

**EFFECTS OF WATER-SOLUBLE FRACTIONS OF USED CRANKCASE
OIL ON SOME PHYSIOLOGICAL PARAMETERS OF THE NILE
TILAPIA (*OREOCHROMIS NILOTICUS*)**

JAMES KPUK MAKPO
B.Sc, M.Sc. (Jos)
PGNS/UJ/13751/02

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DECLARATION

“I hereby declare that this work is a product of my own research efforts, undertaken under the supervision of Professor John W. Wade mni and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged”.

JAMES KPUK MAKPO
PGNS/13751/02

DATE

CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by James Kpuk Makpo (PGNS/UJ/13751/02) were carried out under my supervision.

PROFESSOR JOHN W. WADE, *mni*
Supervisor

Date

DR. DUNG D. PAM
Head of Department

Date

PROF. (MRS.)GEORGINA S. MWANSAT
Dean, Faculty of Natural Sciences

Date

DEDICATION

This work is dedicated to Almighty God my Saviour, Redeemer, Sanctifier, Baptizer, Healer and Deliverer who gave me purpose and hope for living in time and in eternity. And to my loving wife, Gladys Makpo and children: Paul Makpo, Deborah Makpo, Priscilla Makpo, Mercy Makpo, Philip Makpo, Peace Makpo and Perfecta Makpo.

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ABSTRACT

Used crankcase oil is disposed off indiscriminately and it eventually finds its way into the aquatic environment resulting in surface and ground water contamination by complex interacting chemicals and substances. Polycyclic aromatic hydrocarbons (PAHs), heavy metals, additives, antioxidants and trace levels of chlorinated solvents have been detected in used engine oil and these pose a great risk to fish populations and the human consumer of fish. This study (i) investigated the sublethal effects of water soluble fractions of used crankcase oil on feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), length and weight and muscle and liver glycogen of *Oreochromis niloticus* fingerlings, (ii) investigated the sublethal effects of the water soluble fractions (wsf) of used crankcase oil on the condition factor of *O. niloticus* fingerlings, (iii) assessed the bioaccumulation of metals in the liver, gill and muscle of *O. niloticus* fingerlings exposed to wsf of used crankcase oil, (iv) determined the sublethal effects of used crankcase oil on the haematological indices of *O. niloticus* fingerlings, (v) investigated the effects of used crankcase oil on some selected enzymatic activities in *O. niloticus* fingerlings. The water soluble fractions (wsf) of the used crankcase oil was prepared using the method described by Anderson, Neef, Cox & HighTower (1974) while the 96hr static renewal bioassay technique was employed to obtain the median lethal concentration (LC₅₀) from which the sublethal concentrations (definitive values) were made. One-way analysis of variance (ANOVA) was used to interpret the data on the mean concentration of metals in the experimental water and fish organs as well as the variations in mean values of liver and muscle glycogen and the growth performance and feed utilization of the experimental fish. Fish showed significantly reduced weight ($P < 0.05$) with values of 1.1, 1.5, 1.6 and 1.8 g respectively.

However, fingerlings in the control (0.00 ml/L) and the least sublethal concentration of 8.75 ml/L had significant increases in weight from 6.30g initial weight to 20.10g and 16.81g in that order. There was a strong positive correlation between the weight and length of fish with $r = 0.861$. The analysis of variance of growth performance between groups and within groups was significantly different ($P < 0.05$) during the exposure period. Liver glycogen decreased with increasing time in the fish groups in the sublethal concentrations with glycogen values at 0.68, 0.80, 0.75, 0.55 and 0.30 mg/L. Similarly muscle glycogen decreased as exposure time progressed with reduced values at 0.04, 0.04, 0.03, 0.03, and 0.02 in the ascending order of the sublethal concentrations. Conversely, the liver and muscle glycogen values increased significantly ($P < 0.05$) in fingerlings in the control group. Statistically significant ($P < 0.05$) low values of FCR, and PER were observed in the control when compared with the higher sublethal concentrations. These statistical trends show evidence of stress and an impairment of carbohydrate metabolism in the experimental fish as well as a decreased capacity to efficiently utilize protein when exposed to used Crankcase Oil. *O. niloticus* fingerlings had a mean condition value of less than one (< 1) showing a condition below mean average. The concentration of metals and other elements in the used crankcase oil was in the order $\text{Ca} > \text{Zn} > \text{Na} > \text{Fe} > \text{Si} > \text{Al} > \text{Cu} > \text{Mn} > \text{Br} > \text{Pb}$, while the muscles, gills and liver had concentrations of metals in the following order: $\text{Fe} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Pb} > \text{Cr}$. There was a decrease in circulating erythrocytes, from 1.16 to 0.62 μl as well as decreases in MCV from 109.3 to 34.2 μl and blood platelets from 274.0 to 0.0 μl respectively. Conversely, the result showed increases in WBC, LY, MO, GR, and MCHC from 6.5 to 27.8, 4.1 to 20.9, 0.7 to 2.8, 1.8 to 5.9 and 125.5 to 198.9 μl in that order. There was a significant difference ($P < 0.05$), between WBC and RBC of fish in the control tank and those

in the sublethal concentrations. The activities of the enzymes ALP and ALAT revealed a significant increase ($P < 0.05$) in both cases when compared to the control. The concentration levels for ALP in the highest sublethal concentrations were 256.06 ± 0.441 , 200.12 ± 0.831 , and 100.00 ± 0.762 iu in the fish muscle, liver and gills respectively. While in the control, the levels were 35.00, 16.65 and 13.35 iu in the muscle, liver and gills in the same order. ALT concentration levels were 462.41 ± 0.098 , 430.425 ± 0.126 and 398.00 ± 0.056 iu in the fish muscle, liver and gills respectively at the highest sublethal concentration. Concentration levels at the control tank were 5.712 ± 0.031 , 48.137 ± 0.005 and 5.195 ± 0.038 iu in the fish muscle, liver and gills in that order. Exposure of the test fish to the wsf of used crankcase oil resulted in retarded growth as seen in the reduction in weight and constancy of length of fish in the higher sublethal concentrations. *O. niloticus* fingerlings had a poor feed conversion capability in the presence of used crankcase oil evidenced by decreases in muscle and liver glycogen at the higher sublethal concentrations. Metals are capable of accumulating in the tissues of fish when concentration levels are high. The unregulated disposal of used crankcase oil poses a great threat to the health of the environment. Consequently, government at all levels should control this indiscriminate practice through legislation and by creating collection centres for used crankcase oil. Bioremediation, recycling and other processes should be put in place to ensure proper disposal and to prevent the pollution of the environment.

CHAPTER ONE INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Industrialization is considered to be vital to a nation's socioeconomic development, however the unregulated circumstances accompanying such development especially in developing countries have led to events of surface and ground water contamination by complex interacting chemicals and substances (Coors & Frische, 2011, Ekubo & Abowei 2011, Landrum, Chapman, Neff, & Page, 2012; Dahunsi & Oranusi, 2013; Akinsorotan, 2014). Water contamination is generally a very serious problem in the contemporary world. This necessitates the indispensable assessment of the quality of water as it affects aquatic organisms and man who depends on the water sources for his daily requirements (Nwaniet al., 2015).

