death of human beings, aquatic life and also disturbs the production of different crops. In fact, the effects of water pollution are said to be the leading cause of death for humans across the globe, moreover, water pollution affects our oceans, lakes, rivers and drinking water, making it a widespread and global concern.

2.2.1. Sources of Environmental Pollution

2.2.1.1. Industrial Sources of Pollutants.

Industry is economically important to countries and employs millions of workers throughout the world. Industry is not just buildings and factories, but also includes industrialized agriculture, ships and other vessels at sea, refineries and oil drilling platforms in the ocean, trucks used for transportation of goods etc. The industry contributes various kinds of pollutants to the environment. The pollutants are mainly in gaseous, water and solid forms that can cause serious damage to the biosystem (Karade, 2007).

Therefore industry is all around us and plays an important role in our lives. Major industrial activities have the potential for generating air emission, wastewater effluents and solid wastes, (Fig 2.0), all of which may contain a variety of chemical pollutants (Karade, 2007). For example, the environmental discharge of inorganic mercury and subsequent human exposure to methyl mercury. Heavy exposure to chemicals is more likely to be seen in workers working in industries (Table 2.1). This is known as occupational exposure.

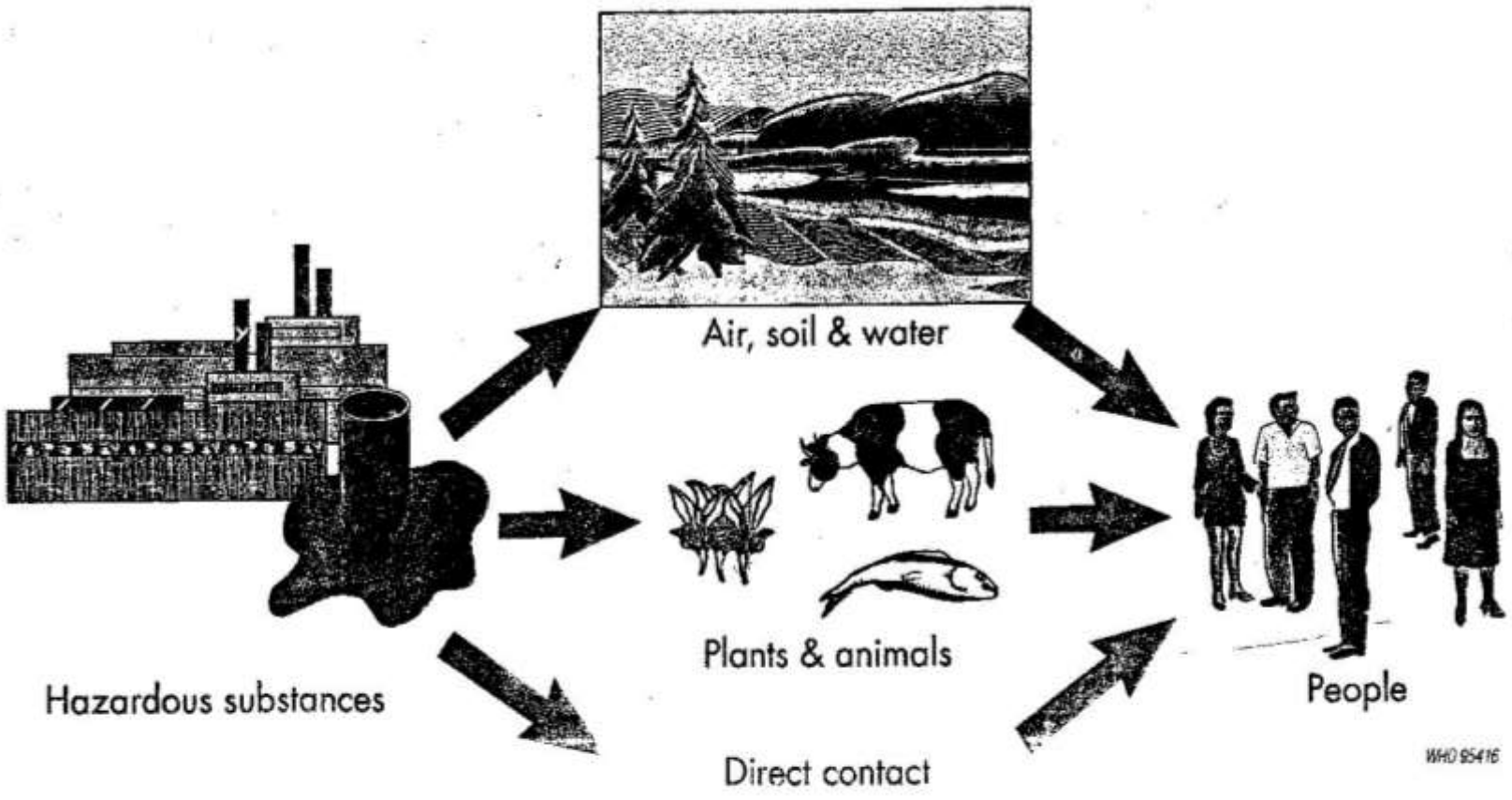


Fig 2: Pathways by which environmental chemicals can reach the general public. WHO, 2000.

2.2.1.2 The Mercury Industry

Metallic mercury is used in the chlorine-alkali industry for electrolytic production of chlorine and sodium hydroxide. It is used to manufacture scientific and electrical apparatus, as a catalyst in chemical processing reactions and to produce thermometers, (USEPA, 2003).

Metallic mercury is a worldwide contaminant, but most mercury poisoning has been due to methylmercury, particularly as a result of eating contaminated fish (Fig. 2). The environmental toxicity of mercury is a good example of three important properties of a substance. These properties are toxicity, volume of use (as an industrial substance, the release of which was poorly regulated and controlled) and mobility together with biological transformation. Methylmercury accumulation in seafood and fish products is a growing global concern that poses severe health risks to the public. In the US, legislation has been passed to reduce mercury pollution from coal-burning power plants and banning certain products that contain mercury, (USEPA, 2003). Mercury is often used in barometers and thermometers. It can also be combined with other metals to create special alloys called amalgams which are used in dentistry for filling and to make mirrors. Mercury can be found in different lamps in the industrial production of chloride and sodium hydroxide. some mercury compounds are used as ingredients in skin creams, antiseptics, and a preservative in vaccines (Clarkson and Magos, 2006). For most people, exposure to mercury occurs when they eat fish or shellfish contaminated with methyl mercury. This compound is found in nearly all fresh water and marine fish (USEPA, 2003).

2.2.1.3 The Textile Manufacturing Industry

The textile industry is one of the largest employers of manpower worldwide. During the last twenty years, much of the textile industry has shifted to many African and Asian countries. The textile industry includes the spinning, weaving, knitting and finishing of all types of natural and synthetic fibres. The textile industry uses vegetable fibres such as cotton, animal

fibres such as nylon, polyester and acrylics. The production of natural fibres is approximately equal to the amount of production of synthetic materials of which polyester accounts for about half. Because textile operations produce so much wastewater, mills may be tempted to assume that they cannot avoid large volumes of wastewater and therefore, they may become lax in pollution prevention. Pollutants in wastewater from textile factories vary greatly and depend on the chemicals and treatment process used. Pollutants that are likely to be present include suspended solids, bio degradable organic matter, toxic organic compounds, for example, phenols and heavy metals (Karade., 2007).

During fabrication, workers can be exposed to a variety of bleaching, scouring and dyeing agents. Exposure to fibre dust is of concern (Table 2.2). Toxic chemicals are used in the manufacture of synthetic fibres. Toxic dangers also exist in the dyeing and finishing sections of the textile industry. In dyeing and printing, workers are frequently exposed to dyes, a variety of acids such as formic, sulfuric and acetic acids, florescent brighteners, organic solvents and fixatives. Workers in the finishing operations are frequently exposed to crease-resistant agents, to flame retardants and to a number of toxic solvents used for degreasing and spotting. Care must be taken in the use of these substances to prevent contact with the skin and suitable measures taken to ensure there is no escape of the material or its vapor into the atmosphere. Skin diseases of the dermatitis type are common in bleaching, dyeing and finishing, in the preparation of flax and in the use of solvents for making synthetic fibres (Karade, 2007).

Chrome eczema arising from chrome poisoning is a hazard from the use of potassium or sodium dichromate in the textile industry. Occupational health effects include byssinosis, chronic bronchitis, dermatitis, cancer of the bladder among dyers and cancer of the bladder and of the nasal cavity among weavers and other textile workers, (Table 2.2).

Table 2.2: Diseases and Symptoms of Textile Workers exposed to chemicals (Karade, 2007).

Technical term of disease	Symptoms
Bysinnosis	Acute tightness of the chest, wheezing, coughing
Chronic bronchitis	Attacks of coughing
Dermatitis	Inflammation of the skin
Bladder/nasal cancer	Bleeding, discomfort and pain

In its overall evaluation, the International Agency for Research on Cancer concluded that working in the textile manufacturing industry entails exposures that are possibly carcinogenic to humans.

These exposures may occur simultaneously with physical hazards, including noise, vibration and heat. The exposure levels and chemicals used in any one country could be quite different from those used elsewhere. Volatile organic emissions (from oils added during spinning and from solvents) are largely uncontrolled and are used in texturizing, heat setting, finishing, dyeing and printing operations (Karade, 2007).

2.2.1.4 The Asbestos Industry

Asbestos is used extensively in roofing felt and insulation, asbestos cements, brake linings, electrical appliances and fire-proofing and counting material. International Labour Organization (ILO) has called on countries throughout the world to eliminate asbestos-related diseases (WHO, 2003). WHO advises that the best way to eliminate such diseases is to stop using all types of asbestos. Numerous countries have adopted national asbestos bans, many other continue to use asbestos at various levels. WHO estimates that 107,000 global annual deaths are caused by mesothelioma asbestos related lung cancer and asbestosis (Stainer, 2013; WHO, 2010; Virta, 2006). In 2005, occupational exposure to asbestos was estimated to cause 43,000 mesothelioma deaths (Driscoll, 2005) and 7,000 deaths due to asbestosis (Driscoll, 2005; Fingerhut, 2005).

Exposure may take place from both natural sources and industrial applications. There are two types of fibers namely chrysotile and crocidolite. Chrysotile ($3MgO.\ 2SiO_{2.}\ 2H_2O$) is the most important commercially and represents about 90% of the total asbestos used. Crocidolite (blue asbestos) is composed of short-rod like fibers, which are more dangerous than chrysolite fibers. Inhalation of asbestos fibers deep into the lungs causes physical damage and is linked to mesothelioma, a form of lung cancer. Asbetosis is a respiratory

disease and is characterized by lung fibrosis and calcification and can lead to cancer (Roggli and Sanders, 2000).

2.2.1.5 The Petroleum Industry

Today, the world's petroleum refining industry produces more than 2500 products including napthes, distillates, residual fuels, asphalts, liquefied petroleum gas, petrol, kerosene/paraffin; aviation fuels, diesel fuels and a variety of other oils and lubricating oil, (Kane 2006). Crude oil is a mixture of thousands of different hydrocarbons with a wide range of boiling points. It contains compound with various amounts of sulfur, nitrogen, oxygen, trace metals and water. Petroleum refineries produce a wide variety of air and water pollutants and hazardous solid wastes. The specific mix of pollutants varies with the activities and processes involved. Frequently emitted pollutants include all the distillation products of refining (fuels, solvents, oil, waxes, greases, asphalt) and specifically hydrogen sulfide, polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, and benzene. Because these facilities are usually sited in large industrial zones involving multiple petrochemical facilities, significant contamination of air and water is usually associated with their presence. Residences of adjoining communities are potentially at risk from the inhalation of polluted air and the ingestion of polluted water. Large volumes of hazardous wastes are generated and must be appropriately disposed of or they may adversely affect health through the contamination of soil and groundwater. Residents living downwind of refineries have been shown to be at greater risk of developing respiratory symptoms (coughing and wheezing) (Kane, 2006).

A wide range of potential occupational health hazards is also present in petroleum refineries. Exposure results from skin contact and the inhalation of gases and vapors, mainly hydrocarbons, which are either naturally present in crude oil or emitted during its refining or are formed and emitted during processing (Kane 2006).

Gaseous sulfur compounds such as hydrogen sulfide and sulfur dioxide and mercaptans are emitted during removal and treatment of sulfur. Exposure to dusts and fumes results mostly from maintenance operation such as abrasive blasting, the use of catalysts and the handling of viscous or solid products such as bitumen. In its overall evaluation, the International Agency for Research on Cancer (IARC) has concluded that occupational exposures in petroleum refining are probably carcinogenic to humans (Kane, 2006).

2.2.1.6 The Solvents using Industry

Organic solvents and their vapors are commonly encountered in our modern environment. Industries use large quantities in the manufacturing process of many different end products. Exposure can be through materials such as gasoline (petrol) vapors, aerosol sprays and paint remover. A good example of the solvent is benzene. Benzene is an excellent solvent of rubber latex.

2.2.2 Agricultural Sources of Pollutants.

Many chemicals are used in agriculture, including nitrogen and phosphorous fertilizers, pesticides, plant growth regulators, disinfectants etc. Of these, pesticides are the chemicals that cause the most health and environmental concerns (Gilden *et al.,* 2010).

2.2.2.1 Uses of Pesticides

Pesticides are used in agriculture, horticulture, forestry and livestock production. By far, the largest source of concentration by pesticides has resulted from their use in agriculture and public health. Pesticides are widely misused, most seriously in countries where legislation, monitoring and enforcement are inadequate. Some pesticides like DDT have been banned or restricted in many countries but are still widely available in others (Gilden *et al.*, 2010).

2.2.2.2 Contamination of Air, Soil and Water due to Pesticides

Air can become contaminated with pesticides during spraying operations. The evaporation of droplets during the spraying of emulsified pesticide formulations may result in the formation of tiny particles that can be carried great distances in air currents. This has been confirmed by studies showing the presence of pesticide in urban smog (Kymisis and Hadjistavrou, 2008). It is also common practice in many countries to treat houses with pesticides to control disease vectors. The pesticide evaporates in the house and may be inhaled by the inhabitants. Further amounts may be taken in through the skin by contact with treated surface or ingested by consuming contaminated food.

Soil may be deliberately treated with pesticides to control insects or nematodes. In addition, a large proportion of the pesticide sprayed on crops or used as an herbicide misses its target and falls on to the soil surface. Some pesticides notably organochlorines are known to persist in soil for years (Kuniuki, 2001).

Water may be polluted by the dumping of excess pesticides left over after spraying operation, accidental spillage of pesticide formulation or by the application of pesticide to rivers or ponds for aquatic weed control. Dumping of pesticides into water bodies may lead to contamination of drinking water.

2.2.2.3 Exposure of Humans to Pesticides

Often pesticides are applied only days or hours before the crops are harvested. Such crops may contain residues that lead to high exposures of the crops to the residues. Other ways in which food can be contaminated is through fish caught in pesticide treated rice paddies which contain significant levels of pesticide residues. Treatment with pesticides to prevent losses of food during storage or bulk transport also creates a hazard (Kymisis and Hadjaistavrou, 2008).

The losses caused by arthropod pests and rodents can be extremely heavy. It is common practice to treat food and grain with pesticides to avoid such losses. Foods treated in this way may contain high concentrations of pesticides. The majority of cases of unintentional pesticide poisoning occur largely among farm workers and their families. Exposure occurs primarily during mixing or using pesticides spraying aircraft or re-entering a previously treated area. Acute occupational exposure may also occur during the manufacture, formulation, packaging and transport of pesticides. Acute effects associated with high occupational exposure to pesticides include chemical burns of the eye, skin damage, neurological effects, and liver effects. Chronic exposures are suspected of leading to reproductive problems and an increased risk of developing cancers delayed neurological and psychological effect etc (Gilden *et al.,* 2010).

2.2.3 Urban Sources of Pollutants

It has been known for thousands of years that human activities and urbanization may lead to air pollution. In fact, air pollution began as soon as humans started to use wood fires for heating and cooking. Many cities are faced with major air pollution problems caused by both urbanization and industrialization. Industries within cities are sometimes the main contributor, while congested streets, poorly maintained motor vehicle engines and high levels of lead in petrol also contribute to the air pollution problems. In some cities, the use of wood or coal as the main household fuel is a major contributor and a cause of respiratory problems in the young and elderly (Kuniuki, 2001).

2.2.3.1 Fossil Fuels as a source of air pollution

The combination of fossil fuel for domestic heating, power generation, transportation and in industrial processes, summarized in Table 2.3 below, contributes to the principal sources of anthropogenic air pollutant emissions in the atmosphere in urban areas. The most common air

Table 2.3: Human Activities and the by-product of fossil fuel combustion (Miller, 2004)

Activity	**Air pollutants**
Power plants used to generate electricity	SOx, NOx, (NO and NO_3) SO_4 and nitrate NO_3
Oil combustion	SOx, Soot
Burning of domestic solid fuels (Coal and wood)	SOx, Soot, Fly ash
Diesel fuel combustion	SOx and Soot, NOx
Petrol fuelled vehicles	NOx, CO, Pb
Cigarette smoking and barbeques	PAHs, and others

pollutants in urban environments include sulfur oxides (SOx) especially sulfur dioxide (SO_2), the nitrogen oxides (NO, NO_2), carbon monoxide (CO), ozone and lead (Miller, 2004).

2.2.3.2 Ozone as a Source of Air Pollution

Although ozone depletion is of concern in the upper atmosphere, at ground level, the reverse problem (excessive ozone levels) can occur under condition of urban air pollution (Rubin, 2001).

Ozone, a photochemical oxidant is formed in the lower atmosphere in the presence of NOx, hydrocarbons and volatile organic compounds (VOCs). Atmospheric temperatures above 18^oC together with plenty of sunlight to catalyse the reactions are also required. The VOCs may be emitted from a variety of synthetic sources including road traffic, production and use of organic chemicals (e.g. solvents), transport and use of crude oil, use of natural gas and to a lesser extent, from waste disposal sites and waste water treatment plants. Cities in warm sunny location with high traffic densities tend to be especially prone to the net formation of ozone and other photochemical oxidants from precursor emissions. High concentrations of ozone at ground level are toxic to plants and cause respiratory problems in the elderly and asthmatics (Nicole, 2003).

2.2.4 Accidental Release of Pollutants

Accidents at production facilities or during the transport of hazardous materials have also contributed to air, water and soil pollution and also to adverse human health effects (USEPA, 2000).

Accidents such as explosions, fires and collisions of transport vehicles can lead to the release of many potentially dangerous chemical agents into the environment. Once this occurs, workers and the general population are subject to exposure. Most chemical accident occurs

because of carelessness, but unskilled engineers, poorly trained operators or lack of communication may also contribute. To avoid accidents, it is important that industries provide training for personnel involved in hazardous industrial processes (USEPA, 2000).

2.3 Routes of Exposure of Heavy Metals.

There are three principal routes of exposure. These are**:** dermal absorption, inhalation and ingestion.

2.3.1 Dermal Route of Exposure

The skin is one of the most common routes of exposure to substances but, fortunately, it is an effective barrier against many chemical agents. If a chemical agent cannot penetrate the skin, it cannot exert a toxic effect by the dermal route. If it cannot penetrate the skin its toxicity depends on the degree of absorption that takes place. The greater the absorption, the greater the potential for a chemical agent to exert a toxic effect. Chemical agents are absorbed much more readily through damaged or abraded skin than through intact skin. A chemical agent must cross a large number of cell layers in the skin before it can reach circulation (Hossin and Mokhtar, 2001).

Once a chemical agent penetrates the skin, it enters the blood stream and is carried to all parts of the body. The ability of a chemical agent to penetrate the skin depends on whether or not the chemical is fat-soluble. Chemicals that can dissolve in fat are much more likely to penetrate the skin than water-soluble chemicals.

Skin irritation and skin allergy are the most common condition resulting from dermal exposure in the workplace in the chemical industry. Of particular concern is dermal exposure of workers to pesticides during mixing and application of these materials. Some pesticide formulation are especially hazardous if they are both toxic and contain fat-soluble solvents such as kerosene, xylene and other petroleum products that make it easier for the pesticide to penetrate the skin (Hossin and Mokhtar, 2001).

Irritation is a skin condition that can be produced by prolonged contact with certain chemicals. This condition is caused by solvents, acids, alkalis, detergents and coolants (Christopher *et al.,* 2004).

Allergic contact dermatitis is a delayed type of skin disease caused by high sensitivity to a chemical. Very small quantities of the chemical which normally would not cause any irritation produce damage to the skin due to increased sensitivity. Symptoms are rash, swelling of the skin, itching and blistering. Allergic contact dermatitis is caused by repeated contact with substances such as chromium (present in cement, leather, rust-proofing agents, etc), cobalt (present in detergents and colour pigments), nickel (nickel plaited objects such as ear rings, keys, coins, tools), (Table 2.4). After a chemical agent has been absorbed through the skin and has entered the systemic circulation, it can travel throughout the body and damage organs and body systems. (Sandau *et al.*, 2000).

Table 2.4: Recognized Skin Effects of Pesticides (Sandau *et al*, 2000)

Pesticide	**Effect**
Parquat	Contact dermatitis
Benomyl, DDT, Lindene, Malathion	Skin sensitization, allergic reaction, rash
Hezachlorobenzene	Photo allergic reactions
Organochlorine pesticides	Chlorcne

2.3.2. Inhalation Route of Exposure

The lung is another common route of exposure but, unlike the skin, lung tissue is not a protective barrier against chemical exposure. The main function of the lung is the exchange of oxygen from air to blood and CO_2 from blood to air. Consequently, the lung tissue is very thin and allows the passage not only of oxygen, but also of many other chemicals directly into the blood. In addition to systemic damage, chemicals that pass through the lung surface may also injure the lung tissue and interfere with its vital role of oxygen supply (St-Pierre *et al.*, 2001).

Chemical can become airborne in two ways, either as tiny particles (i.e. dust) or as gases and vapors. Most of the traditional air pollutants (SO_2, nitrogen oxides, CO, O_3, Pb) directly affect the respiratory (lung) and cardiovascular (heart & blood vessels) systems. Decreased lung function and increased mortality have been associated with elevated levels of SO_2. NO_2 and ozone also affect the respiratory system. Carbon monoxide binds to haemoglobin and is able to displace oxygen in the blood, which in turn can lead to damage to the heart and nervous system. Lead inhibits haemoglobin synthesis in red blood cells, impairs liver and kidney function (Mar *et al.*, 2000).

The human health effects of exposure to air pollutants vary with the amount and length of exposure but also with the health status of the people exposed. Certain people are at a greater risk of damage from inhalation exposure e.g. the young and the elderly together with those already suffering from respiratory and cardiopulmonary disease. In industry, inhalation of chemicals in the form of gases, vapor or particles and absorption through the lungs is the most important route of exposure. A variety of chemicals can become airborne in the workplace, (Mar *et al.*, 2000). The health risks from occupational exposure to airborne contaminants are often higher in small workshops e.g. the recycling and repair of lead-acid batteries in small enterprises have led to heavy exposure of workers to airborne lead.

In order to decrease the risk of the inhalation exposure, it is necessary to have very good ventilation and wear a respirator fitted with a filter. (St – Pierre *et al.*, 2001).

2.3.3. Ingestion as Route of Exposure

This is the principal pathway for entry of compounds that are present in food and drink. Chemicals that are ingested enter the body by absorption from the gastrointestinal tract. The major site for absorption is the small intestine because of its physiological function in absorbing nutrients (Mar *et al.*, 2000).

2.3.3.1 Food

Ingestion of food contaminated with high levels of hazardous chemicals has resulted in severe health damage. It affects the nervous system, immune system, reproductive system, renal system etc. Organomercury compound have been the cause of several major poisoning epidemics in the general population due to either the consumption of contaminated fish or to eating bread prepared from cereals treated with alkyl mercury fungicide (Mar *et al.*, 2000).

2.3.3.2 Water

Thousands of organic and inorganic chemicals have been identified in drinking water around the world. The problems associated with chemical constituents of drinking water arise primarily from their ability to cause adverse health effects after prolonged periods of exposure. Long-term exposure to arsenic in well water in Taiwan has resulted in “Blackfoot” disease and skin-cancer (Christopher *et al.*, 2004). Arsenic contamination of groundwater (the main sources of drinking water) was detected in six districts of West Bengal, India. The contamination is due to the natural soil composition of the region. In West Bengal alone, 200,000 people have been reported to be suffering from arsenical skin lesions (Christopher *et al.*, 2004).

The toxic effects of nitrate in humans depend on the conversion of nitrate to nitrite which is toxic. The major biological effect of nitrite in humans is its involvement in the conversion of

normal blood haemoglobin, which transports oxygen in the blood, to methemoglobin, which is unable to transport blood oxygen to the tissues and organs, (Christopher *et al.*, 2004).

2.4 Environmental Pollutants

Some of the various environmental pollutants relevant to the present study, which are causing pollution in the environment, are described thus:

2.4.1 Cadmium

2.4.1.1 Occurrence, Exposure and Uses

Cadmium has become widely used in the manufacture of alloys and for electroplating. Cadmium is extracted from zinc sulfide ores in the course of smelting and is a constituent of the so called "blue powder", a consideration product that has up to 4 or 5% of cadmium. Cadmium is also found in sludge after the electrolytic recovery of zinc (Maret and Moulis, 2013). It is used in silver, copper and other alloys, but about half the amount produced is used in the electroplating of metals since it resists corrosion better than nickel or steel. (Abde-Sabour, 2001).

It is used extensively as a stabilizer in plastics and in pigments. Minor uses include nickel-cadmium storage batteries, fungicides, insecticides, photography, television picture tubes etc. Other uses for cadmium are in metal bearing ceramics, process engraving, cadmium vapor camps, and for rust proofing tools and other iron and steel articles previously coated with zinc. The presence of cadmium has to be considered, not only in the manufacture of paints, but in the spraying of pigments and in welding processes when the metal or the welding iron contains cadmium (Alven *et al.,* 2002). Cadmium may be sprayed onto graphite or may be used in rods as neutron absorbers in nuclear reactors. The use of diethyl cadmium in the manufacture of tetraethyl-lead as an additive to gasoline has been reported (Hossin and Mokhtar , 2001).

2.4.1.2. Health Effects.

The greatest industrial hazards, involving cadmium were in the smelting of ores, the working up of residues, the handling of "blue powder", production of compounds, spaying of pigments, welding alloys and melting the metal. Solders containing cadmium in varying amounts with copper, lead, tin and silver especially silver solders have been a source of poisoning (Hossin and Mokhtar, 2001). Remelting of scrap and the use of a blow torch in working cadmium plated steel pipe are dangerous, often because of the presence of potentially toxic cadmium (Nordberg, 2010; WHO, 1992).

Industrial and experimental evidence have shown cadmium to be one of the most hazardous metals. It has a significant vapor pressure at its melting point (320.9^{o}C), at which point an air concentration many times the safe limit can be produced. Freshly generated fumes of cadmium have been shown to be more acutely poisonous than "old" settled fumes that are inhaled as dust. Cadmium and its compound may be inhaled or ingested. have pointed out that in industry exposure is mainly via air, while for the general population, exposure is through food. Smoking of tobacco may provide additional exposure in both groups (Hartwig, 2013; Hellstrom *et al.*, 2001; ATSDR, 2008). Inhaled cadmium may be absorbed to the extent of 10 to 50%, but is undoubtedly swallowed after clearance from the lung by the ciliary's ladder in the bronchi and trachea. About 5% of ingested cadmium is absorbed through the gut. Absorbed cadmium is bound and transported by metallothionein, a low-molecular weight compound inducible protein. Cadmium is deposited in the liver from which it is slowly released and subsequently deposited in the kidney, which is the critical organ in long-term exposure (Baselt, 2008). Renal tubular damage is estimated to occur when cadmium concentration reaches 200µg/g wet weight in the cortex. Excretion of cadmium from the body is very slow, the biological half-time in humans being 10 to 40 years (Jarup *et al.*, 2000).

When cadmium exposure has been at low levels for a prolonged period, urine cadmium values reflect the body burden of cadmium. When, however, exposure is more intense, urine cadmium level may be as high as 50µg/g creatinine and will indicate recent exposure rather than body burden (Jarup *et al.*, 2000; Jarup and Berglund, 1998; Jarup, 2003). Cadmium is thought to act biochemically by competing with and displacing other metals and by reacting with –SH groups in enzymes (Alven *et al.,* 2002; Nordberg *et al.*, 2002; Hossin and Mokhtar, 2001; Hellstrom *et al.,* 2001). Cadmium is a known carcinogen. Ingesting very high levels severely irritates the stomach, leading to vomiting and diarrhea. Long term exposure leads to a build up in the kidneys and possibly kidney disease, lung damage and fragile bones (WHO, 2006; Mudgal *et al,* 2010).

2.4.2 Chromium

2.4.2.1 Occurrence, Exposure and Uses

Metallic chromium is relatively inert but its surface is oxidized in damp air and its burns readily in the finely powdered state. Its bivalent salts (e.g. chromous oxide CrO) are unstable. The trivalent compounds that are important in industry are chromic oxide (Cr_2O_3) and chromic sulfate ($Cr_2(SO_4)_3$). The most important industrial compound are the hexavalent e.g. (Chromic acid, CrO_3, the chromates CrO_4 and the dichromates Cr_2O_7 and these are also the most toxic (Kotas and Stascika, 2000). Metallic chromium is alloyed with iron, nickel, molybdenum and manganese to produce a series of stainless steels that are resistant to corrosion and inert to nitric acid.

