DETERMINATION OF WATER POLLUTION USING *CLARIAS GARIEPINUS* (BURCHELL, 1822) OXIDATIVE STRESS ENYZMES AS BIOMARKERS IN TATSAWARKI STREAM, KANO

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA
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DECLARATION

I declare that the work in this Dissertation entitled "DETERMINATION OF WATER POLLUTION USING *CLARIAS GARIEPINUS* (BURCHELL, 1822) OXIDATIVE STRESS ENYZMES AS BIOMARKERS IN TATSAWARKI STREAM, KANO" has been carried out by me in the Department of Biology. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

ABDULLATEEF Habibat ______________________ ______________________

Signature Date

CERTIFICATION

This dissertation entitled "DETERMINATION OF WATER POLLUTION USING *CLARIAS GARIEPINUS* (BURCHELL, 1822) OXIDATIVE STRESS ENYZMES AS BIOMARKERS IN TATSAWARKI STREAM, KANO" by Habibat ABDULLATEEF meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my Parents, Alhaji Abdullateef Abdulsalam and Hajia T. Abdullateef Abdulsalam.

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ABSTRACT

The role of water is indispensable in the sustainability of life. Tatsawarki stream is the main source of water for most inhabitants of kano-central. Physicochemical parameters of Tatsawarki stream and biomarkers oxidative strees in *Clarias gariepinus* from the stream were investigated for 8-months (November, 2015 to June, 2016). Water samples were collected from five sampling Stations. All parameters were determined using standard methods. Heavy metals (Pb, Cr, and Fe) were determined in both water samples and fish tissues (liver, kidney and gill) of caged and free- roaming *Clarias sp*, using atomic absorption spectrophotometer. CAT, GSH, MDA, and SOD and Vitamin C and E were determined in the fish tissues. Temperature (25.33± 4.60^{0}C), pH (8.30± 0.42), Total alkalinity (76.81±56.21mg/l.CaCO3), BOD (0.44±0.66mg/l), Chloride (6.28±4.69mg/l), Nitrate-nitrogen (19.53± 4.50mg/l) and Phosphate-phosphorous (1.55±0.62mg/l) fell within permissible limits for survival of aquatic organisims. High EC (1729.31±540.35µS/cm), TDS (841.06±265.83mg/l), Sulphate (245± 100.71mg/l) in Station 2, could be attributed to organic effluents. Low mean DO (1.01±1.03mg/l) in all the Stations during the study period could be associated to indiscrimante waste water disposal mechanisims by the inhabitants around the stream. The trend of accumulation of heavy metal in water is Iron > Lead > Chromium, however Chromium was not detected in the stream's water. The presence of chromium in the fish tissues was attributed to bioaccumulation of metals by the fish. There was a significant difference in total protein, non-enzymatic (Glutathione, Vitamin C and Vitamin E), and enzymatic oxidative stress biomarkers (CAT, MDA, SOD). The increase in MDA in gill (388.40±312.51nmoles/mg protein), kidney (466.30±340.75nmoles/mg protein), and liver (422.57±357.51 nmoles/mg protein) can be associated to presence of oxidative stress in the stream. Pathological alterations (deformation, diffusion and degeneration of secondary lamella in the gill, degeneration, dissociation, congestion and edema) in kidney and infilteration, vacoulation and architectural structural alteration in the liver of *Clarias sp*, indicates poor water quality of Tatsawarki stream. Recommedations on how to improve the water quality are profferd.

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ACCRONYMS, ABBREVIATIONS AND DEFINITIONS

FAO = Food and Agriculture Organization

WHO = World Health Organization

FEPA= Federeal Environmental Protection Agency

APHA = American Public Health Association

Cr = Chromium

Pb = Lead

CAT = Catalase

SOD = Superoxide Dismutase

MDA = Malondyhyde

MT = Metalothionein

GSH = Glutathione

NO_3-N = Nitrate- Nitrogen

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Aquatic environment has been a sink for many environmental contaminants due to continuous pollution by various anthropogenic activities (Adekunle *et al*., 2007). These contaminants such as: domestic, industrial, and agricultural waste waters have the potential to cause oxidative stress in aquatic organisms, and disturb balance of antioxidant/prooxidant. Contaminants can be absorbed directly from contaminated water or indirectly via the food chain (Lopes *et al.,* 2011; Lushchak, 2011; Ben Ameur *et al*., 2015; Amaeze and Onyema, 2014).

Discharge of contaminants can cause deterioration of the aquatic environment and cause a marked shift in the ability of an aquatic environment to support its constituent's biotic communities such as fish (Livingstone, 2001; Wang, 2002; Lushchak, 2011). In order to prevent these deleterious effects of contaminants, early diagnostic toxicants tools (Biomarkers) were initiated, to provide unique information on ecosystem health (Cajaraville *et al.,* 2000; Ameur *et al*., 2015). Frequently used biomarkers includes: histopathological analysis, DNA integrity and detoxification enzyme status in tissues of organisms, among others.

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants both in laboratory (Thophon *et al*., 2003) and in field studies (Teh *et al*., 1997; Ben Ameur *et al*., 2015). Histopathological biomarkers in fish offer a number of practical advantages in the field such as ease of sample collection, ability to assess many body systems and cell types from the same fish (Ben Ameur *et al*, 2012).

Histopathological analysis also enables the specific sites of cellular injury to be identified (Hinton *et al*., 1992).

Interest on the study of oxidative stresses responses in aquatic organisms induced by toxicants such as: heavy metals was initiated (Soares *et al* .1997; Osuala, 2012) due to its negative effects on the cellular functioning of aquatic organisms, effects on growth and development, and induction of oxidative stress. Ayeloja *et al*., (2014), investigated heavy metal concentration in some fishes including *Clarias gariepinus* in Eleyele reservoir Ibadan, Oyo state. These authors reported that the level of lead in both the water body and fish is less than the European Commission, FAO and WHO, (2004) recommended standards of 0.4, 0.5mg/kg / 0.05mg/l and 2.0 mg/kg respectively. This decrease may affect the growth and development of aquatic organisms (Authman, 2008; Zhu *et al.*, 2004; Pandey and Tripathi, 2014). Najeeb *et al.*, (2014), reported that, if lead level is higher than the recommended standards, formation of free radicals within an organism might be induced hence oxidative stress.

Other heavy metals if present in high levels that could induce oxidative stress are: Copper, Chromium, Zinc, Arsenic and many others (Farombi *et al*., 2007 and Doherty *et al*., 2010).

Nzeve *et al*., (2014), stated that, the mean Cr concentration in *Clarias gariepinus* from Masinga Reservoir, Kenya is higher than the WHO recommended standard limit of 0.15mg/kg / 0.05mg/l (WHO, 2004). The elevated Cr level could lead to severe effects such as induction of oxidative stress.

Oxidative stress has the ability to initiate bimolecular oxidations, disturb cellular metabolism and its regulation and cause death (Lushchak, 2011). Oxidative stress has been implicated in numerous diseases and disorders such as aging, cancer, atherosclerosis, cirrhosis, pulmonary fibrosis, neurodegenerative diseases and cataracts (Halliwell *et al*., 2000; Lushchak, 2011).

The three main factors responsible for oxidative stress are:

(a) Increase in oxidant generation: is the increase in endogenous ROS levels which if enhanced causes oxidative stress.

(b) Decrease in antioxidant protections, which include changes in induction of antioxidant defenses and changes on antioxidant enzyme activity.

(c) Failure to repair oxidative damage, which leads to impaired DNA bases, protein oxidation products, and lipid peroxidation products. (Livingstone, 2001; Nordberg and Arner, 2001).

In order to detoxify these detrimental effects of ROS generation in the normal aerobic metabolism of aquatic organisms, series of cellular antioxidants with both enzymatic and non-enzymatic activities are present in the cells and tissues (Nordberg and Arner, 2001).The levels of the antioxidant enzymes is an indicator of the antioxidant status of the organism and serve as biomarkers of oxidative stress (Livingstone, 2001)

Antioxidants such as Catalase (CAT), Superoxide dismutase (SOD), Glutathione (GSH) Malondialdehyde (MDA), Metallothionein (MT), serve as bio indicators/ biomarkers of oxidative stress, in both marine and freshwater organisms (Bebianno *et al*., 2004; Borković *et al*., 2005; Farombi *et al*., 2007; Yildirin *et al*., 2011; Idowu *et al*., 2014). Other antioxidants are: b-carotene (vitamin A), ascorbate (vitamin C), and atocopherol (vitamin E). They participate in the process of eliminating oxyradicals and hence serve as bioindicators of contaminants (VanDerOost *et al.*, 2003; Yildirim *et al*., 2010, Ahmad *et al*., 2014). The induction of these biomarkers reflects response to pollutants (Karadag *et al*., 2014).

Fish has being frequently subjected to prooxidant effects of pollutants present in the aquatic environment (Jordanoska *et al*., 2008). These pollutants may catalyze reactions that generate reactive oxygen species (ROS) which lead to environmental oxidative stress (Zikic *et al*., 2001; Vindohini and Narayanan, 2009). Due to this ability, many investigations have being made which proposed several fish species as indicators of the quality of aquatic environment (Dautremepuits *et al*., 2004; Lopes, 2001; Velkova-Jordanoska *et al*., 2008; Shahid *et al*., 2014).

Clarias gariepinus due to its hardy nature and ability to tolerates adverse water quality conditions compared to other fish, has been widely used as bio indicator of environmental stress (Doherty *et al*., 2010; Adeogun *et al*., 2012). Thus the evaluation of oxidative stress in *Clarias gariepinus* to determine water pollution in Tatsawarki stream.

1.2 Statement of Research Problem

Unregulated discharge of domestic, industrial and agricultural wastes/ wastewater in to Tatsawarki stream has led to high possibility of oxidative stress in the stream, which could affect the stream's water quality. Akpan, (2013), noted that anthropogenic inputs affect the quality of water bodies.

Elevated levels of heavy metals due to anthropogenic inputs, higher than recommended concentration in the water bodies have been reported in several Nigerian streams (Biney *et. al*. 1991;): Tatsawarki Stream may not be an exception. Heavy metals affect physiological balance and other processes in fish (Alibabic *et al*., 2007). It could also cause histo-pathologhical damage (Andhale *et al*., 2011; Tarasub *et al*., 2012), disruption of reproductive endocrine potential (Drevnickand and Sandheinrich 2003; Kasperczyk *et al*., 2008), generation of oxidative stress (Vinodhini and Narayanan, 2009; Farombi *et al*., 2007; Soundararajan *et al*., 2009) and mortality if present in acute toxicity (Otitoloju and Don-Pedro, 2002)

Clarias gariepinus is of great commercial importance and widely consumed in Nigeria (Olaifa *et al*., 2004; Doherty *et al*., 2010) but its growth is under threat. Among the threats for continued survival of some fishes might be due to oxidative stress (Ekambaram *et al*., 2014). Oxidative stress retards the growth and reproductive rate of aquatic organisms, affects the health of aquatic environment and hence affects per capital income of the fisherman (Wendelaar, 1997; Adeogun *et al*., 2012). The absence of fishing activities in some Stations of Tatsawarki stream could be associated with oxidative stress.

1.3 Justification

The health of a water body is determined by its water quality which could be easily influenced by anthropogenic inputs (Adakole *et al.,* 2015). River Tatsawarki despite its importance in provision of water and water resources to communities within its catchment area receives both organic and inorganic wastes through run-offs from domestic waste, agricultural effluents and industrial waste, which could affects its water quality. Hence the justification for this study.

Organic wastes are usually bioaccumulatory in nature and have the ability to stimulate oxidative stress enzymes production, which is fatal to aquatic living organisms, affects growth, its reproductive rates and affects aquatic habitat. (Kalay and Canil, 1999; Lentech, 2006), hence lead to several research on determination of oxidative stress in aquatic biota (Soares *et al.,* 2008; Osuala, 2012). Commonly determined oxidative stress indices include: CAT, SOD, MDA, MT,GSH (Bebianno *et al*., 2004; Borković *et al*., 2005; Farombi *et a*l., 2007; Yildirin *et al*. 2011; Carvalho *et al*. 2012) thus the evaluation of these parameters in *Clarias gariepinus* in Tatsawarki stream.

The natural physiological functioning of an organism gets disturbed on exposure to stress (Venkata, 2013; Janardana *et al*., 2016). The effects of oxidative stress could be induced at cellular level or even at molecular level, but ultimately it causes physiological, pathological, and biochemical alterations (Gabriel *et al*., 2012; Nagaraju, 2013). Hence the determination of physiological alterations through assessment of histopathological tissues of *Clarias gariepinus* in this work.

1.4 Aim

The aim of this work is to use the response of the antioxidant enzyme biomarkers in *Clarias gariepinus* as determinants of presence or absence of pollution in Tatsawarki stream, Kano.

1.5 Objectives

To determine

1. Temporal and spatial physicochemical parameters of Tatsawarki stream
2. Heavy metals (lead, iron and chromium) in *Clarias gariepinus* and water samples
3. Oxidative stress level using biomarkers, Catalase (CAT), Superoxide Dismutase (SOD), Malondhyde (MDA), Glutathione (GSH) in the gill, liver and kidney, in caged and free roaming *Clarias gariepinus* in Tatsawarki stream and level of vitamin (Cand E) in caged and free roaming *Clarias gariepinus* in Tatsawarki stream.
4. Histopathology of the gill, liver, and kidney of caged and free roaming *Clarias gariepinus* in Tatsawarki stream

1.6 Hypothesis

1. There is no significant difference in temporal and spatial physicochemical parameters in Tatsawarki stream.
2. There is no significant differences in heavy metals (lead, iron and chromium) in *Clarias gariepinus* and water samples
3. There is no significant difference in oxidative stress level using biomarkers, Catalase (CAT), Superoxide Dismutase (SOD), Malondhyde (MDA), Glutathione (GSH) in the gill, liver and kidney, in caged and free roaming *Clarias gariepinus* in Tatsawarki stream and there is no significant difference in the vitamin (Cand E) in caged and free roaming *Clarias gariepinus* in Tatsawarki stream.

4. There is no significance difference in the gill, liver, and kidney in caged and free roaming *Clarias gariepinus* in Tatsawarki stream

CHAPTER TWO

2.0 LITREATURE REVIEW

2.1 Water pollution

Water quality level is a determinant to the utility and survival of aquatic organisms and man (Gleick, 2000). The level of water quality is governed by physicochemical and biological parameters (Singh *et al*., 2013). The physiochemical parameter is a network of variables such as pH, Dissolved oxygen concentration, and temperature, among others. These variables (physical/ chemical) can affect aquatic biota (Kolawole *et al*., 2011). Gulson *et al*., (1996) reported that physiochemical parameters influence biochemical reactions within water body and its concentration serve as indicator of the condition of a water body.

2.2 Physio-Chemical Parameters

Physio-chemical parameters have been widely used for the determination and assessment of environmental pollutants present in the water body (Sangu and Sharma, 1987).

2.3 Physical Parameters

2.3.1 Temperature

The level of temperature influences the rates of metabolism, growth of aquatic organisms, solubility of oxygen in a water body, and organisms' sensitivity to disease, parasites, and toxic materials (Wetzel, 2001; Koeypudsa and Jongjareanjai, 2011). Increase in solubility of dissolved oxygen and toxicity of certain compounds including heavy metals such as lead can be attributed to high temperature (Bhadja and Vaghela, 2013). Aquatic organism’s example” fish adapt to particular water temperature range and abrupt chnge can lead to anaerobic conditions, decrease enzyme activity and dealth (Goel, 2006). *Clarias gariepinus* can tolerate temperatures of 8-35 degrees Celsius.

2.3.2 Electrical conductivity

Electrical conductivity indicates the presence of industrial discharges, ions within the water body. High or Low electrical conductivity in a water can be associated to pollution Higher conductivity can be indicated by saline intrusion on the upstream (Seema, 2015). Agricultural runoff or a sewage leak can lead to high electrical conductivity due to the additional chloride, phosphate and nitrate ions (EPA, 2012), hence makes the water unsuitable for usage.

2.3.3 Total dissolved solids

Total dissolved solids (TDS) in water consist of inorganic salts such as: carbonates, chlorides, sulphates, and nitrates (primarily in ground water), cations such as potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na) and dissolved materials present in proportions that create a balanced solution (Seema, 2015). Additional inputs of dissolved solids can be obtained from both natural and anthropogenic sources. Anthropogenic inputs that cause high total dissolved solids in the water body include: agricultural runoff and domestic waste (Annalakshmi and Amsath, 2012; Seema , 2015) . The increase in the total dissolved solids could alters the balance and composition of the water body hence toxic to aquatic life (Manoj and Padhy, 2015).

2.4 Chemical Parameters

2.4.1 pH

pH is an indicator of the existence of biological life. pH of water body is the measure of hydrogen ion concentration. It is the level of acidity or basicity of water body on a scale of 0-14 in which 7.0 is neutral. The optimum pH for a water body is 7.4. Extreme pH can make water body inhospitable to life. Low pH is harmful to juvenile fish and insects by speeding the leaching of heavy metals. Aquatic organisims function best in a pH range of 6.5 to 9.0 (USEPA, 2005). High pH levels (9-14) can harm fish by denaturing cellular membranes and

Low pH levels can accelerate the release of metals from rocks or sediments in the water body thus affecting the fish's metabolism (Robertson, 2004).

2.4.2 Dissolved oxygen

The level of free, non- compound oxygen present in water or other liquids is known as dissolved oxygen. Dissolved oxygen is important to many forms of life including fish, invertebrates, bacteria and plants. Dissolved oxygen level indicates water quality. Dissolved oxygen level that is too high or too low can harm aquatic life and affect water quality. DO below 5.0 mg/L could adversely affects aquatic life (Sinha and Biswas, 2011). Decrease in DO concentrations in water bodies could be caused by decomposing organic matter, dissolved gases, industrial waste, mineral waste and agricultural runoff (Srivastava *et al*., 2007; Addo *et al*., 2013).

2.4.3 Total alkalinity

One of the best measures of sensitivity of streams to neutralize acidic pollution from rainfall or wastewater to acid inputs is the determination of alkalinity (United States Environment Protection Agency, 2013). Total Alkalinity is the quantitative capacity of an aqueous solution to neutralize an acid (carbonates and bicarbonates). These carbonates and bicarbonates resulted due to the weathering of rocks, waste discharge and microbial decomposition of organic matter into the water body. Alkalinity does not measure the same property as the pH (namely basicity). Alkalinity is important for fish and aquatic life because it protects or buffers against rapid pH changes. Higher alkalinity levels in surface waters will buffer acid rain and other acid wastes and prevent pH changes that are harmful to aquatic life. If alkalinity is naturally low, (less than 20 mg/L.$CaCO_3$) there can be no greater than a 25% reduction in alkalinity.

2.4.4 Biochemical oxygen demand (BOD)

Biochemical oxygen demand is also called biological oxygen demand. It is referred to amount of dissolved oxygen needed (i. e., demanded) by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specific time period (Clair *et al*., 2003). Low BOD concentration can be associated to low level of organic waste and high dissolved oxygen concentration in the water body. Howerver, High BOD concentration is an indication of poor water quality.

2.4.5 Total hardness

Soni *et al*., (2013), classified total hardness in three categories: soft water (0 to 75 mg/L.$CaCO_3$), moderately hard water (76 to 150 mg/L.$CaCO_3$) and hard water (151 to 300 mg/L.$CaCO_3$). High Total hardness values in waters are mainly due to weathering of Ca and Mg-rich rocks in the area around the water body (Zeitoun and Mehana, 2014).

Arash and Homayoon (2009) reported, that total hardness have effects on the level of toxicity and bioavailability of heavy metals.

2.4.6 Chloride

Chloride is an essential electrolyte located in all body fluids, responsible for maintaining acid/base balance, transmitting nerve impulses and regulating fluid in and out of cells.Chloride is a useful indicator of river / groundwater fecal contamination,due to it's a non-reactive solute and ubiquitous to sewage and potable water. Low level of chloride in water body, do not show any significant effects while higher level of chloride present problems (Napgal, N.K., D.A.Levy, and D.D MacDonald, 2003). Anthropogen inputs of Chloride include: septic systems, wastewater treatment facilities, water softening, animal waste, fertilizers, discharge from landfills (United States Geological Survey, 2009). High

Chloride concentration harm aquatic organisims by interfering with its osmoregulation, hence hinder survival growth and reproduction (Molly *et al*., 2012)

2.4.7 Sulphate

Industrial, household discharges, contaminant from tanneries and textiles could lead to increase in sulphate level in water bodies. Increased level of sulphate may lead to decreases in pH of water and increase of bacterial load, i.e. sulphate reducing bacteria. Recommended limits of sulphate for water used as a Domestic Water Supply are below 250 mg/ (NSDWQ, 2005).

2.4.8 Nitrates

The level of nitrates indicates the level of micronutrients in water bodies and the ability of water body to support plant growth. High concentration of nitrate favors the growth of phytoplankton and algae growth hence lead to anoxia in the water body. EPA standard limit of Nitrates- Nitrogen, should not exceed 10mg/l (Mahananda *et al*., 2010).

2.5 Effects of Heavy Metals in Aquatic Ecosystem

Heavy metals released in to water bodies are usually due to anthropogenic activities. These anthropogenic activities include: domestic, industrial, mining, agricultural activities, weathering of rocks and leaching of soils (Adefemi *et al*., 2008; Edem *et al*., 2009). Heavy metals are non-biodegradable and persistence in biological amplification through the food chain (Olojo *et al*., 2005; Erdogrul and Erbilir, 2007; Senthil *et al*., 2008; Honggang *et al*., 2010).

Several authors reported that most heavy metals due to its bioaccumulatory ability could cause intensive and extensive contamination and deleterious effect on natural aquatic systems. (Otitoloju and Don-Pedro, 2002; Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi *et al*., 2007; Agah *et al*., 2009). Elevated heavy metal levels in water body may cause morphological changes in tissues, suppression of growth and development, poor swimming performance, change in enzyme activity and reproduction in aquatic organisms (Majolagbe *et al*., 2013). Heavy metals could lead to immunosuppression (Carey and Bryant, 1995), induction of stress proteins (Piano *et al*., 2004), generation of reactive oxygen species (ROS), and hence cause oxidative stress (Vinodhini and Narayanan, 2009; Farombi *et al*., 2007; Soundararajan *et al*., 2009). Some heavy metals that cause these effects include: cadmium, lead and iron (Bakan and Buyukgungor, 2000; Altas and Buyukgungor, 2007; Kusemiju *et al*., 2012; Bat *et al*., 2012).

The level of iron, if higher than the standard limits (0.3mg/l) could react directly with water to produce ferric hydroxide which leads to oxygen deficiency in the water body, hence resulting to acidity , creation of anaerobic condition and death of aquatic organisms including: fishes (Hovinga *et al.,* 1993).

Lead even at low concentrations is detrimental to fish and human existence. Lead toxicity can reduce intelligence, delay motor development or motor disturbances, affect the nervous system, and impair memory, sensory disturbance such as: hearing problems, and ultimately brain damage (Macrae *et al*., 1993; Sharma *et al.*, 2008). Due to these detrimental effects of excessive intake of metals, its however important to monitor the metal concentration in the aquatic system. Metal concentration in aquatic system can be monitored by measuring their concentrations in water, sediments, fish and biota (Camusso *et al.,* 1995; Ozmen *et al.,* 2005; Figueiredo *et al*., 2007; Öztürk *et al*., 2008; Pote *et al.*, 2008; Praveena *et al*., 2008).

2.6 Effects of Heavy Metals on Fish

Many species of fish, both wild and cultured have the ability to concentrate metals in their body tissues to dangerously poisonous levels (Macfarlane and Burchett, 2000; Yilmaz *et al*., 2007). The concentrated metals may lead to immediate cellular, physiological changes (Alibabic *et al*., 2007), histo-pathologhical damage of livers, kidneys and reproductive system of aquatic organisims (Andhale *et al*., 2011; Tarasub *et al*., 2011), disruption of reproductive endocrine potential (Drevnickand and Sandheinrich 2003; Kasperczyk *et al*., 2008) and mortality if present in acute toxicity in the fish (Otitoloju and Don-Pedro, 2002). Many fish species have being reported as important bio indicators of metal pollution and environmental stress due to the instantaneous changes observed when fish species are exposed to contaminants (Penzo *et al*., 1998; Peakall and Burger, 2003; Indrajith *et al*., 2008; Bhupander *et al*., 2011).

Clarias gariepinus also known as African catfish /African sharp tooth catfish is of commercial importance in Nigeria (Olaifa *et al*., 2004) and widely consumed due to presence of amino acid compositions (Duffus, 1980, Nwuba and Ikpeze 2009; Nwuba *et al*., 2009). *Clarias gariepinus* feeds on a large variety of agricultural byproducts and hardy in nature.it has the ability to tolerate adverse water quality conditions, possess rapid growth, easily reproduces in captivity and tolerates the difficult situation in aquaculture in reference to other fishes. It is therefore a good model to study its response to various environmental contaminants.

The gill is one of the main tissues where uptake and accumulation of heavy metal is obtained. It is a bio indicator of pollution due to sensitivity in response to contaminants, its role as a respiratory site and always in direct contact with water (Bervoets and Blust, 2003; Adeyeye and Ayoola, 2013). This has led to keen interest of several authors to make findings on heavy metal using the tissue gill as bio indicatior (Rashed, 2001; Storelli et al, 2005; Dural *et al*., 2006; Erdogrul and Erbilir, 2007; Farombi *et al*.,2007; Dahunsi *et al*.,2012).

Liver is the the principal organ responsible for the detoxification, transportation and storage of toxic substances (Gbem et *al*., 2001). Adeyeye (2013), reported that an active site of pathological effects induced by contamination storage sites for heavy metals in *Clarias gariepinus* is the liver. Due to the high accumulating ability of liver, several authors recommend liver as the best bio indicator of water pollution (Wu *et al*., 2006; Ploetz *et al*., 2007; Uysal and Kose, 2009; Dural *et al*., 2006; Agah *et al*., 2009). Another tissue that serves as storage sites for metals is the kidney. Due to the importance of these tissues in detoxification of metals and accumulatory ability lead to the study to check the responses in the environment.

2.7 Oxidative Stress

Alinnor (2005) reported that in Nigerian, many water bodies are polluted principally due to the discharge of untreated wastes by many industries. These wastes may increase the level of heavy metals which hence can accumulate in the tissues of aquatic animals (Kalay *et al*., 1999; Ashraf, 2005). The heavy metals accumulated could catalyze reactions that generate reactive oxygen species (ROS), hence lead to environmental oxidative stress (Farombi *et al*., 2007).

Oxidative stress is defined as a situation when steady state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak, 2011).

Many mammalian species including aquatic animals such as fish counteract the impact of ROS due to the presence of antioxidant defensive mechanism. These antioxidants defense enzymes consists of superoxide dismutase. Superoxide dismutase catalyze dismutation of superoxide radical to hydrogen peroxide, catalase acts on hydrogen peroxide, and glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides, generated by organic pollutants such as heavy metals (Tjalkens *et al*., 1998). Low molecular weight antioxidants such as bcarotene (vitamin A), ascorbate (vitamin C), and atocopherol (vitamin E) are important bio indicators (VanDerOost *et al.*, 2003; Yildirim and Asma, 2010, Ahmed *et al*., 2014).

2.8 Antioxidants

2.8.1 Glutathione (GSH)

Glutathione is one of the most important cellular antioxidants (Meister and Anderson.1983).it is made up of thiol group in its cysteine moiety which is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister, 1994). GSH is known to be a substrate for the activity of GST. The adaptive and protective role of biomolecule against oxidative stress induced by the heavy metals as a result of increase in GSH levels with concomitant elevation in the activity of GST in the organs (Farombi *et al*.,2007).

2.8.2 Superoxide dismutase

Superoxide dismutase is an enzyme that is involved in the detoxification pathway, in which oxidative phosphorylation is first converted to hydrogen peroxide and it is then further reduced to give water. SOD provides a defense against oxygen toxicity by catalytically scavenges superoxide radical (Kadar *et al*., 2005).

2.8.3 Catalases

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al*., 2004; Zámocký *et al*., 1999).

2.8.4 Vitamin E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001; Packer *et al*., 2001). Of these, α-tocopherol has been most studied (Brigelius-Flohé, and Traber, 1999)

It has been reported by several authors that α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001; Traber *et al*., 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing.

2.8.5 Vitamin C

Vitamin c is known to have antioxidant activity due to the process it involves in reduction of oxidizing substances such as hydrogen peroxide, (Duarte *et al*, .2007), it also reduces metal ions that generate free radicals through the Fenton reaction (Stohs *et al*., 1995; Carr and Frei., 1999). High levels of ascorbic acid are efficient in reduction of toxicity, preventing disease and enhancing fish tolerance to environmental stress (Abdel–Tawwab *et al*., 2001). The decrease level of ascorbic acid might be their detoxifying effect against the toxicity exerted by the metals (Vinodhini and Narayanan, 2009**)**

2.9 Histopathology

The health of fish exposed to heavy metals and other pollutants under laboratory (Nosakhare *et al*., 2013) and field conditions (Schwaiger *et. al*., 1997) can be evaluated using histopathological alterations in the tissues of fishes(liver, kidney and gills).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kano, is a city in Nigeria on Latitude 12^0 3'N and Longitude $8^0$32E. Kano is one of the main industrial zones in Nigeria hosting industries such as confectionaries, textiles, oil mills, flourmills, breweries, tanneries, food and beverages and toiletries. There are wide spreads, indiscriminate and unregulated disposal of wastes (liquid, solids and gases) emanating from these industries into the ecosystem.

Tatsawarki stream is the major stream that drains Tamburawa and Galinja local government, in Kano (Figure 3.1). Tatsawarki stream also indirectly receives a huge raw wastewater from residential areas of Taurani, Gandun-Albasa, Gyadi Gyadi, Naibawa and Kumbotso.

3.2 Experimental Design

3.2.1 Sampling Stations and sample collection

Five sampling Stations were chosen along the stream. The sampling Stations were taken approximately between 300 meters to 3907 apart respectively.

Station 1 (A): The basic activity that takes place in Station 1 are farming and fishing activities. Effluents discharged in to Station 1 is less in comparison to Station 2 (fig 3.1). The distance between station 1 and 2 was 2554 meters.

Station 2 (B): This Station receives mainly organic waste from residential areas, including feacal matter, and tannery effluents from anthropogenic activities in this Station .The activities practiced in this Station include farming, harvesting of building sand, indiscriminate disposal of waste, illicit open defecation, digging of the upper surface of the soil and many others (fig 3.1). The distance between station 2 and 3 was 3907 meters.

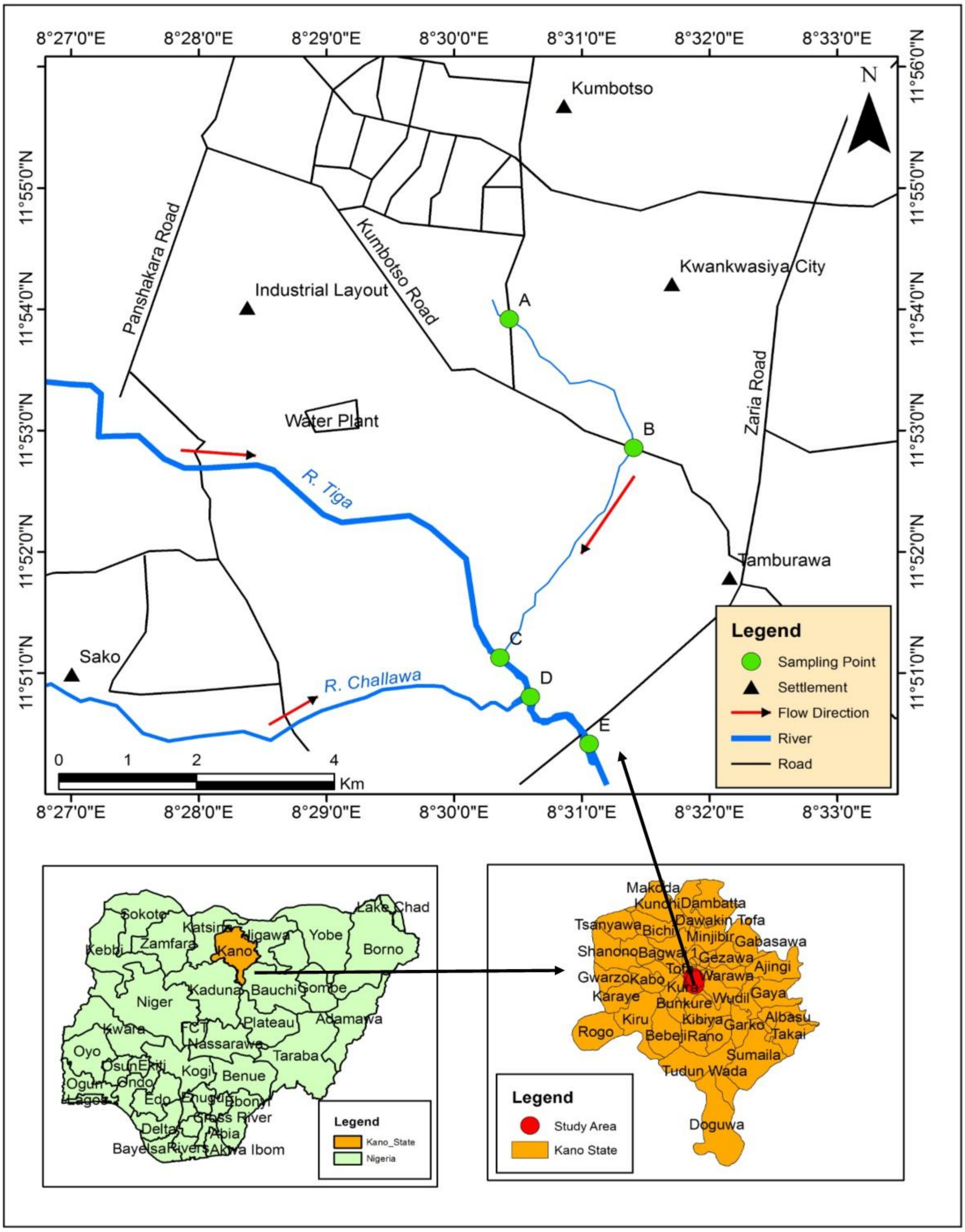


Figure 3.1: Map of the Study area Showing River Tatsawarki.

Source: Google Earth Imagery, 2016

Station 3 (C): Station 3 also receives effluents mainly domestic waste and waste from. The activities done here is mainly fishing and farming (fig 3.1). The distance between station 3 and 4 was 343 meters.

Station 4 (D): Station 4 is the stream course where Tatsawarki stream joins with Tiga River and Challawa River. It receives mainly domestic waste. The activities done in this Station include farming and fishing (fig 3.1). The distance between station 4 and 5 was 985 meters.

Station 5 (E): Most of the water bodies flow around the course of this Station. It receives mainly domestic waste, inorganic waste due to the activities practiced there which include: farming, fishing, canoe riding and some commercial activities around the edges of the water body (fig 3.1).

Monthly collection of water samples were made from each sampling Stations for a period of 8months from the month of November, 2015 to June 2016. The water samples were collected in a 4 litre container and laboratory analysis were made immediately after collection.

3.3 Analysis of Physio-Chemical Parameters

3.3.1 Physical Parameters

3.3.1.1 Temperature

Temperature of the water was determined in the field by using mercury-in glass thermometer. The thermometer was dipped into the water and was allowed to equilibrate for 3 minutes. Readings were taken twice and the average was recorded in the nearest degree Celsius °C.

3.3.1.2 pH

pH was determined by dipping a pH probe (Hanna portable Instrument, model number, HI 98129) into the samples and allowed to equilibrate for 3 minutes. pH readings were taking twice and the average was recorded.

3.3.1.3 Electrical conductivity

About 150ml of water sample was poured in to a Pyrex beaker. Electrical conductivity was determined by dipping the conductivity probe (Hanna portable Instrument, model number, HI 98129) into the water samples and allowed to equilibrate for about 3 minutes. The electrical conductance were taking twice and the average was recorded in micro Siemens per centimeter (µS/cm).

3.3.1.4 Total Dissolved Solids

Total dissolved solids of the water was determined by dipping the probe (Hanna portable Instrument, model number, HI 98129) into the samples and allowed to equilibrate for about 3 minutes. Reading was taken twice and the average was recorded in parts per million (ppm) and then converted to mg/l.

3.3.2 Chemical Parameters

3.3.2.1 Dissolved oxygen

Dissolved oxygen was determined by Azide modification of Winkler method according to (APHA, 2005). Water sample for dissolved oxygen was poured into a 300ml BOD bottle. 2ml $MnSO_4$ solution and 2ml alkali-iodide azide reagent was added in the water sample. The resultant was stoppered to exude air bubbles and mixed gently by inverting the bottle a number of times until a clear supernatant was obtained. The resultant samples were allowed to settle for two minutes and 2ml conc. H_2SO_4 was added by allowing the acid run down the neck of the bottle. The resultant sample was stoppered again and mixed by gentle inversion until dissolution was complete. 100ml of the prepared solution was transferred into a conical flask and 1ml of freshly prepared starch solution was added making the color blue. The sample solution was titrated with 0.0125N of $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow color. Titration was continued until the blue color disappeared.

Dissolved oxygen percentage saturation was calculated according to Lind (1979) and shown in equation (3.1).

DO % Saturation = (DO/ Saturation Level) X 100…………………………… Equation (3.1)

3.3.2.2 Biological Oxygen Demand (BOD)

Biological oxygen demand was obtained through these processes: 300ml of water sample was poured into a 300ml standard BOD bottle and covered carefully to exude air bubbles. The bottle was then kept in an incubator for five days. 2ml of manganese sulphate solution was added followed by 2ml of alkali-iodide azide reagent was added in the five days incubated water samples. The resultant samples bottle was stoppered to exude air bubbles and mixed thoroughly by inverting the bottles several times .The resultant samples was allowed to settle leaving clear supernatant. 2ml of conc. H_2SO_4 was added to the resultant samples. The bottle was stoppered again and mixed with gentle inversion. 100ml of the prepared solution was transferred into a conical flask and 2ml of freshly prepared starch indicator was added. The solution was titrated with 0.0125N of sodium thiosulphate solution until the disappearance of the blue color. BOD was then calculated using equation 3.2.

$(BOD)_5$ in mg/l = DO_1- DO_5……………………………………………………Equation (3.2)

3.3.2.3 Total Alkalinity

Total alkalinity was measured by titration method, according to the method of APHA (2005). 100ml of the water sample collected was transferred into 250ml flask. 2ml of methyl red indicator and 2ml of bromocresol green was added and mixed. The solution was titrated with H_2SO_4 until the sample changed from light blue to light pink .Total alkalinity was calculated using equation 3.3

Total alkalinity in $CaCO_3$ mg/l = Titer value × 10………………………………Equation (3.3)

3.3.2.4 Total Hardness

Total hardness was determined by EDTA titration method as described in APHA (2005). 25ml of water sample collected was transferred into a flask. This was diluted to 50ml with distilled water. 2ml of hardness buffer solution of pH 10.4 was added, followed by addition of 0.1g of Errochrome black T dye. The resultant solution was titrated with EDTA titrant (0.01M) until there was a pale yellow color. Total Hardness was calculated using equation 3.4.

Total hardness in$CaCo_3$/L = ml of Titrant $\times$ 40……………………………Equation (3.4)

3.3.2.5 Nitrate – nitrogen

Phenodisulphonic acid method (APHA, 2005) was used to measure nitrate- nitrogen in the water sample. 100mls of the sample was evaporated to dryness using a clean dry metallic crucible. The residue was kept in an oven set at100˚C till dryness. 2ml of phenol disulphonic acid was added to the residue and the resultant solution was swirled uniformly. The swirled sample was left to stand for 10 minutes, before addition of 10ml distilled water. 5ml of concentrated Ammonia solution was added to the solution and was allowed to cool. The sample absorbance was read in a Hach spectrophotometer model DR-EL/2 at 430nmλ wavelength, where color change was observed. The nitrate-nitrogen concentration was determined from a calibration curve. Nitrate-nitrogen was measured in mg/l.

