ECOTOXICITY ASSESSMENT OF NON-ENZYMATIC ANTIOXIDANTS PRODUCTION BY *CLARIAS GARIEPINUS* (BURCHELL, 1822) IN RIVER GALMA, ZARIA, KADUNA STATE, NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA, FACULTY OF LIFE SCIENCES, DEPARTMENT OF BIOLOGICAL SCIENCE,
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Declaration

I declare that the work in this thesis entitled Ecotoxicity Assessment of Non-Enzymatic Antioxidant Production By *Clarias gariepinus* (Burchell, 1822) In River Galma, Zaria, Kaduna State, Nigeria has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Ozovehe Patrick SAMUEL _____ _____

Signature Date

Certification

This dissertation entitled **"Ecotoxicity Assessment of Non-Enzymatic Antioxidant Production By *Clarias gariepinus* (Burchell, 1822) In River Galma, Zaria, Kaduna State, Nigeria"** by Patrick Ozovehe SAMUEL meets the regulations governing the award of the degree of Master Degree of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This research is dedicated to God Almighty for his un-quantifiable and abundant blessings. It is also dedicated to my daughter, Claire Onyinoyi Patrick.

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Abstract

Ecotoxicity assessment of non-enzymatic antioxidant production by *Clarias gariepinus* in River Galma were investigated in March and August, 2015. 120 specimens (60 per exposure) of 20-45g size range of juveniles were exposed for 14 days in a cage system at five different locations along the river course in an *in situ* bioassay in River Galma. Water samples for physico-chemical determination were collected from the same stations over a period of six months (March - August, 2015). The non-enzymatic antioxidants, Glutathione, Vitamins E and C produced in the fish as well as the histopathology of the organs (liver and kidney) were assayed for following standard methods. Heavy metal (Pb, Cr, Cd, Zn and Mn) concentrations were tested for in digested pooled samples of water, livers and gills of the fish. It showed that the physico-chemical parameters had significant differences ($P<0.05$) in DO (1.43±0.08mg/L to 3.67±0.4mg/L). This was important in generation of reactive oxygen species (ROS) in the environment of the fish culminating in the various antioxidant responses obtained. Significant differences in BOD (1.13±0.12mg/L to 5.43±0.45 mg/L), sulphate (0.02±0.00mg/L) to 0.65±0.07mg/mL), nitrate (0.05±0.00mg/L to 0.38±0.02mg/L), phosphate-phosphorus (0.03±0.01mg/L to 0.46±0.03mg/L) were indicative of the presence of organic pollutants in River Galma. pH (4.45±0.13 to 8.72±0.05) indicated slightly alkaline and acidic environment. Other parameters that were significant include Hardness of water, alkalinity, total dissolved solids, water temperature and electrical conductivity. The glutathione concentrations showed significant differences in the kidney of the fish (19.50±8.60µg (station 1) to 98.51±35.52µg (station 5)). There was also significant difference in the glutathione concentrations of the gill samples (66.78±12.76µg/mL to 121.85±19.16µg/mL). Bio-assay of glutathione in kidney and gill of *Clarias gariepinus* offered good prospect for *in situ* determination of effects of pollution in River Galma. Vitamin C concentrations showed significant difference in the gills (3.67±3.11mg/mL (station 4) to 8.46±8.46 mg/mL (station 1)) and in the liver

(1.08±0.79mg/mL (Station 5) to 6.83±6.30mg/mL (station 4)) of the fish exposed. Mean values of vitamin E production in the gills and livers of the fish ranged from 14.72±14.72mg/mL (station 1) to 68.08±34.27mg/mL (station 5) and 22.26±22.26mg/mL (station 1) to 47.72±10.52mg/mL (station 4) respectively. Vitamin C offers a good prospect as biomarker of oxidative stress than vitamin E. Heavy metal concentrations of water samples of River Galma and samples of organs of fish exposed in the river had significant differences in the zinc and manganese concentrations in all the exposures. Manganese (11.43±0.56mg/L in water samples) and chromium (0.379±0.29 to 0.834±0.17mg/L in fish organs) exceeded the set limits of 2.0-9.0mg/day wet weight and 0.05-0.15 mg/ kg respectively. This therefore, calls for ameliorative actions by relevant authority. Stations 2 and 4 were the most polluted sites along River Galma in terms of DO with the lowest concentrations of 0.8mg/L and 1.3mg/L in stations 2 and 4 respectively. Likewise, the lowest pH values were 4.38 and 4.27 in stations 2 and 4 respectively. Highest values of 140ppm and164ppm were also obtained in stations 2 and 4 respectively. This was also evident in the severe histopathological damages in the kidneys of the fish exposed at these stations. The histopathology of the liver and kidney of *Clarias gariepinus* exposed to River Galma indicated various changes in the architecture. These include vacoulation, constriction, and aggregation of cells, hypertrophy, necrosis and infiltration to congestion of blood vessels.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Ecotoxicology is the study of the effects of toxic chemicals on biological organisms at the population, community and ecosystem levels. It is a multidisciplinary field of study that integrates both toxicology and ecology in evaluation of organisms. The goal of ecotoxicology is the protection of structure and functions of biological communities and ecosystems. Aquatic toxicity refers to the intrinsic property of a substance to elicit detrimental effect on an organism in short-term and/or long-term exposure to that substance. There are two types of aquatic toxicity tests: acute and chronic toxicity tests (ECHA, 2012).

The aquatic medium is usually loaded with myriads of foreign organic and inorganic materials from ever increasing anthropogenic activities and influences which may have deleterious effects on the ecosystem and the living biota within it. Municipal discharges, agricultural run-offs and industrial discharges are amongst the major sources of pollutants to the aquatic ecosystems. These varied discharges are obtained from both point and non-point sources which are either discharged directly or indirectly into water or ultimately through run-offs. Organisms in aquatic environments are usually exposed to a complex mixture of chemicals causing multiple damages at the organisms, population and ecosystem levels (Ginebreda *et al*., 2014; Vorosmarty *et al*., 2010).

Any metal or metalloid may be considered a "contaminant" or "pollutant" if it occurs in places where it is unwanted and in concentration that is detrimental to the environment, biota or to humans (Reena *et al*., 2011). The harmful effects on the aquatic ecosystem become evident especially when such discharges are in large quantities and have exceeded certain environmental thresholds such

that they become deleterious to both aquatic organisms and other organisms dependent on them for survival. For instance, the total dissolved solids set limit by NESREA (Muhammed, 2012) is 500ppm. Also, the set limits for the following metals as indicated by WHO (1994) and FEPA (2003) are: Zn, 75 mg /L; Mn, 0.50 mg /L; Pb, 2.0 mg/L; Cr, 0.15 mg/L; Cd, 2.1µg/g for food and fish safety.

Heavy metals are metallic elements which have a high atomic weight and a density much greater (at least 5 times) than water (Das *et al*., 2010). Essential metals like manganese (Mn), zinc (Zn), copper (Cu), iron (Fe) and nickel (Ni) play a major role in the biological activities of aquatic organisms, and non-essential metals like cadmium, lead and arsenic are the most toxic elements. Even essential metals may be toxic to the biological activities of organisms above certain concentrations (Merciai *et al*., 2014). The non-essential metals (e.g aluminum (Al), cadmium (Cd), mercury (Hg), tin (Sn) and lead (Pb)) have no proven biological function (also called xenobiotics or foreign elements), and their toxicity rises with increasing concentrations (Sfakianakis *et al*., 2015). Heavy metals can be taken up into fish either from ingestion of contaminated food via the alimentary tract or through the gills and skin (Sfakianakis *et al*., 2015).

Antioxidants comprises of enzymatic and non-enzymatic types. The enzymatic antioxidants are commonly represented by super oxide dismustase (SOD), catalase (CAT), Gutathione transferase (GST), acetyl cholinesterase, etc. While non-enzymatic antioxidants are commonly represented by reduced glutathione (GSH), metallothioneine (MT), uric acid, polyphenols, vitamins A, C and E, etc. These antioxidants are known to exhibit varying roles and functions in counteracting the effects of oxidative stress in the environment of organisms. Antioxidant enzymes are crucial in their effort to decrease oxidative stress produced by exposure to toxicants (Saglam *et al*., 2014).

Non-enzymatic antioxidants such as vitamin E (VE) and vitamin C (VC) can also act to overcome oxidative stress, being a part of the total antioxidant system. They prevent the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues. The main biological function of vitamin E is its direct influence on cellular responses to oxidative stress through modulation of signal transduction pathway (Pratt *et al*., 2010). Vitamins E and C supplementation can induce protective effects on certain conditions after free radical-mediated cellular damage or disruption (Yolanda and Maria, 2012). Vitamin E (α-tocopherol) is a fat soluble antioxidant that inhibits the production of reactive oxygen species formed when fat undergoes oxidation. Vitamin E is linked to regulation of various diseases like cancer, atherosclerosis, hypertension, male infertility etc. Antioxidants (both enzymatic and non enzymatic) provide protection against deleterious metal-mediated free radical attacks.

Fishes and other aquatic organisms are sensitive to stress caused by metals and other toxicants. They also possess natural molecules like vitamins and other antioxidants that are involved in scavenging these pollutants, but when the toxicants concentrations outweigh the aquatic organisms' immune ability, it results to stress, injury and low or diminishing levels of these antioxidants (Ozden and Mustafa, 2010; Yildirin *et al*., 2011). Environmental stress has been demonstrated to cause an increase in the oxidative stress, an imbalance in the antioxidant status (Yildirin *et al*., 2010). There is substantial evidence that environmental pollution increases oxidative stress (Olivia *et al*., 2012). Environmental stress as well as variety of physical conditions may lead to the production of certain protein in fish. Some of these proteins are capable of protecting the cells against damages that may result from such environmental perturbations; while others are involved in the regulation of various genes. Cellular antioxidant defense systems in biological systems are impaired when exposed to environmental pollutants, but the levels of

antioxidants in living organisms can increase in order to restore the imbalance caused by oxidative damage. Oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms (Nishida, 2011).

The use of biomarkers has become an important tool for modern environmental assessment as they can help to predict pollutants involved in the monitoring programme. Monitoring changes in biochemical parameters may provide early warning indicators of general as well as specific toxicological responses. Biochemical biomarkers of contamination are important indices used in fish toxicity tests and for field monitoring of aquatic pollution (Haluzova *et al*., 2011). The use of biomarkers has the advantage of ensuring early detection of potentially deleterious xenobiotics that are present in the ecosystem before it is bio-accumulated to the level that would overwhelm the defence system of the organisms. This is important because, recent reviews have demonstrated the low predictive value of preclinical testing in identifying novel pharmaceutics likely to have therapeutic benefits, as well as in detecting potential adverse effects early in drug development (Krauth *et al*., 2014).

Bioaccumulation potentials of aquatic organisms permit the concentration of these contaminants to levels greater than that in the ambient environment. This bioaccumulation tendency of heavy metals in fish and other biota usually results in myriads of physiological damages to the organism so affected. Metal bioaccumulation is a major route, through which increased levels of the pollutants are transferred across the food chain and finally assimilated by human consumers resulting in health risks (Ambedkar and Muniyan, 2011). Among the feasible organisms actually chosen for pollution monitoring, aquatic algae, molluscs and fishes are the organisms mostly used. Various organs and tissues of fish have been implicated in the bio-accumulation of heavy metals.

Some of these organs and tissues that have been investigated include liver, kidney, gills, muscle, skin, bile, heart, etc.

The effects of pollutants can also be seen in the histopathological alterations of the tissues of organs in the polluted environment. This is because metal accumulates in kidneys (and other organs of fish), damaging filtering mechanisms and affecting structure and ultra-structure, depending on the exposure time and dose (Costa *et al*., 2013). For instance, lead acetate was found to induce cellular damage and some structural changes were observed in different organs, especially in the gills of the fishes (Kilikdar *et al*., 2011).

The physico-chemical parameters of the river determine its quality and suitability for both humans and survival of the living biota. Bellingham (2012) stated that, in order to mitigate the impact human societies have on natural waters, it is becoming increasingly important to implement comprehensive monitoring regimes which will quantify water quality, identify impairments and help policy makers make land use decisions that will not only preserve natural areas, but improve the quality of life. This is because maximum productivity of aquatic biota depends on optimum level of physicochemical parameters (Sadia *et al*., 2013).

1.2 Statement of Research Problem

High anthropogenic activities due to increase in human population have imposed various threats to aquatic biota with reference to heavy metals and their bioaccumulation potentials (Zeng *et al*., 2013). River Galma, being an urban river, has various types of anthropogenic activities along its banks. Among various causes of fresh water and river pollution, heavy metals are of considerable importance (Sthanadar *et al*., 2013). High concentration of metal is not a necessity to produce toxic

effect in the fish's body (Edward *et al*., 2013). This is because heavy metal accumulation could occur in animal body tissues gradually and, overtime, can reach toxic concentration levels, greater than permissible limits (Suruchi and Paul, 2011).

Researches dealing with the use of non-enzymatic antioxidants as biomarkers of oxidative stress are relatively scarce. Routine check on fish to ensure that it is safe for human consumption at all time is necessary (Christopher *et al*., 2011). This paucity of knowledge in the use of vitamins C and E, reduced glutathione which are known to play crucial roles in heavy metals detoxification especially in polluted aquatic ecosystem (Saliu *et al*., 2014; Thakur and Kanshere, 2014) like River Galma calls for concern.

The River Galma is currently loaded with run-offs from agricultural and municipal activities of the surrounding communities along its course at various adjoining tributaries (most of which are seasonal); and contain myriads of toxic pollutants (heavy metals inclusive) (Samuel *et al*., 2015). There may also be contribution of toxic pollutants from the few industrial activities in Chikaji and Dakace areas. Thus, this constantly alters the physico-chemical parameters of the river and consequently, biochemical and physiological parameters of the biota.

1.3 Justification

Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health (Zaki *et al*., 2014). Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans (El-Shehawi *et al*., 2013). Thus, the choice of the test fish, *Clarias gariepinus* as a biomarker of oxidative stress in River Galma. The knowledge

of the levels of heavy elements in our environment is necessary for the purposes of setting background values of these elements, routine monitoring of metal accumulation in the biota and estimating the amount of the metals that may possibly get trans-located across the compartments in aquatic ecosystem (Oyekunle *et al*., 2012). River Galma receives variable levels of pollution from different anthropogenic activities along its banks (Butu and Bichi, 2013). The sources of pollutants and xenobiotics to this river are majorly obtained from agricultural and municipal discharges, run-offs from the neighbouring towns and villages along its course. Thus, the use of *Clarias gariepinus* in *in-situ* bioassay in this investigation.

Production of free radicals and reactive oxygen species as a result of oxidative stress usually lead to increased exploitation of non-enzymatic antioxidants such as reduced glutathione, vitamins C and E (Rajagopalan *et al*., 2010). Vitamin C is known to have crucial role in the immunological and antioxidant properties of vertebrates capable of maintaining the integrity, fluidity of membranes and capable of controlling the oxidizing reactions of fatty acids, thus keeping cellular respiration and avoiding cell death (Abdel-Warith *et al*., 2011). Also, vitamin administration is known to be effective in improving the physiological and histological integrity in fishes in polluted aquatic environment (Kumar *et al*., 2014). Combined approach using physico-chemical analyses and bioassays (of reduced glutathione, vitamin C and vitamin E as non-enzymatic antioxidants) for ecotoxicological testing would be extremely important to hazard/risk assessment of polluted aquatic ecosystem (Lai, 2013). Thus, this research has employed these methods in assessing the effects of oxidative stress generated by the presence of xenobiotics on the physiology of fish as biomarkers of aquatic pollutants in River Galma.

1.4 Aim

The aim of this investigation is to assess *in situ* non-enzymatic antioxidant production by *Clarias gariepinus* in River Galma as biomarkers for pollutants induced stress.

1.5 Objectives of the study

The set objectives for the study were to determine the:

i. temporal and spatial variations in physico-chemical parameters and heavy metals (lead, cadmium, zinc, manganese and chromium) concentrations of River Galma;
ii. level of heavy metals in the gills and liver of *Clarias gariepinus* after a 14-day *in-situ* bioassay on River Galma;
iii. histopathological changes of the kidney and liver of *Clarias gariepinus* after a 14-day *in-situ* bioassay on River Galma;
iv. production levels of non-enzymatic antioxidants in *Clarias gariepinus* due to a 14-day *in-situ* bioassay on River Galma.

1.6: Hypotheses

The hypotheses of the study include the following:

i. temporal and spatial variations in physico-chemical parameters and heavy metal concentrations of River Galma are not within accepted limits.

ii. there are no significant changes in the levels of heavy metals in the kidney and liver of *Clarias gariepinus* in a 14-day *in-situ* bioassay on River Galma.

iii. there are no significant histopathological changes in the kidney and liver of *Clarias gariepinus* due to a 14-day *in-situ* bioassay on River Galma.

iv. significant levels of non-enzymatic antioxidants are not produced in *Clarias gariepinus* due to a 14-day *in-situ* bioassay on River Galma.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ecotoxicity and Pollution Management

The term "ecotoxicology" was first coined by Truhaut (1977) who defined it as the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animals, vegetable and microbial, in an integral context. Aquatic toxicology is a multidisciplinary field of study of the effects of manufactured chemicals and other anthropogenic and natural materials and activities on aquatic organisms at various levels of organization, from sub-cellular through individual organisms to communities and ecosystems. Recent studies have been geared towards mitigating the effects of pollution on the biota within aquatic medium. Various organisms have been employed in the ecotoxicological management of pollutants in the aquatic ecosystem especially organisms that live within them and are capable of bio-accumulating the pollutants. This is why evaluating effects on the competence of the fish immune system is important in ecotoxicology (Bols *et al*., 2001).

Many ecotoxicological studies involve pollutants such as heavy metals, pesticides, insecticides and other pollutants; and these come from various sources that finally get to the aquatic ecosystem. Herbicides may enter into the aquatic environment by direct application to attack a particular pest or indirectly through atmospheric precipitation and surface run-offs (Yaji and Auta, 2007). Doherty *et al*. (2010) discussed the role played by some heavy metals in the flesh of some catfishes and tilapias in Nigeria and demonstrated that alteration in the antioxidant enzymes and induction of lipid peroxidation reflects the presence of heavy metals which may cause oxidative stress in fishes and provide a rational use of biomarkers of oxidative stress in bio-monitoring of aquatic

pollution. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms (Storelli *et al*., 2005).

2.2 Effects of Heavy Metals

Heavy metals (such as copper, cobalt, chromium, cadmium, iron, zinc, lead, tin, manganese, nickel, molybdenum, vanadium) are a group of elements with a mass density greater than 4.5g/cm^3 which tend to release electrons in chemical reactions and form simple cations. Metalloids (transitional elements) are antimony, arsenic, astatine, boron, germanium, silicon, tellurium, selenium, among others. Heavy metals are persistent inorganic pollutants of the environment. For instance, Edward *et al*. (2013) observed that though, heavy metals (such as Zn, Mn, Cu, Fe, Pb and Cd) were below tolerable level in the water samples, the levels of bioacumulation in fish parts examined (gill, flesh, kidney and liver) were beyond tolerable levels (WHO and FEPA recommendations) making the fishes unfit for human consumption. Some heavy metals are completely toxic and need to be monitored continuously in the bodies of organisms as they are capable of bioaccumulation, resulting to morbidity and often mortality of the organisms (Ayotunde *et al*., 2011). UNICEF/WHO (2012) observed that close to a billion people most living in the developing world do not have access to safe and adequate water. Most water sources in developing countries are polluted by both organic and chemical pollutants which include heavy elements (Haylamicheal and Moges, 2012).

2.2.1 Manganese

Manganese (Mn) is the 12th most abundant element in the biosphere. It is widely distributed in soil, sediment, water and in biological materials. The element is essential for normal development and body function across the lifespan of all mammals with some 20 identified functions in enzymes and proteins. The role of manganese, as a required co-factor for several enzymes, for example, represents one of the most important functions of this element. Mn contributes to maintain healthy nerves and immune system and helps in blood sugar regulation. It is involved in utilization of Vitamins B1 and E and it is required for normal bone growth and for avoiding blood clotting defects. In mammalian cells, manganese causes DNA damage and chromosome aberrations. Large amounts of manganese affect fertility in mammals and are toxic to the embryo and foetus (Nussey *et al*., 2000). For instance, Rajkowska and Protasowcki (2013) demonstrated the presence of manganese in skin, gills and gonads while zinc was found in the digestive tract and gills.

Although manganese is essential for humans and other species of the animal kingdom as well as for plants, it is toxic at higher levels. In man, chronic manganese excess affects the central nervous system, with the symptoms resembling those of Parkinson's disease. It can also affect the ecosystem negatively, accumulating in the food chain. Excess manganese and iron in drinking water cause staining of kitchen utensils, bath accessories and clothes, as well as a yellowish water appearance and unpleasant taste and odour in food and drinks (Gomez *et al*., 2004).

2.2.2 Cadmium

Cadmium (Cd) is a ubiquitous trace metal, biochemically classified as a non-essential element. The element and its compounds are relatively water soluble compared to other heavy metals. They are therefore, more mobile in the soil and generally more bio-available, and tend to bioaccumulate. Cadmium is a naturally occurring non-essential trace element and its' tendency to bio-accumulate in living organisms often in hazardous levels, raises environmental concern (Kalma *et al*., 2010; Liao *et al*., 2011). Cadmium is a very toxic metal, and also an environmental and industrial pollutant which is present in soil, water, air and food (Kaplan *et al*., 2011).

Chronic manifestations associated with degree of Cd excess include: hypertension, weight loss, microcytic-hypochromic anaemia, lymphocytosis, proteinuria with wasting of beta 2 micro-globulin, emphysema and pulmonary fibrosis (if inhalation was a route of contamination), atherosclerosis, steomalacia and lumbar pain, and peripheral neuropathy (Kazantzis, 2004; Garcia-Rico *et al*., 2007). Acute inhalation of Cd dusts, fumes or soluble salts may produce cough, pneumonitis and fatigue. Also, Roopha and Latha (2013) reported that cadmium exposure induced oxidative stress; delay in sexual maturation and impaired hormones in developing rat ovary. However, manifestations of Cadmium toxicity may be lessened or delayed by an individual's protective and detoxification capacities.

2.2.3 Chromium

Chromium (Cr) is found in rocks, animals, plants, and soil and can be a liquid, solid, or gas. Its compounds bind to soil and are not likely to migrate to ground water but, they are very persistent in sediments and water (WHO, 1998; Louma and Rainbow, 2008). The element is used in metal alloys such as stainless steel; protective coatings on metal (electroplating); magnetic tapes; and

pigments for paints, cement, paper, rubber, composition floor covering and other materials. Its soluble forms are used in wood preservatives.

Chromium (VI) compounds are toxic and known human carcinogens. Where as Cr(III) is an essential nutrient, though breathing high levels can cause irritation to the lining of the nose; nose ulcers; runny nose; and breathing problems, such as asthma, cough, shortness of breath, or wheezing. Skin contact can cause skin ulcers. Allergic reactions consisting of severe redness and swelling of the skin have been noted. Long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation. For instance, a link between DNA damage and concentration of Cr and Mn in soft tissues was observed in mussels in the most polluted site compared to the reference site (Dallas *et al*., 2013). Toxic effects of Cr in fish include: hematological, histological and morphological alterations, inhibition/reduction of growth, production of reactive oxygen species (ROS) and impaired immune function (Reid *et al*., 2011; Vera-Candioti *et al*., 2011).

2.2.4 Lead

As a result of human activities, such as fossil fuel burning, mining, and manufacturing, lead and lead compounds can be found in all parts of our environment. This includes air, soil, and water. The element is used in many different ways. It is used to produce batteries, ammunition, metal products like solder and pipes, and X-ray shielding devices. Lead is a highly toxic metal and, as a result of related health concerns, its use in several products like gasoline, paints, and pipe solder, has been drastically reduced in recent years. The most common sources of lead exposure are lead-based paint and possibly water pipes in older homes, contaminated soil, household dust, drinking water, lead crystal, lead in certain cosmetics and toys, and lead-glazed pottery. Although, Pb is a

naturally occurring substance, its environmental concentrations are significantly increased by anthropogenic sources which include base metal mining, battery manufacturing, Pb-based paints and leaded gasoline (Mager, 2011; Monteiro *et al*., 2011).

Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anaemia. Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage; high level exposure in men can damage the organs responsible for sperm production; absorbed lead is rapidly taken up into blood and soft tissue, followed by a slower redistribution to bone (Ikem and Egiebor, 2005; Garcia -Rico *et al*., 2007). The most susceptible population to lead poisoning is children, particularly soldiers, infants in neonatal periods, and the fetus (Boucher *et al*., 2012).

Lead is a persistent heavy metal which has been characterized as a priority hazardous substance (Sfakianakis *et al*., 2015). In most developed countries, stricter controls have reduced environmental and occupational exposures to lead; however, lead exposure continues to be an issue in developing countries with rapid industrialization, such as China (Gottesfeld and Pokhrel, 2011). Aquatic organisms bio-accumulate Pb from water and diet, although there is evidence that Pb accumulation in fish, is most probably originated from contaminated water rather than diet (Creti *et al*., 2010). For example, Hou *et al*. (2011) monitored the effects of lead on the Chinese sturgeon, *Acipenser sinensis* and observed deformities as body (spinal) curvatures and reduced ability of locomotion and foraging by deformed juveniles. Also, lead poisoning has been documented in six villages in Zamfara State, Nigeria where it claimed 355 people's life across the six villages (Ibrahim and Aliyu, 2010).

2.2.5 Zinc

Zinc (Zn) is the second most abundant trace element after Fe and is an essential trace element and micronutrient in living organisms, found almost in every cell and being involved in nucleic acid synthesis and occurs in many enzymes (Sfakianakis *et al*., 2015). Zn is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of more than 200 enzymes and it plays a role in immune function, wound healing, protein synthesis, DNA synthesis and cell division. The element possesses antioxidant properties, which may protect against accelerated aging and helps speed up the healing process after an injury. The element is a cofactor to more than 300 enzymes involved in important functions such as RNA and DNA metabolism and plays a major role in the stabilization of the structure of a large number of proteins, including signaling enzymes at all levels of cellular signal transduction (Chasapis *et al*., 2012). In addition to this, Zn is involved in more complicated functions, such as the immune system, neuro-transmission and cell signaling (Hogstrand, 2011). Furthermore, application of zinc oxide is known to enhance the healing of chronic and acute wounds and also works as antibacterial and anti-inflammatory agent (Voicu *et al*., 2013).

Although zinc is an essential requirement for good health, excess zinc can be harmful. For instance, Zn was the most accumulated metal, followed by Fe, Cu and Ni, while Pb was the least; and mean of SOD and reduced GSH in the frogs indicate some responses to oxidative stress which varied significantly among sampling areas in the work reported by Taiwo *et al*. (2014). Similarly, highest concentration of the essential metals analysed was found for Zn, especially in fresh and canned mussel in comparison with other essential metals such as copper, manganese and selenium analysed (Olmedo *et al*., 2013).

2.3 Anti-oxidants

Living organisms possess certain biomolecules that protect them against oxidation of their cell molecules due to stress in the environment. These molecules are part of the organisms' strategy to protect self from damage. These biomolecules are generally referred to as antioxidants. Antioxidants are divided into enzymatic and non enzymatic components. These two components work hand in hand to scavenge reactive oxygen species (ROS). The omnipresent nature of both arms of the antioxidant machinery (enzymatic and non-enzymatic) underlies the necessity of detoxification of ROS for cellular survival (Gill *et al*., 2011).

2.3.1 Enzymatic Antioxidants

Enzymatic components include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR), glutathione-S-transferase, glutathione peroxidase, lipid peroxidase, acetylcholinesterase, etc.

2.3.2 Non-enzymatic Antioxidants

Non-enzymatic antioxidants like ascorbic acid (AA), reduced glutathione (GSH), α-tocopherol or vitamin E, carotenoids or vitamin A, flavonoids, metallothionein, uric acid, and the osmolyte proline, endogenous glutathione, and pineal hormone melatonin, have all been shown to be effective in defending the system against free radical mediated tissue injuries. For instance, Osioma *et al*. (2013) observed that blood reduced glutathione was significantly lower in other sites in comparison with the control site (Ethiope River).

2.3.2.1 Glutathione

Glutathione (GSH) is a tripeptide protein made of three amino acids namely cystein, glutamate or glutamic acid and glycine. It was first discovered by J. deRey-Pailhade from human eyeball in 1888. It has many biological roles including protection against reactive oxygen and nitrogen species. As a carrier of an active thiol group in the form of a cysteine residue, it acts as an antioxidant either directly by interacting with reactive oxygen/nitrogen species (ROS and RNS, respectively) and electrophiles or by operating as a co-factor for various enzymes. Reduced glutathione (GSH) is the most abundant cellular thiol and it is involved in several metabolic processes (DeLeve and Kaplowitz, 1991). The chemical structure of GSH determines its potential functions and its broad distribution among all living organisms reflects its important biological role. GSH has been found in all mammalian cells. The reduced glutathione (GSH) is the most important non-enzymatic antioxidant in cell (Sevcikova *et al*., 2011).

2.3.2.2 Vitamins C and E

Ascorbic acid is the most abundant and the most extensively studied antioxidant compound (Hanaa *et al*., 2007). It is considered powerful as it can donate electrons to a wide range of enzymatic and non-enzymatic reactions. However, studies addressing the feasibility of ascorbate (Vitamin C) as a biomarker in fish are very scarce. Antioxidants such as metallothionein, selenium, α- tocopherol, reduced glutathione (GSH), ascorbic acid and carotenoids share a common property that manifest protective influences by scavenging free radicals.

Vitamin E is classified into eight forms which are referred to as tocopherols. Naturally occurring compounds that possess vitamin E activity include α-tocopherol (5, 7, 8 trimethyltocol), β-tocopherol (5, 8-dimethyltocol), γ-methyltocol (7,8-dimethyltocol), δ-methyltocol (8-methyltocol) and their analogous tocotrienols (containing three unsaturated bonds in the chain). α-Tocopherol

is considered to be biologically most active compound. Tocopherol is a useful indicator of exposure to metals and organic contaminants that generate oxidative stress (Palace *et al*., 2005).

These vitamins are capable of ameliorating the effects of xenobiotics on living organisms and in most cases act in synergy. For instance, Layachi and Kechrid (2012) demonstrated how oral exposure to Cd caused reduction in LPO and antioxidant enzyme activities in rat's liver, and vitamin C or vitamin E may have partial ameliorative effects on these disturbances, whereas vitamin C and vitamin E together assured a more efficient protection of the organ against the noticed oxidative stress. In like manner, Thakur and Kanshere (2014) demonstrated how Vitamin C and Vitamin E supplementation played a positive role in detoxification of mercury toxicity, especially the low dose. Also, Das and Sha (2010) observed the powerful immuno-stimulant action of the vitamin C. tocopherol conjugates have proved that they are useful as antioxidants or as pro-drug delivery vehicles. The conjugates are amphiphilic and tend to localize at the interface between water domains and lipid domains (Shea *et al*., 2012). Furthermore, the betterment of humoral, enzymological and histological aspects in vitamin treated fish illustrates the curative and prophylactic role of the vitamins against copper intoxication (Muneesh *et al*., 2014).

2.4 Histopathological effects of pollutants on fish organs

Histopathological lesions provide a reliable, easily quantifiable index of low-level toxic stress to a broad range of environmental pollutants. Histopathological biomarkers can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem (Khoshnood *et al*., 2010). The severity of damage depends on the toxic potentiality of a particular toxicant accumulated in the tissue. Therefore, exposure to polluted water may adversely affect various organs in fish which ultimately could lead to overall toxic impact on organs like gill and liver (Parvathi *et al*., 2011). For instance, remarkable alterations in the activities of glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) in the tissues of Nile tilapia were detected and these alterations were followed by the occurrence of histological lesions in liver and gill tissues of fish collected from the same sites (Alaa and Osman, 2012). These authors also observed that the liver of copper treated fish showed histopathological alterations such as degeneration of hepatocytes, cell necrosis, inflammation with sinusoid dilation, increased incidence of kupffer cells and vacuole formation.

Similarly, Bijoy and Nimila (2011) observed vacuolar degeneration and focal necrosis in hepatocytes of *Etroplus maculatus* exposed to lindane. Glomerular injury, epithelial tubuli contraction, the reduction of renal hematopoietic system and the proliferation of connective tissue related with metal water contaminants have been documented in other reports (Al-Bairuty *et al*., 2013). Al-Balawi *et al*. (2013) also observed that epithelial hypertrophy and epithelial lifting were apparent in the gills of exposed fishes; degeneration of cytoplasm and secondary lamellae; necrosis of hepatocytes; glomerular expansion and gaps between the muscular bundles were found in the fishes exposed to lead acetate.

2.5 Physico-chemical parameters of rivers as water quality index

Water quality index is commonly used for the detection and evaluation of water pollution and can be defined as a rating reflecting the composite influence of different quality parameters on the overall quality of water. These indices include physico-chemical and biological indices. Important physical and chemical parameters influencing the aquatic environment are temperature, rainfall, pH, dissolved oxygen and carbon dioxide. Others are total suspended and dissolved solids, nitrates, sulphates, phosphate -phosphorus, calcium, magnesium, hardness, total alkalinity and acidity and heavy metal contaminants. For instance, Fadaeifard *et al*. (2012) observed significant differences in some water factors such as total hardness, total dissolved solid, total suspended solid, COD, BOD, dissolved oxygen, phosphate, nitrite, nitrate and total ammonia between inlet and outlet water of fish farms where as there were no significant changes in pH, sodium chloride and water temperatures indicative of the effects of fish farm effluents on stream water quality.

The physico-chemical parameters of the river can serve a great deal in determining the adaptability of living organisms to the aquatic environment. Un-favourable environmental conditions evident in fluctuations in these parameters can lead to stress and generation of reactive oxygen species. For instance, Imam and Balarabe (2012) showed how physicochemical fluctuations in temperature, pH and TDS had negative impact on the zooplankton species richness and abundance. Also, heavy metal uptake rate by fish depends on a number of factors such as the concentration of metals, exposure period of fish to metals, surrounding pH, salinity and temperature (Akoto *et al*., 2014). The aquatic environment provides a sink for many environmental contaminants some of which have the potential to cause oxidative stress in aquatic organisms (Amaeze *et al*., 2014). Also, multiple factors including season, physical and chemical properties of water can play a significant role in metal accumulation in different fish tissues (Romeo *et al*., 1999; Hayat *et al*., 2007).

In developing areas and urban centres there are obvious pollution with high levels of faecal coliforms, heavy metals and organic wastes which constitute public health hazards (Ladipo *et al*., 2012). Aquatic resources are exceptionally valuable natural assets enjoyed by millions of people for domestic purposes, animals, transportation and fish production (Ladipo *et al*., 2012). The worldwide deterioration of surface water quality has become a growing threat to human ecosystems, therefore the need to understand the spatial and temporal variabilities of limnological parameters (Adeogun *et al*., 2012).

2.5.1 Dissolved oxygen

The dissolved oxygen is important in the natural self-purification capacity of the river (Zeb *et al*., 2011). Prolonged exposure to low dissolved oxygen levels (less than 5 to 6 mg/dm^3 oxygen) may not directly kill an organism, but will increase its susceptibility to other environmental stresses. Exposure to less than 30% saturation (less than 2 mg/dm^3 oxygen) for one to four days may kill most of the aquatic life in a system. Dissolved oxygen concentrations in unpolluted water normally range between 8 and 10 mg/L and concentrations below 5 mg/L adversely affect aquatic life (DFID, 1999; Rao, 2005). Optimal dissolved oxygen for fish rearing in ponds is atleast 6mg/L (OECD, 2007).