Chromic acid and the dichromates are familiar occupational hazards because of their wide use in industry. The acid is used in chrome plating of a variety of objects, such as household appliances and automobile rim, where brightness, beauty and resistance to wear and rust are advantageous (Gonzalez *et al.*, 2005). Chromium plated directly on aluminum is used in the

chemical, food aviation and electric industries. The dichromates (potassium, sodium, and ammonium) are used as mordant in dyeing and in the quick tanning of leather. The cement industry uses chromium compounds, magnesium foundries use chromic acid solution to give weather resistance and the forestry industry impregnates some timbers with chromium salts. Chromates are also employed in making coal tar dyes, in photography and in photoengraving and chromic acid is used in lithography (Bailey, 2002).

2.4.2.2 Health Effects

A source of chromic acid poisoning arises from exposure to the mist formed in anodizing, an operation whereby a corrosion-resistant coating is formed on aluminum and its alloys. The anodizing solution contains up to 10% chromic acid. The protection of workers against the fine sprays of chromic acid has engaged the best efforts of ventilation engineers in developed countries, (Wani *et al.,* 2006).

Chromic acid and the chromates are oxidizing agents and are irritating to exposed tissues. Consequently, the harmful effects of chromium are largely confined to dermatitis, ulceration of the skin and nasal mucosa and effects on the lungs, principally cancer (Wani *et al.,* 2006).

Chromium is a common cause of contact dermatitis, both as a result of irritation and more frequently from sensitization. Occupation known to have a risk of chromium dermatitis are cement workers, chromium platers, dyers and persons working in printing, photographing and with antirust agents and with wood impregnated with chromium (Pesch *et al.,* 2000).

The lesions of the nasal mucosa are the ones that give the most trouble and usually are the basis for compensation claims made by chrome workers. The chromate dust or the chromic acid spray that is inhaled causes irritation of the nasal mucosa, with inflammation, purulent discharge, formation of crusts and some difficulty in breathing. These chemicals can act alone or with infection and cigarette smoking to cause bronchial disease. There is a higher rate of bronchitis among chromate workers in United States (ATSDR, 2008; Wani *et al.*,

2000). Chromium are toxins and known carcinogens. Breathing high levels can cause irritation to the lining of the nose, nose ulcers, asthma, cough or wheezing. Long term exposure causes damage to the liver, kidney, circulatory and nerve tissues.

2.4.3. Copper

2.4.3.1 Occurrence, Exposure and Uses

Copper occurs widely in nature, both in free form as native copper and in a variety of ores. Extraction from ores involves crushing, roasting and smelting to produce "matte", a mixture in varying proportions of copper and iron sulfides that is further refined to remove sulfur and iron. Copper is used in an extensive range of alloys to which it imparts hardness, principally brass and bronze, as well as special mixtures with other metals such as nickel, beryllium and cobalt. Copper is used in wires, pipes, roof sheeting and vessels and copper salts are used as insecticides, algaecides and parasitoids (Gadd, 2010; Hammond, 2004).

2.4.3.2. Health Effects

Copper is an essential element and because of its distribution in nature, it is normally found in all diets. The daily United States dietary intake of copper ranges from 2-5 mg. Minute amounts of cupric ion are absorbed and stored mainly in the liver, blood and brain. Copper balance is maintained by an efficient homeostatic mechanism that involves the intestine as a barrier and organ of excretion and the liver for storage (Vest *et al.*, 2013; Kim *et al.*, 2005).

Copper is a component of a number of cupro enzymes that catalyse important biochemical and physiological reactions that include iron absorption and heme biosynthesis. Copper deficiency leads to anemia, neutropenia and bone lesions (Jaiser and Winston, 2010).

Copper poisoning on the other hand, leads to a variety of toxic effects that include metallic taste, ptylism, nausea, vomiting, epigastric burning, diarrhoea, hemolysis, hepatic necrosis,

gastrointestinal bleeding, oliguria, hemoglobinuria, hematuria, hypotension, convulsion, coma and death, (Vest *et al.,* 2013; Adeosun *et al.,* 2015).

Scientists have described "Vineyard sprayer's lung" in rural workers in Portugal who applied sprays of Bordeaux mixture (a mixture of aqueous calcium sulphate and calcium hydroxide that involves precipitation of copper hydroxide and several other basic sulfates of copper), there were interstitial pulmonary lesions consisting of copper containing histocytic granulomas and nodular fibrohyaline scars that regressed, remained stationary, or progressed to diffuse pulmonary fibrosis and lung cancer. Copper containing lesions were also found in the nasal mucosa, liver, kidney, spleen and lymph nodes. The copper-related liver lesions included sarcoid-like granulomata, fibrosis, cirrhosis and idiopathic portal hypertension (Jaiser and Winston, 2010). The United States threshold limits value for copper exposure for a 40-hour work week is 0.1mg/m^3 for fumes and 1.0mg/m^3 for the dusts and mists of copper (Jaiser and Winston, 2010).

2.4.4 Manganese

2.4.4.1 Occurrence, Exposure and Uses

Manganese is in demand for the production of alloys especially manganese steel and the making of dry batteries. Such batteries are widely used for flashlights and radios. Manganese is also used in making chlorine gas and potassium permanganates, in the manufacture of paints, varnish, enamel and linoleum, in coloring glass and ceramics, and in making matches and fireworks (Li *et al.,* 2007)

The most hazardous manganese exposures occur in mining and in smelting of ores. Harmful levels may exist in plants manufacturing alloys of manganese and steel. Less dangerous industrial uses of manganese occur in dry battery manufacture, electric arc welding, as well as to some extent in the production of paints, varnishes, enamels, linoleum, fireworks, ceramic glazes and fertilizers. Potentials occupational hazards also exist in the manufacture

of manganese tricarbonyl compounds that are used as additives to fuel oil for inhibiting smoke formation and to gasoline as supplementary antiknock compounds (Boudia *et al.*, 2006; Levy and Nassetta, 2003).

2.4.4.2 Health Effects

The industrial cause of manganism is considered to be the inhalation of dust of manganese ore, or the dioxide, or the fumes from the fusing of manganese in steel manufacture.

Symptoms of manganese poisoning includes: apathy, muscular twitching, stiffness of leg muscle, cramps etc. In addition to these, hematological changes of uncertain importance such as anemia, leucopenia, etc have been reported (Bankovitch and Carrier, 2000; Kim *et al.*, 2005).

There is a poor correlation between urinary manganese levels and severity of the disease (Levy and Nassetta, 2003). Scientist found out that urine manganese concentrations were about 3 times higher in dust-exposed workers in a ferromanganese processing facility than in an unexposed group (Normandin and Beauupre, 2004; Nkwenkeu *et al.*, 2002).

2.4.5 Iron

2.4.5.1. Occurrence, Exposure and Uses

Iron is the fourth most abundant element in the earth's crust (5%) and is mainly extracted from hematite (Fe_2O_3) and limonite (Fe_2O_3. $3H_2O$). Iron is used mainly for structural materials, principally in steel, an iron-carbon alloy. Iron is also used in magnets, dyes, pigments, abrasives and polishing compounds (Boehler and Ross, 2007).

2.4.5.2. Health Effects

Occupational siderosis is caused by exposure to iron oxide such as in paint pigments and jeweler's rouge. Other causes of siderosis include the manufacture of iron oxide, iron shot, sieving and bagging of powder from energy rock and grinding with an energy wheel (Krishna and Grovil, 2004).

Workers using electric arc or oxyacetylene equipment in welding, cutting, grinding, or polishing are at risk, especially when working in closed spaces. The high temperatures of welding operations produce a fume of iron oxide that is readily inhaled in the absence of respiratory protection. The amount of welding fume contaminants (mainly iron oxide) has been determined *in vivo* in arc welders by magnetic measurements. Considerable iron oxide may be caught in the respiratory tract and expectorated in sputum, a process well-known to exposed workers because of the rust colour of the phlegm. The remaining iron oxide accumulates in the lymphoid tissue along the bronchi (Krishna and Grovil, 2004).

2.4.6 Lead

2.4.6.1. Occurrence

Lead (atomic number, 82; relative atomic mass, 207.19, specific gravity 11.34) is a bluish or silvery grey soft metal. The melting point is 327.5^{o}C. It has four naturally occurring isotopes (208/206/207 and 204 in order of abundance) but the isotopic ratios for various mineral sources may differ (WHO, 1995).

The inorganic salts of lead, such as lead sulfide and the oxides of lead are generally poorly soluble in water. However, the nitrate, chlorate and to a much lesser degree, the chloride are water soluble. Some of the salts formed with organic acids are also insoluble e.g. lead oxalate, but the acetate is relatively soluble. Under appropriate conditions of synthesis, stable compounds are formed in which lead is directly bound to a carbon atom. Industrially synthesized lead-carbon compounds which include tetraethylead and tetramethylead are of importance as fuel additives (Mortada *et al.*, 2001).

2.4.6.2 Sources of Lead Pollutants

Soil: Lead is ubiquitous in soil. Levels of 8.20 mg found in non-cultivated soil indicate that it has always been present in man's environment. In cultivated soils levels up to 360mg/kg have

been reported (Tandon *et al.* 2001; Mortada *et al.*, 2001). Sources of lead near industries may reach 10,000 mg/kg or more. Proximity to roads with high traffic density may contribute substantially to soil levels. High levels of lead may occur in dust setting in urban areas and may result in direct contamination of food. Lead is absorbed by edible and non edible vegetation, so that grasses, for instance in highly contaminated areas may attain levels of 20-60 mg/kg (Mortada *et al.*, 2001).Lead enters the food through plants or through accidental ingestion of soil.

Air: Due to urbanization, the amounts of lead in city air range from 1-3µg/m^3 and will occasionally be much higher under peak traffic conditions. Lead containing dusts are present in many manufacturing processes and may add to the lead content in all foods to a small degree. Also contributing to the atmospheric level of leads are industrial lead smelters, disposal of discarded batteries (Tong, 2000).

2.4.6.3 Uses of lead

The patterns of use of refined lead vary from country to country (Table 2. 5). The largest use of lead is for battery production; concentrations of lead in petrol range from zero in such countries as Japan and Thailand to 1.12g/litre in the Virgin Islands (WHO, 2011).

Red-lead containing paint is still widely used to paint structural steel works and can contain up to 66/g lead/kg. Other uses of lead include lead holders (now banned in USA for use in drinking water system), ammunition, foil on wine bottles, cosmetics and folk medicines (Surma in Asia, Kohl in India and Alkohl in Saudi Arabia and Kuwait), (WHO, 2011).

Table 2.5: Principal Uses of Refined lead in Mexico (WHO, 2000)

Types of product	**1988**	**1990**
	%	**%**
Oxides	69.7	56.7
Batteries	9.2	17.9
Tetraethylead	7/9	11.9
Cables	4.0	1.5
Others	9.2	11.9

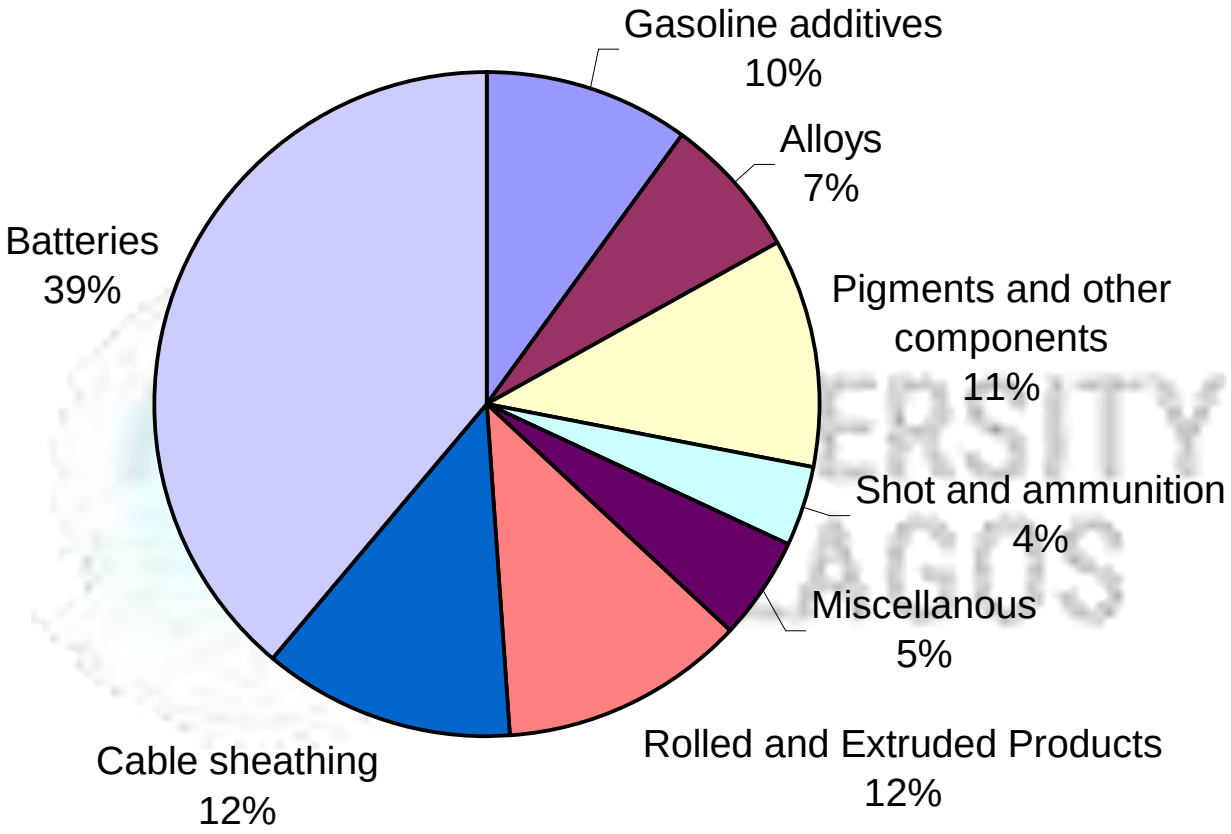


Fig. 2.2: Utilization of lead in European Communities (WHO, 2006)

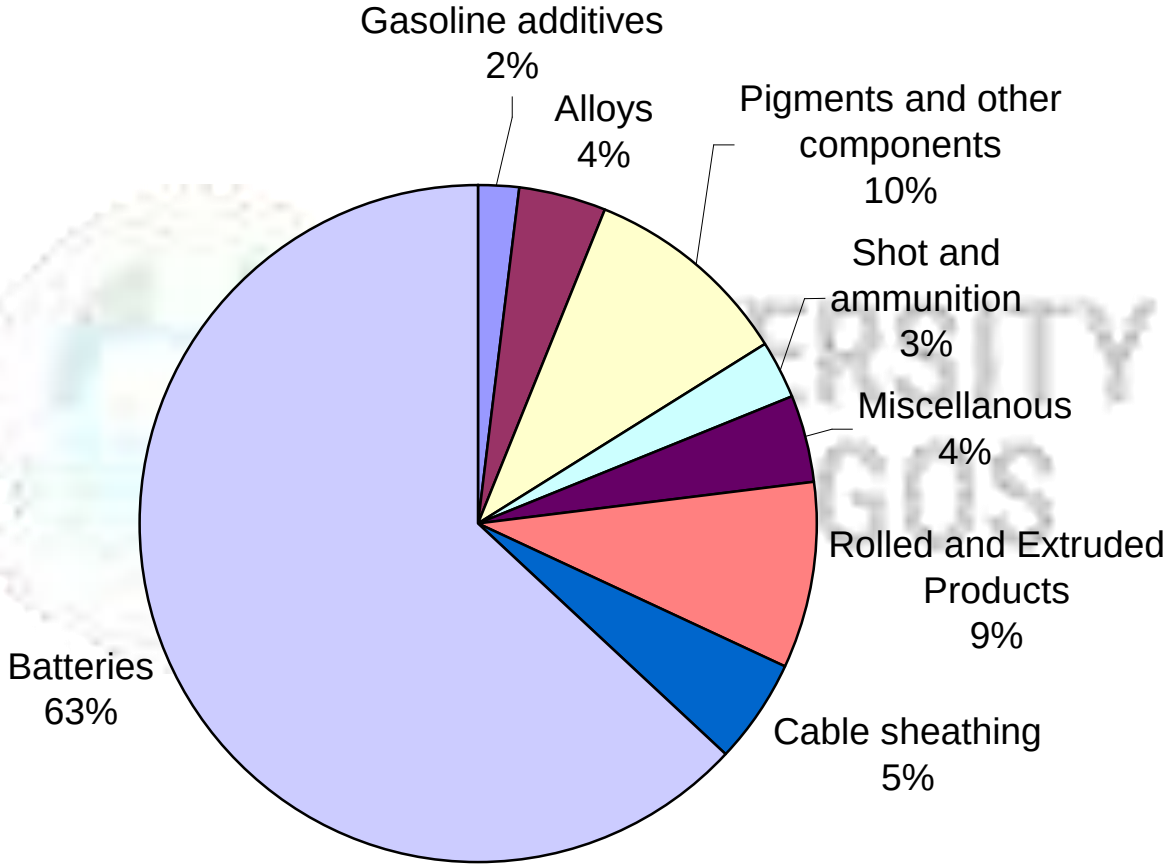


FIG. 2.3: Utilization of lead in European Communities (WHO, 2006)

A source of lead that calls for particular consideration is the lead tetra-alkyls used as petrol (gasoline) additives (Figs. 2.2 and 2.3). The lead derived from petrol additive contributes not only to the intake through inhalation but also to the intake through ingestion as a result of fallout from vehicle exhaust on nearby food crops (Flora and Gupta, 2012 ;Steenland and Boffetta, 2000). Tobacco smoking may also contribute to lead intake by man. While atmospheric lead is present in relatively small concentration, this source assumes considerable importance because greater proportion of lead is likely to be absorbed after inhalation than after ingestion (Klaminder *et al.*, 2006).

Sea water contains lead (0.003 – 0.20mg/litre). Natural water probably contains no more than 0.005mg/litre but in the presence of nitrate, ammonium salts or dissolved carbon dioxide the water becomes lead solvent. This occurs in soft, slightly acid water in older properties where lead piping is still in use. The natural concentration of lead in surface water is about 0.02µg/litre. Lead is not found in surface water or ground water at concentration above 10µg/litre. Over 95% of the lead in offshore surface waters is the result of windborne inputs (Uzu *et al.*, 2010).

2.4.6.4. Agricultural Sources of Lead

Lead occurs naturally in plants and result from both deposition and uptake. Lead has been measured in super phosphate fertilizer at concentrations as high as 92mg/kg. Sewage sludge as a source of nutrients in agriculture may contain even higher levels of lead. The concentration of lead in sewage sludge is typically less than 100mg/kg. Levels as high as 26 g/kg have been measured in the USA. Soils receiving heavy sludge application over long periods of time (years) contained 435mg lead/kg. The concentration in untreated soil was 47mg/kg (Uzu *et al.*, 2010).

2.4.6.5 Industrial Sources of Lead

Lead is used in a large number of industrial sources. Lead containing dusts are present throughout all manufacturing processes and gradually add to the lead content of all foods to a small degree. Lead smelters and dumps where lead-containing material such as old batteries has been discarded or burnt may contribute to localized environmental concentration of the food supply (Steenland and Bofetta, 2000).

2.4.6.6 Anthropogenic Sources of Lead

Lead occurs in a variety of minerals, the most important of which are galena (PbS), cerrusite ($PbCO_3$) and anglesite ($PbSO_4$). Ores containing mainly lead account for about 20% and the remaining 10% is obtained as a by-product from other deposits, such as mixed copper-zinc deposits. Other minor constituents of lead ores are gold, bismuth, antimony, arsenic, cadmium, tin, gallium, thallium, indium, germanium and tellurium (Ayuso and Foley, 2008).

2.4.6.7 Environmental Pollution from Production of lead

Mining operation and the smelting and refining of both primary and secondary lead are known to cause contamination of the nearby environment. Concentrations are usually highest within 3km of the point source. A report from China found that lead levels in ambient air, plants and soil increased proportionally with proximity to a large primary smelter; at 50m from the source, the air lead level was 60µg/m^3, the lead level in plants was 29.1mg/kg and soil lead level was 170mg/kg (Mortada *et al.*, 2001).

Old lead paint on walls and woodworks, and paints on toy may be important sources of excessive lead intake in children. Tinplate cans with soldered seams have been investigated as possible sources of lead contamination for a variety of foods. In a survey carried out in the United Kingdom, the mean lead concentration for canned baby food was about 0.24mg/kg compared with a level of 0.04mg/kg for baby food in jars. The tin coating itself contains little

lead, if any, but the solder used for the seam may contain up to 98% lead (Mortada *et al.*, 2001).

2.4.6.8 Lead Toxicity

At any given time, the blood level of lead represents an expression of a balance between absorption from environmental sources and excretion in urine, feaces, sweat, hair, soft tissues, bone marrow and bone. The majority of occupationally unexposed people have blood lead levels lying between 150-250mg/ml for adults and children. Blood levels of above 360ng/ml in children and above 600-800ng/ml in adults are usually regarded as excessive and indicative of lead absorption (Flora and Gupta, 2012; Rossi, 2008).

Lead significantly inhibits the enzymatic conversion of Aminolevulinic acid (ALA) to prophobilinogen by ALA dehydrase and the final formation of heme by the incorporation of iron into protoporphyrin IX. The inhibitory action results in increased urinary excretion of heme precursors, including porphobilinogen and coproporphyrin III. Erythrocyte coproporphyrin and non-haemoglobin iron stores are increased. Iron passes normally to bone marrow but utilization for haemoglobin formation is decreased. The presence of basophilic stippling in erythrocytes is a feature of lead poisoning (Barbosa *et al.*, 2005).

Mild lead poisoning is associated with slight reduction in nerve conduction velocity. Peripheral nerve damage in lead exposed workers was shown to be related to the degree of anemia. Lead palsy is probably due to direct action of lead on the muscle as well as damage to nerve. Excessive ingestion of lead causes inflammation of the gastrointestinal tract. It results from direct cellular toxic action as shown by intranuclear inclusion, aminoaciduria, glycosuria and other features of the Fanconi syndrome. 50% of cases of chronic plumbism with lead nephropathy have been shown to be associated with gout and with low urinary uric acid excretion. Lead can affect every organ and system in the body and exposure to high lead levels can severely damage the brain and kidneys and

ultimately cause death. In pregnant women high levels may cause miscarriage (Castro-Gonzalez and Mendez-Armenta, 2008).

2.5 Adverse Effects of Pollutants on Humans

Humans are exposed to an array of pollution whether as medicines, industrial or environmental chemicals or naturally occurring substances. Even substances that are essential to our bodies can be toxic at high doses (WHO, 2004; Abubakar and Uzairu, 2015).

An adverse effect can be defined as an abnormal, undesirable and harmful change following exposure to a potentially toxic chemical. There are three types of exposure which are: acute, subchronic and chronic (Sunger, 2001).

Acute exposure to a pollutant is defined as exposure to a chemical for less than 24hours. It usually refers to a single dose. Long-term exposure, known as chronic exposure refers to repeated or continuous exposure to a chemical for a minimum of ninety days. Subchronic exposure is greater than acute but less than chronic. The toxicity of a pollutant could be defined as the capacity to cause a harmful effect in a living organism. Thus, toxicity cannot be defined without reference to the quality (dose) of the pollutant to which the public is exposed, the way in which the pollutant reaches the public (e.g. inhalation, ingestion, dermal) and the duration of exposure (e.g. single dose, repeated doses), the type and severity of adverse effects and the time needed to produce these effects (Sobaszek *et al.*, 2000).

2.5.1 Effects of Pollutants on the Respiratory System

Pollutants absorbed by inhalation have specific properties. They are either (a) gases e.g. CO_2 (b) Vapors or (c) Aerosols, that is, small particles suspended in the air. Gases and vapors may be inhaled directly into the lungs or they may be absorbed unto the surface of aerosols and then inhaled. For example, arsenic released during coal combustion is concentrated on the surface of aerosols. (Sunger, 2001). If the gases are water soluble, they may dissolve in the mucus that covers the respiratory tract causing local irritation. For aerosols, particle size is

the critical factor determining how far down the respiratory tract the particle will go and what part of the respiratory system they will affect. When inhaled, the particles that make up aerosols are deposited all along the respiratory tract. Where the particles are deposited affects the severity of tissue damage, the amount of absorption of the toxicant into systemic circulation and the ability of the lung to remove the particles. The smaller the particle the further they can pass into the respiratory tract (Mar *et al.,* 2000).

The respiratory system can respond in a number of ways to the hazardous gas and particles that are not removed by immune cells. Changes observed in the lung as a result of the inhalation of hazardous gaseous or particulate materials depend on the concentration of the inhaled material, the duration of exposure and the nature of the chemical. Acute changes in the lung include bronchoconstriction, airway oedema and impairment of defense mechanism (Christopher *et al.,* 2004). Acute exposure to sulfur dioxide for as little as 3 minutes can produce bronchoconstriction. Pulmonary oedema is the filling of the alveoli and surrounding tissue with large amounts of fluid. This can be caused by chlorine or SO_2. These gases can damage the blood vessels (capillaries) in the lungs causing fluid to leak out and fill the alveoli (Kymisis and Hadjistavrou, 2008; Christopher *et al.,* 2004; Sunger, 2001; Sobaszek *et al.*, 2000).

2.5.1.1 Respiratory diseases caused by pollutants

Incidence of lung cancer has been found in workers exposed to some forms of nickel, chromium and asbestos. Asbestos is widely used in the construction industry. Important uses include asbestos cement sheets and pipes, insulation materials, taping compounds and floor and ceiling tiles. Contamination of the air inside buildings especially schools, has been a major concern in many countries. Some countries have banned the use of asbestos in buildings (Sobaszek *et al.,* 2000).

Respiratory system disease from exposure to asbestos includes asbestosis, lung cancer and mesothelioma. Other cancers linked to asbestos exposure include, those of the larynx, pharynx, oesophagus, stomach, colon-rectum and the pancreas.

Mesotholioma is a rare type of cancer of the pleura. An increased incidence of mesothelioma has been related to the inhalation of asbestos fibres in the occupational environment (Sobaszek *et al.*, 2000). Emphysema is clearly associated with heavy cigarette smoking and often occurs in combination with chronic bronchitis. Chronic bronchitis is caused by excessive production of mucus in the bronchi and broncholes (Sobaszek *et al.*, 2000).

2.5.2 Effects of Pollutants on the liver

The liver has many different functions. It is involved in digestion, metabolism and synthesis of nutrients needed by the body and it plays a very important role in the detoxification of drugs and chemicals. The primary role of the liver is to receive and process chemicals absorbed from the gastrointestinal tracts before they are distributed to other tissues. After nutrients have been absorbed into the blood from the digestive tract, the nutrient-rich blood passes directly to the liver. (Carle *et al.*, 2005). The cells of the liver remove amino acid, fats, glucose from the blood so that they can be processed.

The liver is the primary site for the metabolism of fat and it stores glycogen which can be converted into energy when it is needed. The hepatocyte (liver cell), the main structural component of the liver, might be likened to a factory (it makes chemical compounds), a warehouse (it stores glycogen, iron and certain vitamins), a waste disposal plant (it excretes bile, urea and other detoxification products); and a power plant (it produces considerable heat during breakdown of complex molecules (Jones and Griffin, 2008; Carle *et al.*, 2005).

Liver damage may be caused by many chemical substances (hepatotoxicants) and it is characterized in two ways: accumulation of fat or death of liver cells. Accumulation of fat in

the liver (steatosis) is a common sign of liver toxicity and can be due to toxic chemicals. Hepatic necrosis (death of liver cells) may result from exposure to a number of chemical agents including phosphorus, carbon tetrachloride, chloroform etc. In cirrhosis, a well known liver condition, large number of liver cells are destroyed and replaced by permanent scar tissue (Siesky *et al.*, 2002). Cirrhosis can be caused by alcohol abuse, viral hepatitis or chemical pollutants that attack liver cells. Liver tumours which can be benign or malignant, have been associated with exposure to arsenic, polychlorinated biphenyls (PCBs) and other pollutants. If too many hepatocytes are killed, the liver will not be able to replace them. This will ultimately lead to liver failure and consequently death (Soto *et al.*, 2002, Wade *et al.*, 2002; Siesky *et al.*, 2002).

2.5.3 Effects of pollutants on the kidney system

The kidney is a complex organ. In addition to the formation of urine to rid the body of wastes, the kidney plays a significant role in the regulation and composition of body fluids. The balance between intake and output of fluids is maintained by the kidneys. The kidney is also a major site of formation for hormones, the formation of ammonia and glucose and the activation of vitamin D. The effect usually reported following exposure to a toxic chemical is decreased elimination of waste (Hodgson *et al.*, 2007). The functional unit of the kidney is the nephron. Each kidney contains over one million nephrons.

Toxicants that affect the kidney (nephrotoxicants) can act in one of three ways.

i. Decrease blood flow to the kidney, which decrease glomerular filtration rate and ultimately the formation of urine; a decease in blood flow would also damage kidney tissue.

ii. Affect the glomerulus directly and hinder its selective ability to filter the blood. Affect the re-absorptive or secretory function of the tubule.

iii. Block the tubule, preventing urine flow (Hodgson *et al.*, 2007).

Decreasing the number of functional rephrons would cause major decreases in renal excretion of water and solvents. Loosing more than 70% of nephrons lead to electrolyte and fluid retention, and ultimately death. Most metals are potent nephrotoxicants. Kidney damage is probably due to a contribution of decreased blood flow resulting in decreased urine production and tissue damage, and the toxicity of the metals on the tubules, resulting in tubule blockage. One such nephrotoxic metal is mercury. Other elements that can damage the kidney include cadmium, chromium, arsenic, gold, lead and iron (Liu *et al*., 2000).