3.3.2.6 Phosphate-phosphorous

Phosphate- phosphorous was determined using the method of APHA (2005). 100ml of sample was poured in to a conical flask, 1ml of Ammonium molybdate reagent was added to make up 50ml mark. The resultant was allowed to stand for 10 minutes and change in color was observed. Absorbance was determined from the resultant with the use of DR-EL/2 Hach spectrophotometer model at 600nmλ wavelength. The phosphate concentration was determined from a calibration curve. The unit of measurement was in mg/l. Phosphate-phosphorous was calculated using equation (3.5)

$MgPO_4/L$= Mg PO_4 in 50 ml vol. Flask×1000/ml of sample used……………..Equation (3.5)

3.3.2.7 Sulphate

Sulphate was determined by turbidimetric method as reported by Golterman and Clymo (1969). 100ml of water sample was placed in a conical flask, and a 1g of Barium chloride crystals was added to the sample. The solution was mixed and then let to stand for 3 minutes, thoroughly until a white turbidity was formed. The turbidity was read from DR-EL/2 Hach spectrophotometer at 430nm wavelength. The sulphate concentration was read from a calibration curve. Sulphate was recorded in mg/l.

3.3.2.8 Chloride

Chloride was determined using, 2-3 drops of potassium chromate in 100ml of water sample in a conical flask .the resultant samples were swirled and titrated against silver nitrate solution. Dirty reddish precipitate was observed and the readings were recorded. Chloride was calculated using equation (3.6).

Cl^- mg/l= volume of $AgNO_3$× 10……………………………………………….Equation (3.6)

3.4 Sources of the Fish Samples and Process of Acclimatization

Post- juveniles of *C. gariepinus* were collected from a private fish farm in Kano, Nigeria, and transported to the outdoor holding tanks in Kano. The tanks were filled with de-chlorinated tap water and the fish was kept for 10 days to allow them to acclimatize to the environmental conditions. The water medium was changed every 48 hours. The fish was fed daily with 3% of their feed and uneaten food was siphoned out regularly to prevent accumulation of metabolites.

3.5 Transportation of Fish Samples to the Sampling Stations

After ten days, post- juveniles were collected from the outdoor tanks and were transported to the sampling Stations of Tatsawarki stream using a 50 liter jerry can containing 30 litre of water which contains between 90 to100 post- juveniles.

After the transportation of the fish samples, 10 fish samples were placed in netted frustum shaped coned wooden localized fish cage with the dimension 28×7.5×15cm and volume 1986.19m^3. The cage is usually known by the northern Hausa fisherman as" mari" and placed into each sampling Stations. Two cages were used in each sampling Stations. The fish samples were exposed in the cage in each sampling Stations for 2 weeks. The samples were then collected and taken to the laboratory for analysis.

3.6 Sample Homogenization

Fish samples were dissected. Liver, gills and kidney were removed and weighed. In a laboratory ceramic mortar; 10 ml of phosphate buffer was added in 1gram of sample weighed and blended together. The resulting homogenate was centrifuged at 2500 rpm speed for 15mins and the supernatant was decanted and stored at -20°C.

3.7 Determination of heavy metals in water body and fish samples

One-liter sample was collected in a polyethylene bottle and preserved with nitric acid (1.0%). The sample was stored in the refrigerator to stabilize the metals for up to 2 weeks. Samples was digested, using concentrated nitric acid and hydrochloric acid ratio (1:3) filtered (same as for water sample and fish sample) and then analyzed using VARIAN (AA240FS) atomic absorption spectrophotometer (AAS), using 1.0%, HNO_3 as blank. The metal concentration was read from a standard curve. The calculation follows the equation (3.7)

Metal concentration, mg/l= A×B/C…………………………………………….. Equation (3.7)

Where A= concentration of metals in digested solution, mg/l

B= final volume of digested solution, ml and

C= sample size, ml

3.8. Determination of Oxidative Stress in gill, kidney and liver of *Clarias sp*

3.8.1 Determination of Catalase activity

Catalase activity was determined using the method described by Aebi's (1974). Exactly 10µl of gill, liver, and kidney homogenates samples were added to a test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). 0.1ml of freshly prepared 30mM H_2O_2 was added to the resultant sample and the absorbance was measured at 240nm within a range of 5 minute on a spectrophotometer. A molar extinction coefficient (E) of 0.041mM^{-1} -cm^{-1} was used to calculate the Catalase activity, which was calculated using equation (3.8a and 3.8b) respectively.

Catalase Conc = Absorbance/E……………………………………………..Equation (3.8a).

Catalase Activity = Catalase Con. /Protein Conc. (mg/ml)……………………Equation (3.8b)

3.8.2 Determination of Glutathione (GSH)

Reduced glutathione concentration measurement was done according to Ellman (1959) as described by Rajagopalan *et al*. (2004).

1.5ml of 10% TCA was added to 150µl of tissue homogenate (in phosphate - saline buffer pH 7.4) and centrifuged at 1500g for 5 minute. 1 ml of the supernatant was treated with 0.5ml of Ellman's reagent and 3ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.The quantity of GSH was obtained from the graph of the GSH standard curve and interpreted based on Table 3.1.

Table 3.1: GSH Standard values for absorbance and concentration.

Absorbance (412nm)	**GSH Conc. (μg/ml)**
0.040	8
0.101	20
0.194	40
0.380	80
0.572	120
0.749	160

3.8.3 Determination of Superoxide Dismutase

Superoxide dismutase (SOD) activity was determined using the method described by Fridovich (1989).

0.1ml tissue homogenate was diluted in 0.9ml of distilled water to make 1:10 dilution of the resultant sample. An aliquant mixture of 0.2ml of the diluted resultant sample was added to 2.5ml of 0.05M carbonate buffer. 0.3ml of 0.3mM adrenaline was added to the resultant sample. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. The Absorbance was measured at 30 seconds up to 150 seconds at 480nm. This was based on equation (3.9a and 3.9b)

Increase in absorbance per minute = (AS-A1)/2.5……………………………… Equation 3.9a

% Inhibition = 100 - {(Increase in absorbance for sample/Increase in absorbance of blank) x 100}…………………………………………………………………….. Equation 3.9b

3.8.4 Determination of Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring thiobarbituric Acid reactive substances according to okhawa *et al* (1979) with slight modification by Atawodi *et al* (2011)

Exactly 2ml of 15% trichloroacetic acid was measured into a test tube, 2ml of thiobabitutric acid was added and 100µl of the tissue homogenate was added. The mixture was incubated at 80^{o}C for 30 minute in a water bath and allowed to cool for some time. The resultant mixture was centrifuged at 3000rpm for 10 minute. A clear supernatant was collected and the absorbance was determined at 535nm in a spectrophotometer.

TBARS Concentrations where expressed in nmol/mg protein calculated as in equation (3.10)

$$\text{Conc. nmol/mg protein} = \frac{\text{Absorbance of sample}}{1.5\text{x}10^{-5}\text{ x protein conc (mg).}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation (3.10)}$$

3.8.5 Determination of Vtamin C and Vitamin E levels in liver, kidney and gill of *Clarias gariepinus*

10mls of methanol was added to each of the samples and it was left for 24hrs to enable extraction to take place. A known standard of the vitamins was serially prepared and ran in a UV-Visible Spectrophotometer model (255OS) to obtain a calibration curve. The unknown samples were run using the same UV-spectrophotometer to obtained concentration values of the vitamin c and e samples.

3.9 Histopathology

Tissues of *Clarias gariepinus which include: g*ills, livers and kidneys were removed. The tissues was fixed in formo –saline or neutral buffered formalin (Park *et al*., 2006).The tissues was washed in running tap water for at least 2hours to remove traces of formalin. This was followed by dehydration using successive percentages of alcohol(30, 50, 70, 90, and

100%).they were cleared inside toluene to remove traces of alcohol and blocked in paraffin wax to allow the wax inside the tissue to displace the xylene.

The gills, liver and kidney was embedded in fresh molten wax using L-shaped embedding moulds. Pairing, orientation and trimming was made. Sections of 4mm or 8µm was cut and stained with hematoxlin and eosin (H&E). Histopathological activity index (HAI) was scored by examining five fields (at 400x) for each slide. Permanent slides were prepared with these sections and photomicrographs taken. This was examined and compared with the control. Portal inflammation, central necrosis and degeneration are histopathological signs graded as zero findings (Park *et al*., 2006)

3.10 Statistical analysis

Data was generated from samples during the course of study. Descriptive analysis and Two-way Analysis of Variance (ANOVA) was used. ANOVA was used to analyze the data to determine the level of significance among the parameters to be measured. Principal component Analysis (Past Software) was used to check the relationship between the different variables. Microsoft Excel spread sheet, 2010 was used for both descriptive and ANOVA test.

CHAPTER FOUR

4.0 RESULTS

The results obtained from this study showed variation in water physiochemical parameters, heavy metals, oxidative stress and histopathology of fish samples.

4.1 Physico-chemical Parameters

The statistical summary of all the physico chemical parameters are presented in Table 4.1

4.1.1 Temperature

The least monthly temperature value (18.5^0C) as well as the least Station mean (24.81± 5.06^0C) obtained during the 8-month study period were both recoded in Station 2 (Table 4.1). December was the coldest month with a mean temperature of 19.90^0C while March was the hottest (31.00^0C) (Fig 4.1).

Analysis of Variance showed that there was a significant difference ($p < 0.05$) in temperature within the monthly means and Station means (Appendix Ia). Principal component of the mean physiochemical parameters revealed that Temperature was positively correlated to TDS s at the month of May (Fig 4.2).

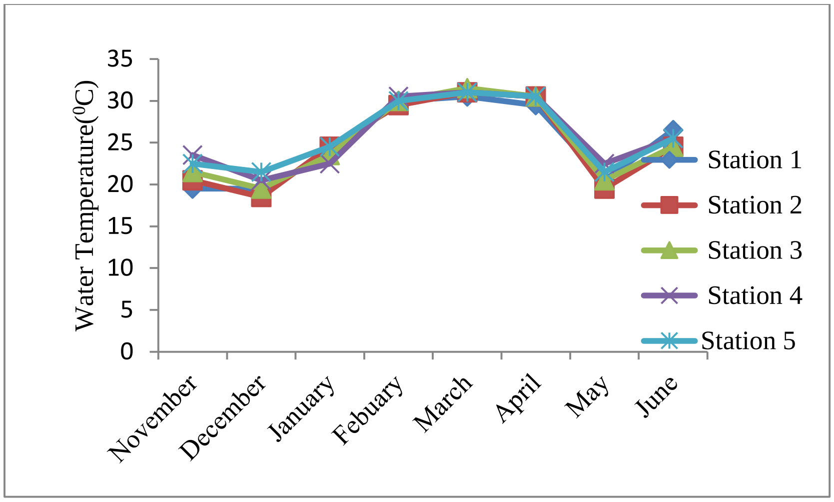
4.1.2 Electrical conductivity

There was no discernible trend in Electrical conductivity during the study period. However, the peak electrical conductivity (2453.00µS/cm) was recorded at Station 2 and highest Station mean electrical conductivity during the 8-month study period (1729.31±540.35 µS/cm).The least monthly mean during the 8-month study period (6.66µS/cm) was obtained in Station 4 as well as the least Station mean (145.13± 604.54µS/cm). December had the highest electrical conductivity with a mean electrical conductivity (920.85µS/cm) while the least was obtained in June with a mean electrical conductivity (282.60 µS/cm) (Fig 4.1).

Table 4.1: Statistical summary of physiochemical parameters obtained from a stretch of Tatsawarki stream.

Physiochemical 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
Temperature (^{0}C)	19.5	30.50	24.94± 4.79	18.5	31	24.81± 5.06	19.5	31.5	25.19±4.82	20.5	31	25.81±4.25	21.5	31	25.88±4.08
pH	7.74	8.16	8.40± 0.38	8.22	8.85	8.56±0.23	7.62	8.93	8.43±0.41	6.66	8.77	8.15±0.70	7.2	8.42	7.96±0.39
Electrical conductivity (µS/cm)	143.5	1030	453.50±279.53	531	2453	1729.31±540.35	66	1920.5	631.63±707.37	104	223.5	145.13±38.34	94	1830	336.69±604.54
Total dissolved solids (mg/l)	72	422	213.94±113.90	262	1216	841.06±265.83	60.5	1139.50	453.56±437.30	50	99	70.31±16.15	46	104	65.5±23.68
Dissolved oxygen (mg/l)	0.35	3.1	1.15± 1.02	0.025	2.45	0.60±1.011	0.025	2.05	0.81±0.89	0.35	3.1	1.24±1.17	0.5	3.3	1.26±1.07
BOD (mg/l)	0.05	2.28	0.54± 0.74	0.01	1.58	0.34±0.62	0.0075	1.58	0.40±0.54	0.05	2.43	0.51±0.81	0.05	1.48	0.43±0.57
Total alkalinity (mg/l.CaC0$_3$)	18	164	87.44±51.07	12.75	354	119.97± 108.71	15	193.5	84.75± 74.42	4	66	39.73±23.23	16	86.5	52.19±23.63
Total hardness (mg/l.CaC0$_3$)	12	132	74.81±53.29	19.5	101	47.88± 30.27	7	131	52.94± 35.91	4	106.5	45.06±40.45	8	124	44.63±36.77
Chloride (mg/l)	2.15	14.7	5.67± 3.87	1.7	26.5	7.59±8.08	1.13	14.25	6.27±4.57	3.7	14.4	6.38±3.42	2.15	12.6	5.51±3.49
Nitrate- Nitrogen (mg/l)	11.75	24.5	18.51±3.84	9.25	24.5	19.38± 5.58	13.25	23.5	19.66±3.19	11.75	25.05	19.85±4.1	9.25	29.5	20.26±5.79
Sulphate (mg/l)	207.5	380	293.75±63.50	77.5	385	245± 100.71	145	335	217.81± 74.48	77.5	370	220.31± 97.97	127.5	315	213.13± 73.80
Phosphate-phosphorous (mg/l)	0.63	2.45	1.7± 0.53	0.5	2.2	1.44±0.58	0.53	2.35	1.67±0.68	0.69	2.35	1.49±0.57	0.22	2.45	1.48±0.73

a



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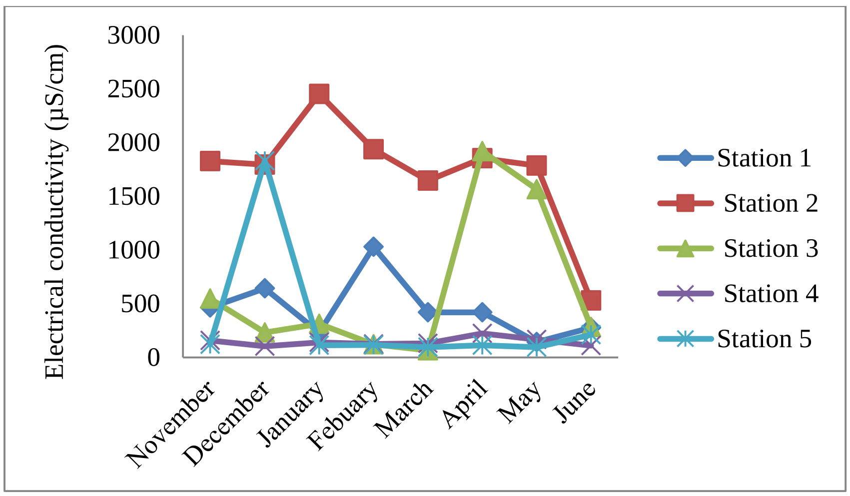

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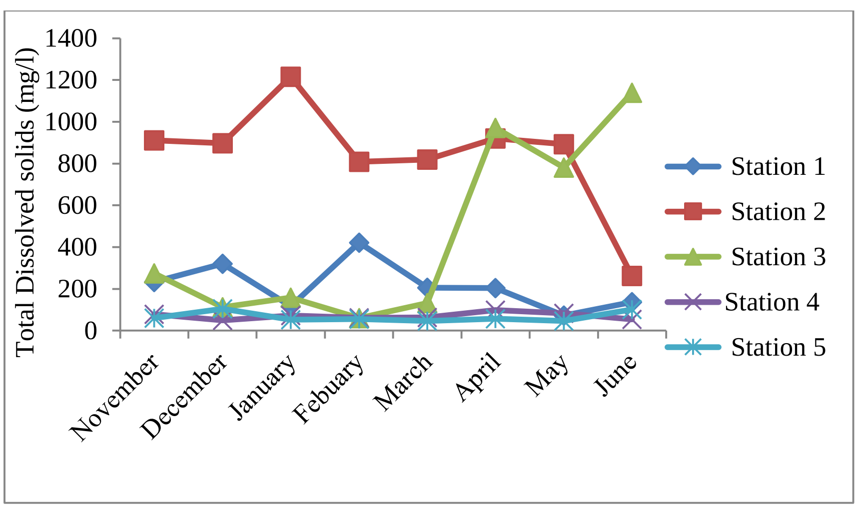


Fig 4.1: Mean monthly variation in a) Water Temperature, b) Electrical Conductivity and c) Total Dissolved solids along Stations on a stretch of Tatsawarki stream

PC	Eigenvalue	% variance
1	3.50398	29.2
2	3.16468	26.372
3	2.22743	18.562
4	1.63072	13.589
5	0.837541	6.9795
6	0.453678	3.7806
7	0.181963	1.5164

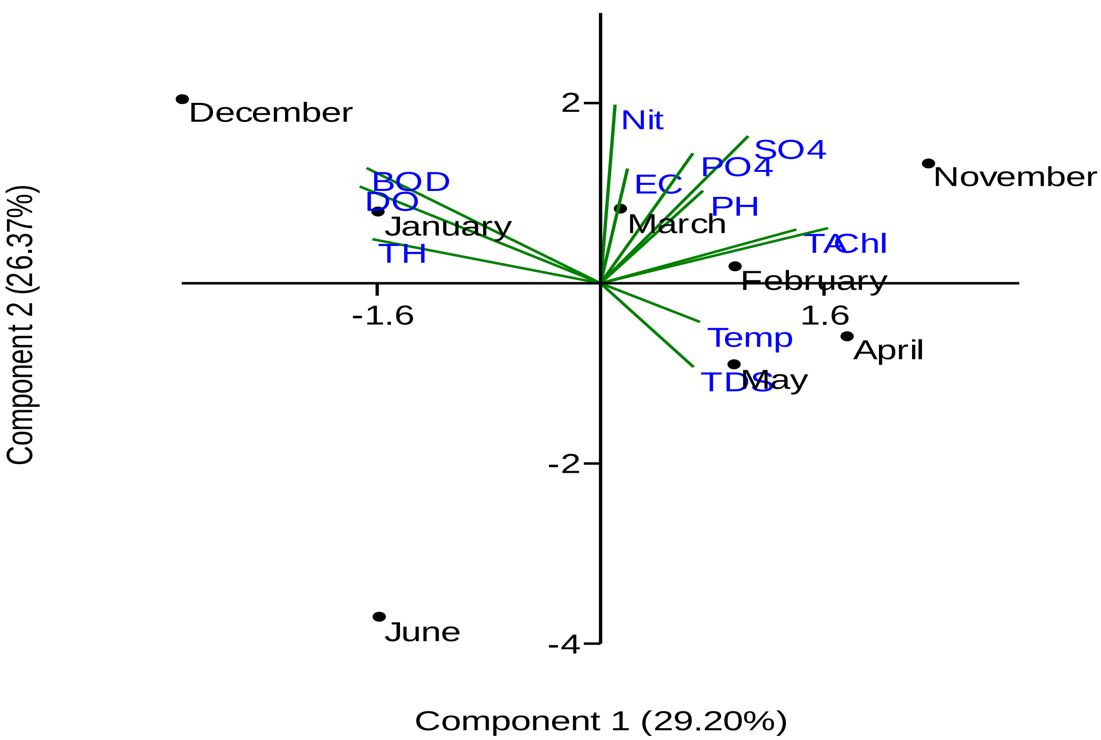


Figure 4.2: Principal component analysis of the mean physiochemical parameters along Station stretch of Tatsawarki stream.

Analysis of Variance using the confidence of interval (0.05) showed that there was no significant difference ($p < 0.05$) in electrical conductivity within the monthly means but there was a significant difference within the Station means (Appendix I b).

Monthly Principal component analysis revealed that electrical conductivity was positively correlated to Total alkalinity, phosphate, nitrate, electrical conductivity, sulphate and chloride at the month of March (Fig 4.2).

4.1.3 Total Dissolved Solids

Variations among the monthly means during the study period shows that Station 2 had the highest Station mean obtained during the 8-month study period (841.06 ± 265.83mg/l) and highest monthly value (1216.00mg/l). However Station 5 was recorded to have the least monthly value (46mg/l) and minimum Station mean obtained during the 8-month study period (65.50 ± 23.68mg/l).The highest Total Dissolved solids was recorded in the month of April with a mean Total dissolved solid (450.50 µS/cm) while the least was obtained in March with a mean (253.20mg/l) (Fig 4.1).

Analysis of variance ($p < 0.05$) revealed that, there was no significant difference in total dissolved solids within the monthly means but there was a significant difference within the Station means (Appendix I c).

Monthly Principal component analysis revealed that Total Dissolved solids are positively correlated to Temperature at the month of May (Fig 4.2).

4.1.4 pH

The Station means of pH during the 8-month study period were circum-neutral. The pH were within the ranges of 6 and 8. The least monthly value was obtained in Station 4 (6.66) while the highest monthly value was recorded in Station 3(8.93). The peak Station mean was recorded in Station 2 with (8.56±0.23) and least in Station 5 (7.96± 0.39) (Fig 4.3). May had the highest pH with the mean (8.72) while June had the least pH (7.80).

However, there was a significant difference ($p < 0.05$) in pH within the monthly means and Station means in Tatsawarki stream (Appendix IIa).

Monthly Principal component analysis revealed that pH is positively correlated to Total alkalinity, phosphate-phosphorous, nitrate-nitrogen, electrical conductivity, sulphate and chloride at the month of March (Fig 4.2).

4.1.5 Dissolved oxygen

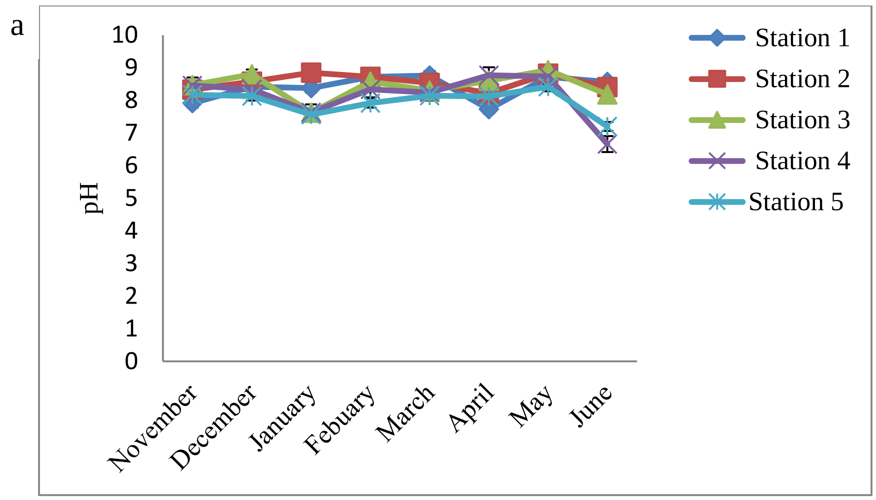
Uniform least monthly values were obtained in dissolved oxygen in Station 2 and Station 3 with (0.03 mg/l) respectively (Fig 4.2). The monthly value and Station mean during the course of study was at its peak in Station 5 (3.3mg/l) (1.26 ±1.07mg/l). Station 2 was recorded with the least Station mean DO (0.60 ± 1.01mg/l). The highest mean DO concentration was recorded in January (2.66mg/l) while the least in November (0.34mg/l) (Fig 4.3).

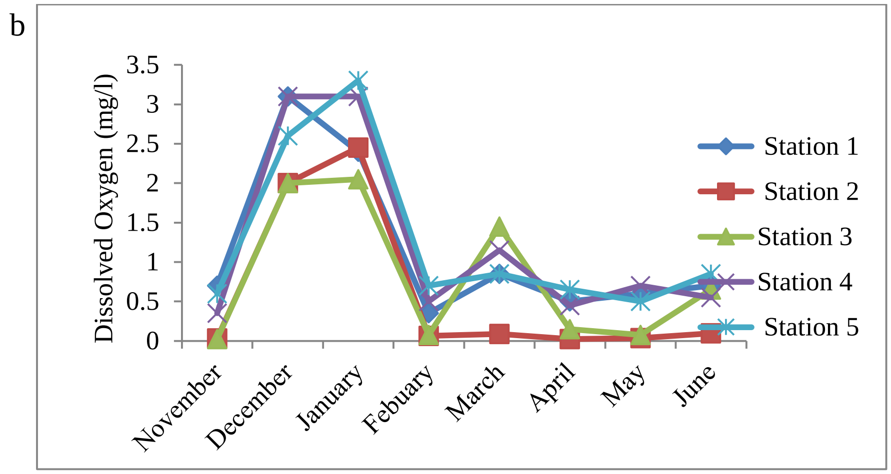
There was a significant difference ($p < 0.05$) in dissolved oxygen within the monthly means and significant difference within the Stations means (Appendix II b).

Monthly Principal component analysis revealed that Dissolved oxygen was negatively correlated to BOD and Total hardness at the month of January (Fig 4.2).

4.1.6 Biological Oxygen Demand (BOD)

There was significant variation in BOD during the study period among Stations in Tatsawarki stream.





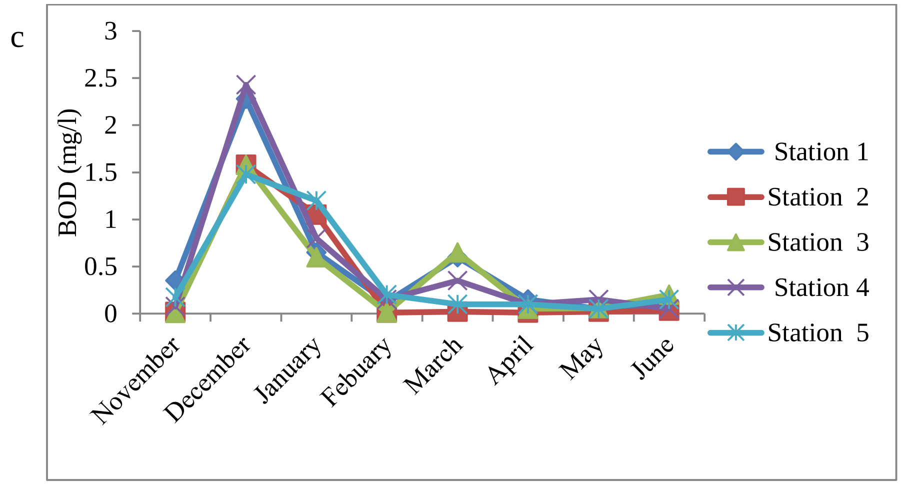


Fig 4.3: Mean monthly variation in a) pH, b) Dissolved oxygen and c) Biological Oxygen Demand along Stations on a stretch of Tatsawarki stream

The Station mean of biological oxygen demand was at its peak in Station 5 (0.43 ± 0.57mg/l) while the least was recorded in Station 2 (0.34 ± 0.62mg/l). Station 3 was recorded with the least monthly value (0.01mg/l) while the highest was in Station 4 (2.43mg/l). December was recorded with the highest BOD mean (1.87mg/l) while May had the least mean BOD concentration (0.07mg/l).

Analysis of Variance (ANOVA) showed significant difference ($p < 0.05$) in biological oxygen demand within monthly means and no significant difference within the Station means (Appendix II c).

Monthly Principal component analysis revealed that BOD was negatively correlated to Dissolved oxygen, and Total hardness at the month of January (Fig 4.2).

4.1.7 Total Alkalinity

Variations among the means during the period of this study shows that Station 2 (354.00mg/l.$CaC0_3$) had the highest monthly value and highest Station mean (119.97 ± 108.71mg/l .$CaC0_3$) while Station 4 (4.00mg/l.$CaC0_3$) had the least monthly mean and least Station mean (39.73 ± 23.23mg/l. $CaC0_3$) (Fig 4.4). November was the month recorded with the peak mean of Total Alkalinity (163.46 mg/l. $CaC0_3$) while the least was recorded in February (31.40 mg/l. $CaC0_3$)

Analysis of variance showed that, there was a significant difference ($p < 0.05$) in total alkalinity within the monthly means but there was no significant difference within the Stations means (Appendix III a). Monthly Principal component analysis revealed that Total alkalinity was positively correlated to phosphate, electrical conductivity, nitrate, sulphate and chloride at the month of March (Fig 4.2).

a

b

c

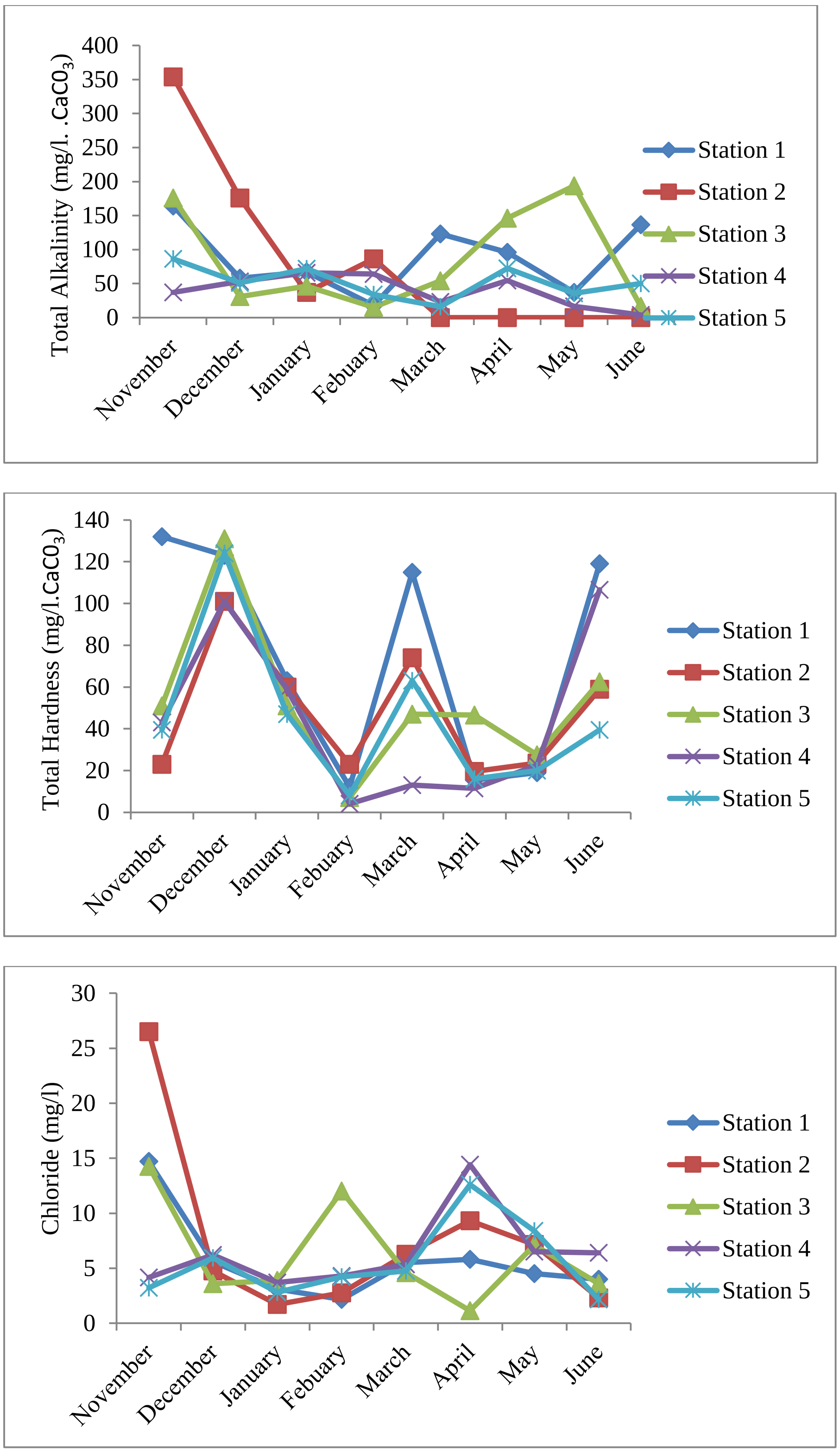


Fig 4.4: Mean monthly variation in a) Total Alkalinity, b) Total Hardness and c) Chloride along Stations on a stretch of Tatsawarki stream

4.1.8 Total Hardness

Uneven distribution of Total Hardness was obtained among the Stations and among the months during this study. The peak of monthly value and Station mean of Total Hardness was obtained in Station 1(132.00mg/l. $CaC0_3$) (74.81 ± 53.29mg/l. $CaC0_3$) while the least monthly value (4.00mg/l.$CaC0_3$) was obtained in Station 4 (Fig 4.4). Consequently the highest Station mean was recorded in Station 5(44.63 ± 36.77mg/l. $CaC0_3$). December was recorded with the highest mean of Total Hardness (116.00mg/l.$CaC0_3$) while February was recorded with the least (10.80mg/l.$CaC0_3$).

Analysis of variance using the confidence interval of 95% showed that, there was a significant difference in Total hardness within monthly means though, there was no significant difference in total hardness within the Stations means (Appendix III b).

Monthly Principal component analysis revealed that Total hardness was negatively correlated to Dissolved oxygen, and BOD at the month of January (Fig 4.2).

4.1.9 Chloride

Chloride mean monthly variations were also observed from the water samples collected along the stretch of Tatsawarki stream. The peak monthly value and Station mean of chloride was obtained at Station 2 (26.5 mg/l) (7.59 ± 8.08mg/l) while the least was obtained in Station 3(1.13mg/l). However the least Station mean was recorded at Station 5 (5.51 ± 4.57mg/l). November was recorded with the highest chloride mean (12.56mg/l) while the least was obtained in January (3.04mg/l) (Fig 4.4).

Analysis of variance ($p < 0.05$) showed a significant difference in chloride concentration within monthly means, although there was no significant difference in chloride within the Stations means (Appendix III c).

Monthly Principal component analysis revealed that chloride was positively correlated to Total alkalinity, phosphate, electrical conductivity, nitrate, and sulphate at the month of March (Fig 4.2).

4.1.10 Nitrate-nitrogen

The mean monthly peak of nitrate/ nitrogen was recorded in Station 5 (29.50 mg/l) while the least was obtained in Station 2 and Station 5 (9.25 mg/l) respectively (Fig 4.5). The highest nitrogen/ nitrate was obtained in Station 5 (20.26 ± 5.79mg/l) while the least was obtained in Station 1 (18.51 ± 3.84 mg/l). The month of December was recorded with the highest mean of nitrogen/nitrate (23.01mg/l) while the least was recorded in June (11.05mg/l).

However there was a significant difference in nitrate/nitrogen within the monthly means but there was no significant difference in Station means (Appendix IV a).

Monthly Principal component analysis revealed that sulphate was positively correlated to Total alkalinity, phosphate, electrical conductivity, chloride and nitrate at the month of March (Fig 4.2).

4.1.11 Sulphate

Identical least monthly value was obtained during this study at Station 2 and Station 4 (77.50mg/l) in June (Fig 4.5). The highest Station mean was obtained in Station 1 (293.75 ± 63.50 mg/l) while the least was obtained in Station 5 (213 ± 73.80mg/l).

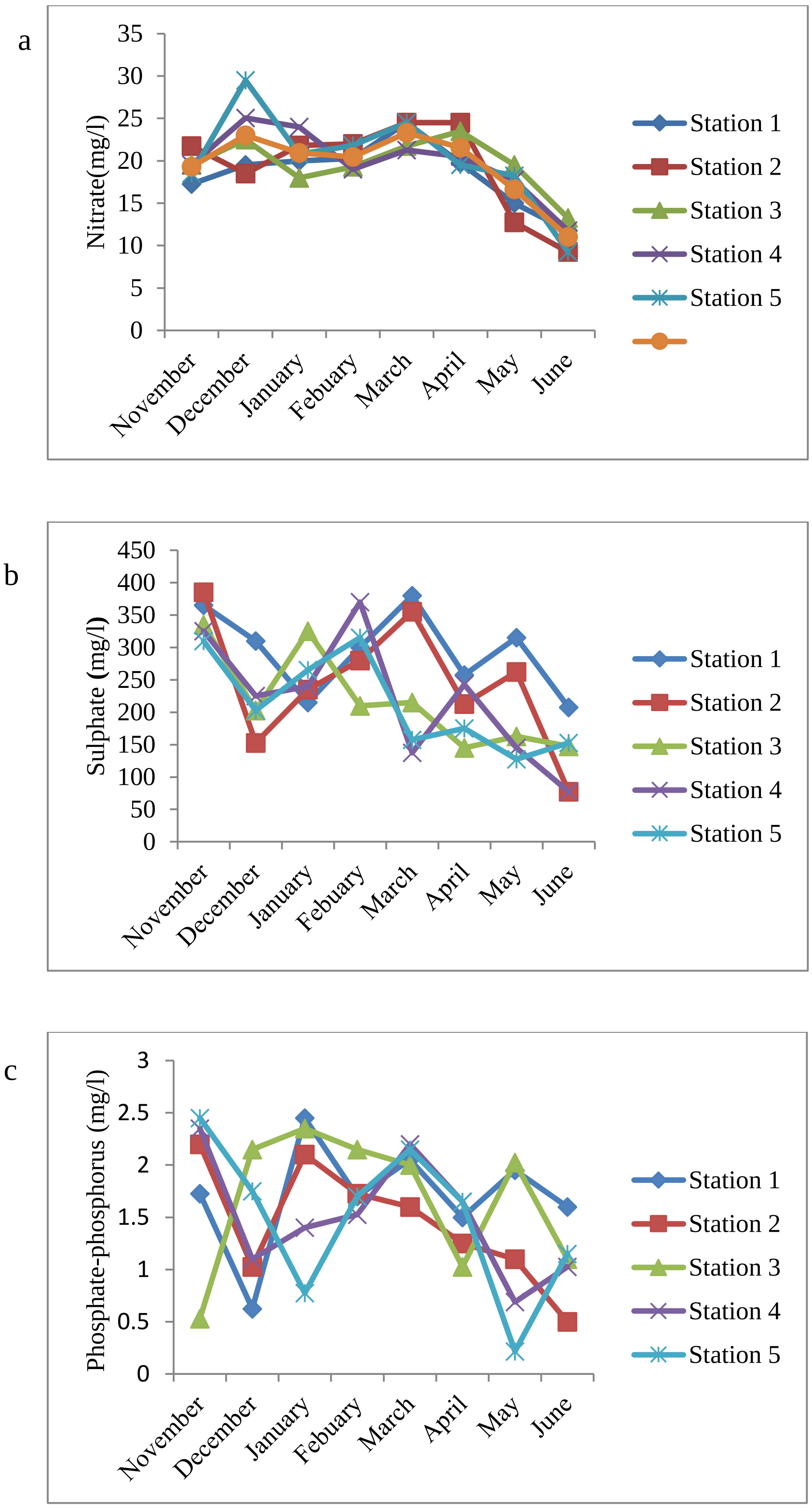


Fig 4.5: Mean monthly variation in a) Nitrate, b) Sulphate and c) Phosphate along Stations On a stretch of Tatsawarki stream.

November was recorded with the highest sulphate mean (344.00mg/l), while June was recorded with the least sulphate mean (132.00mg/l).

There was a significant difference in sulphate within the monthly means although there was no significant difference in sulphate within the Stations means (Appendix IV b).