Used crankcase oil (engine, or motor oil) is a contaminant of concern, with large volumes entering aquatic ecosystems through water runoffs. The major source of petroleum contamination in urbanized settlements comes from used crankcase oil, (Mahaney, 1994 & Batayneh et al., 2012). Polycyclic aromatic hydrocarbons (PAHs), heavy metals, additives, antioxidants and trace levels of chlorinated solvents have been detected in used engine oil, (Mahaney, 1994). He further reported that compounds in runoffs with used crankcase oil may be in water-soluble fractions or may be absorbed to particles in the runoffs. These compounds include metals such as zinc, aluminium, sodium, and calcium and organic compounds such as phenol and chlorophenol. About 64% of this is reported to be in form of settleable solids as Mahaney (1994) and Tamis, Jongbloed, Karman, Koops, and Murk (2011) further observed. Drippings from parking lots, spills on the highways, mechanics garages, improper disposal by users etc.; are major sources of hydrocarbons in urban areas. Naphthalene, benzo(a)pyrene, fluorine

and phenanthrene are common PAH components of used motor oil (Ndimele, Jenyo-Oni, & Jibuike, 2012).

Billiard *et al.* (2002) reported that polycyclic aromatic hydrocarbons (PAHs) are common environmental contaminants that pose a potential risk to fish populations. Hydrocarbons from oil can move to the atmosphere or settle through water to bottom sediments where they may persist for years. The situation is worsened by the fact that shops, which perform oil changes, do not have receptacles for the collection of used oil (Kumari & Abraham, 2012). The further stated that besides PAHs, several thousands of chemicals are used today to meet the technological and economic needs of the society. The introduction of these technological products and by-products into water systems is of much concern to environmental physiologists.

Upshall, Payne and Hellou (1993) reported that while oil is being used in a crankcase, it breaks down to give a wide variety of oxygenated and aromatic hydrocarbons. Like several individual PAHs, waste crankcase oil has been shown to be mutagenic and tetratogenic. Combustion-driven PAHs have been linked to mutagenesis and carcinogenesis. The effects of used crankcase oil are mixed but some immunological, reproductive, fetotoxic and genotoxic effects have been associated with a few of the compounds, (Ayoola & Alajabo, 2012, Vasquez-Duhalt, 2015).

PAHs found in used motor oil are absorbed and distributed to various tissues as indicated by the presence of PAH-DNA adducts in the skin and lungs of male mice that were dermally exposed to used crankcase oil (Satcher, 1997). He also reported that PAHs are lipophilic compounds, they are stored mainly in adipose tissues and secreted in milk. In both humans and animals, lead is stored in the skeletal and soft tissues pool, and accumulated in the kidneys, while molybdenum is stored mainly in the liver and rapidly excreted in the urine and in the bile. Lu (1991) reported that the half-life of

cadmium is 30 years therefore it is excreted slowly and the kidney is usually the primary target organ of cadmium. He further stated that it damages the renal proximal tubules forming lesions and causing urinary excretion of small molecule proteins, amino-acids, and glucose while Chromium also damages the proximal tubules.

A dose-dependent response study by Irwin (1997) showed that when the rainbow trout, *Onchorynchus mykiss* was exposed per os to waste crankcase oil, the enzyme Ethoxyresorufin Odeethylase (EROD) was induced in the liver, kidney and heart of the fish. Upshall *et al.* (1993) also reported an induction of EROD enzymes when the English sole was exposed to PAH compounds.

Irwin (1997) reported that motor oils are manufactured using highly refined heavier, thicker petroleum hydrocarbon base oils and contains up to 20% of a variety of additives such as viscosity index improvers, detergents, dispersants, antiwear additives, pour-point depressants, and antioxidants. He further observed that during use, high temperatures and friction cause changes such as oxidation, nitration and cracking of polymers in the component chemicals. In addition, a variety of substances such as fuel, water, antifreeze, dust, and various combustion products such as PAHs, metals, and metallic oxides accumulate in the oil. Satcher (1997) observed that the degree of chemical changes and accumulation of contaminants in the oil increases with use and varies depending on the type of fuel used and the mechanical properties of the engine. He also stated that the lubricant protects against wear, reduces friction, cleanses the engine of dirt and residues, protects against corrosion, cools the engine, and seals the pistons.

Technology and Lube News Blog (2013) reported that lubricating oil for combustion engine is mainly cycloparaffin base oils and other chemical additives which help to improve various properties of the oil. It observed that viscosity

improvers are long chain polymers of molecular weight between 10,000 to 1,000,000. They are added to crankcase oil to promote easier ignition of the engine when it is cold. Technology and Lube News Blog (2013) observed that the commonly used viscosity improvers are polyacrylates, polymethylmethacrylates, vinylacetate-alkylfumerate copolymers and polyolefines such as poly-isobutylene and that many of these are pour point depressants. Technology and Lube News Blog (2013) also stated that one of the most important properties of motor oil in maintaining a lubricating film between moving parts is its viscosity and that the viscosity of a liquid is said to be its “thickness” or quantity of resistance to flow. Viscosity must be high enough to maintain a satisfactory lubricating film, but low enough such that the oil can flow around engine parts satisfactorily to keep them well coated under all conditions (Technology and Lube News Blog, 2013).

Irwin (1997) stated that oxidation and corrosion inhibitors act against the oxidation of mineral oils at elevated temperatures forming acidic materials which lead to metal corrosion and an increased oil viscosity due to the presence of insoluble oxidation and corrosion products. Heaton (1976) observed that metal derivatives like Benzotriazole or Mecarptobenzothiazole are used as Copper corrosion inhibitors. However, he further reported that there are detergent based inhibitors which contain alkaline earth metal hydroxides or carbonates in colloidal form which neutralize acidic products. And that there are also miscellaneous bases such as amines ethanolamine or Schiff bases which neutralize acids. Antiwear and Extreme Pressure additives prevent seizure and reduce frictional wear in crankcase oil; these include Zincdialkyldithiophosphate and Zincdialkylthio-carbamate. Others like Dithiocarbamates and Nitrophenols serve parallel functions as the first two compounds

above but also have antiseizing properties without increasing the corrosiveness of Copper and Steel (Hewstone,1994a).

Heaton(1976) reported that detergents are used to reduce high temperature in crankcase oil and to keep deposits suspended in the lubricant. He stated that detergents are usually calcium, barium or magnesium salts of (sulphurised) phenols, Salicylic acid, Sulphonic acid, Carboxylic acid, Phosphosulphurised alkenes in the equivalent weight of 400-800. Heaton (1976) further observed that these additives keep carbonaceous sludge materials from settling out of the oil and can even clean such materials out of a dirty engine. He stated that when detergent additives are used up, the lubricant almost immediately appears dark and dirty because the sludge is carried in suspension and that dispersants have similar functions to detergents but are more active in reducing sludge formation under low temperatures. They prevent agglomeration of particles produced by degradation. Common examples are Polyisoalkenyl polyamides in the molecular weight range of 1,000 - 50,000.

Hewstone (1994) reported that several of the oil additives are toxic environmental contaminants e.g Zincdithiophosphate, Zincdialkyldithiophosphates (ZDTPs), Calcium alkyl phenates; Magnesium, Sodium, and Calcium Sulphonates; Tricresyl phosphates, Molybdenum disulfide, heavy metal soaps and other organometallic compounds that contain heavy metals. He stated that hydrocarbons from oil can move to the atmosphere or settle through water to bottom sediments where they persist for years. Metals from oil may build up in various media.