2.5.2 Biological oxygen demand

Biological oxygen demand (BOD) is often used as a measurement of pollutants in natural and waste waters and to assess the strength of waste, such as sewage and industrial effluent waters (Zeb *et al*., 2011). BOD is an important parameter of water indicating the health scenario of freshwater bodies (Bhatti and Latif, 2011). Biochemical Oxygen Demand, or BOD, is a measure of the quantity of oxygen consumed by microorganisms during decomposition of organic matter.

BOD is the most commonly used parameter for determining the oxygen demand on the receiving water of a municipal or industrial discharge. BOD can also be used for evaluation of the efficiency of treatment processes, and it is an indirect measure of biodegradable organic compounds in water. High BOD is an indication of poor water quality. The lower the BOD the less organic matter is present in water. A high BOD is often accompanied by a low DO level. For optimal out-put in fish ponds, BOD of $3mg0_2/L$ is required (OECD, 2007).

2.5.3 Total dissolved solids

The total dissolved solids usually vary from season to season depending on temperature of the aquatic environment. The level of dissolution of substances from various anthropogenic sources increases with increase in temperature of the medium. The maximum allowable TDS in fish pond is 0.13mg/L (Ezeanya *et al*., 2015).

2.5.4 Electrical conductivity

Conductivity is a measure of the capacity of an aqueous solution to carry an electrical current. Conductivity depends on the presence of ions (cations and anions) in water, their total concentration, mobility and valence, and on temperature of water. The FEPA acceptable limit for conductivity in domestic water supply is 70µS/cm (DWAF, 1996). Acceptable limit set for fish rearing in ponds is 30-5000 µS/cm (Stone and Thomforde, 2004).

2.5.5 Total alkalinity

Alkalinity refers to the capability of water to neutralize acids. Generally, the basic species responsible for alkalinity in water are bicarbonate ion, carbonate ion and hydroxide ion, whereas pH is an intensity factor and alkalinity is a capacity (Manahan, 1993). For protection of aquatic life the buffering capacity should be at least 20mg/dm^3 (especially carbonates) within the water matrices. Alkalinity due to the presence of bicarbonate, carbonate and hydroxides of calcium, sodium and potassium (Murhekar, 2011; Lawson, 2011) is vital in neutralizing the acidity of water which increases with dissolved carbon dioxide (Lawson, 2011). The total alkalinity range allowed for optimal fish farming is 50-200mg/L (Bhatnagar and Devi, 2013).

2.5.6 Total hardness

The hardness of water is the concentration of ions that will react with a sodium soap to precipitate an insoluble residue. Water hardness is the result of dissolved minerals' presence, usually total concentration of cations of calcium Ca^{2+}, magnesium Mg^{2+}, iron Fe^{3+} and manganese Mn^{2+}. Acceptable water hardness for drinking water - should not exceed 5mmol/dm^3 (500 mg $CaCO_3$/dm^3). Acceptable limit set for pond water fish rearing should be atleast 20mg/L (Bhatnagar and Devi, 2013).

2.5.7 pH

Acid rain, mining waste and industrial discharges are among some of the factors that can alter the pH of an aquatic environment. According to WHO (2011), there is no health-based guideline value for pH, although 6.5 – 8.5 is proposed for drinking water. Most aquatic animals prefer a pH range of 6.5 - 8.0 which is slightly acidic and slightly alkaline. For instance, Lawson (2011) reported that

aquatic shrimps and crabs require optimum pH range of 6.8 - 8.7 for maximum growth and reproduction. For optimal fish pond performance a pH range of 6.5-8.5 is required (OECD, 2007).

2.5.8 Water and air temperatures

Distribution of temperature is different for surface waters and groundwater. Temperature of surface waters depends mainly on water origin, climatic zone, season, altitude, degree of riparian coverage, inflow of industrial and municipal sewage (power plants, industrial cooling). Temperature increase decrease the amount of dissolved oxygen (DO), increase biochemical oxygen demand (BOD), acceleration of nitrification and oxidation of ammonia to nitrates (III) and (V) which eventually lead to oxygen deficit in water. Higher temperature also increases toxicity of many substances (pesticides, heavy metals) and susceptibility of organisms to toxicants. Temperature of the water is probably the most important environmental variable since it affects metabolic activities, growth, feeding, reproduction, distribution and migratory behaviours of aquatic organisms (Suski *et al*., 2006).

2.5.9 Sulphate

Sulphates are one of the least toxic anions and large quantities would have to be ingested in order for health disorders to occur (especially diarrhoea type symptoms). The presence of sulphate in drinking water can result in noticeable bitter taste. Acceptable concentration of sulphates (VI) for drinking water is 200mg/dm^3. Sulphate concentration range of 0-100mg/L is optimal for fish rearing in pond (Stone and Thomforde, 2004).

2.5.10 Phosphate-phosphorus

Phosphorous is an algal nutrient often contributing to excessive algal growth and eutrophication (Manahan, 1993). Acceptable concentration of phosphates in surface waters is 0.2mg/dm^3. Elevated concentration of phosphorus may result in fouling of natural water and production of toxic cyanobacteria (Omaka, 2007). Acceptable concentration range of phosphate-phosphorus in pond fish rearing is 0.03-2mg/L (Bhatnagar and Devi, 2013).

2.5.11 Nitrate-nitrogen

Nitrate occurs in water naturally as a result of plant or animal material decomposition, but can also be introduced into water due to human activities, e.g. food production, where used as a preservative; use of agricultural fertilizers and manure; disposal of domestic and industrial sewage. Nitrates stimulate the growth of macrophytes and phytoplankton but simultaneously they make up for the nutrient load in water, leading to eutrophication. Some studies have shown there may be a relation between nitrates presence in water and gastric cancer and methemoglobinemia (which in infants is often referred to as blue baby syndrome). In developing areas and urban centres there are obvious pollutions with high levels of faecal coliforms, heavy metals and organic wastes which constitute public health hazards (Arimoro *et al*., 2007; Ladipo *et al*., 2012). Acceptable limit for nitrogen concentration in surface and drinking water is 1.5mg/dm^3. Unpolluted natural waters usually contain only minute amounts of nitrate (Jaji *et al*.,

2007). Maximum allowable concentration of 40mg/L is optimal for pond fish rearing (OECD, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

River Galma is one of the main tributaries of River Kaduna. It has its headwaters near the north western edge of the Jos Plateau and falls near the Magami village into Kaduna plains. The main tributaries of River Galma are River Shika in the middle course and the Rivers Kinkiba and Likarbu in its lower course. The Galma reservoir which is popularly called Zaria dam was constructed across the River Galma in 1975. The major land uses in the catchment areas are farming and animal rearing. There are also some industrial and municipal activities in the surrounding towns and villages such as Chikaji, Dakace and Sabon Gari areas, that produce majorly farming and domestic wastes that ultimately get to the river on the long-run through run-offs and seepages. There are few industries located in Chikaji and Dakace. The sampling areas are all located in Zaria, Sabon Gari Local Government Area of Kaduna State, Nigeria (Fig.3.1).

Five (5) sites were selected along the river. The first site (station 1) was located at Shika Reservoir (Zaria Dam). Shika reservoir was used as reference site because it serves as the upper course of the river and relatively located far from the industrial areas and the municipal waste point of discharge. The second site (station 2) was located at Kakeyi village at about 2,000 metres away from Shika dam. This site receives municipal wastes and effluents and agricultural run-offs from the neighbouring villages and town such as Farin Kasa, Sabon Gari and Kakeyi village.

The third and fourth sites (stations 3 and 4) were located around Federal College of Education, Kongo, Zaria, Kaduna State. Station 3 was located at about 1,500 metres away from Kakeyi site.

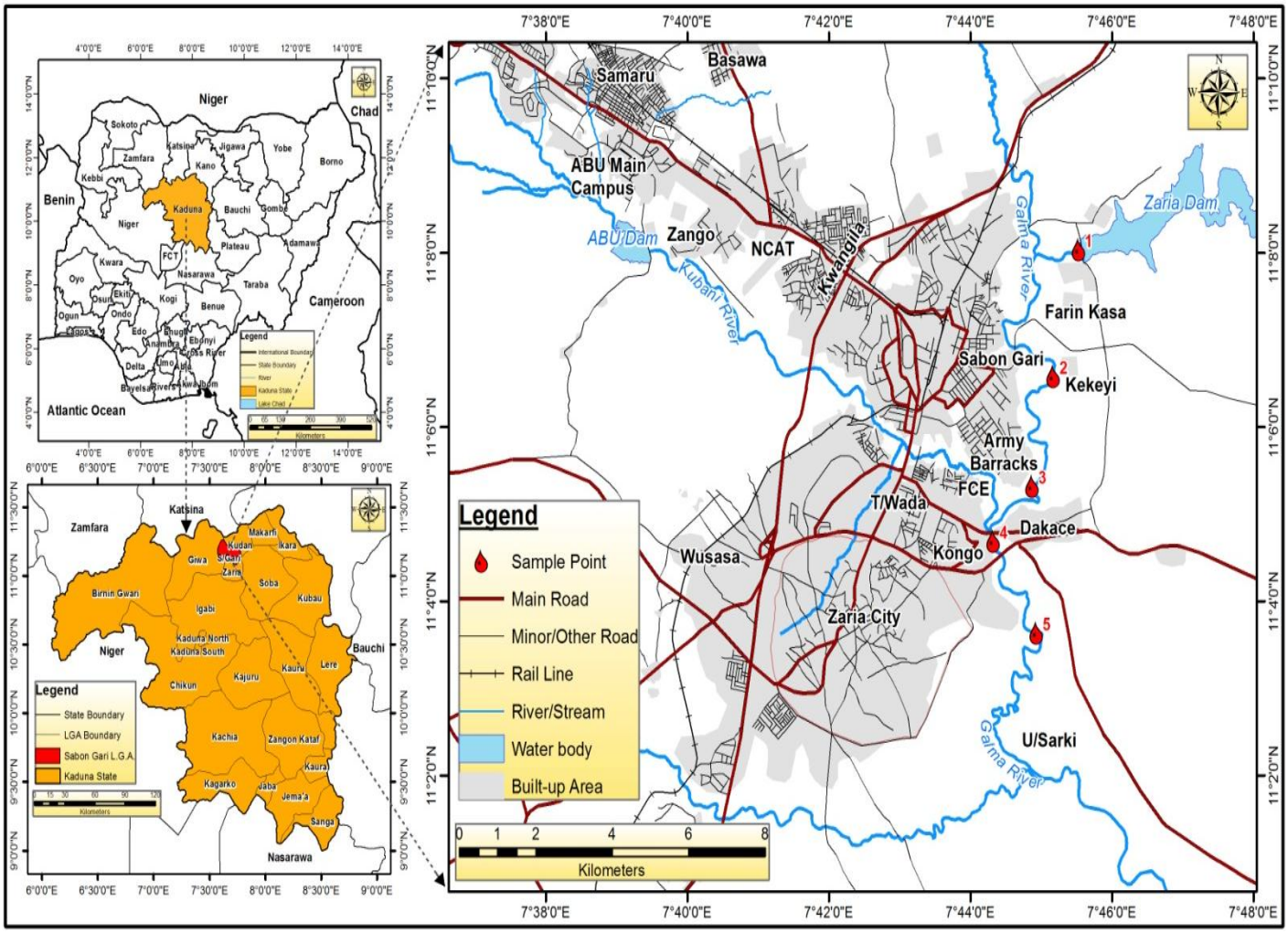


Figure 3.1: Map of Zaria showing Sample Stations
Source: Modified from the Administrative Map of Zaria

Station 3 receives municipal wastes and effluents, agricultural run-offs from some parts of Sabon Gari, Chikaji, Kakeyi and Farin Kasa villages. Station 4 was located at about 500 metres away from station 3. Station 4 receives municipal wastes and effluents, agricultural run-offs from various parts of the town including Tudun Wada, PZ, Kongo via the Kubani stream which adjoins the main stream at this point. Waters from Kakeyi along the stream also joins here.

The fifth site (station 5) was located at Dakace village at about 1000 metres apart from station 4 and receives municipal and agricultural wastes and effluents from Dakace village and the few industries located in the area (Fig.3.1). The few industries include the Sun Seed Company, Zaria Pharmaceutical Company, FAN Milk depot located around Dakace villages. These companies are not producing at full capacity at the moment. Farming and other agricultural activities take place in both wet and dry season through-out the year. The choice of these sites were made base on security reasons and ease of accessibility.

3.2 Collection and Acclimatization of *Clarias gariepinus*

A total of one-hundred and fifty specimens (that is, 75 per exposure) of *Clarias gariepinus* of 20-45g size range were purchased from the commercial fish farm (Bagauda, Kano) and kept in concrete tank for a period of two weeks during which they were fed Coppens (2mm size) feed morning and afternoon on a daily basis. The holding facility was aerated with Blagdon aerator (KOI Air, KA25).

3.3 Cage Construction

Five cages were constructed using polyvinyl chloride (PVC) pipes of various sizes as modification of methods of cage construction described by Palace *et al*. (2005). One inch pipes were used to form the upper and lower frames of the cage. These were measured and cut to size (50cm). PVC of half inch were used as the side and basal bars. Each bar was 50cm long. Holes were made in each frame with the aid of red-hot iron rod wide enough to accommodate the bar conviniently. Holes were also made along the central and side axes of the two lower frames to accommodate the vertical and horizontal bars while the other PVC frames were punctured only on the central axis. A minimum of sixteen holes at regular interval per frame were created.

The PVC frames were connected with the aid of L-shaped elbow joints. For proper and firm fitting of the elbow joints, an adhessive was applied at each joint ready to be fitted into the frame. Shredded foams were placed in the PVC frame to ensure lightness and floatation. The adhesive was also applied on the bars for proper fitting into the holes. The length of each frame was 50cm in-to-in. Holes were also created at the joints to accommodate more bars to fill up the spaces. A lid was constructed at the upper end of the cage. An inch PVC pipes were used with the four sides connected to the frame at one side via T-shaped PVC connector joint after two holes were made at the side.

A net of 1cm mesh size was attached to the inside of the cage with the aid of an adhesive and the free ends of the nets were tack on the side bars through the use of fish twine thread and needle. An iron mesh was used to cover the inner layer of the cage to avoid attack on the content of the cage from predators such as crabs. Floaters in form of foams were attached to the cage on the outside through the use of thread at either sides of the cage. Another foam was attached on the

lid to increase buoyancy and floatation capacity of the cage. Another PVC of two inches were used to cover the four edges of the upper frame of the cage in order to hold the nets firmLy at the upper base (Plate I). The capacity of the cage was 50cm (height) by 50cm (width).

3.4 Setting-up of the Cage for the Bioassay of *Clarias gariepinus* in River Galma

Five cages (one at each site) were set-up in the river for 14 days *in situ* bioassay in the months of March and August, 2015 for the peak of the dry and rainy season exposures respectively. Sixty specimens of *C. gariepinus* (twelve per site) were transported to the river in a special mesh using cage system for each sampling site and then suspended into the water in which the fish were left to fend for themselves within the natural ecosystem for fourteen (14) days. Their feeding was supplemented with coppens feed, 2mm once at dawn after the first 24hours of exposure in the river for the remaining days of the exposure period. The whole process was repeated in August for the rainy season exposure. Also, the cages were moved from point to to the other within the same station on daily basis whenever the waters receded to the main river course during the wet season.

Plate I: Top view of the Cage immersed in River Galma.
Source: Field pictures, 2015.

3.5 Sample collection and preparation

The fish specimens were removed from each site after the first 7 days of exposure and then, 14 days after exposure and kept in a large plastic container and transported to the laboratory in each case. Each specimen was dissected and gills, livers and kidneys removed and homogenized in physiological saline solutions the same day and kept in the test tube meant for each organ from each fish and for each station and then refrigerated prior to analyses for non- enzymatic antioxidants and heavy metals. The specimens were obtained from each sampling station the same day and in the early hours of the day. Prior to exposure, non-enzymatic antioxidants were tested to serve as control from which comparison were made with the outcome of the various sites. The exposure was done at the peak of the dry season in March, 2015 and the whole process (experiment) was repeated at the peak of wet season in August, 2015 for comparison between the seasons.

3.6 Water physico-chemical parameters

Water samples from each site were collected in 2L plastic containers from each site for physico-chemical parameters, nutrients (sulphate, phosphate-phosphorus and nitrate) and heavy metals (Cadmium, lead, zinc, chromium and manganese) analyses. Water samples were collected at dawn between the hours of 6 and 8 on a monthly basis from March to August, 2015 (six months).

3.6.1 Water and Air temperature

Water temperature at various sites was determined *in situ* using mercury-in-glass thermometer which was immersed in water 6cm below the water surface and left to stabilize for five minutes

before it was read and recorded. The ambient air temperature was also determined at each location along the river course.

3.6.2 Dissolved oxygen

Water samples were collected from each sampling site and transported to the Hydrobiology Laboratory of the Department of Biological Sciences for dissolved oxygen analysis according to Winkler's Azide method (APHA, 2010). Water samples were transferred into a 300mL BOD bottle. 2mL $MnSO_4$ solution and 2mL alkali-iodide azide reagents were added, which was then stoppered with care to exclude air bubbles. It was then mixed gently by inverting the bottle a number of times until a clear supernatant was obtained. It was allowed to settle down for two minutes after which 2mL concentrated H_2SO_4 was added by allowing the acid run-down the neck of the bottle. It was stoppered again and mixed by gentle inversion until dissolution was complete. 100mL of the prepared solution was transferred into a conical flask and titrated with 0.0125 of $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow colour. 2mL of freshly prepared starch solution was added and the colour changed to blue. Titration was continued by adding thiosulphate drop-wise until the blue colour disappeared.

3.6.3 Biological oxygen demand

The biological oxygen demand (BOD) was determined according to Winkler's Azide method (APHA, 2010). 300mL of water sample was poured into a 300mL standard BOD bottle and covered carefully to exclude air bubbles. The bottle was then kept in an incubator for five days. After five days in the incubator, the bottle was brought out and 2mL of manganese sulphate solution was added followed by 2mL alkali-iodide azide reagent. The bottle was stoppered carefully to exclude air bubbles and then mixed thoroughly by inverting the bottle several times.

The precipitate was allowed to settle down leaving clear supernatant after which 2mL concentrated H_2SO_4 was added. The solution was titrated with 0.0125N of sodium thiosulphate solution until the blue colour disappeared. The BOD was calculated using the formular: $(BOD)_5$ in mg/l = DO_1-DO_5.

3.6.4 pH

The pH was determined using HANA instrument (HI98129) in the Laboratory. The instrument was immersed into the sample of water from each site and held for about 2minute until a stable reading was obtained and recorded.

3.6.5 Electrical conductivity

HANA instrument (HI98129) was used to measure the electrical conductivity of the water samples. The power key and the conductivity key meter were switched on. The probe was dipped into the water and the stable reading on the meter recorded in µS/cm.

3.6.6 Total dissolved solids

HANA instrument (HI98129) was used to measure the total dissolved solids of the water samples. The power key and the TDS key metre were switched on. The probe was dipped into the water and the stable reading on the metre recorded in mg/L.

3.6.7 Total alkalinity

Total alkalinity (mg/$CaCO_3$L) was determined by acid titration using methyl-orange as an indicator (Arain *et al*., 2008). Hundred mililitre of water sample was transferred into a conical flask. Then, 2 drops of bromocresol green and 2 drops of methyl red were added respectively. The

mixture was swirled and titrated with solution of H_2SO_4 until colour changed. Total alkalinity in $CaCO_3$ mg/L was determined by: Titer value ×10.

3.6.8 Total hardness

Distilled water (25 mL) was added to 25mL of water sample collected from the sampling points. Two (2mL) of buffer solution of pH 10.4 was then added; followed by addition of 0.1g Erochrome black T dye. It was titrated with EDTA titrant (0.01M). The titrant value was multiplied by 40, as $CaCO_3$/L.

3.6.9 Sulphate

Water sample (100mL) was transferred into a conical flask. BaCl (1g) was added and the content shaken. It was left to stand for 3minutes and readings were made at 430nm wavelength using the colorimeter 257 in the laboratory.

3.6.10 Nitrate-nitrogen

Water sample (100mL) was poured into a clean dry metallic crucible and kept in an oven at 100°C till dryness. It was then removed and allowed to cool after which 2mL of phenol disulphonic acid was added and swirled round uniformLy with stirring rod. It was then left to stand for 10 minutes and 10mL distilled water added. After this, 5mL of ammonia solution was added and allowed to cool. Colour change was read at 430nm wavelength using the colorimeter 257 in the laboratory.

3.6.11 Phosphate-phosphorus

Water sample (100mL) was transferred into a conical flask. Ammonium molybdate (1mL) reagent (Dennig's reagent) was added and followed by 1 drop of stannous chloride. It was allowed to stand for 12 minutes and reading was taken at 600nm using the colorimeter 257 in the laboratory.

3.7 Heavy Metal Analyses

Heavy metal contents of the water, liver and gill of the fish exposed in River Galma were determined.

3.7.1 Heavy metal in fish organs

A sample (3g) of the fresh organs (gill and liver) of *Clarias gariepinus* from the various stations were homogenized in 0.9% physiological saline buffer with a ceramic mortar and pestle and then digested in containers containing nitric acid. The digestion was done by heating 1mL of the homogenized organ of the sample of the various stations in each case, with 7.5mL of concentrated nitric acid, 2.5mL of concentrated hydrochloric acid in 50mL graduated beaker at 150°C until near dryness. After which the beaker was brought down and allowed to cool. The content in each beaker was made up to 50mL with distilled water. Samples drawn and digested after 7 day period of exposure and those drawn after 14 day period were mixed together and a pooled sample was obtained for each station and organ. The same preparation was done for the samples obtained during the wet season, August, 2015 exposure. The pooled samples of digested tissues were analyzed for lead, zinc, cadmium, chromium and manganese concentrations via Graphite Furnace Atomic Absorption Spectrophotometry (AA WIN PRO) in Soil Science Department of Ahmadu Bello University, Zaria.

3.7.2 Water heavy metal

The water sample from each station were digested by heating a combination of 1mL of the water sample, 7.5mL of concentrated nitric acid and 2.5mL of concentrated hydrochloric acid in 50mL graduated beaker at 150^0C to near dryness. The beaker was then taken down and allowed to cool, after which the content of the beaker were made up to 50mL with distilled water. Pooled sample (made from mixture of digested water sample monthly for a period of three month) from each station was used for the analyses of heavy metals of interest. The water samples were analyzed for lead, zinc, cadmium, chromium and manganese by Graphite Furnace Atomic Absorption Spectrophotometry (AA WIN PRO) in Soil Science Department of Ahmadu Bello University, Zaria.

3.8 Non-enzymatic antioxidant production level

3.8.1 Glutathione bio-assay

A sample (3g) of liver pieces, kidneys and gills used for measurements of reduced glutathione (GSH) concentrations in *Clarias gariepinus* from each station were homogenized in 0.9% physiological saline buffer using ceramic mortar and pestle.

The following reagents were used for the analysis: 0.2M phosphate buffer (8.40g of NaH_2PO_4 and 9.94g of Na_2HPO_4 was dissolved in distilled water and made up to 1000mL mark in a volumetric flask. The buffer was adjusted to pH8.0); 10% Trichloroacetic acid (10g of TCA was dissolved in distilled water and made up to 100mL in the volumetric flask); and Ellman' reagent (19.8mg of 5,5'-dithiobis nitro benzoic acid (DTNB) in 100mL of 0.1% sodium nitrate).

To 150µL of the tissue homogenate (in phosphate-saline pH 7.4), 1.5mL of 10% TCA was added, and centrifuged at 1500g for 5min. 1.0mL of the supernatant was treated with 0.5mL of Ellman's reagent and 3.0mL of phosphate buffer (2.0m pH 8.0). The absorbance was read at 412nm. Estimation of Reduced Glutathione was determined by the method of Ellman (1959) as described by Rajagopalan *et al*. (2010). The amount of glutathione was calculated using a GSH standard curve and expressed as microngrams of GSH formed/mg protein in each case.

3.8.2 Vitamins C and E

An isocratic liquid chromatographic method for the separation and simultaneous determination of ascorbic acid (vitamin C) and α-tocopherol (vitamin E) from 3g of liver and gill of *Clarias gariepinus* from each station which were already homogenized was as follow: Samples were analysed by means of a reverse-phase column (LiChrospher 100 RP-18), using methanol as mobile phase. The UV–Vis detector used was set at a wavelength of 300 nm and switch to 450 nm at 17 min. These vitamins were separated within 25 min and the detection limits ranged from 7 (β-carotene) to 65 ng mL^{-1} (ascorbic acid). Visible spectrophotometre (UV2550) was used for the analysis in National Research Institute for Chemical Technology (NARICT), Zaria.

3.9 Histopathological examination of fish organs

The histopathology of the kidneys and livers from each station were carried-out in comparison with the reference site and those samples that are not yet exposed. These organs were preserved in 10% formalin until analysis. These organs were also analysed histopathologically prior to exposure of the fish to the river environment after two weeks of acclimatization. These organs were excised

from specimens of *Clarias gariepinus* from each station, and permanent slides prepared for histological studies.

The liver was dissected quickly, sliced into 3mm thick slabs, and immersed in Bouin's fixative for 24 hours; it was dehydrated in successive percentages of alcohol and then embedded in paraffin wax. Sagittal sections (5µ of thickness) were cut and mounted on glass slides. The sections were de-paraffinized in xylene, hydrated in ethanol and stained with hematoxylin-eosin (HE). The same procedure was used for the kidneys of the fish. Photomicrographs were prepared for livers and kidneys of *Clarias gariepinus* after 14 days *in situ* bioassay in River Gama.

3.10 Data analyses

Graphical representation of the various heavy metal concentrations in water, gills and livers were indicated using error bar charts. Same was also done for the non-enzymatic antioxidant concentrations in the various stations. Concentrations of the metals (mg/L) were plotted against (vertical) sampling locations (horizontal). Graphical representation of the physico-chemical parameters were also made.

Descriptive analysis (Mean±SD) was performed using a computer program of IBM SPSS Inc. (version 20.0 for Windows). One way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was used for the physico-chemical parameters to determine the differences between the reference station samples and the samples from other stations at the different periods of exposure using SPSS statistical package at $P \leq 0.05$ level of significance.

Spearman's Correlation Coefficient was used to determine the relationship between heavy metals concentration and non-enzymatic antioxidant production levels. The relationship among the physico-chemical parameters, heavy metal concentrations and non-enzymatic antioxidant production levels were determined by carrying out Principal Components Analyses (PCA) and Canonical Component Analyses using SPSS version 20.

CHAPTER FOUR

4.0 RESULTS

Clarias gariepinus exposed in River Galma displayed variations in the production of non - enzymatic antioxidants as well as histopathological alterations in the architecture of the fish tissues. The physic-chemical paramers and the heavy metal concentration of the river and the organs of the fish also varied from season to season.

4.1 General Observations and Reports on the Exposure of *Clarias gariepinus* in River Galma

The *in situ* exposures took place in March and August, 2015 for dry and wet seasons respectively. The general trend was that mortality occured in few days after exposure. Those that acclimatized to the river environment survived till the last day of exposure. The non-enzymatic antioxidants produced in the fish, physico-chemical parameters and heavy metal concentrations varied widely from station to station, and from season to season.

4.2 Physico-chemical parameters

4.2.1 Air temperature

There was slight monthly variation in the ambient temperature. The highest air temperature of 27^0C was obtained in station 2 in the month of May, 2015. While the lowest air temperature of 19^0C spanned through various months and locations (Fig.4.1a). The ambient temperature mean values ranged from 19.20±0.20 ^{0}C (July, 2015) to 25.00±0.63^{0}C (May, 2015). There was no

significant difference ($P > 0.05$) in the air (ambient) temperature amongst the sampling locations (Table 4.1). There was however, significant difference among the months of sampling ($P \leq 0.05$) (Table 4.2).

4.2.2 Water Temperature

The water temperature had its peak in May with 27^0C at station 2 and the lowest value of 21^0C in the month of July at station 4 (Fig 4.1b). And then, slightly vary from month to month and from station to station. Water temperature mean values measured ranged from 21.60 ± 0.24^0C (July, 2015) to $26.80\pm0.20\ ^0$C (May, 2015). There were no significance differences ($P>0.05$) in the water temperature amongst the sampling locations and months of sampling (Table 4.1 and 4.2).

Water temperature correlated negatively with GSH concentrations in gill, kidney and liver. It also had high negative correlations (-0.7 and -0.8 respectively) with both Cr and Mn concentrations. Water temperature also had negative correlations with vitamin E concentration in both gill and liver of the fish. On the other hand, water temperature positively correlated with vitamin C concentration in gill (Table 4. 3).

4.2.3 Dissolved Oxygen

The dissolved oxygen varied widely from month to month and from location to location. Both the peak (5.10mg/L) and lowest (0.8mg/L) dissolved oxygen concentrations occurred in the month of June, 2015 at stations 1 and 2 respectively (Fig.4.2a). The dissolved oxygen mean values ranged from 1.43±0.08 mg/L (April, 2015) to 3.67±0.4 mg/L (March, 2015). There was no significant difference ($P>0.05$) in the dissolved oxygen amongst the sampling locations. There was significant difference in the months of sampling ($P\leq0.05$) (Table 4.1 and 4.2).

Table 4.1: Physico-chemical Parameters of the Sampling Stations Obtained from River Galma, Zaria

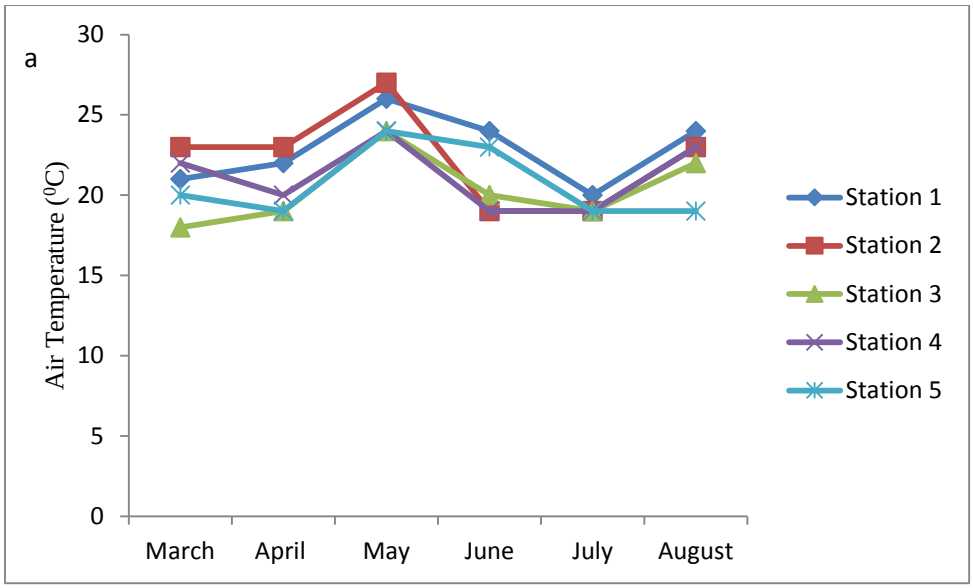
Parameters	Station 1			Station 2			Station 3			Station 4			Station 5		
	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD
Air Temp (°C)	20	26	22.83±0.91	19	27	22.33±1.23	18	24	20.33±0.92	19	24	21.17±0.87	19	24	20.67±0.92
Water Temp (°C)	22	27	23.67±0.71	21	27	23.50±0.81	22	27	23.67±0.71	21	26	23.67±0.67	22	27	23.50±0.81
DO (mg/L)	1.75	5.1	3.22±0.57	0.8	3.5	1.91±0.39	1.3	4.1	2.50±0.41	1.3	3.8	2.39±0.39	1.45	3.2	2.58±0.37
BOD (mg/L)	1.4	6.75	3.82±0.89	0.75	5.05	2.30±0.59	0.95	3.9	2.01±0.41	0.95	6.4	2.49±0.81	1.3	5.55	2.41±0.64
Nitrate (mg/L)	0.058	0.327	0.14±0.05	0.033	0.366	0.17±0.06	0.049	0.414	0.15±0.06	0.054	0.473	0.2±0.08	0.044	0.359	0.15±0.05
Sulphate (mg/L)	0.012	0.463	0.15±0.07	0.017	0.872	0.32±0.12	0.011	0.617	0.25±0.08	0.028	0.72	0.25±0.10	0.013	0.593	0.21±0.09
PO_4 (mg/L)	0.001	0.376	0.11±0.06	0.035	0.546	0.20±0.09	0.043	0.478	0.15±0.07	0.032	0.461	0.15±0.07	0.022	0.426	0.16±0.07
Total Hard. Water (mg/L.$CaCO_3$)	44	96	96.67±19.08	48	140	96.67±16.51	48	100	75.33±8.29	64	164	98.67±14.95	56	140	99.33±14.55
Total Alkalinity (mg/L.$CaCO_3$)	31	75	58.33±6.45	27	105	72.33±11.84	38	105	79.33±9.83	18	124	70.67±15.22	20	131	72.33±14.79
TDS (mg/L)	25	57	45.83±5.72	30	135	83.33±15.20	34	136	88.17±15.40	35	158	89.17±17.21	37	159	89.33±18.78
EC (µS/cm)	52	112	92.50±10.91^{bc}	30	135	83.33±15.20^{c}	67	247	172.17±25.86^{ab}	71	271	181.17±39.82^{a}	86	316	180.00±36.23^{a}
pH	4.65	8.67	7.30±0.61	4.38	8.81	7.28±0.63	4.1	8.82	7.29±0.69	4.27	8.76	7.31±0.64	4.83	8.67	7.34±0.54

Mean values with different alphabets are significantly different ($P \leq 0.05$) from each other.