Certain individuals, owing to hereditary or environmental factors, can be usually susceptible to toxic substances that affect the kidney. For example, some individuals are unusually susceptible to copper nephrotoxicity because of their inability to maintain normal copper's concentration in the body (Wilson's disease). People with kidney damage from diabetes exhibit cadmium nephrotoxicity at doses of cadmium that would not normally affect people. Other factors can cause some individuals to be more sensitive to the effects of these substances; these include nutritional status, alcohol consumption, smoking, genetic background and medications. (Griffin *et al.,* 2000; Liu *et al.,* 2000).

2.5.4 Effects of pollutants on the Nervous System

The nervous system is divided into two parts: The central nervous system (CNS) and the peripheral nervous system. The CNS consists of the brain and spinal cord which primarily interpret incoming sensory information and issue instructions based on past experience. The peripheral nervous system (PNS) consists of nervous system structure outside the CNS, which carries impulses to and from the brain and spinal cord. The nerves serve as communication lines. They link all parts of the body by carrying impulses from the sensory receptors to the CNS and commands from the CNS to the appropriate gland or muscles (Kampa and Castanas, 2008).

Neurotoxicity is the capacity of chemical, biological or physical agents to cause adverse effects on the nervous system. A list of compounds that are neurotoxic include: aluminum, carbon monoxide, carbon tetrachloride, hydrogen sulfide, lead, manganese, methanol etc. (Kampa and Castanas, 2008). Moreover, in addition to toxicants acting directly on the nervous system, the nervous system is greatly affected by any changes in blood circulation.

All cells require oxygen but for the nervous system, a constant supply is essential. Any decrease in blood flows can be reflected in adverse effects on the nervous system before other systems would be affected (Abel Sotin, 2002). Certain toxicants/pollutants are specific for neurons (neurotoxicants) or a certain part of a neuron, resulting in their injury or death (necrosis) and the loss of a neuron is irreversible.

Neurotoxicants can act on the axon, protective myelin, or the transmission of the nerve impulse. Ultimately, destroyed neurons result in a break in communication between the nervous system and the rest of the body.

The amount of function lost from damage to the nervous system depends on the number of neurons permanently damaged and where they are located. Some neurons may be slightly but not permanently damaged and in time, may return to normal function. Permanent damage can result in loss of sensation and paralysis. Because the nervous system controls many functions of the body, almost any function controls speech, sight, memory, muscle, strength and coordination can be inhibited by neurotoxicants (Kazim *et al*., 2008).

The residents of Minamata Bay in Japan whose diet was mostly fish from the bay were exposed to large doses of methyl mercury when industrial waste that contained high amounts of mercury was dumped into the bay. Even more people were injured by methylmercury in Iraq. More than 400 people died and 6,000 people were hospitalized after eating grain that had been coated with methyl mercury. Another neurotoxicant, carbon disulfide ($CS_{2)}$ destroys axons. This chemical has been used for a variety of industrial purposes particularly vulcan

rubber and viscose rayon production (Kazim *et al.*, 2008; Sandau *et al.*, 2000). That the metal lead is toxic to the nervous system has been known for centuries. By destroying myelin, lead slows the transmission of impulses between neurons and can eventually stop them. People can be exposed to lead occupationally if they work at a lead smelting plant, or at home through lead pipes and lead based paints. (Ribas-Fito *et al.*, 2001).

2.6 Management of toxic pollutants in the environment

The environmentally sound management of toxic pollutants requires proper management of the chemical from when it is first manufactured to when it is disposed (often referred to as cradle to grave or life cycle management). The chemical may pose serious risks through occupational exposure, air and water pollutions, ground water and food contamination, or generation of hazardous wastes, (USEPA, 2001).

Strategies for the environmentally sound management of toxic pollutants are as varied as the pollutants themselves. However, certain broad strategic principles and action are common. They generally apply to the safe manufacture, storage, transport, use and disposal of hazardous chemicals to prevent or reduce their adverse effects on human health and the environment. (USEPA, 2001).

2.6.1 Prevention

The strategy of choice in a national programme for the sound management of hazardous chemicals is first and foremost that of anticipating and preventing the release of toxic pollutants into the environment rather than relying on an "after-the-fact" approach of remediation and treatment (USEPA, 1993a; 1991). Several pollution prevention strategies can be adopted to protect human health and prevent environmental degradation.

These include

- Encouraging and promoting greater efficiency in the use of energy.
- Using fuel low in sulfur content.

- Recycling in industrial processes to reduce hazardous waste generation thereby reducing the cost of disposal.
- Reducing wasteful packaging of products; which also reduces the cost of disposal or unnecessary packaging,
- Developing alternative manufacturing technologies to minimize the use of pesticides through good agricultural practices and integrated pest management.
- Promoting cars with catalytic converters to reduce the quantity and toxicity of gaseous emissions
- Promoting adequate public transportation systems to reduce the use of individual cars;
- Legislation and enforcement to provide meaningful incentives to achieve the above objectives and to prevent imports of hazardous chemicals that have been banned or severely restricted in exporting countries (USEPA, 2003).

In order to predict reliably and to prevent potential adverse effects from hazardous chemicals, a Health and Environmental Impact Assessment (HEIA) must be a prerequisite for any major industrial development project. The HEIA is a comprehensive study to evaluate, anticipate and prevent the ways in which a chemical production facility will affect the local community and environment. It concerns not only the media (air, water, soil) but also traffic patterns and aesthetic consideration in the community (USEPA, 1995). The assessment should provide opportunities:

- To include health and environmental consideration in project plans.
- To identify the most appropriate location for a plant and its design characteristics.
- To choose a process to minimize wastes and, hence reduce costs.
- To incorporate control measures to prevent pollution rather than to control it after the act.

- To provide for emergency response where appropriate (USEPA, 1995).

The HEIA must include a preliminary appraisal of the sources and levels of emissions from the proposed facility, an essential step towards the development of techniques to control environmental pollution and to protect workers. It is more cost-effective to force and plan for the effective control of the release of chemicals into the environment than to subsequently fit an operating plant to control such a release (USEPA, 1995).

Examples of information required in an HEIA are:

- Explosion and fire hazards of the product and raw materials used
- Rate and amount of expected release of hazardous chemicals in air, water and land.
- Expected exposure of workers and the public to such chemicals.
- The range of magnitude of health and environmental risks
- And the likelihood of failure of equipment, explosion and natural disasters at the selected site.

Where air or water quality standards exist, it is important that measures be incorporated into the operation of the plant to ensure compliance with such standards. Regular monitoring should be carried out to ensure continuity compliance with appropriate standards (USEPA, 1995).

2.6.2 Control Technology

Controlling a particular pollutant requires the selection of economically -feasible technology that will reduce exposure (and hence risks) to acceptable levels. Strategies to reduce exposure to chemicals and hence risks, must be cost-effective, health and environmental quality objectives must be realistic if they are to be achieved. There are many different technologies available to control emissions of hazardous pollutants from high –pollution industries such as iron and steel, chemicals, textile manufacture and energy production (USEPA, 2001). For

instance, sulfur and nitrogen compounds are amenable to "scrubbing" from smokestack gases; chromium can be removed from leather tannery wastewater by electrodeposition or by coagulation with chemical addition followed by sedimentation, and dust from iron foundries or cement manufacturing can be removed by fabric filters, electrostatic precipitators and wet collectors of various types. Each industry has available its own specific physical, biological and/or chemical treatment methods to control or prevent, at a reasonable cost, hazardous chemical emissions (USEPA, 2001).

2.6.3 Regulations, Incentives and Standards for the Environment

The primary aim of establishing regulations and standards is to protect public health and to eliminate or reduce to an acceptable level exposure to toxic pollutants. Regulations should be clear, simple to understand and govern such matters as treatment requirements for industrial waste streams, USEPA, 2001. They should prevent air and water pollution, setting standards for chemicals in air, food and water, and they should set exposure limits for workers and limits on the quantity to toxic pollutants that may be present in solid wastes to be discharged on land. However, standards and regulations prevent nothing unless they can be implemented and enforced. This requires facilities, technical knowledge and expertise, and the appropriate legislative framework (USEPA, 1991).

Several criteria can be applied in determining the priority for chemical regulation. These include:

- Severity and frequency of observed or suspected adverse health effector:
- Extent of production and use
- Abundance and persistence in the environment
- Population exposed; attention should be paid to exposure involving a large proportion of the general population and to exposures of highly sensitive groups

such as pregnant women, newborn children, the infirm and the elderly (USEPA, 1993b).

Incentives such as the "Polluter Pays" principle have convinced many industrial establishments to control their hazardous emissions. The principle consists of requiring that the polluters pay the cost of pollution damage, including damage to health.

The charges for the full health and environmental costs should be reasonable but sufficiently high that polluters do not simply consider the cost as the price for doing business (USEPA, 1993b).

Any industry generating hazardous chemicals must comply with regulations and standards established by the government. It is essential to monitor compliance with these limits and regulations. Various means can be used, such as permits, to operate with inspection of the industrial facility, or environmental monitoring of the regulated chemical. In the end, it is always important to remember that the best solution is prevention and that it is almost always more effective and less costly to prevent an environmental disaster than correct one.

Regulations can include the phasing-out or banning of toxic chemicals that pose an unacceptable and unmanageable risk to human health or the environment.

Regulations must also be established to prevent accidents and should include specific plans for emergency response procedures (USEPA, 1993b).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area: Sampling Stations

Six surface water locations were chosen for water sampling as shown in Fig. 3.1

They are:

1. Makoko extension of the Lagos Lagoon
   (situated in Yaba, Lagos state)
2. Carter Bridge extension of the Lagos Lagoon
   (situated around Gbogbaniyi market, Lagos state)
3. Maroko extension of the Lagos Lagoon
   (situated in Lagos Island in Lagos state).
4. Majidun River
5. Molatori extension of the Lagos Lagoon
   (situated in Abuja town in Ikorodu, Lagos state).
6. Ibeshe extension of the Lagos Lagoon
   (situated in Ibeshe town in Ikorodu, Lagos state).

Two markets were selected for the purchase of the fish samples:

1. Makoko market, close to Makoko water site.
2. Carter Bridge Market, close to Carter Bridge water site.
   This market is also known as Gbogbaniyi market.

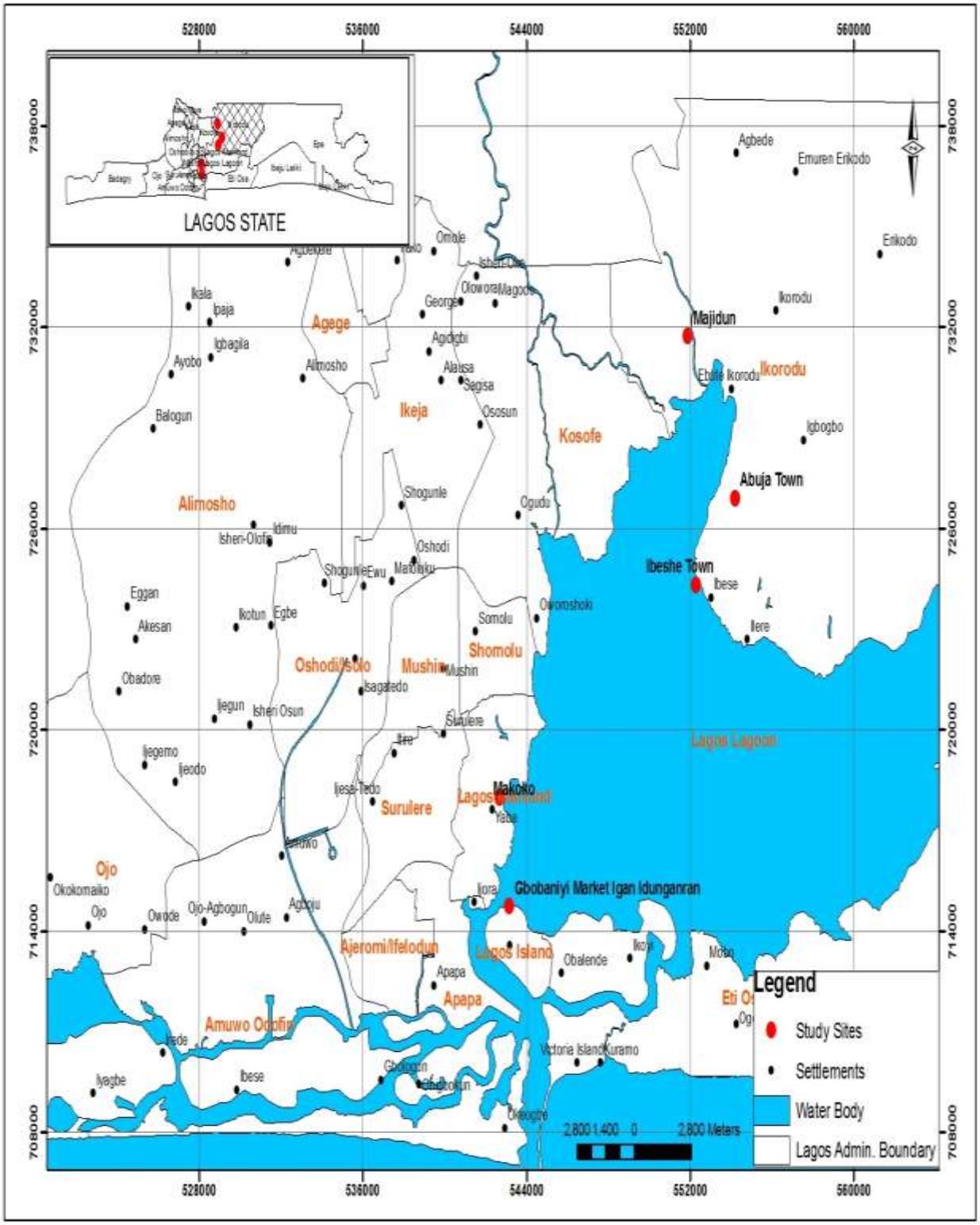


Fig. 3.1: Lagos Lagoon showing the study sites (Lawson, 2011).

3.2 Collection of water samples: Water from the water bodies were collected early in the morning, in September, in sterile 500ml round bottom plastic containers. The containers were dipped inside the water bodies. The samples were taken immediately to the laboratory for storage in a refrigerator.

PHASE ONE

Physiochemical analysis

3.3 Determination of Physio-chemical parameters of water samples:

These were determined according to United State Environmental Protection Agency (USEPA) standard method, (USEPA 1988). Total solids, total dissolved solids, total suspended solids, alkalinity and acidity were all determined.

3.3.1 Total Solids

An evaporating dish was placed in an oven at 103-105^{o}C for 1hr. After an hour, it was cooled and weighed. Water sample (100ml) from one of the water bodies were measured into it. The dish was placed on a steam bath and evaporated to dryness. The dish was cooled and weighed.

Total residue was calculated using the equation below:

$$\text{Total residue (mg/L)} = \frac{\text{Mass of solids in dish (mg)}}{\text{Volume of sample (litre)}}$$

The procedure was repeated for all the water samples collected from the other water bodies.

3.3.2 Total Dissolved Solids (TDS)

A clean evaporating dish was heated to 105^{o}C in an oven and cooled in a desiccator. Water sample (150ml) from one of the water bodies was filtered through a filter paper (weight of filter paper known).

A known volume (100ml) of the filtered sample was transferred to the weighed evaporating dish. The sample was evaporated to dryness on a steam bath and dried for 1 hour in an oven set at 105^{o}C. The residue was placed in a desiccator to cool. The dish was weighed.

The evaporation and cooling steps were repeated until a constant weight was obtained.

Total dissolved solids were calculated using the equation below:

$$\text{TDS (mg/L)} = \frac{\text{A – B (mg) x 100}}{\text{Volume of sample (ml)}} \times 1000$$

A = Weight of dried residue with dish

B = Weight of dish

The procedure was repeated for all the water samples from the other water bodies tested.

3.3.3 Total Suspended Solids (TSS)

The suspended solids are those retained by a standard filter (dried to constant weight).

The pre-weighed filter paper was placed on a holder (the filter paper was the same one used in the methodology for total dissolved solids).

The filter was removed and dried for 1hr at 105^{o}C. It was cooled in dessicator and weighed. The cycle was repeated until a constant weight was obtained and TSS calculated from the equation:

$$\text{TSS (mg/L)} = \frac{\text{A – B x 100}}{\text{Sample volume (ml)}}$$

A = Weight of filter paper and dried residue

B = Weight of filter paper

3.3.4 Acidity

A few drops of phenolphthalein solution were added to 100ml of each water sample in conical flasks.

The mixture was titrated with 0.02M sodium hydroxide solution until a pink colour was obtained. Acidity was calculated from the equation:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times 100}{\text{Volume of water}}$$

3.3.5 Alkalinity

Three drops of phenolphthalein indicator was added to 100ml of each water sample in conical flasks. The mixture was stirred and titrated with 0.1M HCl until the pink colour disappeared. This end point represents phenolphthalein alkalinity. A few drops of methyl indicator was added to the sample and titrated with acid until the colour changed from yellow to orange. This end point represents the total alkalinity.

$$\text{Total alkalinity} = \frac{50{,}000 \times B \times M}{V}$$

B = mls of acid used to reach methyl orange end point

M = molarity of acid

V = volume of sample (ml)

3.4 Analysis of metallic constituents in fish and water samples.

This was determined according to the method of USEPA, (1988).

3.4.1 Digestion of Water Samples: The water was acidified at the time of collection with conc. HNO_3 by adding 5ml of acid to one litre of sample. Each water sample (100ml) was transferred to a beaker and 5ml of conc. HNO_3 was added to it.

The solution was heated on a hot plate until the volume reduced to 15-20ml. The mixture was filtered with filter paper. The sample pH was adjusted to pH 4 by the drop wise addition of 5.0M Na0H standard solution. It was mixed and the pH checked, the sample was transferred

to a 100ml volumetric flask and the volume made up to 100ml with deionized water. A reagent blank was carried through the process described above.

3.4.2 Collection of Fish Samples

The fish samples (Tilapia fish, Bonga fish and Croaker fish) were bought from the market women at Carter Bridge market also known as Gbogbaniyi market and Makoko market. They were taken to the Department of Marine Sciences, University of Lagos and identified by Dr. Emmanuel of Marine Department, University of Lagos, with the aid of texts by Schneider (1990) and Emmanuel (2009).

3.4.3 Digestion of Fish Samples

Fishes collected from each sampled river were dried in the oven and homogenized to powder form. Conc. HNO_3 (100ml) were added to 5g of fish sample before 100ml of H_2O were added. The mixture was placed on a hot plate in a fume cupboard and digested until the volume reduced to 10ml and the solution became colourless. The mixture was cooled and the volume made up to 100ml with deionized water. It was then filtered and the pH adjusted to 4.0. The levels of Pb, Cu, Cr, Cd, Fe, Mg, Mn were determined as described by USEPA, 1988.

3.4.4 Determination of Lead

The lead standard solutions were first prepared into different concentrations in test tubes, using 37mg of lead sulphate dissolved in 250ml of deionized water. This was used to prepare the standard solution ranging from 0.0mg/l to 100mg/l. A cathode lead lamp was fitted into the spectrophotometer (Perkins Atomic Absorption Spectrophotometer (AAS), England) and the wavelength adjusted to 283.31nm. The standard solutions were then aspirated by the spectrophotometer resulting in the absorbance reading and a graphical diagram showing absorbance and concentration was obtained.

The sample mixture was then aspirated by the spectrophotometer, analyzed and the concentration of lead was recorded.

3.4.5 Determination of Copper

The copper standard solutions were first prepared into different concentrations in test tubes using 63mg of copper sulphate dissolved in 250ml of deionized water. This was used to prepare the standard solution ranging from 0.0mg/l to 100mg/l.

A cathode copper lamp was fitted into the spectrophotometer and the wavelength adjusted to 324.73nm. The standard solutions were then aspirated by the spectrophotometer resulting in the absorbance reading of the standard concentration and a graphical diagram were obtained. The sample mixture was then aspirated by the spectrophotometer, analyzed and the concentrations of copper was recorded.

3.4.6 Determination of Iron

The iron standard solutions were first prepared into different concentrations in test tubes using 72.5mg of iron (III) chloride dissolved in 250ml of deionized water. This was used to prepare the standard solution ranging from 0.0mg/l to 100mg/l. A cathode iron lamp was fitted into the spectrophotometer and the wavelength adjusted to 248.33nm.

The standard solutions were then aspirated by the spectrophotometer and the absorbance reading plus a graphical reading were obtained. The sample mixture was then aspirated by the spectrophotometer. The iron concentrations of the samples were determined against the standards containing the appropriate range of iron concentration in 0-10mg/L iron.

3.4.7 Determination of Cadmium

The cadmium standard solutions were first prepared into different concentrations in test tubes using 32.5mg of cadmium sulphate dissolved in 250ml of deionized water. This was used to prepare the standard solution ranging from 0.0mg/l to 100mg/l.

A cadmium cathode lamp was fitted into the spectrophotometer and the wavelength adjusted to 228.80nm.

The standard solutions and the sample were then aspirated by the spectrophotometer and the absorbance readings plus the graphs were obtained. The sample mixture was then aspirated by the spectrophotometer, and the concentration of copper was recorded.

3.4.8 Determination of Magnesium

The magnesium standard solutions were first prepared into different concentrations in test tubes using 67.5mg of magnesium sulphate dissolved in 250ml of deionized water. This was used to prepare the standard solution ranging from 0.0mg/l to 100mg/l. A magnesium cathode lamp was fitted into the spectrophotometer and the wavelength adjusted to 285.21nm. The standard solutions were then aspirated by the spectrophotometer resulting in the absorbance reading being obtained. In addition, a graphical diagram showing absorbance against concentrations for magnesium standard solution was computed. The sample mixture was then aspirated by the spectrophotometer, analyzed and the concentration of magnesium was determined.

3.5 Determination of Anions

This was determined according to the method of USEPA, (1988).

3.5.1 Total Chloride

A clean sample cell was filled to the 25ml mark with test sample. The entire contents of one chlorine reagent powder (Hach product) was added and swirled to mix. The sample was allowed to stand for 6min in order that the full color might develop. The absorbance was then measured.

3.5.2 Nitrite

A clean sample cell was filled to the 25ml mark with the test sample. The entire content of one Hach Nitrite reagent powder was emptied into the water sample in the cell. The mixture was shaken vigorously and left for 5 min before measuring the absorbance.

PHASE TWO

Biochemical effects of the consumption of heavy metal contaminated fish.

3.6 Animal Studies I

The biochemical effects of the consumption of heavy metal contaminated fish (Tilapia fish) using laboratory animals were determined.

3.6.1 Experimental animals.

Twenty-one experimental rabbits were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos. The rabbits were kept in metallic cages and fed commercial pellets and water for two weeks in order to acclimatize them at room temperature while adhering to ethical conditions.

3.6.2 Rabbit Feed:

The rabbits were fed with rabbit pellets and water (*ad libitum*) manufactured by Pfizer Livestock Feeds Nigeria plc, Ikeja, Lagos State.

3.6.3 Fish samples:

Tilapia fish samples were obtained from Carter Bridge market and Makoko market. These two study sites were selected because they are commercial fishing sites. These fishes were bought from the market women at the two sites. The fishes were dried in the oven and weighed.

3.6.4 Experimental Design I

Twenty-one rabbits of mean weight 6.3kg were allowed to acclimatize to laboratory condition in well-ventilated cages for two weeks. The animals were grouped into three with each group containing seven rabbits.

The first group was fed with 100g of fish (obtained from Carter Bridge site) added to their feed on a daily basis for a period of three months.

The second group was fed with 100g of fish (obtained from Makoko site) added to their feed on a daily basis for a period of three months.

The third group served as the control with no fish added to them. Control group (no fish in their diet). They were fed with rabbit's pellet.

After three months, the animals were anaesthetized and blood samples collected through the jugular vein into heparin bottles. The blood sample collected was spun using a centrifuge at 4000 rpm for 35min and the serum was collected using a pipette for analyses. The rabbits were dissected and assays were conducted on the liver, heart, kidney.

The various organs were collected, blotted dry with filter paper and quickly weighed. The organs were then stored at -10^{O}Cuntil further analysis.

3.6.4.1 Extraction of Tissue Total Protein

Tissue total protein was extracted according to the method of Plummer (1988).

Tissue (1g) was homogenized with 5ml of deionized water. The homogenate was collected and centrifuged at 10,000g for 30 mins. The supernatants were collected and termed tissue total proteins.

3.6.6 Total Protein Determination

Total protein was determined by the method of Buiret as described by Bishop *et al*, (2009).

One millilitre of tissue protein extract from each of the samples obtained above in 3.6.4.1 was pipette into different test tubes. To each of these, 5ml of Buiret reagent was added. The mixture was mixed thoroughly and allowed to stand at room temperature for 30 mins. The absorbance was read at 550nm against a reagent bank, which consists of 1ml of deionized water and 5ml of Buiret reagent. Alongside with this, protein standard solution was prepared containing 1% egg albumin (5mg albumin/ml).

3.6.4.3 Determining of Serum Albumin Concentration

The method described by Doumas *et al*. (1971) and modified by Bishop *et al*. (2009) was employed for the determination of serum albumin concentration. One millilitre of serum sample was pipetted into a test tube. The volume was made up to 5ml with deionized water. Into another test tube, 1ml of standard albumin was pipetted and the volume also made up to 5ml with water. The blank tube had only 5ml of water sample. Bromocresol green (5ml) was added to the three tubes. Absorbance was read at 630nm.

Albumin concentration was calculated as follows:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.6.4.4 Quantitative Determination of Glucose

This was determined using Anthrone method as described by Bishop *et al*., (2009).

The anthrone reaction is the basis of a rapid and convenient method for the determination of sugars. The blue-green solution shows an absorption maximum at 620nm. Four millilitres of anthrone reagent (2g/litre in concentrated sulphuric acid) were added to 1ml of serum in

covered tubes. The tubes were placed in boiling water bath for 10minutes.The mixture was cooled and the absorption read at 620nm against a reagent blank.

Standard curves were also prepared for glucose solutions ranging from 0.0 to 1.0g/litre.

3.6.4.5 Estimation of Serum Cholesterol

Serum total cholesterol was estimated by using the method described by Plummer, (1988). Acetic anhydride reacts with cholesterol in a chloroform solution to produce a characteristic blue-green colour. Serum was extracted with an alcohol-acetone mixture which removes cholesterol and precipitates proteins. The organic solvent was removed by evaporation on boiling water bath and the dry residue dissolved in chloroform. The cholesterol level was then determined colorimetrically using the Liebermann-Burchard reaction (Plummer, 1988).

Ten milliliters of the alcohol-acetone solvent was placed in a centrifuge tube and 0.2ml of serum sample was added to it. The tube was immersed in a boiling water bath and shaken until the solvent began to boil. The tube was removed and the mixture shaken for a further 5 min. The mixture was cooled to room temperature and centrifuged. The supernatant was decanted into a test tube and evaporated. The residue was cooled and dissolved in a tube containing cholesterol. A blank test tube with 2ml of chloroform was set up.

Two millilitres of acetic anhydride sulphuric acid mixture was added to all the tubes and thoroughly mixed. The tubes were left in the dark at room temperature and the absorbance read at 680nm against a reagent blank.

3.7 Determination of Heavy Metal Content.

This was done by using atomic absorption spectroscopy method (Monisov, 1992).

3.7.1 Digestion of Serum Samples

Serum was diluted 1:99 with deionized water for metallic estimation. A measured volume (100ml) of deionized water was transferred into a beaker. Conc. HNO_3 (5ml) was added to it and the beaker covered with a watch glass. Boiling chips was added to aid boiling. The mixture was boiled and evaporated to the lowest volume on a hot plate (between 10-20ml) before precipitation occurs. Concentrated nitric acid was added until digestion was completed as shown by a light-coloured, clear solution. The mixture was cooled and diluted to 100ml with deionized water. Portions of this solution was taken to the Atomic Absorption spectrophotometer for metal determination.

3.7.2 Analysis of Metallic Constituents in Serum Samples

Using cathode lamps specific for the metal being determined at their specific wavelength, the metals were determined using Perkins Atomic Absorption spectrophotometer, Analyst 200, England as earlier described in 3.4.4- 3.4.8.

3.7.3. Determination of Catalase Activity

Catalase activity was determined according to the method of Luck (1971) and modified by AACC, 1984. Serum (0.1ml) was added to the reaction mixture which contained 1ml of 0.01M phosphate buffer (pH 7.0) and 0.4ml of $2MH_2O_2$. The reaction was stopped by the addition of 2ml of dichromate acetic reagents (5% potassium dichromate gacial acetic acid mixed in ratio 1:1). The activity of catalase was assayed colorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed/min per mg protein.

3.7.4. Determination of Glutathione Level

Glutathione level in serum was determined according to the method of Sedlak and Lindsay (1968) and modified by AAAC, 1984. To the serum, 10% TCA was added. The supernatant (1ml) was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5 – dithiobisnitro benzoic acid

[DTNB] in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was need at 412nm.

3.7.5. Determination of Serum Aspartate Aminotransferase (AST) Activity

This was determined using Merck reagent kit. The reagents used are:

Solution (1) which consists of potassium dihydrogen phosphate, sodium L-aspartale, di-potassium hydrogen phosphate (dissolved in water).

Solution (2) - $NADH_2$ (dissolved in water)

Solution (3) - Ketoglutarate solution

Bottle (4) - Malate dehydrogenase and LDH.

Procedure

Solution (1) was just warmed to 25^{o}C. The contents of bottle (4) was added to the contents of solution (1) and mixed together to make solution (A).

Solution (A), [3ml], was added to 0.5ml of serum in a cuvette. To this mixture, 0.05ml of solution (2) was added, mixed together and allowed to stand for 1min. the absorbance was measured at 340nm.