Ayejuyo, Raimi and Moisili (2005) stated that advancement in technology and growth in population have led to high level of industrialization and urbanization which in turn have led to environmental pollution arising from the indiscriminate discharge of industrial effluents. These effluents may contain most common heavy metals such as

mercury, zinc, copper, etc. Industrial manufacturers may endanger public health by discharging toxic substances, including heavy metals into water which may cause taste, and odour problems, contaminating irrigated food crops and killing fishes and other natural life in rivers, (Oni, 1987). Sastra and Tyaji (1982) reported that water pollution by heavy metals has become a health hazard in recent years while human activities have increased the quantity and distribution of heavy metals in the seas. Heavy metals are common components of natural waters, though some are essential for living organisms; these may be toxic when present beyond tolerant limits, (Lehninger, Nelson & Cox (1982), Voleslay, 1990). These metals generally remain for long periods in sea food and usually set up a series of reaction mechanisms which accumulate in them, and in large concentrations through food chain in animals or humans when consumed.

Malins (1989) observed that the assessment of ecological impacts in chemically contaminated marine environments is vitally necessary for the evaluation of risk to ecosystems and the health of the human consumer of fish and shell fish. Because fish can rapidly metabolize petroleum hydrocarbons, standard chemical analyses are of little use for assessing exposure of fish to oil (Collier *et al.*, 1993). However, this problem is solved by present-day methods for measuring the metabolites of petroleum hydrocarbons in fish. Analysis with symptomatic pre-spawn coho had significantly elevated concentrations of metabolites of PAHs in their bile compared to levels in non-symptomatic pre-spawn coho as reported by Ylitalo, Buzzitis, Krahn, Scholz and Collier (2002). Once a form of toxic waste affects an organism, it can be quickly passed along the food chain during various physiological and metabolic processes, thus causing various problems to consumers of the affected food organism.

Water pollution by heavy metals has become a health hazard in recent years (Sastra & Tyaji, 1982); while human activities have increased the quantity of and

distribution of heavy metals in the seas. Heavy metals are common components of natural waters, though some are essential for living organisms, these may be toxic when present beyond tolerable limits (Lehninger *et al.*,1993,Voleslay,1990). These metals generally remain in sea and other aquatic foods setting up a series of reaction mechanisms which accumulate in them; they are subsequently transported in large concentrations through food chain to animals or humans when consumed.

Incardona and Collier (2002) reported that urbanization contributes fossil fuel – derived polycyclic aromatic hydrocarbons (PAHs) to aquatic and estuarine environments while Malins (1989) stressed the importance of understanding the types and concentrations of potentially toxic substances in sediments, water and the tissues of organisms. To many people, heavy metal pollution is a problem associated with areas of intensive industrial activity, however, roadways and automobiles now are considered to be among the largest sources of heavy metals (Abedi, Khalesi, Eskandari & Rahmani, 2012, Abdulali, Othman & Ahmad,2013). Zinc, copper and lead are the most common heavy metals released from road travels accounting for about 90% of the total metals from road run - offs (USEPA, 2006).

An automobile lubricating oil is one used in an automobile engine to enable it move smoothly and to reduce friction. It is a complex mixture of hydrocarbons (80 - 90% by value) and performance enhancing additives (10– 20% by volume) as observed by CONCAWE (1996). An understanding of the bioavailability of these substances to aquatic organism, and their metabolism is very important. Moles and Norcross (1998) stated that hydrocarbons are presented to aquatic environment as dissolved or dispersed toxic materials adsorbed into particulate matter, or as small floating tar balls; they enter aquatic food webs by such routes as: (i) Active up – take of dissolved or dispersed fractions by aquatic environment (ii) Absorbed onto particles, both living and dead,

followed by ingestion of these particles by aquatic organisms (iii) Passage into the gut of fish which gulp or drink water (iv) Passage through gills of aquatic organisms during the process of respiration.

1.2 STATEMENT OF THE PROBLEM

Mahaney (1994) reported that organic compounds found in waste oil include toluene, benzene, xylene and ethylbenzene. Also present are organic and inorganic compounds of chlorine, sulphur, phosphorus, bromine, nitrogen and some heavy metals such as zinc, magnesium, barium, and lead resulting from oil additives and contamination during use or disposal (Irwin, 1997). In rural areas, a considerable portion of PAHs in streams or rivers comes from highways. Compounds in runoffs of waste crankcase oil may be in the water-soluble fractions, or may be absorbed to particles in the runoffs. Mahaney (1994) also observed that 64% of the total hydrocarbons generated from parking lots and in runoffs are in form of settleable solids. Improper disposal of used motor oil is another major source of its entrance into the aquatic environment.

Irwin (1997) defined used crankcase oil as lubricating oils removed from the crankcase of internal combustion engines. It is recognized that the major components consist of aliphatic and aromatic hydrocarbons (such as phenol, naphthalene, benz(a)anthracene and fluoranthene). New motor oil contains fresh oil and lighter hydrocarbons that would be of greater concern for short-term (acute) toxicity to aquatic organisms, whereas used motor oil contains more metals and heavy Polycyclic Aromatic Hydrocarbons (PAHs) that contribute to chronic (long term) hazards including carcinogenicity, (Ayoola & Alajabo, 2012). Aromatics are considered to be the most acutely toxic component of petroleum products, and are also associated with chronic and carcinogenic effects. They are often distinguished by the number of rings

they possess, which may range from one to five. Lighter, mono-aromatics (one ring) compounds include benzene, toluene, ethylbenzene and xylene (NIOSH/OSHA, 2010). Upshall *et al.* (1993), observed that aromatics with two or more rings are referred to as Polyaromatic Hydrocarbons (PAHs) and also stated that crankcase oil contain several toxic components including up to 30% aromatic hydrocarbons, with as much as 22 ppm benzo(a)pyrene as a PAH. They further reported that used motor oil has much higher concentrations of PAHs than new motor oil.

Moles (2001) stated that chemical exposure especially to petroleum hydrocarbons can result in altered immune responses as well as decreased resistance to pathogens. He further observed that monocyclic aromatic hydrocarbons (BTEX) are assumed to account for most of the toxicity which induces narcosis. Vasquez-Duhalt (2015) reported that the BTEX are the most abundant aromatic hydrocarbons in most oils, but they are the least persistent because of their relatively high vapour pressures. The bulk of a typical motor oil consists of hydrocarbons with between 18-34 carbon atoms per molecule, (Short, Rice, Heintz, Carls, & Moles, 2003).

Vasquez-Duhalt (1989) reported that the important difference between new and used crankcase oil or motor oil is the heavy metal content. This difference is extremely important because many of the metals are harmful to human health and living organisms. These metals originate from fuel and from motor wear. Used motor oil contains high concentrations of lead, zinc, calcium, barium and magnesium along with lower concentrations of iron, sodium, copper, aluminium, chromium, manganese, potassium, nickel, tin, silicon, boron, molybdenum (Vasquez-Duhalt, 1989). Rai and Pal (2002) observed that to many people, heavy metal pollution is a problem of areas with high industrial density, however; roadways and automobiles are now considered to be among the largest sources of pollution by heavy metals released from road travels

accounting for about 90% of the total metals in road runoffs. They further reported that heavy metals are often dissolved in water and are often consumed either through food, drinking of water or even respiration by fishes. These metals accumulate in food chain and their effects manifest in humans when fishes are consumed.

The extent of the growing crisis in the contamination of the environment and food through food chain by endocrine disrupting chemicals is reflected by the growing list of health advisories regarding eating fish and wildlife which serves as a warning that similar bioaccumulation and effects are occurring in people as in fish and wildlife as Rai & Pal (2002) again observed. Milda and Audrone (2006) stated that low concentrations of heavy metals can cause a chronic stress which may not kill individual fish but lead to a lower body weight and smaller size, however; a mixture of heavy metals differ in their toxicity on living organisms from the effects of single components.