Table 4.2: Physico-chemical Parameter Amongst the Sampling Months at River Galma, Zaria

Parameters	March			April			May			June			July			August		
	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean±SD
Air Temp (°C)	18	23	20.80±0.86^{bc}	19	23	20.60±0.81^{bc}	24	27	25.00±0.63^{a}	19	24	21.00±1.05^{bc}	19	20	19.20±0.20^{c}	19	24	22.20±0.86^{b}
Water Temp°C	22	24	23.00±0.32^{cd}	23	24	23.60±0.24^{bc}	26	27	26.8±0.20^{a}	23	24	23.80±0.20^{b}	21	22	21.60±0.24^{e}	22	23	22.80±0.20^{d}
DO (mg/L)	2.45	4.80	3.67±0.45^{a}	1.3	1.75	1.43±0.08^{c}	1.70	2.50	2.05±0.14b^{c}	0.8	5.10	2.90±0.72^{ab}	1.65	2.20	1.88±0.10^{bc}	2.95	3.50	3.19±0.09^{a}
BOD (mg/L)	1.40	2.80	2.03±0.26^{bc}	0.75	1.40	1.13±0.12^{c}	1.60	3.25	2.19±0.29b^{c}	0.95	6.75	2.73±1.03^{b}	3.90	6.40	5.43±0.45^{a}	2.00	2.45	2.12±0.09^{bc}
Nitrate (mg/L)	0.33	0.44	0.38±0.02^{a}	0.07	0.13	0.11±0.01^{c}	0.21	0.47	0.30±0.05^{b}	0.03	0.07	0.06±0.01^{c}	0.05	0.12	0.09±0.01^{c}	0.03	0.06	0.05±0.00^{c}
SO_4 (mg/L)	0.01	0.03	0.02±0.00^{d}	0.12	0.21	0.17±0.02^{bc}	0.04	0.18	0.12±0.02^{cd}	0.00	0.36	0.19±0.06^{bc}	0.46	0.87	0.065±0.07^{a}	0.22	0.32	0.27±0.02^{b}
PO_4(mg/L)	0.06	0.10	0.07±0.01^{cd}	0.00	0.04	0.03±0.01^{d}	0.01	0.06	0.04±0.01^{d}	0.00	0.38	0.15±0.06^{bc}	0.38	0.55	0.46±0.03^{a}	0.15	0.32	0.20±0.03^{b}
Total Hard. water(mg/L. $CaCO_3$)	88	140	112.80±11.27^{ab}	56	112	75.20±9.91^{c}	48	92	70.40±8.26^{c}	100	184	142.40±14.73^{a}	44	124	86.40±14.78^{bc}	48	92	72.80±8.80^{c}
Total Alkalinity (mg/L.$CaCO_3$)	50	105	77.60±9.37^{b}	64	105	82.00±7.69^{ab}	61	84	72.40±4.83^{b}	69	131	103.20±11.35^{a}	18	38	26.80±3.65^{c}	40	75	61.60±6.27^{b}
TDS (mg/L)	55	136	106.40±14.93^{a}	54	111	92.20±10.18^{ab}	53	73	64.80±4.26^{bc}	57	159	120.80±18.85^{a}	30	47	35.40±3.04^{c}	25	79	55.40±10.34^{c}
EC (µS/cm)	107	271	185.60±29.41^{ab}	93	221	166.20±27.43^{abc}	56	158	122.80±18.33^{bcd}	107	316	219.40±46.80^{a}	30	94	65.40±10.26^{d}	52	148	91.60±15.72^{cd}
pH	7.66	8.67	7.97±0.18^{b}	6.87	7.98	7.18±0.21^{c}	7.72	8.04	7.92±0.05^{b}	8.55	8.82	8.72±0.05^{a}	7.07	8.10	7.56±0.19^{bc}	4.10	4.83	4.45±0.13^{d}

Mean values with the same superscript (s) are not significantly different ($P > 0.05$) from each other.



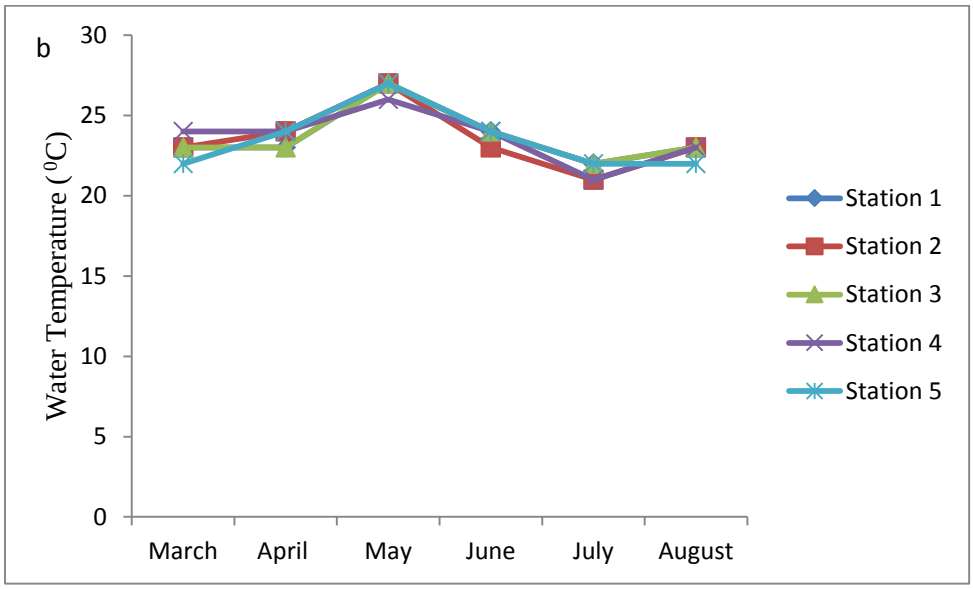


Fig.4. 1: Mean monthly variations in a) air temperature and b) water temperature among sampling stations on a stretch of River Galma, Zaria.

The dissolved oxygen (DO) was negatively correlated with chromium level. It had high negative correlation (-0.8 and -0.9 respectively) with both GSH concentration in liver and zinc concentration. Dissolved oxygen correlated negatively with both vitamin C and E concentrations in liver of the fish. There was also positive correlation with water temperature (Table 4.3).

4.2.4 Biological Oxygen Demand

There was temporal and spatial variation in the biological oxygen demand. BOD had a peak of 6.75mg/L in the month of June, 2015 in station 1. While the lowest value of 0.95mg/L was obtained in station 3 in the month of April, 2015 (Fig. 4.2b). The biological oxygen demand ranged from 1.13±0.12mg/L (April, 2015) to 5.43±0.45 mg/L (July, 2015). There was no significant difference ($P>0.05$) in the biological oxygen demand amongst the sampling stations. There was however, significant difference in the sampling months ($P\leq0.05$) (Table 4.1 and 4.2).

The biological oxygen demand (BOD) had high negative correlation (-0.9) with GSH concentration in liver. It also had negative correlation with both GSH concentration in gill and zinc concentration. There was high positive correlation (0.8) with DO. Biological oxygen demand positively correlated with vitamin C concentration in gill. BOD also negatively correlated with both vitamin E concentrations in gill and liver (Table 4.3).

4.2.5 Nitrate Concentration

The peak nitrate concentration was 0.435mg/L in station 4 in the month of March, 2015. On the other hand, the lowest value of 0.033mg/L was obtained in station 2 in the month of August,

2015 (Fig.4.3a). The nitrate concentration mean values ranged from 0.05±0.00 mg/L (August) to 0.38±0.02mg/L (March). There was no significant difference (P>0.05) in the nitrate concentration of the sampling locations. There was however, significance difference in the nitrate concentration in the months of sampling (P≤0.05) (Tables 4.1 and 4.2).

Nitrate concentration had positive correlation with both chromium and zinc concentrations. Nitrate negatively correlated with both manganese concentration and DO. On the other hand, nitrate concentration correlated positively with both vitamins C and E concentrations in liver of the fish. It also had negative correlation with vitamin C concentration in gill (Table 4.3).

4.2.6 Sulphate Concentration

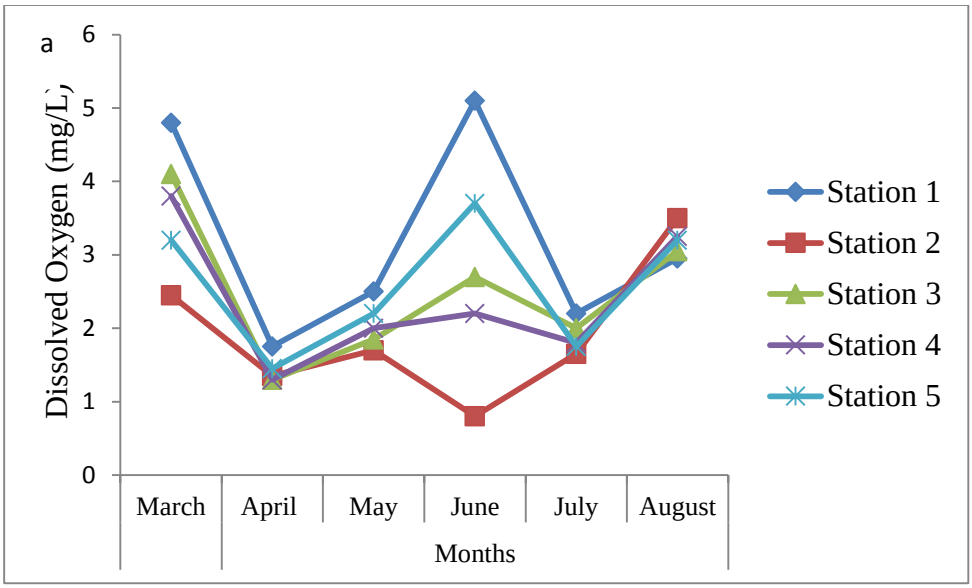
The highest sulphate concentration value of 0.872mg/L was obtained in station 2 in the month of July, 2015. While the sulphate concentration had its lowest in station 1 in the month of June with 0.003mg/L (Fig.4.3b). The sulphate mean concentration values ranged from 0.02±0.00 mg/L (March) to 0.65±0.07 mg/L (July). There was no significant difference in sulphate concentration (P>0.05) amongst the sampling locations. There was significant difference in the sulphate concentration of the sampling months (P≤0.05) (Table 4.1 and 4.2).

Sulphate had high positive correlations (0.8 and 0.9 respectively) with both GSH concentration in liver and zinc concentration. There were also positive correlations with both Cr and N. It had negative correlation with BOD. Sulphate also had negative correlation with DO. However, sulphate concentration positively correlated with both vitamin C and Econcentrations in the liver of the fish (Table 4.3).

Table 4.3: Spearman's Correlation Coefficients of Non enzymatic antioxidant production levels in exposed fish and the physico-chemical parameters of water samples of River Galma, Zaria

	VitCG	*VitCL*	*VitEG*	*VitEL*	GG	GL	GK	Pb	Cr	Cd	Zn	Mn	WTM	AT	DO	BOD	N	S	PO4P	Hard	Alka	TDS	EC	WTH	pH
VitCG	1.0																								
VitCL	-0.2	1.0																							
VitEG	-0.7*	-0.3	1.0																						
VitEL	-0.7*	0.7*	0.3	1.0																					
GG	-0.7*	-0.3	1.0	0.3	1.0																				
GL	-0.4	0.3	0.6*	0.4	0.5*	1.0																			
GK	-0.5*	-0.5*	0.9*	0.2	0.9*	0.3	1.0																		
Pb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0																	
Cr	-0.4	0.2	0.5*	0.6*	0.6*	0.4	0.7*	0.0	1.0																
Cd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0															
Zn	-0.7*	0.7*	0.5*	0.9*	0.5*	0.8*	0.3	0.0	0.6*	0.0	1.0														
Mn	0.3	-0.7*	0.4	-0.5*	0.4	0.1	0.6*	0.0	0.3	0.0	-0.3	1.0													
WTM	0.0	0.3	-0.5*	0.0	-0.6*	-0.6*	-0.7*	0.0	-0.7*	0.0	-0.3	-0.8*	1.0												
AT	0.9*	0.0	-0.8*	-0.4	-0.7*	-0.5*	-0.4	0.0	-0.1	0.0	-0.5*	0.2	0.0	1.0											
DO	0.3	-0.6*	-0.3	-0.7*	-0.3	-0.8*	-0.2	0.0	-0.7*	0.0	-0.9*	0.0	0.5*	0.2	1.0										
BOD	0.7*	-0.4	-0.6*	-0.6*	-0.6*	-0.9*	-0.3	0.0	-0.4	0.0	-0.9*	0.2	0.3	0.7*	0.8*	1.0									
N	-0.5*	0.7*	0.0	0.9*	0.1	0.1	0.1	0.0	0.6*	0.0	0.7*	-0.5*	0.1	-0.1	-0.6*	-0.4	1.0								
S	-0.3	0.7*	0.2	0.6*	0.1	0.8*	0.0	0.0	0.5*	0.0	0.9*	-0.1	-0.4	-0.2	-1.0	-0.8*	0.5*	1.0							
PO4P	-0.2	0.4	0.4	0.5*	0.4	0.9*	0.4	0.0	0.7*	0.0	0.8*	0.2	-0.7*	-0.1	-1.0	-0.7*	0.4	0.9*	1.0						
Hard	0.2	-0.2	0.0	0.1	0.2	-0.4	0.4	0.0	0.7*	0.0	-0.2	0.5*	-0.4	0.5*	0.0	0.4	0.3	-0.1	0.1	1.0					
Alka	-0.7*	0.4	0.6*	0.5*	0.5*	0.9*	0.3	0.0	0.2	0.0	0.8*	-0.2	-0.2	-0.8*	-0.7*	-1.0	0.2	0.7*	0.6*	-0.6*	1.0				
TDS	-0.9*	0.4	0.8*	0.8*	0.7*	0.8*	0.5*	0.0	0.6*	0.0	0.9*	-0.1	-0.3	-0.8*	-0.8*	-1.0	0.5*	0.7*	0.7	-0.2	0.9*	1.0			
EC	-0.9*	-0.1	0.7*	0.5*	0.7*	0.2	0.5*	0.0	0.2	0.0	0.4	-0.3	0.2	-0.9*	0.0	-0.5*	0.3	-0.1	-0.1	-0.2	0.6*	0.7*	1.0		
WTH	0.7*	-0.2	-0.8*	-0.7*	-0.8*	-0.8*	-0.7*	0.0	-0.8*	0.0	-0.9*	-0.2	0.6*	0.6*	0.8*	0.9*	-0.5*	-0.7*	-0.8*	-0.1	-0.8*	-0.9*	-0.5*	1.0	
pH	-0.5*	-0.7*	0.7*	0.0	0.8*	-0.2	0.8*	0.0	0.4	0.0	-0.1	0.4	-0.2	-0.4	0.3	0.0	-0.1	-0.5*	-0.2	0.4	-0.1	0.2	0.6*	-0.2	1.0

Note: VitCG, VitCL, VitEG, VitEL represents vitamin C concentration in gill and liver; and vitamin E concentration in gill and liver respectively. GG, GL, Gk represents glutathione production levels in gill, liver and kidney. WTM and WTH stands for Water temperature measured with thermometer and HANA instrument, AT_ Ambient Temperature, TDS_ Total Dissolved Solids, EC_ Electrical Conductivity, Alka_ Alkalinity, BOD_Biological Oxygen Demand, DO_ Dissolved Oxygen, Hard_ Hardness, PO_4P_ phosphate-phosphorus.



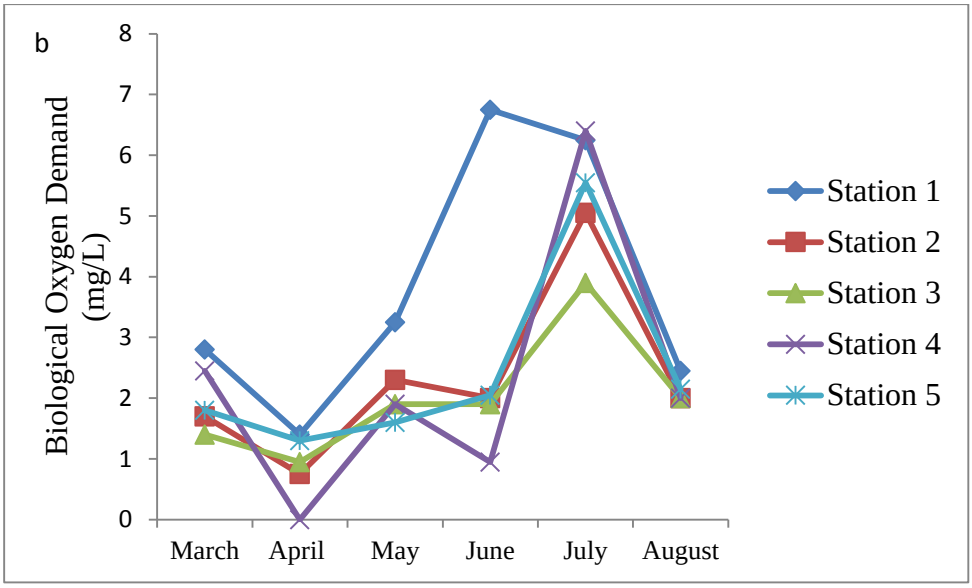


Fig.4.2: Mean monthly variations in a) Dissolved Oxygen and b) Biological Oxygen Demand among sampling stations on a stretch of River Galma, Zaria.

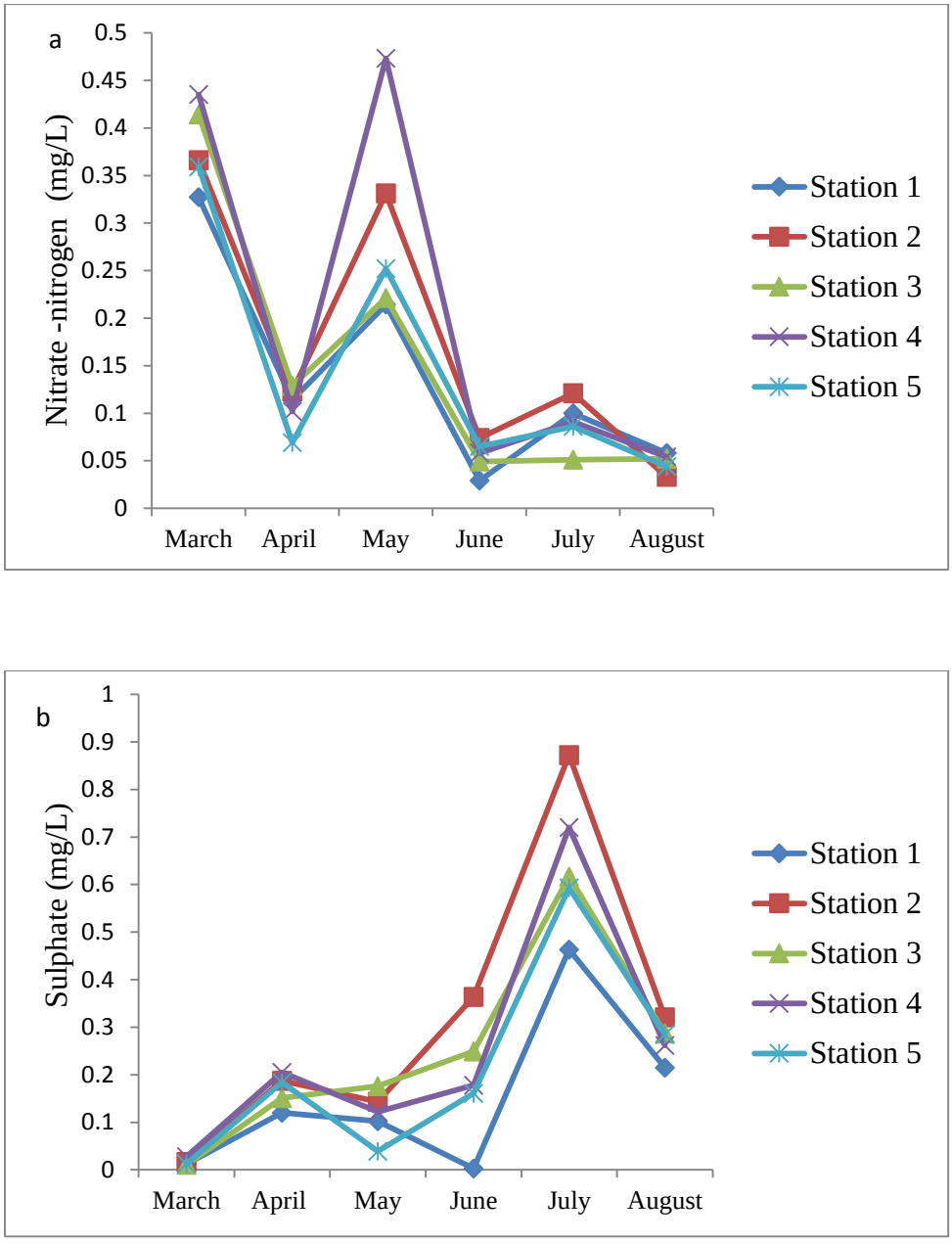


Fig.4. 3: Mean monthly variations in a) Nitrate and b) Sulphate concentration among sampling stations on a stretch of River Galma, Zaria.

4.2.7 Phoshate-phosphorus

The phosphate-phosphorus concentration had its peak value of 0.546mg/L in station 2 in the month of July, 2015. The lowest value of 0.001mg/L was obtained in station 1 in the month of June (Fig.4.4a). The phosphate-phosphorus mean value ranged from 0.03±0.01 mg/L (April) to 0.46±0.03 mg/L (July). There was no significant difference ($P>0.05$) in the phosphate-phosphorus concentration of the sampling locations. On the other hand, there was significance difference in the phosphate-phosphorus concentration in the months of sampling ($P\leq 0.05$) (Table 4.1 and 4.2). Phosphate-phosphorus had high positive correlations (0.9 and 0.9) with both GSH concentration in liver and sulphate concentration. There were also positive correlations of phosphate-phosphorus with both water temperature and BOD. Phosphate-phosphorus positively correlated with vitamin E concentration in liver (Table 4.3).

4.2.8 Hardness of Water

The hardness of water varied widely from month to month and from station to station. It had its highest value of 184mg/L in station 2 in the month of June, 2015. The lowest value of 44mg/L was obtained from station 1 in the month of July, 2015 (Fig. 4.4b). The hardness mean value ranged from 70.40±8.26 mg/L (May, 2015) to 142.40±14.73 mg/L (June, 2015).There was no significant difference ($P>0.05$) in the hardness of water among the sampling stations. There was however, significant difference in the hardness of water in the months of sampling ($P\leq 0.05$) (Table 4.1 and 4.2). Hardness of water had positive correlation with chromium concentration (Table 4.3).

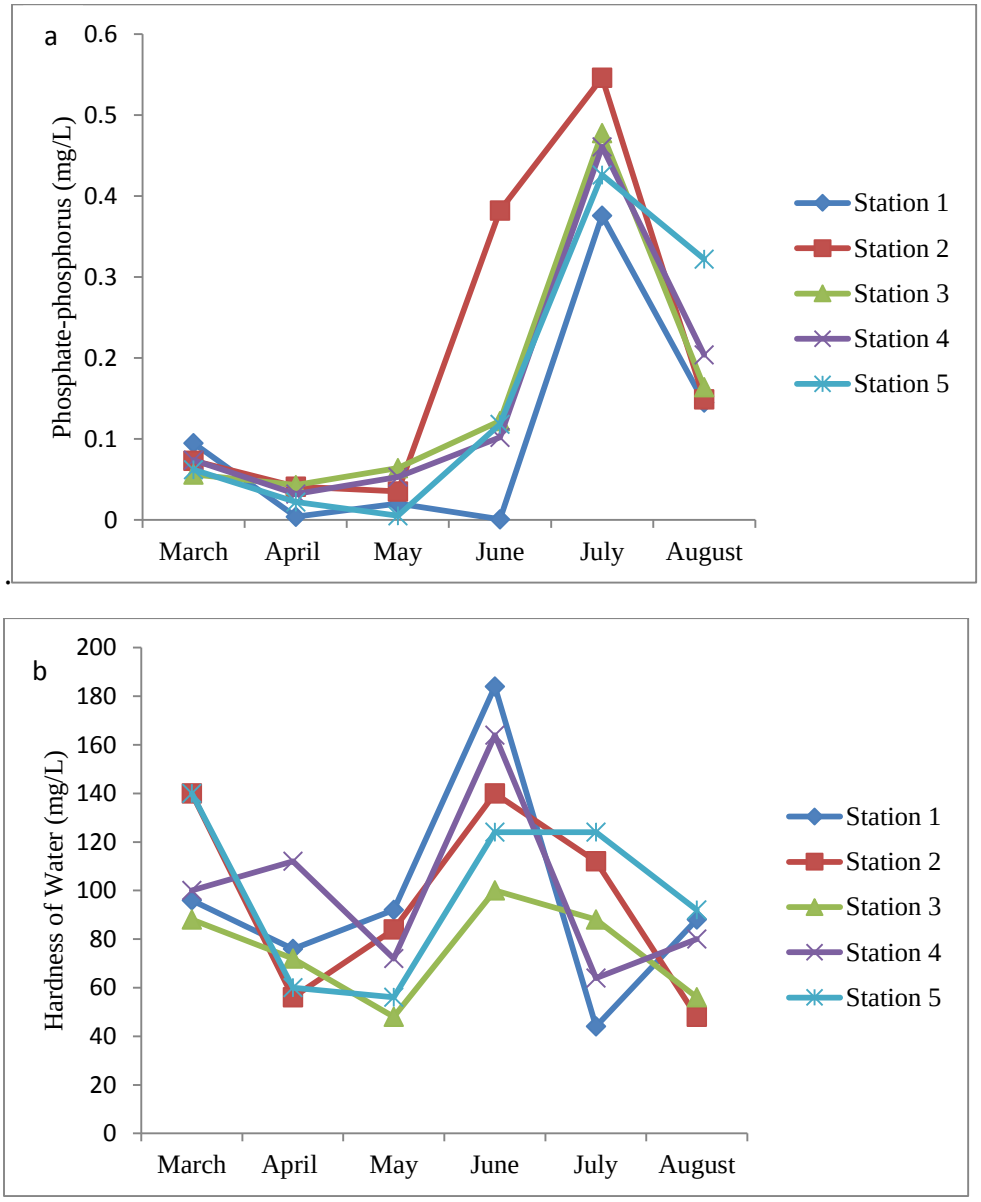


Fig. 4.4: Mean monthly variations in a) Phosphate-phosphorus and b) Hardness of water among sampling stations on a stretch of River Galma, Zaria.

4.2.9 Total Alkalinity

The highest value of 131mg/L was obtained in station 5 in the month of June, 2015. The lowest alkalinity value of 27mg/L was also obtained in the same station but in the month of July, 2015 (Fig.4.5a). The alkalinity mean value ranged from 26.80 ± 3.65 mg/L (July) to 103.20 ±11.35 mg/L (June). There was no significant difference ($P>0.05$) in the total alkalinity of the water amongst sampling locations. There was however, significance difference in the total alkalinity of the sampling months ($P\leq 0.05$) (Table 4.1 and 4.2).

Alkalinity had high positive correlation with both GSH concentration in liver and zinc concentration. There were also positive correlations with GSH concentration in gill, sulphate and phosphate levels. It had negative correlations with DO and hardness of water. There was also high negative correlation (-0.7) with BOD. Alkalinity negatively correlated with vitamin C concentration in gill. It had positive correlations with both vitamin E concentrations in gill and liver of the fish (Table 4.3).

4.2.10 Electrical conductivity

The Electrical Conductivity also varied widely from month to month and from station to station. Bimodal peak EC values of 316µS/cm were obtained from stations 4 and 5 in the month of June, 2015. The lowest value of 52µS/cm was obtained in station 1 in the month of August, 2015 (Fig.4.5b). The electrical conductivity mean value ranged from 65.40 ± 10.26 µS/cm (July, 2015) to 219.40 ± 46.80 µS/cm (June, 2015). Likewise, electrical conductivity mean values ranged from 83.33 ± 15.20 µS/cm (station 2) to 181.17 ± 39.82 µS/cm (station 4). There were significant

differences in the electrical conductivity of both the sampling locations and months ($P \leq 0.05$) (Table 4.1 and 4.2).

The Electrical conductivity had positive correlations with GSH concentration in gill, alkalinity and TDS. There was also negative correlation with BOD. Electrical conductivity had high negative correlation (-0.9) with vitamin C concentration in gill. It was also positively correlated with both vitamin E concentrations in gill and liver (Table 4.3).

4.2.11 Total dissolved solids

The peak value of 158mg/L was obtained for total dissolved solids in station 4 in the month of March, 2015. While the lowest value of 31mg/L in the month of July, 2015 in station 1 (Fig.4.6a). The total dissolved solids mean values ranged from 35.40±3.04mg/L (July) to 120.80±18.85mg/L (June). There was no significant difference ($P>0.05$) in the total dissolved solids among the sampling locations. There was however, significant difference in the total dissolved solids in the months of sampling ($P \leq 0.05$) (Table 4.1 and 4.2).

Total Dissolved Solids (TDS) of the water had high positive correlations (0.8, 0.9 and 0.9 respectively) with GSH concentration in liver, zinc and alkalinity. There were also positive correlations with GSH concentration in gill, kidney, Cr, N, sulphate and phosphate-phosphorus concentrations. On the other hand, TDS was negatively correlated with DO. Total dissolved solids had high negative correlation (-0.9) with vitamin C concentration in gill. On the other hand, it had positive correlations with both vitamin E concentrations in gill and liver (Table 4.3).

The principal component analyses of the physico-chemical parameters of River Galma indicated positive loading of the water temperature, pH, electrical conductivity, and phosphate-phosphorus

to right upper quarter of component 1 concentrating around the month of August, 2015. The dissolved oxygen and total dissolved solids were negatively correlated (Fig.4.7).

4.2.12 pH

There were temporal and spatial variations in pH of River Galma during the period under study. The highest pH value of 8.82 was obtained in station 3 in the month of June, 2015. While the lowest pH value of 4.1 was obtained in the month of August in the same station (Fig. 4.6b). The hydrogen ion concentration (pH) mean value ranged from 4.45±0.13 (August) to 8.72±0.05 (June). There was no significant difference ($P>0.05$) in pH mean values amongst the sampling locations. While there was significance difference in the months of sampling ($P\leq 0.05$) (Table 4.1 and 4.2).

The pH correlated positively with both electrical conductivity and GSH concentrations in gill. It also had positive correlation with GSH concentration in kidneey. pH negatively correlated with both vitamin C concentrations in gill and liver. It was also positively correlated with vitamin E concentration in gill (Table 4.3).

The combine canonical correspondence analyses of all the parameters except the heavy metals only indicated positive interactions in the physico-chemical parameters such as water and air temperatures, alkalinity, biological oxygen demand, dissolved oxygen, electrical conductivity, nitrate and harness of water (Fig. 4.8). Likewise, when all the parameters were analyzed similar results were also obtained (Fig. 4.10).

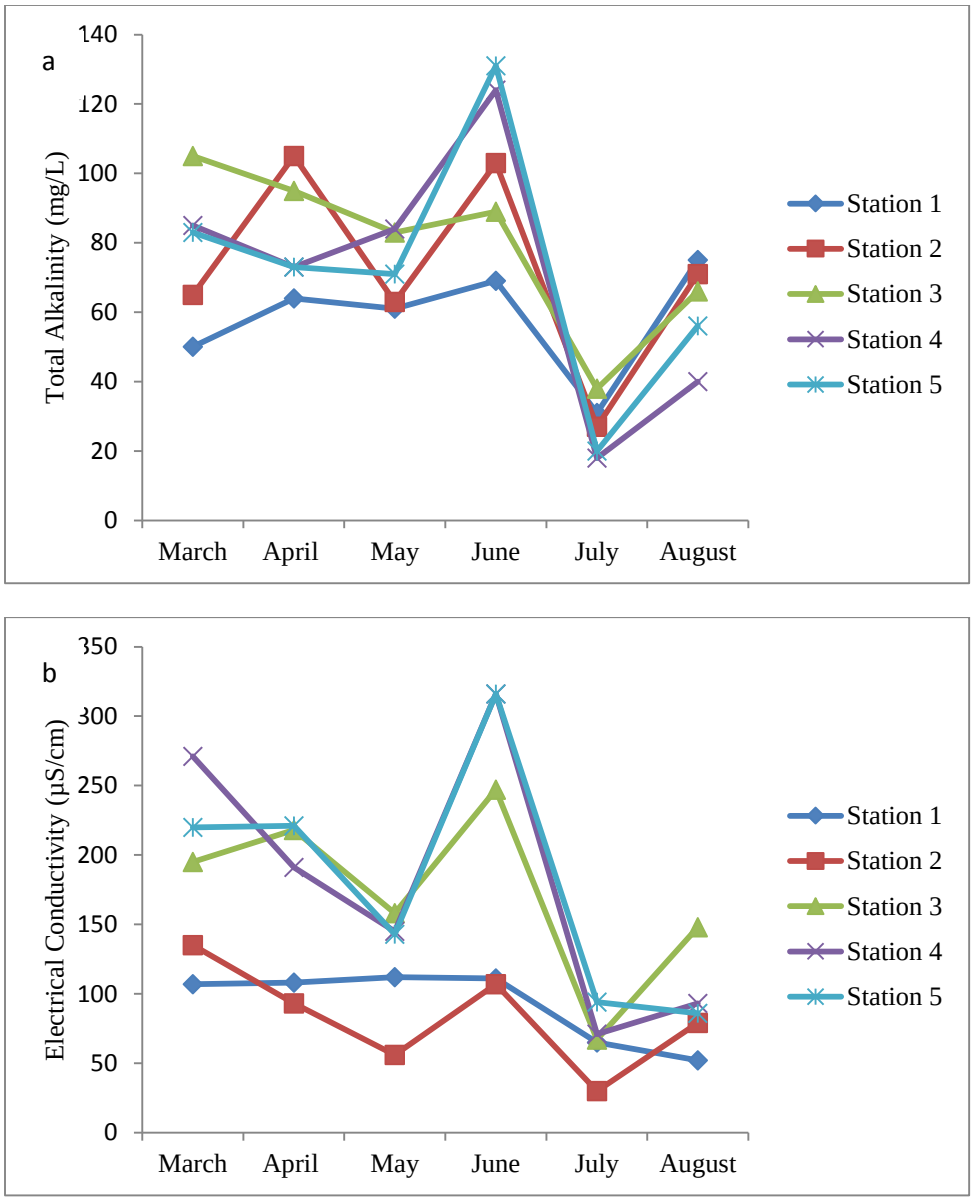
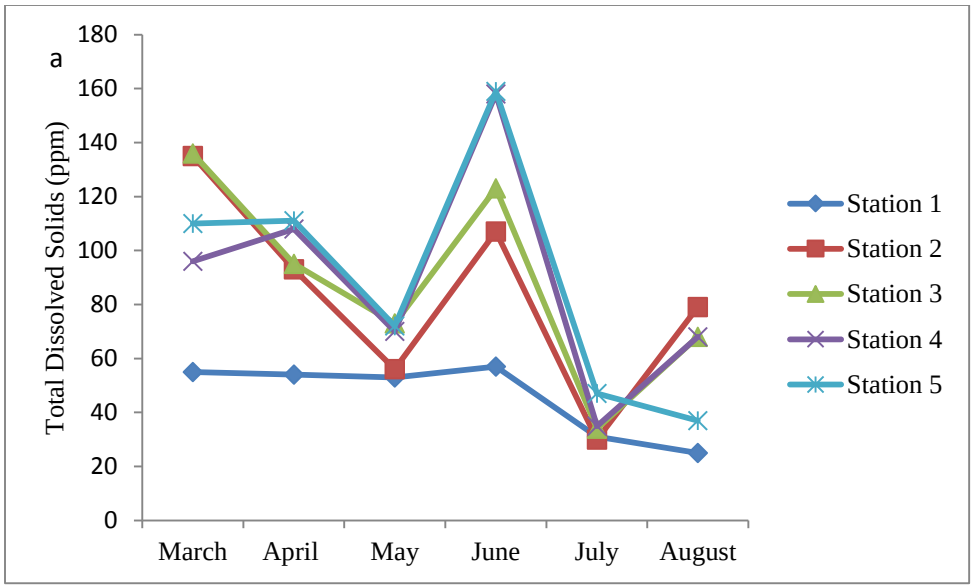


Fig. 4.5: Mean monthly variations in a) Total Alkalinity and b) Electrical conductivity among sampling stations on a stretch of River Galma, Zaria.