4.1.12 Phosphate- phosphorous

Phosphate-phosporous means showed a wide spread of variation among the Stations in this study. The peak monthly value of phosphate- phosphorous was recorded at Station 1 and Station 5 (2.45mg/l) respectively (Fig 4.5). The highest Station mean of phosphate- phosphorous was obtained in Station 1 (1.70 ± 0.53mg/l) while the least was recorded in Station 2 (1.44 ± 0.58 mg/l). March was recorded with the highest phosphate mean of (2.00mg/l) while the least was observed in June (1.075mg/l).

Analysis of Variance (ANOVA) using confidence interval of 95% ($p < 0.05$) showed that, there was no significant difference in phosphate- phosphorous within the monthly means and the Stations means (Appendix IV c).

Monthly Principal component analysis revealed that phosphate was positively correlated to Total alkalinity, electrical conductivity, nitrate, chloride, and sulphate at the month of March (Fig 4.2).

Principal component analysis for all the month studied have been shown in Figure 4.6- 4.13.

PC	Eigenvalue	% variance
1	6.33975	52.831
2	4.07638	33.97
3	1.38274	11.523
4	0.201133	1.6761

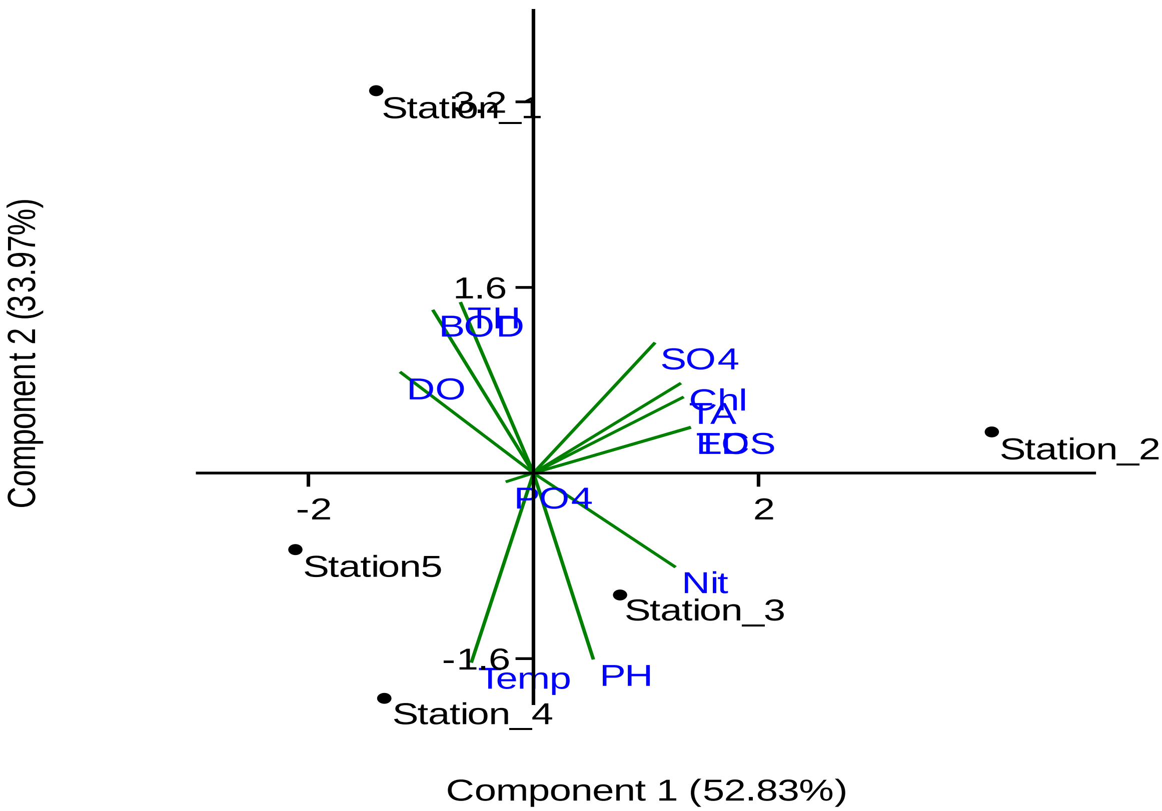


Figure 4.6: Principal component analysis of the physiochemical parameters of the month of November

PC	Eigenvalue	% variance
1	4.58676	38.223
2	3.81574	31.798
3	2.66704	22.225
4	0.930456	7.7538

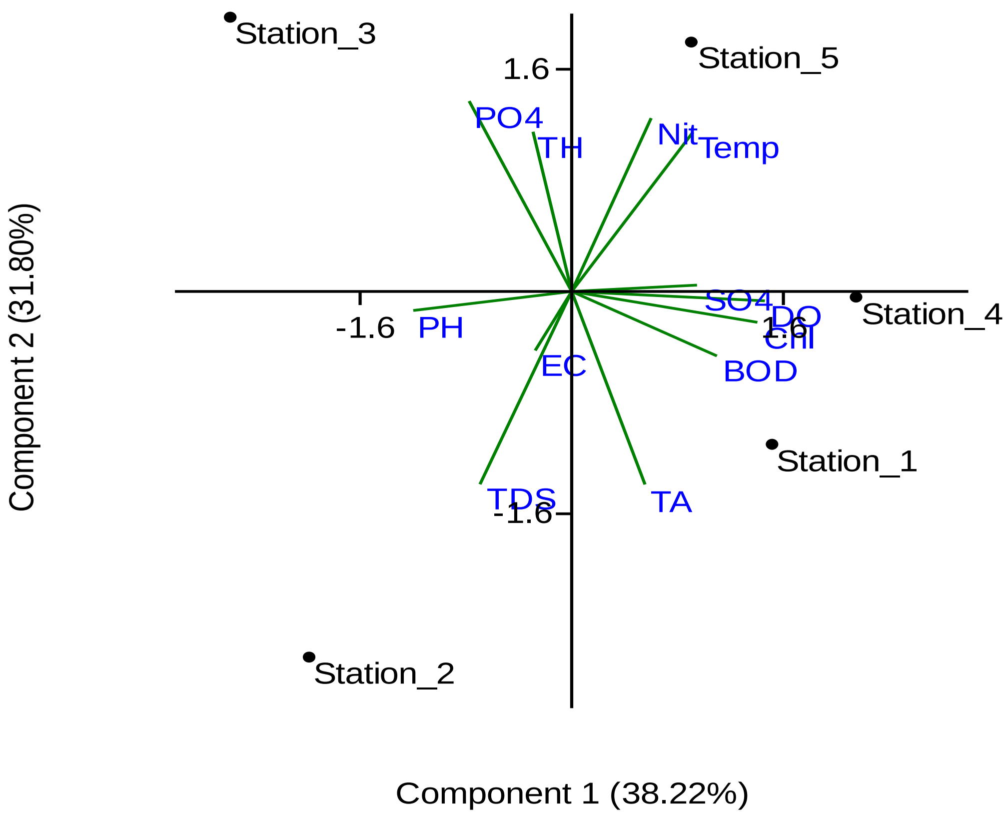


Figure 4.7: Principal component analysis of the physiochemical parameters of the month of December

PC	Eigenvalue	% variance
1	5.2407	43.673
2	3.69665	30.805
3	2.39759	19.98
4	0.665059	5.5422

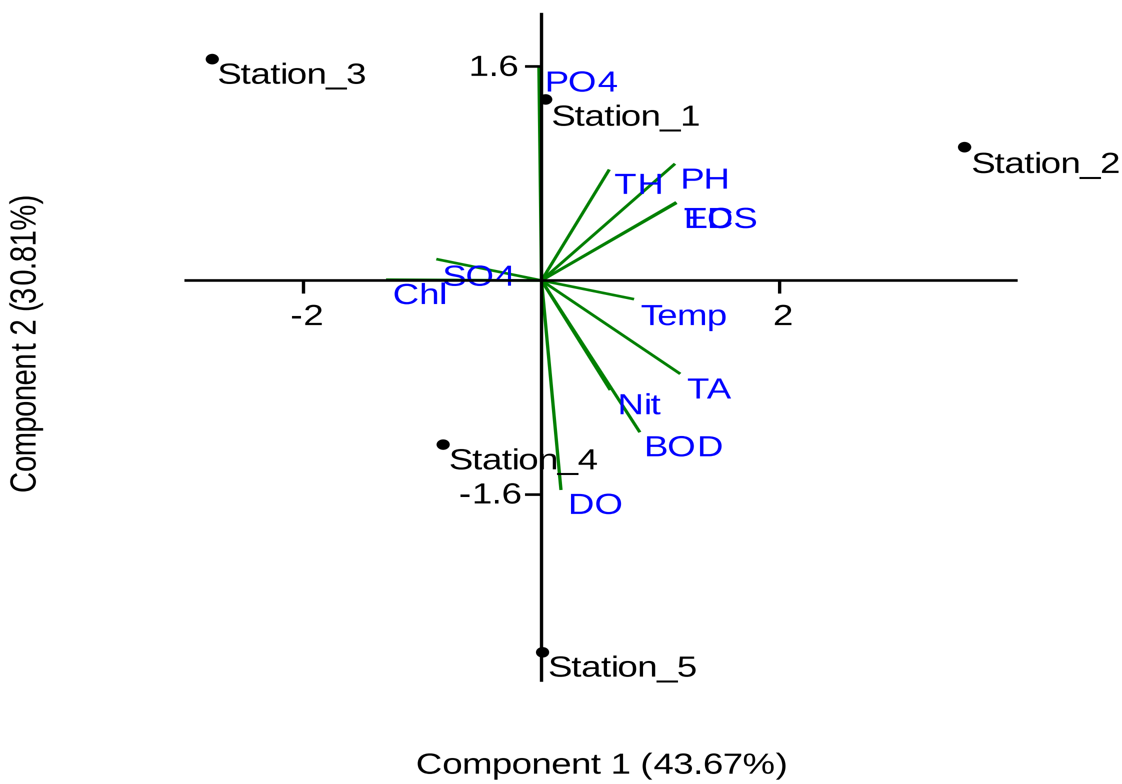


Figure 4.8: Principal component analysis of the physiochemical parameters of the month of January

PC	Eigenvalue	% variance
1	6.20585	51.715
2	3.85936	32.161
3	1.49275	12.44
4	0.442042	3.6837

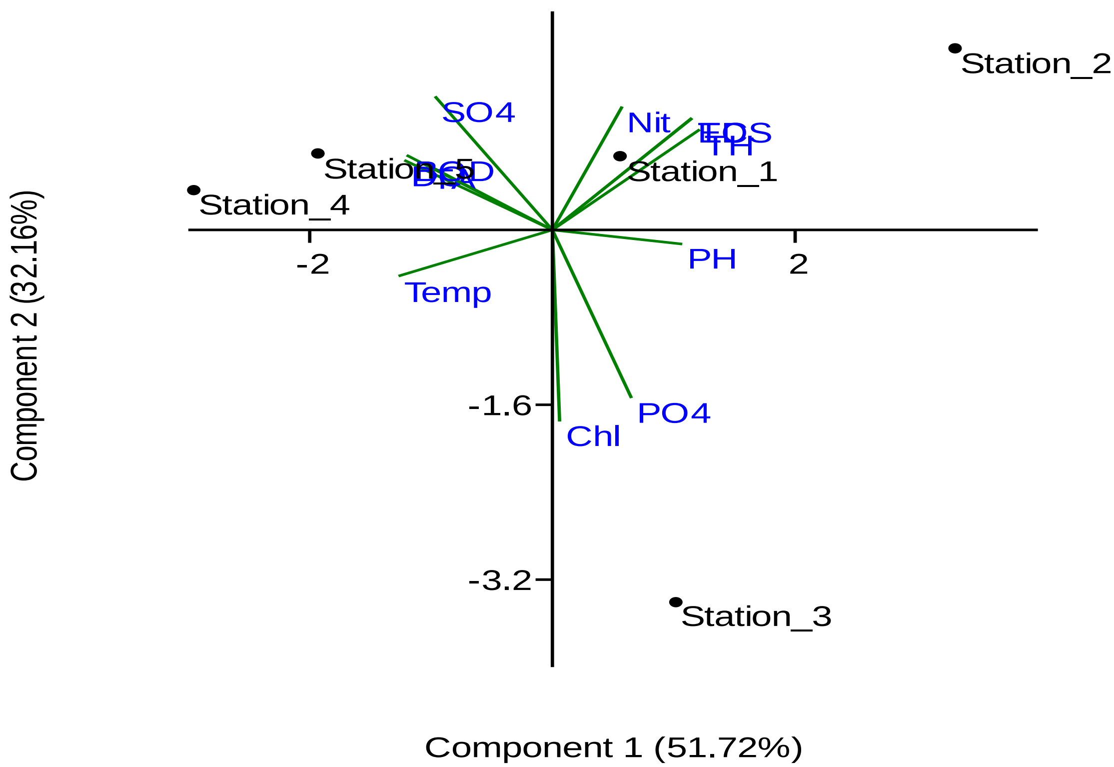


Figure 4.9: Principal component analysis of the physiochemical parameters of the month of February

PC	Eigenvalue	% variance
1	7.4069	61.724
2	2.48213	20.684
3	1.39885	11.657
4	0.712126	5.9344

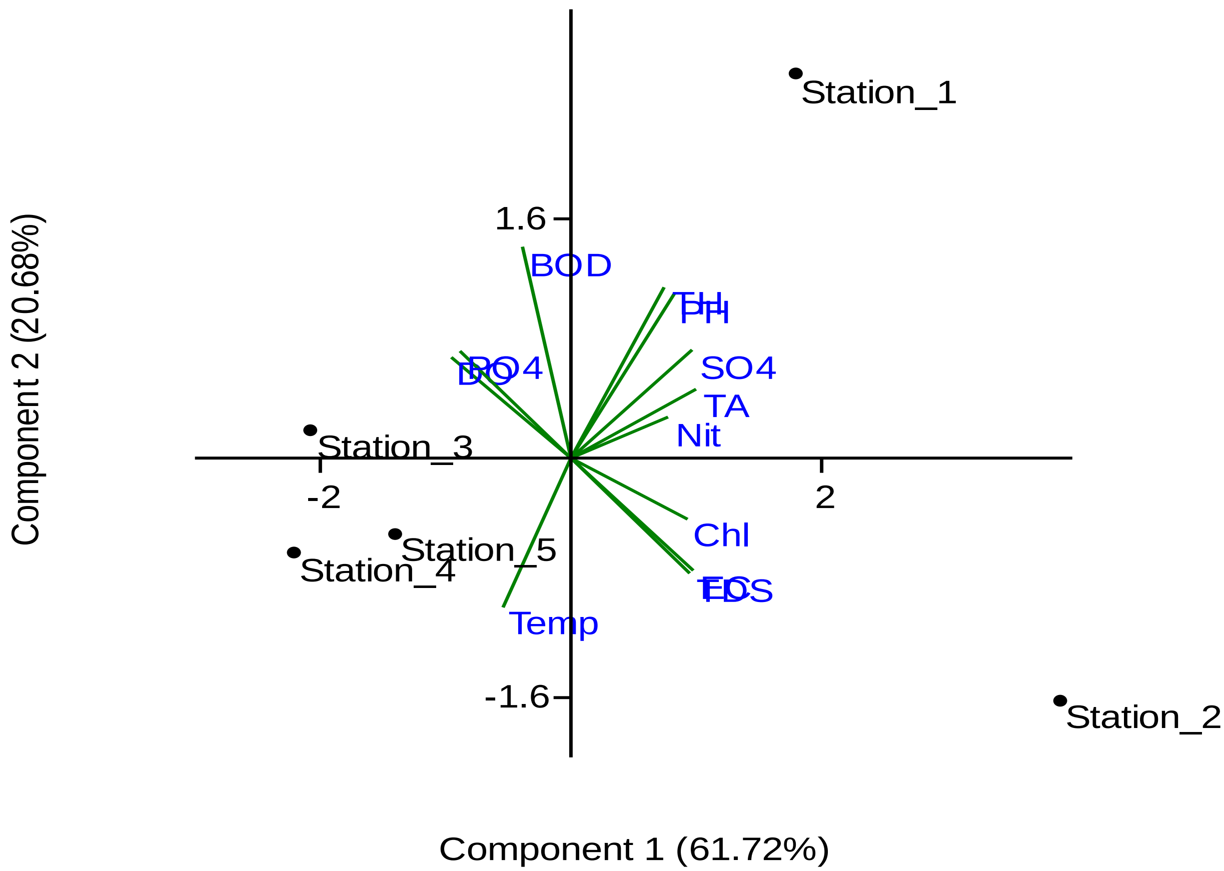


Figure 4.10: Principal component analysis of the physiochemical parameters of the month of March

PC	Eigenvalue	% variance
1	7.87052	65.588
2	2.3157	19.297
3	1.27886	10.657
4	0.534929	4.4577

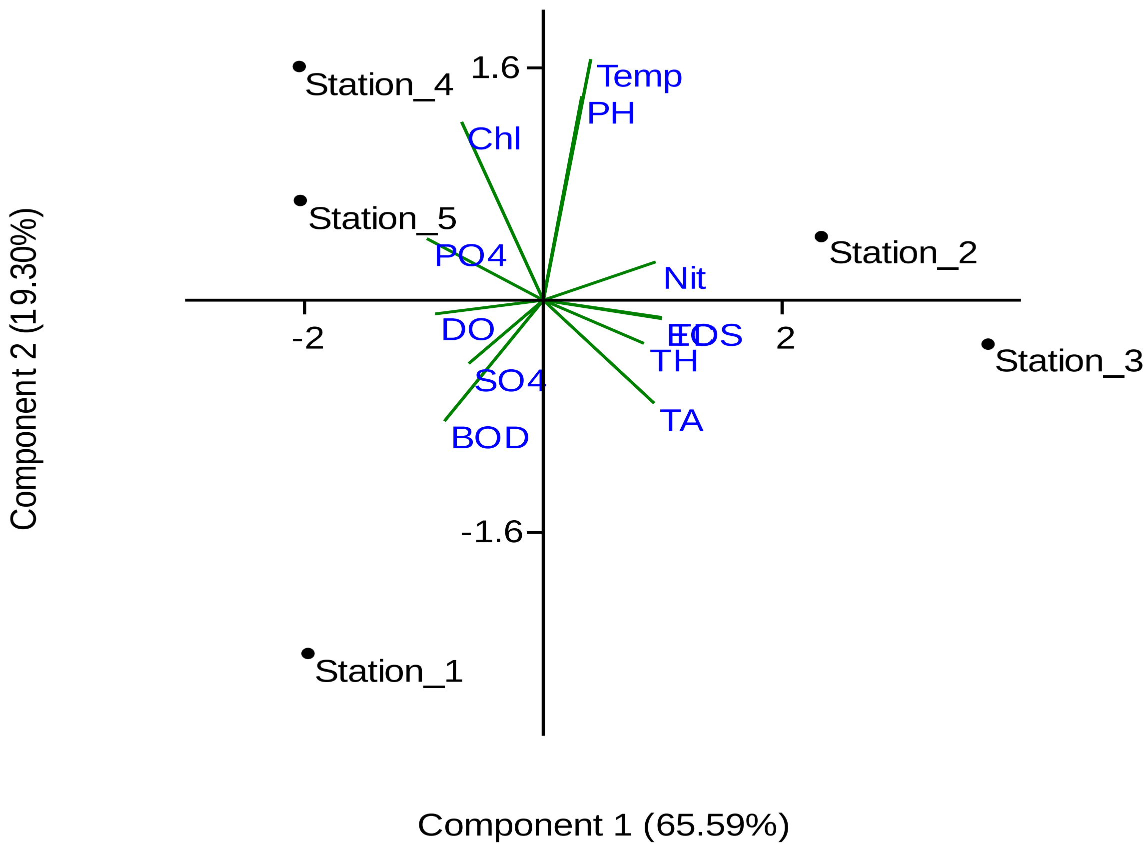


Figure 4.11: Principal component analysis of the physiochemical parameters of the month of April

PC	Eigenvalue	% variance
1	6.41463	53.455
2	3.05937	25.495
3	1.8928	15.773
4	0.633202	5.2767

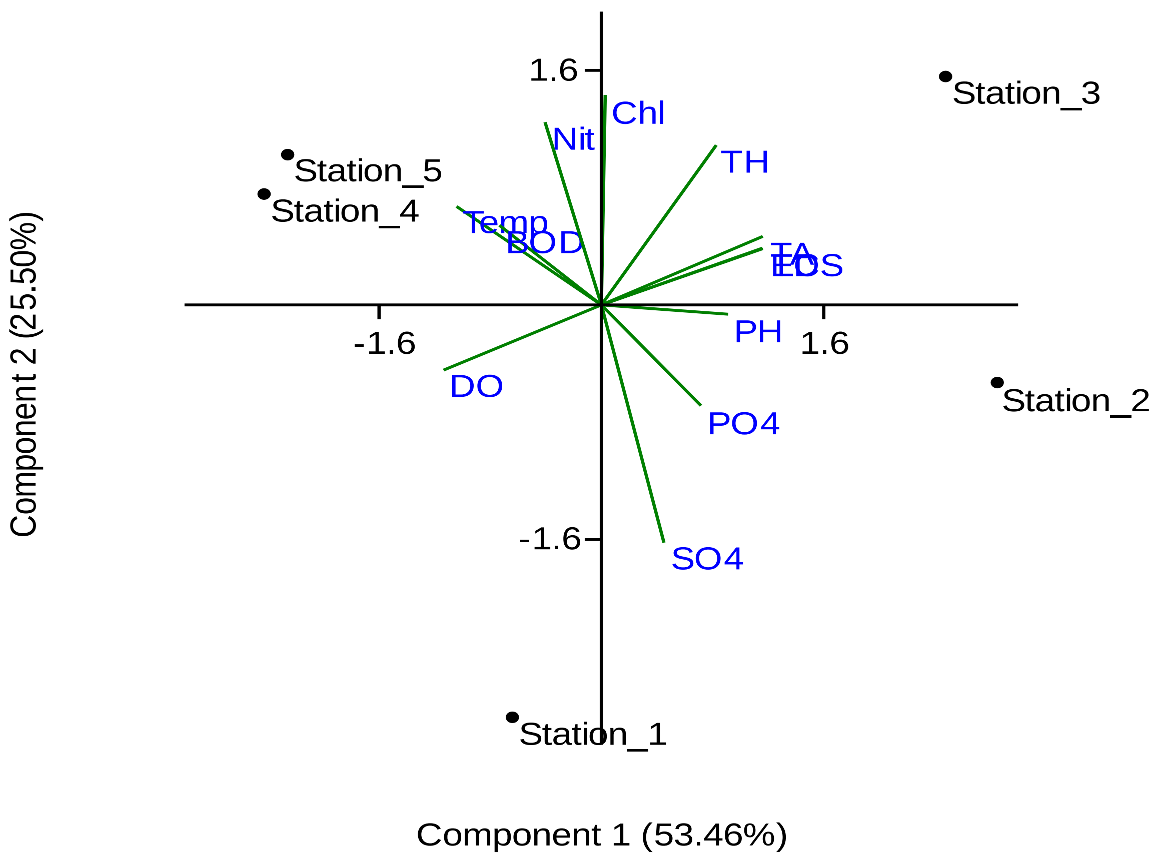


Figure 4.12: Principal component analysis of the physiochemical parameters of the month of May

PC	Eigenvalue	% variance
1	4.87786	40.649
2	2.94138	24.512
3	2.44916	20.41
4	1.7316	14.43

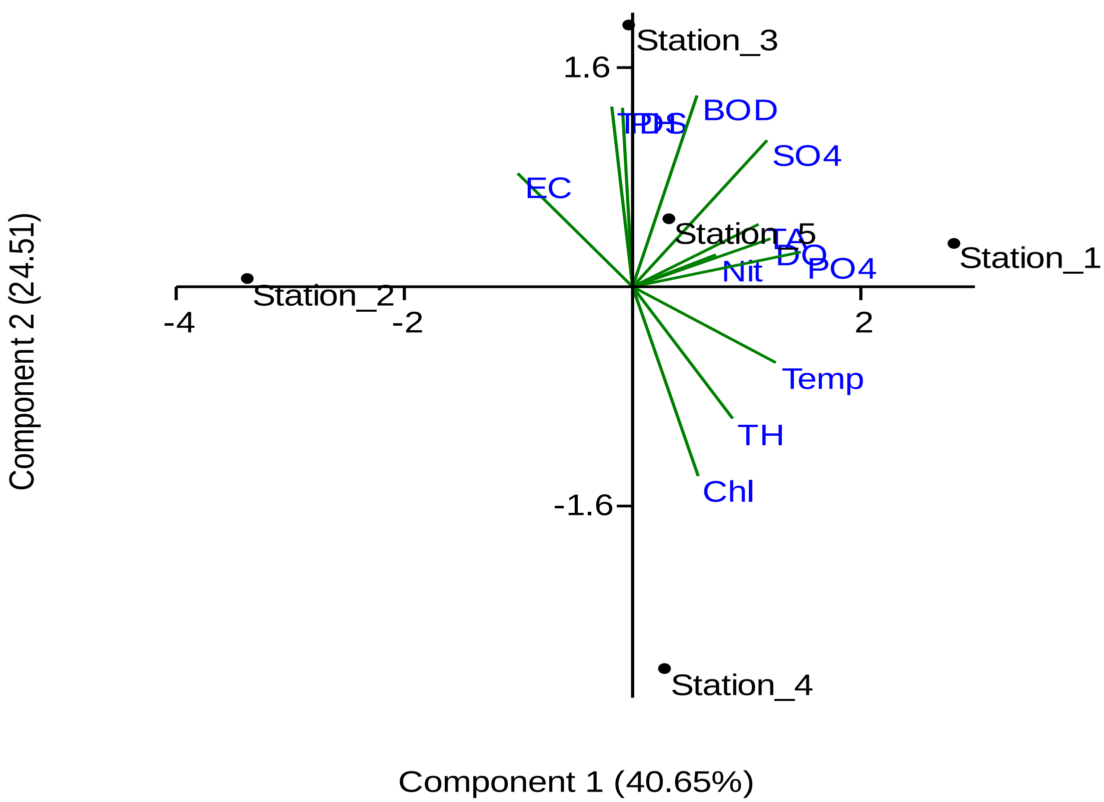


Figure 4.13: Principal component analysis of the physiochemical parameters of the month of June

4.2 Heavy Metals Concentrations in Water

4.2. 1 Water Samples

4.2.1.1 Lead

The highest lead concentration (0.74mg/l) was recorded in Station 5 while at Station 3, lead was not detected. Similarly, in November, Station 3 had the least (0.03mg/l) mean lead concentration while Station 1 had the highest (0.08mg/l). In June, which is the onset of raining season in Kano, the least mean concentration was obtained in Station 1(0.07mg/l) while the highest was recorded in Station 5(0.70mg/l) (Fig 4.14). The highest Station mean during the study period was recorded in Station 5 (0.42± 0.32mg/l) and least in Station 1 (0.08± 0.04mg/l) (Table 4.2). Analysis of variance (ANOVA) showed significant difference in lead among Stations and among months. The interaction of lead concentrations among Stations and among months was significant ($P \leq 0.05$) (Appendix V a).

4.2.1.2 Iron Concentration

The highest mean iron concentration was recorded in Station 1(8.00 mg/l). The highest iron value was obtained in Station 3(5.25mg/l) while the least was obtained in Station 2 (0.11mg/l) in November, apart from Stations 1 and 5, there was gradual increase of Fe from Station 2 (0.69mg/l) to 4 (0.89mg/l) in June (Fig 4.14). The highest study period was recorded in Station 1 with (4.66± 3.62mg/l) (Table 4.2). There was a significant difference ($P \leq 0.05$) in iron concentrations among Stations and between the months (Appendix V b).

4.2.1.3 Chromium Concentration

Chromium concentration was not detected in all the sampling Stations in June unlike in the month of November. The highest chromium concentration was obtained in Station 3 (0.07mg/l) while the least was in Station 4 with (0.03mg/l) in November (Fig 4.14).

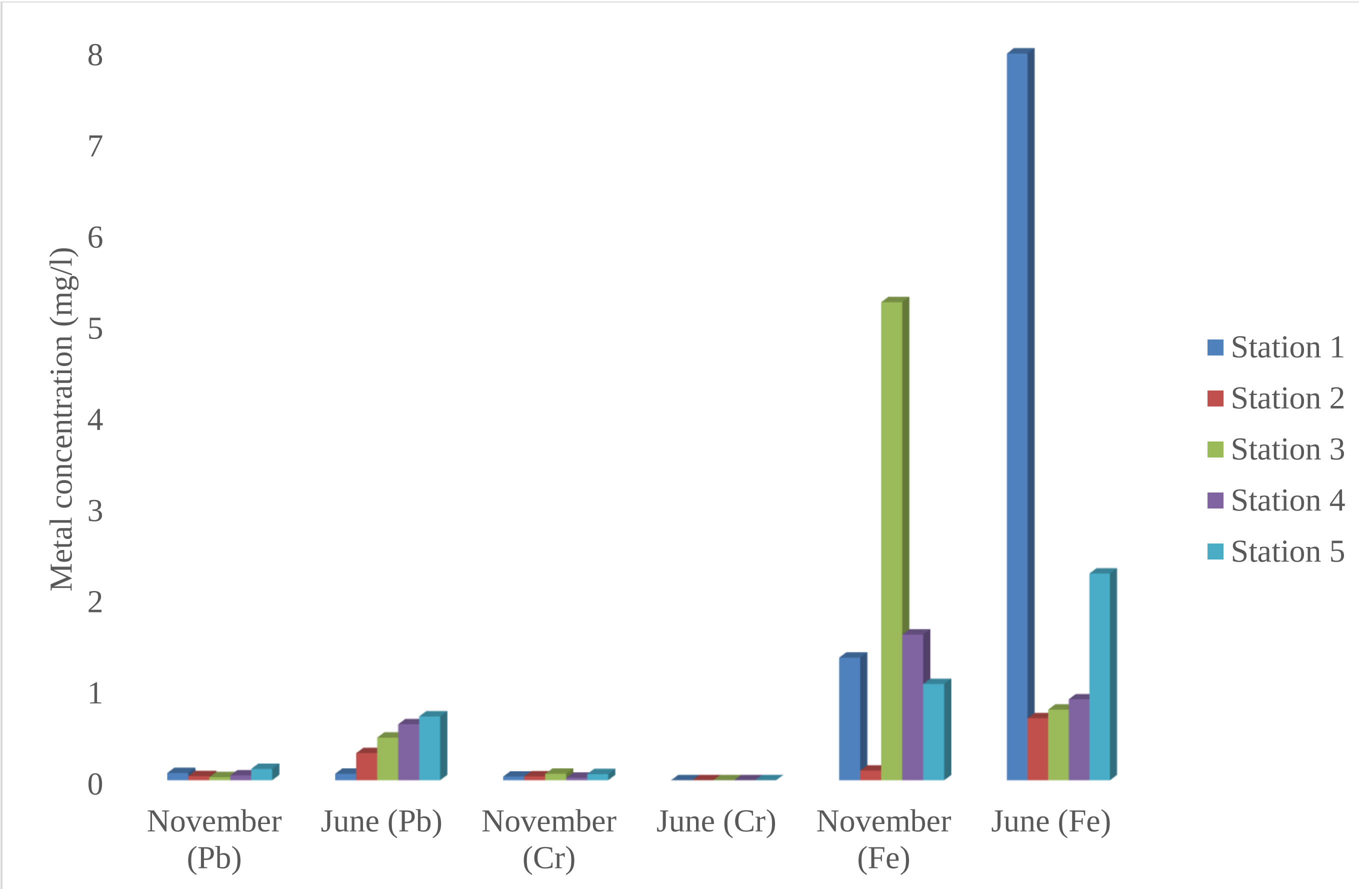


Fig 4.14: Heavy metals (Iron, Lead and chromium), in the month of November and June along a stretch of Tatsawarki stream.

Table 4.2: Statistical summary of heavy metal concentrations (mg/l) in water samples collected from Tatsawarki stream

Heavy metals concentrations(mg/l)	Station 1			Station 2			Station 3			Station 4			Station 5			FEPA (highest limit)	WHO (highest limit)	USEPA	NESREA	SON	NAFDAC
	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ± SD	Min	Max	Mean± SD	Min	Max	Mean±SD	FEPA	WHO	USEPA	NESREA	SON	NAFDAC
Lead	0.01	0.14	0.08± 0.04	0.01	0.34	0.17± 0.15	ND	0.51	0.25 ±0.24	0.02	0.71	0.34± 0.32	0.10	0.74	0.42±0.32	<1.00	0.01	0.01	0.01	0.01	0.01
Iron	1.34	8	4.66 ±3.62	0.09	0.78	0.40± 0.33	0.73	5.28	3.01 ±2.45	0.88	1.63	1.25 ± 0.39	1.05	2.35	1.67±0.67	20	0.30	-	0.30	0.30	0.30
Chromium	ND	0.06	ND	ND	0.12	ND	ND	0.09	ND	ND	0.15	ND	ND	0.10	ND	<1.00	0.05	0.05	0.05	0.05	0.05

ND--- Not Detected

There was a significant difference in chromium between months but there was no significant difference in chromium concentration among Stations. However, there was a significant difference in the interaction of chromium concentrations among the sampling Stations and among the month studied (Appendix V c).

4.3 Heavy Metals Concentration in Fish Tissues

4.3.1 Gill

4.3.1.1 Lead

There were discrepancies in the lead concentration of the gill of caged and free roaming *Clarias gariepinus.* The least (0.04mg/kg) Pb concentration for the months and the least Station mean lead (0.18 ±0.09mg/kg) concentrations in the gill of the caged *Clarias gariepinus* was obtained in Station 5. Similarly, the lead concentration in the gill (0.48 ± 0.24mg/kg) before exposure was higher than the concentration of caged *Clarias gariepinus*. The highest value for lead concentration in the months and Station mean lead concentrations was recorded in Station 4(0.64mg/kg) (0.39 ± 0.15mg/kg) (Table 4.3). In November, Station 1 gill of caged *Clarias sp*, had the highest lead concentration (0.30mg/kg), while the least was recorded in Station 5 with (0.12mg/kg) (Fig 4.15). In June, the peak lead concentration in caged fish was obtained in Station 4(0.51mg/kg), while the least was obtained in Station 1 (0.21mg/kg). However, there was a significant difference in gill among Stations and between the months and there was a significant difference in the interaction between the lead concentrations in the gill of caged fish in Stations and between the months (Appendix V a)

Station 5 was recorded with the least value (0.07mg/kg) and mean lead concentration (0.22 ±0.10mg/kg), in the gill of free roaming *Clarias sp*.

Table 4.3: Heavy metals concentration (mg/kg) in the gill of caged and free roaming *Clarias gariepinus* at Tatsawarki stream, Kano.

Heavy metals concentration(mg/kg)	Type of fish samples	Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5			WHO (highest limit for consumption)
		Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	WHO
Lead	Caged	0.19	0.76	0.48 ±0.24	0.14	0.33	0.25 ±0.07	0.17	0.41	0.27 ±0.09	0.03	0.36	0.24 ±0.12	0.27	0.64	0.39 ±0.15	0.04	0.27	0.18 ±0.09	0.29mg/kg
	Free roaming			0.48 ±0.24	0.13	0.36	0.26 ±0.10	0.19	0.47	0.32 ±0.04	0.24	0.33	0.28 ±0.10	0.19	0.49	0.28 ±0.03	0.07	0.37	0.22 ±0.10	
Chromium	Caged	ND	0.37	ND	ND	0.24	ND	ND	0.45	0.00 ±0.39	ND	0.085	ND	0.04	1.77	0.96 ±0.68	ND	0.11	ND	0.15mg/kg
	Free roaming				ND	0.96	ND	ND	1.65	0.75 ±0.76	ND	0.94	0.37 ±0.45	ND	0.37	0.06 ±0.24	0.30	1.85	0.99 ±0.71	
Iron	Caged	1.58	6.19	3.86 ±2.42	2.27	3.33	2.86 ±0.43	1.34	3.22	2.36 ±0.90	2.03	2.38	2.21 ±0.15	3.25	5.02	4.10 ± 0.90	2.15	7.08	4.59 ±2.59	< 1.00
	Free roaming				3.82	6.94	5.30 ±1.30	3.90	7.30	5.50 ± 1.52	7.16	17.78	12.58 ±5.35	5.32	8.67	7.04 ±1.65	2.97	4.74	3.84 ±0.90	

ND-------------------------Not Detected

a

b

c

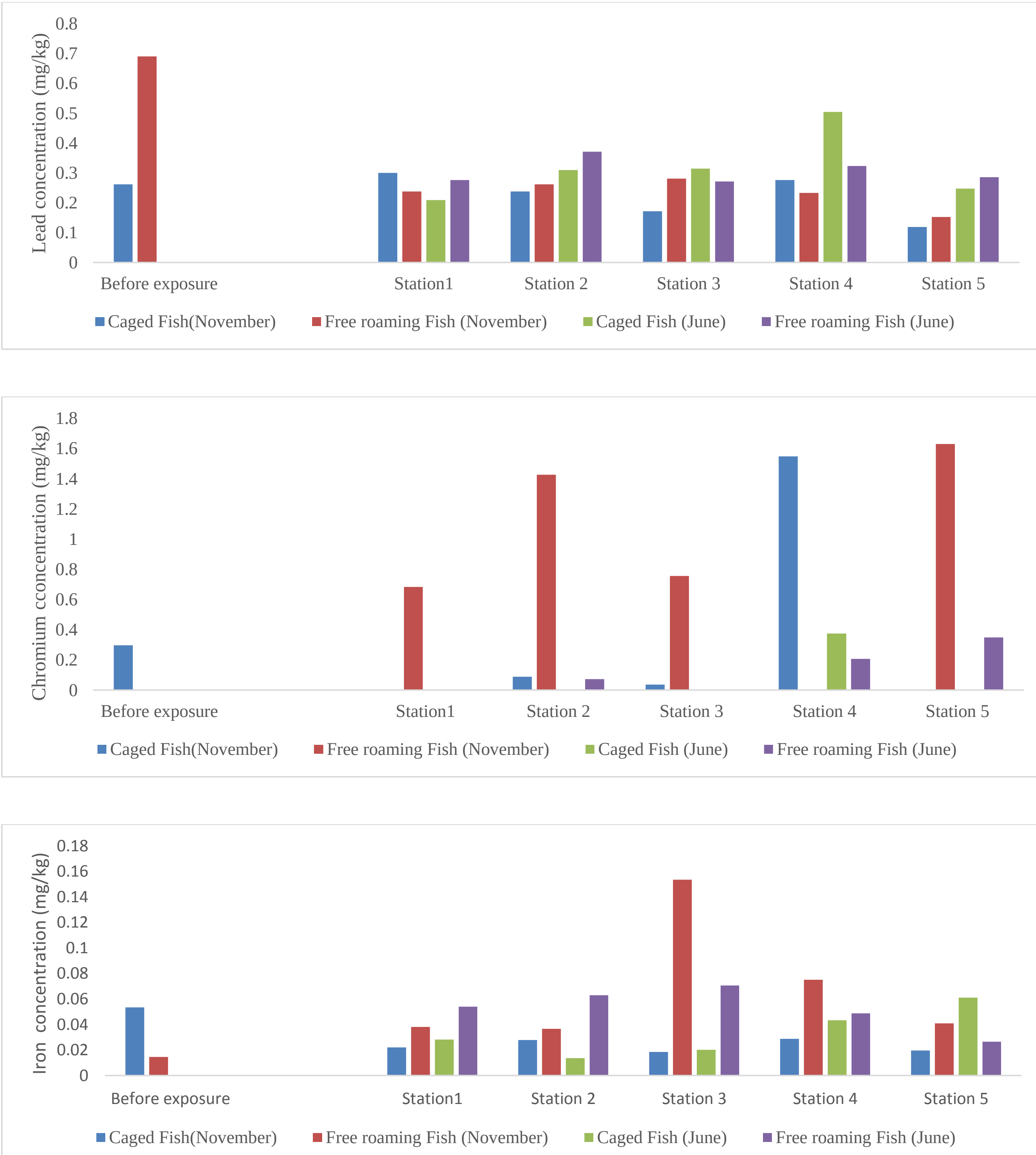


Fig 4.15: Monthly variation of Heavy metals a) Lead b) Chromium c) Iron concentrations in the Gill of caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki Stream.