3.7.6. Determination of Serum Alanine Aminotransferase (ALT) Activity

This was determined using Merck reagent kit. The reagents used are:

Solution (1) which consists of potassium dihydrogen phosphate, DL-alanine, potassium hydrogen phosphate (dissolved in water).

Solution (2) $NADH_2$ (dissolved in double distilled water).

Solution (3) – ketoglutarate solution bottle

Bottle (4) – lactate dehydrogenase.

Procedure

Solution (1) was just warmed to 25^{o}C. The contents of bottle (4) was added to the contents of solution (1) and mixed together to make solution (B).

Solution (B), [3ml], was added to 0.5ml of serum in a cuvette. To this mixture, 0.05ml of solution (2) was added, mixed together and allowed to stand for 5min. the absorbance was measured at 340nm.

3.8 Hormonal Analysis

3.8.1. Determination of Progesterone in Serum Samples

Radioimmunoassay method of Berson and Yalow, (1990) was used for determination of progesterone in serum samples.

Principle: Radioimmunoassay depends on the ability of an antibody to bind its antigens. In order to quantify the antigen, a radioactive antigen and a non-radioactive antigen compete for a limited number of binding sites on a specific antibody. As more non-radioactive antigens are introduced into the system, less binding sites are available for the radioactive antigen yielding a method for quantitation. In the progesterone assay, the antibody is covalently bound to the inner-surface of a poly-propylene tube. Thus the antibody bound antigen is also bound to the tube.

This eliminates the need of a second antibody, or other type of precipitating agent and eliminates the need for centrifugation. At the conclusion of the assay the reaction solution was either aspirated or decanted from the tube leaving only antibody bound antigen. The tube was then placed in a gamma counter calibrated for ^{125}I and the level of ^{125}I-progestrone (or other hormones) was determined. Levels of progesterone in the samples were then graphically determined from a standard curve.

- 100µl of each standard, sample and control was pipetted into respective coated tubes.
- 1.0ml of progesterone ^{125}I (progesterone tracer or any other hormone tracer) was added to all the test tubes.
- All the tubes where incubated for 2 hours.
- The contents of the tubes were aspirated or decanted.
- The tubes were placed in a gamma counter for counting. The counter was calibrated for ^{125}I.
- The result was calibrated and plotted. Any type of RIA data reduction system can also be used. The standard curve is linear over its entire range.

3.8.2 Determination of Serum Prolactin (using ELISA KIT)

The micro plate used in this assay was pre-coated with a monoclonal antibody specific for prolactin. Blood samples were added to the micro plate wells and if prolactin in the sample binds to the antibody pre-coated on the wells. Prolactin quantification was done by the preparation of a standard horseradish peroxidase (HRP) – conjugated polyclonal antibody which is specific for prolactin. This standard was added to each well to "sandwich" the immobilized prolactin on the plate. The micro plate was incubated and the wells washed thoroughly to remove all unbound components. A TMB substrate solution (3, 3' , 5, 5' tetramethyl – benzidine) was added to each well.

The enzyme (HRP) and substrate were allowed to react over a short incubation period. Only the wells that contain prolactin and enzyme-conjugated antibody exhibited a change in colour. The reaction was then terminated by the addition of sulphuric acid solution and the colour change was measured spectrophometrically at a wavelength of 450nm.

3.8.3 Determination of Serum Luteinizing hormone (using ELISA KIT)

Luteinizing standards was added to streptavidin coated wells.

Biotinylated monoclonal and enzyme labelled antibodies was added and the reactants were mixed forming a complex. The complex was deposited simultaneously to the well through the high affinity reaction of streptavidin and biotinylated antibody.

After equilibrium was attained (observed by a colour change), the antibody-bound fraction was separated from unbound antigen.

3.8.4 Determination of Serum Follicle stimulating hormone (using ELISA KIT)

FSH standards was added to streptavidin coated wells. Biotinylated monoclonal and enzyme labelled antibodies were added and the reactants mixed together forming a complex. The complex was deposited simultaneously to the well through the high affinity reaction of streptavidin and Biotinylated antibody.

After equilibrium was attained (observed by a colour change), the antibody-bound fraction was separated from unbound antigen.

PHASE THREE

3.9: Effect of zinc supplementation on hormonal profile of rabbits fed contaminated fish.

3.9.1 EXPERIMENTAL DESIGN II

This phase was designed to investigate the effects of zinc supplemention on the hormonal profile (progesterone) of female rabbits.

21 female rabbits were randomly divided into 3 groups:

Group A, group B and group C

The animal in group A were fed with fish contaminated with heavy metals from Carter Bridge market (100g of powdered fish mixed with 500g of pellets)

Group B: The animals in group B were fed with a mixture of rabbit pellets and fish together with 0.133g of zinc supplements.

Group C: The control group with no fish nor supplements in diet.

3.9.2 Assay Determination,

After three months, the animals were sacrificed and hormonal assays were conducted on the serum as described by Bishop *et al*., 2009.

3.10 ANIMAL STUDIES III

This is to check the effects of the consumption of heavy metal contaminated fish *(Sarotherodon melanotheron*) on biochemical parameters and the histology of the rats using laboratory animals rats.

3.10.1 EXPERIMENTAL ANIMALS

Experimental animals.

Twenty-one experimental rats were obtained from the laboratory Animal Centre, Idi-araba, Lagos, Nigeria.

3.10.2 Feed

The rats were fed with rat chow manufactured by Pfizer Livestock Feeds Nigeria, Plc, Ikeja, Lagos State.

3.10.3 Fish samples

Twenty pieces of fish samples were obtained from Carter Bridge site and Makoko site. These two sites were chosen because they are commercial fishing sites. Fishes were bought from the fishermen at the two sites. The fishes were dried in the oven and weighed.

3.10.4. Experimental Design

Twenty- one rats (190.5 $\pm$ 6.27g) were allowed to acclimatize to laboratory condition in well-ventilated cages for two weeks. The animals were grouped into three with each group containing six rats.

Group 1: This group was fed with 100g of fish (obtained from Carter Bridge site) added to their feed on a daily basis.

Group 2: This group was fed with 100g of fish (obtained from Makoko site) added to their feed on a daily basis.

Group C: Control group (no fish in their diet).

After three months, the animals were anaesthetized and blood samples collected by cutting the jugular vein with a sterile sharp blade into heparin bottles. The rats were dissected and assays (glucose, protein, cholesterol and enzyme analysis) were conducted on the liver, heart, kidney, lung, serum and ovary.

Histological tests were carried out on the tissues while hematological tests were carried out on the blood samples.

3.10.5. Histological Analysis of Tissues.

Histological studies were done at the Department of Anatomic and Molecular Pathology, College of Medicine, University of Lagos.

The tissues were preserved in formalin. The sample areas were cut and placed inside well labeled tissue embedding cassette. The tissues were then processed using a 24hours automatic tissue processor. The tissue processor contains 12 beakers, 10 glass beakers and 2 thermostatically controlled electric metal beakers containing paraffin wax.

The tissues after being processed were embedded using an automatic embedding centre. Embedding is a process of submerging a tissue in a metal disposable embedding mould containing molten paraffin wax. The paraffin wax becomes solidified when it is cold. This

forms a support medium for the tissues during sectioning. Sections of the tissues were cut using a microtome. The sections were placed in a clean grease free slide which was then placed on a hot plate for 30 minutes in order for the sections to adhere to the slides. A staining method using haematoxylin and eosin was used to demonstrate the general structure of the tissue.

3.11 Statistical Analysis of Results

Data entry and analysis were done using graph pad prism software version 5.04. Data for the study were expressed as mean $\pm$ S.E.M. The Students t-test for independent samples and one way analysis of variance (ANOVA) were used to analyze the difference between the mean. Probability value less than 0.01 ($p < 0.01$) was considered as highly significant. The interaction between the metals and the hormones were done using Pearson Correlation test.

CHAPTER FOUR

RESULTS

PHASE ONE

4.1: Physiochemical Parameters

In the sites sampled, pH values ranged from 7.6 to 9.7. Total solids, total dissolved solids and total suspended solids were all within the Maximum Concentration Limit MCL of the USEPA. The waters sampled showed an alkaline trend (Table 4.1).

4.2: Heavy Metals in Water Samples

There was a significantly high level of lead, cadmium, copper and iron in the water samples studied. Chromium was particularly high in Carter Bridge water site. Magnesium and manganese were still within the Maximum Contaminant Level range of the USEPA (Table 4.2).

4.3: Anions in Water Samples

Sulphate levels were significantly higher than the MCL values permitted for water samples. This may be the reason why the waters showed an alkaline trend (Table 4.3).

4.4: Proximate Composition of Fishes

From the proximate analysis of the fishes carried out, it was discovered that Tilapia fish is the most nutritious of all the fishes analyzed. It has a high concentration of protein. Bonga fish has a very low lipid content and a high moisture content, hence it will be good for people on cholesterol watch (Table 4.4).

4.5&4.6: Concentration of Metals in Fish Samples

Tilapia fish has a significantly high concentration of cadmium, lead, copper, manganese and iron. Traces of chromium were found, while magnesium was also present. Bonga fish and Croaker fish also contains traces of the heavy metals (Tables 4.5 & 4.6).

4.0 RESULTS

PHASE ONE

Table 4.1: Physiochemical parameters of water samples collected from different sites of the Lagos Lagoon

Study Location	PH	Acidity (mg/L)	Alkalinity (mg/L)	Total Solids (mg/L)	Total dissolved solids (mg/L)	Total soluble solids (mg/L)
Makoko	6.7 ± 0.12	48.67 ± 0.10	303.00± 27.84*	2.00 ± 0.20	0.30 ± 0.56	0.19 ± 0.02
Carter Bridge	6.3 ± 0.20	18.00 ± 0.05*	131.67± 16.07*	38.00 ± 0.31	23.00± 0.10	1.13 ± 0.05
Maroko	5.7 ± 0.03	12.67 ± 0.58	165.00± 20.00*	34.00 ± 0.11	20.00± 0.90	1.27 ± 0.06
Ibeshe	6.4 ± 0.25	32.50 ± 0.76	128.67± 11.85*	34.00 ± 0.21	21.00± 0.30	0.78 ± 0.10
Majidun river	6.5 ± 0.17	21.00 ± 1.00	106.67 ± 5.77*	15.00 ± 0.17	12.00± 0.11	1.10 ± 0.12
Molatori	9.7 ± 0.6	0.00 ± 0.00	981.67 ± 30.00***	3.00 ± 0.01	2.00 ± 0.02	0.16 ± 0.0
EPA limit	6-8	25.00	250.00	500.00	500.00	500.00

Values are expressed as mean ± (n=3) *p< 0.05, ***p<0.01 ANOVA

Table 4.2: Concentration of Heavy Metals in Water Samples obtained from different sites of the Lagos Lagoon

Study Location	Magnesium (mg/L)	Iron (mg/L)	Cadmium (mg/L)	Copper (mg/L)	Lead (mg/L)	Manganese (mg/L)	Chromium (mg/L)
Maroko	1.57 ± 0.05	8.30 ± 0.02**	0.32 ± 0.02	0.58± 0.10**	0.31 ± 0.02	1.03 ± 0.02	0.01 ± 0.05
Ibeshe	2.29 ± 0.08	6.18 ± 0.02**	1.92 ± 0.03	0.38 ± 0.02	0.95 ± 0.02	0.58 ± 0.07	0.01 ± 0.02
Carter Bridge	2.34 ±0.06**	4.14 ± 0.04	2.82±0.03**	0.40 ± 0.09	1.58± 0.05**	0.32± 0.01**	0.06 ± 0.08
Makoko	2.33 ± 0.03**	2.83 ± 0.02	2.72± 0.04**	0.49 ±0.02**	1.42± 0.02**	0.13 ± 0.04	0.03 ± 0.02
Majidun river	2.05 ± 0.02	10.86± 0.06***	0.89 ± 0.01	0.38 ±0.04	0.48 ± 0.07	4.11 ±0.02	0.02 ± 0.09
Molatori	1.25 ± 0.02	13.52± 0.04***	1.13 ± 0.07	0.39 ± 0.04	0.29 ± 0.04	0.60± 0.06**	0.04 ± 0.07
Maximum contaminant level (MCL)	2.00	0.30	0.01	0.01	0.05	0.05	0.01

Values are expressed as mean ± S.E.M (n=3)

*p < 0.05; **p<0.01, ANOVA

Table 4.3: Concentration of Anions in Water Samples obtained from different sites of the Lagos Lagoon

Study Location	Sulphate (mg/L)	Nitrite (mg/L)
Makoko	2.00 ± 0.20^{a}	0.17 ± 0.04^{b}
Ibeshe	660.00 ± 0.10^{a}	0.08 ± 0.06^{b}
Carter bridge	900.00 ± 0.58^{a}	0.07 ± 0.02^{b}
Maroko	690.00 ± 1.00^{a}	0.05 ± 0.01^{b}
Majidun river	410.00 ± 0.17^{a}	0.04 ± 0.03^{b}
Molatori	240.00 ± 0.34^{a}	0.04 ± 0.02^{b}

Values are expressed as mean $\pm$ S.E.M. of 3 determinations.

Values carrying different superscripts horizontally are significantly different ($p < 0.01$).

Table 4.4: Proximate Composition of Tilapia, Bonga and Croaker fish obtained from Makoko and Carter Bridge markets

Proximate composition (%)	Tilapia fish from Makoko	Bonga fish from Makoko	Croaker fish from Makoko	Tilapia fish from Carter	Bonga fish from Carter	Croaker fish from Carter
Lipid	18.60± 1.40	7.60± 1.80	2.40± 1.60	19.48± 1.20	7.92± 1.70	2.80± 1.40
Protein	38.19± 4.70	16.29± 3.30	28.09± 4.80	30.84± 5.30	12.56± 4.30	30.20± 5.70
Moisture	27.66± 2.20	63.04± 5.70	62.80± 6.70	37.72± 4.30	67.72± 5.50	60.90± 5.80
Ash	1.76± 0.80	10.30± 2.50	5.90± 1.40	1.37± 0.60	8.09± 3.20	4.60± 1.20
Carbohydrate	10.41± 1.70	0.98± 0.20	0.634± 0.90	7.46± 1.50	1.30± 1.60	1.30± 1.10

Values are expressed as mean ± S.E.M. of 3 determinations

Table 4.5: Concentrations of Metals in Tilapia Fish and Water samples from Carter Bridge and Makoko markets

S/N	Sample type	Study Location	Cadmium	Lead	Copper	Manganese	Chromium	Magnesium	Iron
1.	Water (mg/ml)	Makoko	0.32 ± 0.02	0.31 ± 0.02	0.58 ± 0.02	1.03 ± 0.09	0.01 ± 0.02	1.57 ± 0.05	8.29 ± 0.06**
	Fish (mg\g)		0.24 ± 0.08	0.30 ±0.16	0.44 ± 0.05*	0.09 ± 0.04	0.02 ± 0.03	0.37 ± 0.03	3.59 ± 0.14**
2.	Water (mg/ml)	Carter Bridge	2.82 ± 0.03	1.58 ± 0.09	0.40 ± 0.04	0.32 ± 0.07	0.06 ± 0.04	2.35 ± 0.09	4.14 ± 0.02**
	Fish (mg\g)		0.90 ± 0.04*	0.65 ± 0.05*	0.27 ± 0.06*	0.19 ± 0.05	0.01 ± 0.02	0.35 ± 0.02	2.09 ± 0.04*
3.	Maximum contaminant level (MCL)		0.01	0.05	0.01	0.05	0.01	2.00	0.30

*p < 0.05; **p<0.01, ANOVA

Values are expressed as mean ± S.E.M.of three determinations

Table 4.6:Mean concentrations of Metals in *E. fimbriata* (Bonga fish) obtained from Carter & Makoko markets

Sample	Location	Lead (mg/g)	Cadmium (mg/g)	Copper (mg/g)	Iron (mg/g)	Mn (mg/g)	Chromium (mg/g)	Mg
Bonga	Makoko	3.80± 0.05*	3.51± 0.09*	5.17± 1.02*	4.65± 0.03	0.15± 0.01	0.02± 0.01	1.85± 0.03
Bonga	Carter	3.04± 0.03*	3.31± 0.03*	2.00± 0.26	9.94± 1.35*	0.27± 0.01*	0.04± 0.02	1.89*± 0.02

Values are expressed as mean ± S.E.M.of three determinations

*p<0.05, ANOVA

Table 4.7: Mean concentrations of Metals in *P. senegalensis* (Croaker fish) obtained from Carter & Makoko markets

Sample	Study Location	Lead (mg/g)	Cadmium (mg/g)	Copper (mg/g)	Iron (mg/g)	Mn (mg/g)	Chromium (mg/g)	Mg (mg/g)
Croaker	Makoko	1.24± 0.02	4.04± 0.03*	0.84± 0.07	7.32± 0.49*	0.22± 0.02	ND	1.73± 0.13*
Croaker	Carter	2.55± 0.16*	2.19± 0.35	0.82± 0.36	6.13± 0.26*	0.17± 0.01	ND	1.62± 0.22

Values are expressed as mean ± S.E.M. of three determinations

*p<0.05, ANOVA

4.8: Concentration of Anions in Fish Samples

A significant level of sulphate was also found in the fish samples while traces of zinc was detected (Table 4.8).

4.9: Proximate Composition of Fish Diet

The fish diet which composed of rabbits pellets and dried ground fish (mixed together) has a very high protein concentration. The carbohydrate and ash concentrations were very low (Table 4.9).

4.10: Mineral Composition of Fish Diet

All the heavy metals analyzed were present in the fish diet with a high amount of magnesium and manganese. Zinc was present in minute concentrations (Table 4.10).

PHASE TWO

4.11: Relative Weights of Organs of Rabbits fed with Tilapia Fish Diet

The weights of the liver, kidney and lungs decreased significantly in the rabbits fed with Tilapia fish diet when compared with the control. The weight of the heart increased while there was no significant change in the brain weight of the rabbits (Table 4.11).

4.12: Serum Protein, Albumin, Glucose, Bilirubin, Direct Bilirubin and Cholesterol Concentrations in Rabbits

There was a significant decrease in the concentration of serum protein when compared with the control while glucose concentration in the serum increased. Cholesterol levels in the serum decreased significantly (Table 4.12).

Table 4.8a: Concentration of Sulphate, Nitrite and Zinc in Tilapia Fish and Water samples obtained from Carter and Makoko water sites and markets

Sample Type	**Study Location**	**Sulphate (mg/L)**	**Nitrite (mg/L)**	**Initial levels of zinc**
Water (mg/ml)	Makoko	2.20 ± 0.26 [a]	0.18 ± 0.04 [a]	0.58 ± 0.04
Fish (mg/g)	Makoko Market	35.30 ± 3.60 [a]	0.23 ± 0.02 [a]	0.89 ± 0.07
Water (mg/ml)	Carter bridge	900.33 ± 10.58 [a]	0.06 ± 0.02 [a]	ND
Fish (mg/g)	Carter Bridge Market.	280.01 ± 10.07 [a]	0.03 ± 0.01 [a]	0.082 ± 0.01
Rats pellets mg/g).				0.08 ± 0.09

**p<0.05, ***p<0.01 ANOVA

Table 4.8b: Concentration of Sulphate and Nitrite in Tilapia Fish and Water samples

Sample Type	Locations	Sulphate (mg/L)	Nitrite (mg/L)
Water	Makoko site	2.20 ± 0.26 [a]	0.18 ± 0.04 [a]
Fish (mg/g)	Makoko market	35.30 ± 3.60 [a]	0.23 ± 0.02 [a]
Water	Carter bridge	900.33 ± 10.58 [a]	0.06 ± 0.02 [a]
Fish (mg/g)	Carter market	280.01 ± 10.07 [a]	0.03 ± 0.01 [a]
Maximum contaminant level (MCL) of water		250.00	0.01

Values are expressed as mean ± S.E.M of three determinations.

Values carrying the same superscripts horizontally are significantly different ($p<0.01$)

Table 4.9: Proximate Composition of Tilapia Fish Diet given to Laboratory Animals

Proximate composition	%
Carbohydrate	6.30 ± 0.35
Protein	66.30 ± 4.22
Crude Fat	7.14 ± 0.75
Moisture	12.22 ± 1.45
Ash	2.45 ± 0.37
Crude fibre	5.59 ± 0.97

Table 4.10: Mineral Composition of Tilapia Fish Diet

Mineral	Concentration (mg/g)
Lead	0.674 ± 0.04
Cadmium	0.900 ± 0.03
Chromium	0.010 ± 0.01
Zinc	0.970 ± 0.52
Magnesium	48.170 ± 5.90
Manganese	11.790 ± 2.30
Copper	0.252 ± 0.07

PHASE TWO

RESULTS OF ANIMAL STUDIES 1(IN PHASE TWO)

Table 4.11: Relative Weights of organs of rabbits fed with Tilapia fish obtained from Carter Bridge and Makoko water sites

Study location	Brain (g/kg bdy wt)	Heart (g/kg bdy wt)	Liver (g/kg bdy wt)	Kidney (g/kg bdy wt)	Lung (g/kg bdy wt)
Carter bridge	1.07± 0.04	1.31 ± 0.03	4.68 ±0.7 **	1.54 ±0.07 *	0.77 ± 0.02 *
Makoko	1.08± 0.06	1.08 ± 0.16	4.40± 1.1**	1.82± 0.08*	1.24 ± 0.07*
Control	1.07± 0.04	0.84 ± 0.09	6.55± 1.9	1.84 ± 0.02	1.43 ± 0.03

Values represent mean ± S.E.M of 5 rabbits.

*p<0.05, **p<0.01, ANOVA

Table 4.12: Serum Protein, Albumin, Glucose, Bilirubin, Direct Bilirubin and Cholesterol Concentrations in Experimental and Control Rabbits

Study location	Protein (mg/ml)	Albumin (mg/100ml)	Bilirubin (mg/100ml)	Direct Bilirubin (mg/100ml)	Cholesterol (mg/100ml)	Glucose (mg/ml)
Carter Bridge	25.35±0.44*	3.30±0.42*	2.02 ± 0.07	1.10 ±0.05	97.00±4.2***	0.65±0.03*
Makoko	25.71±1.14*	4.60±0.12*	3.60 ± 0.20	1.80 ± 0.10	111.00±12.9***	0.59±0.03*
Control	34.33±1.02	6.10±0.13	2.30 ±0.30	1.25 ± 0.40	220.00±16.7	0.49±0.07

Values are expressed as mean ± S.E.M of 5 rabbits. ***p<0.01, *p<0.05 ANOVA

4.13: Enzyme Activity in the Serum of Rabbits fed with Tilapia Fish Diet

There was a high level of enzyme activity (alkaline phosphatase, serum aspartate amino transferase and serum alanine amino transferase), (Table 4.13).

PHASE TWO (B)

Result of Second Animal Studies

Body weight of the rat fed the Tilapia Fish diet increased initially, but after some few weeks. It started decreasing gradually while the body weight of the control rat remains consistently increased (Fig 4.1 & 4.2).

The feed intake graph showed a decline in feed intake for the rat fed the Tilapia Fish diet.

4.14: Protein, Glucose and Cholesterol Levels of rat Fed the Tilapia Fish Diet

Glucose concentrations increased significantly in the serum of the rat fed the Tilapia Fish diet, while it decreased in the organs studied. Cholesterol concentration was high in all the organs studied, particularly in the heart, but it was reduced significantly in the serum. Protein concentration was high in the serum of the rat (Table 4.14).

4.15: Enzyme Activities of rat fed the Tilapia Fish Diet

Enzyme activity in the serum was significantly high (Table 4.15).

4.16: Serum Hematological Parameter in the Rat fed the Fish diet

The concentration of hemoglobin decreased significantly in the blood of rat exposed to the fish diet. White blood cells was significantly increased (Table 4.16).

Table 4.13: Enzyme Activity in the Serum of Control and Experimental Rabbits fed contaminated Fish obtained from Carter Bridge and Makoko markets

Study location	Alkaline phosphatase (µ/l)	AST (µ/l)	ALP (µ/l)
Carter bridge	287.50 [a] ± 14.78 [a]	100.00 [a] ± 8.20	31.00 ± 3.21 [b]
Makoko	216.50 ± 14.25 [a]	105.00 ± 9.50 [a]	33.00 ± 2.58 [b]
Control	131.25 ± 12.30	66.00 ± 7.20	27.30 ±2.14

Values are expressed as mean ± S.E.M of 5 rabbits.

RESULTS OF ANIMAL STUDIES 2

(IN PHASE II)

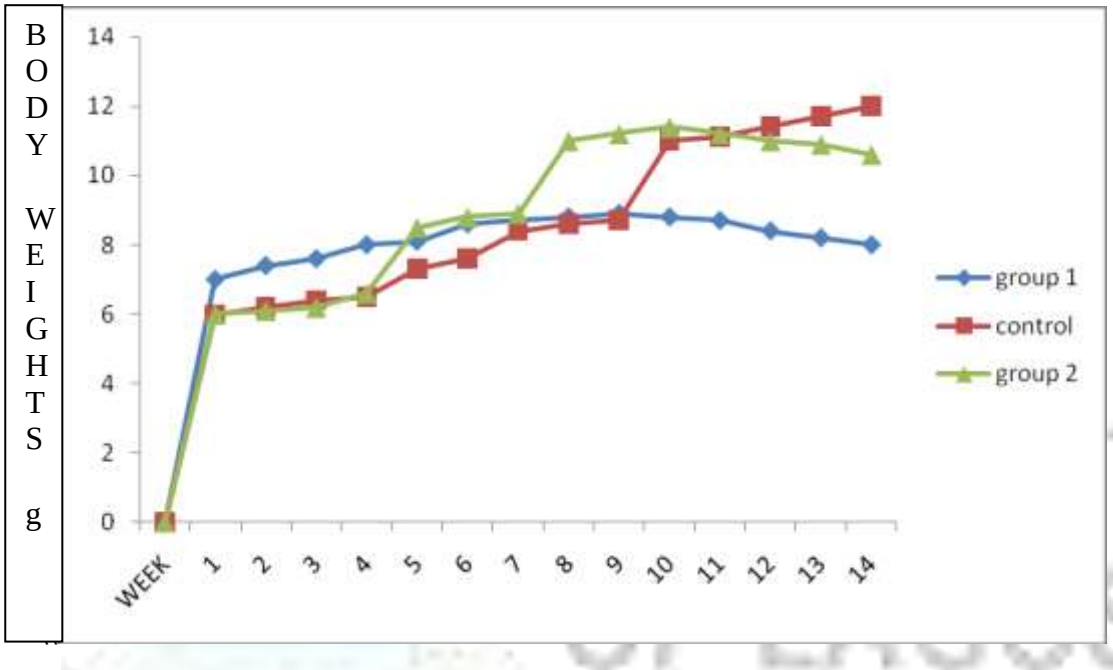


Fig. 4.1: Bodyweights of the rats during the experimental period.

Fig 4.2: Feed intake of rats fed with fishes from Makoko and Carter Bridge markets.

Table 4.14: Serum Protein, Glucose and Cholesterol levels of Rats fed Contaminated Tilapia fish from Carter and Makoko markets

	Protein Concentration (mg/100ml)			Glucose Concentration (mg/100ml)			Cholesterol Concentration (mg/ml)		
	Makoko	Carter	Control	Makoko	Carter	Control	Makoko	Carter	Control
Serum	3.10± 0.70[a]	2.99± 0.42[a]	5.03± 0.61	0.82±0.63[a]	0.78± 0.51[a]	0.51± 0.41	0.40± 0.32[a]	0.47±0.33[a]	0.60± 0.48
Heart	2.80± 0.98[a]	5.00± 0.74[a]	1.80± 0.97	0.20±0.43[a]	0.14± 0.11[a]	0.58± 0.61	0.60± 0.24[a]	0.70±0.52[a]	0.40± 0.41
Lung	3.60± 0.91[a]	1.50± 0.48[a]	1.30± 0.54	0.62±0.55[a]	0.23± 0.42[a]	0.86± 0.76	0.35± 0.51[a]	0.40±0.55[a]	0.20± 0.41
Ovary	1.80± 0.62[a]	2.00± 0.41[a]	1.60± 0.32	0.34±0.51[a]	0.32± 0.48[a]	0.38± 0.53	0.27± 0.41[a]	0.30±0.52[a]	0.10± 0.24
Liver	5.35± 0.73[a]	9.75± 0.81[a]	3.35± 0.63	0.06±0.02[a]	0.04± 0.01[a]	0.18± 0.12	0.70± 0.25[a]	0.60±0.22[a]	0.50± 0.20
Kidney	10.10± 0.64[a]	6.92± 0.73[a]	2.50± 0.43	0.15±0.21[a]	0.20± 0.16[a]	0.17± 0.19	0.50± 0.22[a]	0.60±0.23[a]	0.40± 0.18

1. ($p > 0.05$)
2. Values represent mean ± S.E.M ;
3. Values carrying the same superscripts horizontally are not significantly different ($p>0.01$); t- test for independent samples.

Table 4.15: Alanine aminotransferase and Aspartase aminotransferase Activities of rats fed Contaminated Tilapia Fish from Carter and Makoko markets

Study location	Alanine amino transferase (U/L)	Aspartate amino transferse (U/L)
Carter	113.50 ± 5.21[a]	76.00 ± 3.45[a]
Makoko	70.00 ± 3.13[a]	111.00 ± 5.10[a]
Control	64.00 ± 2.02	50.40 ± 1.30

Values represent mean ± S.E.M. (n=5)

Values carrying the same superscripts vertically are not significantly different (P> 0.01), T- test for independent samples.