The toxicity of a mixture of metals depends on their concentrations, specific composition and duration of fish exposure (Vosyliene, Kazlauskienė, & Svecevičius, 2003). Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing in some parts of the world; in particular in less developed countries though emissions have declined in most developed countries in recent time, (Batayney *et al.*, 2012). Recent data indicate that adverse health effects of cadmium exposure may occur at lower exposure levels than previously anticipated, primarily in the form of kidney damage but possibly also bone effects and fractures. Elleta and Adekola (2005) reported that the general human population is primarily exposed to mercury via food, fish being a major source of methyl mercury exposure and certain groups with high fish consumption may attain blood levels associated with a low risk of neurological damage

to adults. They also stated that heavy metals gain access into river systems from both natural and anthropogenic sources and these get distributed into the water body, habitats and sediments during the course of their transport. Osman (2012) reported the use of lead in battery accumulators, gunpowder, soldering lead materials among others. Heavy pollution of aquatic environments pose serious health hazards if their concentrations exceed allowable limits. Even when those limits are not exceeded, there's still the potential of a long term poisoning, since they are known to bioaccumulate within biological systems (Queck & Foster, 1998, Osman, 2012).

The bioaccumulation of cadmium in humans is of concern as it has a long half-life in the human body and chronic exposure has been linked to kidney dysfunction as reported by Wilks, Kwizera and Bach (1990) and prostate tumours (Ekman, 1999). Chan, Black and Hale (2000) observed that the potential for transfer of cadmium to humans from diet is of particular interest as its concentrations in agricultural soil can be elevated due to their amendment with applications of phosphate fertilizers, animal manures and sewage sludge as well as long-range transport of anthropogenic emissions containing metals. The USEPA (2010) has classified lead as being potentially hazardous and toxic to most forms of life and Awofolu, Mbolekwa, Mshemia and Fatoki (2009) reported that it is responsible for quite a number of ailments in humans such as chronic neurological disorders especially in fetuses and children. Automobile exhaust fumes have been reported to account for about 50% of the total inorganic lead absorbed by human beings (USEPA, 2012).

Al-nagaawy (2008) observed that heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches substantially high levels. Accumulation levels vary considerably among metals and species. Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer

able to balance and/or counter uptake; this capacity however, also varies between different species and different metals (Heath,1987). Lead, the most toxic metal is detectable in practically all phases of the inert environment and all biological systems because it is toxic to most living things at high exposure level including tissues as well as bones which act as storage sites for lead in fishes, (Sorensen, 1991).

Samir and Shaker (2008) reported that the aquatic environment and its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing serious threat to the survival of aquatic organisms including fish. The most important anthropogenic sources of metals are industrial, petroleum contamination and sewage disposal, (Santos, Silva-Filho, Schaefer, Albuquerque-Filho, &Campos, 2005).

Clinical examinations to assess human health have traditionally involved studies of the blood with many techniques used to assess the health of fish adopted from work in humans. Fish blood is essentially the same as that of humans and most other vertebrates. Clausen and Rastogi (1977) reported elevated levels of lead in blood of 52% of mechanics exposed to used motor oil when compared with controls not employed in the auto industry and that in several cases, the elevated lead correlated with decreases in the haematocrit and mean corpuscular haemoglobin (MCH). High blood lead levels have been associated with anaemia observed in cattle that had ingested an unknown amount of used mineral-based crankcase oil while grazing in a pasture, (Sas 1989, Fazioet al.,2013).

1.3 JUSTIFICATION OF THE STUDY

Used crankcase oil being part of the by-products of technology that are carelessly disposed into the environment pose a great risk to the human consumer of fish and other aquatic organisms as Malins (1989) explained, it is very important to understand the types and concentrations of these potentially toxic substances in sediments, water and the tissues of fish and other organisms. This knowledge will enable us devise safer methods of disposing these contaminants to ensure a healthier environment and poison-free fish and other aquatic foods.

1.4 AIM OF THE STUDY

This investigation is designed with the aim of assessing some physiological parameters of *Oreochromis niloticus* fingerlings exposed to water-soluble fractions (wsf) of used crankcase oil.

1.4.1 The Objectives of the Study Were To:

1. investigate the sublethal effects of water soluble fractions of used crankcase oil on crude protein and lipid, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), length and weight and muscle and liver glycogen of *Oreochromis niloticus* fingerlings.
2. investigate the sublethal effects of the water soluble fractions (wsf) of used crankcase oil on the condition factor of *O. niloticus* fingerlings.
3. assess the bioaccumulation of metals in the liver, gill and muscle of *O. niloticus* fingerlings exposed to wsf of used crankcase oil.
4. determine the sublethal effects of used crankcase oil on the haematological indices of *O. niloticus* fingerlings.
5. investigate the effects of used crankcase oil on some selected enzymatic activities in *O. niloticus* fingerlings exposed to wsf of used crankcase oil.

1.5 NULL HYPOTHESES

1. There is no significant effect of the sublethal concentrations of used crankcase oil on the crude protein and lipid, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), length and weight and muscle and liver glycogen of *Oreochromis niloticus* fingerlings.
2. The sublethal concentrations of used crankcase oil have no significant effect on the condition factor of *O. niloticus* fingerlings.
3. There is no significant effect on the bioaccumulation of metals in the used crankcase oil on the liver, gill and muscle of *O. niloticus* fingerlings.
4. The sublethal concentrations of the wsf of used crankcase oil have no significant effects on the haematological indices of *O. niloticus* fingerlings.
5. There is no significant effect of the sublethal concentrations of the used crankcase oil on the enzymatic activities in *O. niloticus* fingerlings.

CHAPTER TWO LITERATURE REVIEW

2.1 EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE ENVIRONMENT

Man today is facing one of the most horrible ecological crises in his cultural history-the problem of pollution of the environment particularly with petroleum products and their activities, the cry of contemporary time is growing louder all over the world (Imanpour & Taghizadeh, 2013). Rai and Pal (2002) observed that pollution has become a major threat to the very existence of mankind on planet earth. The release of water soluble fractions of used automobile lubricant from an industrial plant, a drum, a gallon or from the engine of vehicles onto bare land causes it to enter the environment and is subsequently washed into different water bodies.

Satcher (1997) reported that exposure to used crankcase oil through breathing, eating, drinking or skin contact depending on the duration, route, and dose of exposure causes health risks. He further added that the risk of exposure may be determined by individual characteristics such as age, gender, nutritional status, family traits, lifestyle, and state of health. Okoli-Anunobi, Ufodike and Chude (2002) observed that the deleterious effects of these pollutants on aquatic organisms may be neurophysiological, behavioral and reproductive causing acute or chronic conditions. Rai & Pal (2002) observed that water has been referred to as "life," the entire existence of most; if not all living organisms depend on water, even single celled organisms depend on water for existence and continuity of life. They further stated that man depends directly or indirectly on water for life and in spite of these immeasurable benefits derived from water, man's actions have continued to pose a grave danger to himself and to the aquatic organisms as water bodies are continuously polluted. Although natural phenomena such as volcanoes, algal blooms, storms and earthquakes also cause major changes in water

quality and ecological status of water, nevertheless, man has been his own greatest enemy.