The highest lead value was obtained in Station 4 (0.49mg/kg), but the highest Station mean was recorded in Station 2(0.32 ±0.04mg/kg). In November, the highest lead concentration in the gill of the free roaming *Clarias gariepinus* was obtained in Station 3(0.28mg/kg) and the least was in Station 5(0.15mg/kg). Station 2(0.37mg/kg), gill of the free roaming fish was recorded to have the highest lead concentration, while the least was obtained in Station 3(0.27mg/kg) in June. (Fig4.15). Analysis of variance revealed that, there was a significant difference in lead concentration in the gill of free roaming *Clarias gariepinus* between the months, though there was no significant difference in lead concentration in free roaming gill between Stations. There was no significant difference in the interaction between the lead concentrations in the gill of free roaming fish among Stations and in the months studied (Appendix V b).

4.3.1.2 Chromium

Chromium concentrations in Stations 1 and 5 were not detected in the gill of caged *Clarias sp* in both seasons. The highest Station mean of chromium concentrations in the gill of the caged fish was recorded in Station 4 (0.96 ±0.68mg/kg), which was higher than the gill of the fish before exposure (Table 4.3). Station 4 gill had the highest chromium concentrations in both November (1.55mg/kg) and June (0.37mg/kg) (Fig 4.15). There was a significant difference in chromium concentrations in gill between months and between Stations. Also, there was a significant difference in the interaction between the chromium concentrations in the gill of caged fish in Stations and during the months (Appendix VII a).

From the free roaming gill of *Clarias gariepinus*, Station 5 was recorded to have the highest Station mean throughout the study period (0.99 ±0.71mg/kg). Similarly, Station 5 gill was recorded to have the highest chromium concentrations, in November (1.63mg/kg) and in June (0.35mg/kg) (Fig 4.15). However, there was a significant difference in chromium concentrations in gill between Stations and between months and there was a significant difference in the interaction between the chromium concentrations in the gill of free roaming fish in Stations and in the months (Appendix VII b).

4.3.1.3 Iron

The highest Station mean of Iron in the gill of caged *Clarias gariepinus* was obtained in Station 5 (4.56 ±2.59mg/kg) which was higher than mean of iron concentration before exposure (3.82 ±2.42mg/kg). The least Station mean of iron concentration in the gill of caged *Clarias gariepinus* was obtained in Station 3(2.21±0.15mg/kg) (Table 4.3). In November, the gill of Station 4(3.29mg/kg) had the highest iron concentration among the gill of caged fish and the least was obtained in Station 3(2.11mg/kg). In June, Station 5 gill was recorded with the highest iron concentration (4.92mg/kg) and the least was obtained in Station 3(1.55mg/kg). Analysis of Variance showed that, there was a significant difference in iron concentrations in gill of caged *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in iron concentrations in the gill of caged fish among Stations and in the months during the course of study (Appendix VIII a).

Station 3, gill of free roaming *Clarias gariepinus*, was recorded with the highest Station mean (12.58 ±5.35mg/kg), whereas the least concentration was recorded in Station 5(3.84 ±0.90mg/kg). Station 4 was recorded with the highest iron value concentration (17.45mg/kg) in the gill of the free roaming *Clarias gariepinus* and the least was obtained in Station 1 (4.16mg/kg) in November. Thus in June, Station 2 (8.10mg/kg), was recorded with the highest iron concentration and the least was recorded in Station 4(5.55mg/kg) (Fig 4.15). Analysis of Variance showed that there was a significant difference in gill between Stations and months and there was a significant difference in the interaction between the iron concentrations in the gill of free roaming fish in Stations and in months (Appendix VIII b).

4.3.2 Kidney

4.3.2.1 Lead

The highest Station mean lead concentration in the kidney of the caged *Clarias gariepinus*, was obtained in Station 1(0.26 ±0.08mg/kg), which was less than the mean of *Clarias gariepinus* before exposure (0.63 ±0.25mg/kg). The least Station mean of lead in the caged kidney of the fish was recorded in Station 2(0.15 ±0.04mg/kg) (Table 4.4). In November, the highest lead concentration in the kidney of caged *Clarias gariepinus* was obtained in Station 5(0.23mg/kg), while the least was recorded in Station 4(0.09mg/kg). In June, the highest lead concentration in the kidney of the caged fish was obtained in Station 1(0.31mg/kg), while the least was recorded in Station 2(0.16mg/kg). There was a significant difference in lead concentrations in kidney of the caged *Clarias gariepinus,* between months but there was no significant difference among Stations. There was no significant difference in the interaction between the lead concentrations in the kidney of caged fish among Stations and in the months during the course of study (Appendix IX a).

In the kidney of the free roaming *Clarias gariepinus*, the peak lead concentration was recorded in Station 2 (1219.00 ±1.07mg/kg), while the least lead concentration in the kidney of free roaming *Clarias gariepinus* was recorded in Station 1(0.24 ±0.11mg/kg). Station 2 kidney of *Clarias gariepinus* was recorded with the highest lead concentration in November (0.25mg/kg) and June (2.19mg/kg), while the least was obtained in Station 4(0.09mg/kg). Station 2 free roaming kidney was recorded with the highest lead concentration, while the least was obtained in Station 1(0.30mg/kg) in June (Fig 4.16).

Table 4.4: Heavy metals concentration (mg/kg) in the kidney of caged and free roaming *Clarias gariepinus* at Tatsawarki stream, Kano.

Heavy metals concentration(mg/kg)	Type of fish samples	Before exposure			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	Min	Max	Mean ± SD
Lead	Caged	0.33	0.91	0.63±0.25	0.1	0.33	0.26±0.08	0.1	0.19	0.15±0.04	0.00	0.23	0.16±0.09	0.06	0.41	0.19±0.13	0.13	0.34	0.23±0.09
	Free roaming				0.33	0.91	0.24±0.11	0.11	2.29	1219.00±1.07	0.029	0.614	0.33±0.26	0.06	0.56	0.33±0.24	0.14	0.53	0.32±0.15
Chromium	Caged	ND	ND	ND	ND	0.17	ND	0	1.78	0.93 ±0.84	ND	0.82	0.37±0.34	ND	2.01	0.78±0.99	ND	0.59	0.18±0.25
	Free roaming				0.07	0.32	0.16±0.10	ND	0.51	0.08 ±0.32	ND	1.20	0.49±0.68	ND	1.54	0.80±0.78	0.23	1.68	1.01±0.64
Iron	Caged	0.80	18.98	9.55±9.50	1.40	4.53	2.98±1.64	2.01	2.5	2.30 ±0.21	1.07	5.61	3.24±2.34	2.56	3.38	2.95±0.27	0.86	6.21	3.48±2.77
	Free roaming				0.09	3.90	1.84±1.89	2.07	6.61	4.34±2.38	6.07	7.74	6.88±0.51	1.67	2.15	1.93±0.17	1.92	12.59	7.22±5.56

ND-------------------Not Detected

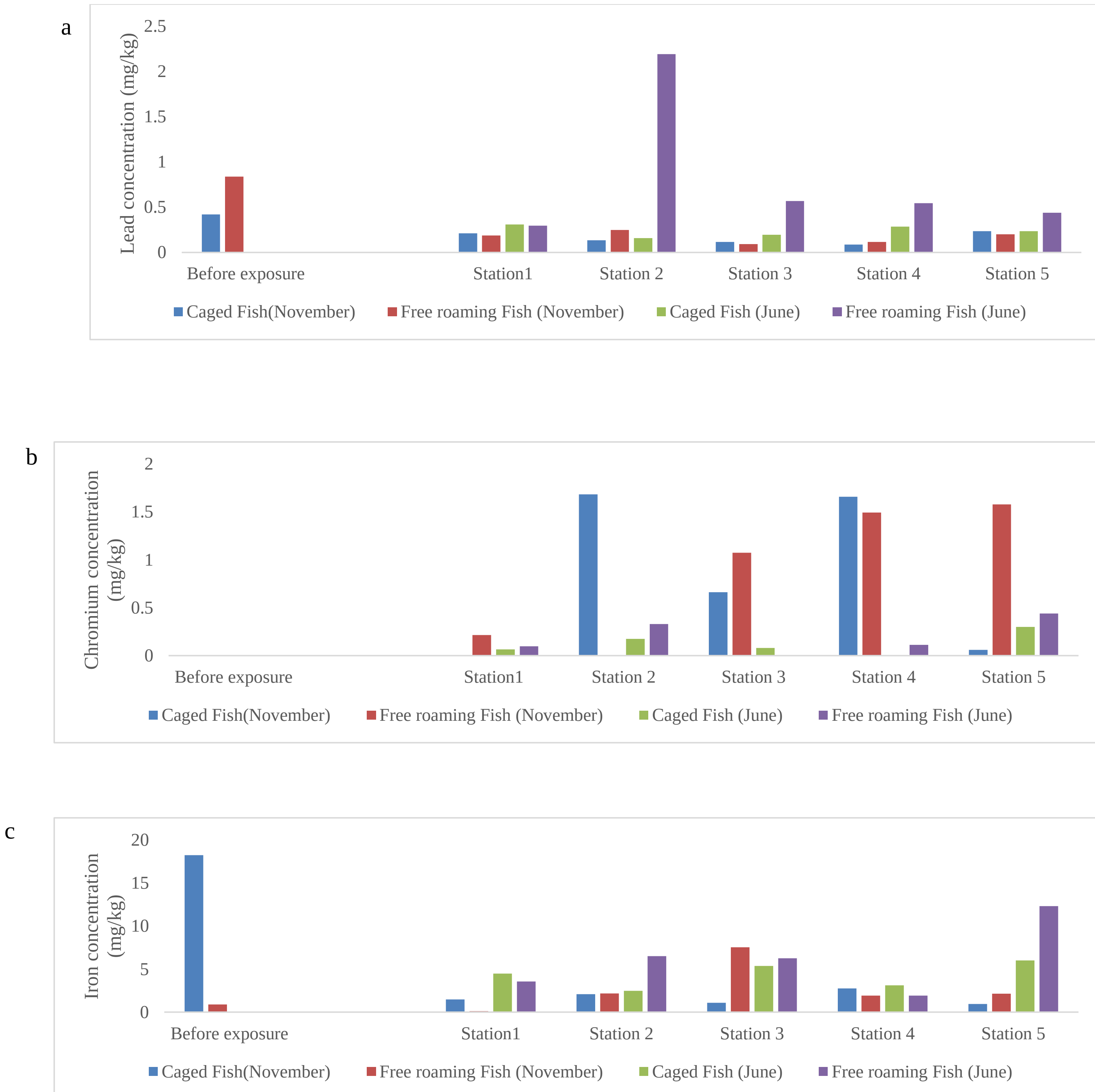


Fig 4.16: Seasonal variation of Heavy metals a) Lead b) Chromium c) Iron concentrations in the Kidney of caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki Stream.

Analysis of variance showed that, there was a significant difference in lead concentration in free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction between the lead concentrations in the kidney of free roaming fish in Stations and between the months (Appendix IX b).

4.3.2.2 Chromium

There were variations in chromium concentrations in some Stations and months. The peak of chromium concentration in the kidney of caged *Clarias gariepinus* was obtained in Station 4 (2.01mg/kg), whereas, the highest Station mean was recorded in Station 2 (0.93 ±0.84mg/kg) (Table 4.4).

In November, the peak of chromium concentration in the kidney of caged *Clarias gariepinus* was in Station 2(1.68mg/kg) while, in June the highest concentration was obtained in Station 5 (0.30mg/kg) (Fig 4.16). However, there was a significant difference in chromium concentrations in kidney of the caged *Clarias gariepinus*, between months and among Stations and a significant difference in the interaction between the chromium concentrations in the kidney of caged fish in Stations and between months (Appendix Xa).

The highest Station mean in the kidney of free roaming, *Clarias gariepinus*, was recorded in Station 5(1.01 ± 0.64mg/kg) while the least Station mean was recorded in Station 3 (0.08 ±0.32mg/kg) (Fig 4.16). In November, the highest value of chromium concentration in the kidney of free roaming *Clarias gariepinus* was recorded in Station 5(1.58mg/kg), whereas in June, the highest mean was obtained in Station 5(0.44mg/kg). Analysis of variance (ANOVA) showed that there was a significant difference in chromium concentration in the kidney of free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction between the chromium concentrations in the kidney of free roaming fish in Stations and between months (Appendix X b).

*4.3.2.3 Iron Concentrations in the Kidney of Caged and Free Roaming Clarias gariepinu***s.**

Station 5 kidney of caged *Clarias sp* (3.48 ±2.77mg/kg) was recorded with the highest Station mean iron concentration, whereas the least was obtained in Station 2 (2.30 ±0.21mg/kg) ,which are less than the mean iron concentration of *Clarias gariepinus* before exposure (9.55±9.50mg/kg).The highest iron concentration in the kidney of caged *Clarias gariepinus* was obtained in Station 4(2.77mg/kg) while the least was obtained in Station 5(0.95mg/kg) in November.Similarly in June, the highest iron concentration in the kidney of the caged *Clarias gariepinus,* was obtained in Station 5(6.00mg/kg) while the least was obtained in Station 2(2.48mg/kg).However, there was a significant difference in iron concentrations in kidney of the caged *Clarias gariepinus*, between months and among Stations and a significant difference in the interaction between the iron concentrations in the kidney of caged fish in Stations and between months (Appendix XI a).

In free roaming fish, the highest iron concentration was recorded in Station 3 (7.74mg/kg), while the least was obtained in Station 1(0.09mg/kg). The highest iron Station mean was recorded in Station 5(7.22±5.56mg/kg), while the minimum Station mean was obtained in Station 1(1.84 ±1.89mg/kg) (Table4.4). In November, the highest concentration of iron in the kidney of free roaming fish was obtained in Station 3(7.52mg/kg), while the least was obtained in Station1 (0.12mg/kg) (Fig 4.16). However in June, the highest iron concentration was obtained in Station 5(12.29mg/kg), while the least was obtained in Station 4(1.94mg/kg) (Fig 4.15).Analysis of variance showed that, there was a significant difference in iron concentrations in the kidney of *Clarias sp* among Stations and between months and a significant difference in the interaction between the iron concentrations in the kidney of free roaming *Clarias gariepinus* in Stations and between months (Appendix XI b).

4.3.3 Liver

4.3.3.1 Lead

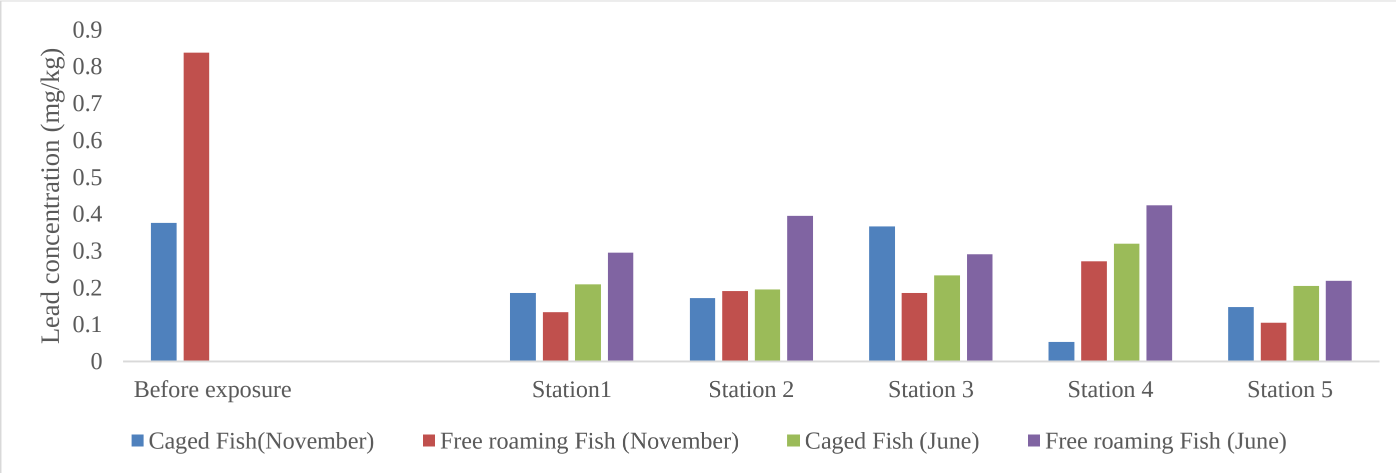
The highest Station mean of lead in the liver of Caged *Clarias sp* was recorded in Station 3(0.3 ± 0.14mg/kg). However the least Station mean in the caged fish was obtained in Station 5(0.18 ±0.08mg/kg) which are less than the mean of lead concentration in the liver of fish before exposure to the field (0.61 0.27mg/kg) (Table 4.5). Station 3 liver was observed to have the highest lead concentration (0.37mg/kg) in November, while the least was recorded in Station 5 (0.15mg/kg) (Fig 4.17).Consequently, in June, the peak lead concentration in the liver of caged fish was recorded in Station 4 (0.32mg/kg), while the least was obtained in Station 2 (0.20mg/kg). However, there was no significant difference in lead concentration in liver of caged *Clarias gariepinus* within Stations and between months but there was a significant difference in the interaction between the lead concentrations in the liver of caged fish in Stations and in the months studied (Appendix XII a)

Among the free roaming *Clarias gariepinus*, the highest Station mean of lead in the liver of free roaming fish was recorded in Station 4(0.35 ±0.09mg/kg) and least was obtained in Station 5(0.16 ±0.13mg/kg) . In November, Station 4 liver was recorded with the highest lead concentration (0.27mg/kg) while the least was recorded in Station5 (0.10mg/kg) (Fig 4.17). Similarly, in June, the highest lead concentration in the liver of free roaming fish was recorded in Station 4(0.42mg/kg), while the least was obtained in Station 5 (0.22mg/kg).

Table 4.5: Heavy metals concentration in the liver of caged and free roaming *Clarias gariepinus* at Tatsawarki stream, Kano.

Heavy metals concentration(mg/kg)	Type of fish samples	Before exposure			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	Min	Max	Mean ± SD
Lead	Caged	0.31	0.91	0.61 ± 0.27	0.16	0.24	0.20 ± 0.04	0.14	0.24	0.18 ± 0.04	0.13	0.46	0.30 ±0.14	ND	0.39	0.19 ±0.16	0.04	0.26	0.18 ±0.08
	Free roaming				0.10	0.36	0.21 ±0.10	0.11	0.44	0.29±0.13	0.07	0.4	0.24 ±0.10	0.23	0.47	0.35 ±0.09	ND	0.31	0.16 ±0.13
Chromium	Caged	ND	ND	ND	ND	0.38	0.01 ± 0.24	ND	1.5	0.70± 0.82	ND	0.66	0.22± 0.30	ND	1.05	0.48 ± 0.58	ND	0.02	ND
	Free roaming					0.98	0.37 ± 0.50	ND	0.20	ND	ND	0.90	0.50 ±0.41	0.00	1.00	0.46 ±0.46	0.12	1.31	0.80 ±0.54
Iron	Caged	2.19	5.91	3.99 ± 1.93	0.63	3.71	2.15 ± 1.6	1.81	4.15	3.06 ±1.12	1.90	14.91	8.11 ±6.76	2.24	3.22	2.79 ±0.45	0.83	31.20	15.84 ±16.28
	Free roaming				1.5	20.8	11.04± 10.35	5.99	11.76	8.95± 3.03	1.86	5.59	4.10± 1.71	4.72	16.97	11.07± 6.42	3.63	4.42	4.15± 0.30

a



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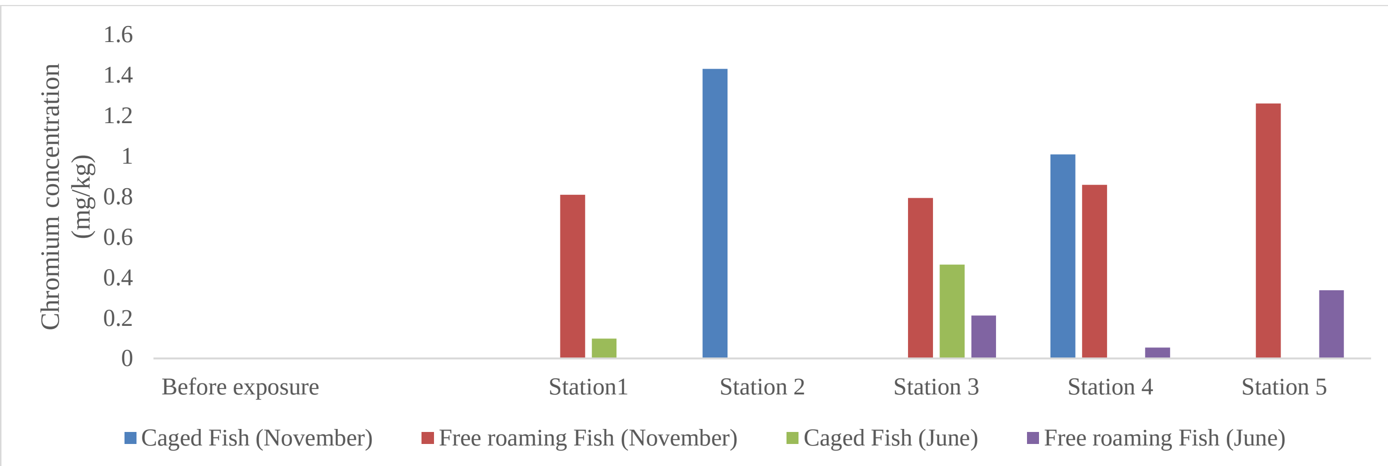

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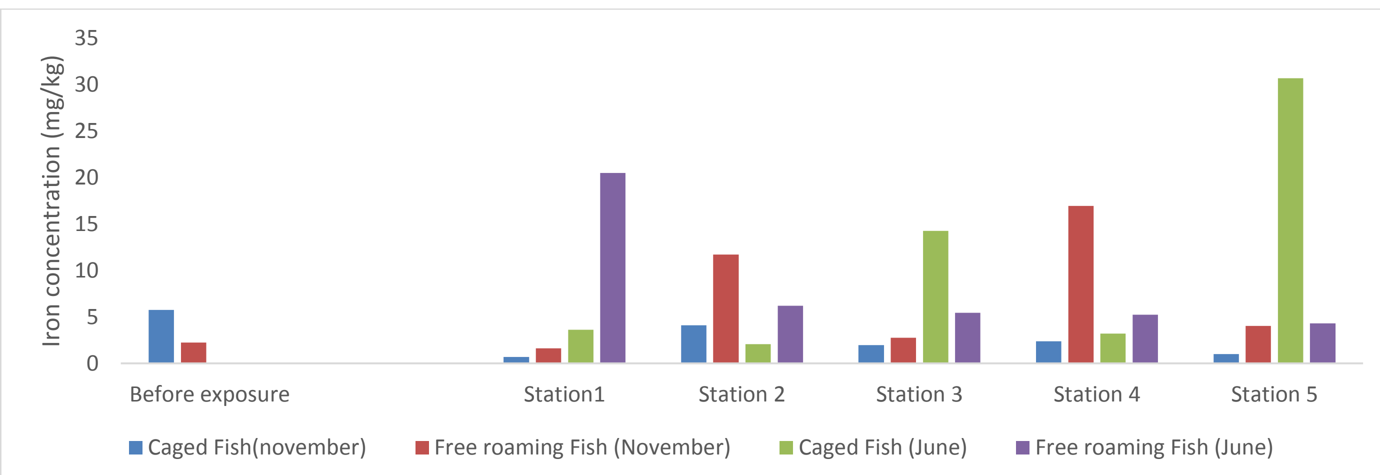

Fig 4.17: Monthly variation of Heavy metals a) Lead b) Chromium c) Iron concentrations in the liver of caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki stream.

Analysis of variance shows that, there was a significant difference in lead concentration in liver of free roaming *Clarias gariepinus* within Stations and between months but there was no significant difference in the interaction between the lead concentrations in the liver of free roaming in Stations and between months (Appendix XII b).

4.3.3.2 Chromium

Station 2 liver of caged *Clarias sp* had the highest mean of chromium concentrations (0.70±0.82mg/kg), whereas the least was observed in Station 1(0.01 ± 0.24mg/kg) (Table 4.5). In November, Station 2 liver had the highest chromium concentrations of (1.43mg/kg), while in June, Station 3 had the highest chromium concentration (0.46mg/kg) (Fig 4.17). There was a significant difference in chromium concentrations in liver among Stations and between months and a significant difference in the interaction between the chromium concentrations in the liver of caged fish within Stations and between months (Appendix XIII a).

The highest Station mean of chromium, in the liver of the free roaming *Clarias gariepinus*, was obtained in Station 5 (0.80 ± 0.54mg/kg). Station 5 liver (1.26mg/kg) was recorded with the highest chromium concentration among the free roaming fishes in November. Similarly in June, Station 5(0.34mg/kg) was also recorded to have the highest chromium concentration (Fig 4.17). However, there was a significant difference in chromium concentrations in the liver of free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction between the chromium concentrations in the liver of free roaming fish in Stations and in months during the course of study (Appendix XIII b).

4.3.3.3 Iron

Station 5 liver of caged *Clarias gariepinus* (15.84 ±16.28mg/kg) was recorded with the highest Station mean of iron, thus, higher than mean of iron concentration of *Clarias sp* before exposure (3.99 ±1.93mg/kg). The least Station mean iron concentration in the liver of the caged fish was obtained in Station 1(2.148±1.6mg/kg) (Table 4.5). Similarly, the least iron value obtained throughout the 8-month study period in the liver of caged *Clarias gariepinus* was recorded in Station 1(0.63mg/kg). In November, Station 2(4.08mg/kg) liver was observed with the highest iron concentration among the caged fish and the least was recorded in Station 1(0.69mg/kg).In June, Station 5 was recorded to with the highest iron value during the study (30.70mg/kg) and the least was obtained in Station 2(2.05mg/kg). Analysis of variance showed that, there was a significant difference in iron concentrations in liver of caged *Clarias gariepinus* within Stations and between months and there was a significant difference in the interaction between the iron concentrations in the liver of caged fish in Stations and months (Appendix XIV a)

The peak of Iron concentration in liver of free roaming *Clarias sp* was recorded in Station 1 (20.80mg/kg), whereas the least was obtained in Station 1 (1.50mg/kg). The highest Station mean was obtained in Station 4(11.07±6.42mg/kg) while the least was obtained in Station 3(4.10± 1.71mg/kg) (Table 4.5). In November, Station 4 liver of free roaming *Clarias gariepinus* was recorded with the highest iron value throughout the 8-month of study (16.92mg/kg) and the least was obtained in Station 1 (1.59mg/kg). In June, Station 1(20.49mg/kg), was recorded with the highest iron concentration and the least was recorded in Station 5(4.28mg/kg) (Fig 4.17). Analysis of Variance showed that, there was a significant difference in the liver of the free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction between the iron concentrations in the liver of free roaming fish within Stations and months (Appendix XIV b).

4.4 Oxidative Stress Biomarkers of Caged and Free Roaming *Clarias Gariepinus.*

4.4.1 Total protein in Fish Tissues

4.4.1.1 Gill

The highest Station mean concentration of Total protein in the gills of caged *Clarias gariepinus* was recorded in Station 4 (29.37mg/ml), furthermore the least was obtained in Station 2(13.86±3.98mg/ml) (Table 4.6). In November, total protein in the gills of caged fish was at its peak in Station 5(26.60mg/ml), which was however higher than concentration before exposure (24.15mg/ml) and the least was obtained in Station 2 (17.30mg/ml). Consequently In June the total protein concentration was highest at Station 4 (29.32mg/ml) while the least at Station 2 (10.42mg/ml) (Fig 4.19). Analysis of variance showed that there was a significant difference in total protein concentrations in gills of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction between the total protein concentrations in the gill of caged fish within Stations and between months (Appendix XV a).

In the free roaming *Clarias gariepinus* gill, the highest Station mean protein was obtained in Station 2 (36.94±24.00mg/ml), whereas the least Station mean was obtained in Station 5 (12.38±7.18mg/ml). The highest mean was recorded in Station 3 (26.55mg/ml) in November. In June, the highest mean was obtained in Station 2 (57.74mg/ml) (Fig 4.19).

Table 4.6: Total Protein Concentration (mg/ml) in Caged and Free Roaming *Clarias gariepinus*

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
Proteins	Type of fish samples	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean± SD
Protein (Gill)	Caged	21.25	24.5	22.70 ±1.70	18.08	21.5	19.77 ± 1.94	10.41	17.4	13.86 ±3.98	20.61	21.4	20.89 ±0.37	19.9	29.37	24.84 ±5.19	10.5	26.7	18.56± 9.29
	Free roaming	21.25	24.5	22.70 ±1.70	13.5	14.32	13.93 ± 0.39	16.1	57.79	36.94 ±24.00	12.85	26.6	19.73 ± 7.88	13.55	15.58	14.93 ± 0.935	6.11	18.7	12.38 ± 7.18
Protein(Kidney)	Caged	8.4	24.3	16.23 ± 9.06	5.7	16.11	10.95 ± 5.96	8.00	10.63	9.36± 1.40	11.70	15.47	13.61 ±2.14	11.15	17.1	14.1± 3.40	19.0	21.6	20.29± 1.46
	Free roaming	8.4	24.3	16.23 ± 9.060	6.5	13.37	10.01 ±3.83	2.1	15.16	8.68± 7.48	13.76	15.80	14.74 ±1.11	12.4	16.00	14.18 ±2.04	20.0	56.95	38.69± 21.00
Protein(Liver)	Caged	30.0	41.3	35.71 ±6.35	20.5	28.9	24.63 ±4.72	10.0	21.9	15.89 ±6.73	19.2	29.0	24.05 ±5.48	21.6	37.68	29.45 ±8.95	16.6	69.7	43.14± 30.56
	Free roaming	30.0	41.3	35.71 ±6.35	13.7	23.05	18.41 ±5.33	6.0	34.8	20.38 ±16.54	8.19	21.6	14.88 ±7.71	10.19	19.3	14.68 ±5.17	20.3	24.7	22.48± 2.45

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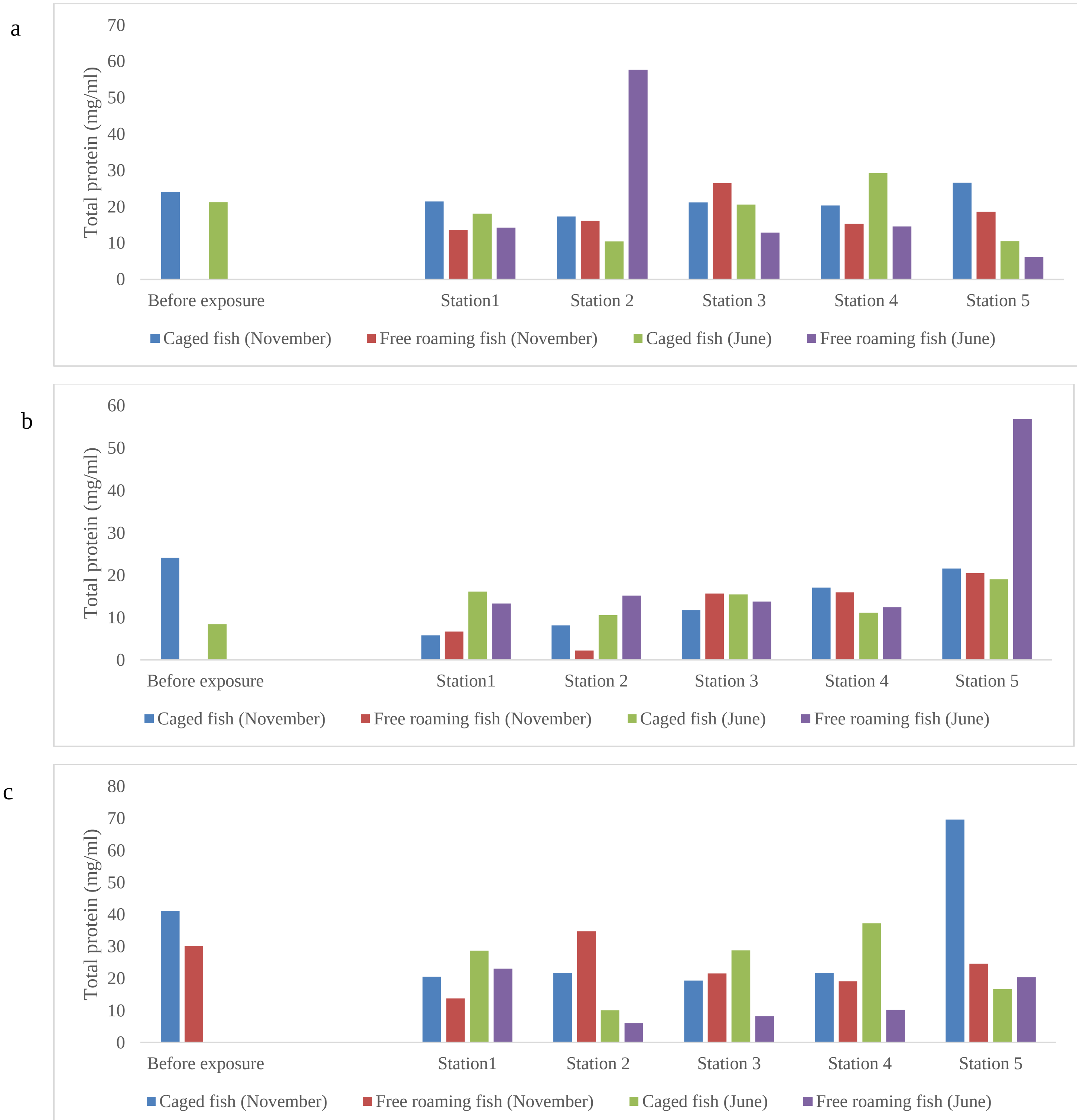


Fig 4.18: Monthly variation in the concentration of Total protein a) Gill b) Kidney and c) Liver in caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki stream.

Analysis of variance revealed that there was a significant difference in the gills of free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction of total protein concentrations in the gills of free roaming fish within Stations and months (Appendix XV b).

4.4.1.2 Kidney

Station 5, kidney of caged *Clarias gariepinus* was recorded to have highest total protein concentration (20.29±1.46mg/ml), while Station 2 had the least (9.36±1.40mg/ml).The total protein concentration in *Clarias gariepinus* before exposure was higher than the Station means after exposure (Table 4.6). In November, the peak total protein was recorded in Station 5 (21.55mg/ml) and the least was obtained in Station 1 (5.80mg/ml) (Fig 4.19). However In June the highest mean concentration was obtained in Station 5 (19.03mg/ml). Analysis of variance showed that there was a significant difference in total protein concentrations in kidney of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in total protein concentrations in the kidney of caged fish within Stations and between months (Appendix XVI a).

The highest station mean total protein concentration in kidney of free roaming *Clarias gariepinus* was recorded in Station 5 (38.69±21.00mg/ml), furthermore the least Station mean concentration was recorded in Station 2 (8.68±7.48mg/ml). The highest mean total protein concentration was obtained in Station 5 (20.50mg/ml), while the least was recorded at Station 2(2.20mg/ml) in November. In June the peak of total protein was recorded in Station 5 (56.88mg/ml) (Fig 4.19).There was a significant difference in the kidney of free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction of total protein concentrations in the kidney of free roaming *Clarias gariepinus* in Stations and between months (Appendix XVI b).

4.4.1.3 Liver

The highest Station mean, total protein concentration in the liver of caged *Clarias gariepinus* was recorded in Station 5 (43.14±30.56mg/ml), whereas the least was recorded in Station 2 (15.89±6.73mg/ml) (Table 4.6). In November the topmost total protein concentration was obtained in Station 5 (69.6mg/ml). Subsequently In June, the peak mean in caged fish was obtained in Station 4 (37.19mg/ml), while the least was recorded at Station 2 (10.055mg/ml) (Fig 4.19). ANOVA showed significant difference in total protein concentrations in liver of *Clarias gariepinus* among Stations and between months and significant difference in the interaction in total protein concentrations in the liver of caged fish among Stations and between months (Appendix XVII a).

Station 5, was recorded with the highest Station mean concentration of total protein in the liver of free roaming *Clarias gariepinus* (22.48±2.45mg/ml), while the least was at Station 3 (14.88±7.71mg/ml). In November, the highest mean concentration was obtained in Station 2 (34.70mg/ml). However, in June, the peak concentration in liver of free roaming *Clarias gariepinus* was recorded in Station 1 (23.03mg/ml) (Fig 4.19). Analysis of variance showed that there was a significant difference in the liver of the free roaming *Clarias gariepinus* within Stations and between months and there was a significant difference in the interaction in total protein concentrations in the liver of free roaming *Clarias gariepinus* in Stations and between months (Appendix XVII b).

4.5 SOD Activity in Fish Tissues

4.5.1 Gill

Station 3 gill of caged *Clarias sp* was observed to have the highest Station mean (12.27±6.02U/ml), while the least was recorded in Station 1(10.00±7.49 U /ml) (Table 4.7).The Sod activity in June was higher than the Sod activity in November .

Similarly, the highest SOD activity in caged *Clarias gariepinus* in November was obtained in Station 4 (8.175 U /ml) in caged *Clarias sp*, while the highest SOD in June was obtained in Station 2 (17.91 U /ml) (Fig 4.19a). ANOVA showed significant difference in SOD activity in the gill of *Clarias gariepinus* among Stations and between months and significant difference in the interaction of SOD activity in the gill of caged fish among Stations and between months (Appendix XVIII a).

In the free roaming gill of *Clarias gariepinus*, similar highe SOD activity was observed in June in comparison to November. The highest SOD activity In November and June was obtained in Station 3 (8.23 U /ml) (18.21 U /ml) (Fig 4.19). Furthermore, the highest Station mean SOD activity was recorded in Station 3 (13.22±5.76 U /ml), whereas the least was recorded in Station 1 (10.03±6.18 U /ml) (Table 4.19a). However, there was a significant difference in the gill of free roaming *Clarias gariepinus* within Stations and between months and there was a significant difference in the interaction of SOD activity in the gill of free roaming *Clarias sp* in Stations and between months (Appendix XVIII b).