Table 4.16: Serum Hematological Parameters in Rats fed contaminated Tilapia Fish from Carter and Makoko Markets

Parameter	Makoko	Control	Carter
PCV %	24.00±1.20[a]	29.00±1.10	26.00±1.50[a]
WBC (m).	7000.00±36.00[a]	6900.00±23.00	7700.00±45.00[a]
RBC (millions/mm)	4.86±0.61[a]	6.68±0.58	5.96±0.53[a]
Platelets (/L)	457.00±20.00[a]	265.00±15.00	334.00±19.00[a]

PHASE THREE

(Zinc Supplementation)

4.17 Weight of Organs in Rat fed with Tilapia Fish Diet & Zinc Supplements

There was a decrease in the weight of the heart which was formerly bloated after the administration of zinc supplements. Zinc supplementation, boosted the weight of the lung, kidney, liver and ovary leading to an increase in their weight (Table 4.17).

4.18 & 4.19: Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and iron levels in Serum and Organ of rat fed with Tilapia Fish Diet (from Makoko & Carter Bridge Market)

Cadmium, Copper, Chromium, Manganese and Magnesium were present in the serum and organs of the rats fed with Tilapia Fish diet (Tables 4.18 & 4.19).

4.20: Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron levels in Serum and Organs of rat fed with Zinc Supplements

Feeding with Zinc supplements, chelated cadmium out of the system due to the absence of cadmium in the serum and significantly decreased the levels of the other metals (Table 4.20).

4.21: Serum hematological parameters in rats fed Tilapia Fish Diet and Zinc supplements

Zinc supplementation boosted the hematological profile of the rats by increasing the PCV and RBC levels (Table 4.21).

4.22: Serum lipid profile in rats fed Tilapia Fish diet and Zinc supplements

Zinc supplements increase HDL concentration in the rats fed with it. LDL concentration was also reduced (Table 4.23).

4.23: Catalase and Glutathione activities in rats fed with Tilapia fish diet

Zinc supplementation significantly increased catalase and glutathione activities in the rat fed with it (Table 4.24).

4.3: Effects of Zinc Supplementation on Organs of Rats fed contaminated Fish

The rats fed with zinc supplemented diets had significant increase in body weights, increase in weights of organs (Table 4.17), a significant decrease in the levels of heavy metals in the serum and organs (Tables 4.18-4.20), increase in hormonal levels (Tables 4.25-26). Feeding with zinc tablets also boosted the hematological profile of the rats (tables 4.21-22), increased HDL levels in the lipid profile (Table 4.23) and also significantly increased catalase and glutathione levels (Table 4.24). It also reduced aberrations in the structure of the tissues.

PHASE THREE:

Table 4.17: Weight of Organs in rats fed with Tilapia Fish obtained from Makoko and Carter Bridge markets and Zinc Supplements

Organs/Group	Makoko	Carter	Zinc supplemented	Control
Heart (g)	0.66 ± 0.03	0.64 ± 0.06	0.57 ± 0.09	0.69 ± 0.07
Lung (g)	1.32 ± 0.57*	1.33 ± 0.75	1.53 ± 0.43	1.59 ± 0.44*
Kidney (g)	1.03 ± 0.87	1.03 ± 0.91*	1.17 ± 0.61*	1.17 ± 0.95
Ovary (g)	0.22 ± 0.91	0.21 ± 0.63	0.32 ± 0.86	0.31 ± 0.62
Liver (g)	5.79 ± 0.47*	6.35 ± 0.84*	7.50 ± 0.57*	7.64 ± 0.77*

Values represent mean of 5 rats *p< 0.05 T-test

Table 4.18: Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron Levels in serum and tissues of rats fed with Tilapia fish from Carter Bridge market

Carter bridge group	Pb	Cd	Cu	Cr	Mn	Mg	Fe
serum mg/ml	ND	0.003± 0.01*	0.041±0.40*	0.014 ±0.04*	0.024 ± 0.09	0.168 ±0.76*	0.361 ±0.54
liver mg/g	ND	0.001 ±0.01	0.021 ±0.60	0.019 ±0.02*	0.028±0.20 *	0.101 ±0.30	0.581±0.70*
heart mg/g	ND	0.001 ±0.07	0.029 ±0.40	0.009 ±0.07	0.036±0.73 *	0.089 ±0.43	0.496 ±0.64
ovary mg/g	ND	0.002 ±0.04*	0.031* ±0.90	0.006 ±0.08	0.019 ±0.59	0.124 ±0.71*	0.360 ±0.74
kidney mg/g	ND	0.001 ±0.02	0.036* ±0.50	0.003 ±0.05	0.025 ±0.07	0.169 ±0.59*	0.392 ±0.58
lung mg/g	ND	0.002 ±0.06*	0.029 ±0.08*	0.006 ±0.04	0.063±0.53 *	0.047 ±0.27	0.493±0.38*
Control							
serum mg/ml	ND	ND	0.029 ±0.069	ND	0.027±0.02 8	0.146 ±0.468	0.391 ±0.582
liver mg/g	ND	ND	0.015 ±0.034	ND	0.019 ±0.043	0.050 ±0.071	0.397 ±0.490
heart mg/g	ND	ND	0.027 ±0.073	ND	0.020 ±0.046	0.341 ±0.587	0.298 ±0.749
ovary mg/g	ND	ND	0.028±0.092	ND	0.019±0.08 9	0.029±0.024	0.261±0.547
kidney mg/g	ND	ND	0.029 ±0.045	ND	0.011 ±0.047	0.024 ±0.076	0.049 ±0.095
lung mg/g	ND	ND	0.025 ±0.096	ND	0.075 ±0.052	0.040 ±0.037	0.049 ±0.068

Values represent mean ± S.E.M of 5 rats. * $p<0.05$, t-test for independent sample

ND: Not determined.

Table 4.19: Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron Levels in serum and tissues of rats fed with Tilapia fish from Makoko market

Makoko Group	Pb	Cd	Cu	Cr	Mn	Mg	Fe
serum mg/ml	0.002± 0.03*	0.029± 0.024*	0.049± 0.074*	0.010 ±0.032*	0.030 ±0.062	0.096 ±0.019	0.268 ±0.786
liver mg/g	0.006± 0.039*	0.010 ±0.061	0.041± 0.029*	0.002 ±0.058	0.086± 0.073*	0.062 ±0.054*	0.246 ±0.622
heart mg/g	0.009± 0.001*	0.006 ±0.027	0.044± 0.082*	0.006 ±0.037*	0.044 ±0.057	0.086 ±0.018*	0.469 ±0.790
ovary mg/g	0.001± 0.051	0.002 ±0.074	0.030 ±0.052	0.009 ±0.075*	0.046 ±0.059	0.088 ±0.052*	0.586± 0.654*
kidney mg/g	0.001± 0.063	0.009± 0.076*	0.039± 0.062*	0.001 ±0.079	0.074± 0.069*	0.102 ±0.052	0.349 ±0.091
lung mg/g	0.001± 0.004	0.001 ±0.065	0.019 ±0.084	0.007 ±0.077*	0.087± 0.051*	0.054 ±0.069	0.597± 0.085*
Control							
serum mg/ml	ND	ND	0.029 ±0.069	ND	0.027 ±0.028	0.146 ±0.368	0.391 ±0.582
liver mg/g	ND	ND	0.015 ±0.034	ND	0.019 ±0.043	0.050 ±0.071	0.397 ±0.490
heart mg/g	ND	ND	0.027 ±0.073	ND	0.020 ±0.046	0.341 ±0.587	0.298 ±0.749
ovary mg/g	ND	ND	0.028 ±0.092	ND	0.019 ±0.089	0.029 ±0.024	0.261 ±0.547
kidney mg/g	ND	ND	0.029 ±0.045	ND	0.011 ±0.047	0.024 ±0.076	0.049 ±0.095
lung mg/g	ND	ND	0.025 ±0.096	ND	0.075 ±0.052	0.040 ±0.037	0.049 ±0.068

Values represent mean ± S.E.M of 5 rats. * $p<0.05$, t-test for independent sample

ND: Not determined.

Table 4.20: Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron Levels in Serum and Tissues of rats fed with Zinc Supplements

Zinc Group	Pb mg/g	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
serum mg/ml	ND	ND	0.021±0.065	0.011 ±0.069*	0.019 ±0.039	0.049 ±0.073	0.281 ±0.867
liver mg/g	ND	ND	0.021±0.012*	0.010 ±0.086*	0.020±0.063*	0.051 ±0.572*	0.686±0.978*
heart mg/g	ND	ND	0.027 ±0.687	0.009 ±0.043	0.016 ±0.072	0.068 ±0.063*	0.681±0.074*
ovary mg/g	ND	ND	0.029±0.047*	0.006 ±0.058	0.026±0.073*	0.068 ±0.084	0.481 ±0.642
kidney mg/g	ND	ND	0.024±0.062*	0.010 ±0.071*	0.021 ±0.081	0.046 ±0.077	0.496 ±0.094
lung mg/g	ND	ND	0.021 ±0.053	0.004 ±0.070	0.053±0.082*	0.038 ±0.074	0.612±0.864*
Control							
serum mg/ml	ND	ND	0.029 ± 0.069	ND	0.027 ± 0.028	0.146 ± 0.368	0.391 ± 0.582
liver mg/g	ND	ND	0.015 ± 0.034	ND	0.019 ± 0.043	0.050 ± 0.071	0.397 ± 0.490
heart mg/g	ND	ND	0.027 ± 0.073	ND	0.020 ± 0.046	0.341 ± 0.587	0.298 ± 0.749
ovary mg/g	ND	ND	0.028 ± 0.092	ND	0.019 ± 0.089	0.029 ± 0.024	0.261 ± 0.547
kidney mg/g	ND	ND	0.029 ± 0.045	ND	0.011 ± 0.047	0.024 ± 0.076	0.049 ± 0.095
lung mg/g	ND	ND	0.025 ± 0.096	ND	0.075 ± 0.052	0.040 ± 0.037	0.049 ± 0.068

Values represent mean ± S.E.M of 5 rats.

* $p<0.05$, T-test for independent sample

Table 4.21: Serum Hematological Parameters II in Rats fed contaminated Tilapia fish from Carter and Makoko markets, Zinc supplements (for 3 months) and Control

Parameter/group	Makoko Group	Carter Group	Zinc Group	Control
RBC/(pl)	2.47 ± 0.32	6.26 ± 0.53*	7.76 ± 0.29*	7.39 ± 0.71
Hb (g/dl)	4.20 ± 0.98	10.60 ± 0.76	14.10 ± 0.88*	12.60 ± 0.95
Haematocrit (L/L)	0.13 ± 0.61	0.21 ± 0.97	0.45 ± 0.65*	0.41 ± 0.21
MCV (Fl)	58.00 ± 8.64*	56.00 ± 9.22	58.40 ± 4.67*	55.40 ± 1.42
MCH (Pg)	17.20 ± 3.76	17.40 ± 4.54	18.20 ± 3.65*	17.10 ± 3.68
MCHC (g/dl)	31.80 ± 9.82	31.00 ± 7.41	31.10 ± 6.43*	30.80 ± 7.84
RDW	14.70 ± 7.64	15.30 ± 8.52*	15.10 ± 6.52*	15.90 ± 3.21
Platelets (/L)	$323x10^9$ ± 67.98	$795x10^9$ ± 76.32	$753x10^9$ ± 28.90	$952x10^9$ ± 76.53

Values represent mean ± S.E.M. (n=5)

(*p>0.05); t- test

Table 4.22: Serum Differential Leucocytes Counts of rats fed contaminated Tilapia fish from Carter and Makoko markets, Zinc Supplements (for 3 months) and Control

Differential leucocytes count	Makoko	Carter	Zinc supplemented	Control
Leucocytes count	$6.54 \times 10^9 \pm 0.48$*	$6.78 \times 10^9 \pm 0.77$	$6.25 \times 10^9 \pm 0.38$*	$6.23 \times 10^9 \pm 0.29$
Neutrophils/L	$0.57 \times 10^9 \pm 0.71$	$1.07 \times 10^9 \pm 0.57$	$1.57 \times 10^9 \pm 0.28$*	$1.11 \times 10^9 \pm 0.33$
Lymphocytes/L	$4.00 \times 10^9 \pm 0.73$	$4.37 \times 10^9 \pm 0.95$*	$5.87 \times 10^9 \pm 0.52$*	$3.98 \times 10^9 \pm 0.61$
Monocytes/L	$0.36 \times 10^9 \pm 0.01$	$0.89 \times 10^9 \pm 0.09$	$1.29 \times 10^9 \pm 0.62$*	$0.99 \times 10^9 \pm 0.08$
Eosinophils/L	$0.03 \times 10^9 \pm 0.04$	$0.12 \times 10^9 \pm 0.08$	$0.18 \times 10^9 \pm 0.07$*	$0.13 \times 10^9 \pm 0.05$
Basophils /L	$0.01 \times 10^9 \pm 0.01$	$0.01 \times 10^9 \pm 0.02$	$0.03 \times 10^9 \pm 0.04$*	$0.02 \times 10^9 \pm 0.01$

Values represent mean $\pm$ S.E.M. (n=5)

(*$p>0.05$); t- test

Table 4.23: Serum Lipid Profile in rats fed Contaminated Tilapia fish from Carter and Makoko markets, Zinc supplements and Control

Study location	Cholesterol mmol/l	Triglyceride mmo/l	HDL mmol/l	LDL mmol/l
Makoko	1.30 ± 0.27*	0.40 ± 0.09	0.60 ± 0.03	0.80 ± 0.06
Carter	1.50 ± 0.94*	0.50 ± 0.07	0.80 ± 0.06	0.85 ± 0.06*
Zinc supplemented	1.70 ± 0.76*	0.40± 0.07	0.90 ± 0.08*	0.60± 0.07
Control	1.90 ± 0.52	0.50 ± 0.04	0.80 ± 0.02	0.70 ± 0.09

Values represent mean ± S.E.M. (n=5)

(*p>0.05); t- test

Table 4.24: Catalase Activity and Glutathione Level in Rats fed contaminated Tilapia fish from Carter and Makoko markets, Zinc Supplements and Control

Study location	Glutathione	Catalase
Control	1.27 ± 0.49	0.48 ± 0.09*
Makoko	1.26 ± 0.27	0.35 ± 0.01
Carter	1.25 ± 0.44	0.34 ± 0.06
Zinc supplemented	1.28 ± 0.28*	0.49 ± 0.04*

Values represent mean ± S.E.M. (n=5)

(*p>0.01); t- test

4.25: Effects of Zinc Supplementation on Hormonal Levels of rabbits fed with Tilapia fish from Carter Bridge and Makoko River

The rabbits fed with the fishes obtained from Carter Bridge River (group A), had a poor appearance and exhibited loss in weight, loss of fur, scaly growths on skin and eyelids together with scaly growths on mouth tips.

The weight of the rabbits fed with zinc supplemented diet appreciated gradually with no sign of hair loss nor scaly growth on eyelids. This may be due to the fact that there was an increase in appetite as evident from their feeding pattern.

Follicle stimulating hormone level was reduced to 1.40 Iu / ml and 1.10 Iu /ml when compared to the control value of 1.60Iu / ml. Prolactin level was reduced to 1.20 ng / ml and 1.10 ng /ml when compared to the control value of 1.80ng / ml. Lutenizing hormone level was increased to 3.80 Iu / ml and 4.00 Iu / ml.

Table 4.25: Serum Prolactin, Progesterone, Lutenizing Hormone and Follicle Stimulating Hormone in Rabbits fed contaminated Tilapia Fish

Groups	Prolactin (mg/ml)	Progesterone (mg/ml)	Lutenizing hormone (Iu/ml)	Follicle stimulating hormone (Iu/ml)
Carter site group	1.20 ±0.03	0.03±0.01*	3.80±0.10*	1.40 ±0.01
Makoko site group	1.10±0.05*	0.30 ±0.05*	4.00 ± 0.02	1.10 ±0.05*
Control	1.80 ± 0.02	1.60 ± 0.03	1.10 ± 0.05	1.60 ± 0.01

Values represent mean ± S.E.M. (n=5).

* $p < 0.05$, ANOVA.

Table 4.26: Progesterone Levels in Serum of Rats and Rabbits fed with Tilapia fish diet from Carter Bridge and Makoko markets, Zinc Supplements and Control

Study location	Progesterone (ng/ml) Rats	Progesterone (ng/ml) Rabbits
Makoko	13.00 ± 1.67*	0.30 ± 0.05 [a]
Carter	8.00 ± 0.54	0.03 ± 0.01 [a]
Zinc	27.00 ± 2.98*	1.80 ± 0.09
Control	17.00 ± 1.88	1.60 ± 0.03

Values represent mean ± S.E.M. (n=5).

* $p < 0.05$, ANOVA.

Table 4.27: Serum Progesterone Levels of Rabbits fed contaminated Tilapia fish, Zinc Supplemented Diet and Control

Study location	Progesterone (ng/ml)
A-fed with Carter fish diet alone	$0.03^{a} \pm 0.02$
B- fed with Makoko fish diet Alone	0.05 ± 0.04
B -fed with fish diet and zinc supplement	$6.80^{a} \pm 0.70$
C – fed with rabbit pellet alone	6.00 ± 0.50

Values represent mean $\pm$ S.E.M (n=5) $^{a}p < 0.05$ T-test

Histological Studies

Plates 1-6: Histological Studies results (Phase Two)

Photomicrograph of the lungs showed interstitial inflammation, congestion and edema in rats fed with Tilapia Fish diet when compared with the control. Photomicrographs of the liver showed cytoplasmic vacuolation in the rats fed with Tilapia Fish diet when compared with the control.

Plates 7-15: Histological Studies (Phase Three)

Photomicrograph of kidneys of rats fed with Tilapia Fish diet showed congested glomeruli and chronic inflammation when compared with the control. Photomicrographs of ovaries of rats fed with Tilapia Fish diet showed mild congested vessels. The zinc supplemented ovary showed no abnormalities. Photomicrographs of lungs tissues in rats fed with Tilapia fish diet showed interstitial abnormality, inflammation and pneumonia. Photomicrographs of lung tissues in the control group and zinc supplemented group showed no abnormality

Fig. 4.9: HISTOLOGICAL STUDIES 1

Photomicrographs of lung, liver tissues of rats fed with fishes from Makoko Site and Carter Site.

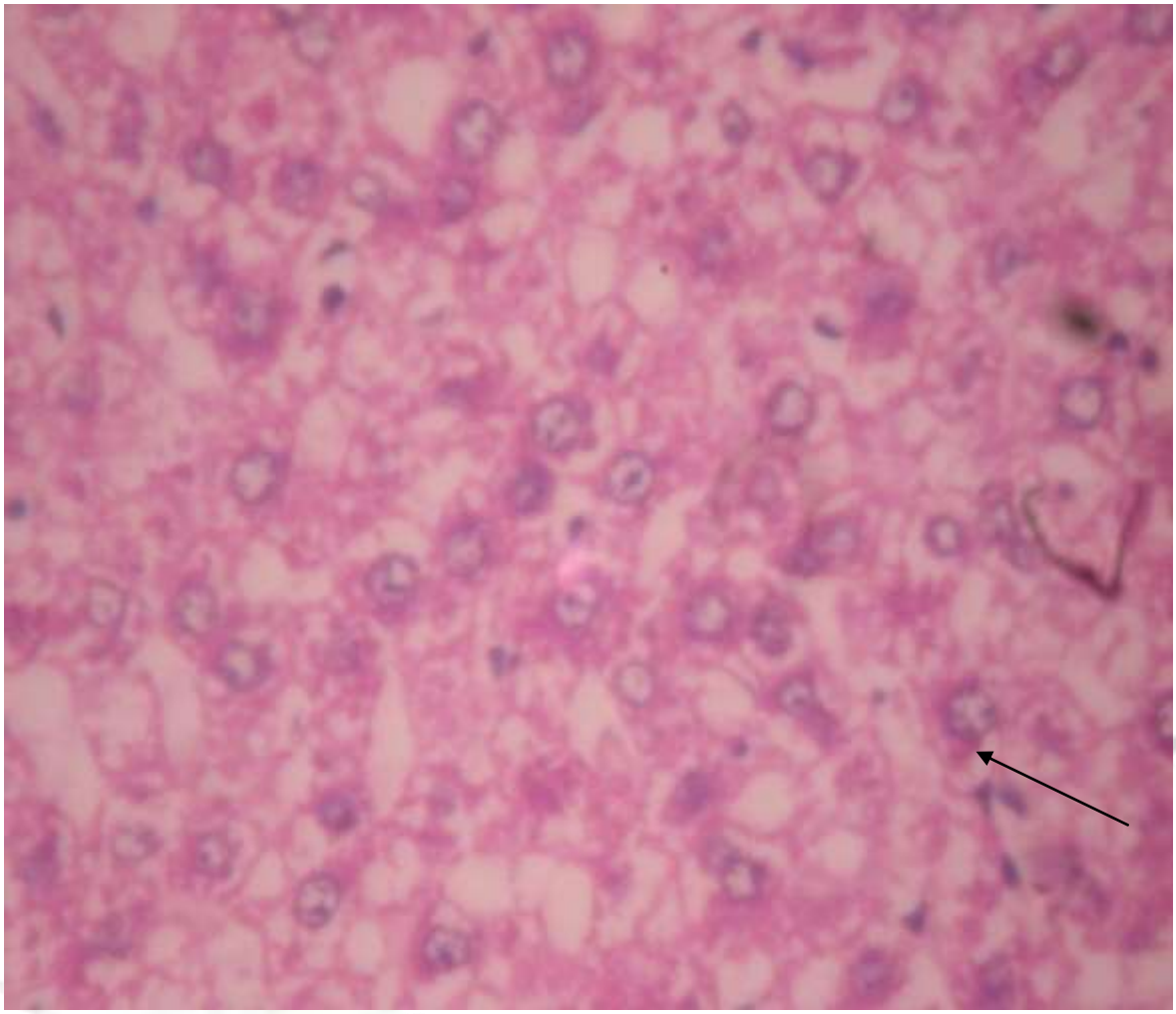


Plate 1: Photomicrograph of Liver of rats fed with fishes from Makoko Site showing cytoplasmic vacuolation (x400)

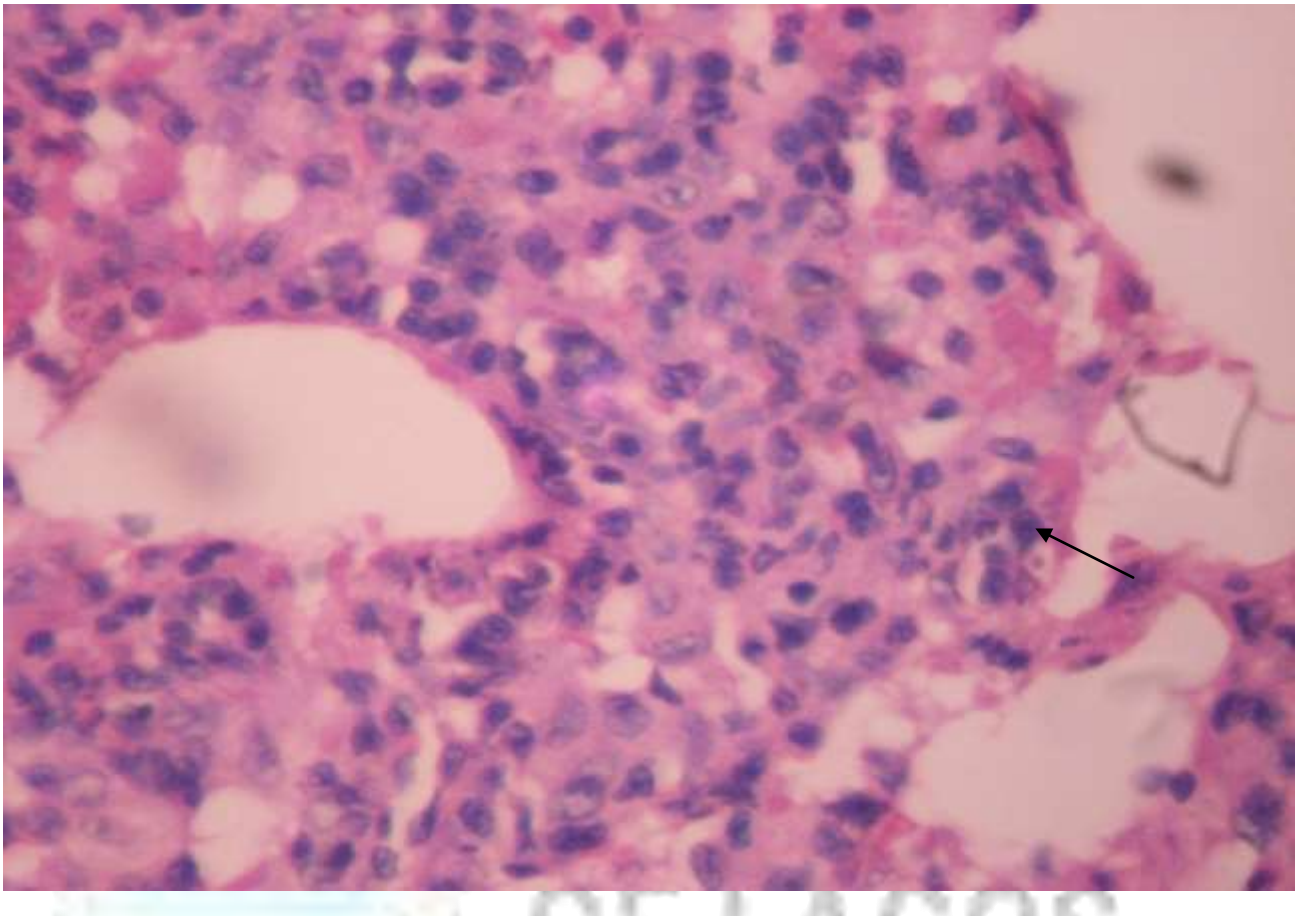


Plate 2: Photomicrograph of Lungs of rats fed with fishes from Makoko Site showing interstitial inflammation (x400)

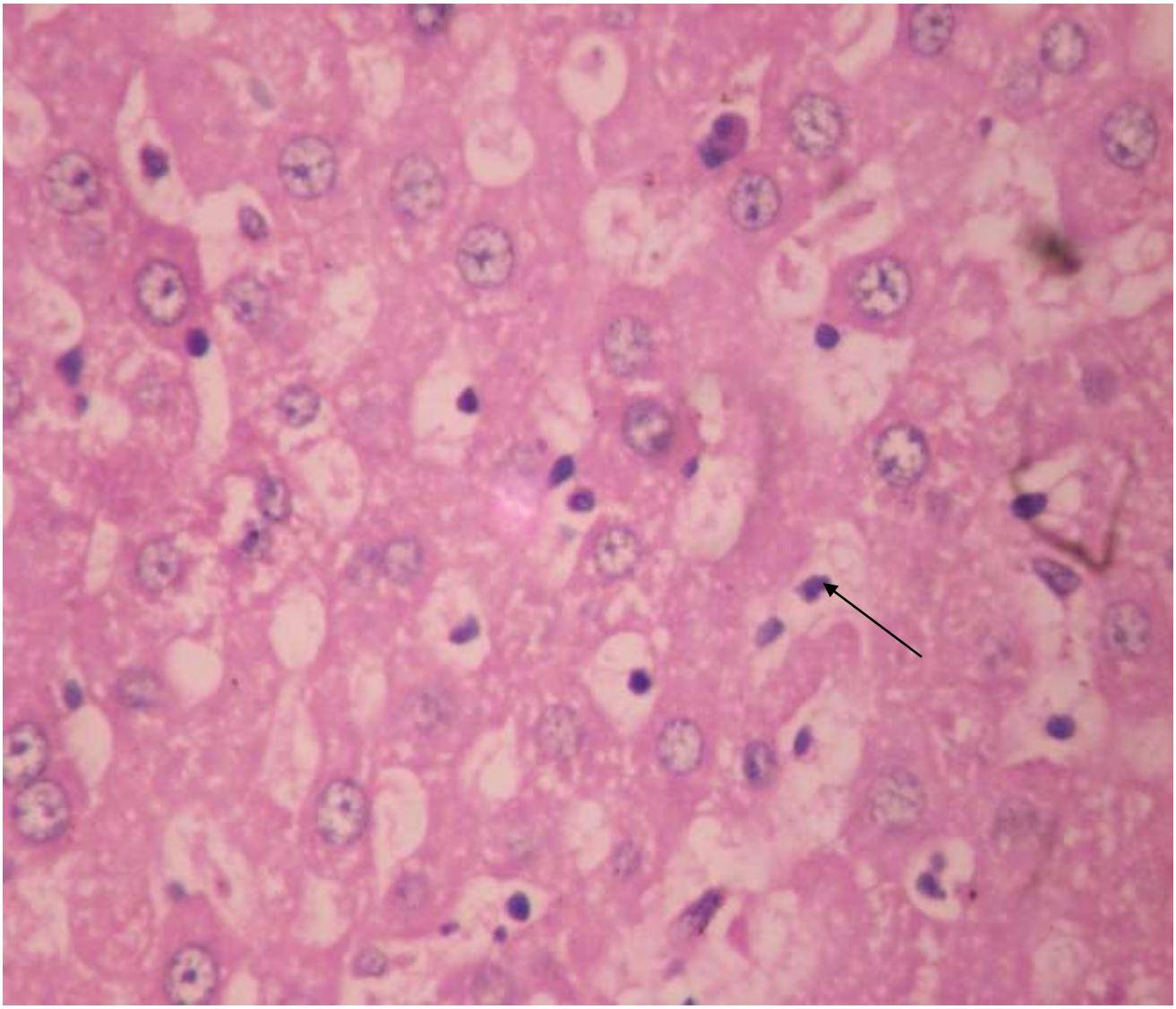


Plate 3: Photomicrograph of Liver of rats fed with fishes from Carter Bridge Site showing cytoplasmic vacuolation (x400)