Water pollution is a major problem globally, Pink and Daniel (2002), Dahunsi and Oranusi (2012) reported that it is the leading cause of diseases and death worldwide, while West-Larry (2006) observed that it accounts for the deaths of more than 14,000 people daily. It is understood that water quality standard for drinking water has been defined by the World Health Organization (WHO, 1993), the standard for certain agricultural and industrial uses are also well-defined. Water has often been considered adequate for fish as long as there is no obvious mortality which can be ascribed to known pollutants. The degradation of aquatic habitats through pollution has often passed unnoticed (Avengebe, 1999). Kester, Osofero and Daramola(2007) observed that Nigeria is a country endowed with many large bodies of inland water and that there is the need for proper management of this fresh water at suitable quality for the use of Nigerians, animals and aquatic organisms. The attainment and maintenance of this suitable water quality can only be sought through pollution abatement or control (Oruc & Uner,1998). They further stated that the attainment and maintenance of the recommended water quality is becoming difficult everyday because of the use of agro chemicals, insecticides, herbicides and especially the discharge of effluents from industries and oils from mechanic workshops.

Oruc and Uner(1998) further stated that in Nigeria, water is taken to be the means of clearing up engine oil as well as refuse and that most of the used engine oils, especially the soluble fractions that contain different toxic substances such as heavy metals are allowed to flow into the water bodies untreated and this leads to changes in water quality.Omoregie and Ufodike (2000), Omoregie and Okunsebor (2003) andOmoregie, Okunsebor and Audu (1986) reported that damage caused by

hydrocarbon pollution to the aquatic environment is irreversible. For example, Malins (1989) stated that sediment chemistry data collected from Eagle Harbour, Washington State revealed that the pollution problem there was caused by long – standing inputs of aromatic hydrocarbons and other compounds resulting from the use of creosote in the area; studies of the brains of English sole from Eagle Harbour showed complex profiles of chlorinated hydrocarbons revealing that fish readily concentrate toxic chemicals such as hydrocarbons from water.

Billiard *et al.* (2002) observed that exposure of the early life stages of fish to PAHs under field and laboratory conditions can mimic the embryo – toxic effects of planar halogenated hydrocarbons (PHHs) the most potent of which is 2, 3, 7, 8 – tetrachlorodibenzo-p-dioxin. Carlson and Zelikoff (2002) reported that Benso(a) pyrene (a hydrocarbon) when injected (2µg/gBW) into the Japanese medaka (*Oryzias latipes*) suppressed lymphocyte proliferation. At concentrations of 20 and 200µg/g BW, it suppressed antibody – forming cell (AFC) numbers, superoxide production and host – resistance against bacteria. Incardona and Collier (2002) stated that studies have detected PAHs in both marine and anadromous fish species, and a common site of morphological defects, including oedema, and dorsal curvature of the body axis have been observed in marine and fresh water embryos exposed to hydrocarbons in the laboratory and in the field. They also observed that PAHs act on specific targets in the excitatory conduction induced by PAHs which are secondary to cardiac dysfunction. Lanno, Hickie and Dixon (1989) explained that physiological parameters are affected by nutritional status and further observed that many effects of nutritional status on test organisms are intimately related to nutritional effects on metabolic rate. For example, Das, Ayyapan and Jena (2004) discovered that a sublethal concentration of ammonia may reduce growth, damage various organs and predispose fish to disease.

Collvin (1985) observed reduced feed conversion efficiency in perch (*Perca fluviatilis* L.) exposed to waterborne copper; this decrease was attributed to the increased metabolic energy demands of copper detoxication. Borgmann and Raph (1986) reported decreases in feed conversion efficiency in larval white sucker (*Castostomous commersoni*) and young common shiners (*Notropis cornutus*) exposed to sublethal levels of cadmium, 2, 4-dichlorophenol or pentachlorophenol.

2.2 EFFECTS OF THE METAL CONTENT OF USED CRANKCASE OIL

Metals are often found dissolved in water and are often consumed either through food, drinking water, or even respiration by fish; these metals accumulate in food chain and their effects manifest in humans when the fish is consumed. Batayneh *et al.* (2012) observed that heavy metals from oil are diluted and affected by various surface water components such as carbonates, sulphates, and organic compounds. After entering natural water bodies insoluble salts or complexes are formed which are presumed not to be harmful to aquatic organisms (Eister, 1998). Part of these salts and complexes sink and get accumulated in bottom sediments. When the pH of water declines, (during acid rains); heavy metals can be mobilized and released into the water making it toxic to aquatic biota (Taylor, Branch, Halls, Owen & White, 1998). On the other hand, low concentrations of heavy metals can cause a chronic stress which may not kill individual fish but may lead to a lower body weight and smaller size (Milda & Audrone, 2006).

In most ecotoxicological studies, effects of a single metal on fish have been evaluated, while studies of biological responses of fish to a mixture of heavy metals (more than three components) are scarce as observed by Reddy & Reddy (2013). However, the effects of a mixture of heavy metals differ in their toxicity on living organisms from the effects of single components (Milda & Audrone, 2006). The toxicity

of a mixture of metals depends on their concentrations, specific composition and duration of fish exposure (Vosyliene *et al.*, 2003). Heath (1987) reported that accumulation levels vary considerably among metals and species. He further observed that toxic effects occur when excretory metabolic storage and detoxification mechanisms are no longer able to counter uptake, this capacity however; often varies between different species and different group of metals. Lead, the most toxic metal, is detectable in practically all phases of the inert environment and all biological systems because it is toxic to most living things at high exposure level: tissues as well as bones act as storage sites for Lead in fishes (Sorensen, 1991).

Samir & Shaker (2008) reported that the aquatic environment and its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing a threat to the survival of aquatic organisms including fish. The most important anthropogenic metals are those of industrial, petroleum contaminated and sewage origin (Santos *et al.*, 2005). Many techniques used to assess health in fish have been adopted from work in humans. Fish blood is essentially the same as that of humans and most other vertebrates. Clausen & Rastogi (1977) and Batayneh *et al.* (2012) also reported elevated blood lead levels in 52% of mechanics exposed to used motor oil when compared with two levels in controls not employed in the auto industry. In several cases, the elevated lead levels correlated with decreases in packed cell volume (PCV) and mean corpuscular haemoglobin, (MCH) (Dahunsi & Oranusi, 2012). High blood Lead levels have been associated with shortage of blood or anaemia. Anaemic condition was also observed in cattle that had ingested an unknown amount of used mineral-based crankcase oil while grazing in a pasture (Sas, 1989).

Satcher (1997) observed that PAHs found in used motor oil are absorbed and distributed to various tissues as indicated by the presence of PAH-DNA adducts in the skin and lungs of male mice that were dermally exposed to used crankcase oil. He further reported that PAHs are lipophilic compounds, which are mainly stored in adipose tissue and secreted in milk. In the skeletal and soft tissue, cadmium (Cd) is accumulated in the kidneys, while Molybdenum is stored somewhat in the liver and rapidly excreted in the urine and bile. The half-life of Cadmium is 30 years and hence, is excreted very slowly. The kidney is the primary target organ of Cadmium. It damages the renal tubules forming lesions and causing urinary excretion of small-molecule proteins, amino-acids and glucose; Chromium also damages the proximal tubules (Lu, 1991).

2.3 ACTIVITIES OF SOME ENZYMES IN *NO. NILOTICUS* FINGERLING EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

A dose-response study by Irwin (1997) showed that when the rainbow trout, *Onchorynchus mykiss* was exposed orally to waste crankcase oil and analysed Ethoxyresorufin Odeethylase (EROD) enzymes were induced in the liver, kidney and heart. Upshall *et al.*, (1993) also reported an induction of these enzymes in the organs of fish exposed to oil. Peterson, Rice, Short, Balachey and Iron (2003) confirmed higher levels of the detoxification enzyme, cytochrome (CYPIA) in various aquatic animals exposed to persistent oil pollution. They reported that suspension – feeding clams and mussels concentrate and only slowly metabolize hydrocarbons, which leads to chronically elevated tissue contamination as was observed in the clams, *Protothaca staminea*. After chronic exposure of pink salmon fry to PAHs, Peterson *et al.* (2003) reported that their growth became stunted and their survival rate reduced

leading to enhanced mortality. This also led to abnormal development and reproductive impairment due to endocrine disruptions.