Table 4.7: SOD Activity (U/ml) in Caged and Free Roaming *Clarias gariepinus*

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
SOD	Type of fish samples	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
SOD (Gill)	Caged	12.90	16.84	14.86±2.24	3.51	16.50	10.00±7.49	4.71	17.92	11.31±7.61	7.05	17.54	12.27±6.02	8.11	16.14	12.16±4.60	7.06	16.84	11.93±5.62
	Free roaming				4.65	15.44	10.03±6.18	3.53	18.95	11.26±8.83	8.21	18.35	13.22±5.76	3.53	13.68	8.6±5.84	4.65	16.14	10.40±6.61
SOD (Kidney)	Caged	5.78	10.90	8.19±2.74	2.35	8.42	5.39±3.48	7.06	11.62	9.33±2.61	8.21	14.74	11.48±3.76	8.2	16.84	12.53±4.97	9.45	10.62	10.03±0.66
	Free roaming				11.76	14.4	13.09±1.50	1.75	11.93	6.82±5.82	2.35	14.04	8.2±6.73	8.2	9.82	9.02±0.92	5.87	15.8	10.83±5.72
SOD (Liver)	Caged	8.77	11.76	10.23±1.68	5.76	8.77	7.29±1.70	4.65	12.63	8.65±4.58	5.85	17.19	11.52±6.53	5.7	14.04	9.91±4.75	5.68	6.67	6.22±0.51
	Free roaming				5.78	12.63	9.22±1.96	4.5	9.82	7.21±3.01	5.7	16.14	10.96±5.97	1.15	10.43	5.79±5.34	11.21	12.94	12.05±0.95

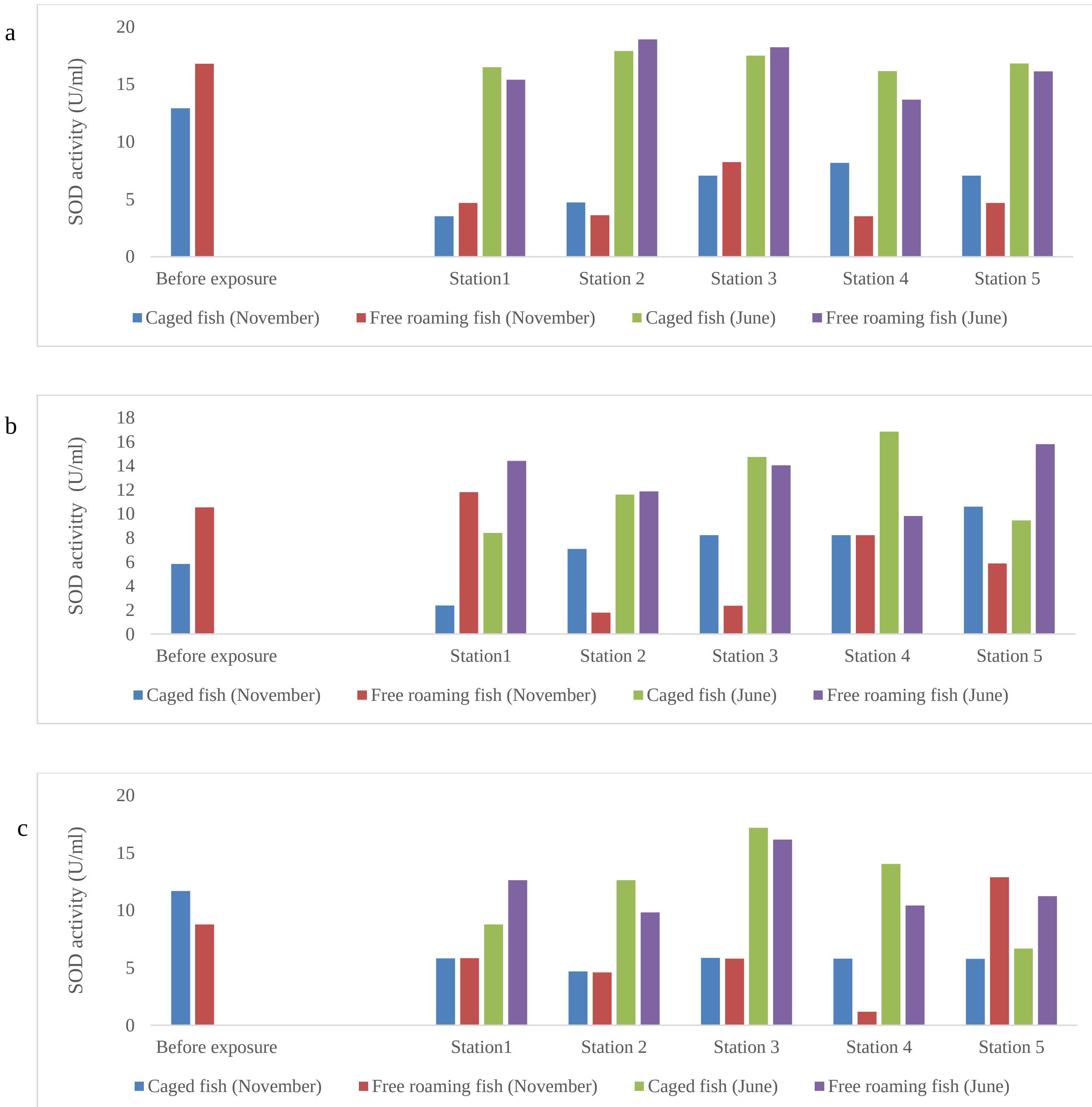


Fig 4.19: Monthly variation in SOD activity in a) Gill b) Kidney and C) Liver in caged and free roaming *Clarias gariepinus* in Tatsawarki stream.

4.5.2 Kidney

The highest Station mean of SOD concentration in the kidney of caged *Clarias sp* was obtained in Station 4 (12.53±4.97U /ml), whereas the least was recorded in Station 1 (5.39±3.48U /ml) (Table 4.19b).There was continuous increase in trend of SOD concentration In June in respect to November with exception to Station 5. The peak of SOD activity recorded in Station 4 (16.83U /ml), in comparison to the peak in November in Station 5(10.61U /ml) in caged *Clarias gariepinus* (Fig 4.19b). The SOD concentration in the kidney of caged fish was however higher than the concentration before exposure both in November (5.83U /ml) and June (10.54 U /ml) (Fig 4.19b). Analysis of variance using the confidence interval of 95% revealed that, that there was a significant difference in SOD concentrations in the kidney of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in SOD concentrations in the kidney of caged fish among Stations and between months (Appendix XIX a).

Station 1, kidney of free roaming *Clarias sp* was recorded with the highest Station mean of SOD activity (13.09±1.50U /ml) (Table 4.19). The kidney of the free roaming *Clarias gariepinus* of all the stations was recorded with higher SOD activity in June in comparison to the November. Station 1(11.80 U /ml) had the highest SOD activity in November and Station 5(15.80 U /ml) (Fig 4.19b), in June.

Apart from Station 1, higher SOD activity was recorded in the kidney of caged *Clarias sp* in contrast to the kidney of free roaming In November , whereas in June, Station 3 and 4 kidney of free roaming *Clarias gariepinus* had a higher activity in respect to the kidney of the caged fish.There was a significant difference in the kidney of free roaming *Clarias gariepinus* within Stations and between months and there was a significant difference in the interaction in SOD concentrations in the kidney of free roaming *Clarias sp* in Stations and between months (Appendix XIX b).

4.5.3 Liver

The peak Station mean of SOD concentration in the liver of caged *Clarias gariepinus* was obtained in Station 3 (11.52±6.53 U /ml), thus higher than the SOD concentrations before exposure (10.23±1.68 U /ml) (Table 4.19). The least Station mean was however recorded in Station 5 (6.22±0.51 U /ml) (Table 4.19). The highest SOD value in the liver of caged *Clarias sp*, in November and June was obtained in Station 3(5.87 U /ml), (17.18 U /ml), respectively (Fig 4.19c). However, there was a significant difference in SOD activity in the liver of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in SOD activity in the liver of caged *Clarias sp* among Stations and between months (Appendix XX a).

Station 5 (12.05±0.95 U /ml), liver of free roaming *Clarias gariepinus*, was recorded to have the highest SOD activity, while the least Station mean was recorded in Station 4 (5.79±5.34 U /ml) (Table 4.19). SOD activity in June was observed to be higher during the course of study than the month of November. The highest SOD activity In November was obtained in Station 5 (12.87 U /ml), with the least value in Station 4(1.17 U /ml). In June, the peak SOD activity was obtained in Station 3 (16.14 U /ml) (Fig 4.19c).

With exception to Station 3 and 4, the liver of free roaming *Clarias gariepinus* was recorded to have the highest SOD activity in November.

Analysis of variance ($P \leq 0.05$), showed that , there was a significant difference in the liver of free roaming *Clarias gariepinus* within Stations and between months and there was a significant difference in the interaction in SOD concentrations in the liver of free roaming fish, in Stations and between months (Appendix XX b).

4.6 Catalase Activity in Fish Tissues

4.6.1 Gill

Station 5 gill (88.98±100.15 U/mg protein) of caged *Clarias sp* had the highest Station mean, catalase activity hence higher than the CAT concentration in *Clarias gariepinus* before exposure (45.12±49.06 U/mg protein). The least was observed in Station 4 (25.08±27.10 U/mg protein) (Table 4.8). Distinct variation was obtained in months. In November , Station 1 gill of caged *Clarias sp* (4.61 U/mg protein) was recorded with the highest Catalase value, while in June, Station 5 had the highest (175.72 U/mg protein) (Fig 4.20a). Uniform least values of Catalase activity was observed in both caged and free roaming *Clarias gariepinus*, throughout the months. Analysis of variance showed that there was a significant difference in CAT activity in the gill of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in CAT activity in the gill of caged *Clarias sp* among Stations and between months (Appendix XXI a).

Among the gill of the free roaming *Clarias gariepinus,* Station 3 (62.54±69.58 U/mg protein) was recoded to have the highest CAT activity, while the least was obtained in Station 2 (16.02±15.47 U/mg protein) (Table 4.8). In November, the least CAT activity was recorded in Station 3 (2.29 U/mg protein), while the peak value was recorded in Station 5 (3.50 U/mg protein).

Table 4.8: Catalase Activity (U/mg protein) in Caged and Free Roaming *Clarias gariepinus*

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
CAT	Type of fish samples	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean±SD
CAT(Gill)	Caged	2.58	87.64	45.12±49.06	4.56	75.84	39.91±40.76	3.35	165.00	81.68±90.53	2.00	77.79	39.88±43.71	1.58	48.58	25.08±27.10	2.2	176.33	88.98±100.15
	Free roaming				3.2	104.27	53.17±57.70	2.56	29.42	16.02±15.47	2.27	123.39	62.54±69.58	2.78	110.22	53.99±59.23	3.48	121.39	62.14±67.72
CAT (Kidney)	Caged	2.49	255.17	125.82±142.49	4.11	84.66	44.37±46.47	2.91	153.94	78.11±86.83	1.84	166.92	84.30±95.20	1.42	239.14	119.78±136.66	2.25	112.00	56.75±62.93
	Free roaming				2.10	153.26	77.18±86.69	10.52	90.91	50.70±46.38	2.30	118.68	60.46±67.15	2.39	155.32	78.36±87.70	2.30	49.98	26.10±27.46
CAT (Liver)	Caged	2.51	67.21	34.62±37.06	1.59	77.85	39.68±43.95	2.00	148.44	74.68±83.92	1.85	117.05	58.96±65.92	1.65	54.43	27.78±30.15	1.87	134.51	67.44±75.71
	Free roaming				3.15	106.12	54.39±59.16	1.5	222.80	147.27±167.41	1.5	222.8	111.90±127.36	3.85	289.04	145.96±164.05	2.16	81.81	41.79±45.75

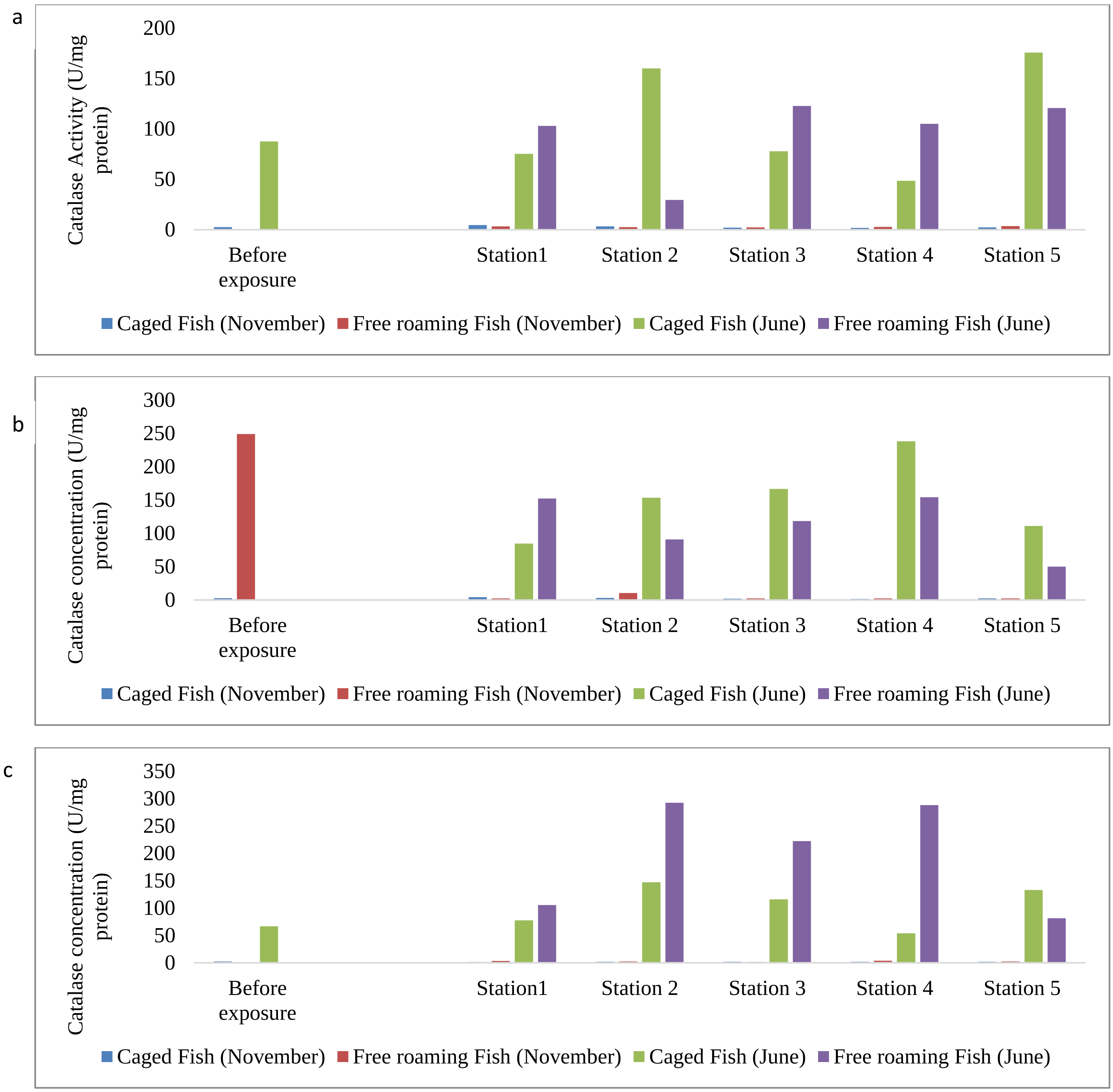


Fig 4.20: Monthly variation in CAT activity in a) Gill b) Kidney and C) Liver in caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki stream.

In June, the highest CAT value in the gill of free roaming *Clarias sp*, throughout the study period was obtained in Station 5 (120.79 U/mg proteins) (Fig 4.20a). Exemption of Station 2 and 5, observation showed that free roaming *Clarias sp*, had a higher CAT activity than the caged *Clarias sp* In June. There was a significant difference in the gill of free roaming *Clarias gariepinus* within Stations and between months and there was a significant difference in the interaction in CAT activity in the gill of free roaming fish, in Stations and between months (Appendix XXI b).

4.6.2 Kidney

Station 4 kidney of caged *Clarias gariepinus* (119.78±136.66 U/mg protein), was recorded with the highest Station mean of CAT activity, whereas the least was recorded in Station 1 (44.37±46.47 U/mg protein) (Table 4.8). The peak Station mean of CAT activity was obtained in Station 4 which was howerver, less than the CAT activity in *Clarias sp*, before exposure. Marked variation was observed in months. The month of June showed high CAT activity relatively throughout the Stations in respect to November. The peak CAT activity in November in caged *Clarias sp* was obtained in Station 1 (4.13 U/mg protein), while the least was recorded in Station 3(1.85 U/mg protein) (Fig 4.20b). Furthermore, In June, the highest CAT activity value was observed in Station 4 (154.31 U/mg protein), while the least was obtained in Station 5 (49.88 U/mg protein) (Fig 4.20b). Analysis of variance ($P \leq 0.05$), showed significant difference in CAT activity in the kidney of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in CAT activity in the kidney of caged *Clarias sp* among Stations and between months (Appendix XXII a).

Similarly, Station 4 kidney (78.36±87.70 U/mg protein), of free roaming *Clarias sp*, was recorded with the highest Station mean CAT activity, and the least was obtained in Station 5(26.10±27.46 U/mg protein) (Table 4.8). The highest CAT activity value during the study period was observed in Station 2 (10.53 U/mg protein), while the least was recorded in Station 1(2.11 U/mg protein), in November (Fig 4.20b). In June the peak CAT activity throughout the study was recorded in Station 4 (154.31 U/mg protein), whereas the least was obtained in Station 5 (49.88 U/mg protein) (Fig 4.20b). Apart from Station 1, similar observation of higher catalase activity value in caged *Clarias gariepinus* (84.61-238.13 U/mg protein) in respect to free roaming *Clarias sp* was recorded (Fig 4.20b). However, there was a significant difference in the kidney of free roaming *Clarias gariepinus* within Stations and between months. (Appendix XXII b).

4.6.3 Liver

The highest Station mean, catalase activity in the liver of caged *Clarias sp* was recorded in Station 2 (74.68±83.92 U/mg protein), hence higher than the CAT activity, before exposure (34.62±37.06 U/mg protein). The least Station mean was obtained in Station 4 (27.78±30.15 U/mg protein) (Table 4.8). During the 8-month study, discernible observations in the months were obtained, with the peak of CAT activity in the liver of caged *Clarias gariepinus* in November and June recorded in Station 2 (2.01 U/mg protein), (292.25 U/mg protein) (Fig 4.20c).However, there was a significant difference in CAT activity in the liver of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction of CAT activity in the liver of caged *Clarias sp* among Stations and between months (Appendix XXIII a).

Station 2 liver of free roaming *Clarias gariepinus* was recorded with the highest CAT activity (147.27±167.41 U/mg protein), whereas, the least was observed in Station 5 (41.79±45.75 U/mg protein). In November, the peak of CAT activity was obtained in Station 4 (3.89 U/mg protein) (Table 4.8). Observations made in June, showed that with exception of Station 5 (81.41 U/mg protein), the free roaming liver *Clarias sp* recorded higher CAT activity, than the caged fish (Fig 4.20c). Analysis of variance ($P \leq 0.05$), showed that, there was a significant difference in CAT activity in the liver of *Clarias gariepinus* among Stations and between months (Appendix XXIII b).

4.7 Malondehyde Concentration (lipid peroxidation) in Fish Tissues

4.7.1 Gill

Station 1(381.12±244.80nmols/mg protein) gill had the highest Station mean, MDA concentration, while the least was recorded in Station 4 (135.95±11.83nmols/mg protein) (Table 4.8).There was marked variation in MDA concentration within the months, with higher concentrations in the onset of raining season in June. The peak of MDA concentration in June during the 8-month study was obtained in Station 5 (629.65nmols/mg protein), whereas in November was recorded in Station 2 (210.27nmols/mg protein) (Fig 4.21a). There was a significant difference in MDA concentrations in gill of caged *Clarias gariepinus* among Stations and between months (Appendix XXIV a).

The highest Station mean, in the gill of free roaming *Clarias sp* was recorded in Station 5 (1511.26±1646.31nmols/mg protein), while the least was obtained in Station 4 (186.13±104.17nmols/mg protein (Table 4.8)

Table 4.9: Malondehyde Concentration (nmols/mg protein) in Caged and Free Roaming *Clarias gariepinus*

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
MDA	Type of fish samples	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
MDA(Gill)	Caged	103.55	345.19	224.57±139.17	168.10	593.20	381.12±244.80	210.15	387.53	298.51±101.91	67.00	276.52	171.76±119.81	125.30	146.24	135.95±11.83	96.57	630.29	363.13±307.75
	Free roaming				125.47	617.93	371.93±284.01	90.87	309.48	199.08±124.91	108.57	420.84	265.17±179.59	95.89	277.00	186.13±104.17	85.51	2941.00	1511.26±1646.31
MDA (Kidney)	Caged	97.53	720.00	408.61±359.19	271.78	695.53	484.61±245.92	253.17	927.00	590.02±388.26	117.56	1099.88	585.52±540.55	191.67	1421.90	806.32±709.71	77.21	524.86	300.77±258.13
	Free roaming				220.67	998.00	609.38±448.39	236.82	249.13	242.60±6.43	137.89	608.97	372.97±271.37	111.16	643.54	377.35±306.78	91.78	495.28	293.25±231.97
MDA (Liver)	Caged	52.76	129.59	90.79±43.89	58.7	110.11	84.21±29.27	127.67	1049.85	576.34±517.83	91.97	765.15	427.95±387.55	99.90	186.26	142.80±49.46	20.16	342.79	181.17±185.88
	Free roaming				175.29	670.15	421.44±284.22	61.89	603.72	332.04±311.57	228.67	1495.11	862.37±729.98	201.87	1243.06	720.23±597.92	59.6	894.63	477.18±481.38

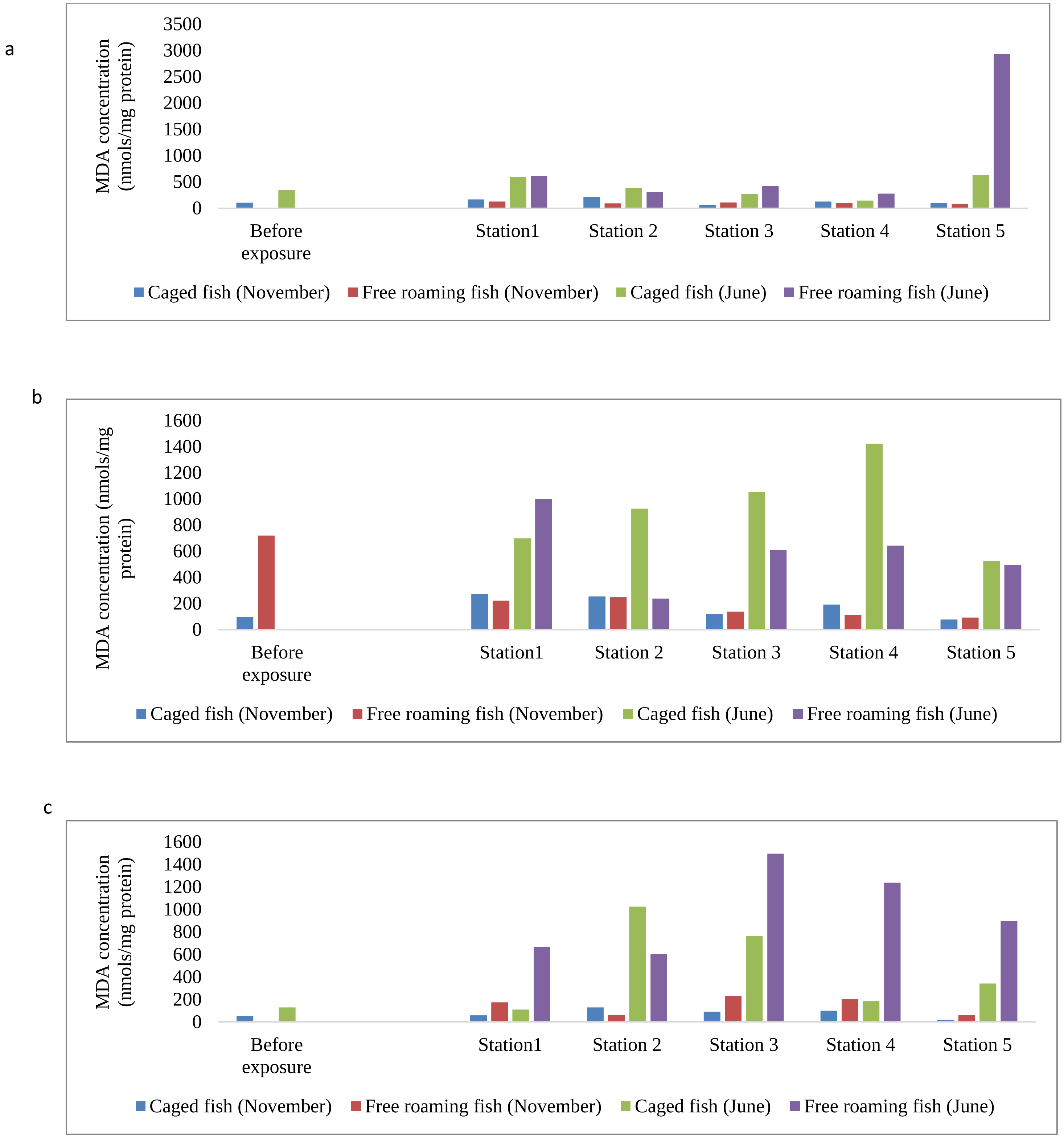


Fig 4.21: Monthly variation in the concentration of MDA in a) Gill b) Kidney and C) Liver in caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki stream.

Apart from Station 3, in November, higher MDA concentration was obtained in caged *Clarias gariepinus* in comparison to free roaming *Clarias sp*, with. The peak of MDA concentration in gill of free roaming *Clarias gariepinus,* in November, was recorded in Station 2 (210.26nmols/mg protein) (Fig 4.21a). Furthermore, in June, the gill of free roaming *Clarias sp* with exemption of Station 2 had a higher MDA concentration than the caged fish. However, Station 3 (420.695nmols/mg protein), was recorded with highest MDA concentration in June (Fig 4.21a).

Analysis of variance using 95% confidence interval showed that, there was a significant difference in MDA concentrations in the gill of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction of MDA concentration in the gill of free roaming *Clarias sp* among Stations and between months (Appendix XXIV b).

4.7.2 Kidney

Station 4 kidney of caged *Clarias gariepinus* (806.32±709.71695nmols/mg protein) had the highest Station mean, which is higher than the MDA concentration in the kidney of *Clarias sp* before exposure. The least was obtained in Station 5 (300.77±258.13nmols/mg protein) (Table 4.8). In November, the peak of MDA concentration was observed in Station 1(271.82nmols/mg protein), and the least in Station 5 (77.23nmols/mg protein) (Fig 4.21b). In June, apart from Station 1, MDA concentrations obtained in the kidney of caged *Claris sp* was higher than that of the free roaming, with the peak MDA concentration recorded in Station 4(1420.95nmols/mg protein) (Fig 4.21b). There was a significant difference in MDA concentrations in the kidney of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in MDA concentrations in the kidney of caged *Clarias sp* among Stations and between months (Appendix XXV a).

Among the kidney of the free roaming *Clarias gariepinus*, the highest Station mean was observed in Station 1 (609.38±448.39nmols/mg protein), while the least was obtained in Station 2 (242.60±6.43nmols/mg protein) (Table 4.8). In November, Station 2 (248.12nmols/mg protein) was recorded with the highest MDA concentration and Station 4 (643.03nmols/mg protein), in June (Fig 4.21b). Analysis of variance revealed significant difference in MDA concentrations in the kidney of free roaming *Clarias gariepinus* among Stations and between months (Appendix XXV b).

4.7.3 Liver

The highest Station mean, MDA concentration in the liver of caged *Clarias gariepinus* was recorded in Station 2 (576.34±517.83nmols/mg protein), whereas the least in Station 1 (84.21±29.27nmols/mg protein) (Table 4.8). In November, the peak of MDA concentration in the liver of caged *Clarias sp*, was obtained in Station 2 (128.25nmols/mg protein). Furthermore in June, highest concentration was recorded in Station 3 (1494.56nmols/mg protein) (Fig 4.21c). There was a significant difference in MDA concentration in the liver of *Clarias gariepinus* among Stations and between months and significant difference in the interaction of MDA concentration in the liver of caged *Clarias sp* among Stations and between months (Appendix XXVI a).

Station 3 liver of free roaming *Clarias sp* (862.37±729.98nmols/mg protein), was recorded with the highest Station mean of MDA concentration, while the least was observed in Station 2 (332.04±311.57nmols/mg protein) (Table 4.8).Apart from Station 2, the MDA concentrations of the free roaming liver of *Clarias sp,* was recorded with an increasing trend, in comparison to the MDA concentrations in the caged *Clarias sp*. Similarly the highest MDA concentration in the liver of free roaming fish in November was recorded in Station 3 (230.19nmols/mg protein) (Fig 4.21c). Furthermore, in June, with exception of Station 2, the MDA concentration in the liver of free roaming *Clarias sp* in all the Stations was however, higher than the MDA concentration in the caged *Clarias sp* (Fig 4.21c).However, there was a significant difference in MDA concentrations in the liver of free roaming *Clarias gariepinus* among Stations and between months (Appendix XXVI b).

4.8 Glutathione Concentration in Fish Tissues

4.8.1 Gill

The gill of caged *Clarias sp* with the highest Station mean, glutathione concentration during the study period was recorded in Station 5 (70.93±41.27µg/), thus higher than the *Clarias gariepinus*, before exposure (60.68±41.19µg/ml). The least Station mean in the gill of caged *Clarias sp* was obtained in Station 1 (19.94±9.63µg/ml) (Table 4.9). In November , Station 1(28.28 µg/ml),and Station 5 (35.2µg/ml) gills of caged fish was recorded with the highest GSH concentrations in comparison to the free roaming *Clarias sp* in respect to other Stations (Fig 4.22a). In June, the highest GSH concentration was recorded in Station 5 (106.65 µg/ml). However, there was a significant difference in GSH concentrations in gill of caged *Clarias gariepinus* among Stations and between months (Appendix XXVII a).

Station 3 gill of the free roaming fish was recorded with the highest Station mean of GSH concentration during the study period (92.07±39.02µg/ml), while the least was obtained in Station 5 (34.13±1.14 µg/ml) (Table 4.9).

Table 4.10: Glutathione Concentration (µg/ml) in Caged and Free Roaming *Clarias gariepinus*

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
GSH	Type of fish samples	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean ±SD	Min	Max	Mean± SD
GSH(Gill)	Caged	25.00	96.70	60.68 ±41.19	11.50	28.33	19.94 ±9.63	16.67	97.00	56.34 ±45.80	18.00	36.67	27.39 ±10.67	25.00	91.70	58.25 ±38.22	35	108.30	70.93± 41.27
	Free roaming				18.00	62.00	40.01 ±25.22	33.20	64.00	48.46 ±17.55	58.23	126.70	92.07 ±39.02	48.20	62.30	55.13 ±7.93	33.00	35.20	34.13± 1.14
GSH (Kidney)	Caged	35.00	107.30	71.05 ±41.51	41.65	117.00	78.83 ±42.93	33.00	93.30	63.03 ±34.49	35.00	353.00	193.30±182.68	41.60	293.30	166.89±144.64	1.70	33.33	17.48± 18.11
	Free roaming				21.67	88.30	54.94 ±38.40	11.65	18.30	14.91 ±3.75	38.20	130.00	82.88 ±51.56	40.00	197.00	118.05±90.01	33.21	138.30	85.64± 60.47
GSH (Liver)	Caged	26.67	167.00	96.77 ±80.92	15.00	28.30	21.68 ±7.48	16.67	248.30	131.67±132.78	23.00	183.00	102.76±91.91	28.31	176.70	102.09±85.18	28.20	327.00	177.13 ±171.90
	Free roaming				33.2	186.00	108.63±87.03	31.67	201.70	116.54±97.93	31.67	275.00	150.84±137.65	35.00	252.00	143.08±124.62	25.00	250.00	137.13 ±129.36

a b c

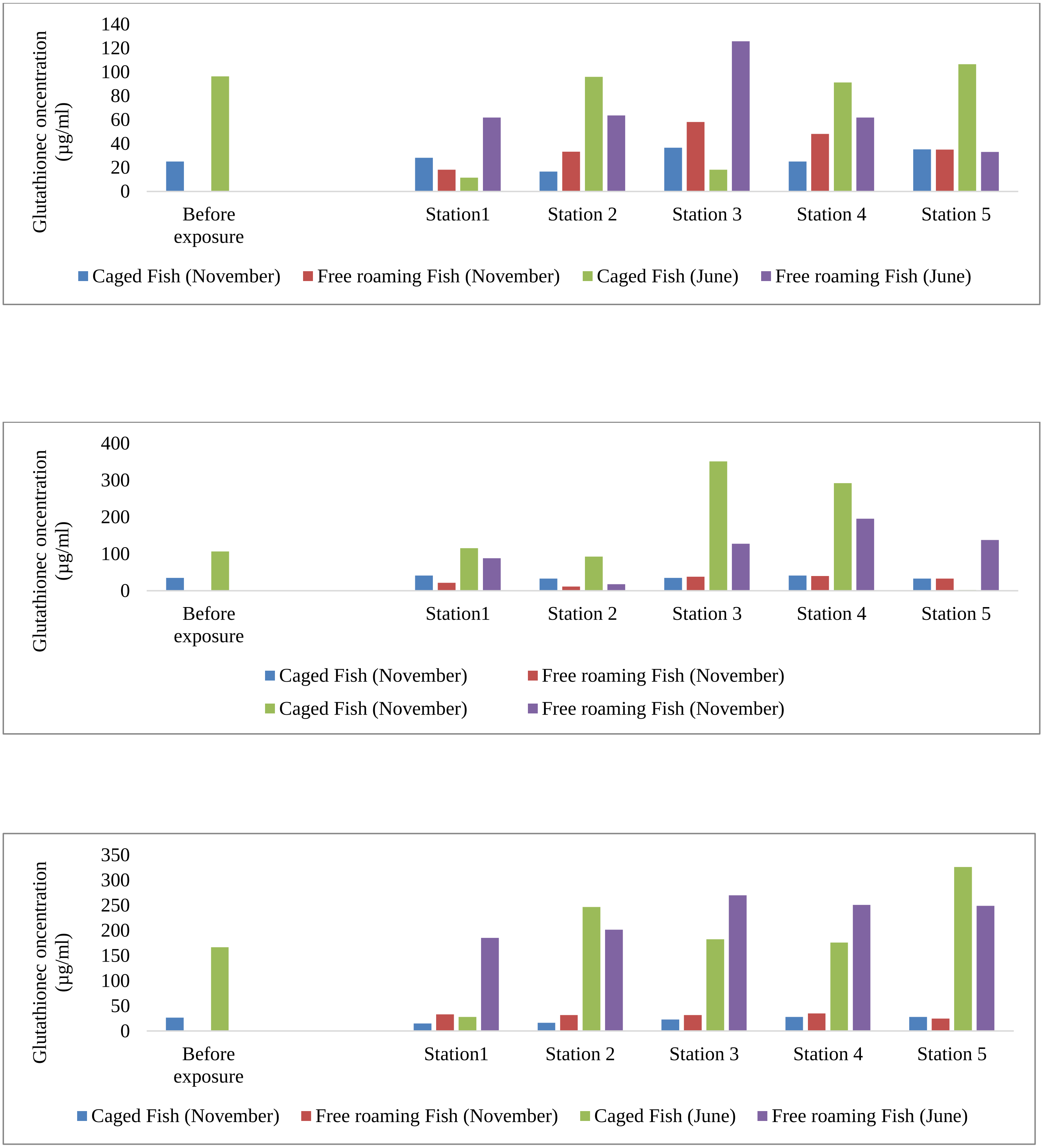


Fig 4.22: Monthly variation in the concentration of GSH in a) Gill b) Kidney and C) Liver in caged and free roaming *Clarias gariepinus* along the stretch of Tatsawarki stream.

In November, the peak of GSH concentration was observed in Station 3 (58.28µg/ml), whereas in June it was obtained in Station 2 (63.65 µg/ml) (Fig 4.22a). Analysis of variance revealed that, there was a significant difference in GSH concentrations in the gill of free roaming *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction of GSH concentration in the gill of free roaming *Clarias sp* within Stations and between months (Appendix XXVII a).

4.8.2 Kidney

Station 3 kidney (193.30±182.68µg/ml) of caged *Clarias gariepinus* was observed with the highest station mean of GSH concentration, while the least was recorded in Station 5 (17.48±18.11µg/ml) (Table 4.9). In November, the highest GSH concentration was recorded in Station 1 (41.66µg/ml), whereas in June, the peak of GSH concentration was recorded in Station 3 (351.50µg/ml) (Fig 4.22b). Analysis of variance, using the confidence interval of 95%, showed that, there was a significant difference in GSH concentrations in the kidney of caged *Clarias gariepinus* among Stations and between months (Appendix XXVIII a).

Among the kidney of free roaming *Clarias sp*, the highest Station mean during the 8- month study was obtained in Station 4 (118.05±90.01µg/ml) (Table 4.9). Furthermore, the least was recorded in Station 2 (14.91±3.75 µg/ml). In November, and June, Station 2 was recorded with the least GSH concentration (11.66µg/ml), (18.15µg/ml), respectively (Fig 4.22b). However, there was a significant difference in GSH concentrations in the kidney of free roaming *Clarias gariepinus* among Stations and between months and a significant difference in the interaction within Stations and between months (Appendix XXVIII b).

4.8.3 Liver

The highest Station mean of GSH concentration in the liver of caged *Clarias sp*, was recorded in Station 5 (177.13±171.90µg/ml), while the least was obtained in Station 1 (21.68±7.48 µg/ml) (Table 4.9). Among all the sampled Stations, Station 5 liver (28.265µg/ml) of caged fish had the highest GSH concentration than the free roaming *Clarias sp*, in November (Fig 4.22c). In June, Station 5 liver was recorded with the highest GSH concentration (326.00µg/ml) (Fig 4.22c). There was a significant difference in GSH concentration in the liver of caged *Clarias gariepinus* among Stations and between months (Appendix XXIX a).

In the free roaming *Clarias gariepinus*, the highest Station mean was recorded in Station 3 (150.84±137.65 µg/ml), whereas the least was recorded in Station 1 (108.63±87.03µg/ml) (Table 4.9). In November, all the Stations were recorded with higher GSH concentrations in comparison to the liver of caged *Clarias sp*, apart from Station 5 (25.10µg/ml) (Fig 4.22c). Station 2 (201.35µg/ml) and Station 5 (249.15 µg/ml), were recorded with minimum GSH concentration in comparison to the liver of caged *Clarias sp*, in that Stations (Fig 4.22c). However, there was a significant difference in GSH concentrations in the liver of free roaming *Clarias gariepinus* among Stations and between months and significant difference in the interaction within Stations and in seasons (Appendix XXIX b).

4.9 Vitamin C Concentration in Fish Tissues

4.9.1 Gill

The highest Station mean of vitamin C concentration in gill of caged *Clarias gariepinus* was recorded in Station 4 (12.61±0.51mg/ml), while the least was recorded in Station 3(1.24±0.78mg/ml) (Table 4.10). The concentration of vitamin C before exposure

(15.34±9.40mg/ml) was higher than the highest Station mean. Station 5 (12.17mg/ml) was recorded with the highest vitamin c concentration in comparison to the other Stations and the free roaming *Clarias gariepinus* in that Station in November. In June, the peak of vitamin c was recorded in Station 4 (23.42mg/ml) (Fig 4.23a). Analysis of variance showed significant difference in vitamin c concentration in the gill of caged *Clarias gariepinus* among Stations and between months also significant difference in the interaction in vitamin C in the gill of the caged Fish within Stations and between months (Appendix XXX a).

Station 1 gill (13.31±2.47mg/ml) of free roaming *Clarias sp*, was recorded with the highest Station mean of vitamin c, during the study period, furthermore, the least concentration was obtained in Station 2 (1.72±1.57mg/ml) (Table 4.10). In November, the highest vitamin C concentration was obtained in Station 1 (11.72mg/ml), whereas in June, the highest concentration was obtained in Station 4 (16.46mg/ml). The least vitamin c concentration in November and June were obtained in Station 2 (0.36mg/ml), (3.08mg/ml), respectively (Fig 4.23a). However there was a significant difference in vitamin c concentration in the gill of free roaming *Clarias gariepinus* among Stations and between months. The interaction between vitamin C in the gill of the free roaming fish is also significant in Stations and in seasons (Appendix XXX b).