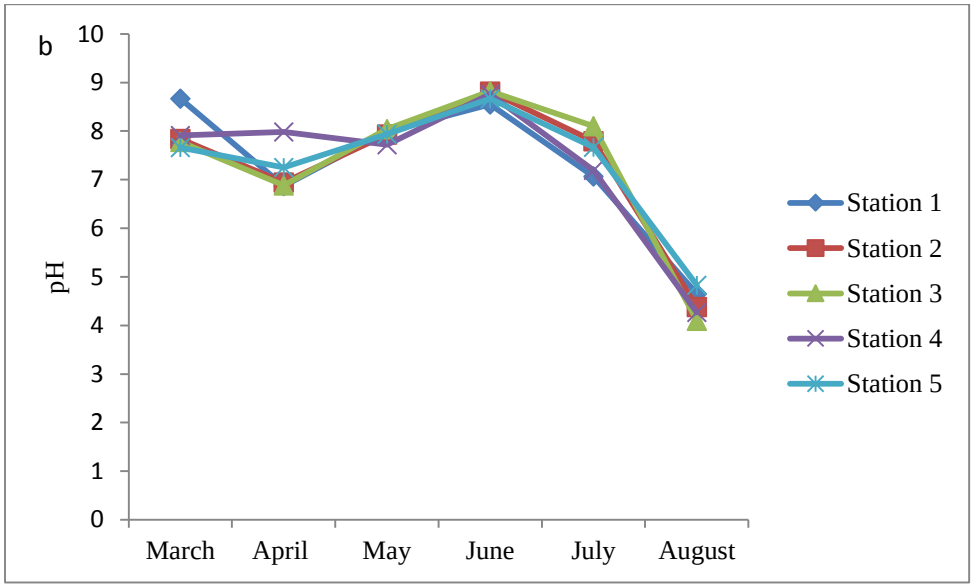


Fig. 4.6: Mean monthly variations in a) Total Dissolved Solids and b) pH among sampling stations on a stretch of River Galma, Zaria.

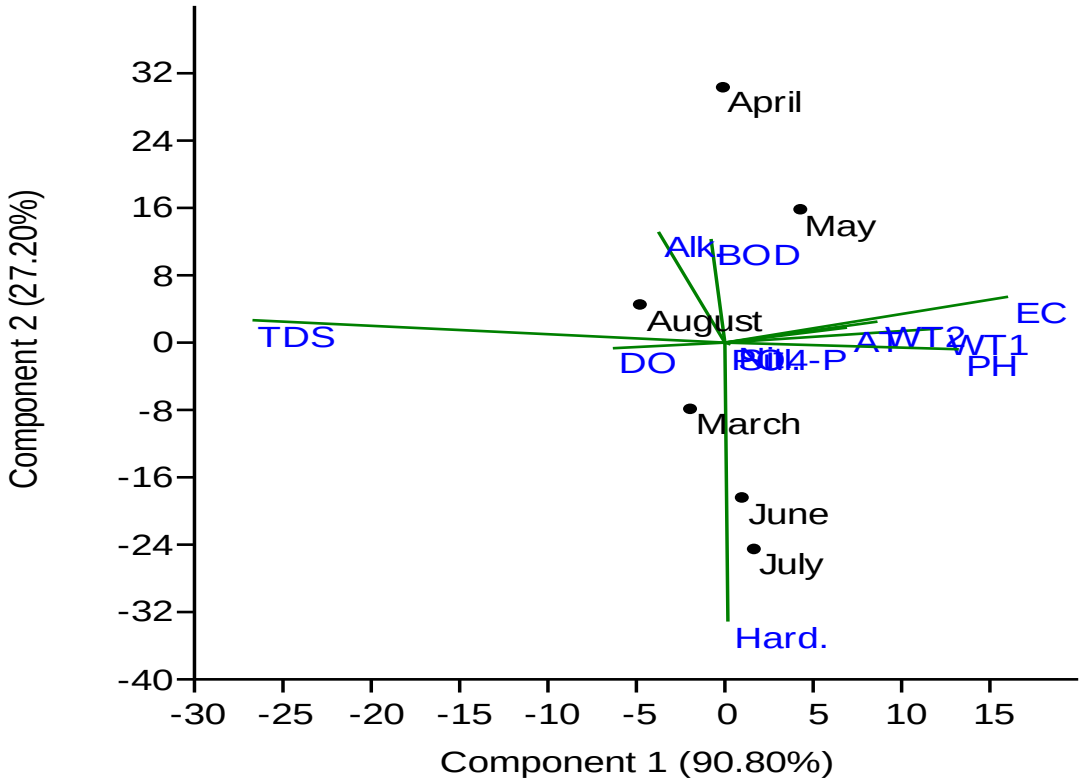


Fig. 4.7: Principal Component Analysis of Physicochemical Parameters obtained from river Galma, Zaria

Key: WT = Water Temperature, DO = Dissolved Oxygen, Nit. = Nitrate, Hard. = Hardness, Sul. = Sulphate, Alk. = Alkalinity, PO4-P = Phosphate Phosphorous, TDS = Total Dissolved Solid, EC = Electrical Conductivity.

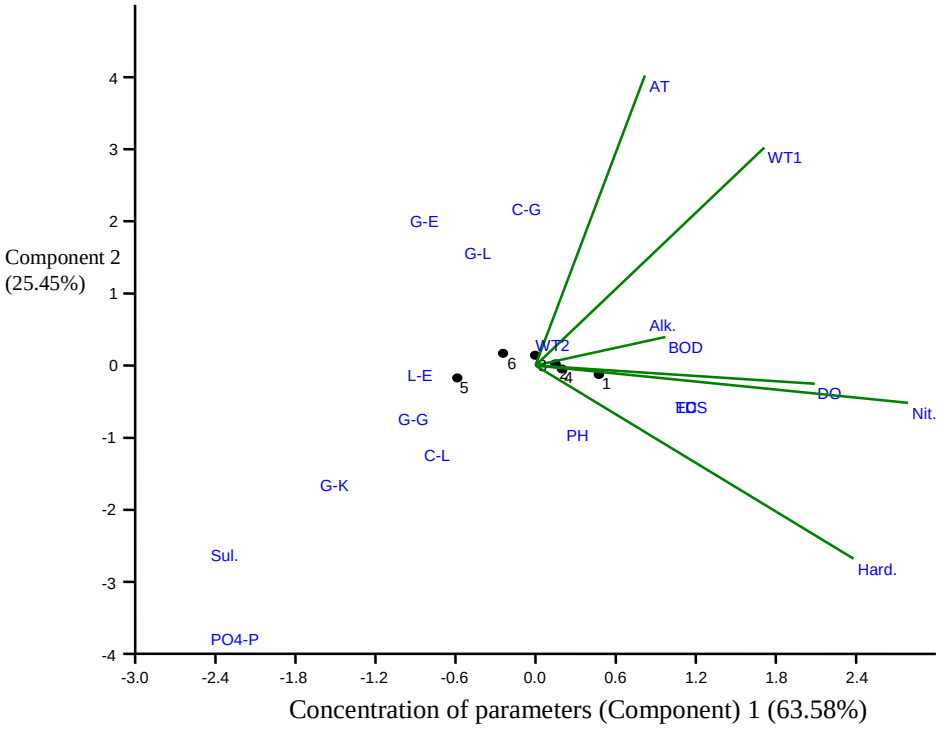


Fig. 4.8: Combined Canonical Correspondence Analysis of Physico-chemical parameters, Vitamins C and E and Glutathion concentrations

NB: WT = Water Temperature, DO = Dissolved Oxygen, Nit. = Nitrate, Hard. = Hardness, Sul. = Sulphate, Alk. = Alkalinity, PO4-P = Phosphate Phosphorous, TDS = Total Dissolved Solid, EC = Electrical Conductivity.G-K (glutathione concentrations in kidney), G-L (glutathione concentrations in liver), G-G (glutathione concentrations in gill), C-L (vitamin C concentration in liver), C-G (vitamin C concentrations in gill), L-E (vitamin E concentration in liver and gill), G-E (vitamin E concentration in gill).

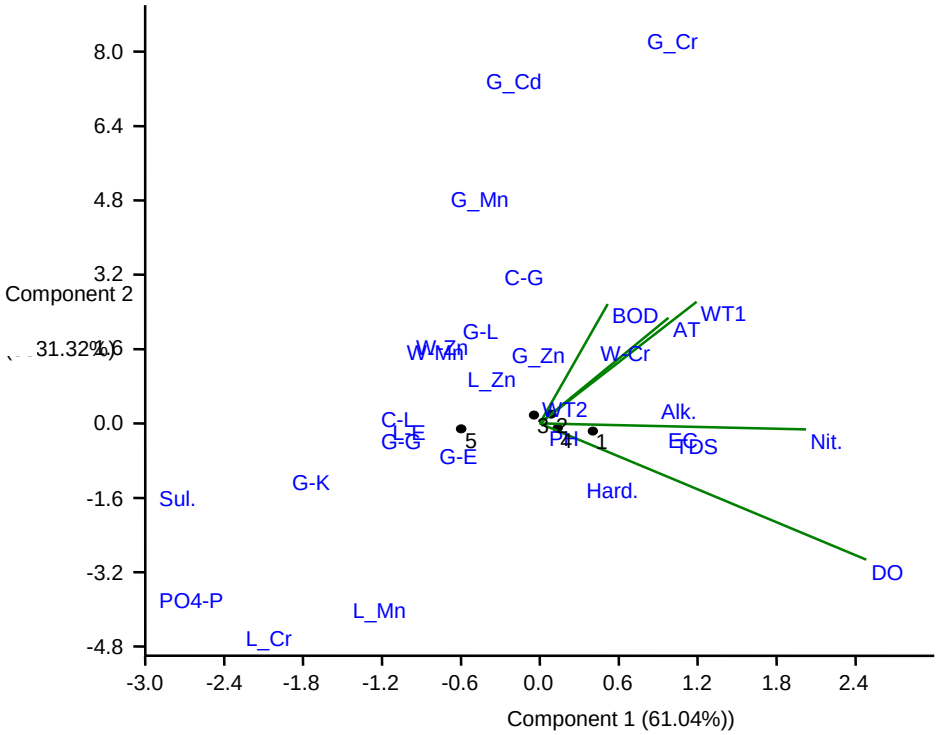


Fig. 4.9: Combined Canonical Correspondence Analysis of Heavy metals, Physico-chemical parameters, Glutathione and Vitamins C and E

NB: WT = Water Temperature, DO = Dissolved Oxygen, Nit. = Nitrate, Hard. = Hardness, Sul. = Sulphate, Alk. = Alkalinity, PO4-P = Phosphate Phosphorous, TDS = Total Dissolved Solid, EC = Electrical Conductivity. G-K (glutathione concentrations in kidney), G-L (glutathione concentrations in liver), G-G (glutathione concentrations in gill), C-L (vitamin C concentration in liver), C-G (vitamin C concentrations in gill), L-E (vitamin E concentration in liver and gill), G-E (vitamin E concentration in gill). G_Mn (Mn concentration in gill), G_Cr (Cr concentration in gill),G_Zn (Zinc concentration in gill), L_Mn (Mn concentration in liver), L_Zn (concentration in liver), L_Cr (Cr concentration in liver), W_Cr (Cr concentration in water), W_Mn (concentration in water), W_Zn (Zn concentrations in water).

4.3 Heavy Metal Components of River Galma and Fish Organs

4.3.1 Lead

Lead was not detected in samples of water collected from River Galma during the period of the study. Likewise, lead was not detected in gill and liver of the samples of fish in the August and March, 2015 exposures (Figs. 4.10, 4.11 and 4.12; and Table 4.4).

4.3.2 Cadmium

Cadmium was not detected in water samples obtained from River Galma both in dry and wet seasons. Cadmium was only detected in the gills and livers of the fishes exposed in March. The highest concentration in the gill of the fish was 0.009 mg/L in station 3. The same concentration of 0.008mg/L in the gill of the fish was obtained in stations 2 and 4. The lowest concentration of 0.006mg/L was obtained in station 1. Cadmium was however, not detected in the gill of the fish exposed in station 5. On the other hand, the liver of the fish exposed in the dry season had the highest concentration of 0.008mg/L in both stations 2 and 3. Stations 3 and 4 had 0.007mg/L concentration in the liver of the fish within the same period. Cadmium was not detected in fishes from wet season exposure. There was significance difference ($P \leq 0.05$) in the cadmium concentration in both liver and gill of the sample (Table 4.4). There were significant differences ($P \leq 0.05$) in Cd concentrations during the months of exposure. There were no significant differences ($P>0.05$) in heavy metals of the water samples in all the locations and Months of sampling (Fig. 4.13).

Table 4.4: Seasonal heavy metal concentrations in of organs *Clarias gariepinus* in an *in situ* exposure in River Galma, Zaria

Fish organ/Months	**Pb (mg/L)**	**Cr (mg/L)**	**Cd (mg/L)**	**Zn (mg/L)**	**Mn (mg/L)**
Gills					
March (Dry season)	0.00±0.00	0.03±0.02	0.01±0.00	0.06±0.02	0.05±0.04
August (Wet season)	0.00±0.00	2.00±2.00	0.00±0.00	2.02±0.36	0.13±0.13
Total	**0.00±0.00**	**1.02±1.00**	**0.00±0.00**	**1.04±0.37**	**0.09±0.07**
P value		**0.353ns**	**0.005***	**0.001***	**0.562ns**
Liver					
March (Dry season)	0.00±0.00	0.02±0.02	0.01±0.00	0.05±0.01	0.05±0.05
August (Wet season)	0.00±0.00	4.00±2.45	0.00±0.00	2.11±0.35	0.27±0.11
Total	**0.00±0.00**	**2.01±1.33**	**0.00±0.00**	**1.08±0.38**	**0.16±0.07**
P value		**0.143ns**	**0.004***	**0.000***	**0.105ns**

Mean values are significant at $P \leq 0.05$ and not significant (ns) at $P > 0.05$

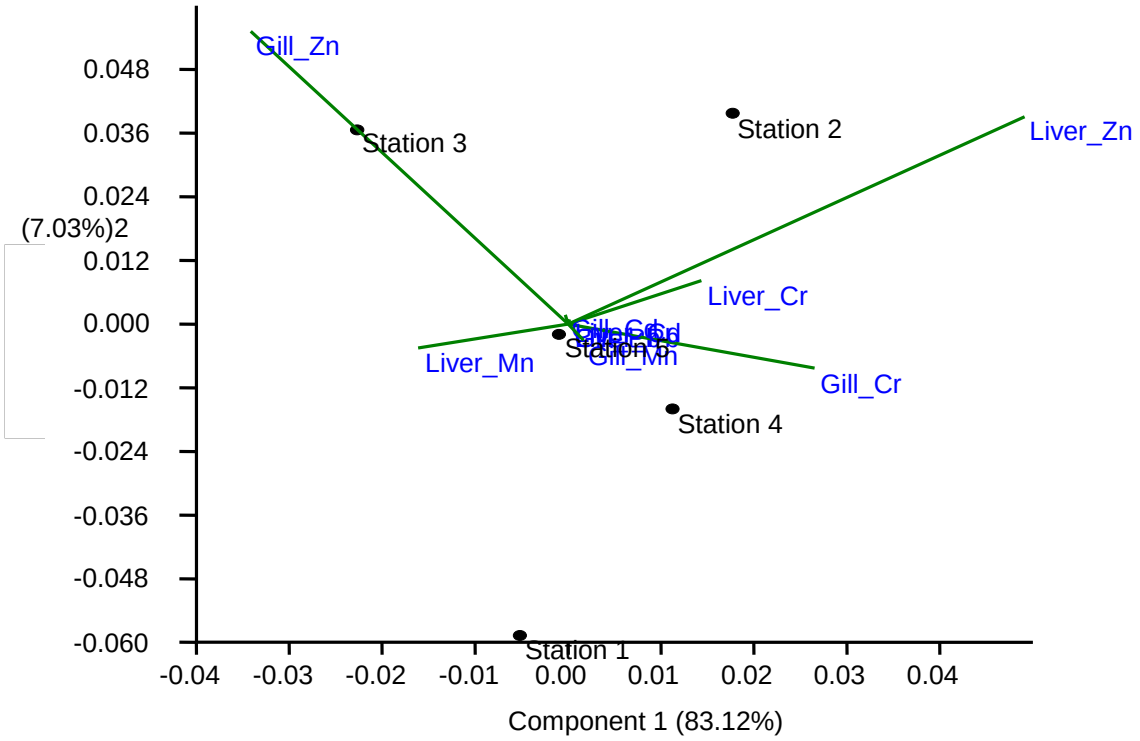


Fig. 4.10: Interraction of heavy metals in fish organs of *Clarias gariepinus* exposed in March, 2015.

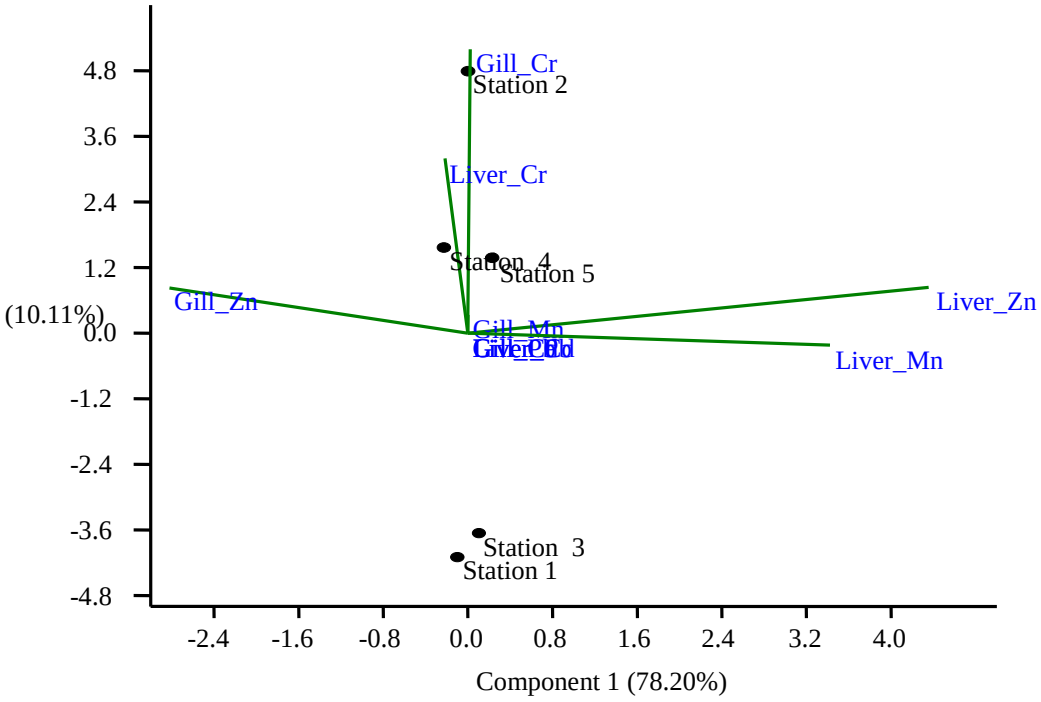


Fig. 4.11: Interraction of heavy metals in fish organs of *Clarias gariepinus* exposed in August, 2015.

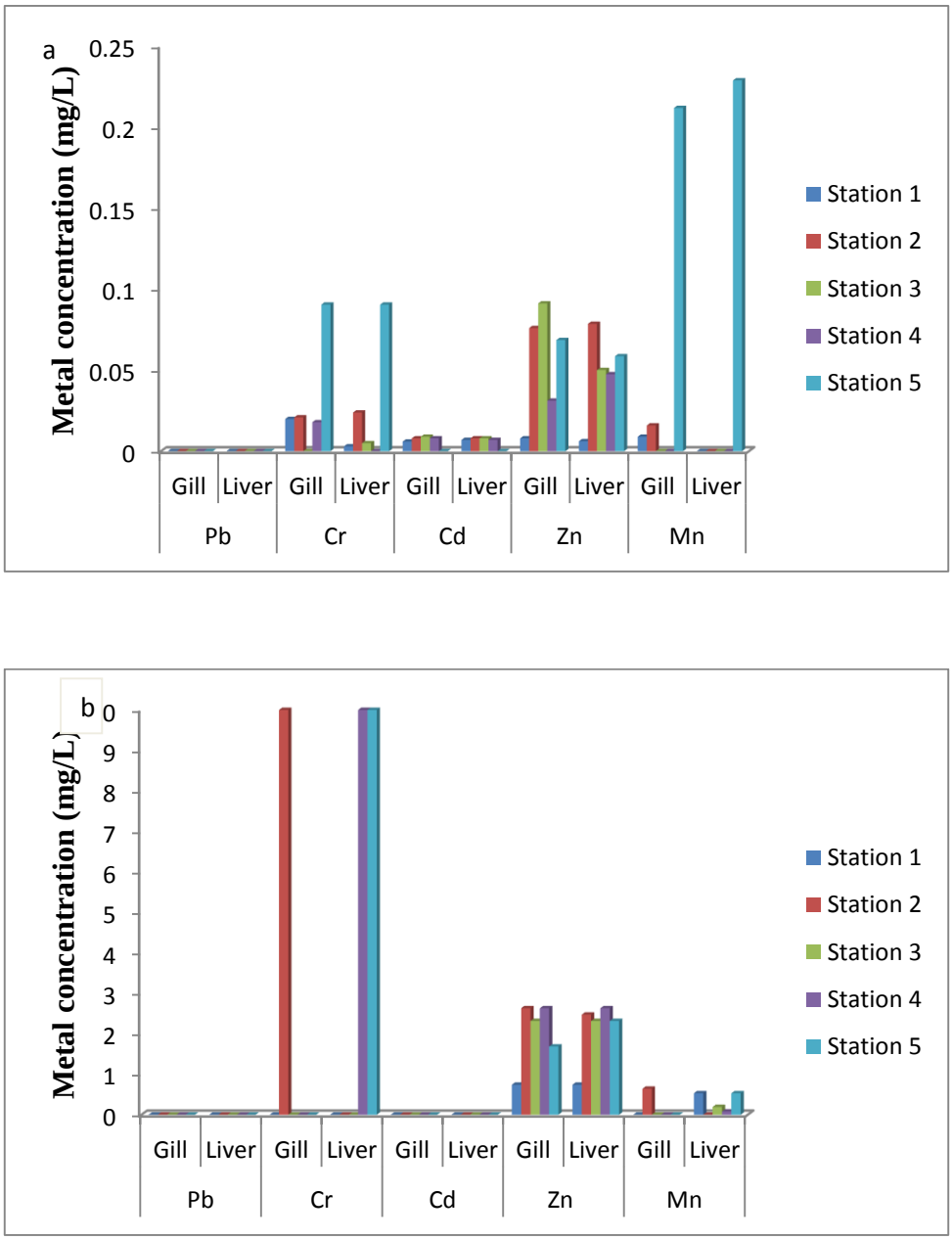


Fig. 4.12: Combined heavy metal concentrations in organs of *Clarias gariepinus* exposed for 14 days in a) March and b) August in an *in situ* bioassay along a stretch of River Galma, Zaria

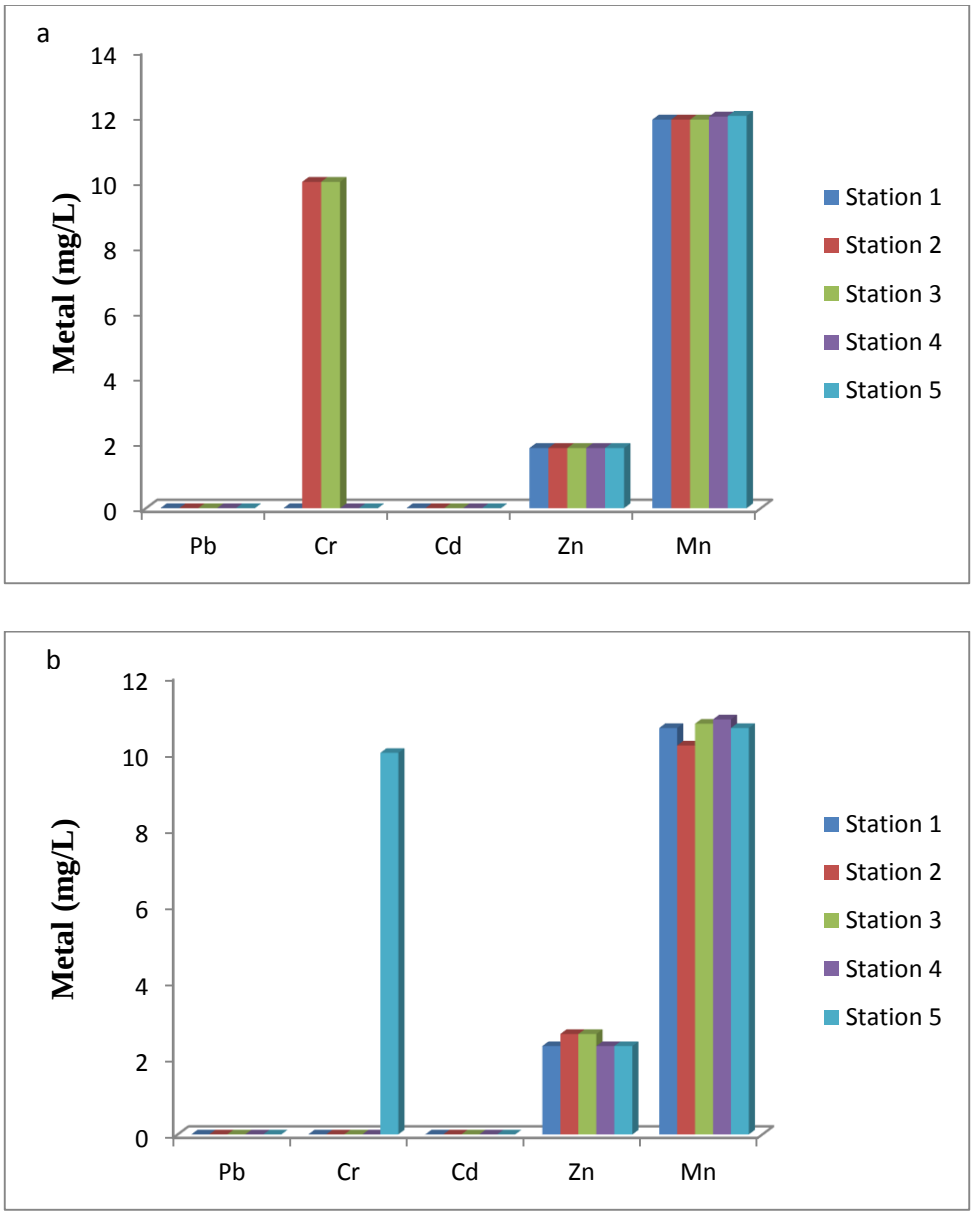


Fig. 4.13: Seasonal Heavy metal concentrations in water samples along a stretch of River Gaalma in a) dry season (March-May) and b) wet season (June-August)

4.3.3 Chromium

Cr had a uniform concentration of 10mg/L in water samples from stations 2 and 3 during the dry season and the same concentration at station 5 during the wet season. The same concentration of 10mg/L was detected in the gill of the fish exposed at station 2 during the wet season. The highest concentration of 0.091mg/L was detected at station 5 in the gill of the fish while 0.018mg/L at station 4 was the lowest during the dry season. Likewise, 0.091mg/L at station 5 was the highest in the liver of the sample. The lowest concentration of 0.024mg/L was detected at station 2 during the dry season. Stations 4 and 5 had concentration of 10mg/L during the wet season. Cr was not detected in station 3 in wet seasons. There were no significance differences ($P>0.05$) in chromium concentrations in all the fishes exposed in both March and August. Cr mean values were uniform in the sites (stations 2, 4 and 5) where they were detected (Figs. 4.12 and 4.13). Also, there were no significant differences ($P>0.05$) in the pooled water samples in all the locations and Months of sampling (Table 4.4).

The Cr concentration was positively correlated with glutathione concentration in gill. It was also positively correlated with GSH concentration in kidney. Cr had positive correlations with vitamin E concentrations in gill and liver (Table 4.3).

4.3.4 Zinc

Zinc concentration in water had its highest concentration at station 2 and 3 with 2.651mg/L while stations 1, 4 and 5 had a uniform concentration of 2.33mg/L during the wet season. Similarly, stations 1-5 had uniform zinc concentration of 1.857mg/L during the dry season. The gills of the fishes exposed in River Galma during the dry season had 0.0917mg/L in station 3 as the highest and 0.031mg/L in station 4 as the lowest. The highest zinc concentration of 2.651mg/L was

obtained in stations 2 and 4 with 0.746mg/L in station 1 as the lowest in the gill of the fish during the wet season. Similarly, the highest zinc concentration in the liver of the fish was 2.651mg/L at station 4 with 0.746mg/L in station 1 as the lowest during the wet season. However, the highest concentration of 0.0791mg/L in station 2 with 0.0062mg/L in station 1 as the lowest was obtained in the liver of the fish during the dry season. There were significance differences ($P \leq 0.05$) in the zinc concentration in all the exposure. However, there were no significance difference ($P > 0.05$) in all the locations of the exposure of the fish in both August and March, 2015 (Table 4.4). There were significance differences ($P \leq 0.05$) in Zn concentrations during the months of exposure.

There were no significant differences ($P > 0.05$) in the concentration of heavy metals in all the fish organs. The zinc concentration mean values in gill ranged from 0.06±0.02mg/L (March) to 2.02±0.36mg/L (August). Likewise Zinc concentration mean values in liver ranged from 0.05±0.01mg/L (March) to 2.11±0.35mg/L (August). There were significance differences ($P \leq 0.05$) in ZN concentrations in the water samples from all the locations during the Months of sampling. Highest mean values of zinc concentrations were obtained from stations 2 and 4 (Figs. 4.12 and 4.13).

Zinc was positively correlated with GSH concentration in liver. It was also positively correlated with Cr concentration. Zinc negatively correlated with vitamin C concentration in gill. On the other hand, zinc positively correlated with both vitamin C concentration in liver and vitamin E concentration in gill. It also had high positive correlation (0.9) with vitamin E concentration in liver (Table 4.3).

4.3.5 Manganese

Manganese concentrations in water samples of River Galma were relatively high both in the dry and wet seasons. The highest concentration of 12.01mg/L was recorded in station 5; stations 1-3 had uniform concentration of 11.89mg/L during the dry season. The highest concentration of 10.88mg/L was obtained in station 4 during the wet season. The lowest concentration of 10.19mg/L was obtained in station 2. Stations 1 and 5 had a uniform concentration of 10.648mg/L during the same period. On the other hand, manganese concentrations detected in the fish organs were relatively low both in the dry and wet seasons. The highest concentration obtained in the gill of the fish was 0.212mg/L at station 5 with the lowest concentration of 0.009mg/L at station 1 during the dry season. Manganese was not detected in stations 3 and 4 during the same period. Manganese was only detected in station 2 with 0.648mg/L concentration in the gill of the fish during the wet season. Likewise, manganese was only detected in station 5 with concentration of 0.229mg/L in the liver of the fish during the dry season. The highest concentration of 0.534mg/L was obtained in stations 1 and 5 with lowest concentration of 0.008mg/L in station 4 in the liver of the fish during the wet season. Manganese was not detected in station 2 during the same period. There were significant differences ($P \leq 0.05$) in Mn concentrations of water samples from all the locations during the Months of sampling. The highest mean values of manganese (mg/L) were detected in station 2 (Table 4.4, Fig. 4.12).

Manganese (Mn) was positively correlated with concentration of GSH in kidney. Manganese negatively correlated with both vitamins C and E concentrations in liver (Table 4.3). The heavy metals exhibited strong interaction in the gills of the fish exposed in station 5 in March, 2015 (Fig. 4.10). There was positive uptake of heavy metals in gills (Mn and Zn) around stations 3 and 5 in August, 2015 exposure with other metals showing little interactions (Fig. 4.11).

4.4 Non-enzymatic Antioxidants Production Levels in Organs of *Clarias gariepinus*

4.4.1 Glutathione

The levels of glutathione production in organs of *Clarias gariepinus* in *in situ* bio-assay in River Galma exhibited variations both in the dry and wet seasons. During the dry season, the gill of the fish after 7-day exposure, produced peak concentration of 187.23µg/mL at station 5. The lowest concentration of 79.43µg/mL was produced in fish exposed at station 3. There was general increase in the concentration of glutathione produced at the 14th day with the exception of station 1 which produced the lowest on this date with 22.7µg/mL. The production level at station 5 increased to 289.21µg/mL. On the other hand, during the wet season the highest glutathione production of 60.99µg/mL was obtained at station 3 on the 7th day in the gill of the fish. The lowest was obtained from the samples from station 1 at 4.26µg/mL concentration. However, on the 14th day of the exposure the glutathione production level at station 3 increased to 113.48µg/mL. The lowest concentration of 96.45µg/mL was obtained in station 2. There was increase in the glutathione production on the 14th day of exposure (Table 4.5, Fig. 4.16). The glutathione concentration in the gills of the samples ranged from 43.265±29.250µg/mL (station 1) to 154.570 ± 29.250µg/mL (station 5) (Table 4.6, Fig. 4.16).

From the Spearman's Correlation Coefficient Analysis the glutathione (GSH) concentration in gill of the fish (*Clarias gariepinus*) was positively correlated (0.9) with glutathione concentration in kidney (Table 4.3). The principal component analyses showed that the gills exhibited negative correlations in stations 5 and 4 in the March, 2015 exposure (Fig. 4.12). There were positive correlations in the gills at the 7th day and positive interaction in both gill and liver at the 14th day in stations 3, 4 and 2 in the August, 2015 exposure. On the other hand, there were negative interactions among gill glutathione and Cr and Mn concentrations (Fig. 4.14).

Table 4.5: Non- Enzymatic Antioxidants Produced in *Clarias gariepinus* Exposed *in situ* in River Galma, Zaria (µg/mL)

Parameters	Station 1			Station 2			Station 3			Station 4			Station 5		
	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD
Gill cont.			**104.9±0.00**												
			84.4±87.32												
GSH (dry sn_Gill)	22.7	146.1		96.45	116.31	106.4±14.04	79.43	95.04	87.2±11.04	80.85	139.01	109.9±42.13	187.23	289.21	238.2±72.11
			117.73±0.00												
Liver cont.															
GSH (dry sn_Liver)	15.6	69.5	42.6±38.11	48.23	177.3	112.8±91.26	29.79	217.02	123.4±132.39	25.53	68.09	46.8±30.09	76.59	131.92	104.3±39.12
Kidney co.			**130.49±0.00**												
			27.7±19.05												
GSH (dry sn_Kidney)	14.18	41.13		35.46	76.6	56.0±29.09	15.6	25.53	20.6±7.02	21.28	48.23	34.8±19.05	87.8	99.15	93.5±8.03
GSH (Wet sn_Gill)	0	4.26	2.1±3.01	8.51	96.45	52.5±62.18	60.99	113.48	87.2±37.11	56.74	109.22	82.9±37.11	41.13	100.71	70.9±42.19
GSH (Wet sn_Liver)	0	5.6	2.8±3.95	1.42	96.45	48.9±67.19	15.6	43.97	29.8±20.06	26.95	70.92	48.9±31.09	15.6	45.39	30.5±21.06
GSH (Wet sn_Kidney)	0	22.7	11.4±16.05	9.93	48.23	29.1±27.08	11.35	56.74	34.0±32.09	39.72	75.18	57.5±25.07	17	190.07	103.5±122.38
Vc gill			**0.98±0.00**												
Vitamin C (Gill)	0	16.919	8.5±11.96	0.398	13.666	7.0±9.38	0.595	8.654	4.6±5.69	0.564	6.774	3.7±4.39	0.449	8.149	4.3±5.44
Vc liver			**1.39±0.00**												
Vitamin C (liver)	0	5.183	2.6±3.66	0.516	12.152	6.3±8.23	0.577	10.179	5.4±6.79	0.529	13.121	6.8±8.90	0.294	1.871	1.1±1.11
Ve gill			**13.58±0.00**												
Vitamin E (Gill)	0	29.434	14.7±20.81	30.793	34.749	32.8±2.79	35.236	49.462	42.3±10.06	33.202	41.96	37.6±6.19	33.809	102.35	68.1±48.47
Ve liver			**13.68±0.00**												
Vitamin E (Liver)	0	44.523	22.3±31.48	33.843	38.559	36.2±3.33	32.092	35.911	34.0±2.70	37.201	58.237	47.7±14.87	25.641	38.978	32.3±9.43

Bolded mean standard values are the non-enzymatic antioxidants produced in *Clarias gariepinus* prior to exposure in River Galma.