Stegemann, Schlezinger, Gradock & Tillit (2001) reported the detection of cytochrome P450 1A (CYPIA) in the gill, heart, kidney and liver of several fishes from the Western North Atlantic. They implicated PAHs as the causative agents for lesions in these organs observed in the English sole (*Parophrys vetulus*) and starry flounder (*Platichthys stellatus*). The activities of several hepatic enzymes such as Aryl Hydrocarbon Hydroxylase (AHH), Epoxide Hydrolase (EH), Glutathione S-transferase (GST) and Alkaline Phosphatase (ALP) were also observed. These enzymes are involved in the activation and detoxification of PAHs. Liver lesions have been identified as biomarkers for exposure to PAHs, Prasad and Veeraiah (2002) observed an elevation in the activities of Glutamate dehydrogenase, Aminotransferase, Aspartate aminotransferase and Alanine aminotransferase in the kidney, muscle, brain and liver of *Labeo rohita* (Hamilton) exposed to cypermethrin pollution.

2.4 EFFECTS OF THE WSF OF USED CRANKCASE OIL ON THE HAEMATOLOGICAL INDICES OF *O. NILOTICUS* FINGERLINGS EXPOSED TO USED CRANKCASE OIL

Haematological assessments are meant to test the possible presence of anaemia, presence and intensity of a disease, among other factors. These parameters help to assess the health status of the individual organism. Salazar and Salazar (2002) observed that bioaccumulation is the ultimate link between environment and organism, and a necessary element for evaluating environmental quality and ecosystem health. They explained that the expose-dose-response triad emphasizes the measurement of tissue chemistry and associated biological responses. Haematological studies are also used to detect physiological changes following different stress conditions of fish exposed to engine oil. Thus haematology can be considered as an essential index to the general

health status. The most common haematological variables measured during stress include red blood cells (RBC) and white blood cells (WBC) count, haemoglobin content and haematocrit value and indices (Jairajpuri, Rana and Jetley, 2014). Blaxhall and Daisley(2005) reported that haematological parameters are often determined as an index of their health status. Haematological variables are used more often when clinical diagnosis of fish physiology is applied to determine the sublethal concentrations of pollutants (Reddy & Reddy 2013).

The use of haematological variables as indicators of stress in fish has been illustrated by Ovuru and Ekweozor (2004)Ayoola (2008), Reddy and Reddy (2013).Jairajpuri *et al.*,(2014) reported that haematological parameters can provide information on the physiological responsesfish make to a changing external environment.Changes in haematological indices can also result from the close association of the circulatory system with the external environment (Opara, Udevi & Okoli, 2010).Performing blood chemistry analysis often provides vital information aiding the diagnosis for health assessment and management of cultured fish (Daka & Ekweozor, 2004, Ekubo & Abowei, 2011). Haematological indices are very important parameters for the evaluation of fish physiological status. Dahunsi and Oranusi (2013) and Luskova (1997) as reported in Milda and Audrone (2006) observed that physiological changes depend on fish species, age, the cycle of sexual maturity of spawners and diseases. These changes are said to be related more to the response of the whole organism to its survival, reproduction and growth (Milda and Audrone, 2006).

Studies on specific indices reflecting the effect of some substances on the organism, such as changes in the blood serum cholinesterase under the influence of insecticides or changes in methaemoglobin under the influence of nitrites have been determined (Ready & Ready 2013). However, Fazio *et al.*, (2013) and Nwani *et*

al.,(2015) reported that the complex unspecified biochemical indicators of blood reveals more fully the general effect of pollutants on fish and makes possible a forecast of the consequences of long-term exposure to chemical pollutants. Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural fresh water resources in the tropics as observed by Saliu and Salami (2010). Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Saliu&Salami, 2010) and animals generally (Etim, Williams, Akpabio & Offiong,2014). The counts of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation as Saliu and Salami (2010) further observed. Fish haematological parameters are often determined as an index of their haematological variables used more often when clinical diagnosis of fish physiology is applied to determine the sublethal concentrations of pollutants as observed by Wedemeyer and Yasutake (1997). Jairajpuri *et al.*,(2014) stated that it can provide information on the physiological responses of the fish.

2.5 GONADAL DEVELOPMENT IN *O. NILOTICUS* FINGERLING EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

Sexual maturity in Tilapia is a function of age, size and environmental conditions. Jegede (2008) stated that stages of gonad development are dynamic and could vary from time to time. While Duponchelle & Panfili (1998) reported that the Nile Tilapia, *O. niloticus* attains first sexual maturity at a total length of between 9 – 15cm at the age of 5 – 10 months. They further observed that it matures at about 10 to 12 months or 350 to 500g in several East African lakes. The Mozambique tilapia, *T. mozambicus* reaches sexual maturity at a smaller size and younger age than the Nile

and Blue tilapia, *O. niloticus* and *O. aureus* respectively (Nico & Neilson, 2015). They also observed that Tilapia populations in large lakes mature at a later age and larger size than the same species raised in small farm ponds. For example, the Nile Tilapia matures at about 10 – 12 months and at a weight of about 350 – 500 grams (Balubid, 2003). Omotosho, Fagade and Adebisi (1990) also reported that under good growth conditions, the Nile Tilapia will reach sexual maturity in farm ponds at an age of 5 – 6 months at 150 – 200 grammes. When growth is slow, sexual maturity in the Nile Tilapia is delayed a month or two but stunted fish may spawn at a weight of less than 20 grammes (Dos Santos, Mareco & Pai-Silva, 2013). Under good growing conditions in ponds, the Mozambique Tilapia and Nile Tilapia may reach sexual maturity in as little as 3 months of age when they seldom weigh more than 60 – 100 grams (Invasive Animals CRC, 2012). In poorly fertilized pond culture, tilapia may be as small as 15 grams as Nico and Neilson (2015) observed. The gonadosomatic index is essentially an indicator of the state of gonadal development and maturity of the *O. niloticus* fingerlings under investigation. It is a known fact that the GSI increases with the maturation of the fish. Omeje, Olufemi and Madu (2009) observed that the understanding of the breeding biology of fish species under natural conditions is essential in developing techniques for the artificial breeding and rearing of such species.

2.6 THE ESSENTIAL COMPONENTS OF CRANKCASE OIL: BENZENE, TOLUENE, ETHYLBENZENE AND XYLENE (BTEX)

Aromatics (Table 1) are considered to be the most acutely toxic components of petroleum products and are also associated with chronic and carcinogenic effects, (Irwin, 1997). Lighter monocyclic aromatic hydrocarbons which consist primarily of benzene, toluene, ethylbenzene and xylene (BTEX) are assumed to account for most of

Table 1
The Main Composition of Oil (Virgin)

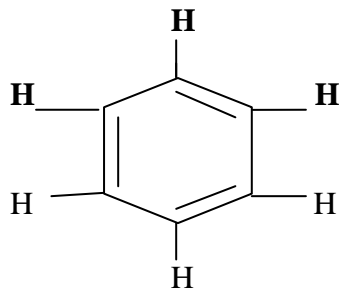
(a) Hydrocarbon groups	(weight, mg/L)
Saturates	86.3
Aromatics	12.9
Polars	0.8
Asphaltenes	0.0
Volatiles	3.2
(b) Metals	
Aluminium	15
Copper	18
Iron	220
Lead	18500
Silicon	17
Antimony	6
Sodium	59
Calcium	688
Barium	177
Zinc	1360
Magnesium	410

Source: Satcher (1997)

the toxicity and are the most abundant aromatic hydrocarbons in most crankcase oils though they are the least persistent because of their relatively high vapour pressures, (Short *et al.*, 2003). They also observed that petroleum hydrocarbon products are complex mixtures of chemicals therefore, risk assessment for these products in general, focuses on specific toxic constituents. Short *et al.* (2003) further stated that the petroleum constituents of primary interest to human health have been the aromatic hydrocarbons like benzene, ethylbenzene, toluene and xylene. Human exposure to benzene is a global health problem, (Satcher, 1997).