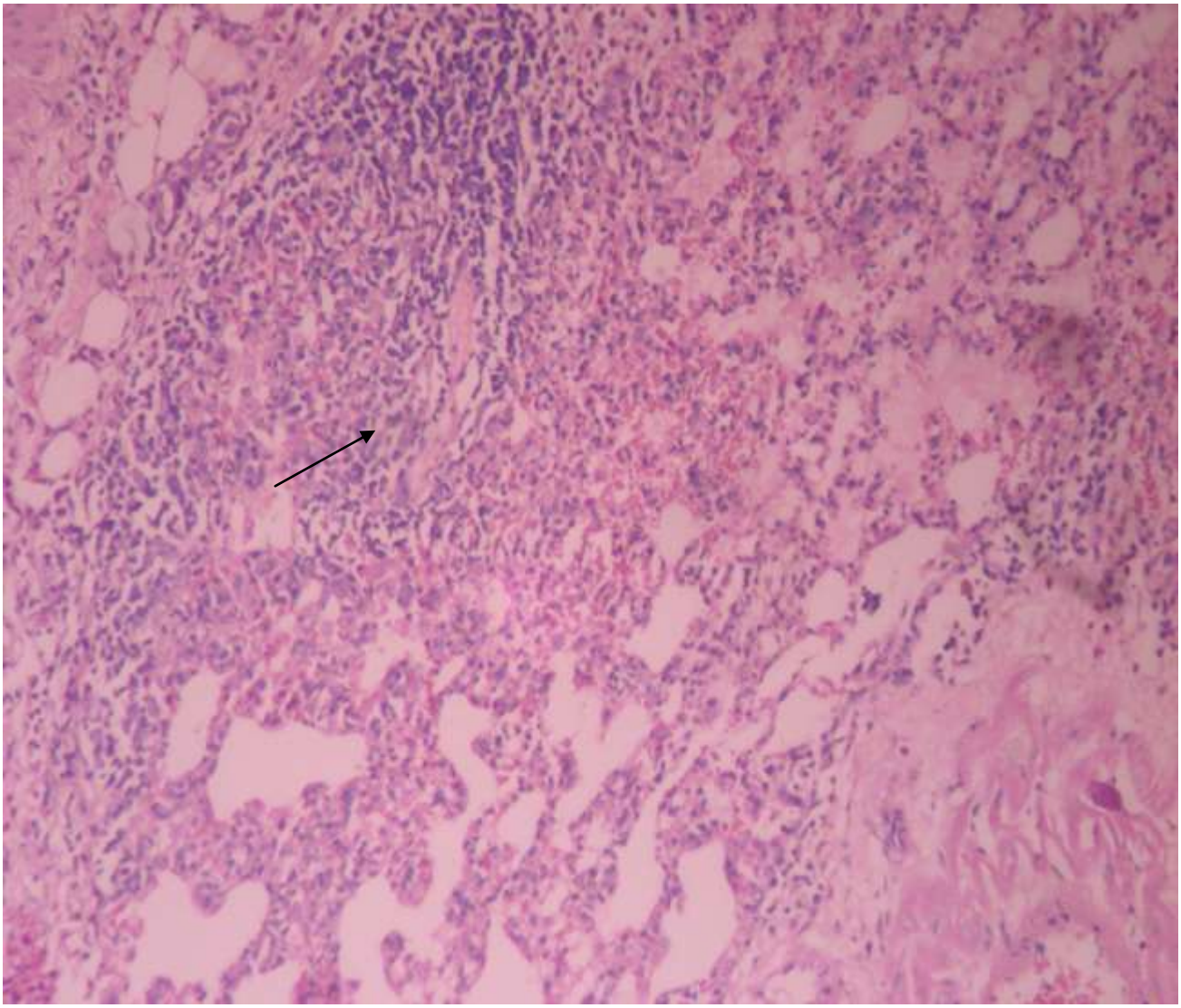


Plate 4: Photomicrograph of Lungs of rats fed with fishes from Carter Bridge Site showing congestion, Inflammation and edema (x400)

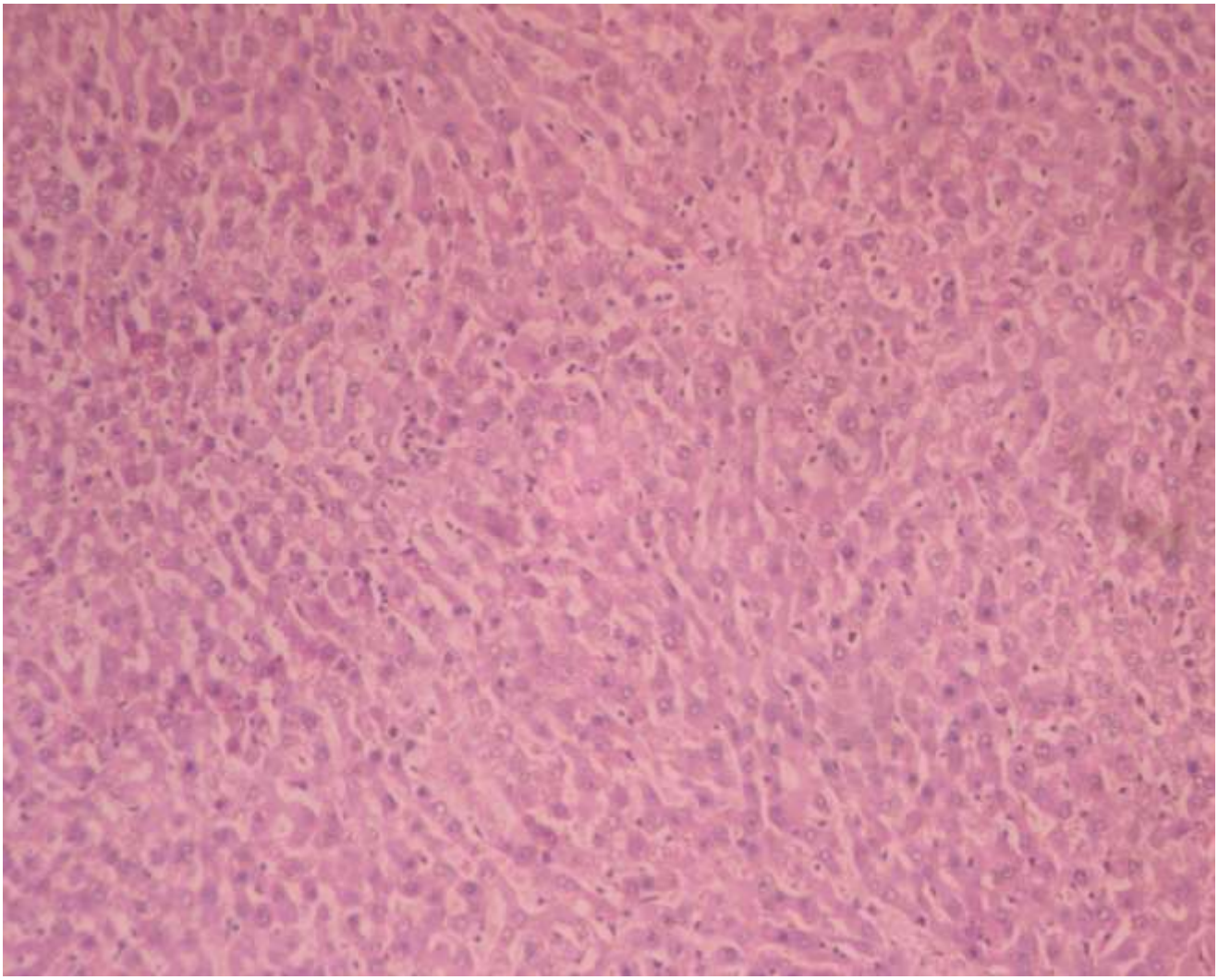


Plate 5: Photomicrograph of Liver of rats from the control group showing no abnormality (x400)

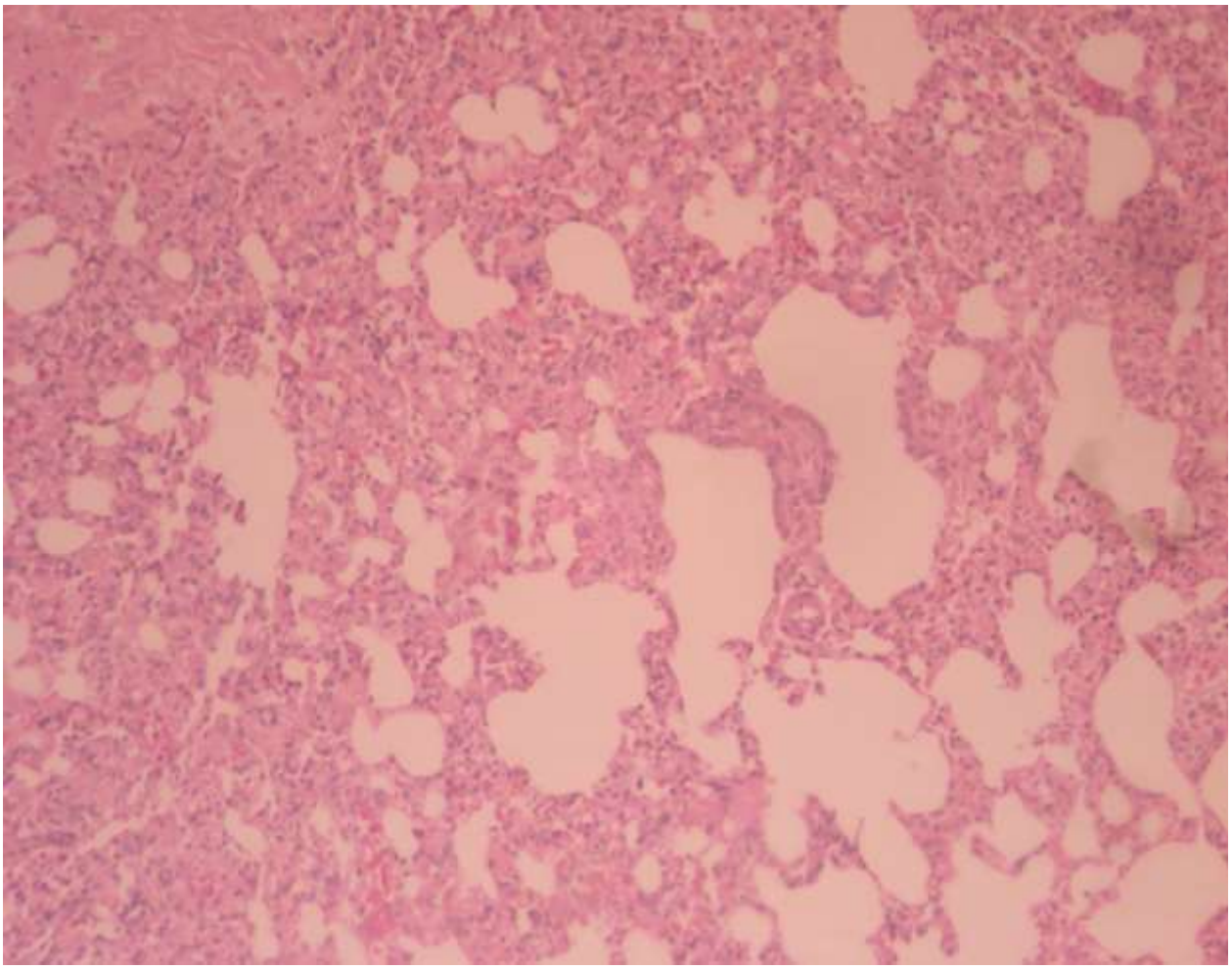


Plate 6: Photomicrograph of Lungs of rats from the control group showing no abnormality (x400)

Fig. 4.10: HISTOLOGICAL STUDIES II

Photomicrographs of liver tissues from rats fed with fishes from Carter sites and Makoko sites

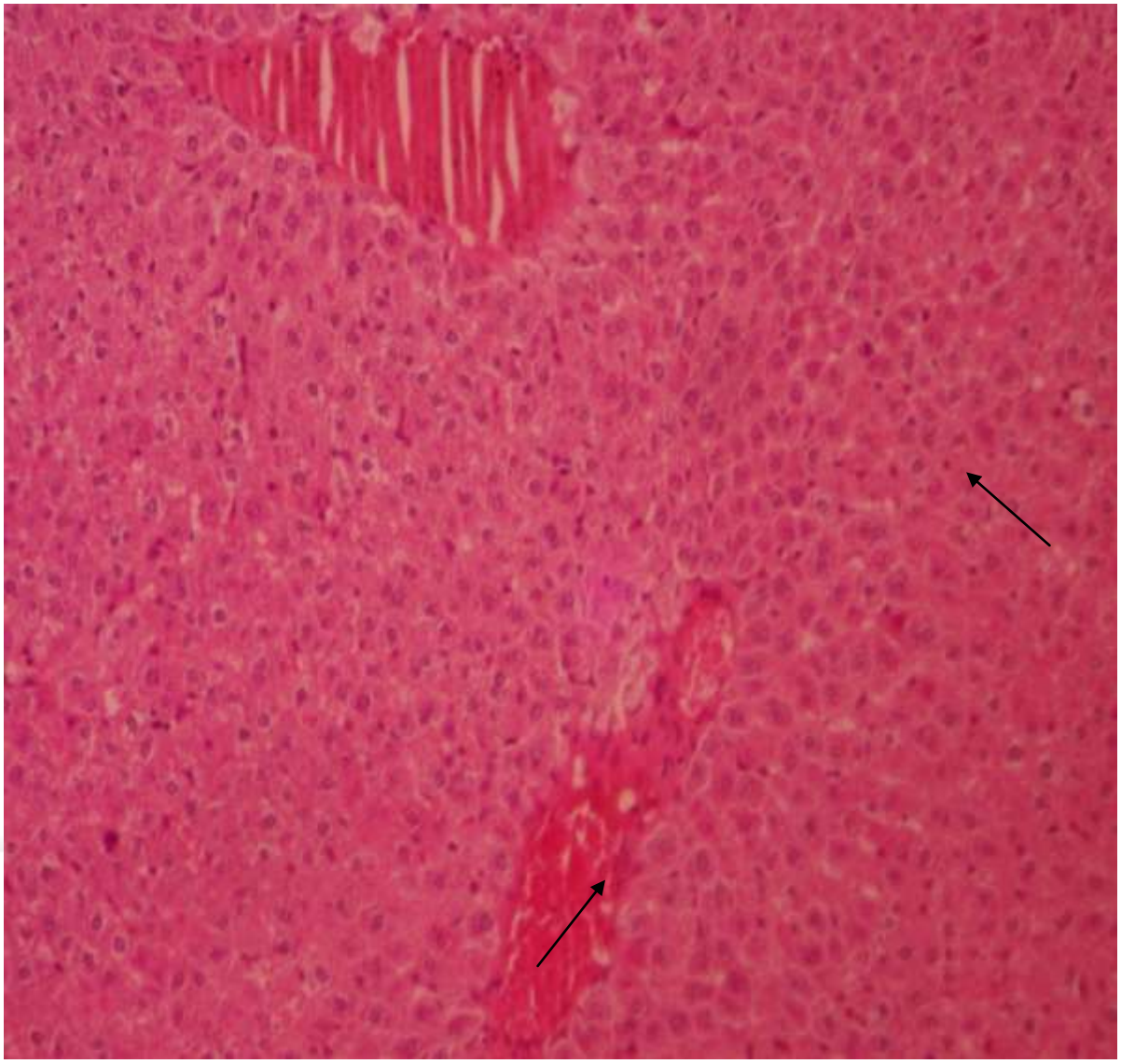


Plate 7: Photomicrograph of Liver of rats fed with fishes from Carter Bridge Site hepatocytes and severely congested blood vessels(x400)

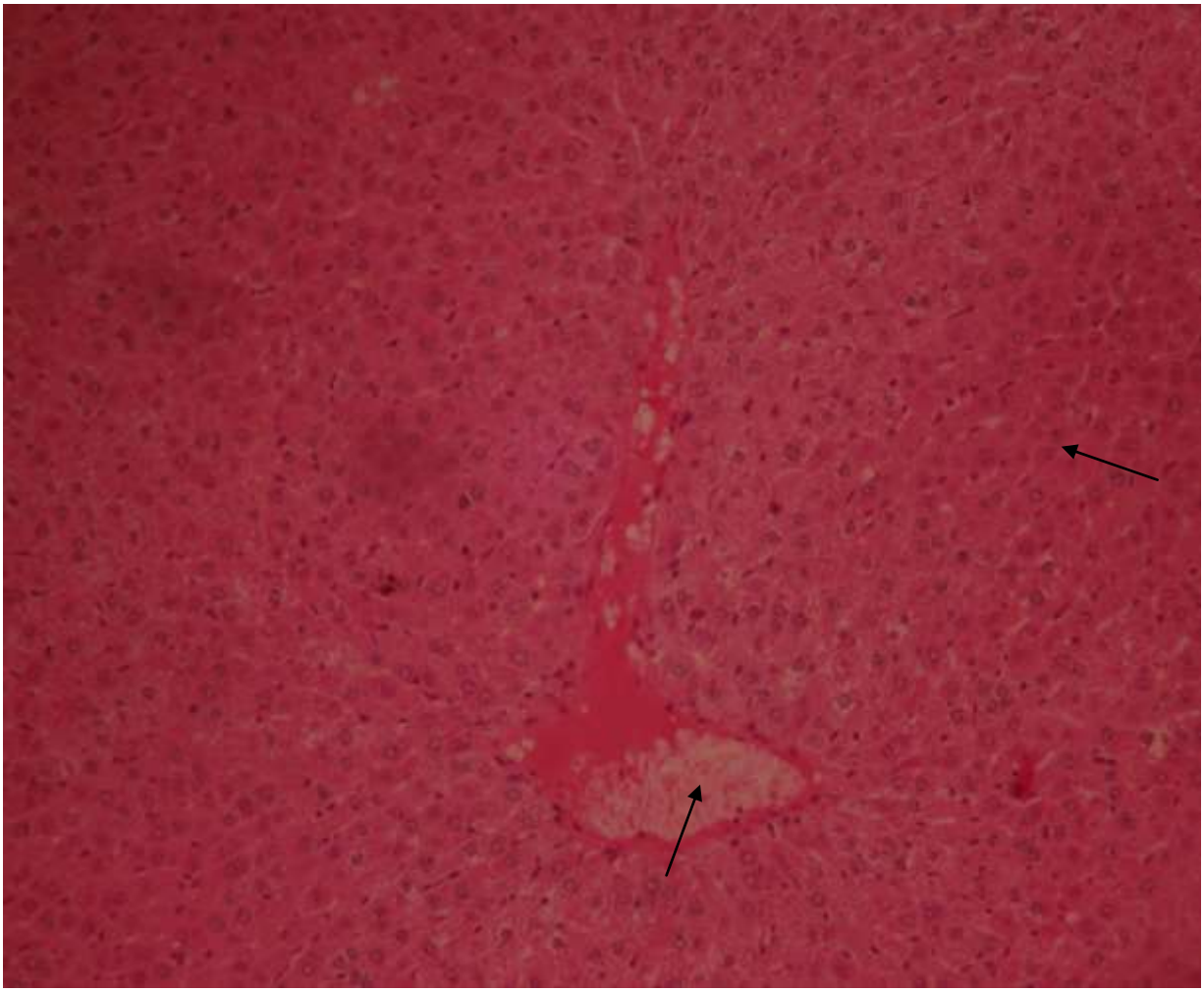


Plate 8: Photomicrograph of Liver of rats fed with fishes from Makoko Site showing mildly congested blood vessels (x400)

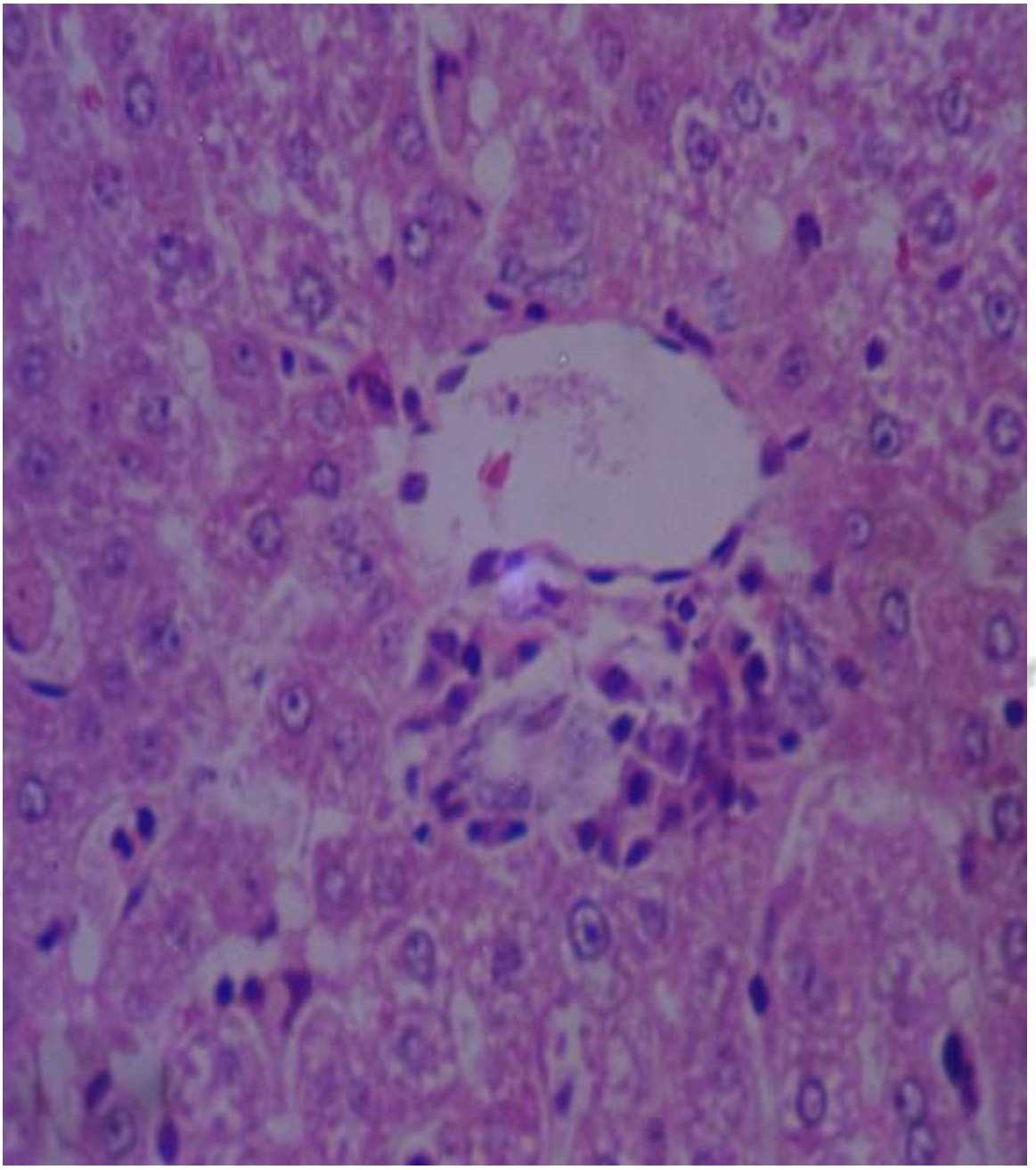


Plate 9: Photomicrograph of Liver of rats from the control group showing no abnormality (x400)

Fig 4.11: Photomicrographs of kidney tissues from rats fed with fishes from Carter sites and Makoko sites

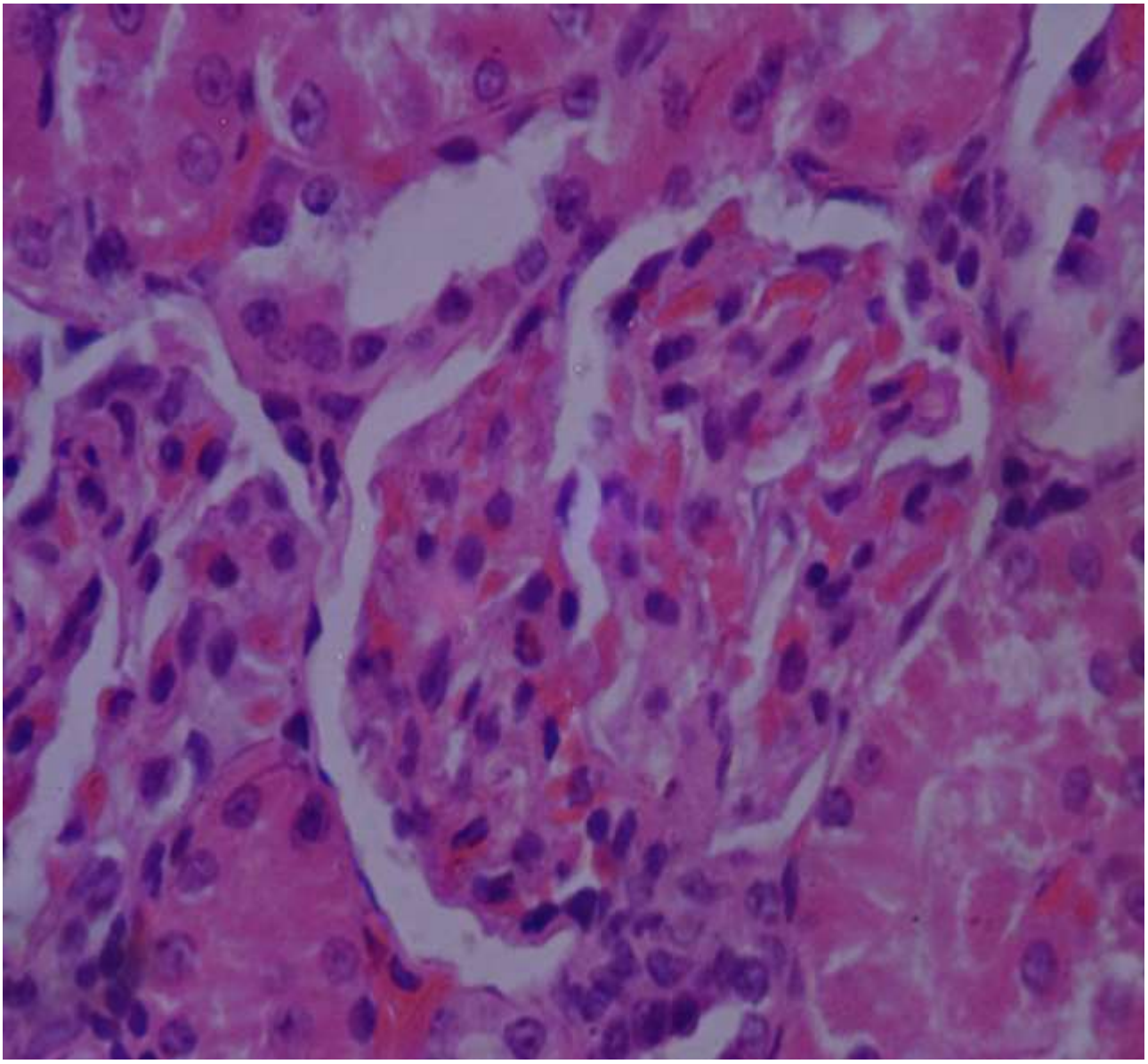


Plate 10: Photomicrograph of kidney of rats fed with fishes from Carter Site showing congested glomeruli (x400)

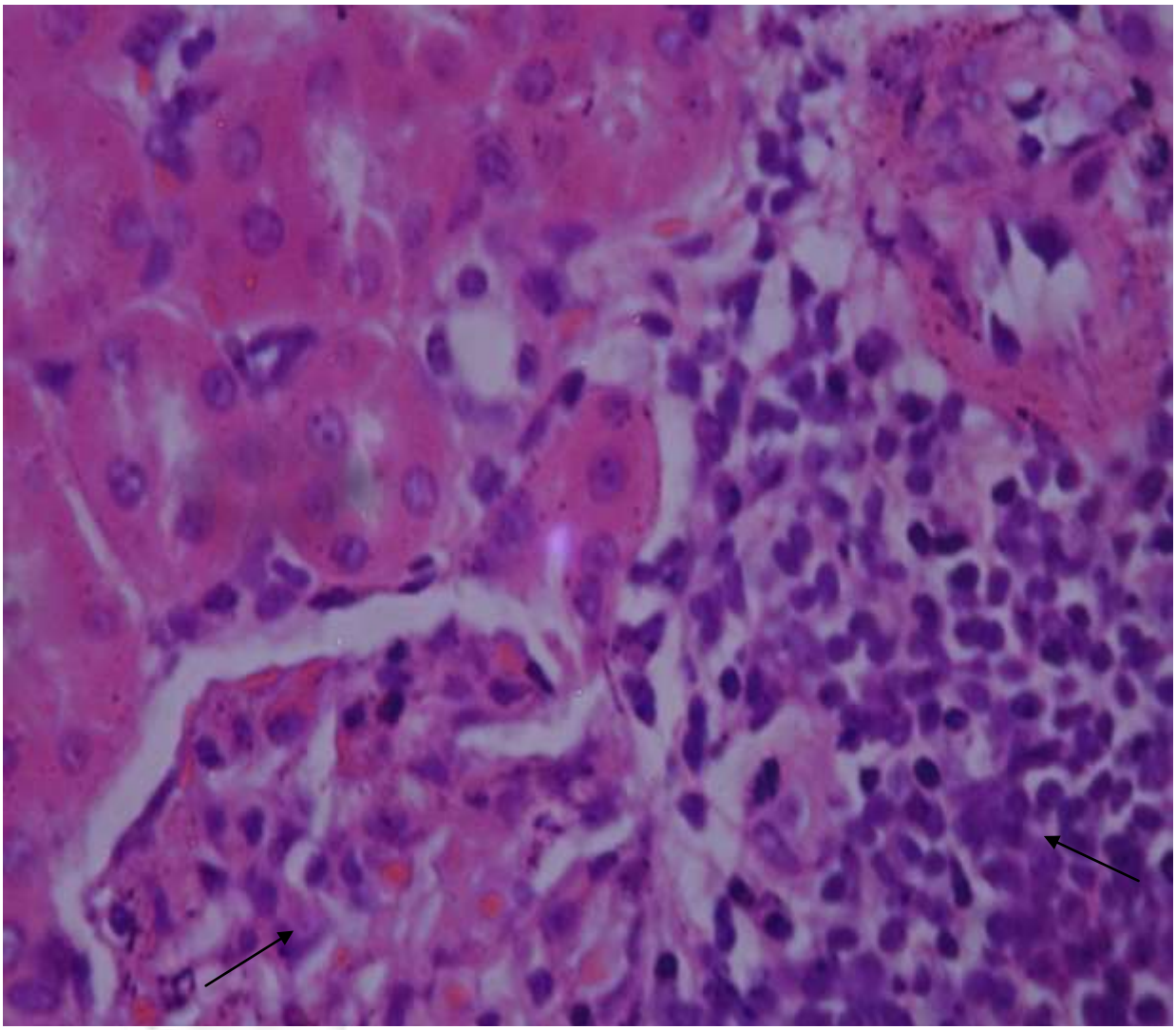


Plate 11: Photomicrograph of kidney of rats fed with fishes from Makoko Site showing chronic inflammatory cells within the interstitium of the kidney (interstitial nephritis) (x400)