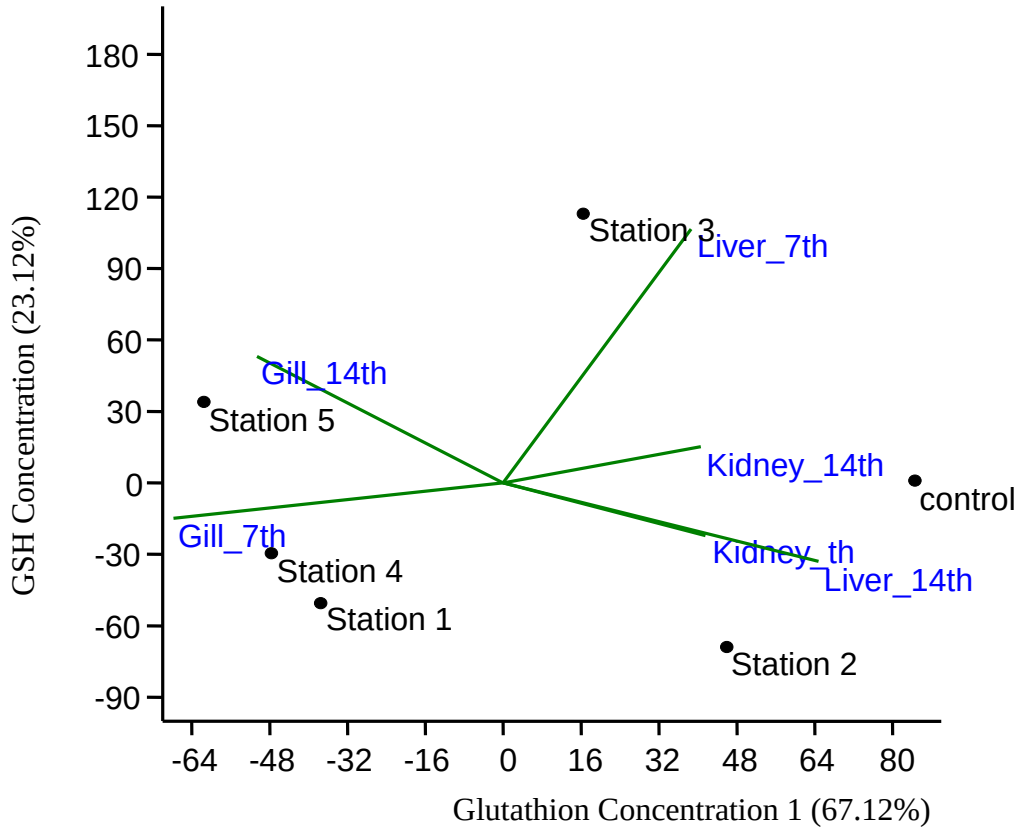


Fig. 4.14: Principal Component Analysis of Glutathion Production Levels in *Clarias gariepinus*Fish Exposed *in situ* in River Galma for 7 and 14 days in March.

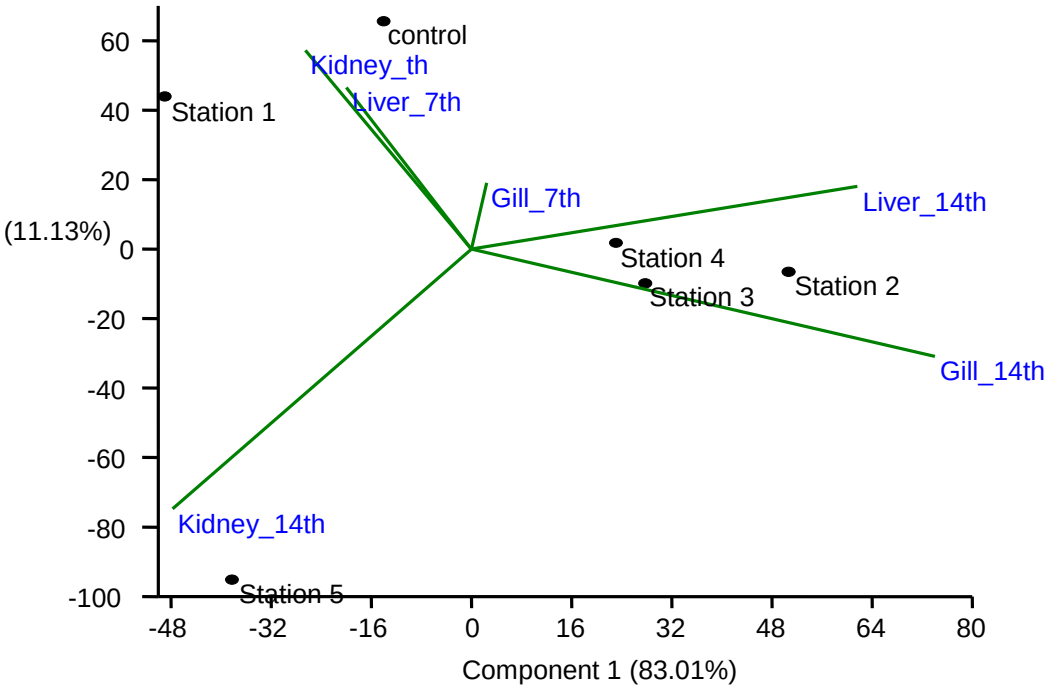


Fig. 4.15: Principal Component Analysis of Glutathion Production Level in *Clarias gariepinus* Exposed *in situ* in River Galma for 7 and 14 days in August.

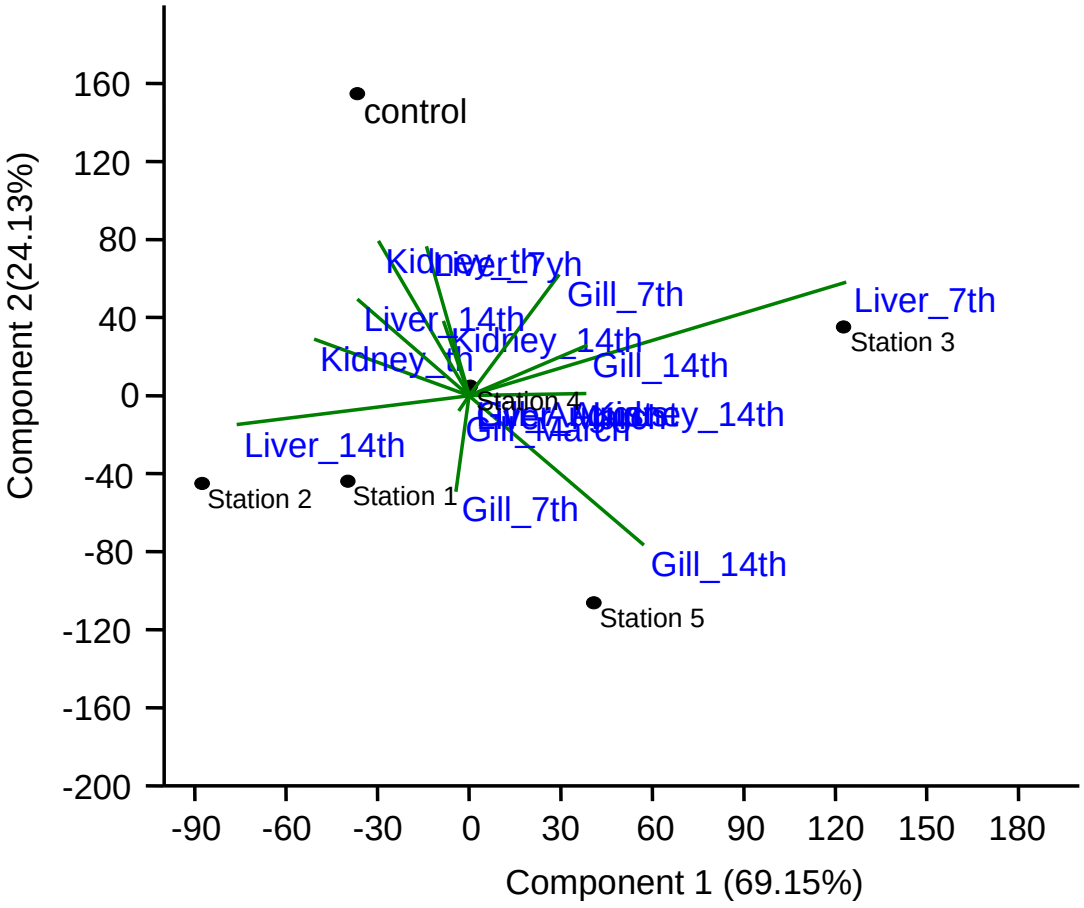


Fig. 4.16: Combined Principal Component Analysis of Glutathione Production Levels in *Clarias gariepinus* in *in situ* exposure in River Galma for both March and August.

In the liver of the fish, the highest concentration of 217.02µg/mL was obtained from station 3 on the 7th day during the dry season. The lowest concentration of 68.09µg/mL was obtained in station 4 during the same period. There were drastic reduction in the glutathione production levels in stations 1, 3 and 4 on the 14th day of exposure. The glutathione level decreased from 69.503µg/mL to 15.603µg/mL in station 1, 217.023µg/mL to 29.793µg/mL in station 3, 68.093µg/mL to 25.533µg/mL in station 4 respectively. At station 2 however, the level of production increased from 48.23µg/mL to 177.3µg/mL. There were generally low levels of glutathione production level during the wet season in comparison to what were obtained during the dry season. The lowest concentration of 1.42µg/mL was obtained in station 2. The peak concentration of 26.95µg/mL was obtained in samples from station 4 on the 7th day. There were however, increases in the levels of glutathione production on the 14th day. The highest concentration of 96.45µg/mL was recorded in station 2 while the lowest was obtained in station 5 with a concentration of 45.39µg/mL in the wet season (Table 4.6, Fig.4.17). The glutathione concentration in the liver of the sample ranged from 22.693±27.877µg/mL (station 1) to 80.850±27.877µg/mL (station 2) (Table 4.12).

The principal component analyses indicated that the glutathione production in the liver at the 7th day exposure exhibited strong positive correlation in station 3. There were positive interactions among the glutathione concentrations in the liver and the heavy metals_ Zn, Cr and Mn. It also had negative interactions among gill glutathione and Cr and Mn (Fig. 4.14). There were also interactions of glutathione produced in the liver with both nitrate concentration and dissolved oxygen (Fig. 4.16).

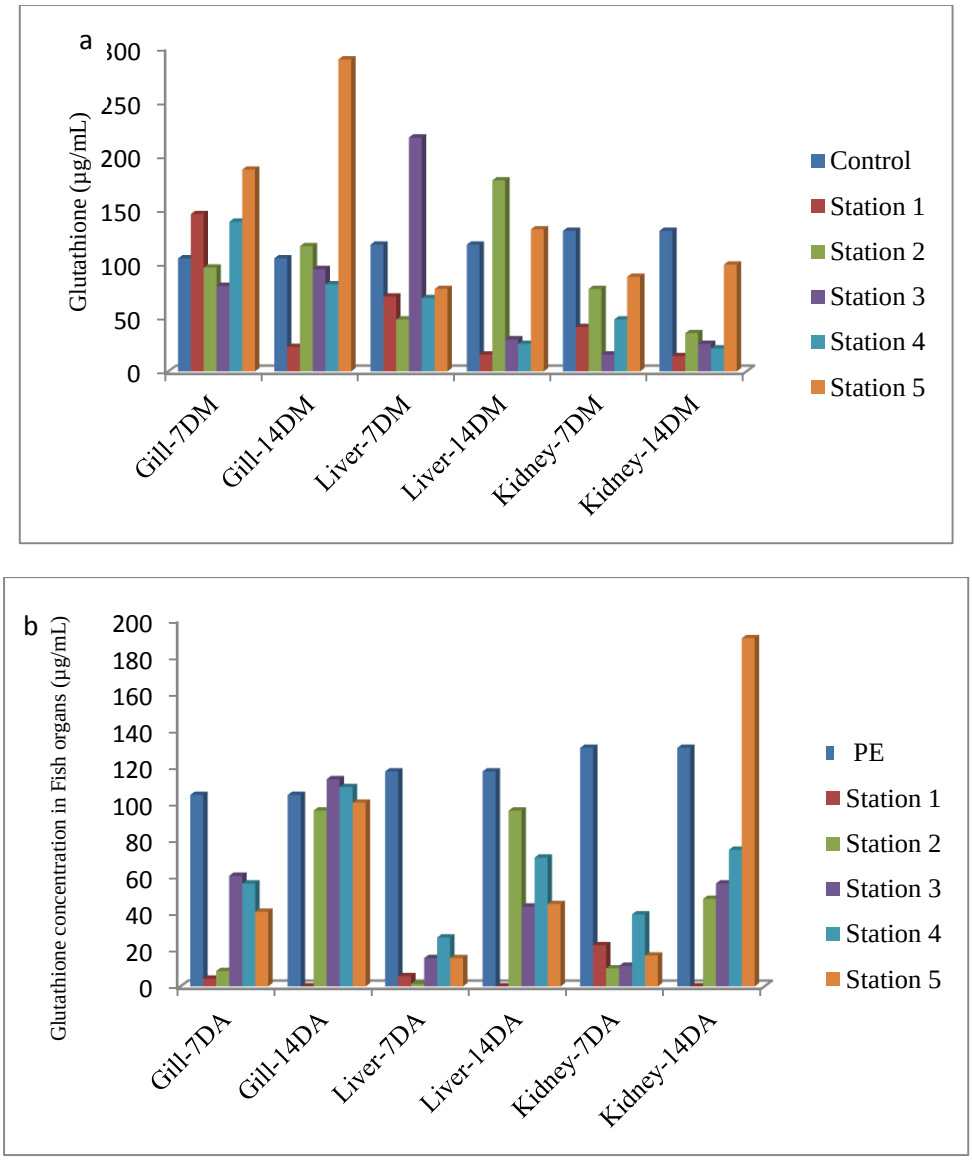


Fig.4.17: Seasonal 7th and 14th day glutathione concentrations in organs of *Clarias gariepinus* a) March for dry season and b) August for wet season.

NB: Gill-7DM, Gill-14DM refers to the GSH production level at the 7th and 14th day of exposure of the fish in the month of March. Liver 7-DM, Liver 14DM represent the GSH production levels at the 7th and 14th day exposure of the fish in the month of March. Kidney-7DM Kidney-14DM Represent the GSH production levels in the 7th and 14th day exposure o the fish in the month of March.

Gill-7DA, Gill-14DA refers to the GSH production level at the 7th and 14th day of exposure of the fish in the month ofAugust. Liver 7-DA, Liver 14DA represent the GSH production levels at the 7th and 14th day exposure of the fish in the month ofAugust. Kidney-7DA Kidney-14DA Represent the GSH production levels in the 7th and 14th day exposure o the fish in the month ofAugust.

PE stands for GSH production level in the organs prior to exposure

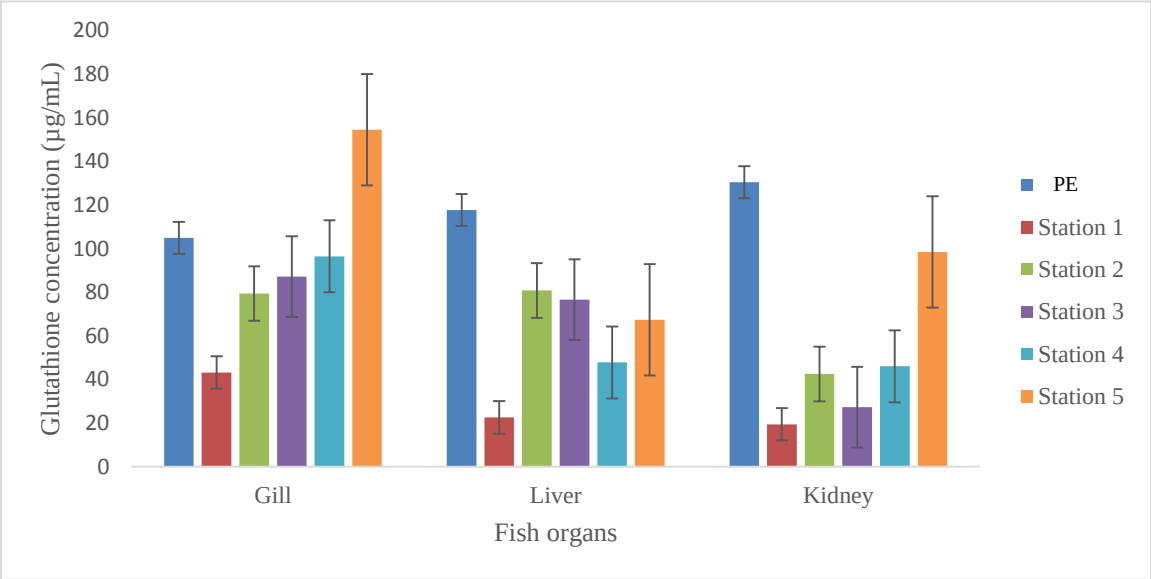


Fig. 4.18: Combined variations in Glutathione production levels in organs of *Clarias gariepinus* in an *in situ* bioassay in River Galma, Zaria.

PE stands for GSH production level in the organs prior to exposure

The kidney of the fish had its highest concentration of 87.8µg/mL at station 5 and lowest concentration of 15.6µg/mL in station 3 on the 7th day of exposure during the dry season. There were decreases in the concentration of glutathione on the 14th day with the exception of station 5 that increased to 99.15µg/mL which also constituted the highest concentration on this day. The lowest concentration of 14.18 was recorded at station 1. On the other hand, there were increases in the glutathione production levels on the 14th day during the wet season. The lowest glutathione production level of 9.93µg/mL was recorded from station 2 on the 7th day which increased to 48.23µg/mL on the 14th day. The highest glutathione production on the 7th day in the kidney of the fish was 39.72µg/mL at station 4. The highest concentration of 190.07µg/mL was obtained at station 5 on the 14th day (Table 4.5, Fig. 4.18). The glutathione concentration in the kidney of the sample ranged from 19.502±17.120µg/mL (station 1) to 98.505±17.120µg/mL (station 5).

Glutathione concentration in kidney of the fish had negative correlation with both vitamin C concentrations in gill and liver. It also had high positive correlation (0.9) with vitamin E concentration in the gill (Table 4.3). There were also positive interactions between kidney and liver (Fig. 4.13). There were also negative interactions in the kidneys and liver at the 7th day at station 1 (Fig.4.14). There were both negative and positive interactions in all the organs around station 4 in the combined analyses of fish organs in both March and August, 2015 exposures (Fig. 4.15). On the other hand, glutathione concentration in liver and kidney of the fish exhibited positive correlation with the dissolved oxygen in both March and August exposures (Fig. 4.17).

4.4.2 Vitamin C

The peak vitamin C concentration of 16.919mg/mL in the gill of the fish was obtained in stations 1. The lowest concentration of 6.774mg/mL of vitamin C was obtained in station 4 during the

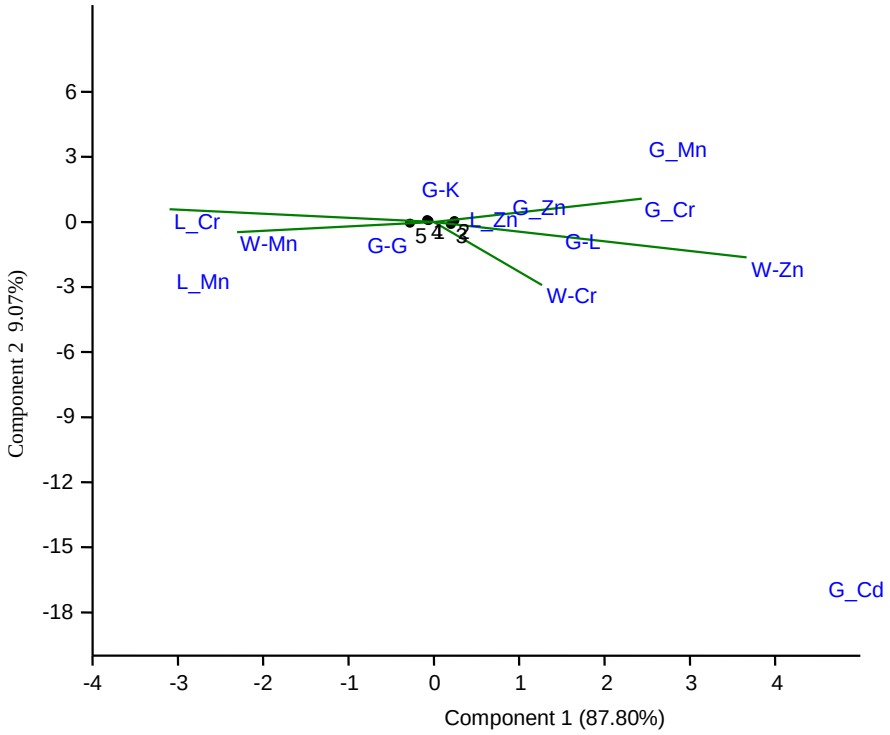


Fig. 4.19: Canonical Correspondence Analysis of interaction of Heavy metals with Glutathione Concentration

NB: G-K (glutathione concentrations in kidney), G-L (glutathione concentrations in liver), G-G (glutathione concentrations in gill), C-L (vitamin C concentration in liver), C-G (vitamin C concentrations in gill), L-E (vitamin E concentration in liver and gill), G-E (vitamin E concentration in gill). G_Mn (Mn concentration in gill), G_Cr (Cr concentration in gill),G_Zn (Zinc concentration in gill), L_Mn (Mn concentration in liver), L_Zn (concentration in liver), L_Cr (Cr concentration in liver), W_Cr (Cr concentration in water), W_Mn (concentration in water), W_Zn (Zn concentrations in water).

dry season. The wet season had its peak in station 3 with 0.595mg/mL. The lowest concentration of 0.398mg/mL was obtained in station 2.

The vitamin C concentration in the liver of the fish had its highest in station 4 with 13.121mg/mL. The lowest was obtained in station 5 with 1.87mg/mL during the dry season. On the other hand, the peak concentration of 0.577mg/mL was obtained in station 3; while the lowest was obtained from station 5 with a concentration of 0.294mg/mL during the wet season (Table 4.6, Fig. 4.19a and 4.20a). The highest mean value in vitamin C concentration (8.492±2.952mg/mL) was obtained in the gills of fish exposed in station 1. While vitamin C production level in the gill of the fish had its lowest in station 3 with 3.670±2.952mg/mL (Table 4.6). Station 5 had the lowest concentration (1.083±2.396mg/mL) in the liver of the fish and the highest was obtained in station 2 with 6.334±2.396mg/mL (Table 4.6, Fig.4. 19a and 4.20a).

From the PCA the fish organs exhibited no interactions in the vitamin C concentrations in both March and August exposures (Fig. 4.19).

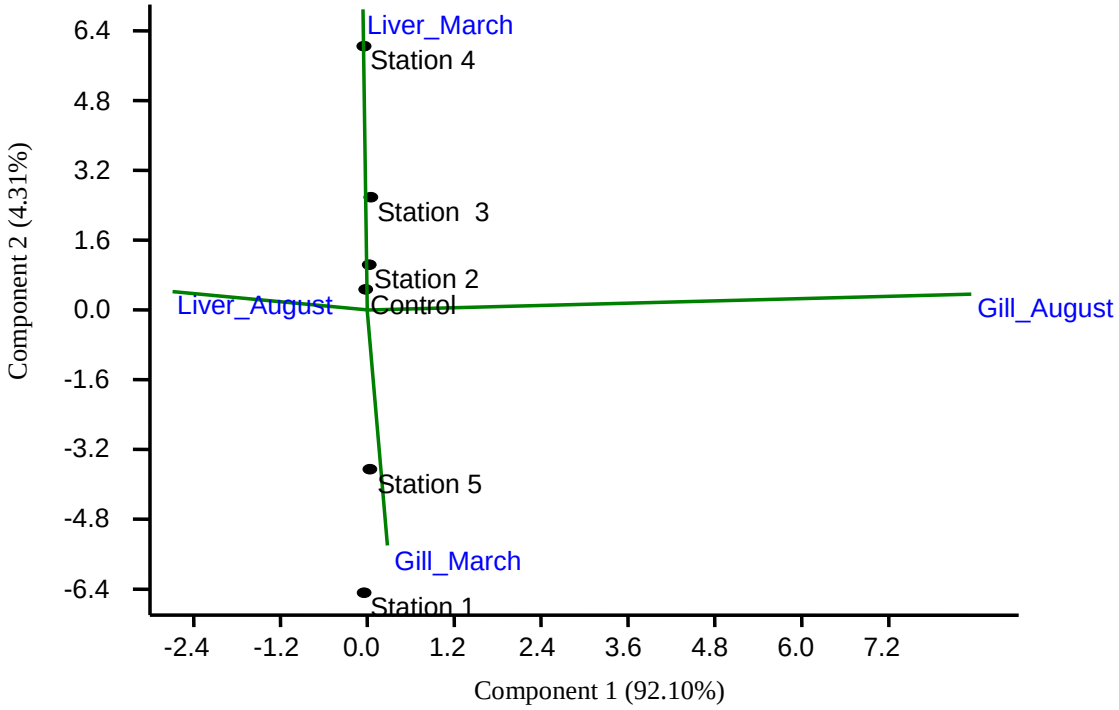
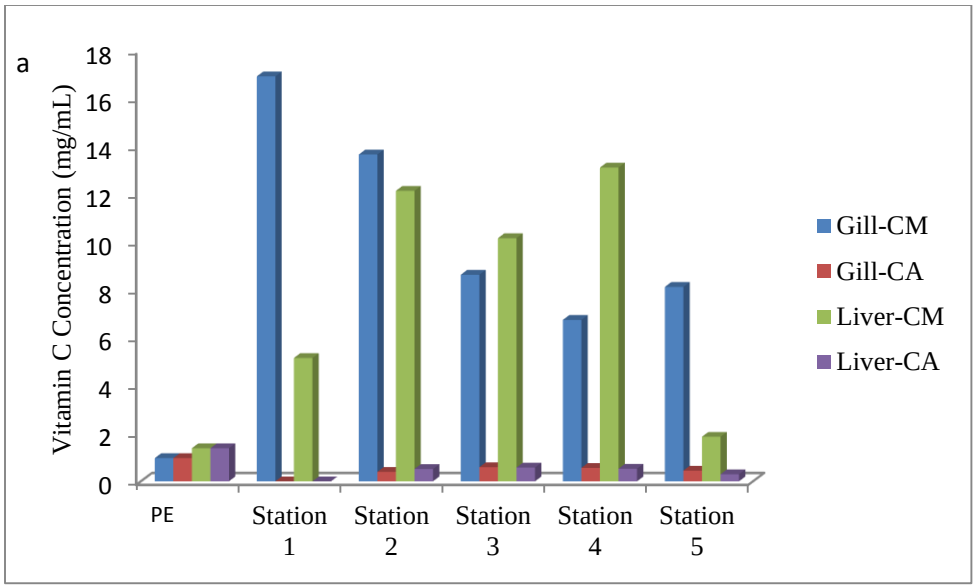


Fig. 4.20: Principal Component Analysis of Vitamin C concentration in organs of *Clarias gariepinus* for both March and August exposures.



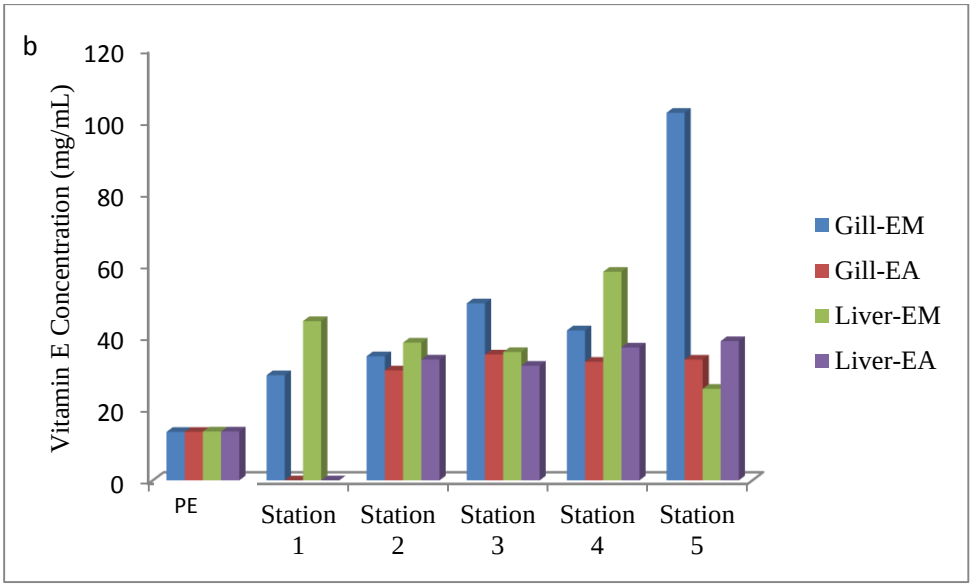
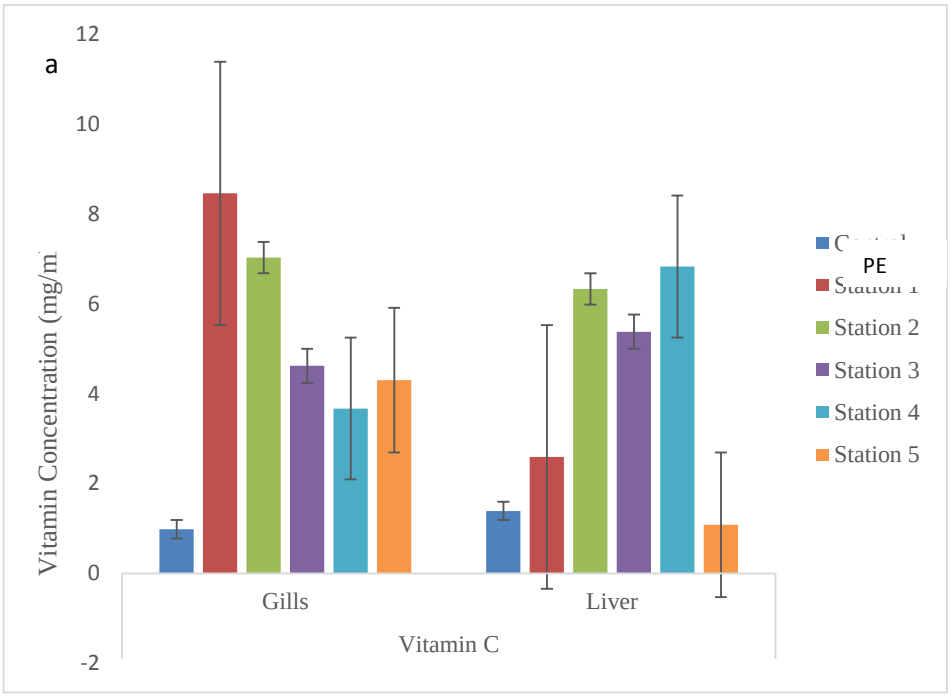


Fig. 4.21: Seasonal Variation in a) Vitamin C and b) Vitamin E Levels in organs of *Clarias gariepinus* exposed on a stretch of River Galma, Zaria.

NB: a) Gill-CM (Vitamin C concentration in gill of the fish in the months of March), Gill-CA (Vitamin C concentration in gill of the fish in the months of August), Liver-CM (Vitamin C concentration in liver of the fish in the months of March), Liver-CA (Vitamin C concentration in gill of the fish in the months of August).

b) Gill-EM (Vitamin E concentration in gill of the fish in the months of March), Gill-EA (Vitamin E concentration in gill of the fish in the months of August), Liver-EM (Vitamin E concentration in liver of the fish in the months of March), Liver-EA (Vitamin E concentration in gill of the fish in the months of August).

PE stands for GSH production level in the organs prior to exposure


a
12
10
8
6
4
2
0
-2
Vitamin Concentration (mg/m
Gills
Liver
Vitamin C
PE
Station 2
Station 3
Station 4
Station 5

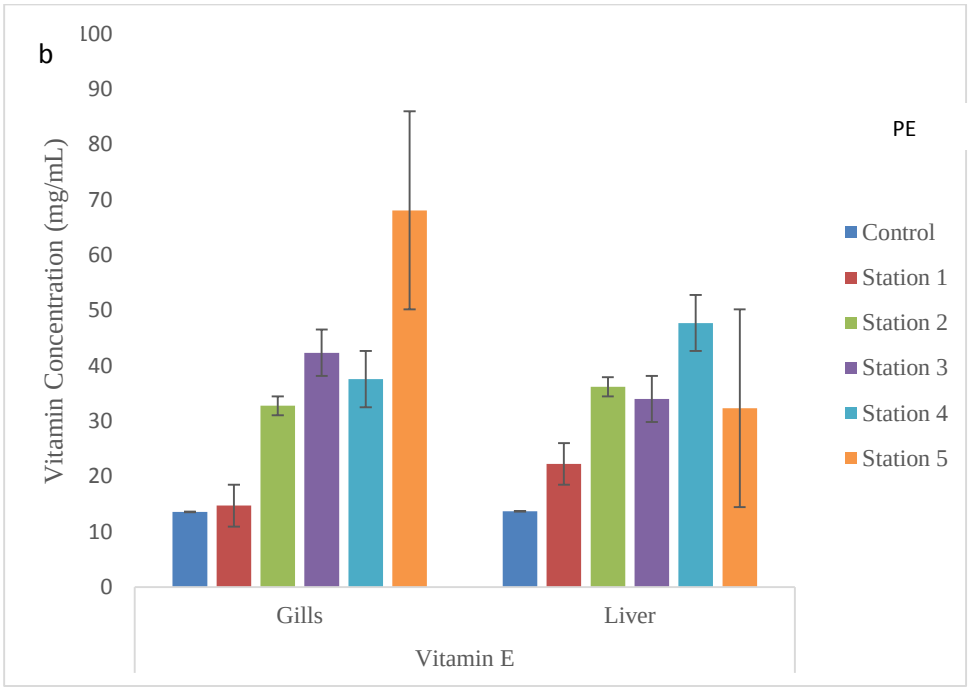

b
100
90
80
70
60
50
40
30
20
10
0
Vitamin Concentration (mg/mL)
Gills
Liver
Vitamin E
PE
Control
Station 1
Station 2
Station 3
Station 4
Station 5

Fig. 4.22: Combined variations in a) Vitamin C and b) Vitamin E production levels in organs of *Clarias gariepinus* in *in situ* bio-assay in River Galma, Zaria.