2.6.1 Benzene

Wilcox and Greenbaum (1965), Pauling (1987) and March (1992) reported the following structural description of Benzene (Benzol cyclohexa -1,3,5- triene):



Structure of Benzene Molecule, Source: Pauling (1987).

Pauling (1987) observed that Benzene is an organic chemical compound composed of 6 carbon atoms in a ring with 1 hydrogen atom attached to each carbon atom. It is a natural constituent of crude oil, and is one of the most basic petrochemicals. It is a cyclic hydrocarbon with a continuous pi-bond sometimes abbreviated as Ph-H. Benzene is colourless, highly inflammable and has a sweet smell. It is mainly used as a precursor to heavy chemicals such as ethylbenzene and cumene which are produced on a billion kilograms scale. March (1992) stated that because of its high

octane number, it is an important component of gasoline (petrol) and its carcinogenicity has limited most non-industrial applications. He further observed that benzene is used mainly as an intermediate component making other chemicals.

Huff (2007) reported that about 80% of benzene is consumed in the production of three chemicals: ethylbenzene, cumene, cyclohexane and that its most widely produced derivative is ethylbenzene, a precursor to styrene, which is used to make polymers and plastics. Cumene is converted phenol from resins and adhesives. Cyclohexane is used in the manufacture of nylon. Smaller amounts of benzene are used to make some types of rubbers, lubricants, dyes, detergents, drugs, explosives and pesticides (Huff, 2007). Smith (2010) and ATSDR (2012) observed that as a petrol additive, benzene increases the octane rating and reduces engine knock. ATSDR (2012) also reported that in the USA, concerns over its negative health effects and possibility of entering into groundwater have led to stringent regulation of petrol's benzene content with limit around 1%. ATSDR (2012) further noted that European petrol specifications now contain the same 1% limit on benzene content. The United States Environmental Protection Agency has new regulations that lowered benzene content of petrol to 0.62% in 2011. Occupational Safety And Health Administration [OSHA] (2010) observed that benzene is an excellent liquid in the organometallic chemistry of low-valent metals. Benzene causes cancer and other illness which include bone marrow failure, aplastic anemia, acute leukemia, myeloid leukemia, myelodysplastic syndrome (MDS), acute lymphoblastic leukemia and chronic myeloid leukemia, (OSHA, 2010).

Environmental Health News (2010) reported that a short-term breathing of high levels of benzene can result in death, low levels can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion and unconsciousness. Environmental Health News

(2010) also observed eating or drinking foods containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness sleeping, convulsions and death.

National Institute for Occupational Safety and Health (NIOSH, 2012) stated that the major effects of benzene are manifested through chronic exposure through blood. NIOSH (2012) further reported that Benzene damages the bone marrow, causes decrease in red blood cells leading to anaemia and can also cause excessive bleeding and depress the immune system and increase the risk of infection. It is associated with blood cancers and pre-cancer of the blood. ATSDR (2012) Environmental Health News (2010) and OSHA (2010) stated that benzene targets the liver, kidney, lung, heart and the brain and can cause DNA strand breaks, chromosomal damage and causes cancer in both animals and humans.

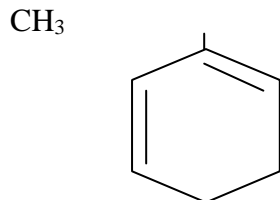
The International Agency on Cancer Research (IACR, 2012) reported that benzene causes cancer of the lungs in human and that some women having breathed high levels of benzene for many months had irregular menstrual periods and a decrease in the size of their ovaries. Exposure to benzene has been linked directly to neural birth defects, spinal bifida and anencephaly. Men in exposed to high level of benzene are more likely to have an abnormal amount of chromosome in their sperm which affect fertility and foetal development.

Water and soil contamination are important pathways of concern for transmission of benzene. United State Environmental Protection Agency has set a maximum contamination level (MCL) for benzene in drinking water at 0.05 mg/L as promulgated through, the National Primary Drinking Water Regulations (US EPA, 2012). Rana and Verma (2005) and Baselt (2008) reported that the measurement of benzene in humans can be accomplished via urine, blood and breath test, however, all these have limitations because benzene rapidly metabolizes in the human body into by-

products called metabolites. Baselt (2009) further noted that the maximum allowable amount of benzene in a work room air during an eight-hour workday, 40-hour workweek is 0.1 ppm. Because benzene causes cancer, the American National Institute for Occupational Safety and Health (NIOSH, 2010) recommended that all workers wear special breathing equipment's when they are likely to be exposed to benzene level exceeding the 8hr recommended exposure limit of 0.1 ppm.

2.6.2 Toluene

March (1992) and Wade (2003) gave the following description of toluene: formerly known as toluol; toluene is a clear, water insoluble liquid with the typical smell of paint thinner; it is a mono-substituted benzene derivative, one in which a single hydrocarbon from the benzene molecule is replaced by a univalent group, in this case the methyl group, CH_3 .



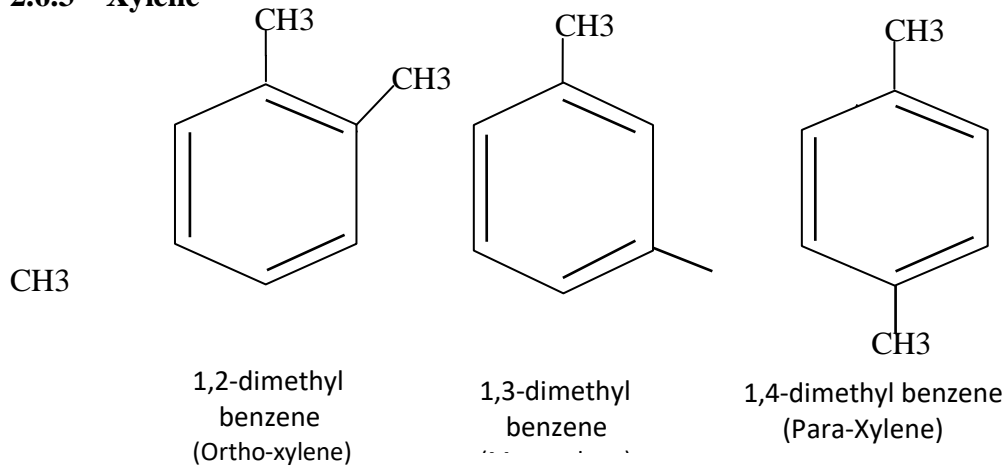
Chemical structure of Toluene: Source: March (1992)

Streicher, Gabow, Moss, Kono and Kaehny (1981), Devathanan, Low, Teoh, Wan, and Wong (1984) and Hogan (2011) observed that toluene is an aromatic hydrocarbon that is widely used as an industrial feedstock and as a solvent. It is also used as an inhalant drug like other solvents for its intoxicating properties. Inhaling toluene has a potential of causing severe neurological harm. Hogan (2011) further reported that toluene is a common solvent able to dissolve paint, paint thinner, many chemical reactants, rubber, printing ink, adhesives (glues), lacquers, leather tanners and disinfectants. Hogan (2011) also noted that toluene is a raw material for the manufacture of TNT and toluene

dissoyanate (used to produce polycyrene foam) and that toluene is also used as cement for fivepolystyrene kits and as an octane booster in gasoline fuels (petrol).