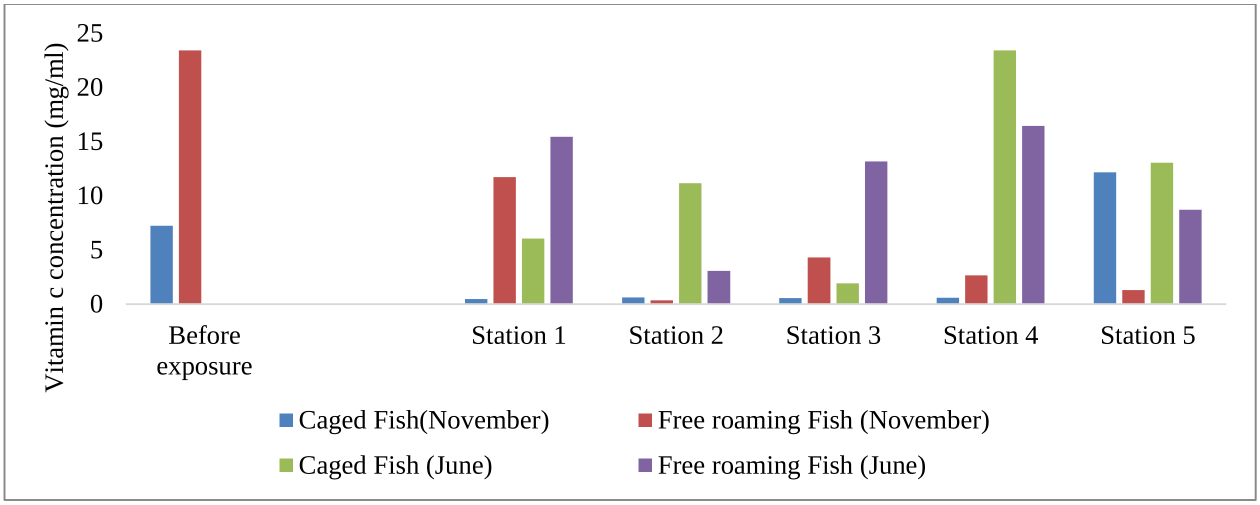
4.9.2 Kidney

Station 4 kidney of caged *Clarias gariepinus* (5.55±5.45mg/ml) had the highest Station mean of vitamin c concentration. This concentration in Station 4 was however, higher than the vitamin c concentration of Clarias *sp* before exposure (1.15±0.20mg/ml).The least vitamin c concentration was obtained in Station 2 (0.42±0.17mg/ml) (Table 4.10).

Table 4.11: Vitamin C concentrations in caged and free roaming *Clarias gariepinus* of Tatsawarki stream.

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
Vitamins	Type of fish samples	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean ± SD	Min	Max	Mean± SD
Gill	Caged	7.20	23.43	15.34± 9.34	0.43	6.09	3.26± 3.22	0.61	11.18	5.90± 6.10	0.555	1.94	1.24± 0.78	0.59	23.43	12.01± 13.18	12.11	13.04	12.61± 0.51
	Free roaming				11.12	15.51	13.31± 2.47	0.36	3.08	1.72± 1.57	4.25	13.22	8.75± 5.11	2.66	16.47	9.56± 7.97	1.29	8.72	5.00± 4.29
Vitamin C (Kidney)	Caged	0.98	1.38	1.15± 0.20	0.26	0.58	0.42± 0.17	0.47	0.68	0.57± 0.12	0.29	2.44	1.37± 1.24	0.83	10.32	5.55± 5.45	0.41	5.85	3.11± 3.12
	Free roaming				1.20	8.32	4.76± 4.11	6.00	6.55	6.27± 0.31	0.36	2.28	1.32 ±1.10	0.97	4.35	2.66 ±1.95	1.61	6.63	4.11 ±2.88
Vitamin C (Liver)	Caged	1.87	23.43	12.65±12.45	0.56	6.94	3.75±3.68	0.60	6.47	3.53± 3.38	2.81	3.49	3.15 ± 0.39	0.70	2.52	1.61 ±1.05	1.46	2.18	1.82 ±0.41
	Free roaming	1.87	23.43	12.65±12.45	0.42	4.61	2.512±2.37	0.55	3.51	2.04±1.70	0.55	4.81	2.67± 2.44	0.45	10.08	5.25±5.54	1.21	5.31	3.26±2.36

a



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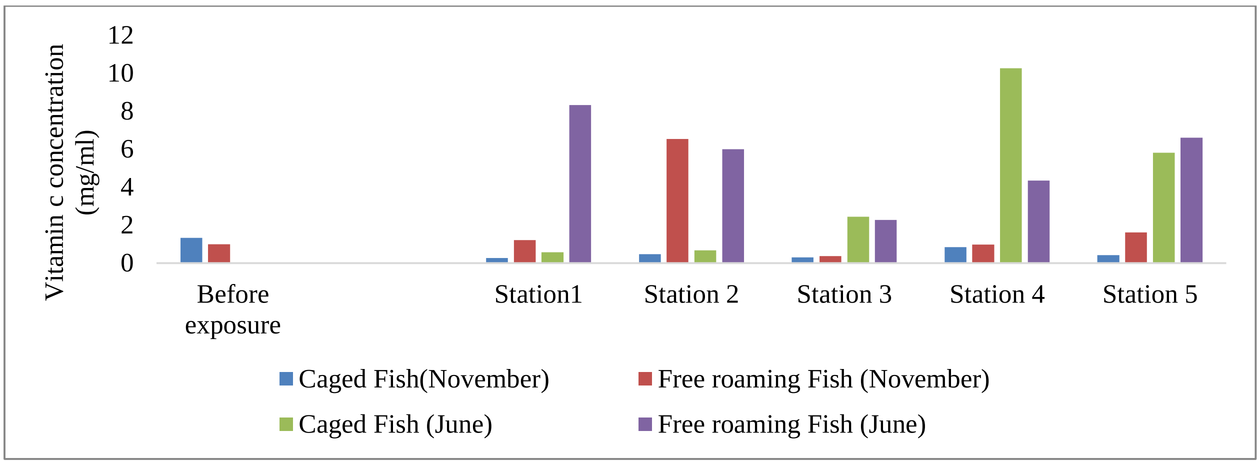

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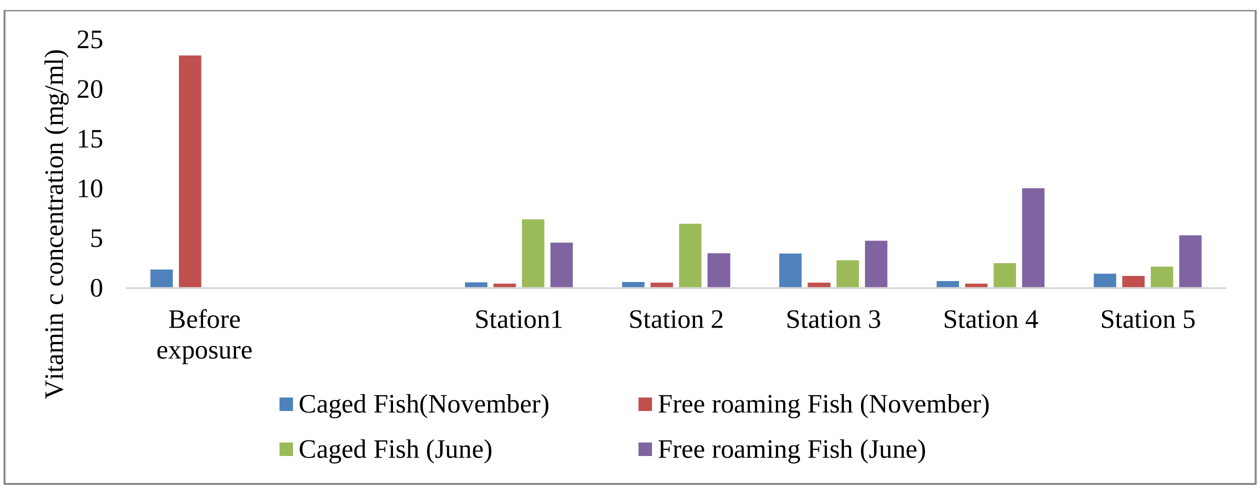


Fig 4.23: Monthly variation of Vitamin C a) Gill b) Kidney c) liver of caged and free roaming, *Clarias gariepinus* along the stretch of Tatsawarki stream.

In November, there was uniform least concentration in kidney of caged *Clarias sp* in all the stations, in comparison to the free roaming fish studied in all the Stations. Similarly the least vitamin c value throughout the study period in the dry season (November) and onset of raining season (June) was recorded in Station 1 (0.27mg/ml), (0.57mg/ml) (Fig 4.23b). However, there was a significant difference in vitamin c concentration in the kidney of caged *Clarias gariepinus* among Stations and between months (Appendix XXXI a).

Among the kidneys of free roaming *Clarias gariepinus* in all the Stations studied, the highest Station mean of vitamin c concentration was recorded in Station 2 (6.27±0.31mg/ml), while the least was obtained in Station 3 (1.32±1.10mg/ml) (Table 4.10). The peak of vitamin c was recorded in Station 2 (6.54mg/ml) in November, whereas in June Station 1, had the highest vitamin c concentration (8.32mg/ml) (Fig 4.23b). Consequently in November and June the least concentration was recorded in Station 3 (0.36mg/ml), (2.27mg/ml) (Fig 4.23b). Analysis of variance (ANOVA) showed significant difference in vitamin c concentration in kidney of free roaming *Clarias gariepinus* between Stations and between months (Appendix XXXI b).

4.9.3 Liver

The highest Station mean of vitamin c concentration in the liver of caged *Clarias gariepinus* was recorded in Station 1 (3.75±3.68mg/ml), hence less than the vitamin c concentration in the liver of the fish before exposure (12.65±12.45mg/ml) (Table 4.10). The least Station mean of vitamin c was obtained in Station 4 (1.61±1.05mg/ml) (Table 4.10). The peak of vitamin c concentration in the liver of caged *Clarias sp* was obtained in Station 5 (1.46mg/ml) in November, whereas in June, the highest vitamin c value was recorded at Station 1 (6.94mg/ml) (Fig 4.23c). There was a significant difference in vitamin c concentration in the liver of caged *Clarias gariepinus* among

Stations and between months also in the interaction in within Stations and in the month studied (Appendix XXXII a).

Station 4 liver of free roaming *Clarias gariepinus* had the highest Station mean, vitamin c concentration (5.25±5.54mg/ml), while the least was obtained in Station 3 (2.04±1.70mg/ml) (Table 4.10). In November, the highest vitamin c concentration in the free roaming fish was recorded in Station 5 (1.22mg/ml) while the least was recorded in Station 4(0.45mg/ml). In June, Stations 1 and 2 (3.51mg/ml) liver had the least vitamin c concentration in comparison to the liver of the caged *Clarias gariepinus*, in these respective Stations (Fig 4.23c). Analysis of variance revealed, significant difference in vitamin c concentration in the liver of free roaming *Clarias gariepinus* among Stations and between months (Appendix XXXII b).

4.10 Vitamin E Concentration in Fish Tissues

4.10.1 Gill

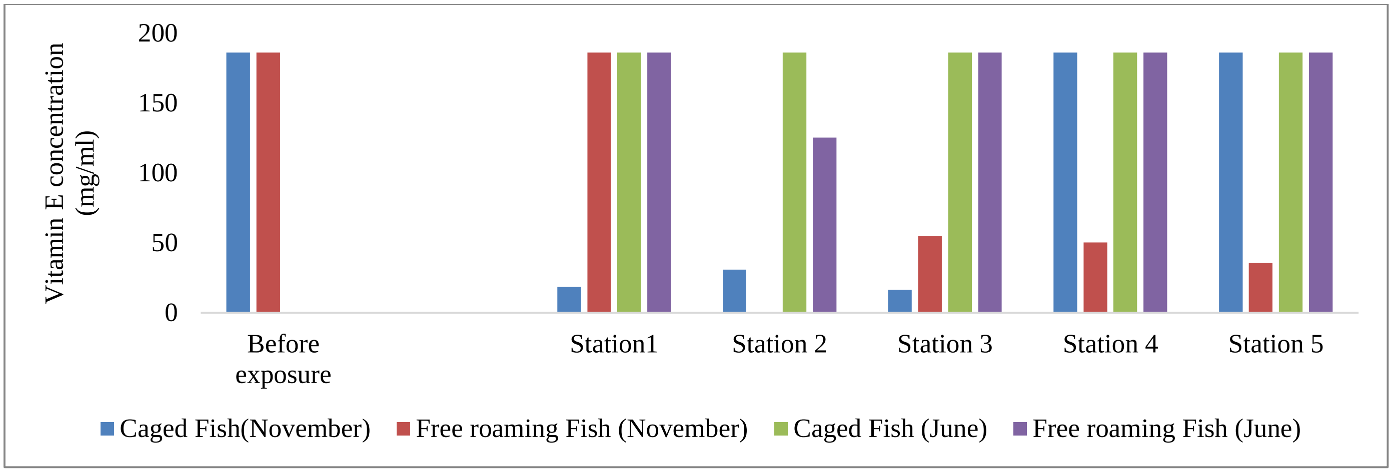
Among the gills of caged *Clarias gariepinus*, the highest Station mean of vitamin e concentration was obtained in Stations 4 and 5 (186.15±00mg/ml) while the least was recorded in Station 3 (101.36±97.91mg/ml) (Table 4.11). In November, Stations 4 and 5 gill among the caged fish had the highest vitamin E concentration (186.15mg/ml) while the least was obtained in Station 3 (16.57mg/ml). Identical concentrations (186.15mg/ml) in the gills of *Clarias sp* in all Stations were obtained (Fig 4.24a). However there was a significant difference in vitamin e concentration in the gill of caged *Clarias gariepinus* among Stations and between months (Appendix XXXIII a).

Station1 gill of free roaming *Clarias gariepinus* had the highest Station mean vitamin e concentration, (186.15±00mg/ml).

Table 4.12: Vitamin E concentration in caged and free roaming *Clarias gariepinus*

		Before exposure			Station 1			Station 2			Station 3			Station 4			Station 5		
Vitamin	Type of fish samples	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean ± SD	Min	Max	Mean± SD
Vitamin E (Gill)	Caged	186.15	186.15	186.15 ±00	18.49	186.15	102.32 ± 96.80	30.86	186.15	108.51 ± 89.66	16.52	186.15	101.36 ± 97.91	186.15	186.15	186.15 ±00	186.15	186.15	186.15 ±00
	Free roaming				186.15	186.15	186.15 ±00	ND	125.90	60.65 ± 74.79	53.83	186.15	120.49 ±75.82	50.21	186.15	118.22 ± 78.44	35.706	186.15	110.95± 86.83
Vitamin E(Kidney)	Caged	30.85	113.85	72.06± 47.6	ND	86.49	34.32 ± 59.63	54.73	93.56	74.17 ± 21.82	14.54	186.15	100.37 ± 99.05	7.811	186.15	96.98 ± 102.96	3.32	186.15	94.76± 105.53
	Free roaming				ND	186.15	85.925 ± 115.7	ND	186.15	85.55± 116.16	ND	105.53	44.01± 71.03	41.87	136.99	89.42 ± 54.29	17.211	186.15	101.68± 97.54
Vitamin E(Liver)	Caged	28.25	186.15	107.20± 91.16	ND	106.70	42.23 ± 73.89	ND	186.15	88.59 ± 112.65	ND	129.12	58.68 ± 80.76	9.03	186.15	97.84 ± 101.97	22.78	106.70	64.62 ± 47.63
	Free roaming	28.25	186.15	107.20± 91.16	45.11	186.15	115.91 ± 81.11	0.82	186.15	93.51± 106.97	64.11	186.15	125.16 ± 70.42	64.22	186.15	125.19 ± 70.39	42.90	111.02	76.74± 39.06

a



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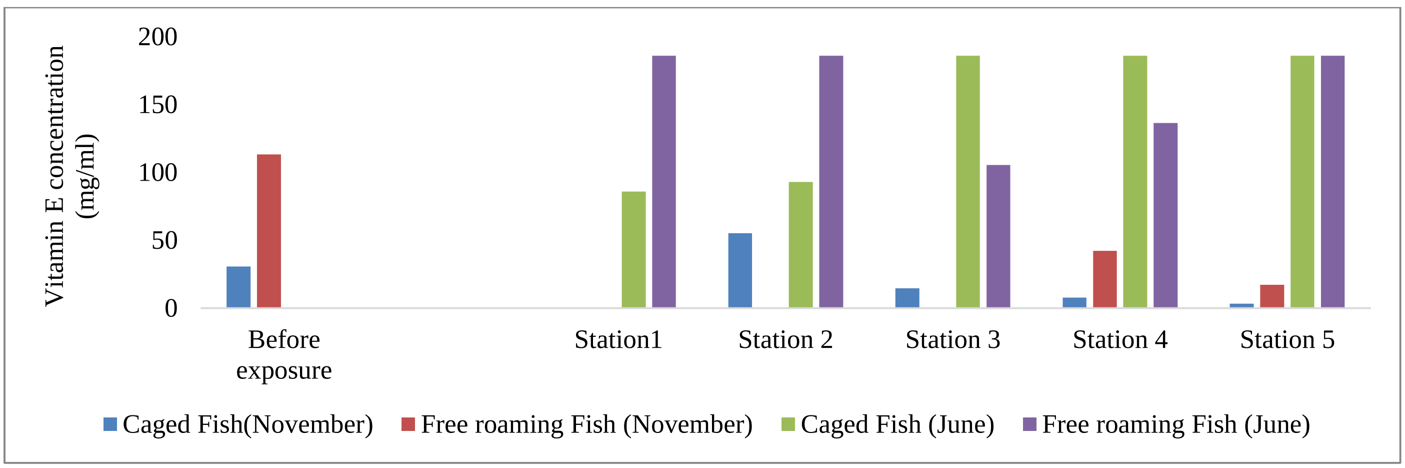

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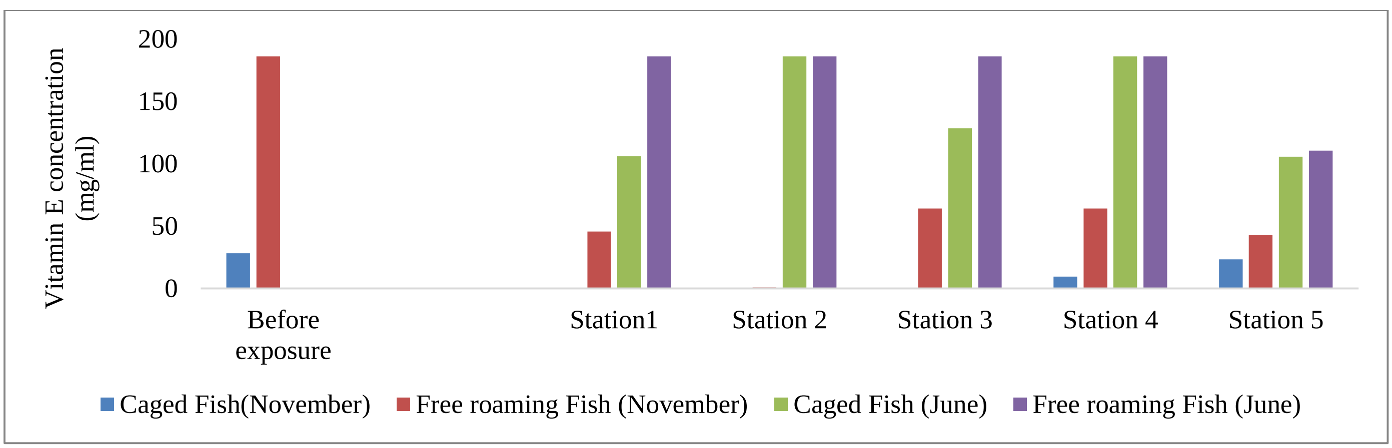

Fig 4.24: Monthly variation of Vitamin E a) Gill b) Kidney c) Liver of caged and free roaming, *Clarias gariepinus* along the stretch of Tatsawarki stream.

The least concentration was obtained in Station 2 (60.65±74.79mg/ml) (Table 4.11). In November, the least vitamin e concentration was recorded in Station 3 (30.86 mg/ml), whereas in June, the least was obtained in Station 2 (125.42mg/ml) (Fig 4.24a). Analysis of variance showed that significant difference in vitamin e concentration in the gill of free roaming *Clarias gariepinus* among Stations and between months. The interaction between vitamin e in the gill of the free roaming fish is significant within Stations and in seasons (Appendix XXXIII b).

4.10.2 Kidney

Station 3 (100.37±99.05mg/ml) kidney of caged *Clarias gariepinus* had the highest Station mean of vitamin e concentration during the 8-month study. Station 3 kidney vitamin e concentration was however higher than the vitamin e in *Clarias sp* before exposure (72.06±47.60mg/ml). The least vitamin e concentration was obtained in Station 1 (34.32±59.63mg/ml) (Table 4.11). In November, the highest vitamin e concentration in the caged kidney was obtained in Station 2 (55.28). Similarly In June, the highest concentration of vitamin e in the kidney of caged kidney was obtained at Stations 3, 4, and 5 (186.15mg/ml), while the least was recorded in Station 1 (85.96mg/ml) (Fig 4.24b). However, there is significant difference in vitamin e concentration in the kidney of caged *Clarias gariepinus* among Stations and between months (Appendix XXXIV a).

Station 5 (101.68±97.54mg/ml) had the highest Station mean of vitamin e, while the least was recorded in Station 3 (44.01±71.03mg/ml) (Table 4.11). In November, the highest vitamin e concentration in kidney of free roaming *Clarias gariepinus* was recorded in Station 4 (42.40mg/ml) (Fig 4.24b). Consequently, in June, Stations 1, 2, 5 (186.15 mg/ml), were recorded with the highest vitamin e concentration, while the least was recorded in Station 3 (105.52mg/ml) (Fig 4.24b). However, there is significant difference in vitamin e concentration in the kidney of caged *Clarias gariepinus* among Stations and between months (Appendix XXXIV a).

Station 5 (101.68±97.54mg/ml) had the highest Station mean of vitamin e, while the least was recorded in Station 3 (44.01±71.03mg/ml) (Table 4.11). In November, the highest vitamin e concentration in kidney of free roaming *Clarias gariepinus* was recorded in Station 4 (42.40mg/ml) (Fig 4.24b). Consequently, in June, Stations 1, 2, 5 (186.15 mg/ml), were recorded with the highest vitamin e concentration, while the least was recorded in Station 3 (105.52mg/ml) (Fig 4.24b). Analysis of variance showed that there was a significant difference in vitamin e concentration in the kidney of free roaming *Clarias gariepinus* among Stations and between months and significant difference in the interaction in vitamin e concentration in the kidney of free roaming *Clarias sp* among Stations and in months (Appendix XXXIV b).

4.10.3 Liver

Station 4 had the highest Station mean concentration of vitamin e among the caged *Clarias gariepinus* (97.83±101.97) during the period of study. Station 4 concentration was however less than the vitamin E concentration of *Clarias sp* before exposure (107.20± 91.16mg/ml). The least vitamin e concentration was recorded in Station 1 (42.25±73.89mg/ml) (Table 4.11). In November, the highest vitamin e value was recorded in Station 5 (23.38mg/ml). Similarly in June Stations 2 and 4 had the highest vitamin e concentration value (186.15mg/ml) (Fig 4.24c). However there was a significant difference in vitamin e concentration in the liver of caged *Clarias gariepinus* among Stations and between months (Appendix XXXV a).

Among the livers of free roaming *Clarias gariepinus* in the 8-month study, Station 4 liver was recorded as the Station with the highest Station mean , vitamin e concentration

(125.19±70.39mg/ml), while the least was in Station 5 (76.74±39.06 mg/ml) (Table 4.11). In November, the highest vitamin e value was recorded in Station 4 (64.23mg/ml) while the least was obtained in Station 2 (0.88mg/ml) (Fig 4.24c). In June, the highest vitamin e concentration was recorded in Station in all the Stations (186.15mg/ml) respectively, apart from Station 5 (110.57mg/ml) (Fig 4.24c). There was a significant difference in vitamin e concentration in the liver of free roaming *Clarias gariepinus* among Stations and between months and significant difference in the interaction within Stations and between months (Appendix XXXV b).

4.11 Histopathology in Fish Tissues

4.11.1 Gill

There was marked variation between the gill of the *Clarias sp* before exposure and after exposure. The gill of fish before exposure had normal lamella and filaments (Plate Ia) , thus the gill of *Clarias gariepinus* exposed to Tatsawarki stream showed deformed secondary lamella (Plate I b), Difffusion of secondary lamella (Plate Ic) and Degeneration and epithelia lifting of secondary lamella (Plate I d)

4.11.2 Kidney

Section of the kidney of *Clarias gariepinus* before exposure showed normal haematopoietic tissue, renal tubules, renal corpuscles and glomerulus (Plate II a). The section of kidney of *Clarias sp* after exposure showed: degeneration, dissociation, edema of renal tubules and congestion of haematopoietic tissue ((Plate II b).

4.11.3 Liver

The liver of *Clarias gariepinus* before exposure show unexposed central vein, while the liver of *Clarias sp* exposed had vacoulation and fatty degeneration, infiltration of the liver, hepatocyte necrosis, cellular infiltration and architectural and structural alteration with hemolysis of blood vessels.

Microscopic examination are shown in plate1- 4 (at×450)

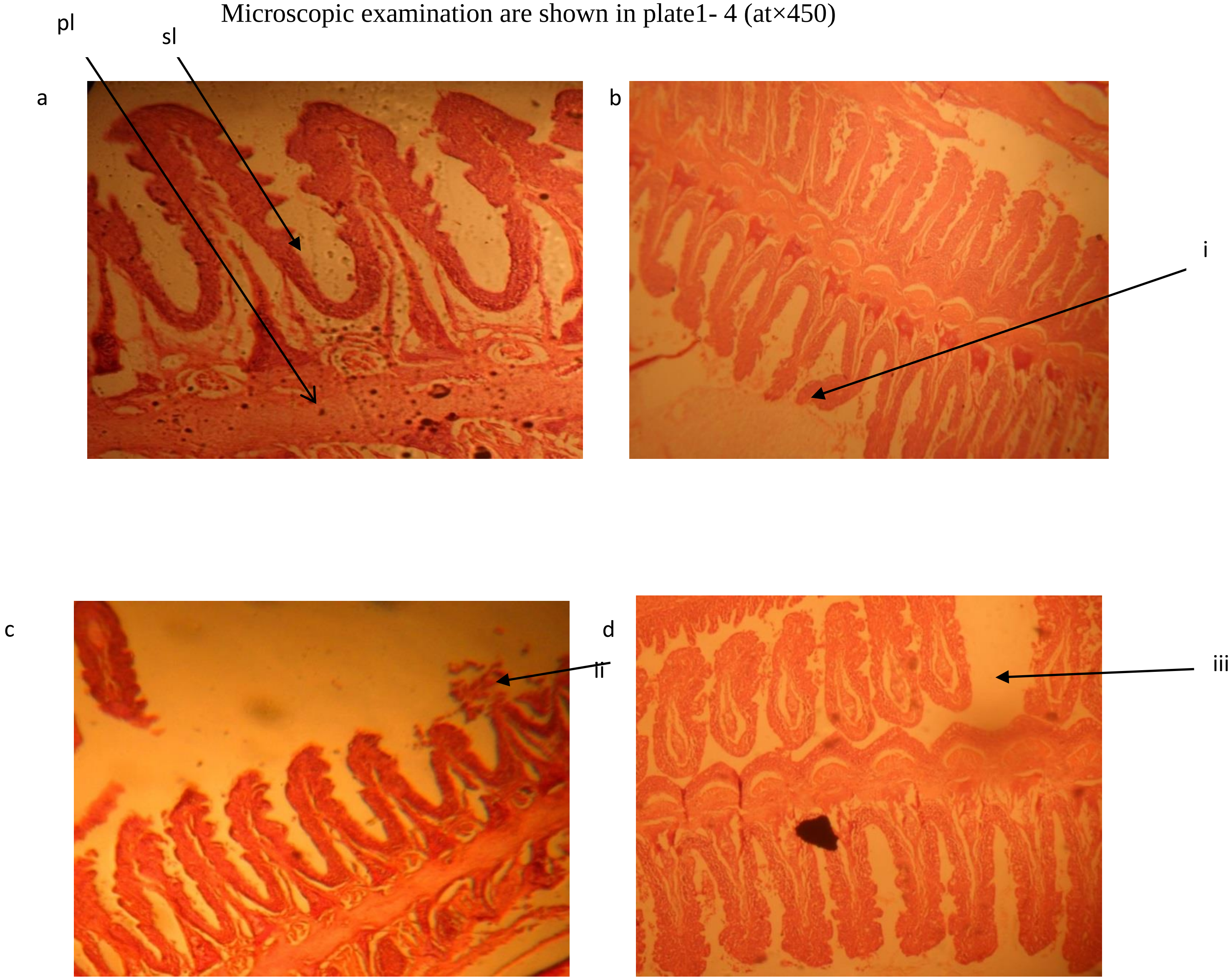

Plate I: × 100 a) Gill of *Clarias gariepinus before* exposure showing pl (primary lamella), sl (secondary lamella b) i. deformed secondary lamella c) ii. Diffusion of secondary lamella d) iii. Degeneration and epithelia lifting of secondary lamella

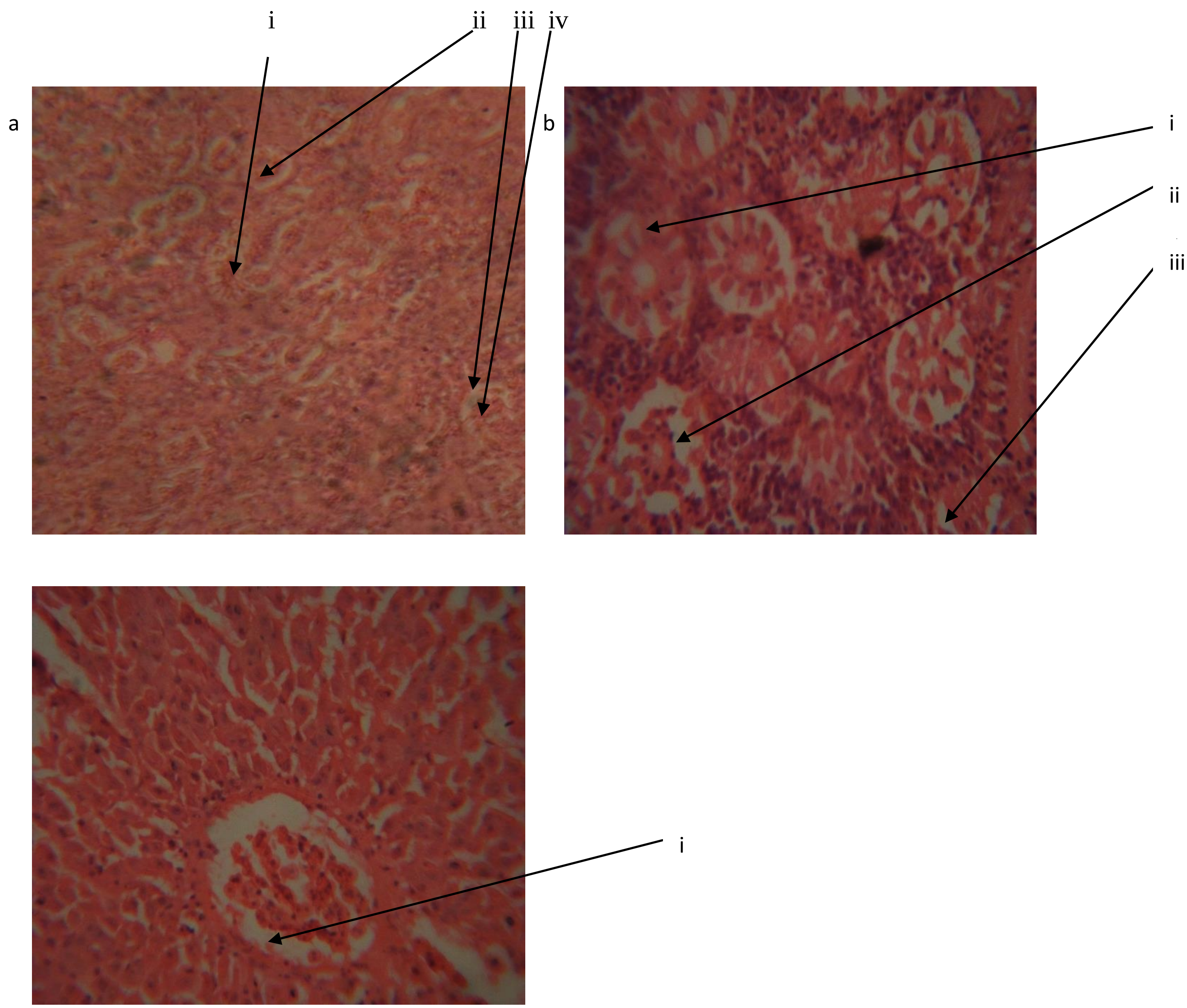

Plate II: × 100 a) section of the kidney of *Clarias gariepinus*, i) haematopoietic tissue ii) renal tubules iii) renal corpuscles and iv) glomerulus b) i) degeneration ii) dissociation iii) edema of renal tubules and congestion of haematopoietic tissue c) dissociation and congestion of haematopoietic tissue

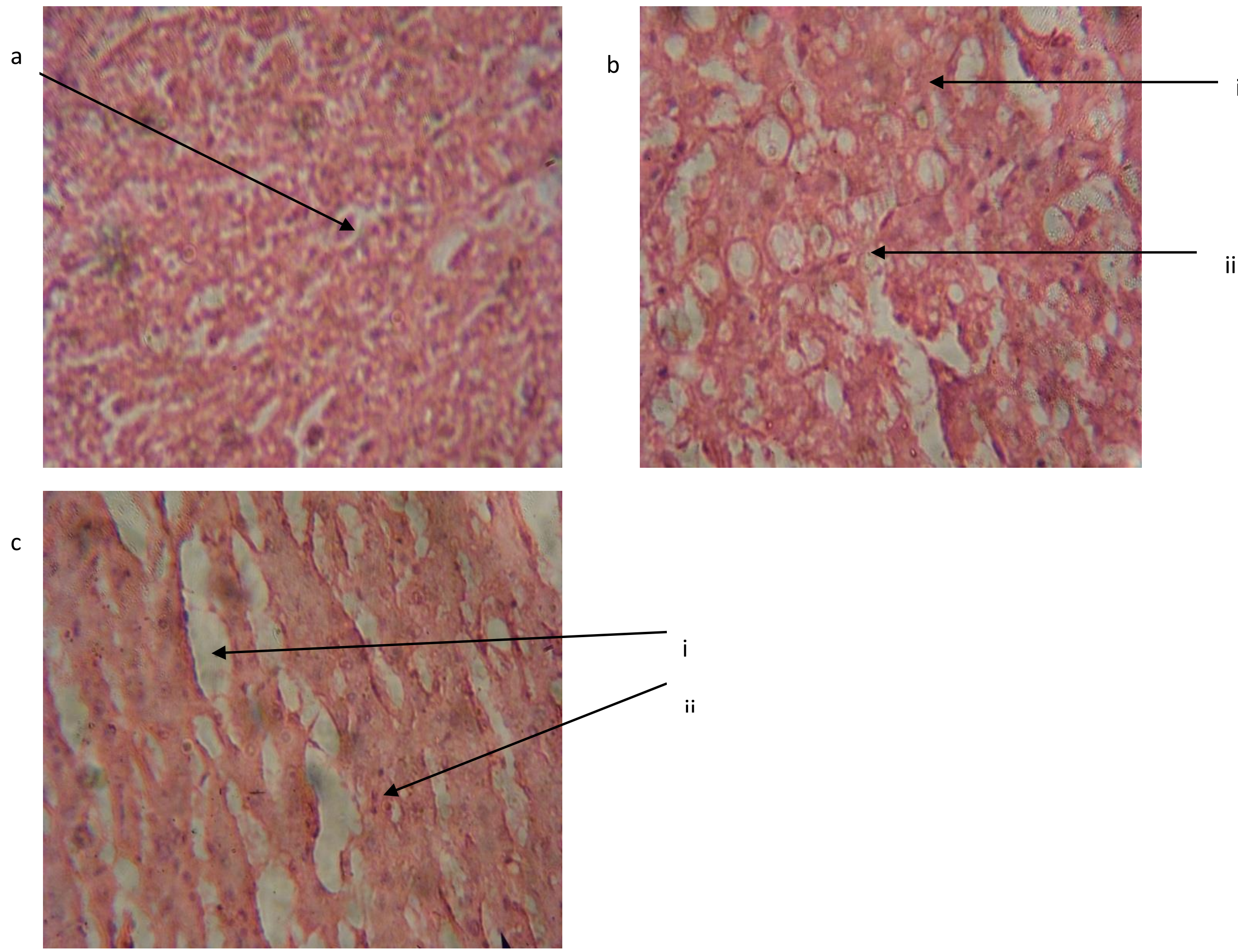

Plate III: × 100 a) section of the liver of unexposed central vein *Clarias gariepinus*, b) i) vacoulation and fatty degeneration ii) infiltration of the liver c) i) hepatocyte necrosis ii) cellular infiltration and architectural and structural alteration with hemolysis of blood vessels.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physiochemical Parameters of Tatsawarki Stream

The quality of water has tremendous impact on the standard and wellbeing of aquatic organisms. Singh *et al*., (2013) reported that the level of water quality is governed by physicochemical and biological parameters.

Temperature influences solubility of dissolved oxygen in the water body, hence affects the survival of aquatic organisms (Ubwa *et al*., 2013). The least monthly temperature during the course of this study was 18.5^0C while the grand mean during the same period was 25.33^0C (Figure 4.1a). This falls within the acceptable temperature range (16^0C and 27^0C), suggested by (FEPA, 1983) for *Clarias gariepinus* and other biota survival in aquatic habitats. Temperature in water bodies changes daily, monthly, seasonally and even yearly, due to changes in surface water temperature and activities around the water body (United Nations Environment Programme Global Environment Monitoring System, 2006).

The electrical conductivity during the study period were within the range of 145.13 and 1729.31 µS/cm. Freshwater streams usually have a conductivity between 150 to 500.00 µS/cm which supports diverse aquatic life (Behar, 1997). Some Stations exceeded the EPA standard limit of 1500.00 µS/cm. This can be attributed to presence of high concentration of ions through influx of urban run-offs (Chapman, 1993; Addo *et al*., 2011). The increase in electrical conductivity in Tatsawarki stream during this study could be attributed to agricultural runoff, domestic waste and other human activities in and around the water body. Similar inferences had been reported

(Deepali *et al.,* 2009; Essumang *et al.*, 2011; Annalakshmi and Amsath, 2012; Gopalkrushna, 2011; Seema, 2015). These increase could affects aquatic life (Verla *et al.*, 2007).

The pH during this 8-month study was circum-neutral falling within the range of pH 6-8, that is suitable for the survival of aquatic organisms (DAWF, 1996; Chapman, 199). The pH obtained, was also within the Nigerian standard for Drinking Water Quality (NSDWQ, 2007) safety range of (6.5-8.5) and the WHO standards (2011). Extreme pH can make water body hostile to aquatic life and alter the toxicity of other pollutant in one form or the other (Morrison *et al*. 2001).

Total dissolved solids means recorded during the study, falls within the range of 65.5 and 841.06mg/ml. Station 2 (841.06mg/ml) TDS was above the highest permissible limit of NSDWQ, (2005), (500mg/l), which implies the water is unfit for drinking. This result disagree with the report of Akaahan *et al*., (2015), on TDS concentration in River Benue (46.90 5.52mg/l), high TDS concentration could arise due to domestic waste dumps, industrial discharges, or probably soil weathering (Efe, 2005; Ubwa *et al*., 2013), this could alter the balance and composition of water body.

The highest station mean dissolved oxygen was 1.26 ± 1.07 mg/l, although the highest DO value was 2.66mg/l which was lower than WHO permissible limit of 4 mg/L, FEPA (1999) standard (6.0mg/l) and recommendation of the Federal Ministry of Environment required for fish growth and its survival and other aquatic organisms. Low DO affects aquatic life and stress fishes, and may result in anaerobic conditions that could cause bad odour (Sinha and Biswas, 2011; Ubwa *et al*., 2013). Low DO concentrations in water bodies can be caused by decomposing organic matter, dissolved gases, industrial waste, mineral waste and agricultural runoff (Srivastava *et al*., 2011; Addo *et al*., 2013). This observation is similar to the report made by Aina *et al*., (2012), that low DO could be associated to high level of nutrients, organic loads, total solids contents from industrial effluents which may affects aquatic organisms. Water with low DO from 0.2 - 0.5 mg/L are considered hypoxic; waters with less than 0.5 mg/L are anoxic.