PE stands for GSH production level in the organs prior to exposure

4.4.3 Vitamin E Concentrations

Vitamin E production levels in the organs of *Clarias gariepinus* in *in situ* bio-assay in River Galma were very high in comparison with vitamin C concentrations and control. Station 5 recorded the highest concentration of 102.35mg/mL in the gill of the fish. The lowest concentration of 29.43mg/mL was obtained from station 1 during the dry season. The wet season recorded its peak at station 3 with 35.24mg/mL. The lowest was obtained from station 2 with 30.79mg/mL in the gill of the fish during the wet season (Table 4.5). Vitamin E concentration in station 5 had the highest mean value of 68.082±9.021mg/mL while the lowest was recorded in station 1 with 14.717±9.021mg/mL in the gills of the fish (Table 4.5).

However, the liver of the fish had its peak at station 4 with 58.237mg/mL. The lowest concentration of 25.641mg/mL was obtained in station 5 during the dry season. However, the highest concentration of 38.978mg/mL was obtained from the same station 5 during the wet season. The lowest concentration during this season was obtained from station 3 with 32.092mg/mL (Table 4.5, Figs. 4.20b and 4.21b). The mean range values of 22.262±6.055mg/mL (station 1) to 47.720±6.055mg/mL (station 4) were obtained in the liver of the fish (Table 4.5).

Vitamin E concentration in gill was negatively correlated with vitamin C concentration in gill. Vitamin E concentration in liver was negatively correlated with vitamin C concentration in gill. It also had positive correlation with Vitamin C concentration in liver. Vitamin E concentration in gill was positively correlated with glutathione production level in liver (Table 4.3).

From the PCA, the liver showed negative correlation with the vitamin E concentrations in August exposures. On the other hand, the gill showed positive interaction in vitamin E concentration in August exposure. The organs of the fish exposed in March exhibited no interaction (Fig. 4.22). Likewise, the vitamins exhibited no interactions with heavy metals (Fig.4. 23). Also, the vitamins exhibited no interaction with the physico-chemical parameters (Fig. 4.25).

4.5 Histopathology of Kidney and Liver of *Clarias gariepinus* exposed for 14 days in River Galma

Histopathological damages in the kidney and and liver of the fish exposed in River Galma for 14 days varied from station to station in comparison with the photomicrograph obtained prior to exposure. The histopathological alteration observed in station 1 showed vacoulation and constriction of the tissues of the kidney. Similar alterations were observed in the liver of the fish exposed at the same station but more severe in the kidney (Plates IV and V). Vacoulation, constriction and aggregation of cells were also observed in the kidney and liver of the fish exposed in stations 2 and 3 with greater severity. In addition to this, necrosis and infilteration of the cells also occurred (Plates VI-IX).

The histopathological damages observed in station 4 showed that in addition to other feature highlighted above, there was congestion of blood vessels in the kidney of the fish. The severity of the damage was greater than what were observed in all the other stations in both kidney and liver of the fish exposed at this station. At station 5 however, the tissue alterations were minimal (Plates X-XII).

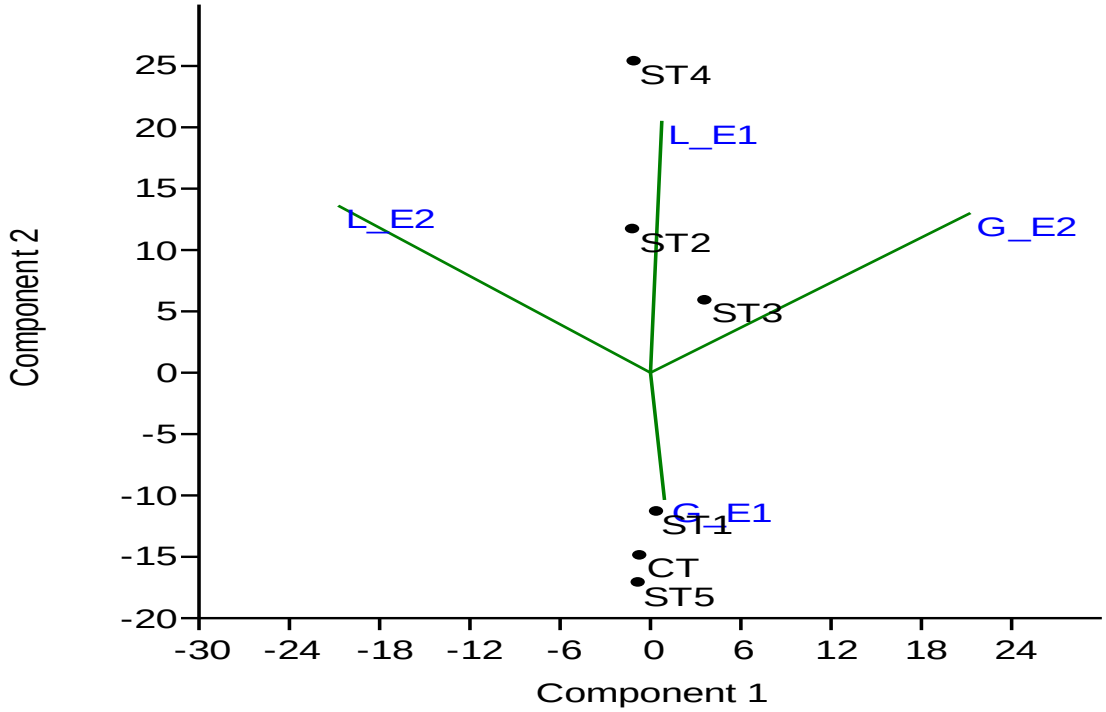


Fig. 4.23 Principal Component Analysis of Vitamin E Concentrations in Liver and Gill of *Clarias gariepinus* exposed in River Galma, Zaria.

NB: G_E1, G-E2, L_E1, L-E2, CT, ST 1-5 stands for vitamin E concentrations in gill and liver in March and August exposures, control, and the stations respectively.

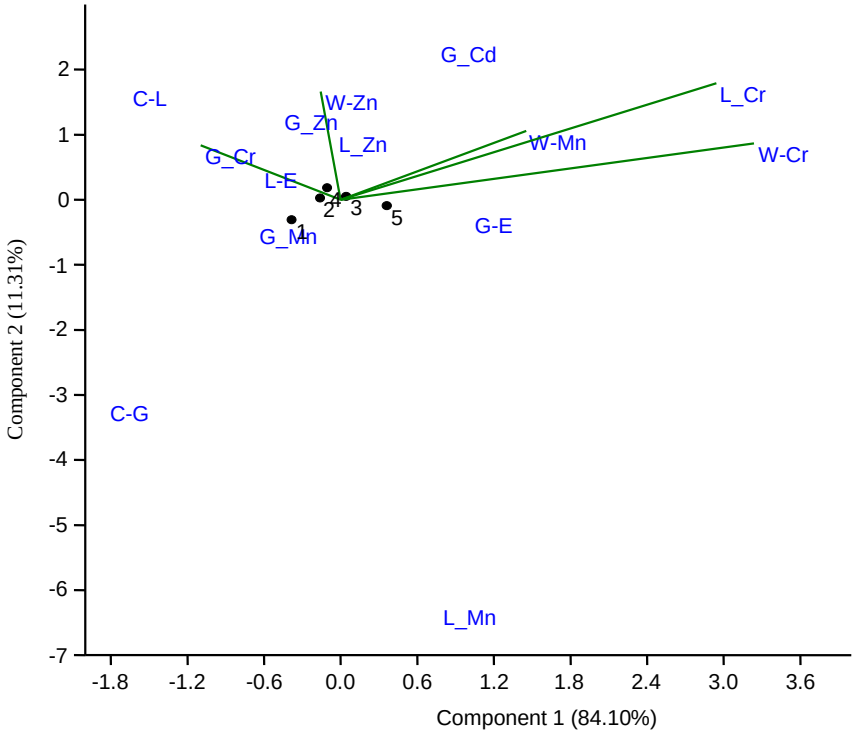


Fig. 4.24 Canonical Correspondence Analysis of interaction of Heavy metals with Vitamin E and C Concentrations in *Clarias gariepinus* exposed in River Galma, Zaria.

NB: C-L (vitamin C concentration in liver), C-G (vitamin C concentrations in gill), L-E (vitamin E concentration in liver and gill), G-E (vitamin E concentration in gill). G_Mn (Mn concentration in gill), G_Cr (Cr concentration in gill),G_Zn (Zinc concentration in gill), L_Mn (Mn concentration in liver), L_Zn (concentration in liver), L_Cr (Cr concentration in liver), W_Cr (Cr concentration in water), W_Mn (concentration in water), W_Zn (Zn concentrations in water).

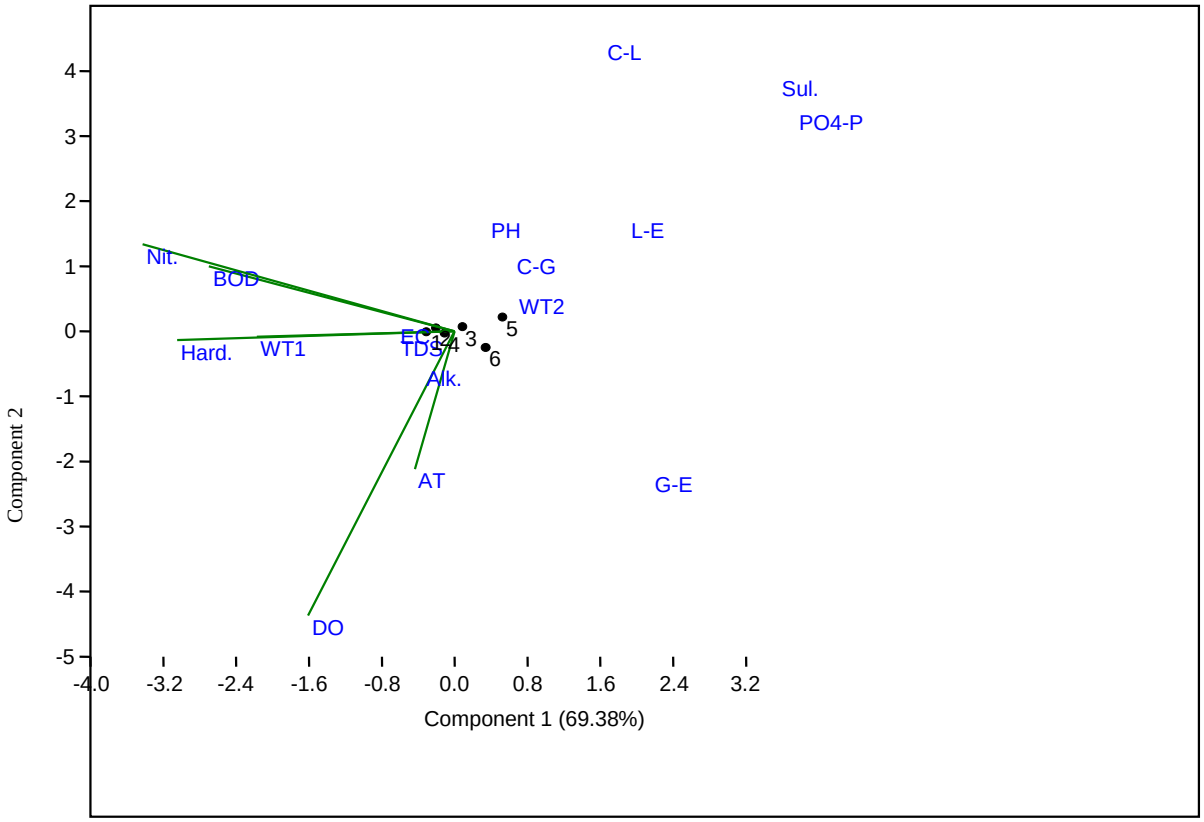


Fig. 4.25: Canonical Correspondence Analysis of interaction of Physico-chemical parameters with Vitamin E and C Concentrations in *Clarias gariepinus* exposed in River Galma, Zaria.

NB: WT = Water Temperature, DO = Dissolve Oxygen, Nit. = Nitrate, Hard. = Hardness, Sul. = Sulphate, Alk. = Alkalinity, PO4-P = Phosphate Phosphorous, TDS = Total Dissolve Solid, EC = Electrical Conductivity. C-L (vitamin C concentration in liver), C-G (vitamin C concentrations in gill), L-E (vitamin E concentration in liver and gill), G-E (vitamin E concentration in gill).

a

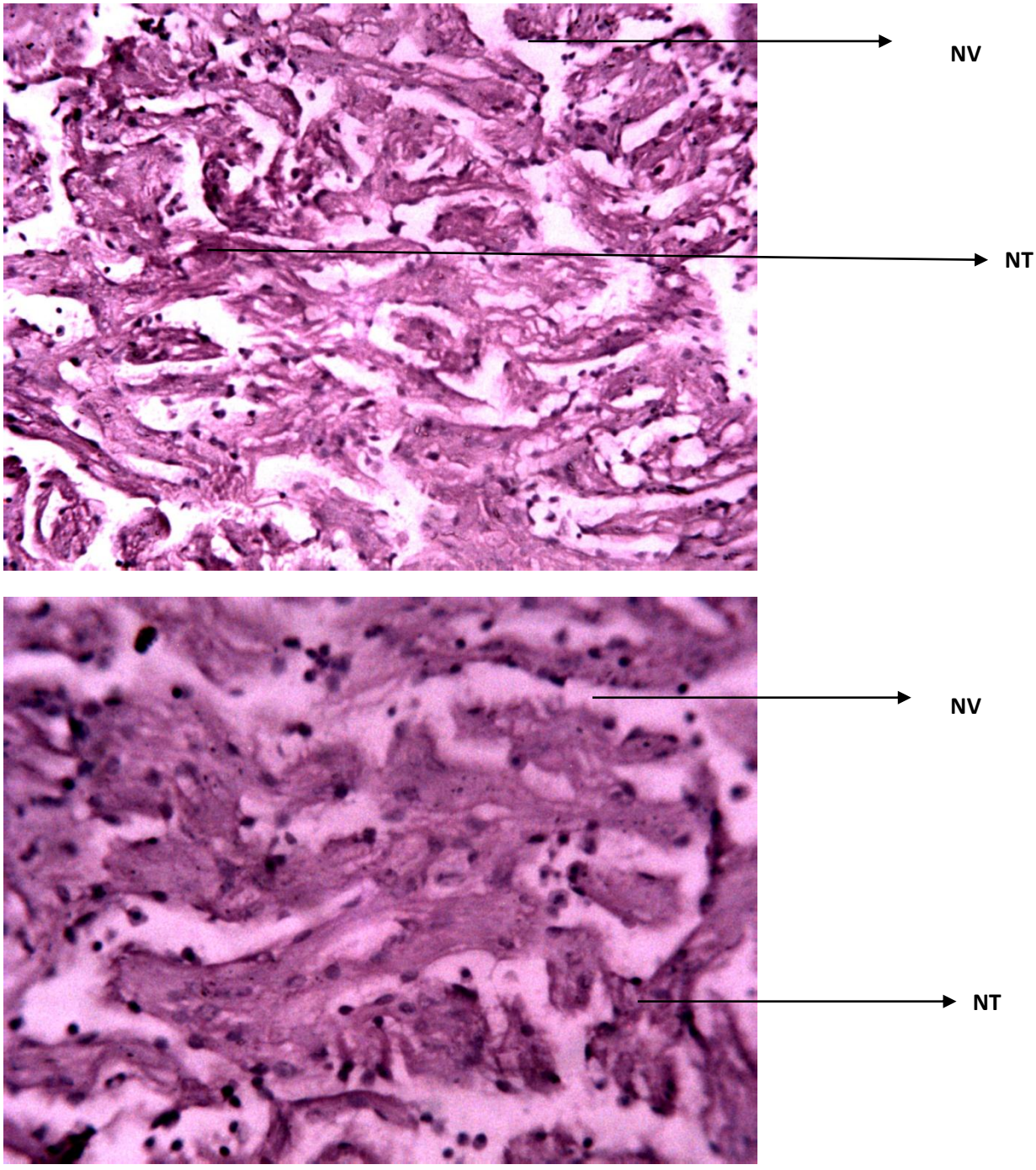
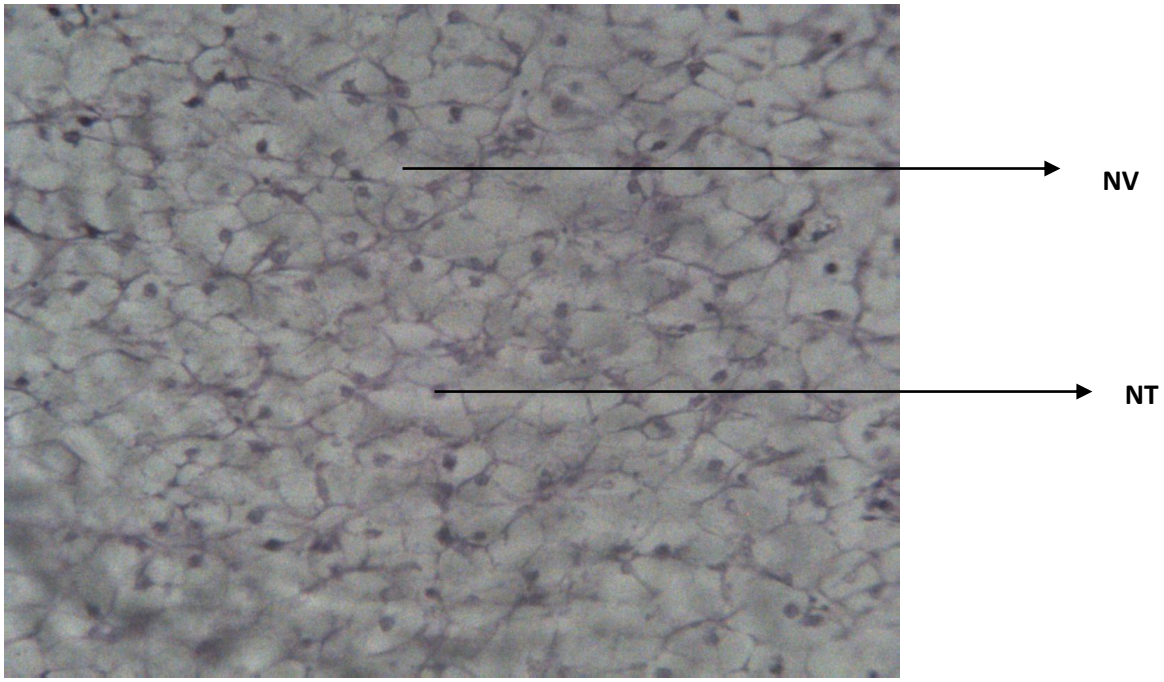


Plate II: Photomicrograph of Kidney of *Clarias gariepinus* prior to exposure at a) x 250 and b) x400. NV and NT indicate the normal tissue vacuoles and normal tissue cells respectively.

a



b

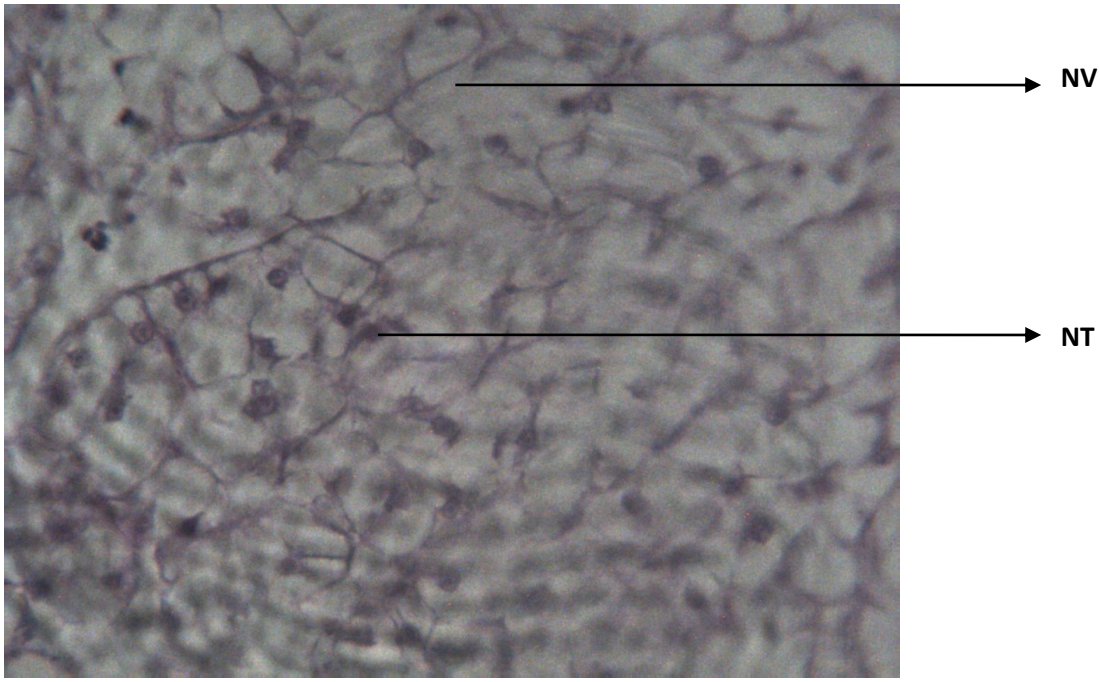


Plate III: Photomicrograph of Liver of *Clarias gariepinus* prior to exposure at a) x 250 and b) x400. NV and NT indicate the normal tissue vacuoles and normal tissue hepatocytes respectively.

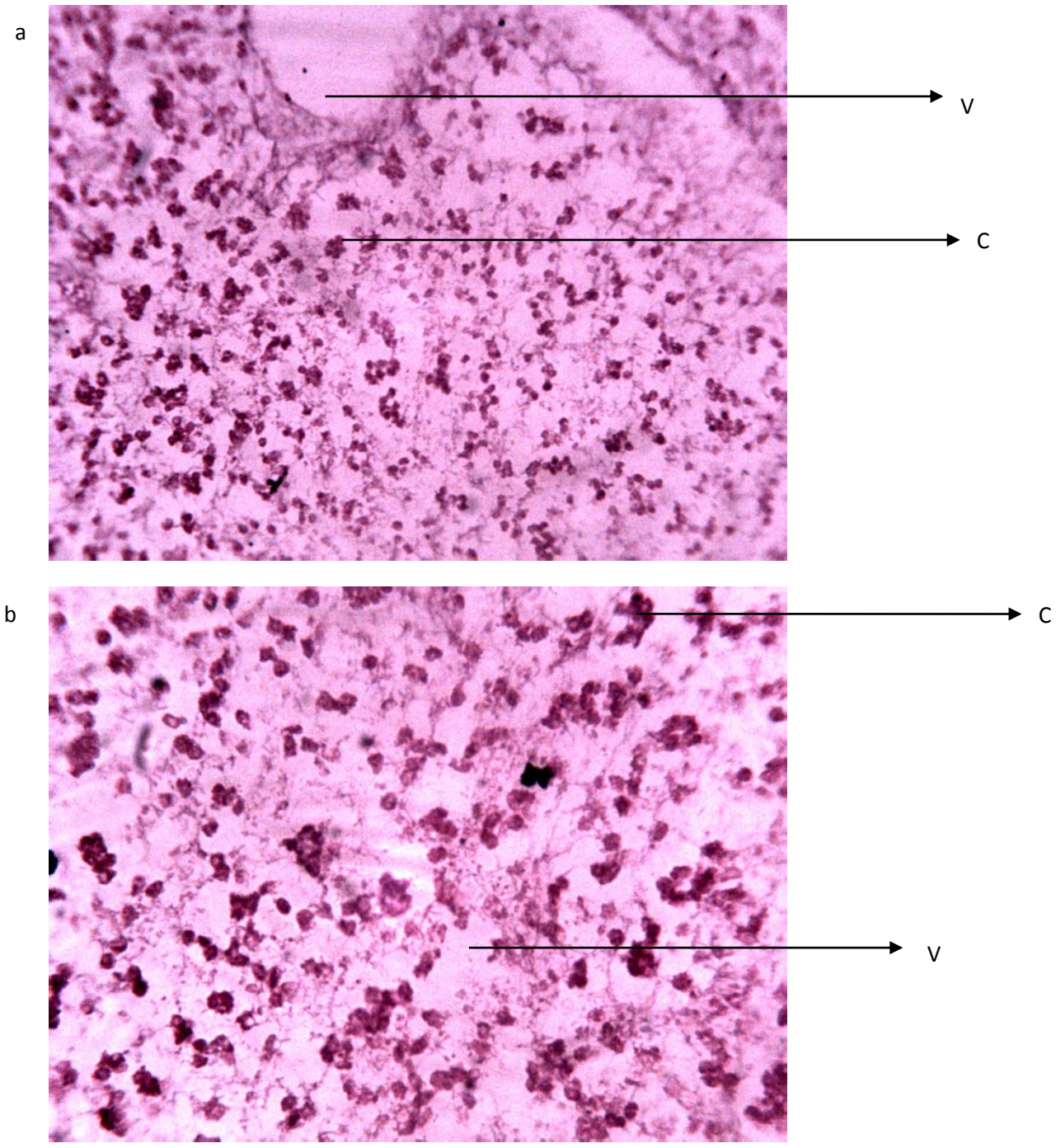


Plate IV: Photomicrograhof Kidney of *Clarias gariepinus* obtained from Station 1 after 14 days bio-assay in River Galma a) x 250 and b) x400. The letters V and C indicate severe vacoulation of the tissue and, constriction and aggregation of cells respectively.

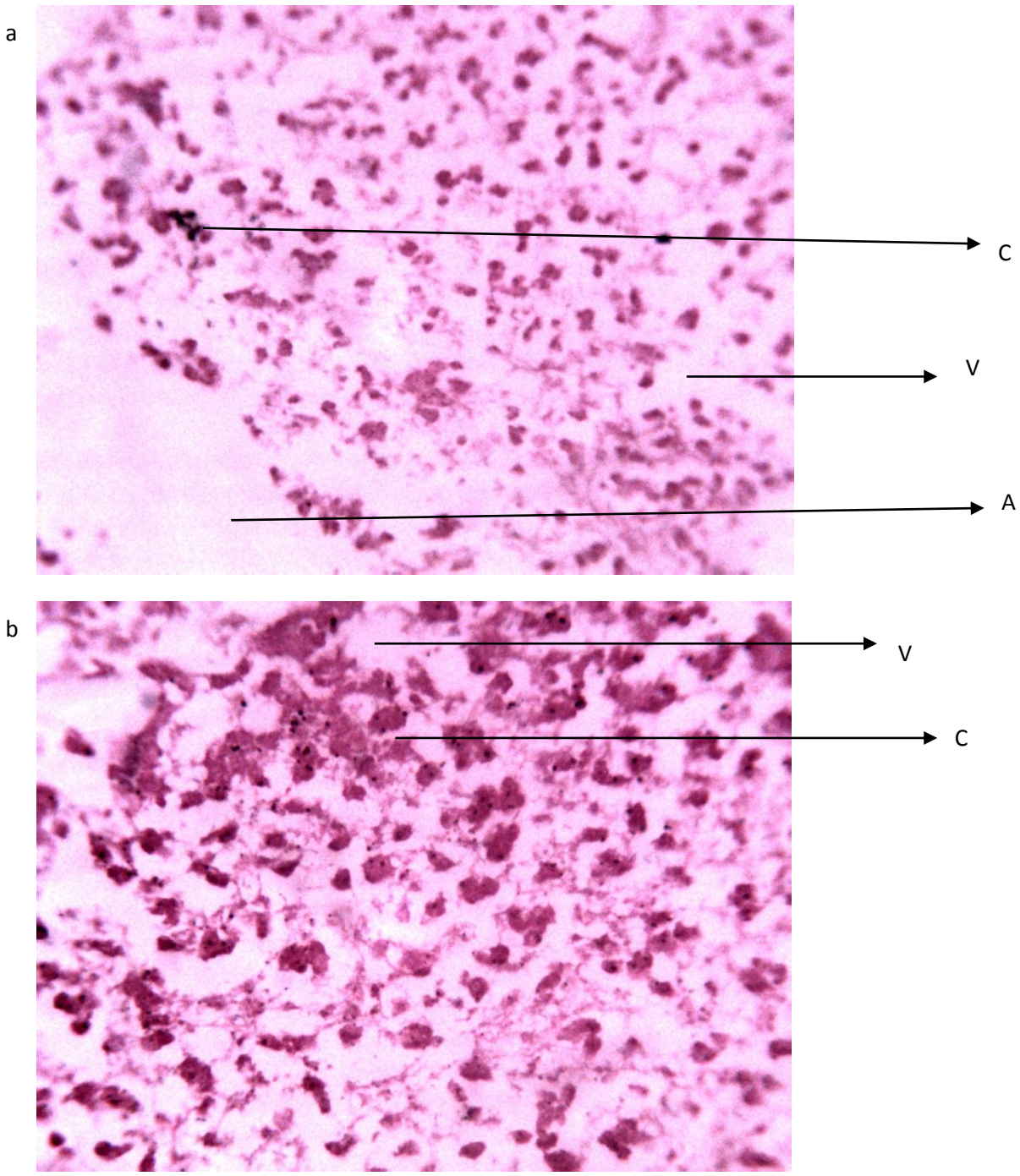
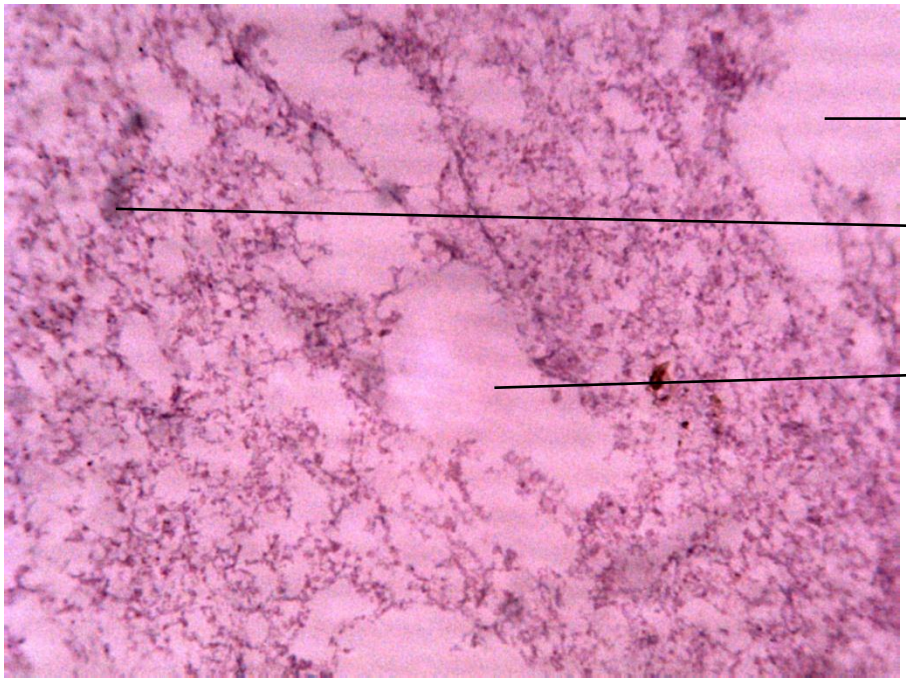


Plate V: Photomicrograh of Liver of *Clarias gariepinus* obtained from Station 1 after 14 days bio-assay in River Galma a) x 250 and b) x400. The letter V indicates vacoulation of the tissue. The letter C indicates constriction, hypertrophy and aggregation of hepatocytes. The letter A indicates area of necrosis and infiterations of the hepatocytes.

a



b

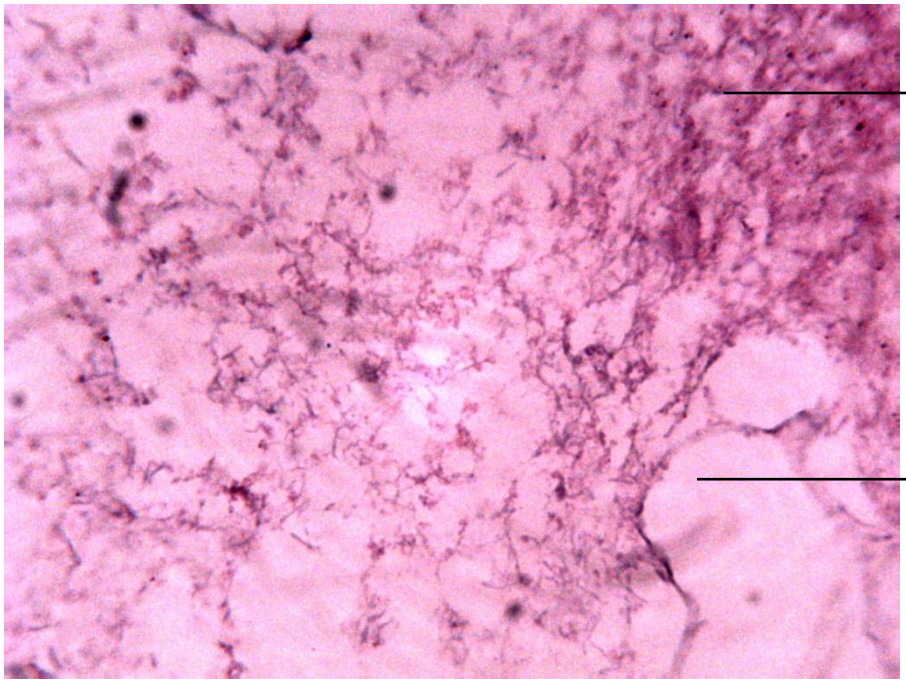


Plate VI: Photomicrograh of Kidney of *Clarias gariepinus* obtained from Station 2 after 14 days bio-assay in River Galma a) x 250 and b) x 400. The letter V indicates severe vacoulation of the tissue. The letter C indicates constriction, hypertrophy and aggregation of cells. (A) stands for necrosis and infilteration of the cells.