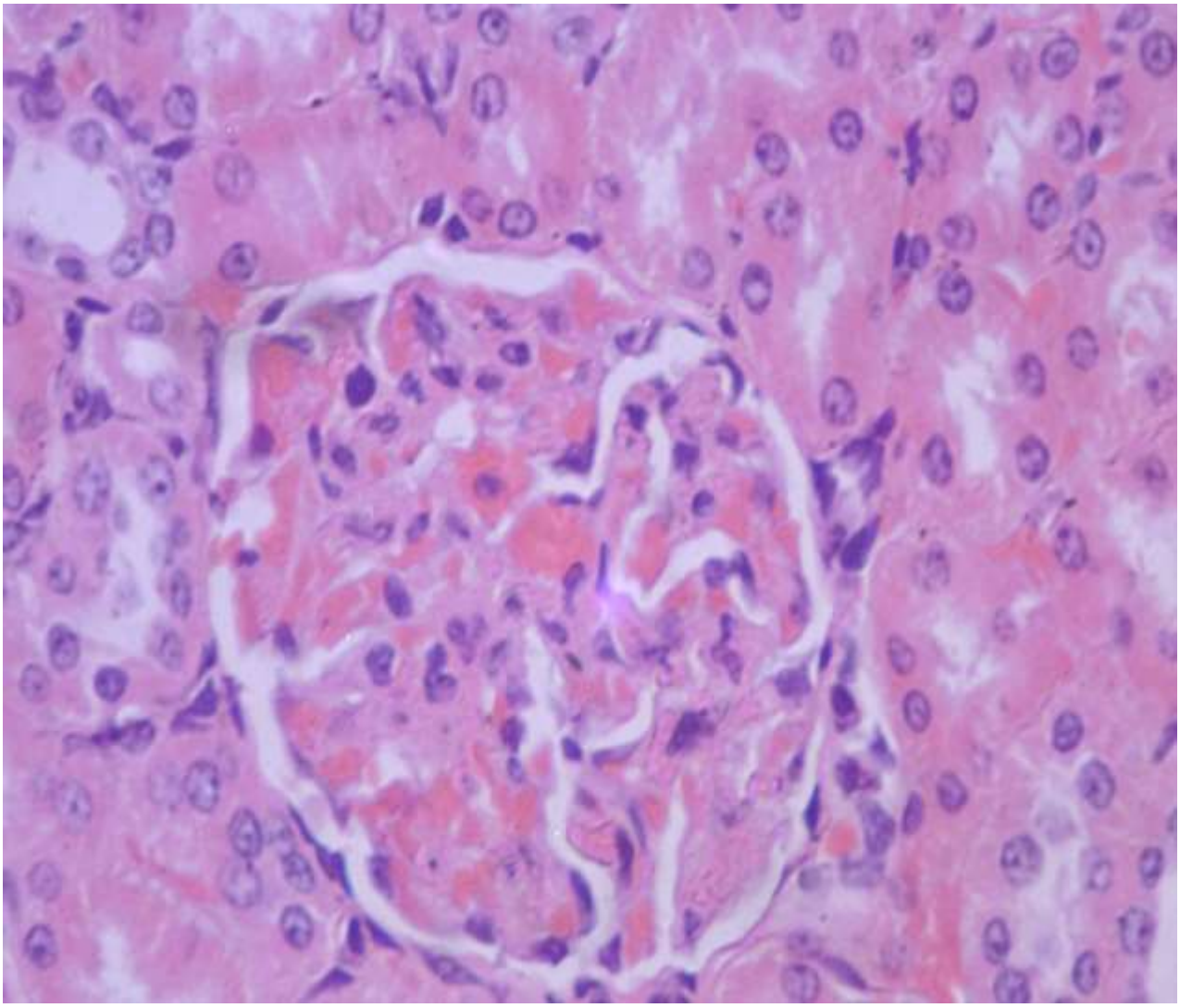


Plate 12: Photomicrograph of kidney of rats from the control group show glomeruli with no abnormality(x400)

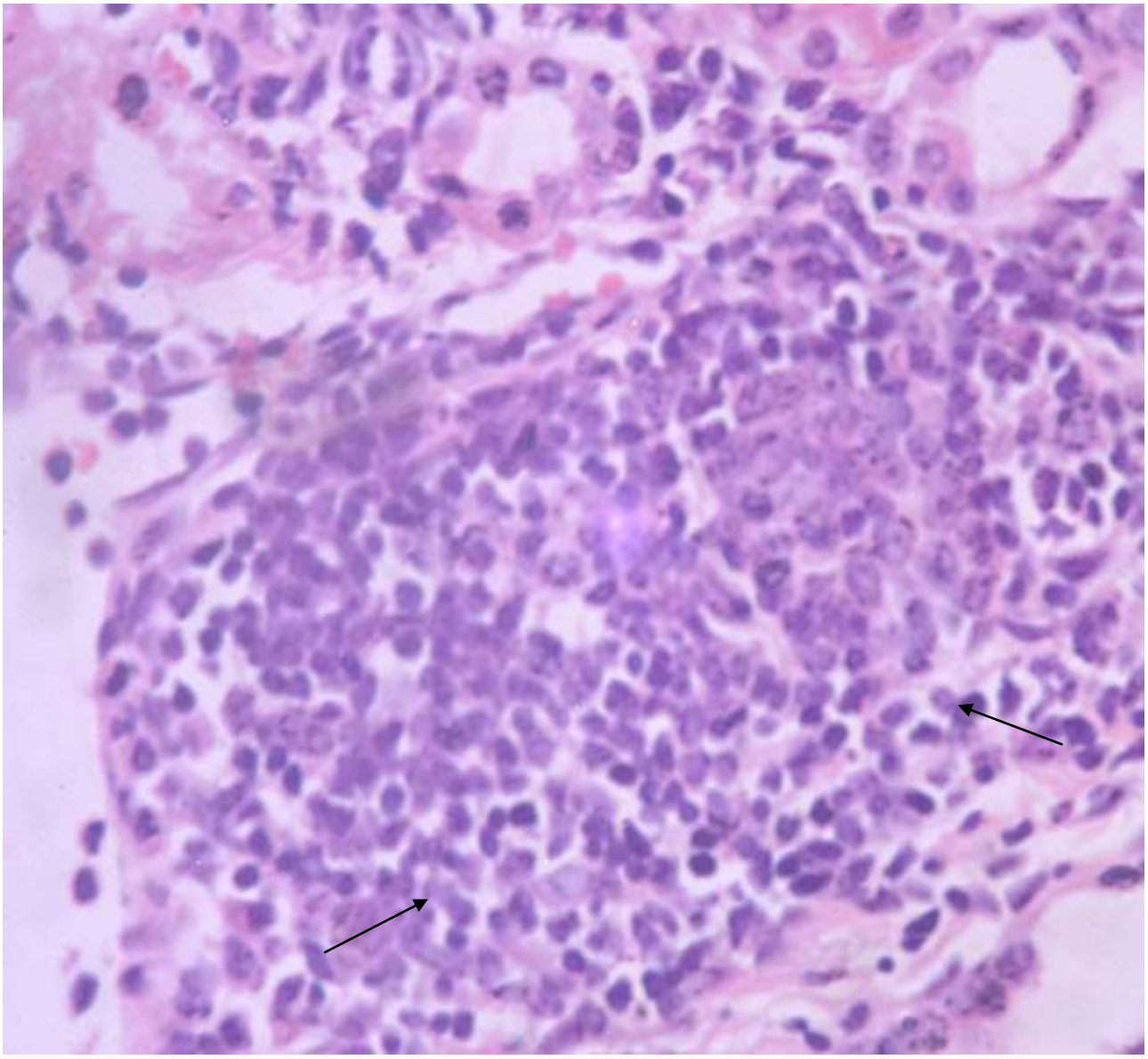


Plate 13: Photomicrograph of kidney of rats fed with fishes and zinc supplements showing Interstitial infiltration (interstitial nephritis) (x400)

Fig.4.12: Photomicrographs of ovary tissues from rats fed with fishes from Carter sites and Makoko sites

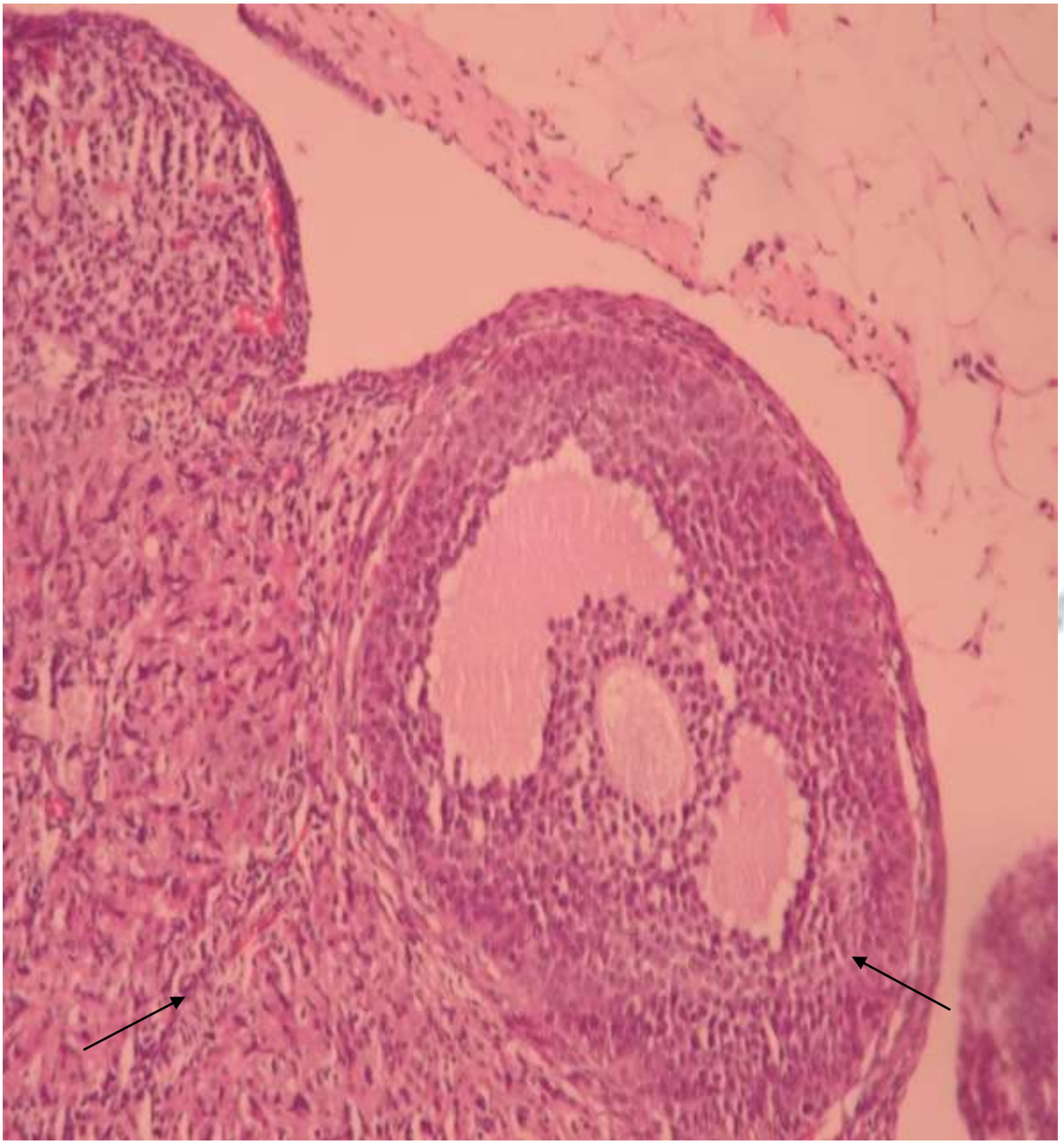


Plate 14: Photomicrograph of ovary of rats fed with fishes from Carter Site showing developing follicles and mild congestion (x400)

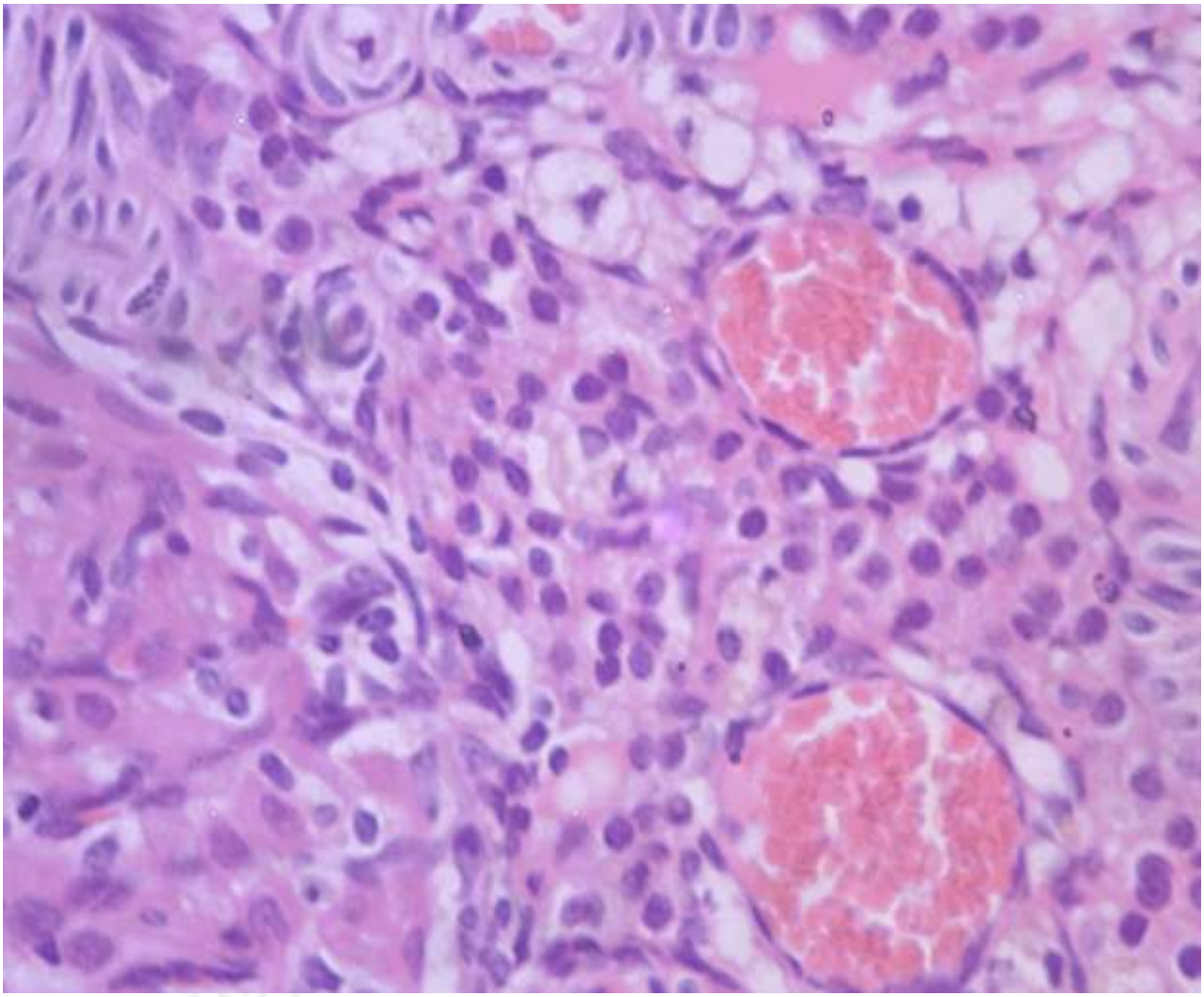


Plate 15: Photomicrograph of ovary of rats fed with fishes from Makoko market showing congested vessels (x400)

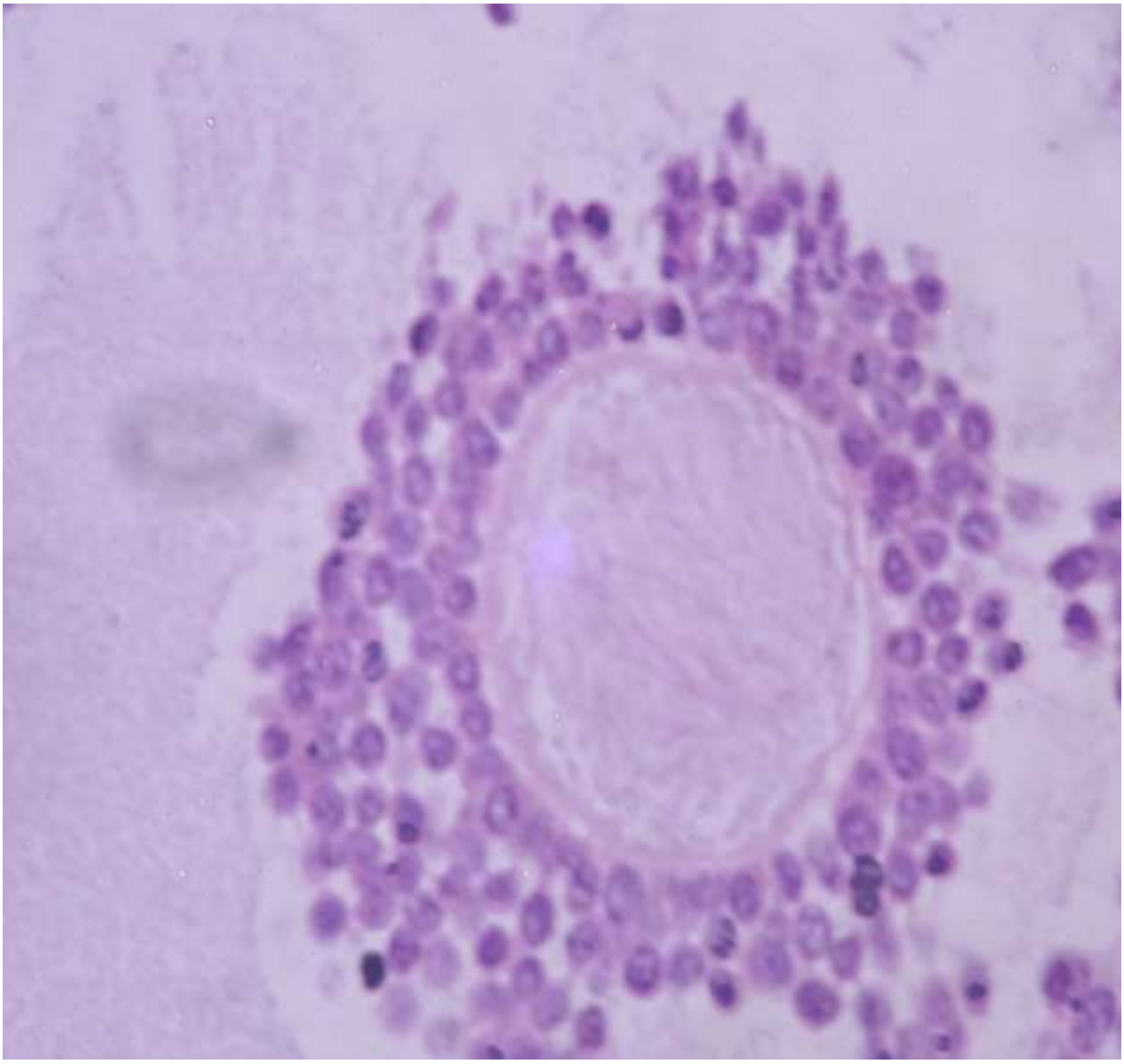


Plate 16: Photomicrograph of ovary of rats from the control group showing developing follicles with no abnormalities (x400)

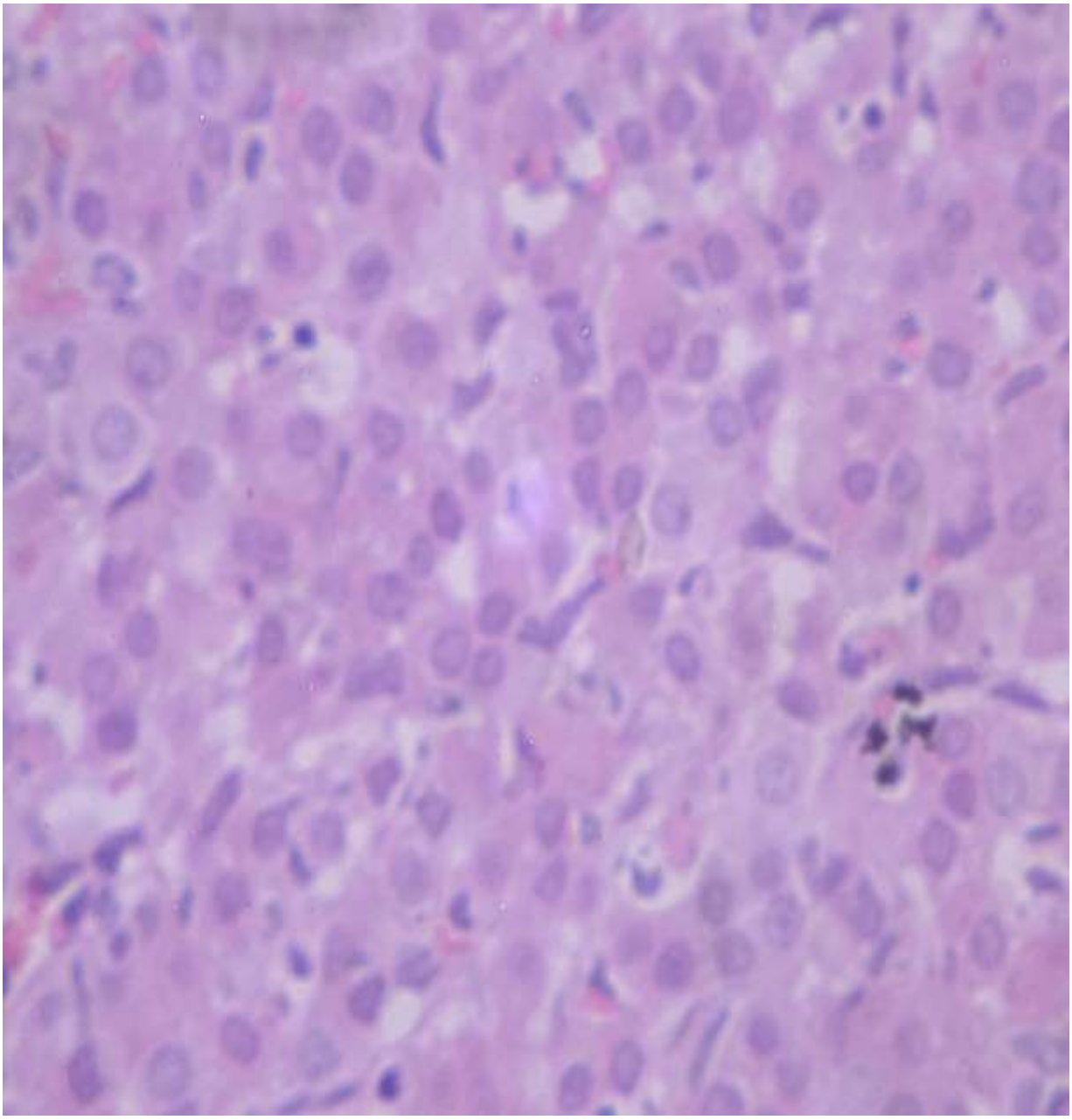


Plate 17: Photomicrograph of ovary of rats fed with fishes and zinc supplements showing no abnormalities (x400)

Fig 4.13: Photomicrographs of lung tissues from rats fed with fishes from Carter sites and Makoko sites

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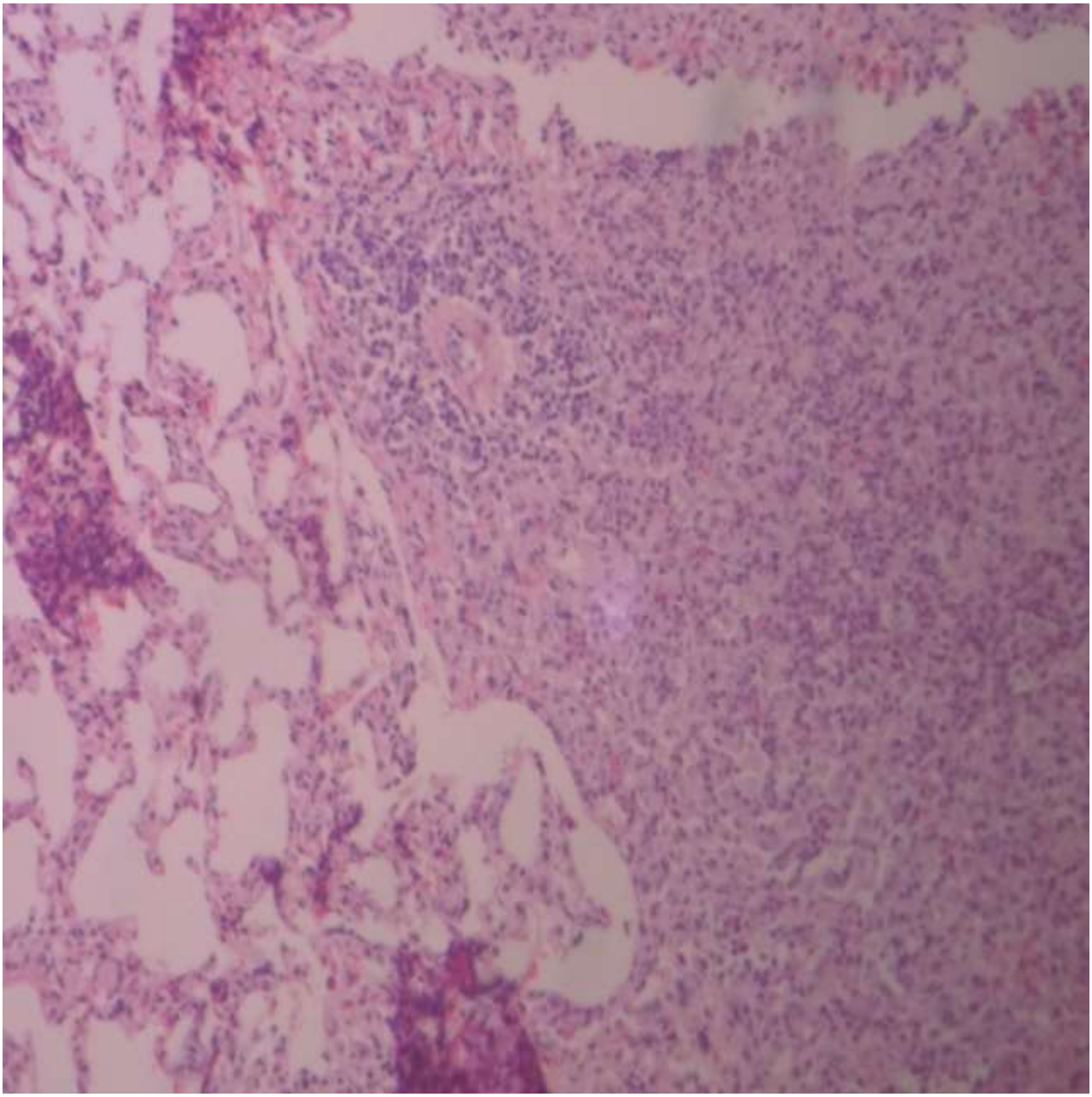

Plate 18: Photomicrograph of lungs of rats fed with fishes from Carter Site showing interstitial inflammatory infiltrate and pneumonia (x400)