During the period of this study, the highest Station mean and least was within the ranged from 39.73mg/l.$CaCO_3$ to 119.97mg/l.$CaCO_3$. However the highest monthly value was 354mg/l.$CaCO_3$. The WHO limit for Total Alkalinity for drinking water is 200mg/l and permissible limit is 600mg/l. All the Stations were able to meet the WHO standard limit for drinking water.

BOD Station means during the 8-month study period were between 0.34 and 0.0075mg/l, which was less than EU permissible limit (3.0 to 6.0 mg/L), FEPA (1991) limits (10mg/l) and the universal water quality index of 3.00 mg/l for the protection of fisheries, aquatic life and for domestic water supply (Boyacioglu, 2007; Joseph *et al*., 2012; Sayar *et al*., 2015). Although the peak of BOD obtained during this study was 2.43mg/l. Clair *et al*, .2003, reported that high BOD indicates poor water quality while lower BOD indicates low levels of organic waste. Adakole *et al*., (2012), reported that BOD of (<1.0 –unpolluted), moderately polluted between (2-9mg/l) and heavily polluted (10 mg/l). According to this inference, some of the Stations can be reported as unpolluted and some moderately polluted.

The Station means of Total Hardness ranged from 44.63mg/l to 74.81mg/l. Although the peak of total hardness was 116.00 mg/l. Lind, (1979), classification on Total hardness: soft (0-60mg/l), moderately hard (61-120mg/l), hard (121-180mg/l) and very hard water (180mg/l) and above. On the basis of Lind classification, the grand mean during the 8-month study showed that Station 2,3,4,5 are soft water while Station 1 is moderately hard.

The WHO permissible limits for chloride for drinking water is 250 mg/l. Thus Chloride Station means during the course of the study ranged from 5.51mg/l to 7.59 mg/l., which was less than the concentration in the study made in Lagos State, Nigeria water body (2.84-13.47 mg/L) (Gopalkrushna, 2011; Longe and Balogun, 2010) and the report by Imaobong Udousoro and Ini Umoren in Uruan stream, Akwa Ibom state (12.8-15.60mg/L). Similarly, this result disagrees with the report of Bichi *et al*., (2013), on the study of chloride in Tatsawarki stream (303.99mg/l) at point A, (101.1mg/l) at B and (128mg/l) at point C. However, Essumang, (2011), reported that low chloride concentration might be an indication of absence of intrusion of seawater and may not show any significant effects unlike higher level of chloride which present problems.

The non-significant difference in Station NO_3-N means might be attributed to the influx of sewage, fertilizers, domestic wastes and other organic wastes into most of the sampling Station of Tatsawarki stream. Gopalkrushna, 2011, Mahananda *et al*., 2010 and Imaobong Udousoro and Ini Umoren reported that nitrates- nitrogen is one of the basic indicator of the degree of organic pollution of a water source. The mean NO_3-N of the stretch of Tatsawarki stream during the study period is 19.53mg/l. The highest monthly NO_3-N mean (23.01mg/l) was obtained in December while the lowest monthly mean was obtained in June (11.05mg/l). This observation might be attributed to evaporation and dilution of the stream water in December and June respectively.

Phosphate/phosphorous had the least mean range of 1.44 and 1.7mg/l, in comparison to highest WHO standards (5 mg/L). Increase in phosphate level, could lead to eutrophication and can be caused by sources such as: phosphorus rich bedrock, industrial effluents and fertilizer run-off

(Esry, 1991; Ubwa *et al*., 2013). It may lead to death of aquatic organisms if present in high concentrations (DAWF, 1996; Joseph *et al*., 2012).

During the 8 months study, the sulphate Stations mean concentration ranged from 213mg/l and 293mg/l, although the highest mean value was 344mg/l. Even though some Station mean concentration is low, some Stations analyzed, had values higher than the WHO set standards (250 mg/L). According to the National standard for drinking water quality in Nigeria (NSDWQ, 2005), the recommended concentration is (100mg/l). During the course of this study, the sulphate concentration still did not meet these standards. The result of this study disagrees with the previous report of sulphate concentration in Tatsawarki stream by Bichi *et al*., 2013, that all the sampling points, point A (248mg/l), point B (73mg/l) and point C (102mg/l) conforms with the WHO guidelines. These might be due to contaminants such as: Industrial effluents, household discharges, contaminant from tanneries and textiles discharged in to water bodies and may lead to decreases in pH of water and increase of bacterial load, i.e. sulphate reducing bacteria.

5.2 Heavy Metals Concentration in Tatsawarki stream and *Clarias gariepinus.*

Heavy metals have being known to pose problems globally due to its effects on man, aquatic environment and aquatic organisims (Ashraj, 2005; Yoon *et al*., 2006; 2011; Vosyliene, 2006; Oyekunle *et al*., 2011; Vosyliene; Farombi *et al*., 2007; Tay *et al*. 2008). its toxic ability, persistence and bio-accumulative ability in the food chain (Ikem *et a*l. 2003; Patrick, 2015); may lead to many works on it’s to check and balance its effects.

5.2.1 Water samples

5.2.1.1 Lead

The mean lead concentrations obtained during the study period ranged from (0.08± 0.04mg/l) to (0.42± 0.32mg/l), however these concentrations does not conform with the permissible standard limit of 0.01mg/l of EU, (1998, 2006); WHO (2004, 2006); SON, (2003, 2007); NAFDAC, (2007), for drinking water and USEPA, (2005) standards. The high concentration of lead in Tatsawarki stream corresponds to the report made by Mwegoha, 2010 in the water body of Dar'es Salaam city, Tanzania (0.113±0.104mg/L). Other findings of high concentration in lead were made by Joseph *et al*., (2012) in Lake Chad, Baga, North Eastern Nigeria (1.23 ± 0.12 μg/g). He reported that the increase in lead in Lake Chad could lead to pollution. Bichi *et al*., (2013) reported that high lead concentration in sample point A (1.1mg/l) and point B (1.4mg /l) in Tatsawarki stream could be associated to the fact that the water joint with river Challawa, and hence receive metals from the wastewater from Tamburawa water works.

Salawu *et al*., (2014) findings in a Seasonal River in Maru town, Zamfara state, Nigeria (0.177±0.0004ppm), attributed that the increase in lead could be due to huge amounts of raw sewage, agricultural and industrial wastewater (Abdel-Moati and El-Sammak, 1997). Egbe *et al*., (2016), reports on high lead concentration in River Gora, Kaduna (0.82mg/ml) and emphasize on the bioaccumulatory and toxic effects of lead in organisms. High lead concentration of Tatsawarki stream can be attributed toinflux of tannery industrial waste and domestic effluents around the catchment region. High lead concentration in water bodies has being reported by several authors in its process of production of free radical and hence inducing oxidative stress (Nordberg and Arner, 2001; O'Brien *et al*., 2003; Farombi *et al*., 2007 and Doherty *et al*., 2010; Najeeb *et al*.,2014).

5.2.1.2 Chromium

There was a significant difference in chromium concentration between months, but there was no significant difference among sampling Stations at the stretch of Tatsawarki stream. Similarly, chromium concentration was not detected in June (onset of raining seasons), but however detected in November. The highest chromium mean concentration was reported in Station 3 (0.07mg/l) while the least was in Station 4 with (0.03mg/l). This results showed that some Stations were able to fall within WHO, (2004), USEPA, (2005), SON, (2007), NAFDAC, (2007), set standards, while some Stations were above the standard limits (0.05mg/l), this result was in accordance with Aliyu *et al*.,(2016) findings in River kaduna (2.41mg/l), which he implicated as the cause of pollution in the water body. Previous work on Tatsawarki stream by Bichi *et al*., (2013) also reported of high chromium concentration in the surface and ground water of the stream (8.8mg/l) on average and (3.3mg/l) respectively. The non –detectable concentration of chromium In June, can be attributed to dilution and evaporation during the season. High chromium, along with other heavy metals such as zinc, arsenic, iron, lead has being reported by several authors to cause toxicity to aquatic life, disruption of aquatic food chain, susceptibility of aquatic organisms to infection and also induction of oxidative stress (WHO 1995; Nordberg and Arner, 2001; O'Brien *et al*., 2003; Farombi *et al*., 2007 and Doherty *et al*., 2010).

5.2.1.3 Iron

Iron mean concentration in the stretch of Tatsawarki stream was at its peak in November (5.25mg/l) and June (0.89mg/l). Though iron is known for its essentiality in the nutrition of organisms but level above the national/ international standard have been reported severally by different authors to cause numerous effects. In all the Stations at the stretch of Tatsawarki stream, iron concentration was far above the highest contaminant set standard of WHO, (2004); USEPA, (2002); SON, (2007); NAFDAC, (2007) (0.30mg/l). This findings is in accordance with the investigation made by Joseph *et al*., (2012), (20.92 ± 1.55 mg/l) in the work on Lake chad, Baga, North Eastern Nigeria, which he associated the increased in iron concentration could lead to pollution in the water body. High iron concentration in Tatsawarki stream can be attributed to the anthropogenic inputs in the water body which consists of the illicit discharge of untreated sewage contaminants, non- biodegradable wastes and agricultural run -off of pesticides and herbicides. Several toxic effects of iron, which include resulting to oxygen deficiency in water body, hence resulting to acidity, creation of anaerobic condition, oxidative stress and death of aquatic organisms including: fishes have being reported by several authors (Hovinga *et al.*, 1993; Bakan and Büyükgüngör, 2000; Tüzen, 2003; Nelson *et al*., 2005; Altas and Büyükgüngör, 2007; Kusemiju *et al*., 2012; Bat *et al*., 2012).

5.3 Fish samples

Heavy metals might directly accumulate in the tissue or indirectly by transferring through the food chain in fish which may be due to anthropogenic inputs such as: industrial effluents (Allinson *et al*., 2009; Oyekunle *et al*., 2012; Dirican *et al.*, 2013). These heavy metals could affects cellular and reproductive system of fish (Parrott & Blunt, 2005; Thorpe *et al*., 2007; Eruola and Adedokun, 2012). Elevated level of heavy metals could also lead to mortality of aquatic organisms (Otitoloju and Don-Pedro, 2002). Due to these effects, tissues such as liver, kidney, gill in the fish has being used by several authors to determine heavy metal concentration (Olaifa, 2004; Storelli *et al*., 2005).

5.3.1 Gill

During the study period, the accumulation of heavy metals in gill were in the trend of Iron > Chromium > Lead in the caged and free roaming *Clarias gariepinus*. Iron in the gill of free roaming > Iron in the gill caged *Clarias sp* > Chromium in the gill of free roaming > Chromium in the gill caged *Clarias sp*, this can be attributed to difference in the duration of exposure to the Tatsawarki stream.

5.3.1.1 Lead

Apart from the Station mean, lead concentration in the caged gill of Station 4 *Clarias sp* (0.39 ± 0.15mg/kg), all the other station sampled gill were within the EU set standards (0.30mg/kg), WHO and FAO, (2006) and USEPA, (1986) acceptable standard for fish consumption (0.30mg/kg) . In November, all the gill of the caged *Clarias sp* were within the EU set standards, unlike in June, this may be attributed to influx of wastes from other waterbodies during the onset of raining season . Station 4 (0.51mg/kg) had the highest lead concentration which was far above the EU set standards In June, Similar inference was reported by Eneji *et al*., (2011) and Jenyo and Oladela, (2016). High lead concentration in Station 4 in June in Tatsawarki stream could be associated to influx of run-off wastes/ contaminants, erosion of lead containing materials from other water bodies in to the stream and drainage of agro-chemical containing lead compounds from agricultural activities around Tatsawarki stream. Baturh and Daniel, (2015), reported that high lead concentration in the gill of *Clarias grapienus* in Lake Akpoko and River Benue Nigeria could be attributed to increased quantity of sediment deposits in the water bodies. They also implicated extreme lead concentration above the national/ international detrimental to fish and human existence.

Station 2 gill of free roaming *Clarias sp* was recorded with the highest Station mean (0.32 ± 0.04mg/kg) and only Station above the EU set standards (0.30mg/kg). Correspondingly, to the caged gill of *Clarias gariepinus*, in November, the gill of the free roaming fish was within the set standards, in contrary to onset of raining season (June). This indicates, higher lead concentration, hence higher contaminants bio accumulated in both caged and free roaming gill of *Clarias sp* which could be associated to high inflow of waste water from other Stations in June.

5.3.1.2 Chromium

Chromium concentrations in the gill of Stations 1 and 5 were not detected in November and June, indicating drastic reduction of pollutants in the sampling Station. The highest Station mean of chromium concentrations in the gill of the caged fish was recorded in Station 4 (0.96 ±0.68mg/kg), which was higher than the WHO, 2008 standard for fish and fish products (0.15mg/kg). Station 4 gill had the highest chromium concentrations in November (1.55mg/kg) and June (0.37mg/kg). Chromium concentration in the gill of *Clarias sp* obtained during this study after exposure, was less than the findings of Baturh *et al*., (2015) on the gill of *Clarias gariepinus* exposed in River Benue (13.80mg/kg) and the findings of Eneji *et al*., (2011). These results of high chromium concentration in the gill of *Clarias sp*, disagrees with the findings on chromium concentration in the sampling Station during the 8-month study. Jenyo and Oladela, (2016), reported that highe concentrations of metals in fish in respect to the water body could be attributed to strong variations of flow rate, pollutant input and transport and also sedimentation of metals in the water body. This inference is in in consonance with the findings of Haslam, (1990). In Tatsawarki stream the high chromium concentration in the gill of the fish indicates bioaccumulation of chromium in respect to the sampling station and can be attributed to tannery wastes.

In the free roaming gill of *Clarias gariepinus,* in contrast to caged gill of *Clarias gariepinus,* the highest station mean, and chromium concentration in November and June was obtained in Station 5(0.99 ±0.71mg/kg), (1.63mg/kg) and (0.35mg/kg) respectively. Unlike the findings of caged *Clarias sp* in respect to difference in stations can be attributed to duration of exposure.

5.3.1.3 Iron

The highest Station mean of Iron in the gill of caged *Clarias gariepinus* was obtained in Station 5 (4.56 ±2.59mg/kg) was higher than mean of iron concentration before exposure (3.82 ±2.42mg/kg). This can be attributed to presence of iron in the water body. Iron concentration in the gill of caged *Clarias sp* was within the FAO, 1989 set standards (100.00mg/kg). This iron concentration during the study period was less than the findings of Udiba *et al*., (2014) on the study of River galma (31.14 ± 4.92mg/kg) and River kubanni (31.14 ± 5.40mg/kg). High iron concentration in the gill of caged *Clarias gariepinus* obtained in June in Station 5(4.92mg/kg) in comparison to Station 4(3.29mg/kg) to November could be attributed to high influx of untreated sewage and industrial waste water. Similar inference was observed by Jenyo and Oladela, 2016 on there study in Lake Asejire, Nigeria.

In the gill of the free roaming fish the highest iron value in November was obtained in Station 4 (17.45mg/kg), thus Station 2 (8.10mg/kg), was recorded with the highest iron concentration In June, which were below the set FAO, 1983 set standards. Some authors also reported trace concentration of Iron in fish tissues below the set standards (Adewoye *et al*., 2005)

5.3.2 Kidney

The level of heavy metal accumulation was on the trend Iron > Chromium > Lead in the kidney. Similarly the iron in the kidney of free roaming *Clarias sp* > Iron in the kidney of caged *Clarias* *gariepinus* > Chromium in the kidney of free roaming fish > Chromium in the kidney of caged *Clarias gariepinus* > Lead in the kidney of free roaming *Clarias sp*> Lead in the kidney of caged *Clarias gariepinus*. This could be attributed to the limited period of exposure of the caged *Clarias gariepinus*.

5.3.2.1 Lead

In November, the highest lead concentration in the kidney of caged *Clarias gariepinus* was obtained in Station 5 (0.23mg/kg), which was within the WHO (1992), WHO and FAO, 2006, USEPA (1986) set standards for consumption, in disparity to the month of June Station 1 (0.31mg/kg) (Figure 4.16a). This can be attributed to presence of contaminants during the onset of raining season (June) in respect to November and due to higher agricultural activities around and adjacent to the sampling Station in Tatsawarki stream.

Similar findings was obtained in the kidney of free roaming *Clarias sp*, with higher lead concentration in June and November. Station 2 (2.19mg/kg), (0.25mg/kg), but dissimilar in respect to Station. This, dissimilarities in Stations in caged and free roaming liver of *Clarias sp* could be attributed to the duration of exposure.

5.3.2.2 Chromium

Station 1 kidney of caged *Clarias sp* is the only Station, chromium was not detected. Other Stations were recorded with high concentration above the WHO, 2008 standards for fish products and consumption (0.15mg/kg). The highest Station mean was recorded in Station 2 (0.93 ± 0.84mg/kg). Nzeve *et al*., 2014 attributed high chromium concentration to municipal and tannery wastes.

In the free roaming fish, Station 5 kidney in November and June was recorded with highest chromium concentration, (1.58mg/kg) (0.44mg/kg) respectively. It consequently exceeded the WHO, 2008 standards. This could be associated to some activities around the station and influx of waste from other water bodyin to the station.

5.3.2.3 Iron

Iron Station mean in the kidney of caged *Clarias sp* during the 8-month study ranged from (2.30 ±0.21mg/kg) to (3.48 ± 2.77mg/kg). These concentrations are below the FAO, 1989 set standards for consumption (100mg/kg). Udiba *et al*., (2012) attributed low iron concentration in respect to set national/ international standards poses no significant toxicological risk or iron intoxication. Similar less iron concentration in *Clarias gariepinus* have being reported in River Niger (1.263mg/kg) and River Warri (1.340mg/kg) (Ezemonye and Egbroge and Egbroge *et al*., 1992; Oboh, and Edema, 2007)

In free roaming fish, the highest iron Station mean was recorded in Station 5 (7.22±5.56mg/kg), while the least was obtained in Station 1 (1.84 ±1.89mg/kg), this indicates higher effluents from activities around Station 5 in respect to Station 1.

5.3.3 Liver

Heavy metal accumulation, during the study period was on the trend Iron > Chromium > Lead in the liver of *Clarias sp*. The Iron in the liver of free roaming *Clarias sp* > Iron in the liver of caged *Clarias gariepinus* > Chromium in the liver of free roaming fish > Chromium in the liver of caged *Clarias gariepinus* > Lead in the liver of free roaming *Clarias sp*> Lead in the liver of caged *Clarias gariepinus*. This could be attributed to the limited period of exposure of the caged *Clarias gariepinus*.

5.3.3.1 Lead

The non- significant difference in lead concentration in liver of caged *Clarias gariepinus* among stations and between months could be attributed to the influx of contaminants discharged in to Tatsawarki stream. The highest Station mean of lead in the liver of caged *Clarias sp* was recorded in Station 3 (0.30 ± 0.14mg/kg), which was higher than the WHO, 1992, WHO and FAO, 2006 and USEPA, 1986 standard (0.29mg/kg). Stations 1,2,3,5 were within the international standards for fish consumption. Similar inference of higher lead concentration in the liver of *Clarias gariepinus* was made by Farombi *et al*., 2007. Lead concentration in the liver of caged *Clarias sp* was higher in November than in June, may be due to dilution and evaporation obtained in June.

Among free roaming *Clarias gariepinus*, the highest Station mean of lead in the liver of free roaming fish was recorded in Station 4 (0.35 ±0.09mg/kg), (Table 4.5) this is contrary to the findings obtained in the liver of caged fish with the highest station mean recorded in Station 3 (0.30±0.14mg/kg). This could be associated to the fact that the duration of exposure of the caged fish was less than the free roaming fish in the stream. It could also be associated to the inference made by Bichi *et al*., (2013), on high lead concentration in Tatsawarki stream due to the joint with River Challawa and activities around the River Challawa including the water works. This joint was obtained in Station 4 of the Tatsawarki stream. Unlike in the liver of caged *Clarias gariepinus*, high lead concentration of lead was recorded in June than in November, this can be attributed to high inflow of lead containing materials and run-off or flooding of agrochemicals from agricultural activities during the onset of rain in June.

5.3.3.2 Chromium

Chromium concentration in the liver of caged *Clarias sp*, ranged from (0.01 ± 0.24mg/kg) to (0.70±0.82mg/kg). Stations 1 and 5 liver of *Clarias sp* were the only stations recorded with least chromium concentration within the WHO set standards (0.15mg/kg) for consumption. Stations 2, 3, and 4 liver, chromium concentration were above the set standards. The highest chromium concentration was obtained in Station 2 (0.70±0.82mg/kg). This indicates tannery wastes discharged from industries in the sampling Station in Tatsawarki stream.

The highest chromium concentration in the liver of free roaming *Clarias sp* obtained in November (1.26mg/kg) and in June (0.34mg/kg) could be attributed to less evaporation and dilution in November in respect to onset of raining season in June.

5.3.3.3 Iron

In November, Station 2 (4.08mg/kg) liver of caged *Clarias sp* had the highest iron concentration and the least was recorded in Station 1 (0.69mg/kg), thus, indicating higher anthropogenic input in Station 2. In June, Station 5 was recorded with the highest iron value during the study (30.70mg/kg), indicating influx of effluents and flooding of contaminants in to Station 5.

The highest Station mean during the study period was obtained in Station 4 (11.07±6.42mg/kg), which is within the FAO, (1989) set standards (100mg/kg). Higher iron concentration was recorded in June (20.49mg/kg), than in November (16.92mg/kg). This can be attributed to higher inflow of waste from neighboring water or areas, or agricultural run-off in to Tatsawarki stream.

5.4. Oxidative Stress Biomarkers

The decreasing trend of oxidative stress biomarkers in gill is MDA > Vitamin E > CAT > GSH > SOD > Vitamin C, during the 8-month study while the order of oxidative stress biomarkers in kidney during the study period MDA > GSH > Vitamin E > CAT > SOD > Vitamin C. Furthermore, the liver of *Clarias sp* during the study period, the other of antioxidants were MDA > GSH > Vitamin E > CAT > SOD > Vitamin C.

5.4.1 Total protein

The total protein concentration during the course of study was higher in the kidney (17.26±7.10mg/ml) and liver (18.16±7.44mg/ml) of free – roaming *Clarias gariepinus*, than the kidney (13.66±2.87mg/ml) and liver (27.43±11.29mg/ml) of caged *Clarias gariepinus*, this can be attributed to variation of duration of exposure between the caged and free roaming *Clarias sp* and adaption and telorance of the free roaming *Clarias sp* in respect to the introduced caged *Clarias sp.* Total protein in liver > Total protein in gill > Total protein in kidney during the course of this study.

5.4.1.1 Gill

In November, total protein in the gills of caged fish was at its peak in Station 5 (26.60mg/ml), and the least was obtained in Station 2 (17.30mg/ml). Consequently in June the total protein concentration was highest at Station 4 (29.32mg/ml) while the least at Station 2 (10.42mg/ml). The least concentration in Station 2 in months can be attributed to contaminants present in the Station. Janardana *et al*., (2016), attributed decrease of protein to presence of pollutant that impair the process of protein synthesis in the tissues of fishes.

In the free roaming *Clarias gariepinus* gill, the highest Station mean protein was obtained in Station 2 (36.94±24.00mg/ml), whereas the least Station mean was obtained in Station 5 (12.38±7.18mg/ml). Dissimilarities in Station in gill of caged and free roaming *Clarias sp*, can be attributed to duration of exposure.

5.4.1.2 Kidney

Station 5, kidney of caged *Clarias gariepinus* was recorded with the highest total protein concentration (20.29±1.46mg/ml), while Station 2 had the least (9.36±1.40mg/ml).The total protein concentration in *Clarias gariepinus* before exposure (16.23±9.06mg/ml) was higher than the Station means after exposure. Janardana *et al*., (2016), observations on decrease in protein level during exposure might be due to increased catabolism and decreased anabolism of proteins and may be attributed to toxic stress.

The highest total protein, Station mean concentration in kidney of free roaming *Clarias gariepinus* was recorded in Station 5 (38.69±21.00mg/ml), furthermore the least Station mean concentration was recorded in Station 2 (8.68±7.48mg/ml). Decrease in protein in station 2 might be attributed to inhibition of protein synthesis (Janardana *et al*., 2016).

5.4.1.3 Liver

The highest Station mean, total protein concentration in the liver of caged *Clarias gariepinus* was recorded in Station 5 (43.14±30.56mg/ml), whereas the least was recorded in Station 2 (15.89±6.73mg/ml). The least station mean of total protein in Station 2 can be attributed to pollution of the site.

In November the highest mean concentration was obtained in Station 2 (34.70mg/ml). However, in June, the peak of total protein concentration in the liver of free roaming *Clarias gariepinus* was recorded in Station 1. Dissimilarities in Stations can be attributed to duration of exposure to contaminants in Tatsawarki stream.

5.4.2 Superoxide dismutase (SOD)

SOD concentration in gill of caged *Clarias sp* (11.54± 6.27U/ml) and kidney (9.75±3.10U/ml) was higher than the gill (10.70±6.65U/ml) and kidney (9.59±4.14 U/ml) of free roaming *Clarias sp* in the stretch of Tatsawarki stream. This could be attributed to duration of exposure of caged *Clarias sp* to the pollutants discharged/ and stress in the stretch of Tatsawarki stream. SOD concentration during the study period increase in this trend SOD in Gill > SOD in kidney > SOD in Liver. The decrease of SOD in liver could be attributed to detoxification role of Liver in related to stress. Similar reports of high SOD concentration in gill in fish was reported by Ergul *et al*., (2009). Bebianno *et al*., (2004); Farombi *et al*., (2007), attributed elevated levels in the activities of SOD in the gills to uninterrupted contact with contaminated water body and possession of thinnest epithelium structure which hence induce ease accumulation of metal ions.

5.4.2.1 Gill

Station 3 (12.27±6.02U/ml) gill of caged *Clarias sp* had the highest Station mean of SOD concentration. This concentration was less than the SOD concentration before exposure (14.86±2.24 U/ml). Similar inference was observed by Olagoke, (2008) and Saliu *et al*., (2014). Similarly, the highest SOD concentration in caged *Clarias gariepinus* in June recorded in Station 2 (17.91 U /ml) was higher than the SOD concentration in November 4 (8.175 U /ml). High SOD concentration in the stretch of Tatsawarki stream can be attributed to high level of stress, which may be attributed to run-off effluents/ flooding of contaminants from other water body in to the stream in June than in November. Doherty, *et al*., (2010) attributed increase in SOD to response to the stress of the sampling Stations.

In the free roaming gill of *Clarias gariepinus*, similar observation of higher SOD concentration, In June 3(8.23 U /ml) in comparison to the month of November (18.21 U /ml) was obtained. This can be associated to higher effluents discharged in to the water body In June than the dry season and higher stress level. Station 3 was recorded with higher SOD concentration during the two seasons, this can be attributed effluents from other water bodies and municipal waste and domestic waste and activities around the water body mainly fishing and farming. The least Station mean was recorded in Station 1(10.03±6.18 U /ml), indicating less response to stress and less contaminants.

5.4.2.2 Kidney

The SOD concentration in the kidney of caged fish was however higher than the concentration before exposure both in November (5.83U /ml) and June (10.54 U /ml). The decrease in SOD concentration in the kidney of caged fish during the months studied in respect to *Clarias sp* before exposure may be attributed to the effects of discharged pollutants in the stretch of Tatsawarki stream. Similar inference was made by Otitoloju and Olagoke, (2011) and Saliu *et al*., (2014). Increase of SOD is an indication of increase in production of superoxide anion radicals (Ergul *et al*., 2009).

The kidney of free roaming *Clarias gariepinus* in all the stations had higher SOD activity in June in comparison to November, with Station 1 (11.80 U /ml) with the highest SOD concentration in November and Station 5 (15.80 U /ml) in June. Increase in SOD in Station 1 and 5 during this study can be attributed to drastic reduction and effects of contaminants in the water body. Similar findings was reported by Otitoloju and Olagoke, (2011).

5.4.2.3 Liver

There was a significant difference in SOD concentrations in the liver of *Clarias gariepinus* among Stations and between months and there was a significant difference in the interaction in

SOD concentrations in the liver of caged *Clarias sp* among Stations and between months. This can be attributed to the contaminants discharged and the toxic effects of these pollutants, hence lead to oxidative stress in the stretch of Tatsawarki stream.

Station 5 (12.05±0.95 U /ml), liver of free roaming *Clarias gariepinus*, had the highest SOD concentration during this study, hence, can be linked to response to oxidative stress. Bebianno *et al*., (2004) and Yildirin *et al*., (2011), reported that increase levels of antioxidant defense enzyme may be associated to possibility of pollutants initiating the production of antioxidant defenses to overcome stressful conditions generated by such pollutants.

5.4.3 Catalase

Catalase concentration in gill (55.10±60.45U/mg protein) and kidney (76.66± 85.62U/mg protein) of caged *Clarias sp* was higher than gill (49.57±53.94U/mg protein) and kidney (58.56±63.08U/mg protein) in free roaming *Clarias sp*, during the 8-month study period.CAT activity in Liver > CAT activity in Kidney > CAT activity in gill. Farombi *et al.*, (2007), associated elevated level CAT activity in the liver to the role of liver as center for detoxification of metabolites.

5.4.3.1 Gill

Station 5 gill (88.98±100.15 U/mg protein) of caged *Clarias sp* had the highest Station mean, catalase activity during the 8-month of study, hence higher than the CAT activity in *Clarias gariepinus* before exposure (45.12±49.06 U/mg protein). Dautremepuits *et al*., (2004), reported that increase in CAT activity is usually observed in the face of environmental pollutants, due to the function of CAT activity as the first site for defence against oxidative stress. This finding disagrees with the findings of Farombi *et al*., (2007) In November, the least CAT activity was recorded in Station 3 (2.29 U/mg protein), while the peak value was recorded in Station 5 (3.50 U/mg protein). In June, the highest CAT activity value in the gill of free roaming *Clarias sp*, throughout the study period was obtained in Station 5 (120.79 U/mg proteins).The peak CAT activity recorded in Station 5 in all the months can be attributed to activities such as agricultural activities, harvesting of sand and other activities around the sampling Station.

5.4.3.2 Kidney

The peak Station mean CAT activity in kidney of caged *Clarias sp* was recorded in Station 4 (119.78±136.66U/mg protein) (Table 4.8). This CAT activity in Station 4 was less than the CAT activity in *Clarias sp*, before exposure (125.82±142.49U/mg protein). Similar inference was made by Farombi *et al*., (2007), which he attributed to flux of superoxide radicals, which have been shown to inhibit CAT activity.

Similarly, Station 4 kidney (78.36±87.70 U/mg protein), of free roaming *Clarias sp*, was recorded with the highest Station mean CAT activity. The similarities in Station 4 kidney in both caged and free-roaming *Clarias sp* can be attributed to the agricultural activities around the Station of Tatsawarki stream.

5.4.3.3 Liver

The highest Station mean, Catalase activity in the liver of caged *Clarias sp* was recorded in Station 2 (74.68±83.92 U/mg protein), hence higher than the CAT activity, before exposure (34.62±37.06 U/mg protein). Ergul *et al*., (2009), reported that high levels of CAT in the liver can be attributed to high production of peroxide radicals.

Station 2 liver of free roaming *Clarias gariepinus* was recorded with the highest CAT activity (147.27±167.41 U/mg protein), whereas, the least was observed in Station 5 (41.79±45.75 U/mg protein). In November, the peak CAT activity, was obtained in Station 4 (3.89 U/mg protein). Observations made in June, showed that with exception of Station 5 (81.41 U/mg protein), the free roaming liver of *Clarias sp* had higher CAT activity, than the liver of caged *Clarias sp* this cab be association to duration of exposure.

5.3.4 Malondehyde

MDA concentration in the 8-month study showed that the MDA grand mean concentration in the gill (506.71± 467.80nmols/mg protein) and liver (562.65±481.01nmols/mg protein) of free-roaming was higher than the MDA concentration in the gill (270.09±157.22nmols/mg protein) and liver (282.49±233.10nmols/mg protein) of caged *Clarias gariepinus*, hence oxidative damage in free roaming tissue. This can be attributed to duration of exposure of free roaming *Clarias sp*. The bioaccumulation of MDA was in the trend of MDA in Kidney> MDA in Liver > MDA in Liver. Farombi *et al*., (2007) and Pavlović *et al.*, (2010); Doherty *et al*., (2010), attributed increase in MDA activity in the kidney to high antioxidant (CAT, SOD and GSH). Similar observation was made by Aina *et al*., (2012).

5.3.4.1 Gill

The marked variation in MDA concentration within the months, with higher concentrations in June could be attributed to high erosion and flooding of effluents in to the stream The peak of MDA concentration in June was obtained in Station 5 (629.65nmols/mg protein), whereas in November it was recorded in Station 2 (210.27nmols/mg protein) (Figure 4.21a). Farombi *et al*., (2007) and Saliu *et al*., (2014), also made similar observation of high MDA concentration, and they associated to oxidative damage. MDA concentration was higher in June than in November. High MDA concentration in Station 5 is an indication of stress in the Station and hence oxidative damage of the gill. Station 5 receives flooded effluents from all the other Stations studied, which include domestic effluents, municipal, fecal wastes, agricultural run-off and other contaminants.

Similarly to the gill of caged *Clarias sp* , the highest MDA concentration in the gill of free roaming *Clarias sp* during the 8-month study was recorded in Station 5 (1511.26±1646.31nmols/mg protein), while the minimum was obtained in Station 4 (186.13±104.17nmols/mg protein). These findings in Station 5, indicates pollutants and stress in the sampling station.

5.3.4.2 Kidney

Station 4 kidney of caged *Clarias gariepinus* (806.32±709.71695nmols/mg protein) had the highest Station mean of MDA concentration, which was higher than the MDA concentration in the kidney of *Clarias sp* before exposure (408.61±359.19nmols/mg protein). Doherty *et al.*, (2010), attributed high MDA concentration to increase of pollutant and stress in the water body. Eboh *et al*., (2014), attributed increase in MDA concentration to degradation of an environment due to poor water quality. Similar observation of high MDA concentration in tissues of fish exposed in comparison to fish not exposed was made by Charissou and Vasseur, (2004)

In November, station 2 (248.12nmols/mg protein) kidney of free roaming *Clarias sp* was recorded with the highest MDA concentration and Station 4 (643.03nmols/mg protein), in June. MDA concentration in the kidney was higher in June than tin November, this can be attributed to influx of wastes from other water bodies and wastes from residential/ agricultural activities in to the stretch of Tatsawarki stream.

5.3.4.3 Liver

The highest Station mean, MDA concentration in the liver of caged *Clarias gariepinus* was recorded in Station 2 (576.34±517.83nmols/mg protein), whereas less than MDA concentration before exposure (90.79±43.89nmols/mg protein). Similar reports of elevated concentration of MDA in liver of fishes exposed to a particular environment in comparison to fishes before exposure or control was reported by Farombi *et al*., (2007) with elevated level (177%) in the liver of *Clarias gariepinus* obtained from the Ogun River in comparison to the control fish from the Agodi fish farm. Other reports on elevated MDA in fish exposed to effluents in a water body in response to control were by Doherty *et al*., (2010) and Eboh *et al*., (2014). They however, attributed the increase to pollution of the test site. Station 2 in the stretch of Tatsawarki stream receives effluents from organic waste, domestic waste including feacal matter, and tannery effluents, this could have contributed to the level of stress in that Station. The least Station mean of the liver of caged *Clarias sp* was recorded in Station 1 (84.21±29.27nmols/mg protein). This concentration is less than the liver of *Clarias sp* before exposure; this can be attributed to less stress in Station 1.

Station 3 liver of free roaming *Clarias sp* (862.37±729.98nmols/mg protein), was recorded with the highest Station mean of MDA concentration. High MDA concentration, in Station 3 is attributed to presence of oxidative stress in the water body (Joseph and Kafilat, 2012). Patrick-Iwuanyanwu *et al*., (2007), reported that increase in MDA levels in the liver is an indication of elevated levels of lipid peroxidation.

Dissimilarities in Stations in caged and free roaming of *Clarias sp* can be linked to duration of exposure of caged *Clarias gariepinus*.

5.3.5 Glutathione (GSH)

GSH concentration was recorded higher in gill (53.96±18.17µg/ml) and liver (131.39±115.49µg/ml) of free roaming *Clarias sp*, than the gill (46.57±29.12µg/ml) and liver (107.06± 97.85µg/ml) of caged *Clarias sp*. GSH concentration in the liver > GSH concentration in kidney > GSH concentration in gill, during the course of this study. Similar report of lower GSH concentration was reported by Farombi *et al*., (2007) which he attributed to sensitivity of the system as biochemical indicator of environmental pollution

5.3.5.1 Gill

The gill of caged *Clarias sp* with the highest Station mean, glutathione concentration during the study period was recorded in Station 5 (70.93±41.27µg/ml), thus higher than the *Clarias gariepinus*, before exposure (60.68±41.19µg/ml). This can be attributed to less effect of effluents that could lead to stress in Station 5 at the stretch of Tatsawarki stream.

Station 3 gill of the free roaming fish was recorded with the highest station mean of GSH concentration during the study period (92.07±39.02µg/ml), while the least was obtained in Station 5 (34.13±1.14 µg/ml). This is in contrast to the GSH concentration in the gill of caged *Clarias sp*. This disparity can be attributed to duration of exposure of the caged *Clarias gariepinus*

5.3.5.2 Kidney

Station 3 kidney (193.30±182.68µg/ml) of caged *Clarias gariepinus* was observed with the highest Station mean of GSH concentration, while the least was recorded in Station 5

(17.48±18.11µg/ml). The least concentration of GSH in caged *Clarias sp* in Station 5 can be attributed to protection of biomolecule against oxidative stress. This is an indication of presence of contaminants and oxidative stress in Station 5 in the stretch of Tatsawarki stream. Similar observation was made by Otitoloju, A and Olagoke, (2011).

Among the kidney of free roaming *Clarias sp*, the highest station mean was obtained in Station 4 (118.05±90.01µg/ml). Furthermore, the least was recorded in Station 2 (14.91±3.75 µg/ml). These results are associated to pollution at the Station 2 in the stretch of Tatsawarki stream. Stress from Station 2 can be contributed to the activity around the station such as: farming, collection of sand by the camel riders, indiscriminate disposal of waste, illicit open dumping of feacal waste, digging of the upper surface of the soil and many others

5.3.5.3 Liver

The highest Station mean of GSH concentration in the liver of caged *Clarias sp*, was recorded in Station 5 (177.13±171.90µg/ml). Station 5 GSH concentration was higher than the control (96.77±80.92µg/ml). Similar findings of higher GSH concentration were reported by several authors (Olagoke 2008; Farombi *et al*., 2007). Farombi *et al*., (2007), attributed increase in the levels of GSH in liver of *Clarias sp* as compared to their control, to adaptive and protective role of the biomolecule against oxidative stress induced by heavy metals. Similar findings in fish from polluted waters having higher concentration than the control or before exposure were reported by several authors (Di Giulio *et al*., 1993; Pandey et *al*., 2003; Sahan *et al*., 2010; Aina *et al*., 2012). Joseph and Kafilat, (2012), findings on decrease in GSH concentration disagrees with this findings. Joseph and Kafilat, (2012), findings were observed in the laboratory, while this study was observed in the field.

In comparison to GSH concentration in liver of caged fish , higher GSH concentrations was recorded in the liver of free roaming *Clarias sp* in November, apart from Station 5 liver of caged *Clarias sp* (25.10µg/ml). This increase can be attributed to duration of exposure of contaminants by the free roaming *Clarias sp* in respect to the caged fish with exception to Station 5. Joseph and Kafilat, (2012), reported that increase in activity of the GSH defense system helped the organisms to regulate bioaccumulation of the heavy metals to levels the body can tolerate and overcome oxidative stress.