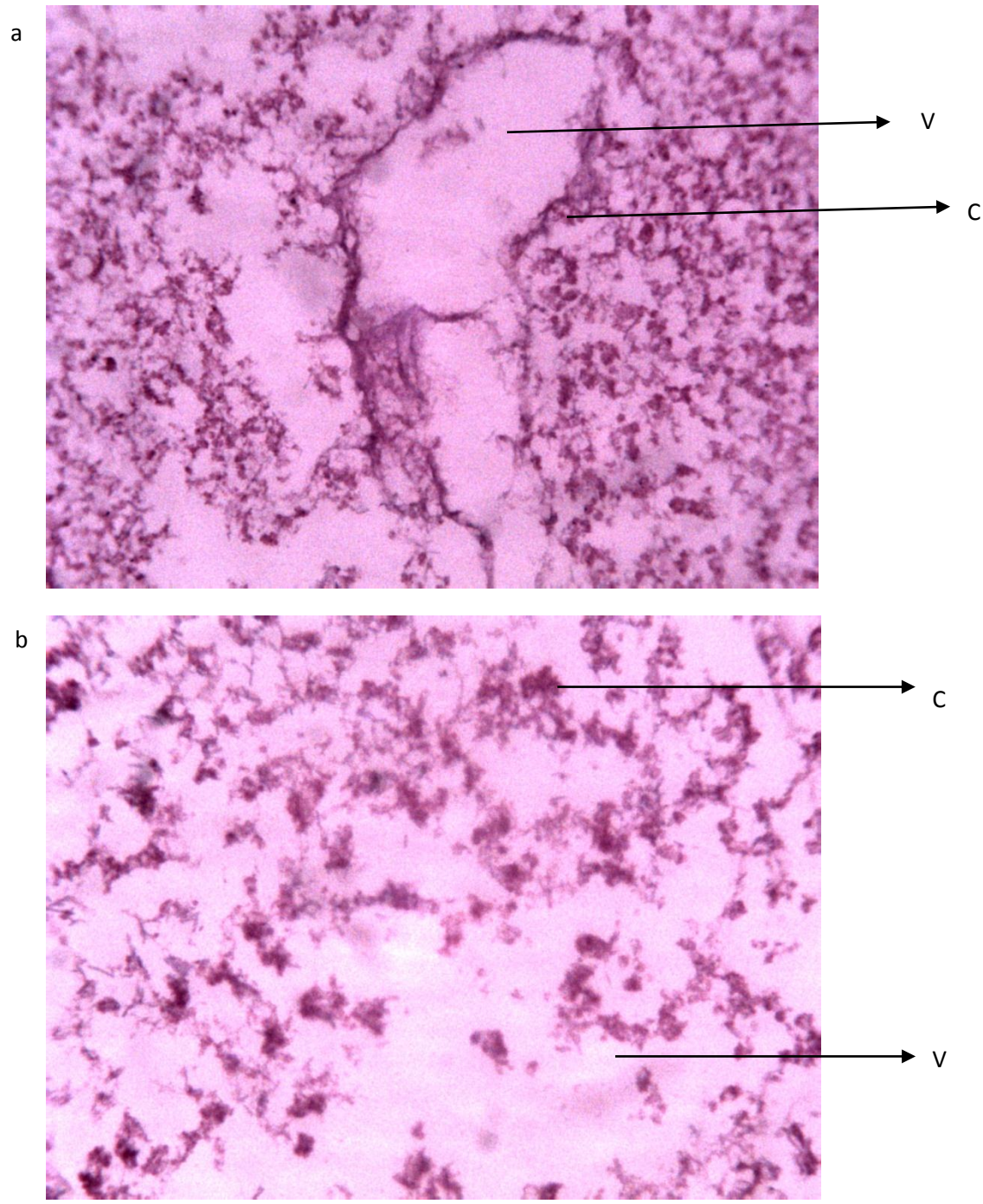


Plate VII: Photomicrograh of Liver of *Clarias gariepinus* obtained from Station 2 after 14 days bio-assay in River Galma a) x 250 and b) x 400. The letter V indicates severe vacoulation and necrosis of the tissue. The letter C indicates constriction, hypertrophy and aggregation of hepatocytes.

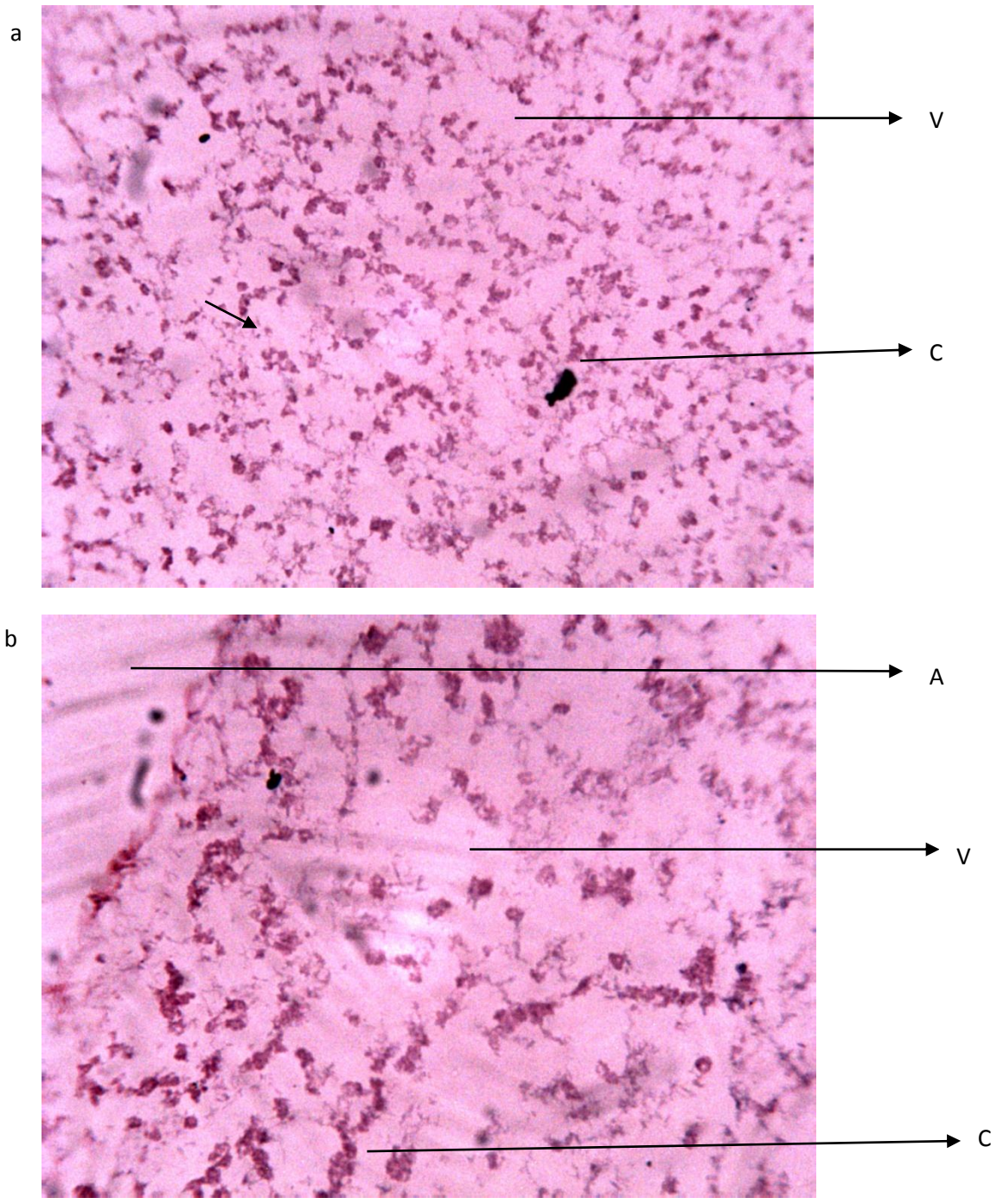
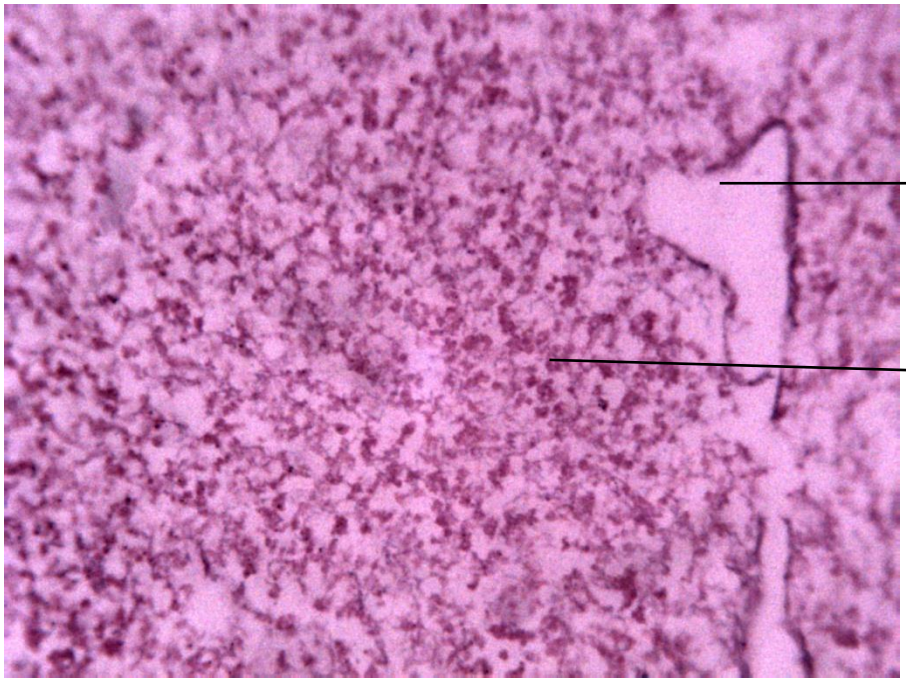


Plate VIII: Photomicrograh of Kidney of *Clarias gariepinus* obtained from Station 3 after 14 days bio-assay in River Galma a) x 250 and b) X 400.The letter V indicates severe shattering, vacoulation and necrosis of the tissue. The letter C indicates constriction, hypertrophy and aggregation of cells. The letter A stands for necrosis and infilteration of the cells.

a



b

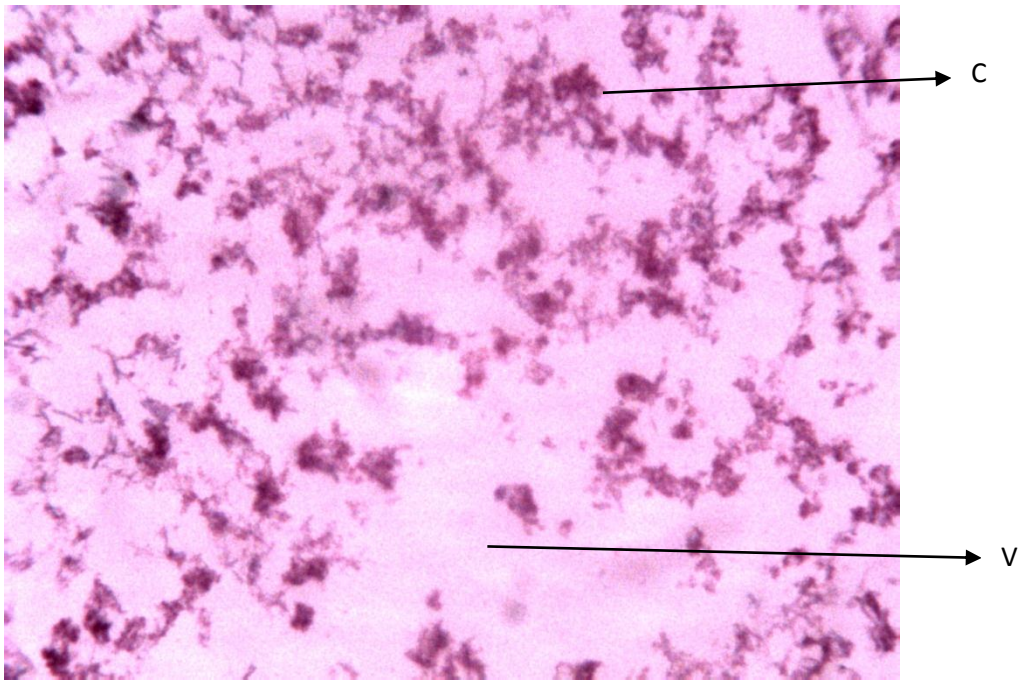
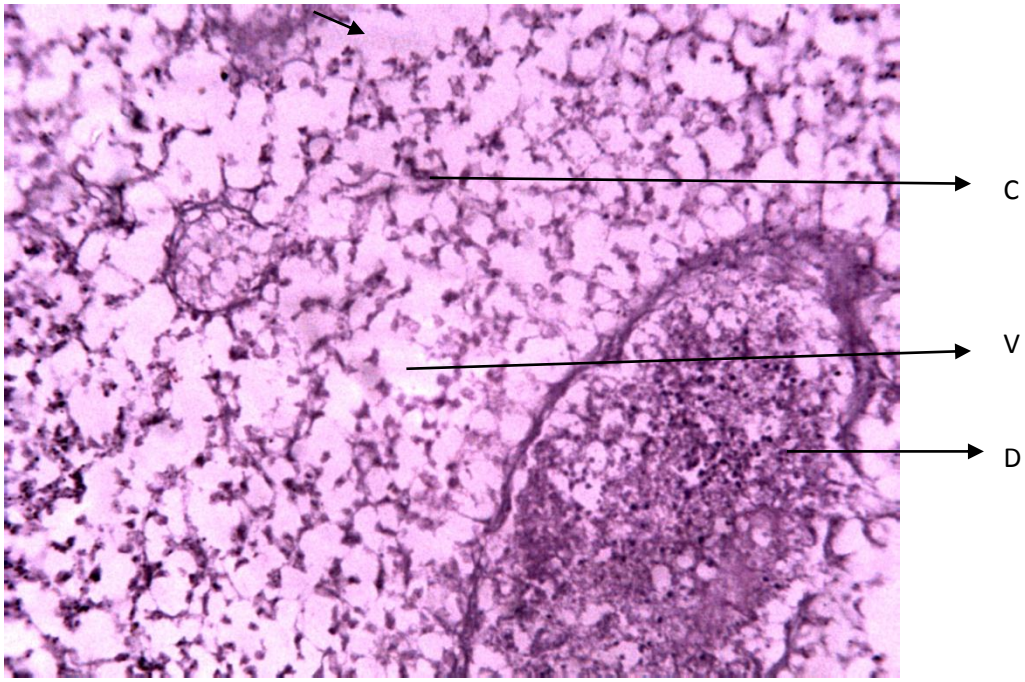


Plate IX: Photomicrograh of Liver of *Clarias gariepinus* obtained from Station 3 after 14 days bio-assay in River Galma a) x250 and b) x 400. The letter V indicates vacoulation of the tissue. The letter C indicates constriction and aggregation of hepatocytes.

a



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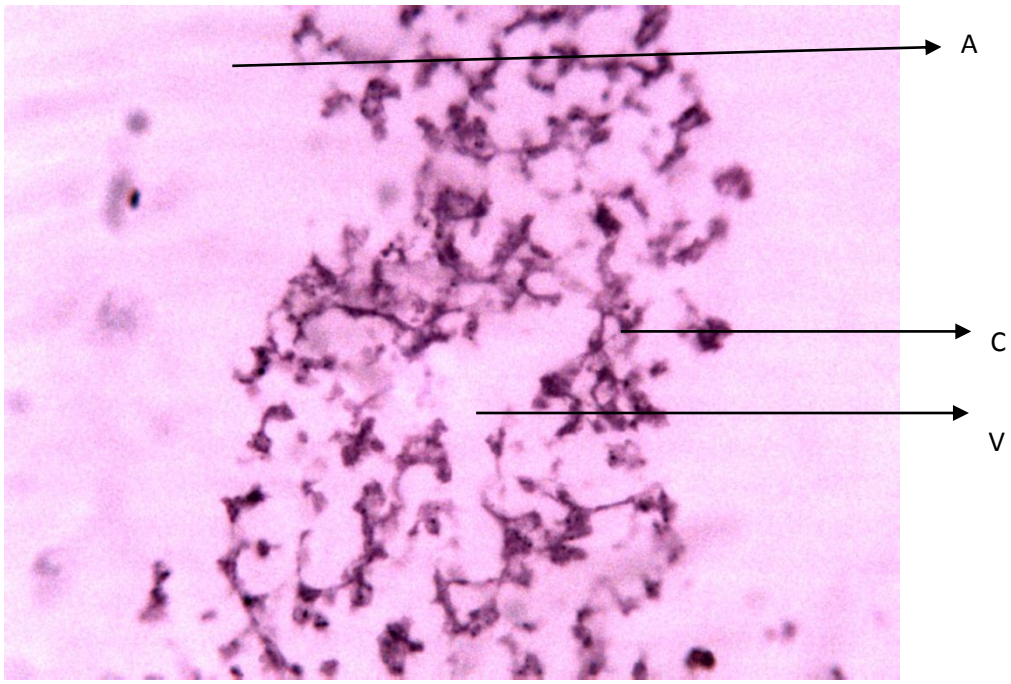
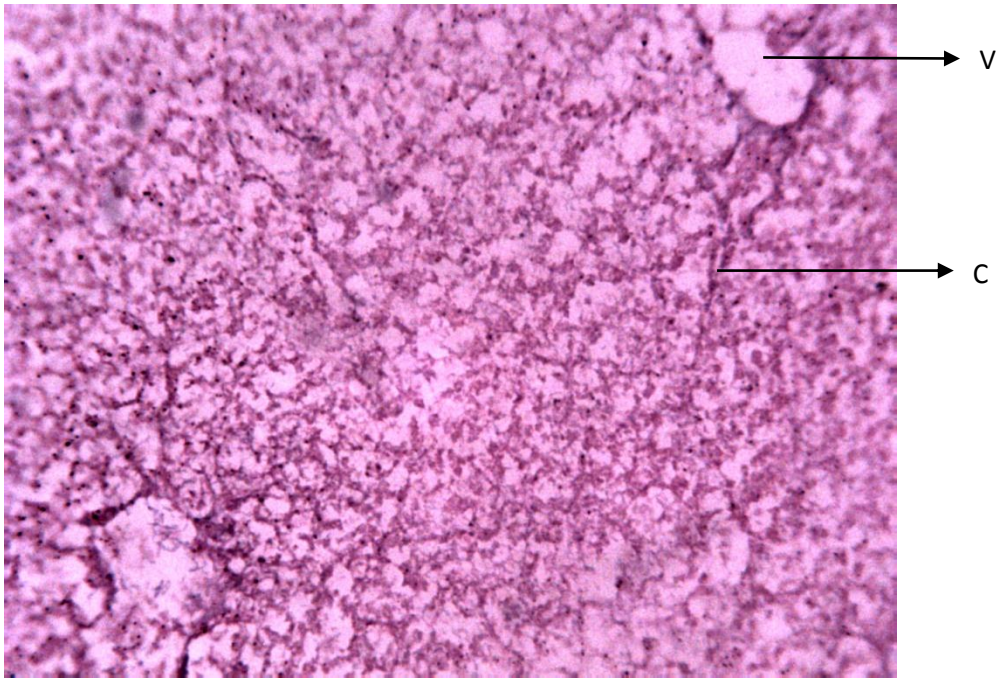


Plate X: Photomicrograh of Kidney of *Clarias gariepinus* obtained from Station 4 after 14 days bio-assay in River Galma a) x 250 and b) x 400 . The letter V indicates severe shattering and vacoulation of the tissue. The letter C indicates constriction, hypertrophy and aggregation of cells. Letter D stands for congestion of blood vessels, necrosis and infilteration of the cells. Letter A stands for necrosis and infilteration of the cells.

a



b

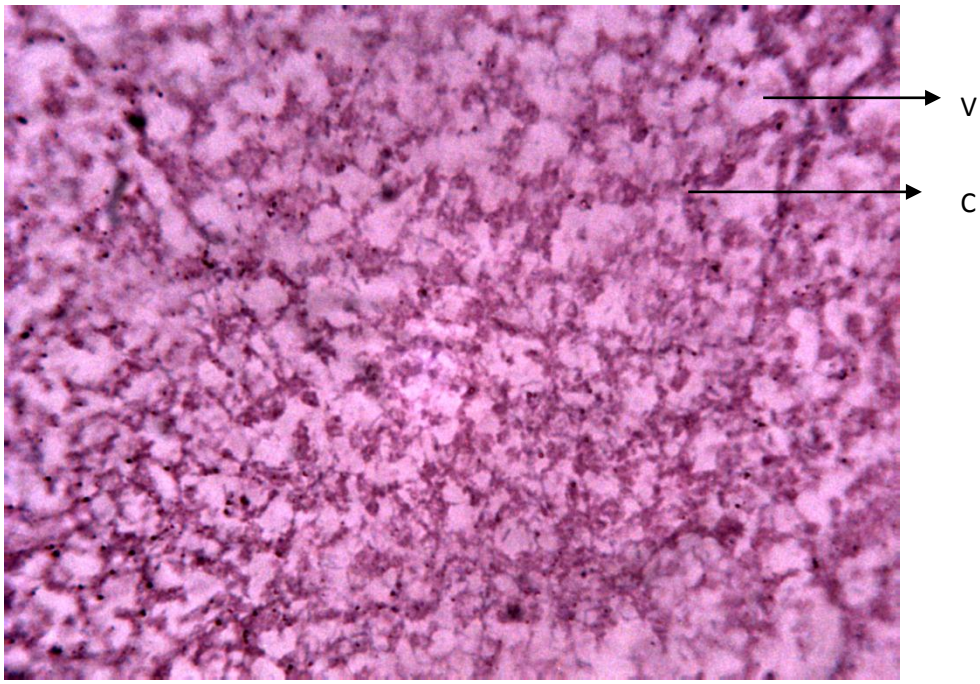


Plate XI: Photomicrograh of Liver of *Clarias gariepinus* obtained from Station 4 after 14 days bio-assay in River Galma a) x 250 and b) x 400. The letter V indicates vacoulation of the tissue. (C) Indicates constriction and aggregation of cells.

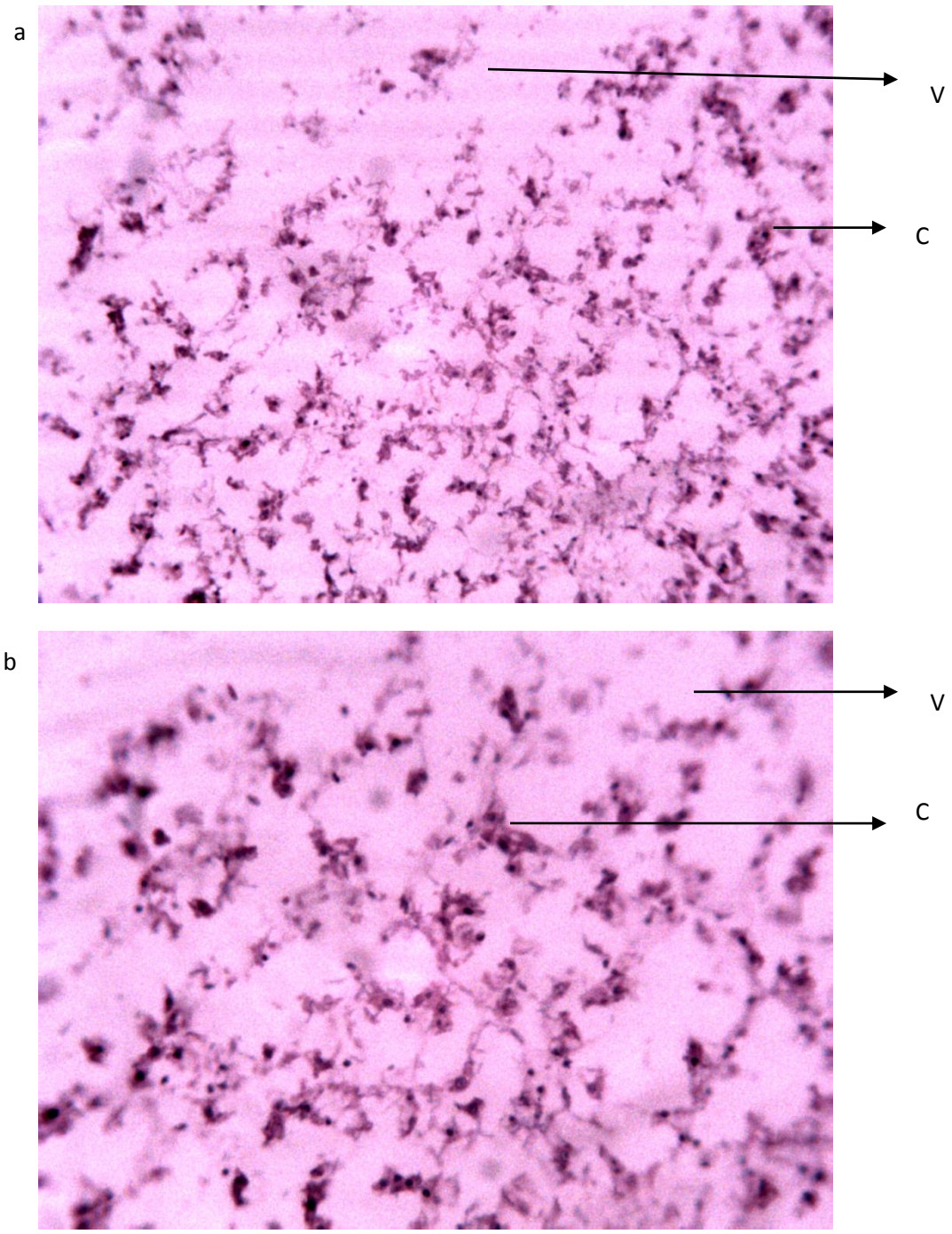


Plate XII: Photomicrograh of Kidney of *Clarias gariepinus* obtained from Station 5 after 14 days bio-assay in River Galma a) X 250 and b) X 400. The letter V indicates severe shattering and vacoulation of the tissue. The letter C indicates severe constriction, hypertrophy and aggregation of cells.

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Physico-chemical Parameters of River Galma

The highest ambient temperature was 27.00°C in the month of May while the lowest of 18.00°C was obtained in the month of March, 2015. This can be attributed to the intermittent rainfall followed by intense scourge of the sun during these months. Similar temperature ranges were obtained by Ahamefula *et al*. (2014) who reported that the air temperature ranged between 26.3 and 28.0°C in Lagos coastal water.

The highest water temperature of 27.00°C was obtained in the month of May. Water temperature was lowest (21.00°C) in the month of July. The high water temperature in the month of May, just like what was obtained in ambient temperature can be attributed to intermittent rainfall during the period. The low temperature obtained in the month of July may be because the water level was high. Slightly higher temperature values (28.3 and 31.0°C.) were obtained by Ahamefula *et al*. (2014) in Lagos coastal water. The changes in temperature values may also be due to run-offs, seepages and anthropogenic activities from the neighbouring communities. Murhekar (2011) observed that warm water discharged from factories, the removal of trees and vegetation that shade streams, and water that runs off city streets can cause temperature changes that may threaten the balance of aquatic systems. Water temperature mean values measured ranged from 21.60 ± 0.24^{0}C (July, 2015) to $26.80\pm0.20\ ^{0}$C (May, 2015). The initial high temperature in May was reduced in the month of July probably because the water level of the river was high. Omaka *et al*. (2014) obtained a similar range of values (28.60–30.00 °C) in Abakaliki River.

Both the highest (5.10mg/L) and lowest (0.8mg/L) dissolved oxygen concentrations occurred in the month of June, 2015 at stations 1 and 2 respectively. This is probably because of the peculiarities of these parts of the river. Station 2 is where the major anthropogenic pollutants enter the river. This agrees with Butu and Bichi (2013) who reported that River Galma receives variable levels of pollutant from different sources of anthropogenic activities along its banks. This is also in line with the findings of Samuel *et al.* (2015) on River Galma who observed the lowest dissolved oxygen of 3.46±0.55mg/L from the same station. Also, while rainfall may dilute and weaken the effects of point source pollution, it also increases the contribution of non-point sources or diffuses pollution through land run-off from agricultural fields and leaches from refuse dumps (Jaji *et al*., 2007). Station 1is located at the upper course of the river and is relatively free from municipal discharge. This may have accounted for the highest level of the dissolved oxygen recorded at this station. The highest mean values of dissolved oxygen (3.67±0.4 mg/L) in the month of March may be due to increased aeration level of all the constituents of the river depth; and the concentration of the chemical constituents of water body since the water level was low during this period. Similar range of values were reported by Omaka *et al*. (2014) who recorded 1.40–3.53 mg/L in Abakaliki River. The effect of waste discharge on a surface water source is largely determined by the oxygen balance of the system and its presence is essential in maintaining biological life within a system (DFID, 1999). For healthy fresh water bodies at least 5 mg/L DO is essential for the survival of aquatic organisms (Adakole and Abolude, 2012). Judging from the results in comparison with these standards River Galma can be said to be polluted and therefore not fit for direct human consumption without treatment. However, the river may not pose serious threat to the survival of the aquatic biota and may be used for other domestic activities.

Biological oxygen demand (BOD) had a peak of 6.75mg/L in the month of June, 2015 in station 1. While the lowest value of 0.95mg/L was obtained in station 3 in the month of April, 2015. Lager part of the month of June was without rain and as such, the initial municipal discharge and run-off from rainfall may have brought in a lot of organic matter. The decomposition of the organic matter may have accounted for the high BOD value obtained in the month of June. The BOD range (1.13±0.12mg/L (April, 2015) to 5.43±0.45 mg/L (July, 2015)) of the sampling months may have been due to the presence of xenobiotics from all the various locations along the river course that have rendered the living organisms vulnerable. The washed off organic pollutants from the surrounding and the discharged wastes into the river body may have been retained within the water body both in the dry and rainy seasons. This is because poor water qualities may be caused by low water flow, municipal effluents and industrial discharges (Chitmanat and Traichaiyaporn, 2010). Similar BOD range (1.20–7.03mg/L) was obtained by Omaka *et al*. (2014) in Abakaliki River.

The highest nitrate concentration was 0.435mg/L in station 4 in the month of March, 2015. The low water level during the dry season might be responsible for the high nitrate concentration. In addition, the peculiarity of station 4 along the stretch of River Galma especially the reception of wastes via Kubani stream may have had significant contribution to the high concentration. The lowest value of 0.033mg/L was obtained in station 2 in the month of August, 2015. This may be attributable to the dilution factor of the river since the river was in flood. The nitrate concentration mean values ranged from 0.05±0.00 mg/L (August) to 0.38±0.02mg/L (March). Farming activities were noticed during the dry and rainy seasons along the river banks. These anthropogenic activities of the farmers and the neighbouring communities may have contributed to the nitrate load of the river. It is well known that high concentration of nitrate in drinking water sources can be fatal to humans and animals (Monteiro *et al*., 2003). Acceptable limits for nitrate concentration in drinking

water is 5-50mg/L (WHO, Murhekar, 2011) hence, the nitrate concentration of River Galma is within acceptable limits.

The highest sulphate concentration value of 0.872mg/L was obtained in station 2 in the month of July, 2015. The increased sulphate concentration in the month of July may have arisen from the agricultural activities of farmers along the river course. This was also probably because there was discontinuity in rainfall during the larger part of the month of June. The run-offs that followed after this period of drought may have contributed new set of xenobiotics to the river body. Sulphate concentration mean range was 0.02±0.00 mg/L (March) to 0.65±0.07 mg/L (July). Lower concentration mean ranges of Sulphate concentration (0.05±0.01mg/L to 0.24±0.01mg/L) were obtained by Samuel *et al*. (2015) on the same river. Acceptable concentration of sulphate (VI) in drinking water is 200mg/dm^3 (US EPA, 2010) and the values obtained in this study are within the acceptable limits.

Just like sulphate concentration, the highest phosphate-phosphorus concentration value (0.546mg/L) was obtained in station 2 in the month of July, 2015. Similar reasons adduced for sulphate concentration in River Galma would also be apt for the phosphate-phosphorus concentration. Phosphate-phosphorus concentration ranged from 0.03±0.01 mg/L (April) to 0.46±0.03 mg/L (July). Lower range of values (0.01±0.01mg/l to 0.20±0.01mg/l) were reported by Samuel *et al*. (2015) from the same river. The high values obtained in this research call for concern since elevated concentrations of phosphorus may result in fouling of natural water and production of toxic cyanobacteria (Omaka, 2007). Acceptable concentration of phosphates in surface waters is 0.2 mg/dm^3 (US EPA, 2010). The phosphate-phosphorus concentration (which had its highest concentration mean value of 0.46±0.03mg/l) obtained in this research is above the acceptable limit.

Hardness of water is a measure of total concentration of cations of calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{3+}) and manganese (Mn^{2+}). The hardness mean values ranged from 70.40±8.26 mg/L (May, 2015) to 142.40±14.73 mg/L (June, 2015). The short period of drought in the month of June might have caused increase in the concentration of these elements. Similar ranges of values (72.67±21.27mg/L to 115.67±29.35mg/L) were recorded by Samuel *et al*. (2015). Water hardness had its highest value of 184mg/L in station 2 and its lowest value of 44mg/L from station 1. The peculiarities of stations 1and 2 as stated before may have accounted for the discrepancies in their concentration values. Acceptable water hardness for drinking water - should not exceed 5mmol/dm^3 (500mg $CaCO_3/dm^3$) (US EPA, 2010). The values obtained in this research are within this limit.

Alkalinity is an indirect measure of concentration of anions in water. The alkalinity mean value ranged from 26.80±3.65 mg/L (July) to 103.20±11.35 mg/L (June). Reasons adduced for fluctuation in alkalinity might be due to discontinuity of rainfall in the month of June. The dissolved anions according to McNeely *et al*. (1979) may be sourced from bicarbonates, carbonates, hydroxides, phosphates, borates or silicates which may be derived from industrial wastes, dissolved rocks, salts, soils or bottom sediments. Lower range of alkalinity values (20.5-90.0 to (43.33±18.67 mg /L) were obtained by Lawson (2011) in Lagos lagoon. Likewise, lower mean range (30.75±2.94mg/L- 37.33±5.86mg/L) were obtained by Samuel *et al*. (2015) from River Galma. Alkalinity levels between 30 and 500 mg /L is generally acceptable to fish and shrimp production (Mcneely *et al*., 1979; Abowei and George, 2010). Alkalinity levels between 20 and 50 mg /L according to Boyd (1982) will permit plankton production for fish culture. The mean range values obtained in this research are within the acceptable range for normal survival of the aquatic biota of River Galma.

The significant difference ($P \leq 0.05$) observed in the electrical conductivity in River Galma with mean value range of 65.40±10.26 µS/cm (July, 2015) to 219.40±46.80 µS/cm (June, 2015) may be as a result of imbalances in the anion and cation levels of the water due to influx of xenobiotics into the aquatic medium from various run-offs. This is also possible since the physical, chemical and biological characteristics of freshwater systems change with size (Chételat *et al*., 2006). Also, Mbalassa1 *et al*. (2014) attributed the significant difference and high value of EC to runoffs from upstream which brings sediment loads and organic matter from the heavily agricultural lands and deforested areas from adjacent headwaters of Ishasha River in East Africa. The highest concentrations of electrical conductivity, nitrates and total dissolved solids in station 4 further buttresses this. In addition to this, the farming activities around the water bodies especially during the dry season (when the river is restricted to the major river course) may have been washed off into the river body during rainy season. This may also have led to increased chemical concentrations of River Galma. The same reasons could be adduced for the bimodal peak of the electrical conductivity (316µS/cm) in both stations 4 and 5 as well as for the mean range of values (83.33±15.20 µS/cm (station 2) to 181.17±39.82 µS/cm (station 4) given the peculiarities of these stations. Also, the highest mean value of electrical conductivity obtained during the month of June may have been as a result of concentration of the water body after initial rainfall that may have washed off xenobiotics into the river body. This is in conformity with Samuel *et al*. (2015) who reported mean range of 82.33±4.5 µS/cm to 134.17±23.08 µS/cm. The FEPA acceptable limit for conductivity in domestic water supply is 70 µS/cm (DWAF, 1996). The values obtained in this research are above this limit.