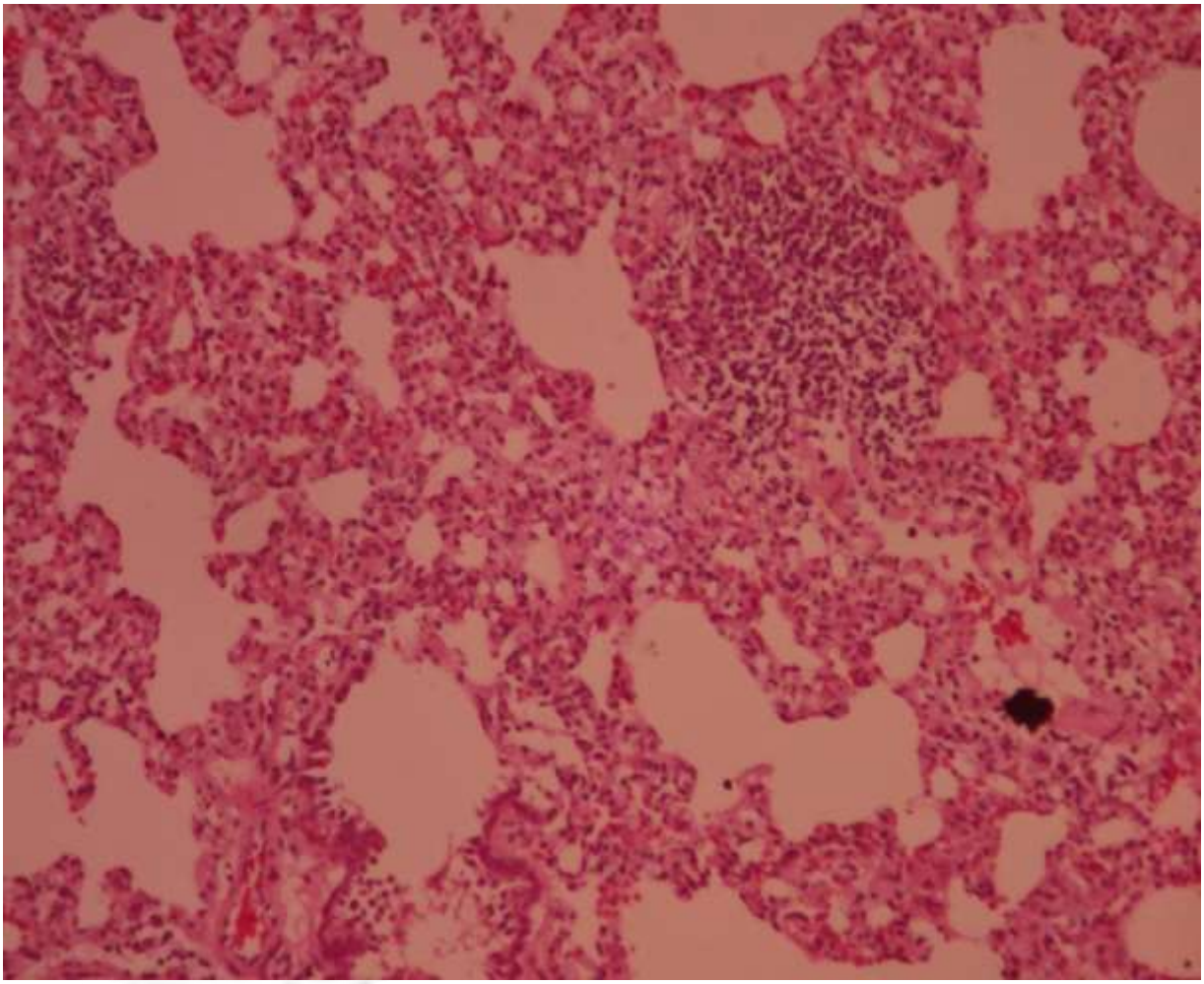

Plate 19: Photomicrograph of lungs of rats fed with fishes from Makoko Site showing no abnormality (x400)

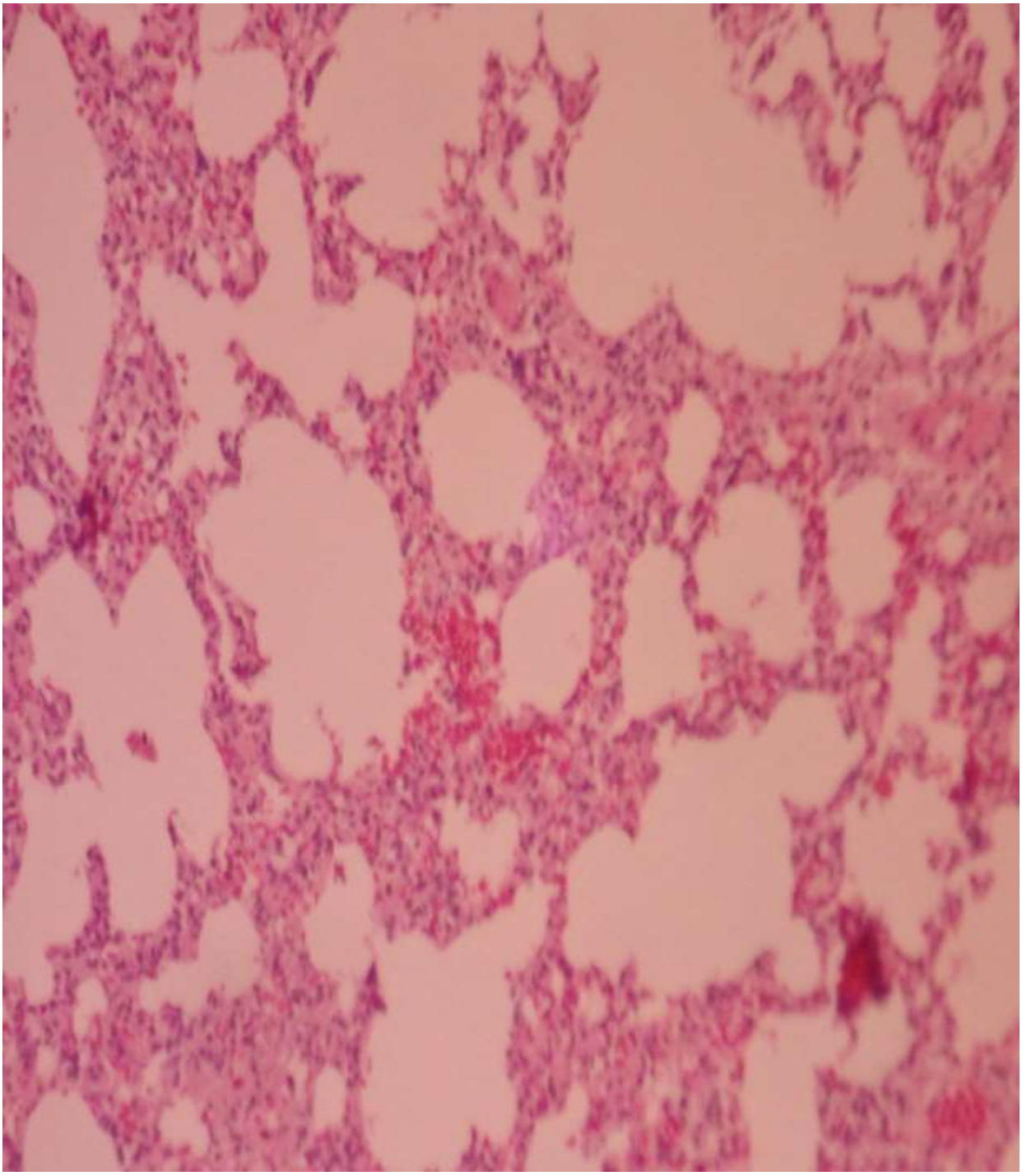

Plate 20: Photomicrograph of lungs of rats from the control group showing no abnormality (x400)

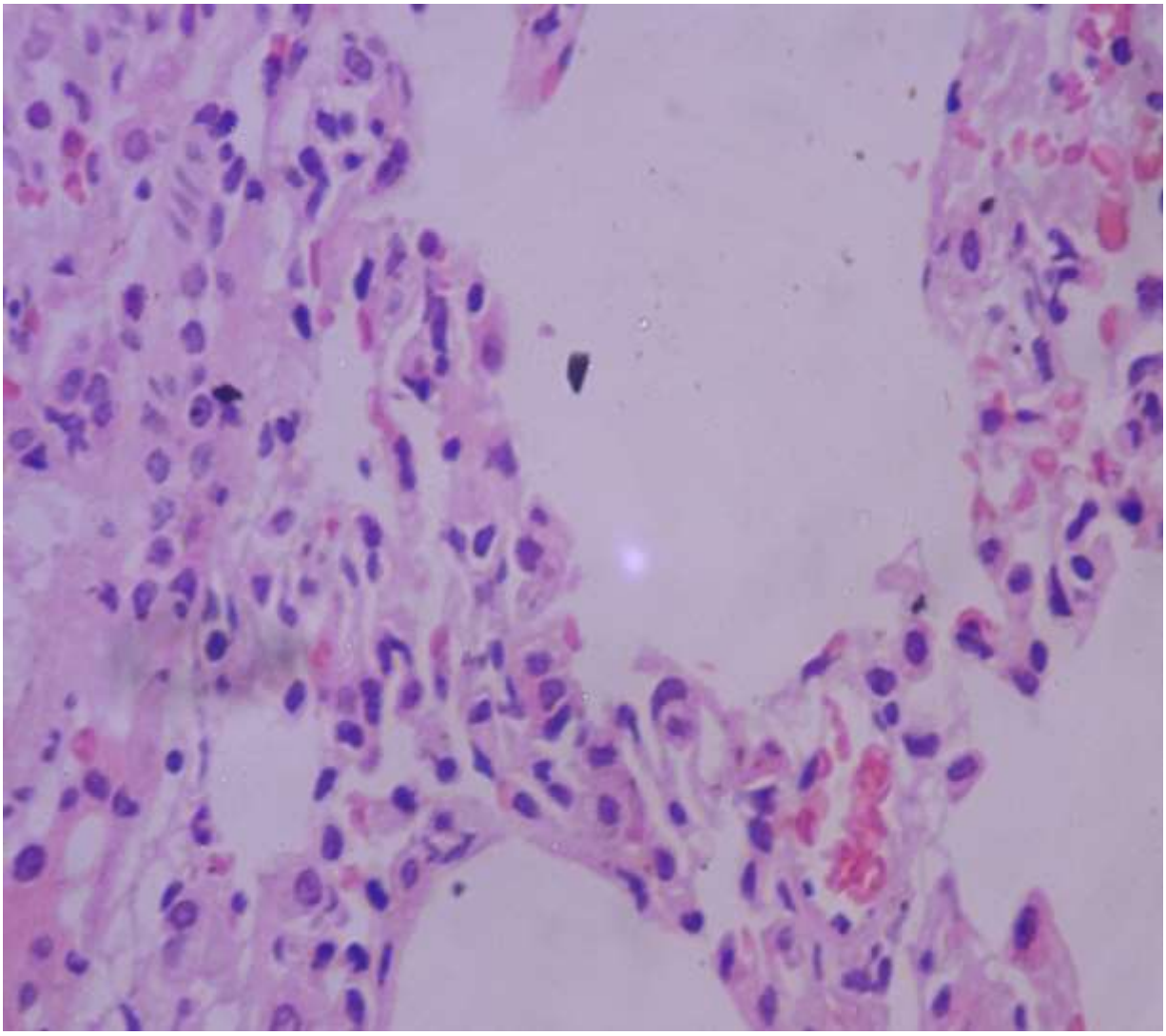

Plate 21: Photomicrograph of lungs of rats fed with fishes and zinc supplements showing no abnormalities (x400)

CHAPTER FIVE

5.0 DISCUSSION

5.1 PHASE ONE

5.1.1 Heavy metals in water and fishes

In the Western part of the country, especially Lagos State, there are numerous industries. Effluents from these industries greatly distress the geochemistry of the soil. The discharged chemicals interact with ground water and alter the pH and other water quality parameters (Nayek *et al.*, 2010; Saeed *et al.*, 2013). Of the five sites studied in Lagos State, pH values ranged from 5.7 to 9.7. Total solids, total dissolved solids (TDS) and total soluble solids were all within USEPA range. This compares favourably with a study, carried out by Shyamala *et al.*, (2008), in India where they reported pH values from 6.5 to 9.5. pH is considered as an important ecological factor and provides an important piece of information in many types of geochemical equilibrium or solubility calculation. pH is an important parameter in water body since most of the aquatic organisms are adapted to an average pH and do not withstand abrupt changes. The pH values obtained in this study fluctuated between 5.7 and 9.7. It showed an alkaline trend. The maximum permissible level of alkalinity in rivers is 600mg/L (US Environmental Protection Agency,1993a).

The mean value of alkalinity in the sites studied is 302mg/L. The value of alkalinity in water provides an idea of natural salts present in water (Shyamala *et al.*, 2008). The cause of alkalinity

is the minerals dissolved in water from soil. The various ionic species that contribute to alkalinity include bicarbonate, hydroxide, phosphate, borate and organic acids. These factors are characteristics of the source of water and natural processes taking place at any given time. Discharge of waste water into rivers tends to increase the alkalinity. The desirable limit of TDS is 500mg/L. High TDS in water may be due to pollution when waste waters from industries are discharged into pits, ponds, rivers and lagoons (Conso, 2000). TDS values observed in all the sites were below the desirable limit.

The variability in metal concentration of marine organisms depends on many factors such as, the position of the fish in the food chain, size, age and characteristics kinetics for elements and their biological half time (Cogun *et al.*, 2006). Pollution accumulated in marine organisms is transferred to man through the food chain (Cogun *et al.*, 2006; Uluozlu *et al.*, 2007). Marine organisms have been recognized as a useful tool for the monitoring of the environment. According to the USEPA (2010), the maximum contaminant levels (MCls) in fishes are 0.01mg/g for cadmium, 0.05mg/g for lead and 0.01mg/g chromium. In this study, lead concentration found in *Tilapia fish* ranged from 0.31mg/g to 0.65mg/g. These values were above the MCL value of the USEPA. Cadmium concentrations found in the fish ranged from 0.23mg/g to 0.90mg/g while chromium concentrations ranged from 0.02mg/g to 0.01mg/g. These values are above the MCL values of the USEPA.

Olowu *et al.*, (2010), determined the heavy metal contents of Tilapia fish obtained from Epe and Badagry Lagoons. It was reported that iron was found mostly abundant in the fish and it ranged from 0.11-6.5µg/g in Epe lagoon and 0.20-8.40µg/g in Badagry lagoon. These values are higher than the iron levels found in this study. Jimoh *et al.*, (2011), examined the concentration of heavy metals in *Macrobrachium vollenhovenii* (herklots) obtained from the Epe lagoon in

Lagos, Nigeria. It was noted that the range of concentrations of copper (9.63-13.56µg/g) recorded in their study was lower than the range of values (copper= 860-1620µg\g) reported by Anetekhai *et al.* (2007) in *M. vollenhovenii* obtained from Ologe lagoon, Lagos,Nigeria. This may be due to the greater metal load in Ologe lagoon because of the presence of Agbara Industrial Estate which discharges its waste into the lagoon (Kusemiju *et al.,* 2001). Values obtained for copper from Ologe lagoon is higher than values obtained for copper in this study. The range of values of copper (9.63-13.56µg/g), and lead (0.45-1.56µg/g) in *M. vollenhovenii* recorded in Jimoh *et al.,* 2011, was lower than the values of these metals reported in *M.vollenhovenii* in previous studies. Abulude *et al.* (2006) reported 2400 and 1300µg/g for copper and lead respectively in *M.vollenhovenii* from the coastal waters of Ondo state, Nigeria; Banjo *et al.* (2010) reported copper (150µg/g) and lead (50µg/g) in *M. vollenhovenii* from different markets in southwest Nigeria. These low concentrations of metals in Epe lagoon might be due to less agro-chemical usage by farmers around Epe town as a whole, which are the major sources of heavy metal contaminants in aquatic environment (Banjo et al, 2010).

According to Ekeanyanwu *et al.* (2011), tilapia fish examined from Okumeshi River in Delta state contained higher concentration of manganese with a value of 7.77mg/kg against 0.13mg/l in water. Studies on the different parts of the fish revealed higher concentrations of 1.97mg/kg manganese in the muscle of tilapia fish. Concentration of 0.62mg/kg cadmium was detected in the muscle of tilapia while a low concentration of 0.04mg/kg was recorded in tilapia bone. In most of the fish samples, cadmium concentration was found to be above the maximum tolerable values provided by international institutions. These values are above the concentrations of manganese and cadmium obtained in this study. Doherty *et al.* (2010) looked at heavy metals in fishes as an indicator of environmental pollution in Lagos, Nigeria. From the results, copper and

lead were highly accumulated in the flesh of *Chrysichthys nigrodigatatus* and *Tilapia guineensis* from the Lagoon. The values of copper (3.92ppm) and lead (0.44ppm) are above the statutory limit set by WHO and FAO. In Turkey, 5 heavy metal concentrations in liver and muscle tissues of *Mugil auratus* were investigated. Chromium and nickel concentration in all tissues were found below detection limits of 0.05 and 0.1µg/g dry weight respectively. Cu, Pb and Cd were detected as mg/kg dry weight within the limits 0.49 -1.30, 0.60 -1.21 and 0.15 0.50 in liver tissue and 0.30 -1.00, 0.57 – 1.12 and 0.10-0.40 in muscle tissues respectively (Mercola, 2002). Pb and Cd concentrations found for *Tilapia Orechromis* in this study were higher than the results of that study. In a previous study, carried out in Turkey, the cadmium levels in liver and muscle tissues of *Sparus aurata* and *Mullus barbatus*, the mean cadmium levels of muscle and liver tissues estimated as mg/kg, dry weight were 3.5 and 5.22 in *S. aurata*; 4.78 and 5.27 in *M. barbatus* respectively (Kaley *et al.*, 2004). The cadmium levels of *T. orechromis* found in this present study were higher than the results of that study.

In China, Liang *et al.* (2004) have reported the values of six heavy metals investigated in veined rapa whelk. The concentrations were (wet weight); 0.05-0.39mg/kg for Co, 0.09-0.66mg/kg for Ni, 0.15-30.61mg/kg for Cd, 0.09-0.75mg/kg for Pb. The cadmium level and lead of fish in this study was found to be higher than the results of that study. On the east coast of the middle Adriatic, heavy metal concentrations in soft tissue of *Mytilus galloprovincialis* were investigated. Measured concentrations were found to be 2-7mg/kg for Pb, 1-2.9mg/kg for Cr; 2-13mg/kg for Mn; 53.4-719mg/kg for Fe; 3.7-11.1mg/kg for Cu (Orescanin *et al.*, 2006). Lead, chromium, manganese and copper concentrations of tilapia fish sample in this study were found to be higher than the result of that study. Heavy metal concentration of marine organisms depends on age,

environment and feeding behavior. Heavy metal concentrations are higher in internal rivers than open seas (Filazi *et al.,* 2003; Adeosun *et al.,* 2015).

5.2 PHASE TWO

5.2.1 Effect of the consumption of heavy metal contaminated fish

Blood lead concentration (Pb-B) at low levels has been cited as causing several retarded conditions in children, among them is growth retardation. To test the accuracy of this observation, the height and weight data of lead poisoned patients (Pb-B, 4.82-22.73µmgl/L) were examined by Sachs and Moel, (1989). While investigating dietary intake, Sachs and Moel in 1989 obtained a height mean of 32 for subjects with a Pb-B of 2.41 to 3.84µmol/L and a height mean of 41 for controls with a Pb-B of 0.48 to 1.120µmol/L. These resports would have been skewed toward short stature if expressed as a mean. Schwartz *et al.* (1986) deduced from their analysis of National Health and Nutrition Examination survey that stature is inversely correlated to blood lead values and Pb-Bs of 0.24 to 1.68µmol/L are statistically significant predictors of children's height, weight and chest circumference. Ronis *et al.* (2001) deduced from their analysis of the skeletal effects of developmental lead exposure in rats that lead exposure reduced somatic growth, longitudinal bone growth and bone strength. Lead has also been reported to cause anorexia among other symptoms in a painter with chronic lead toxicity (Hu, 2001). These findings are in agreement with the findings of this study which found a decrease in weight of the rabbits fed the heavy metal contaminated fish diet. Fujiwara (1980) found remarkable changes in tissues of cadmium treated rats. There was a whitish-yellow colour change of kidneys and considerable degeneration.

According to Morava and Gergely (1980), lead in the kidney reduced the re-absorption of amino-acids, sugars, phosphate and citrate. Cadmium accumulation in the kidney of mammals leads to proteinura, aminoaciduria, glycosuria and hypertension (Castro-Gonzalez and Mendez-Armenta, 2008; Morava and Gergelry, 1980). In this study, there was a great physical degeneration and reduction in weight of the lung, liver, kidney and heart. Also the concentrations of protein in these organs were greatly increased when compared to the control. These findings were in agreement with the results of Fujuwara, (1980) and Morava and Gergely, (1980). Heavy metals have also been shown to cause diabetes. In this study, serum glucose in the sample groups was elevated when compared to the control group.

Contamination of water by heavy metals due to industrial discharges, mining operations or mobilizations of naturally occurring metals in sedimentary aquifers has been reported in China, USA, Utah, Chile, Argentina and Tokyo (Mazumder *et al.*, 1992). Between 1983 and 1985, fourteen villages in South Bengal were affected by chromic heavy metal toxicity. A high level of heavy metal was detected in the water from shallow tube wells. Twenty patients were investigated clinically. The liver biopsy showed moderate portal zone fibrosis with splenomegaly. The patients had jaundice, ascites, low serum albumin, elevated globulin, elevated Serum aspartate aminotransferase, elevated serum alanine aminotransferase and also elevated alkaline phosphatase. A patient died of hepatic encephalopathy after two months of hospitalization while another developed lung abscess. Neuropathic changes were observed in 65% of patients by electromyography (Mazumder *et al.*, 1992; Holta 1989; Chakraborti *et al.*, 1987; Mazumder, 1986; Southunuck, 1983).These findings are in line with this present study which found low serum albumin, elevated aspartate aminotransferase, elevated alanine amino

transferase, and also elevated alkaline phosphatase in rabbits fed the fish diet, which may suggest liver damage (Obianime *et al,* 2013).

5.2.2 Effect on hematological parameters:

The concentration of hemoglobin decreased significantly ($p<0.01$) in the blood of rats exposed to the fish diet. Heavy metals such as cadmium, chromium and lead have been reported to alter hemoglobin levels by decreasing their affinity towards oxygen and its binding capacity rendering the erythrocytes more fragile and permeable (Adakole, 2012; Adeyemo *et al,* 2010) which probably results in cell swelling deformation and damage. The fish diet induced a mean decrease in the hemoglobin, PCV and RBC levels. The results are in good concurrence with earlier works (Vinodhini and Narayanan, 2009; Mastan *et al.,* 2009; Singh *et al.,* 2008; Vutkuru, 2005) that reported a significant decrease in RBC, hemoglobin levels and packed cell volume of fresh water fish exposed to heavy metals.

The related decreases in the hematological indices implicate the toxic effect of the fish diet that affects both metabolic and hematopoietic activities of the rats. The decrease in hematological indices of the rats could also lead to anaemia. The anaemic condition results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells. Reduction in hemoglobin content in the rats exposed to the fish diet could also be due to the inhibitory effects of the toxic substances responsible for the synthesis of hemoglobin. Joshi *et a.l* (2002) suggested that heavy metals exposure also decreased the RBC, hemoglobin and PCV levels due to impaired intestinal absorption of iron. High white blood cell counts indicate damage due to infection of

body tissues, severe physical stress and as well as leukemia. White blood cell counts were found increased following the fish diet. Similar findings were also documented showing significantly higher levels in fishes exposed to increased copper concentrations (Singh *et al* 2008; Mazon *et al*, 2002).

5.2.3 Effect on the hormonal level:

This study also confirms the fact that heavy metals, together with environmental pollutants are endocrine disruptors since they interfere with the levels of progesterone, lutenizing hormone and follicle stimulating hormone. Hormone disrupting effects of environmental pollutants were noticed in serum chromium levels of 0.03-0.06mg/100ml, serum copper levels of 0.32-0.38mg/100ml; serum chloride levels of 1.60-1.70mg/100ml and serum nitrite levels of 0.11-0.12mg/100ml. These levels were all above the MCL levels of pollutants in the serum of rats and rabbits. As the levels of these environmental pollutants increases, the levels of progesterone decreases. This may lead to early menopausal periods and infertility in females (Laufente *et al.*, 2005; Lafuente and Esquifino, 1998).

The findings in this study further suggests that heavy metals are inversely correlated to progesterone levels. Marie *et al.* (2001) investigated the effects of lead on the system in lead smelter workers. Median levels of lead in plasma ranged from 0.04-3.7µg/dl in the active lead workers. FSH levels were lower in the lead workers than in the control group indicating an effect of lead at the pituitary level. Moderate exposure to lead was associated with minor changes in the male endocrine function particularly affecting the hypothalamic-pituitary axis (Lafuente and Esquifino, 1998; Lafuente *et al.*, 2005;Aspotoli and Catalini, 2011).

This work is in agreement with the present study which found low level of FSH in the rabbits being studied. Lafuente and Esquifino, (1998) found out that cadmium modify amine metabolism at the CNS and pituitary hormone secretions. This xenobiotic is associated with deleterious effects on the gonal function and with changes in the secretory pattern of other pituitary hormones like prolactin, ACTH and TSH. Lafuente *et al*. (2004) analyzed the effects of cadmium on plasma levels of LH, FSH in rats. They found out that the metal exposure abolished the daily pattern of plasma LH levels. In 2005, they looked at the effects of cadmium on prolactin secretion in rats and observed that the cadmium exposed animals exhibited variations in plasma prolactin levels. These findings are in line with this present study which found altered levels in LH and prolactin levels in the experimental animals studied. Some of the most dangerous emerging diseases facing society today are directly related to exposures to deadly environmental toxins. For example, 45 states in U.S.A. have used mercury adversaries from coal-fired plants as well as for nine or more water ways in the US from fish. Exposure to heavy metal toxins such as lead is linked with a 46% increase in the mortality rate according to the Centers for Disease Control in Atlanta, USA.

Heavy metals in the environment have both an economic and medical cost. For example, men in Califonia in fishing industry have sued the state over heavy metal contamination due to their livelihood. Pregnant women are now warned not to eat certain fish such as tuna because of its mercury content (Ellithrope and Settieri, 2009). Some of the most toxic and commonly found heavy metals are mercury, lead, cadmium, chromium. Many of these impact the nervous system and are linked to Alzheimer's and other neurodegenerative diseases, while others can harm the kidneys, immune and cardiovascular systems (Goldberg and Burnett, 2001). Heavy metals accumulate in various tissues and are associated with increases in today's biggest killers:

cardiovascular disease and cancer (Elias, 1985). Reducing these heavy metals from the body has been a challenge to modern day medicine. Treatment consists of measuring via blood test, levels of heavy metals in the body, then attempt to remove them from blood using expensive methods. Thus instead of focusing on crisis management and a removal of heavy metals from blood and tissues, there is a need to concentrate on prevention (Goldberg and Burnett, 2001).

5.3 PHASE THREE

5.3.1 Effect of zinc supplementation

From phase 3 of the study, it was observed that the rabbits fed with zinc supplements had an increase in the progesterone and also an increase in body weight as evident from the increase in their feeding pattern. From this study, it can be seen that the zinc supplements do reverse the heavy metal effects in the rabbits. They also increase the production of progesterone thereby enhancing ovulation in female rabbits. The supplementation of zinc boosted the hematological profile of the rats, increased HDL, catalase and glutathione levels, decreased serum glucose levels and also reduced aberrations in the tissues of the animal models. With zinc supplements, adverse effects from the consumption of heavy metal contaminated fish can actually be managed. This also suggests the fact that zinc supplement enhances ovulation as evident from the increase in progesterone levels.

Results showed that the administration of the fish diet produced a marked decrease of liver, heart, lung, kidney and ovary weight. This is in accordance with previous data indicating weight loss in testes, seminal vesicles and epididymis accompanied by a loss of reproductive capacity in rats exposed to cadmium (Saygi *et al.,* 1991). Many possible cellular mechanisms have been hypothesized to

explain heavy metal toxicity, but oxidative stress is a major process responsible for triggering cytotoxicity pathways that lead to the generation of reactive oxygen species ROS (Pinheiro *et al.*, 2008). Khan *et al.* (2008) found that lead induced oxidative stress in exposed human populations. A similar finding was also reported for cadmium (Chater *et al.*, 2008). Interactions between metal ions such as iron, cadmium, chromium or copper could affect the generation of reactive oxygen species. Cadmium, copper and iron have been reported to induce the production of reactive oxygen species in eukaryotic systems (Ghio *et al.*, 2002; Radetski *et al.*, 2004). Zinc is an antioxidant and may prevent the formation of reactive oxygen species and peroxidation in the blood samples. This study found a reduction in the levels of catalase and glutathione in the serum of the experimental animals, but with the addition of zinc in the diet, their levels were increased significantly.

5.4 Contributions to Knowledge

The study was able to establish that:

1. Consumption of the heavy metal contaminated Tilapia fish obtained from Makoko and Carter Bridge market caused severe alterations in biochemical, haematological and oxidative parameters in the experimental animals.

2. It also caused aberrations in the histopathological profile of the tissues of the experimental animals and disrupted their hormonal milieu.

3. Supplementation with dietary zinc effectively ameriolates the deleterious effects of the consumption of heavy metal contaminated aquatic food.

5.6 Conclusion:

The sources of aquatic protein (Tilapia fish) in the Carter Bridge and Makoko Markets are highly contaminated with heavy metals from industrial effluents. Consumption of this fish produces negative metabolic changes. Dietary zinc is effective in ameliorating the biochemical and histomorphology following long term administration.

It is recommended that dietary supplements of zinc be taken daily as prophylactic and therapeutic management of effects of consumption of heavy metal contaminated fish.

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