5.3.6 Vitamin C

Higher vitamin C concentration in all the tissues of free roaming *Clarias sp* in comparison to all the tissues of caged *Clarias sp*. Vitamin c in Gill > Vitamin c in Kidney > Vitamin c in Liver ,during the months study.

5.3.6.1 Gill

The highest station mean of vitamin C concentration in gill of caged *Clarias gariepinus* was recorded in Station 4 (12.61±0.51mg/ml), while the least was recorded in Station 3 (1.24±0.78mg/ml). Low vitamin C concentration in Station 3 can be attributed to higher stress in the Station and higher contaminants. Less vitamin C concentration in Station 3 can be attributed to the effluents such as: domestic waste and also run-off from other water body.

Station 1 gill (13.31±2.47mg/ml) of free roaming *Clarias sp*, was recorded with the highest Station mean of vitamin C, furthermore, the least was obtained in Station 2 (1.72±1.57mg/ml). This can be associated to the observation that Station 2 receives effluents from organic waste, domestic waste including feacal matter, and tannery effluents and higher stress level in comparison to Station 1.

5.3.6.2 Kidney

Station 4 kidney of caged *Clarias gariepinus* (5.55±5.45mg/ml) had the highest Station mean of vitamin C concentration. This concentration in Station 4 was however, higher than the vitamin c concentration of *Clarias sp* before exposure (1.15±0.20mg/ml). This result showed a healthy status of Station 4 and less stress in Station 4 in the stretch of Tatsawarki stream.

In November, the peak of vitamin C concentration was recorded in Station 2 (6.54mg/ml), whereas in June Station 1, had the highest vitamin c concentration (8.32mg/ml). The high concentration of vitamin c in June can be attributed to dilution effects and evaporation.

5.3.6.3 Liver

The highest Station mean of vitamin C concentration in the liver of caged *Clarias gariepinus* was recorded in Station 1 (3.75±3.68mg/ml), hence less than the vitamin C concentration in the liver of the fish before exposure (12.65±12.45mg/ml). Ozden, (2010), reported that low level of vitamins is obtained in polluted areas as compared to the unpolluted reference. Oakes *et al*., (2004), attributed decrease in vitamin C levels in comparison with the control to response of ascorbic acid to ROS generated due to xenobiotics.

Eboh *et al*., (2014), reported similar findings of less vitamin C concentration in the liver of *Clarias gariepinus* exposed in Gbarantoru swamp (33.21 ± 0.37μg/mg protein) than the liver of control fish caught from the Niger Delta University Agricultural Farm (86.24 ± 0.48 μg/mg protein).

In November, the highest vitamin C concentration in free roaming *Claris gariepinus* was recorded in Station 5 (1.22mg/ml) while the highest in June was recorded at Station 4 (10.0414 mg/ml. Low concentration of vitamin C in the liver of free roaming *Claris gariepinus* in

November can be attributed to pollutants In November. Eboh *et al*., (2014), attributed low vitamins to involvement in scavenging of pollutant, that could lead to stress and usually caused, when toxicant concentration outweigh the aquatic ability. Other inference was made by Ortega, (2006) and Yildirin and Danabas, (2011).

5.3.7 Vitamin E

Vitamin E concentration during the 8-month period, in kidney (81.32±90.95mg/ml) and liver (107.30±73.59mg/ml) of free roaming *Clarias sp* was higher than kidney (80.12±77.80mg/ml) and liver (70.40±83.38mg/ml) of caged *Clarias gariepinus* (Table 4.12). Vitamin E concentration during the study period was in this trend vitamin e in gill > vitamin e in liver > vitamin e in kidney.

5.3.7.1 Gill

Among the gills of caged *Clarias gariepinus*, the highest Station mean of vitamin E concentration was obtained in Stations 4 and 5 (186.15±00mg/ml) while the least was recorded in Station 3 (101.36±97.91mg/ml). Less concentration in Station 3 can be attributed to response of oxidative stress in the water body.

In November, the least vitamin E concentration was recorded in Station 3 (30.86 mg/ml), whereas in June, the least was obtained in Station 2 (125.42mg/ml). Higher concentration in June in comparison to November can be associated to less stress in June, and dilution factor.

5.3.7.2 Kidney

Station 3 (100.37±99.05mg/ml) kidney of caged *Clarias gariepinus* had the highest Station mean of vitamin E concentration during the course of study. Station 3 kidney vitamin E concentration was however higher than the vitamin E in *Clarias sp* before exposure (72.06±47.60mg/ml).This can be attributed to less pollutants.

In November, the highest vitamin E concentration in kidney of free roaming *Clarias gariepinus* was recorded in Station 4 (42.40mg/ml). Consequently, in June, Stations 1, 2, 5 (186.15 mg/ml). Vitamin E concentration in June was higher than in November and may be attributed to dilution effects.

5.3.7.3 Liver

Station 4 liver had the highest station mean concentration of vitamin E among the caged *Clarias gariepinus* (97.83±101.97mg/ml)) during the period of this study. Station 4 vitamin E concentration was however less than vitamin E concentration of *Clarias sp* before exposure (107.20± 91.16mg/ml). Similar findings of low concentration of vitamin E in the liver of catfish exposed to Gbarantoru swamp (3.21 ± 0.67µg/mg protein) in respect to vitamin E content in the liver of catfish harvested from Niger Delta University Agricultural Farm (5.76 ± 0.31 µg/mg protein) which was the control was made by Eboh *et al*., (2014).

During the course of this study, Station 4 had the highest Station mean vitamin E concentration (125.19±70.39mg/ml), while the least was recorded in Station 5 (76.74±39.06 mg/ml) .This indicate higher pollutants in Station 5. The less concentration in Station 5 can be attributed to response to oxidative stress and its role as antioxidant.

5.4 Histopathology

There was a significant difference in Gill of *Clarias gariepinus* with pl (primary lamella), sl (secondary lamella before exposure and after exposure showing deformed secondary lamella, diffusion of secondary lamella, degeneration and epithelia lifting of secondary lamella .

Alterations was observed in the gill of *Clarias sp* exposed to Tatsawarki stream. Chavan and Muley, (2014), reported that pathological lessions/ alterations can be reaction of fish to toxicant intake or an adaptive response to prevent entry of heavy metals. Similar observation such as damages/ injury of gills was reported by Navaraj and Yasmin, (2012), which they associated with impairment in gaseous exchange efficiency in fish.

There was a significant difference in Kidney of *Clarias gariepinus* with haematopoietic tissue, renal tubules, renal corpuscles and glomerulus before exposure and after exposure in Tatsawarki stream with degeneration, dissociation, edema of renal tubules and congestion of hematopoietic tissue and dissociation and congestion of hematopoietic tissue. This can be attributed to effluents/ contaminants discharged in to Tatsawarki Stream.

There was a significant difference in Liver of *Clarias gariepinus* before exposure with unexposed central vein and after exposure with vacoulation and fatty degeneration, infiltration of the liver, hepatocyte necrosis, cellular infiltration and architectural and structural alteration with hemolysis of blood vessels. Several authors have made reports on degeneration of liver, and they attributed to exposure to different toxic chemicals (Figueiredo *et al*., 2007). Hepatic alterations such as necrosis supported the findings of Olojo *et al*., (2005), in which they attributed to metal contaminated ecosystem.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The results of this study revealed, that Temperature (25.33± 4.60^{0}C), pH (8.30± 0.42), Total Alkalinity (76.81±56.21mg/l.CaCO3), BOD (0.44±0.66mg/l), Chloride (6.28±4.69mg/l), Nitrate/Nitrogen (19.53± 4.50mg/l) and Phosphate/ phosphorous (1.55±0.62mg/l), during the 8-month study period, were within the permissible level set standards. Thus indicating that some of the physical parameters are suitable for the survival of aquatic life. Total Hardness concentration in the water body during the study, revealed that Stations 2 (47.88mg/l), 3(52.94mg/l) 4 (45.06mg/l) and 5(44.63mg/l) in the stretch of Tatsawarki stream can be classified as soft water, whereas Station 1 (74.81mg/l) is moderately hard in the 8-month study period. High EC in Station 2 (1729±540.35µS/cm), High TDS in Station 2 (841.06 ±265.83mg/l), Sulphate (293.75±63.50mg/l) and Low DO (1.01±1.03mg/l) in all the Station during the study period can be associated to indiscriminate disposal mechanisms and human activities around the catchment area. This contaminants disposed in to some Stations and activities in Tatsawarki stream include: organic waste, domestic contaminants including: feacal waste and non- biodegradable materials like polythene bags and plastics, digging of top soil made by the camel riders, agricultural runoff and industrial effluents, which could affect aquatic life and hence stress aquatic organisms in the water body.

There was a significant difference in monthly means of BOD, Total Alkalinity, Chloride, Nitrate-Nitrogen, Sulphate, but not within Station means. Although, there was a significant difference in Temperature, EC, TDS, pH, DO, in Tatsawarki stream within monthly means and Station means.

Temperature and TDS are negatively correlated to BOD, DO and Total Hardness in Tatsawarki stream.

The lead concentration ranging from (0.08± 0.04mg/l) and (0.42± 0.32mg/l) and Iron concentration with the highest mean concentration (4.66 ±3.62mg/l), in the stretch of Tatsawarki stream during the study were above the permissible standard limit of 0.01mg/l for lead and (0.30mg/l) standards for iron. This indicates presence of pollution in Tatsawarki stream. Chromium concentration was not detectable in June, but present in high concentration in Station 3 (0.07mg/l) and Station 5 (0.07mg/l) in November and above the permissible standards. The non- detectable chromium concentration in June can be attributed to dilution. The Iron concentration and chromium concentration in all the tissues of free roaming *Clarias sp*, was higher than the tissues of caged *Clarias sp*. This can be attributed to limited duration of exposure to the Tatsawarki stream. The grand mean Lead concentration showed lead in kidney (0.34±0.22mg/kg) > lead in liver (0.23±0.10mg/kg) > lead in gill (0.27±0.10mg/kg). Likewise, chromium in kidney (0.48±0.52mg/kg) > chromium in liver (0.34±0.42) > chromium in gill (0.32±0.43mg/kg). This can be attributed to the role of kidney as "storage site" and target organ for heavy metals. Although Iron in liver (7.12±4.80mg/kg) > Iron in gill (5.04±1.57mg/kg) > Iron in kidney (3.71±1.80mg/kg), can be attributed to liver ability to bio-accumulate metals than other tissues due to presence of metal- tie proteins in liver.

The higher GSH, SOD, CAT, MDA activity, Vitamin C and Vitamin E in June can be attributed to influx of urban runoffs into the stretch of Tatsawarki stream.

There was a significant difference in histopathology of Gill, Liver and Kidney of *Clarias gariepinus* before exposure and after exposure. This could be contributed to effluents discharged in to Tatsawarki stream, such effluents/ contaminants consist of urban run- off seepages, sewage, domestic effluents, industrial waste and agricultural waste. Contamination/ pollution in some Stations can be attributed to the alterations, deformation, diffusion and degeneration of secondary lamella in the gill, degeneration , dissociation , congestion and edema in kidney and infilteration , vacoulation and architectural structural alteration in the liver of *Clarias sp* in the stretch of Tatsawarki stream.

6.2 Conclusion

1. Low DO (1.01±1.03mg/l), Nitrogen/Nitrate (19.53±4.5mg/l), Phosphate (1.56±0.62mg/l), concentration at the stretch of River Tatsawarki stream might be attributed to organic effluents, but the level of Chloride (6.28±4.69mg/l) , BOD (0.44±0.66mg/l) and pH (8.3±0.42) were within the permissible level for survival of aquatic organisms.

2. Lead concentration (ranging from 0.08±0.04 to 0.42± 0.32mg/l) in the water sample during the course of the study were within the permissible level of 1.00mg/l recommended by FEPA for survival of aquatic biota. The mean lead concentration in Gill before exposure being higher than the mean of all the Stations after exposure indicate release of metals into Twa stream. Chromium concentration in all the tissues, gill (5.04±1.57mg/kg), kidney (3.71±1.80mg/kg), and liver (7.12±4.80mg/kg) of *Clarias sp* in Tatsawarki stream but were not detectable in the waters of Tatsawarki stream. This is attributed to bioaccumulation of metals in *Claris gariepinus.*

3. The higher GSH, SOD, CAT and MDA activity In June is attributed to influx of urban runoffs into the stream.The higher activity of vitamin C in the gill (15.34±9.34mg/ml) and liver (12.65±12.45mg/ml) before exposure in both the caged and free roaming *Clarias* sp shows that it is a better antioxidant indicator of stress in Tatsawarki stream than vitamin E

4. The histopathology observation in *Clarias* sp observed includes infilteration, vacoulation and structural alteration in the liver, disruption, deformation and disintegration of the secondary lamella in the gill and congestion and edema in kidney.

6.3 Recommendation

1. There should be strict enforcement of laws on proper disposal of effluents in to water bodies and a form of punishment for offenders. Continuous monitoring of effluents into Tatsawarki stream should be made, in order to verify if imposed standards and regulations are met, due to Tatsawarki stream flows into other water bodies and serve as source of water for consumption to people within the catchment area, and serve as a habitat for aquatic organisms including: *Clarias gariepinus*.
2. In order to preserve the biodiversity of aquatic life, further studies/ investigations should be made in order to check other metals present in Nigeria water bodies, in order to verify if the wastes discharge in to the water bodies are properly treated.
3. Further studies should be conducted on distribution of other metals and other organs and tissue such as skin of *Clarias gariepinus* species, since skin is another organ in which effluents in the water body bio accumulate in to the fish and also in order to preserve biodiversity in aquatic life
4. In subsequent determination of oxidative stress, antioxidants specific kit should be used.

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APPENDIX I

Anova Results for Temperature, EC and TDS

a

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	7.775	4	1.94375	2.71785	0.04977	2.71408
Columns	725.475	7	103.639286	144.914	2.8E-20	2.35926
Error	20.025	28	0.71517857			
Total	753.275	39				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	12452008.31	4	3113002.08	12.3133	6.7E-06	2.71408
Columns	1583115.8	7	226159.4	0.89456	0.52409	2.35926
Error	7078849.888	28	252816.067			
Total	21113974	39				

c

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3418516.75	4	854629	13.3066	3.4E-06	2.71408
Columns	131453.475	7	18779.1	0.29239	0.9513	2.35926
Error	1798325.65	28	64225.9			
Total	5348295.875	39				

APPENDIX II

Anova results of pH, DO and BOD

a ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1.88020375	4	0.470051	3.142895	0.02968	2.71408
Columns	2.84868	7	0.406954	2.721013	0.0276	2.35926
Error	4.18767625	28	0.14956			
Total	8.91656	39				

b ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2.7309725	4	0.682743125	8.464729018	0.000131295	2.71408
Columns	35.29728938	7	5.042469911	62.51713099	2.06888E-15	2.35926
Error	2.2584075	28	0.080657411			
Total	40.28666938	39				

c ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	0.2116588	4	0.05291	1.11318	0.37001	2.71408
Columns	14.122285	7	2.01747	42.442	3E-13	2.35926
Error	1.3309738	28	0.04753			
Total	15.664917	39				

APPENDIX III

Anova results Total Alkalinity, Total Hardness and Chloride

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	32161.8377	4	8040.46	2.59286	0.05806	2.71408
Columns	60598.3329	7	8656.9	2.79165	0.02461	2.35926
Error	86828.0443	28	3101			
Total	179588.215	39				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	5081.4375	4	1270.36	2.46758	0.06782	2.71408
Columns	41817.1938	7	5973.88	11.6038	7.9E-07	2.35926
Error	14414.9625	28	514.82			
Total	61313.5938	39				

c ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	21.50146	4	5.37536	0.27622	0.89084	2.71408
Columns	329.9415394	7	47.1345	2.42207	0.04507	2.35926
Error	544.89272	28	19.4605			
Total	896.3357194	39				

APPENDIX IV

Anova result for Nitrate-nitrogen, Sulphate and Phosphate-phosphorous

a

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	13.813375	4	3.45334	0.54405	0.70473	2.71408
Columns	567.47975	7	81.0685	12.7718	3E-07	2.35926
Error	177.729625	28	6.34749			
Total	759.02275	39				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	35969.6875	4	8992.42	2.52026	0.06353	2.71408
Columns	143465	7	20495	5.74404	0.00034	2.35926
Error	99905.3125	28	3568.05			
Total	279340	39				

c

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.45391	4	0.11348	0.33841	0.84971	2.71408
Columns	4.12386	7	0.58912	1.75689	0.13623	2.35926
Error	9.38898	28	0.33532			
Total	13.9667	39				

APPENDIX V

Anova results of Lead, Iron and Chromium concentrations in respect to months in along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.114154	1	3.114154	758.4529	2.23E-17	4.351244
Columns	66.88412	4	16.72103	4072.41	8.45E-29	2.866081
Interaction	95.82296	4	23.95574	5834.425	2.33E-30	2.866081
Within	0.082119	20	0.004106			
Total	165.9033	29				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.004171	1	1.004171	433.6984	4.98E-15	4.351244
Columns	0.426127	4	0.106532	46.01075	8.2E-10	2.866081
Interaction	0.364763	4	0.091191	39.38506	3.25E-09	2.866081
Within	0.046307	20	0.002315			
Total	1.841369	29				

c

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.208946	1	0.208946	16.83396	0.000553	4.351244
Columns	0.018864	4	0.004716	0.379944	0.820273	2.866081
Interaction	0.013133	4	0.003283	0.264523	0.897251	2.866081
Within	0.248243	20	0.012412			
Total	0.489185	29				

APPENDIX VI

Anova results of Lead concentrations in in the gill of caged *Clarias gariepinus* and Lead in gill of free roaming *Clarias sp* along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.06939456	1	0.069394558	10.08004	0.004761	4.351244
Columns	0.13802721	4	0.034506803	5.012352	0.005793	2.866081
Interaction	0.08431293	4	0.021078231	3.061759	0.040407	2.866081
Within	0.13768707	20	0.006884354			
Total	0.42942177	29				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.039292517	1	0.039292517	5.313707	0.032003966	4.351244
Columns	0.03029932	4	0.00757483	1.024379	0.418796999	2.866081
Interaction	0.019959184	4	0.004989796	0.674793	0.617257781	2.866081
Within	0.147891156	20	0.007394558			
Total	0.237442177	29				

APPENDIX VII

Anova results of Chromium concentrations in in the gill of caged *Clarias gariepinus* and Chromium concentration in free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.946703	1	0.946703	13.34234612	0.001582	4.351243503
Columns	5.027037	4	1.256759	17.71211486	2.35E-06	2.866081402
Interaction	1.335743	4	0.333936	4.706315937	0.007695	2.866081402
Within	1.419096	20	0.070955			
Total	8.728579	29				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	4.34312909	1	4.343129	141.2602	1.61E-10	4.351244
Columns	3.244477494	4	0.811119	26.38165	9.92E-08	2.866081
Interaction	2.618699187	4	0.654675	21.29329	5.63E-07	2.866081
Within	0.614911759	20	0.030746			
Total	10.82121753	29				

APPENDIX VIII

Anova results of Iron concentrations in in the gill of caged *Clarias gariepinus* and Iron concentration in free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	9.479408	1	9.479408	412.6393	8.02E-15	4.351244
Columns	27.16402	4	6.791006	295.613	1.76E-17	2.866081
Interaction	32.60862	4	8.152154	354.8639	2.91E-18	2.866081
Within	0.459452	20	0.022973			
Total	69.7115	29				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	26.5023	1	26.5023	212.8254	4.01E-12	4.351244
Columns	276.8761	4	69.21903	555.8599	3.46E-20	2.866081
Interaction	151.5152	4	37.87879	304.1837	1.33E-17	2.866081
Within	2.490521	20	0.124526			
Total	457.3841	29				

APPENDIX IX

Anova results of Lead concentrations in in the kidney of caged *Clarias gariepinus* and Lead concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.04915	1	0.04915	8.540189125	0.008422524	4.351243503
Columns	0.058871	4	0.014718	2.557328605	0.070449541	2.866081402
Interaction	0.036531	4	0.009133	1.586879433	0.216545557	2.866081402
Within	0.115102	20	0.005755			
Total	0.259653	29				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.062863946	1	3.062864	495.3146	1.39E-15	4.351243503
Columns	4.05044898	4	1.012612	163.7558	5.58E-15	2.866081402
Interaction	3.31785034	4	0.829463	134.1375	3.82E-14	2.866081402
Within	0.123673469	20	0.006184			
Total	10.55483673	29				

APPENDIX X

Anova results of Chromium concentrations in in the kidney of caged *Clarias gariepinus* and Chromium concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.571592	1	3.571592	84.25425	1.31E-08	4.351244
Columns	3.792132	4	0.948033	22.36421	3.81E-07	2.866081
Interaction	5.137348	4	1.284337	30.29765	3.12E-08	2.866081
Within	0.847813	20	0.042391			
Total	13.34888	29				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.292807	1	3.292807	83.65554156	1.39E-08	4.351244
Columns	3.848176	4	0.962044	24.44124685	1.86E-07	2.866081
Interaction	3.938677	4	0.984669	25.01605793	1.54E-07	2.866081
Within	0.78723	20	0.039361			
Total	11.86689	29				

APPENDIX XI

Anova results of Iron concentrations in in the kidney of caged *Clarias gariepinus* and Iron concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	51.05516529	1	51.05517	2206.723	6.05E-22	4.351244
Columns	4.702677342	4	1.175669	50.81516	3.36E-10	2.866081
Interaction	28.40467459	4	7.101169	306.929	1.21E-17	2.866081
Within	0.462723829	20	0.023136			
Total	84.62524105	29				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	83.21973571	1	83.21973571	1964.598	1.91237E-21	4.351244
Columns	160.6035038	4	40.15087595	947.8561	1.73278E-22	2.866081
Interaction	119.4042958	4	29.85107395	704.705	3.29249E-21	2.866081
Within	0.847193526	20	0.042359676			
Total	364.0747288	29				

APPENDIX XII

Anova results of Lead concentrations in in the liver of caged *Clarias gariepinus* and Lead concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.017006803	1	0.017007	2.527806	0.127539	4.351244
Columns	0.064122449	4	0.016031	2.38271	0.085826	2.866081
Interaction	0.12292517	4	0.030731	4.567745	0.008772	2.866081
Within	0.134557823	20	0.006728			
Total	0.338612245	29				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.163435	1	0.163435	22.0818015	0.000138	4.351244
Columns	0.123238	4	0.03081	4.16268382	0.012978	2.866081
Interaction	0.00966	4	0.002415	0.32628676	0.856999	2.866081
Within	0.148027	20	0.007401			
Total	0.444361	29				

APPENDIX XIII

Anova results of Chromium concentrations in in the liver of caged *Clarias gariepinus* and Chromium concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.999410073	1	0.99941	35.07324	8.59E-06	4.351244
Columns	2.600158636	4	0.65004	22.81246	3.24E-07	2.866081
Interaction	4.326511997	4	1.081628	37.95859	4.49E-09	2.866081
Within	0.56989887	20	0.028495			
Total	8.495979576	29				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.013742	1	3.013742	64.74188107	1.07E-07	4.351244
Columns	2.032635	4	0.508159	10.91637993	7.36E-05	2.866081
Interaction	0.885159	4	0.22129	4.753797887	0.00736	2.866081
Within	0.931002	20	0.04655			
Total	6.862539	29				

APPENDIX XIV

Anova results of Iron concentrations in in the liver of caged *Clarias gariepinus* and Iron concentration in liver of free roaming along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	573.5229	1	573.5229	5609.905	5.65E-26	4.351244
Columns	805.6843	4	201.4211	1970.197	1.19E-25	2.866081
Interaction	998.4864	4	249.6216	2441.669	1.39E-26	2.866081
Within	2.04468	20	0.102234			
Total	2379.738	29				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.427703168	1	6.427703	27.80479	3.68E-05	4.351244
Columns	297.4916925	4	74.37292	321.7204	7.64E-18	2.866081
Interaction	791.9758351	4	197.994	856.4771	4.75E-22	2.866081
Within	4.623450413	20	0.231173			
Total	1100.518681	29				

APPENDIX XV

Anova results of Total protein concentrations in in the gill of caged *Clarias gariepinus* and Total protein concentration in gill of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	47.55528	1	47.55528	220.8074	3.83E-08	4.964603
Columns	1627.35667	4	406.8392	1889.024	2.42E-14	3.47805
Interaction	2023.80887	4	505.9522	2349.223	8.14E-15	3.47805
Within	2.1537	10	0.21537			
Total	3700.87452	19				

b ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	47.5553	1	47.55528	220.8074	3.83E-08	4.964603
Columns	1627.36	4	406.8391675	1889.024	2.42E-14	3.47805
Interaction	2023.81	4	505.9522175	2349.223	8.14E-15	3.47805
Within	2.1537	10	0.21537			
Total	3700.87	19				

APPENDIX XVI

Anova results of Total protein concentrations in in the kidney of caged *Clarias gariepinus* and Total protein concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	12.816005	1	12.816	1421.631	4.1E-12	4.964603
Columns	279.91277	4	69.97819	7762.417	2.08E-17	3.47805
Interaction	153.99697	4	38.49924	4270.576	4.11E-16	3.47805
Within	0.09015	10	0.009015			
Total	446.815895	19				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	510.05	1	510.05	7977.01	7.58E-16	4.964603
Columns	2404.4011	4	601.1003	9401.005	7.97E-18	3.47805
Interaction	1041.1831	4	260.2958	4070.938	5.22E-16	3.47805
Within	0.6394	10	0.06394			
Total	3956.2736	19				

APPENDIX XVII

Anova results of Total protein concentrations in in the liver of caged *Clarias gariepinus* and Total protein concentration in liver of free roaming fish at Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	197.56898	1	197.569	2476.732857	2.59E-13	4.964602744
Columns	1613.80677	4	403.4517	5057.687006	1.77E-16	3.478049691
Interaction	3136.28937	4	784.0723	9829.163125	6.38E-18	3.478049691
Within	0.7977	10	0.07977			
Total	4948.46282	19				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	422.4643	1	422.46432	33824.2	5.55E-19	4.964603
Columns	186.3193	4	46.5798325	3729.37	8.09E-16	3.47805
Interaction	759.4749	4	189.8687325	15201.66	7.21E-19	3.47805
Within	0.1249	10	0.01249			
Total	1368.383	19				

APPENDIX XVIII

Anova results of SOD concentrations in in the gill of caged *Clarias gariepinus* and SOD concentration in gill of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	589.48082	1	589.4808	333040	6.01E-24	4.964603
Columns	13.87628	4	3.46907	1959.927	2.01E-14	3.47805
Interaction	19.73508	4	4.93377	2787.441	3.46E-15	3.47805
Within	0.0177	10	0.00177			
Total	623.10988	19				

b

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	662.745845	1	662.7458	214828.5	5.38E-23	4.964603
Columns	46.37953	4	11.59488	3758.471	7.78E-16	3.47805
Interaction	19.20043	4	4.800107	1555.951	6.37E-14	3.47805
Within	0.03085	10	0.003085			
Total	728.356655	19				

APPENDIX XIX

Anova results of SOD concentrations in in the kidney of caged *Clarias gariepinus* and SOD concentration in kidney of free roaming fis along the stretch of Tatsawarki stream..

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	120.295125	1	120.2951	229133.6	3.9E-23	4.964603
Columns	119.77765	4	29.94441	57036.98	9.7E-22	3.47805
Interaction	54.29955	4	13.57489	25856.93	5.07E-20	3.47805
Within	0.00525	10	0.000525			
Total	294.377575	19				

b ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	257.18792	1	257.1879	170323.1	1.72E-22	4.964603
Columns	95.09713	4	23.77428	15744.56	6.05E-19	3.47805
Interaction	88.37673	4	22.09418	14631.91	8.73E-19	3.47805
Within	0.0151	10	0.00151			
Total	440.67688	19				

APPENDIX XX

Anova results of SOD concentrations in in the liver of caged *Clarias gariepinus* and SOD concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	195.875405	1	195.8754	40935.3	2.14E-19	4.964603
Columns	70.23478	4	17.5587	3669.529	8.77E-16	3.47805
Interaction	72.18962	4	18.04741	3771.662	7.65E-16	3.47805
Within	0.04785	10	0.004785			
Total	338.347655	19				

b ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	179.28072	1	179.2807	32715.46	6.56E-19	4.964603
Columns	106.61363	4	26.65341	4863.76	2.15E-16	3.47805
Interaction	89.22833	4	22.30708	4070.635	5.22E-16	3.47805
Within	0.0548	10	0.00548			
Total	375.17748	19				

APPENDIX XXI

Anova results of CAT concentrations in in the gill of caged *Clarias gariepinus* and CAT concentration in gill of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	54775.9	1	54775.9	10618.25	1.82E-16	4.964603
Columns	12874.23	4	3218.557	623.914	6.05E-12	3.47805
Interaction	12767.62	4	3191.904	618.7474	6.31E-12	3.47805
Within	51.58655	10	5.158655			
Total	80469.34	19				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	43596.32065	1	43596.32	7909.239	7.91E-16	4.964603
Columns	5938.72928	4	1484.682	269.3509	3.92E-10	3.47805
Interaction	5864.43778	4	1466.109	265.9814	4.17E-10	3.47805
Within	55.12075	10	5.512075			
Total	55454.60846	19				

APPENDIX XXII

Anova results of CAT concentrations in in the kidney of caged *Clarias gariepinus* and CAT concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	109949.6205	1	109949.6	268871.5	1.75E-23	4.964603
Columns	13434.6304	4	3358.658	8213.282	1.56E-17	3.47805
Interaction	14238.8442	4	3559.711	8704.94	1.17E-17	3.47805
Within	4.0893	10	0.40893			
Total	137627.1844	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	59677.81	1	59677.81	144986.3	3.84E-22	4.964603
Columns	7432.755	4	1858.189	4514.44	3.11E-16	3.47805
Interaction	8180.893	4	2045.223	4968.837	1.93E-16	3.47805
Within	4.1161	10	0.41161			
Total	75295.58	19				

APPENDIX XXIII

Anova results of CAT concentrations in in the liver of caged *Clarias gariepinus* and CAT concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	53862.85841	1	53862.86	55636.26	4.61E-20	4.964603
Columns	6099.24682	4	1524.812	1575.015	5.99E-14	3.47805
Interaction	6005.89982	4	1501.475	1550.91	6.47E-14	3.47805
Within	9.68125	10	0.968125			
Total	65977.6863	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	190669.4	1	190669.4	443949	1.43E-24	4.964603
Columns	39831.98	4	9957.996	23185.9	8.74E-20	3.47805
Interaction	39581.39	4	9895.348	23040.03	9.02E-20	3.47805
Within	4.29485	10	0.429485			
Total	270087.1	19				

APPENDIX XXIV

Anova results of MDA concentrations in in the gill of caged *Clarias gariepinus* and MDA concentration in gill of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	370752.8	1	370752.8	435667.2	1.57E-24	4.964603
Columns	197828.6	4	49457.15	58116.51	8.84E-22	3.47805
Interaction	167774.2	4	41943.55	49287.37	2.01E-21	3.47805
Within	8.51	10	0.851			
Total	736364.1	19				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3282452.39	1	3282452	717050.4	1.3E-25	4.964603
Columns	5132165.136	4	1283041	280279.9	3.39E-25	3.47805
Interaction	5266571.924	4	1316643	287620.1	2.98E-25	3.47805
Within	45.77715	10	4.577715			
Total	13681235.23	19				

APPENDIX XXV

Anova results of MDA concentrations in in the kidney of caged *Clarias gariepinus* and MDA concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2750787.295	1	2750787	6101.16	2.89E-15	4.964603
Columns	539478.4498	4	134869.6	299.1366	2.33E-10	3.47805
Interaction	465923.3611	4	116480.8	258.3508	4.81E-10	3.47805
Within	4508.6302	10	450.863			
Total	3760697.736	19				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	940695.3125	1	940695.3	1051514	1.91E-26	4.964603
Columns	316276.9086	4	79069.23	88384.02	1.09E-22	3.47805
Interaction	327270.8269	4	81817.71	91456.28	9.16E-23	3.47805
Within	8.9461	10	0.89461			
Total	1584251.994	19				

APPENDIX XXVI

Anova results of MDA concentrations in in the liver of caged *Clarias gariepinus* and MDA concentration in liver of free roaming fish at along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	820724.5095	1	820724.5	6308.241	2.45E-15	4.964603
Columns	706390.6222	4	176597.7	1357.362	1.26E-13	3.47805
Interaction	546554.7659	4	136638.7	1050.23	4.52E-13	3.47805
Within	1301.0355	10	130.1036			
Total	2074970.933	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3470520	1	3470520	442201.6	1.46E-24	4.964603
Columns	780366.7	4	195091.7	24857.9	6.17E-20	3.47805
Interaction	429276.6	4	107319.2	13674.24	1.22E-18	3.47805
Within	78.48275	10	7.848275			
Total	4680241	19				

APPENDIX XXVII

Anova results of GSH concentrations in in the gill of caged *Clarias gariepinus* and GSH concentration in gill of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6610.248	1	6610.248	8380.2381	5.922E-16	4.9646027
Columns	7608.09435	4	1902.0236	2411.3181	7.144E-15	3.4780497
Interaction	9788.08135	4	2447.0203	3102.2456	2.03E-15	3.4780497
Within	7.8879	10	0.78879			
Total	24014.3116	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	4707.846125	1	4707.8461	22894.187	3.905E-18	4.9646027
Columns	8286.97095	4	2071.7427	10074.855	5.637E-18	3.4780497
Interaction	2881.93895	4	720.48474	3503.7067	1.105E-15	3.4780497
Within	2.05635	10	0.205635			
Total	15878.81238	19				

APPENDIX XXVIII

Anova results of GSH concentrations in in the kidney of caged *Clarias gariepinus* and GSH concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	89679.528	1	89679.528	93255.407	3.488E-21	4.9646027
Columns	86908.226	4	21727.057	22593.4	9.946E-20	3.4780497
Interaction	83265.666	4	20816.417	21646.45	1.232E-19	3.4780497
Within	9.61655	10	0.961655			
Total	259863.04	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	35763.80738	1	35763.807	24195.306	2.962E-18	4.9646027
Columns	23892.68567	4	5973.1714	4041.0325	5.418E-16	3.4780497
Interaction	11938.62607	4	2984.6565	2019.2111	1.733E-14	3.4780497
Within	14.7813	10	1.47813			
Total	71609.90042	19				

APPENDIX XXIX

Anova results of GSH concentrations in in the liver of caged *Clarias gariepinus* and GSH concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	143605.4886	1	143605.49	144711.81	3.877E-22	4.9646027
Columns	51398.17843	4	12849.545	12948.536	1.608E-18	3.4780497
Interaction	45197.21043	4	11299.303	11386.351	3.058E-18	3.4780497
Within	9.92355	10	0.992355			
Total	240210.8011	19				

b ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	200026	1	200026	36855.968	3.614E-19	4.9646027
Columns	5010.4298	4	1252.6075	230.8003	8.402E-10	3.4780497
Interaction	5502.4068	4	1375.6017	253.46271	5.29E-10	3.4780497
Within	54.27235	10	5.427235			
Total	210593.11	19				

APPENDIX XXX

Anova results of vitamin c concentrations in in the gill of caged *Clarias gariepinus* and vitamin c concentration in gill of free roaming fish along the stretch of Tatsawarki stream..

a

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	339.1831885	1	339.1831885	187855.9	1.05E-22	4.964603
Columns	419.3417653	4	104.8354413	58062.88	8.88E-22	3.47805
Interaction	327.2048873	4	81.80122183	45305.43	3.07E-21	3.47805
Within	0.0180555	10	0.00180555			
Total	1085.747897	19				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	274.8815	1	274.8815	102152.2	2.21E-21	4.964603
Columns	316.3922	4	79.09806	29394.65	2.67E-20	3.47805
Interaction	74.7011	4	18.67527	6940.159	3.63E-17	3.47805
Within	0.026909	10	0.002691			
Total	666.0017	19				

APPENDIX XXXI

Anova results of vitamin c concentrations in in the kidney of caged *Clarias gariepinus* and vitamin c concentration in kidney of free roaming fsh along the stretch of Tatsawarki stream.

a

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	61.1730242	1	61.17302	72522.85	1.23E-20	4.964603
Columns	74.4143327	4	18.60358	22055.23	1.12E-19	3.47805
Interaction	61.7157843	4	15.42895	18291.58	2.86E-19	3.47805
Within	0.008435	10	0.000843			
Total	197.3115762	19				

b

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	56.67099	1	56.67099	177310.4	1.4E-22	4.964603
Columns	58.45086	4	14.61272	45719.81	2.93E-21	3.47805
Interaction	34.02348	4	8.505869	26612.9	4.39E-20	3.47805
Within	0.003196	10	0.00032			
Total	149.1485	19				

APPENDIX XXXII

Anova results of vitamin c concentrations in in the liver of caged *Clarias gariepinus* and vitamin c concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	39.6689945	1	39.66899	957032.4	3.07E-26	4.964603
Columns	15.7723185	4	3.94308	95128.58	7.52E-23	3.47805
Interaction	39.5384043	4	9.884601	238470.5	7.6E-25	3.47805
Within	0.0004145	10	4.14E-05			
Total	94.9801318	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	124.5554	1	124.5554	96610.74	2.92E-21	4.964603
Columns	25.1536	4	6.288399	4877.564	2.12E-16	3.47805
Interaction	27.50714	4	6.876785	5333.942	1.35E-16	3.47805
Within	0.012892	10	0.001289			
Total	177.229	19				

APPENDIX XXXIII

Anova results of vitamin e concentrations in in the gill of caged *Clarias gariepinus* and vitamin e concentration in gill of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	48517.36	1	48517.36	119207266	1.02E-36	4.964603
Columns	32465.23	4	8116.308	19941790	1.86E-34	3.47805
Interaction	32465.23	4	8116.308	19941790	1.86E-34	3.47805
Within	0.00407	10	0.000407			
Total	113447.8	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	59867.29291	1	59867.29	244469.1	2.82E-23	4.964603
Columns	31922.0079	4	7980.502	32588.51	1.59E-20	3.47805
Interaction	15235.22346	4	3808.806	15553.32	6.43E-19	3.47805
Within	2.4488695	10	0.244887			
Total	107026.9731	19				

APPENDIX XXXIV

Anova results of vitamin e concentrations in in the kidney of caged *Clarias gariepinus* and vitamin e concentration in kidney of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	90783.77005	1	90783.77	395062.4	2.56E-24	4.964603
Columns	12166.95807	4	3041.74	13236.69	1.44E-18	3.47805
Interaction	15956.09882	4	3989.025	17358.98	3.71E-19	3.47805
Within	2.29796	10	0.229796			
Total	118909.1249	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	124075.7	1	124075.7	704748.8	1.42E-25	4.964603
Columns	7645.846	4	1911.462	10857.09	3.88E-18	3.47805
Interaction	9102.137	4	2275.534	12925.02	1.62E-18	3.47805
Within	1.760566	10	0.176057			
Total	140825.4	19				

APPENDIX XXXV

Anova results of vitamin e concentrations in in the liver of caged *Clarias gariepinus* and vitamin e concentration in liver of free roaming fish along the stretch of Tatsawarki stream.

a ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	104283.4	1	104283.4	246699.6	2.69E-23	4.964603
Columns	8188.684	4	2047.171	4842.922	2.19E-16	3.47805
Interaction	7732.832	4	1933.208	4573.324	2.92E-16	3.47805
Within	4.22714	10	0.422714			
Total	120209.1	19				

b ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	81234.46	1	81234.46	792558	7.87E-26	4.964603
Columns	7347.456	4	1836.864	17921.23	3.17E-19	3.47805
Interaction	7149.08	4	1787.27	17437.37	3.63E-19	3.47805
Within	1.024966	10	0.102497			
Total	95732.03	19				

APPENDIX XXXVI

CAGE (MARI)