The highest value of 158mg/L was obtained for total dissolved solids in station 4 in the month of March, 2015. While the lowest value of 31ppm in the month of July, 2015 was obtained in station

1. The peculiarities associated with these months and stations as described for other parameters above may also be suitable for the total dissolved solids. The significance difference obtained in the total dissolved solids and the mean values range of 35.40±3.04mg/L (July) to 120.80±18.85mg/L (June) may be as a result of the aforementioned reasons for other parameters above. Lawson (2011) obtained the total dissolved solids value as high as 2560mg/L in Lagos lagoon. Lower mean range of values (33.40±1.25mg/L to 78.20±12.18mg/L) was obtained by Samuel *et al*. (2015). EPA Secondary Regulations (US EPA, 2010) advises a maximum contamination level (MCL) of 500mg/liter for TDS. When TDS levels exceed 1000mg/L it is generally considered unfit for human consumption. The results of this research indicate that the values obtained in River Galma are within the acceptable limits.

The mean pH values obtained in this study were significantly different from August (4.45±0.13) to June (8.72±0.05). Omaka *et al*. (2014) obtained a slightly different pH range (6.80–7.93) in Abakaliki River. According to WHO (2011), there is no health-based guideline value for pH, although 6.5 – 8.5 is proposed for drinking water. The pH of River Galma would not pose any negative effects on the biota because most aquatic animals prefer a pH range of 6.5 - 8.0 which is slightly acidic and slightly alkaline. For instance, Lawson (2011) reported that aquatic shrimps and crabs require optimum pH range of 6.8 - 8.7 for maximum growth and reproduction. Likewise, Ramanathan *et al*. (2005) recommended optimum range of pH 6.8-8.7 for maximum growth and production of shrimps and carps.

5.2 Heavy metal components of River Galma and Organs of *Clarias gariepinus* Exposed in River Galma

Lead was not detected in fish organs and water samples during the period of study. This is probably because lead producing and allied companies are not located within the study area. Municpal discharge of leaded gasoline from the neighbouring environment may not have reached detectable level either in the water or the biota of the river.

The mean Cd concentration recorded in the gills of *Clarias gariepinus* in station 3 was 0.009 ± mg/L however, Cd was not detected in the water samples. Similar observation was made by Edward *et al*. (2013) who reported heavy metals concentrations below detectable level in the water while high values were bioaccumulated in fish parts beyond tolerable levels. Low levels of cadmium has been implicated in DNA damage in the liver of Oujiang coloured common carp (*Cyprinus carpio var. color*) (Jia and Zhang, 2011). The Cd detected in the fish organs may have been washed off from the municipal discharge into the river body especially from the carbon corrugated company. The mean Cd value (0.01±0.00mg/L) bioaccumulated by *Clarias gariepinus*' gills in this study are below the recommended (FAO, 1983) permissible limit (0.5mg/L) and the permissible daily intake of Cd (0.1 µg/g wet weight) (FAO/WHO, 1999).

The concentration of chromium in water and gill of the fish at several stations in both wet and dry seasons was 10.00 mg/L. This concentration may have come from run-offs and then retained in the water matrices. Essential heavy metals may become toxic to living organism when their thresholds have been exceeded despite the fact that they have known biological functions (Abadi *et al*., 2014). Also, a link between DNA damage and concentration of Cr and Mn in soft tissues was observed in mussels in the most polluted site (Dallas *et al*., 2013). The permissible limits set for Cr by FEPA (2003) are 0.05 and 0.15 mg/ kg respectively. Cr concentration mean range (0.379±0.29 to 0.834±0.17mg/L) found in organs of the fish exceeded this limit. The higher content of the metal in fish organ probably depict bioaccumulation of the metal in the fish organ.

This agrees with the findings of Ahmed *et al*. (2013) who reported that fish assimilate Cr by ingestion or by the gill uptake tract and accumulation in fish tissues, mainly liver, occurs at higher concentrations than those found in their environment.

The highest concentration of Cr (10mg/L) in the liver of the fish exposed in River Galma was obtained in stations 4 and 5 during the wet season. The heavy metal may have been washed off along with the municipal discharge into the river body during the rainy season through run-offs given the peculiarities of these stations. Liver is the site of multiple oxidative reactions and maximal free radical generation (G¨ul *et al*., 2004; Avci *et al*., 2005). This is probably why the metal was bioaccumulated in this organ. This high concentration of Cr exceeded the set acceptable limits of 0.05-0.15mg/Kg (FEPA, 2003). This calls for concern because essential heavy metals can become toxic when certain thresholds have been exceeded (Abadi *et al*., 2014).

Station 2 had the highest Zn concentration of 0.0791mg/L in the liver of the fish exposed in River Galma during the dry season; and 2.651mg/L was recorded during the wet season in station 4. These concentrations are within acceptable set limit of 60.0mg/L per day (FAO/WHO, 1999). Hence, it would not cause any deleterious effects since Zn is a cofactor to more than 300 enzymes involved in important functions such as RNA and DNA metabolism and plays a major role in the stabilization of the structure of a large number of proteins, including signaling enzymes at all levels of cellular signal transduction (Chasapis *et al*. 2012).

Significant difference ($P \leq 0.05$) was observed in the concentrations of zinc in both dry and wet season's exposures. Zinc is a constituent of most metal containers and corrugated roofing materials. Corrosion of these materials and subsequent run-offs may have contributed to the metal load of the river. Loro *et al*. (2012) reported that despite the fact that zinc is essential, at certain

levels it is a toxic metal whose effects may be mediated by oxidative stress in fish. Likewise, Otitoloju and Don-Pedro (2002) reported that Zn was more toxic than Pb. Furthermore, Doherty *et al*. (2010) observed that the accumulation of Zn was higher than Pb in tissues of both *C. gariepinus* and *O. niloticus*. The recommended daily zinc intake for an adult is 60.0mg/day wet weight for Zn (FAO/WHO, 1999). The highest mean value of 1.36±1.29mg/L in the gills of fish exposed in station 2 and the peak mean value of 2.25±0.40mg/L detected in water samples from stations 2 and 3 are within accepted limits.

Highest Cd concentrations (0.008mg/L) were detected in liver of the fish exposed in stations 2 and 3 during the dry season. Cadmium is a very toxic metal, and also an environmental and industrial pollutant which is present in soil, water, air and food (Kaplan *et al*. 2011). Its presence even in smallest concentration calls for concern because cadmium has been implicated in induction of oxidative stress; delay in sexual maturation and impaired hormones in developing rat ovary (Roopha and Latha, 2013).

The highest manganese concentration detected in the liver of the fish exposed in River Galma were O.229mg/L in station 5 during the dry season exposure and 0.534mg/L in stations 1and 5 during the wet season exposure. Mn is an essential metal and the values bioaccumulated in the liver are within the acceptable limits of 2.0-9.0mg/day wet weight (FAO/WHO, 1999).

The highest concentration of Mn (0.212mg/L) was detected in the gill of the samples exposed in station 5. Mean value as high as 11.43±0.56mg/L was detected in water samples. The low detection of manganese in the fish organs in comparison with their concentration in water samples may be due to the fact that manganese is an essential trace element and probably have low bioaccumulation tendencies unlike other trace elements. This agrees with Dhaneesh *et al*. (2012) who reported that

nearly all the species depicted high concentration of Mn in their gills as these organs are the chief route for metal ion exchange from water because of their large surface areas that assist rapid diffusion of toxic elements. The recommended daily intake for an adult is 2.0-9.0mg/day wet weight for Mn according to FAO/WHO (1999). This limit is exceeded.

5.3 Non-enzymatic Antioxidants Production Levels in River Galma

5.3.1 Glutathione concentration

The highest mean concentration value of glutathione in gills (238.2±72.11µg/mL) of *Clarias gariepinus* was obtained in station 5 during the dry season. Since streams are known to have self-purification capabilities (Adakole *et al*., 2003), the comparatively higher glutathione level at station 5 could be attributed to additional waste discharges being received by River Galma at that station. The high level of production of glutathione in the gill of *Clarias gariepinus* during the dry season exposure in comparison with the wet season may be attributed to stream water evaporation and consequent concentration of wastes. Similarly, the gill's GSH level gradually increased from 104.96µg/mL on day 1 to 187.23µg/mL on day 7; and eventually to 289.21µg/mL in station 5 on the 14th day during the dry season. Different types of wastes have been reported to have higher toxic effects on ROS generation ((Di Giulio *et al*., 1995; Scoullos *et al*., 2007). However, there was no significant variation in GSH between the seasons. This may be attributed to the fact that in most cellular reactions, glutathione must be available in reduced form (GSH) to prevent the harmful effects of ROS induced oxidative stress (Meyer, 2008).

The highest glutathione production level in the liver of *Clarias gariepinus* were obtained in stations 2 (112.8±91.26 µg/mL) and 3 (123.4±132.39µg/mL) respectively. This reiterates the

peculiarity of these parts of the river and the importance of the organ in response to reactive oxygen species generated from the effects of xenobiotics. Livingstone (2001) observed that the amount of reactive oxygen species (ROS) production can be increased by the presence of a wide range of natural and man-made xenobiotics. This is also in conformity with the reports of Lattuca *et al*. (2009) who showed that the liver of *Odontesthes nigricans* exhibited a better control of the oxidative damage than the gills.

There was significant difference ($P \leq 0.05$) in the mean values of the kidney of the fish exposed in March between the 7^{th} and 14^{th} day (which ranged from 20.6±7.02 to 93.5±8.03µg/mL). Vertebrates' kidneys are known to perform important functions related to electrolyte and water balance and maintenance of stable internal environment (Magar and Shaikh, 2013). Since glutathione is a key player in antioxidative system (Meyer and Hell, 2005); increased production is probably needed to counteract the effects of xenobiotics in the environment of the fish.

The GSH production levels in the gills of the fish exposed in River Galma showed an increase from day 0 to day 14 especially during the dry season especially in station 5. The production levels in the liver were equally high but not as high as what were obtained in the liver of the fish. The production levels in the kidney were not that high but significant. This significant difference might be due to the fact that kidney is the major organ of detoxification and offer homeostatic balance in vertebrates (Farag *et al*., 2006). In this study, the organs were all involved in the defence of the body probably due to the specific xenobiotic present in one station or the other. The gills and the kidney offered a better biomarker of oxidative stress in River Galma than the liver.

5.3.2 Vitamins E and C concentrations

The initial vitamin C (VC) concentration in *Clarias gariepinus* prior to exposure in River Galma was (13.58mg/mL). Due to environmental dynamics the highest values of VC production level were obtained in station 4 (6.77mg/mL) and station 5 (8.15mg/mL) in the gill of the fish during the dry season. Gills being the first organ having contact with the environment are potential target for oxidative disruption (Lattuca *et al*., 2013). The reduced VC observed during this investigation may be due to their being utilized in Clarias' gill for dealing with oxidative stress. This observation is similar to EL-Gazzar *et al.* (2014) who reported that Vitamin C scavenges reactive oxygen species and renders a protective effect against Cd toxicity. This is also in conformity with the findings of Oakes *et al*. (2004) who attributed dietary deficiency and utilization of ascorbic acid by *Catostomus catostomus* in response to elevated ROS generation in a stressed aquatic environment.

Just like the gills of the fish, similar scenario played out in the liver of the fish exposed in station 5. Lowest values of 1.87mg/mL and 0.29mg/mL were obtained in station 5 in the liver of the fish during the dry and wet seasons respectively. Hounkpatin (2012) reported that the antioxidant, vitamin C has protective effect against haematotoxicity caused by cadmium and mercury. There were generally low levels of VC production in the liver of the fish during the wet season. This might be due to high water levels due to flood. The reduced VC content in the liver of the fish may have been used up by the fish in defence of itself.

There were increased production levels of VE in both liver and gills during the dry and wet seasons when compared with VC. The highest values of VE, 102.35mg/mL and 33.81mg/mL in gills of the fish during the dry and wet seasons respectively were obtained at station 5. Antioxidant, VE, is efficient in dealing with oxidative stress generated in aquatic environmenrts. Supplementation

of lycopene and vitamin E had been shown to decrease the toxic effect of diazinon (Ahmed and Mahdi, 2014).

The liver of the fish had its peak at station 4 with 58.24mg/mL during the dry season. The VE production levels in liver were generally low in both seasons (when compared to VE production levels in the gill of the fish). This result is in contrast with those found by Lattuca *et al*. (2009) who showed that the liver of *Odontesthes nigricans* exhibited a better control of the oxidative damage than the gills. There was however, no significance difference in the levels of Vitamin E production during the exposure period.

5.4 Histopathology of *Clarias gariepinus* Exposed for 14 days in River Galma

Clarias gariepinus exposed in each of the sampling stations showed alterations in liver and kidney tissues. In stations 1 and 2, these alterations ranged from constriction, aggregation, hypertrophy and vacoulation of the cells of the kidneys. Similarly, pathological changes were also observed in the liver tissues, but with lower severity. The Kidney, being the primary organ of detoxification (Farag *et al*., 2006) was probally engaged in dealing with the xenobiotics. In station 3, necrotic tissues were observed on both liver and kidneys of *Clarias gariepinus*. Similar observation was made by Makinde *et al*. (2015) who observed an advancing hepatic necrosis in the liver of *Clarias gariepinus* exposed to 2, 4-D amine.

In addition to the other alterations observed in the kidney of the fish exposed in other stations, station 4 showed congestion of blood vessels in the kidney. This is in agreement with the work of Chavan and Muley (2014) who observed that the major histopathological changes in liver include loss of cellular architecture, necrosis in hepatocytes and accumulation of fat in parenchymal cells.

Station 5 showed alterations such as vacoulation of tissues, constriction and aggregation of cells and hypertrophy. The lesser pathological damages in *Clarias gaariepinus* tissues may likely be attributed to self-purification at this station down the river course. The effects of xenobiotics on the fish did not result in mortality despite the pathological damages on the tissues. However, in the most polluted stations (2 and 4) the fish showed lethargy and erractic swimming. In most cases some were pale and easy to catch.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The ecotoxicity assessment of non- enzymatic antioxidant production by *Clarias gariepinus* in River Galma was carried out during the months of March and August, 2015. Physico-chemical parameters as well as heavy metal (Pb, Cd, Cr, Zn and Mn) components of the river and the organs of fish that were exposed *in-situ* were determined. The non-enzymatic antioxidants assayed for were the reduced glutathione (GSH), Vitamin E (α-tocopherol) and Vitamin C (ascorbic acid) using standard methods.

All the heavy metals tested for were within acceptable limits except for manganese (11.43±0.56mg/L_detected in water samples) and Cr (0.834±0.17mg/L _detected in the organs of the fish) which exceeded the set limits of 2.0-9.0mg/day wet weight and 0.05- 0.15 mg/ kg respectively. Pb was not detected in water and fish organs of *Clarias gariepinus* exposed in River Galma. Cd was detected in both gills and liver of the fish during the dry season which ranged from 0.006±0.00mg/L (liver) to 0.0062±0.00mg/L (gill). Zn concentration in the liver of the fish ranged from 0.0485±0.00mg/L to 2.1098±0.00mg/L. Mn concentration ranged from 0.0474±0.00mg/L (gill) to 0.2682±0.00mg/L (liver). While Cr concentration ranged from 0.024±0.00mg/L to 4.0±0.00mg/L in the liver of the fish. Stations 2 and 4 along River Galma were the most polluted sites in terms of DO with the lowest concentrations of 0.8mg/L and 1.3mg/L respectively. Likewise, the lowest pH values were 4.38 and 4.27 in stations 2 and 4 respectively. Highest values of total dissolved solids of 140ppm and164ppm were also obtained in stations 2 and 4 respectively. Generally, the physico -chemical parameters of River Galma varied, but with no

significant differences (P>0.05) among the sampling stations. However, there were significant differences (P≤ 0.05) in all the measured parameters among the sampling months. The mean range values for the physico-chemical parameters that were significant are as follow: DO (1.43±0.08 mg/L to 3.67±0.4 mg/L), BOD (1.13±0.12mg/L to 5.43±0.45mg/L), nitrate (0.05±0.00mg/L to 0.38±0.02mg/L), sulphate (0.02±0.00mg/L to 0.65±0.07mg/L), phosphate-phosphorus (0.03±0.01mg/L to 0.46±0.03mg/L), water hardness (70.40±8.26mg/L to 142.40±14.73mg/L), alkalinity (26.80±3.65 mg/L to 103.20±11.35 mg/L), electrical conductivity (65.40±10.26 µS/cm to 219.40±46.80 µS/cm), total dissolved solids (35.40±3.04ppm to 120.80±18.85ppm), Water temperature (21.60±0.24^{0}C to 26.80±0.20 ^{0}C), hydrogen ion concentration (pH) (4.45±0.13 to 8.72±0.05).

The histopathological damages on *Clarias gariepinus* include: vacoulation, constriction, aggregation of cells, hypertrophy, necrosis and infilteration to congestion of blood vessels. The severity and magnitude of these symptoms reflects various impacts of xenobiotics along the stretch of the river.

The mean value of GSH concentrations in the kidney ranged from 19.50±8.60µg/mL (station 1) to 98.51±35.52 µg/mL (station 5) while the mean value of GSH concentrations in the gill ranged from 66.78±12.76 µg/mL (August) to 121.85±19.16 µg/mL (March). The glutathione production level in the liver ranged from 2.8±3.95 µg/mL (station 1) to 123.4 ±132.39 µg/mL (station 3).

The production levels of Vitamins E antioxidants were higher in the dry season (102.35mg/mL) than in the wet season (35.24mg/mL). Vitamin C production level obtained in the gill and liver of the fish in both seasons were 3.67±3.11mg/mL (station 4) to 16.919mg/mL (stations 1) and

0.398mg/mL (station 2) to 13.121mg/mL (station 4) respectively. Mean values of vitamin E produced in the gill and liver of the fish in both seasons ranged from 14.72±14.72mg/mL (station 1) to 102.35mg/mL (station 5) and 22.26±22.26mg/mL (station 1) to 58.237mg/mL (station 4) respectively.

6.2 Conclusions

The mean range value obtained for the dissolved oxygen was 1.43±0.08 mg/L to 3.67±0.4 mg/L. This showed that River Galma is polluted and the disolved oxygen reflected it. This mean range value is below the normal dissolved oxygen required for optimal survival of aquatic biota and could lead to generaltion of reactive oxygen species and consequently, oxidative stress. Heavy metals were detected at varying concentration in the both water and fish organs. All the heavy metals tested for were within acceptable limits except manganese (11.43±0.56mg/L) and Cr (0.834±0.17mg/L) which exceeded the set limits.

The histopathology of the organs of *Clarias gariepinus* exposed in River Galma for 14 days indicated various changes in the architecture of the tissues. The histopathological damages include vacoulation, constriction, aggregation of cells, hypertrophy, necrosis and infilteration to congestion of blood vessels. These alterations in the cellular structures of the fish indicated that tRiver Galma is polluted and needs urgent ameliorative measures.

Kidneys and gills of *Clarias gariepinus* offer a good prospect in *in situ* bio-assays of glutathione in River Galma. The GSH highest mean value of 98.51$\pm$35.52μg/mL obtained in the kidney of the fish expose in the River Galma in comparison with the mean value of 130.49$\pm$0.00μg/mL obtained

in the fish prior to exposure reflected the utilization of the anti-oxidant in dealing with the effect of oxidative stress as a result of pollution.

Vitamin C offers a good prospect as biomarker of oxidative stress generated as a result of pollution than vitamin E in River Galma. The increased production levels of vitamin C (as high as 16.919mg/mL) in the gill of the fish in comparison with the mean value of 0.98 ± 0.00mg/mL prior to exposure; and 13.121 mg/mL obtained in the liver of the fish exposed in River Galma in comparison with 1.39 ± 0.00mg/mL obtained prior to exposure indicated the up-regulation of the vitamins in dealing with the xeno-biotics in the polluted sites.

6.3 Recommendations

Glutathione, vitamin C and E bio-assays in *Clarias gariepinus* should be employed in ecotoxicity assessment of River Galma with special reference to the use of GSH and up-regulation of the production level of ascorbic acid as biomarker of oxidative stress.

Histopathological alterations in tisssues (kidney and liver) should be used for fast determination of health status of the living biota within River Galma.

Further research should take into cognizance and evaluate the heavy metal load of the sediments at each location along River Galma.

Holistic policies and approach should be made and implemented to stop careless dumping of refuse along river bodies by relevant authorities.

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APPENDIX I

Analysis of Variance for Physico-chemical parameters based on Months

	Sum of squares	Df	Mean square	F value	Significance
Water Temperature (°C)	Between group 76.400	5	15.2800	53.929	0.000
	Within group 6.800	24	0.283		
	Total 83.200	29			
Air Temperature(°C)	97.867	5	19.573	6.383	0.001
	73.600	24	3.067		
	171.467	29			
Dissolved oxygen (mg/l)	18.672	5	3.734	6.176	0.001
	14.511	24	0.605		
	33.183	29			
Biological Oxygen Demand (mg/l)	54.550	5	10.910	9.171	0.000
	28.552	24	1.190		
	83.102	29			
Nitrate(mg/l)	0.494	5	0.099	38.933	0.000
	0.061	24	0.003		
	0.555	29			
Sulphate(mg/l)	1.222	5	0.244	31.788	0.000
	0.185	24	0.008		
	1.407	29			
Phosphate-phosphorus(mg/l)	0.653	5	0.131	25.699	0.000
	0.122	24	0.005		
	0.774	29			
Hardness of Water(mg/l)	20554.667	5	4110.933	6.117	0.001
	16128.000	24	672.000		
	36682.667	29			
Total Alkalinity(mg/l)	16222.00	5	3244.400	11.068	0.000
	7035.200	24	293.133		
	23257.200	29			
Total Dissolved Solids (ppm)	26658.167	5	5331.633	7.840	0.000
	16322.000	24	680.083		
	42980.167	29			
Electrical Conductivity (µS/cm)	86267.767	5	17253.553	4.605	0.004
	89924.400	24	3746.850		
	176192.167	29			
pH	55.457	5	11.091	97.879	0.000
	2.720	24	0.113		
	58.117	29			

APPENDIX II

Analysis of Variance Table for Heavy Metals based on Months (Season) in Gill of the Fish

		Sum of square	Df	Mean square	F value	Significance
Pb (mg/l)		Between group 0.000	1	0.000	0.000	0.000
		Within group 0.000				
		Total 0.000	8	0.000		
			9	0.000		
Cr (mg/l)		9.702	1	9.702	0.970	0.353
	80.005		8	10.001		
		89.707	9			
Cd (mg/l)		0.000	1	0.000	14.561	0.005
	0.000		8	0.000		
		0.000	9			
Zn (mg/l)		9.609	1	9.609	29.275	0.001
	2.626		8	0.328		
	12.234		9			
Mn (mg/l)		0.017	1	0.017	0.365	0.562
	0.370		8	0.046		
		0.387	9			

APPENDIX III

Analysis of Variance Table for Heavy Metals based on Months (Season) in Liver of the Fish

		Sum of square	Df	Mean square	F value	Significance
Pb (mg/l)		Between group 0.000	1	0.000	0.000	0.000
		Within group 0.000				
		Total 0.000	8	0.000		
			9	0.000		
Cr (mg/l)		39.510	1	39.510	2.634	0.143
		120.006	8	15.001		
		159.515	9			
Cd (mg/l)		0.000	1	0.000	15.652	0.004
	0.000		8	0.000		
	0.000		9			
Zn (mg/l)		10.635	1	10.635	35.429	0.000
		2.401	8	0.300		
		13.036	9			
Mn (mg/l)		0.124	1	0.124	3.339	0.105
		0.296	8	0.037		
		0.420	9			

APPENDIX IV

Analysis of Variance Table for Heavy Metals in water

	Sum of square	Df	Mean square	F value	Significance
Pb (mg/l)	Between group	4	0.000	0.000	0.000
	0.000				
	Within group 0.000	15	0.000		
	Total 0.000				
		19	0.000		
Cr (mg/l)	30.411	4	7.603	0.510	0.730
	223.784	15	14.919		
	254.196	19			
Cd (mg/l)	0.000	4	0.000	0.773	0.560
	0.000	15	0.000		
	0.000	19			
Zn (mg/l)	2.556	4	0.639	0.422	0.790
	22.724	15	1.515		
	25.280	19			
Mn (mg/l)	0.131	4	0.033	0.704	0.602
	0.699	15	0.047		
	0.830	19			

APPENDIX V

Analysis of Variance Table for Combined Heavy Metals based on Months

	Sum of square	Df	Mean square	F value	Significance
Pb (mg/l)	Between group	1	0.000	0.000	0.000
	0.000				
	Within group 0.000	8	0.000		
	Total 0.000	9			
Cr (mg/l)	10.000	1	10.000	0.400	0.545
	200.000	8	25.000		
	210.000	9			
Cd (mg/l)	0.000	1	0.000	0.000	0.000
	0.000	8	0.000		
	0.000	9			
Zn (mg/l)	0.910	1	0.910	59.968	0.000
	0.121	8	0.015		
	1.031	9			
Mn (mg/l)	4.315	1	4.315	122.675	0.000
	0.281	8	0.035		
	4.597	9			

APPENDIX VI

Analysis of Variance for Vitamin C production levels in gill of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	567.133	1	567.133	16.272	0.000
Stations	138.508	5	27.702	0.795	0.567
Error	627.370	18	34.854		
Total	1333.011	24			

APPENDIX VII

Analysis of Variance for Vitamin C production levels in liver of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	374.855	1	374.855	16.322	0.001
Stations	129.222	5	25.846	1.125	0.382
Error	413.383	18	12.314		
Total	917.466	24			

APPENDIX VIII

Analysis of Variance for Vitamin E production levels in gill of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	29144.079	1	29144.079	89.526	0.000
Stations	8120.678	5	1624.136	4.989	0.005
Error	5859.686	18	325.538		
Total	43124.444	24			

APPENDIX IX

Analysis of Variance for Vitamin E production levels in liver of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	23107.420	1	23107.420	157.578	0.000
Stations	2774.898	5	554.980	3.785	0.016
Error	2639.535	18	146.641		
Total	28521.854	24			

APPENDIX X

Analysis of Variance for Glutathione production levels in gill of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	213506.525	1	213506.525	62.369	0.000
Stations	26505.411	5	5301.082	1.549	0.225
Error	61618.593	18	3423.255		
Total	301630.529	24			

APPENDIX XI

Analysis of Variance for Glutathione production levels in liver of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	113776.002	1	113776.002	36.602	0.000
Stations	20663.941	5	4132.788	1.330	0.296
Error	55951.619	18	3108.423		
Total	76615.560	23			

APPENDIX XII

Analysis of Variance for Glutathione production levels in Kidney of *Clarias gariepinus* exposed in River Galma

Sources of variation	Sum of square	Df	Mean sum of square	F value	Significance
Seasons	88554.061	1	88554.061	75.533	0.000
Stations	38618.556	5	7723.711	6.588	0.001
Error	21102.933	18	1172.385		
Total	148275.550	24			

APPENDIX XIII

Seasonal heavy metal concentrations of organs of *Clarias gariepinus* exposed *in situ* in River Galma base on stations

Fish organs /Stations	Pb (mg/l)			Cr (mg/l)			Cd (mg/l)			Zn (mg/l)			Mn (mg/l)		
Gill	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean
Station 1	0.00	0.00	0.00±0.00	0.02	0	0.01±0.01	0.006	0	0.00±0.00	0.008	0.746	0.38±0.37	0.009	0	0.00±0.00
Station 2	0.00	0.00	0.00±0.00	0.021	10	5.01±4.99	0.008	0	0.00±0.00	0.0765	2.651	1.36±1.29	0.016	0.648	0.33±0.32
Station 3	0.00	0.00	0.00±0.00	0.00	0	0.00±0.00	0.009	0	0.00±0.00	0.0917	2.33	1.21±1.12	0.00	0	0.00±0.00
Station 4	0.00	0.00	0.00±0.00	0.018	0	0.01±0.01	0.008	0	0.00±0.00	0.0314	2.651	1.34±1.31	0.00	0	0.00±0.00
Station5	0.00	0.00	0.00±0.00	0.091	0	0.05±0.05	0.00	0	0.00±0.00	0.069	1.698	0.88±0.81	0.212	0	0.11±0.11
Total	**0.00**	**0.00**	**0.00±0.00**	**0.150**	**10**	**1.02±1.00**	**0.031**	**0**	**0.00±0.00**	**0.2766**	**10.076**	**1.04±0.37**	**0.237**	**0.648**	**0.09±0.07**
P value						**0.485ns**			**0.886ns**			**0.951ns**			**0.516ns**
Liver	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean
Station1	0.00	0.00	0.00±0.00	0.003	0	0.00±0.00	0.007	0	0.00±0.00	0.0062	0.746	0.38±0.37	0.00	0.534	0.27±0.27
Station2	0.00	0.00	0.00±0.00	0.024	0	0.01±0.01	0.008	0	0.00±0.00	0.0791	2.492	1.29±1.21	0.00	0.00	0.00±0.00
Station3	0.00	0.00	0.00±0.00	0.005	0	0.00±0.00	0.008	0	0.00±0.00	0.0504	2.33	1.19±1.14	0.00	0.193	0.10±0.10
Station4	0.00	0.00	0.00±0.00	0	10	5.00±5.00	0.007	0	0.00±0.00	0.0478	2.651	1.35±1.30	0.00	0.08	0.04±0.04
Station5	0.00	0.00	0.00±0.00	0.091	10	5.05±4.95	˚	0	0.00±0.00	0.059	2.33	1.20±1.14	0.229	0.534	0.38±0.15
Total	**0.00**	**0.00**	**0.00±0.00**	**0.123**	**20**	**2.01±1.33**	**0.030**	**0**	**0.00±0.00**	**0.2425**	**10.549**	**1.08±0.38**	**0.229**	**1.341**	**0.16±0.07**
P Value						**0.592ns**			**0.895ns**			**0.962ns**			**0.401ns**

APPENDIX XIV

Combined seasonal means of heavy metal concentrations of organs of *Clarias gariepinus in situ* in River Galma and water samples of River Galma base on fish organs and months of sampling

Fish organs/ water	Pb (mg/l) Dry	Wet	Cr (mg/l) Dry	Wet	Cd (mg/l) Dry	Wet	Zn (mg/l) Dry	Wet	Mn (mg/l) Dry	Wet
Gill	0.00±0.00	0.00±0.00	0.030±0.00	2.0±0.00	0.0.062±0.00	0.00±0.00	0.05532±0.00	2.0152±0.00	0.0474±0.00	0.1296±0.00
Liver	0.00±0.00	0.00±0.00	0.024±0.00	4.0±0.00	0.006±0.00	0.00±0.00	0.0485±0.00	2.1098±0.00	0.0458±0.00	0.2682±0.00
Combined mean	**0.00±0.00**		**1.51±0.82**		**0.00305±0.00**		**1.06±0.26**		**0.12±0.05**	
P value			**0.556ns**		**0.956ns**		**0.934ns**		**0.479ns**	
Water	0.00±0.00	0.00±0.00	4.00±2.45	2.00±2.00	0.00±0.00	0.00±0.00	1.86±0.00	2.46±0.08	11.94±0.03	10.63±0.12
Combined mean	0.00±0.00		**3.00±1.53**		0.00±0.00		**2.16±0.11**		**11.28±0.23**	
P value			**0.545ns**				**0.000***		**0.000***	

APPENDIX XV

Seasonal heavy metal concentrations of samples of water from River Galma base on stations

Water	**Pb (mg/l)**			**Cr (mg/l)**			**Cd (mg/l)**			**Zn (mg/l)**			**Mn (mg/l)**		
	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean	Dry	Wet	Mean
Station 1	0	0	0.00±0.00	0	0	0.00±0.00	0	0	0.00±0.00	1.857	2.33	2.10±0.24	11.898	10.648	11.27±0.63
Station 2	0	0	0.00±0.00	10	0	5.00±5.00	0	0	0.00±0.00	1.857	2.651	2.25±0.40	11.898	10.193	11.05±0.85
Station 3	0	0	0.00±0.00	10	0	5.00±5.00	0	0	0.00±0.00	1.857	2.651	2.25±0.40	11.898	10.761	11.33±0.57
Station 4	0	0	0.00±0.00	0	0	0.00±0.00	0	0	0.00±0.00	1.857	2.33	2.10±0.24	11.989	10.875	11.43±0.56
Station 5	0	0	0.00±0.00	0	10	5.00±5.00	0	0	0.00±0.00	1.857	2.33	2.10±0.24	12.011	10.648	11.33±0.68
Total	0	0	0.00±0.00	20	10	3.00±1.53	0.00±0.00	0.00±0.00	0.00±0.00	9.285	12.292	2.16±0.11	59.694	53.125	11.28±0.23
P value						0.739ns						0.986ns			0.994

APPENDIX XVI

Combined seasonal concentration of Glutathione production levels (µg/mL) in organs of *Clarias gariepinus* exposed on a stretch of River Galma

Fish organs	Dry			Wet		
Gill	7th	14th	Mean	7th	14th	Mean
Station1	146.1	22.7	84.4	4.26	0	4.26
Station 2	96.45	116.31	106.38	8.51	96.45	52.48
Station 3	79.43	95.04	87.24	60.99	113.48	87.24
Station 4	139.01	80.85	109.93	56.74	109.22	82.98
Station 5	187.23	289.21	238.22	41.13	100.71	70.92
Total	**648.22**	**604.11**	**125.23**	**171.63**	**419.86**	**59.58**
Liver						
Station 1	69.5	15.6	42.55	5.67	0	5.67
Station 2	48.23	177.3	112.77	1.42	96.45	48.94
Station 3	217.02	29.79	123.405	15.6	43.97	29.79
Station 4	68.09	25.53	46.81	26.95	70.92	48.94
Station 5	76.59	131.92	104.26	15.6	45.39	30.50
Total	**479.43**	**380.14**	**85.96**	**65.24**	**256.73**	**32.75**
Kidney						
Station 1	41.13	14.18	27.66	22.7	0	22.7
Station 2	76.6	35.46	56.03	9.93	48.23	29.08
Station 3	15.6	25.53	20.57	11.35	56.74	34.05
Station 4	48.23	21.28	34.76	39.72	75.18	57.45
Station 5	87.8	99.15	93.48	17	190.07	103.54
Total	**269.36**	**195.60**	**46.5**	**100.7**	**370.22**	**49.36**

APPENDIX XVII

Seasonal Variations in Vitamin C and E concentrations (mg/mL) in organs of *Clarias gariepinus* in an *in situ* bio-assay in River Galma

Fish organs/Locations	Vitamin C Dry	Wet	Mean	Vitamin E Dry	Wet	Mean
Gills	13.579			13.579		
Control						
Station 1	16.919	˚	16.919	29.43	0	29.43
Station 2	13.666	0.398	7.032	34.75	30.79	32.77
Station 3	8.654	0.595	4.6245	49.46	35.24	42.35
Station 4	6.774	0.564	3.669	41.96	33.20	37.58
Station 5	8.149	0.449	4.299	102.35	33.81	68.08
Total	**54.162**	**2.006**	**7.3087**	**257.95**	**133.04**	**42.042**
Liver	13.678			13.678		
Control						
Station 1	5.183	˚	5.183	44.523	0	44.523
Station 2	12.152	0.516	6.334	38.559	33.843	36.201
Station 3	10.179	0.577	5.378	35.911	32.092	34.00
Station 4	13.121	0.529	6.825	58.237	37.201	47.719
Station 5	1.871	0.294	1.0825	25.641	38.978	32.31
Total	**42.506**	**1.916**	**4.9605**	**202.871**	**142.114**	**38.95**