Streicher *et al.*,(1981) and ATSDR (2000) reported that toluene should not be inhaled as low to moderate levels can cause tiredness, confusion, weakness, drunken type actions, memory loss, nausea, loss of appetite and hearing and colour vision loss. ATSDR (2000) further stated that high levels inhaled within a short time period may cause light headedness, nausea, sleepiness, unconsciousness and death while effects of long term exposure is often associated with psycho-organic syndrome, visual evoked potential (VEP) abnormality, toxic polyneuropathy, cerebella, cognitive and pyramidal dysfunctions, optic atrophy and brain lesion.

2.6.3 Xylene



Xylene Isomers, Source: Klaussen *et al.* (1986)

They further reported that Xylene constitutes about 0.5-1% of crude oil depending on the source, therefore xylenes are found in small amounts in petrol and aeroplane fuels and is produced mainly as part of the BTEX aromatics (Benzene, toluene, ethylbenzene and xylene). It is produced by dehydrocyclodimerization and methylating of toluene and benzene(Klaussen,Amdor,&Doull, 1986).Weast (1984) and Grayson (1985) described Xylene as a solvent which contains a small percentage of ethylbenzene, a colourless mixture sweet-smelling and highly inflammable. Grayson (1985) also

recorded that Xylene is a common component of ink, rubber, adhesive and leather industries and is used for thinning paints and varnishes and can substitute toluene where slower drying is required.

ATSDR (2007) reported that exposure to xylene can occur via inhalation, ingestion, eye or skin contact and that Xylene produces central nervous system depression and irritation of the eyes and skin, it is fetotoxic and teratogenic to animals. ATSDR (2007) observed that the signs and symptoms of acute exposure to xylene include: headache, fatigues, irritability, lassitude, nausea, anorexia, flatulence, irritation of the eyes, nose, throat and motor incoordination and impairment of equilibrium, dizziness, confusion, cardiac irritability etc. Chronic exposure results in conjunctivitis, dryness of nose, throat, skin, dermatitis, kidney and liver damage (NIOSH, 1987a & 1987b).

CHAPTER THREE MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN AND METHODOLOGY

3.1.1 Collection and Preparation of Used Crankcase Oil

The used crankcase oil was collected from automobile mechanics around Total Filling Station along Keffi-Abuja Road Nasarawa State, Nigeria in gallons and brought to the Zoology Laboratory, Nasarawa State University, Keffi Nasarawa State, Nigeria for the preparation of water soluble fractions (wsf). The water soluble fractions (wsf) of the used crankcase oil was prepared using the method described by Anderson, Neef, Cox & High Tower (1974). Ten liters of dechlorinated tap water was taken into an aspirator with one magnetic stirrer of 5.0 cm length and 1 cm in diameter with a hot plate (Bran Scientific And Instrument company, England; Model 78HW-1). One litre of the used crankcase oil was measured and introduced into the aspirator and vigorously mixed together with the water at 1000 revolutions per minute for 20 hours using the magnetic stirrer. Thereafter, the set-up was allowed to cool for 5 hours. It was then siphoned within 8 hours.

3.1.2 Collection and Acclimatization of *O. niloticus* Fingerlings

The first set of 200 mixed sex *Oreochromis niloticus* fingerlings used were collected from Panyam Fish Farm Plateau State, and the second set of 150 fingerlings were from Agric. Bank Fish Farm Nyanya, Abuja Nigeria and transported early in the morning to the Zoology Laboratory, Nasarawa State University, Keffi using oxygenated plastic bags. Ice chips were used to lower the temperature during transportation. The fingerlings were acclimatized in the laboratory for two weeks each time before the commencement of the experiment.

3.1.3 Acute Toxicity/Sublethal Tests of Used Crankcase Oil on *O. niloticus* Fingerlings

A static renewal bioassay technique was employed in which the test media were renewed at the same concentration once every 24 hrs from the stock maintained throughout the experimental period. Preliminary tests were carried out to determine the potency of the serial dilutions that were made for this investigation. Acute toxicity concentrations of 150, 300, 450, 600 and 750 ml/L were used to determine the median lethal concentration (LC50) value of the used crankcase oil. These concentrations served for the range finding test and were measured out with a measuring cylinder in replicate. Clear undiluted dechlorinated tap water served as control. Ten active fish of 5.7 ± 0.3 cm mean length and 6.3 ± 0.1 g mean weight were introduced into each of the test concentrations in the aquaria in replicate. Mortality was assessed constantly over a 96hr. range finding experimental period. Fish was assumed to be dead when there was no body or operculum movement when prodded with a glass rod.

Serial dilutions were made of various concentrations of the water soluble fractions of used crankcase oil based on this preliminary assay. From the acute toxicity test results, sublethal concentrations were determined from the 96hr- LC50 obtained by several dilutions of the wsf of the used crankcase oil. Starting from a sub-lethal concentration of 140ml/L, the preceding concentration was half the succeeding one in descending order according to the method used by Ayoola and Alajabo (2012) and Ashade and Kumoyi (2013). These sublethal concentrations served as the definitive concentrations for the ten weeks investigation period.

3.2 TREATMENTS OF FISH GROUPS FOR TOXICITY TEST

The experimental setup which was replicated and made up of six 100 litre rectangular glass aquaria contained five sub-lethal concentrations and one aquarium

contained clear dechlorinated tap water as control. Each aquarium had 10 mixed sex fingerlings of *Oreochromis niloticus* introduced into it according to the following definitive sublethal concentrations: 140, 70, 35, 17.5, 8.75 and 0.00 ml/L which served as the control. The fingerlings were fed twice daily, morning and evening at 3% body weight on commercial feed (Coupens) between 8 o'clock in the morning and 6 o'clock in the evening according to the method used by Nehemiah, Maganira & Rumisha (2012).

3.3 DETERMINATION OF WATER QUALITY PARAMETERS OF THE EXPERIMENTAL AQUARIA

The following water quality parameters were measured during the period of this investigation: Temperature °C, Free- Carbon dioxide (CO₂), pH, Dissolved Oxygen (DO), Total Alkalinity, Total Hardness, Ammonia and Metallic Ions namely: Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), Chromium (Cr), Lead (Pb), and Aluminium (Al). The determination of water quality parameters was carried out using American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Pollution Control Federation (WPCF) (2005) methods according to the specific parameters assessed.

3.3.1 Temperature

The temperature of water was measured daily morning, afternoon and evening in triplicate for each experimental group using a mercury centigrade dry bulb thermometer. The thermometer was dipped 5 cm below the water and held in that position for 2 minutes to equilibrate before readings were taken according to the standard methods of American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Pollution Control Federation (WPCF) (2005). After every week, average temperatures were calculated and this was used to

obtain single mean temperature values for each experimental group during the period of investigation.

3.3.2 Dissolved Oxygen (DO)

The DO was determined using the Alstebeg (Azide) method. Water samples were collected in 250ml stoppered bottles. The bottle was corked inside the water to avoid any trapping of air bubbles for each round of test carried out. The water was then fixed by adding 2ml of Manganese sulphate and 2 ml of alkaline-iodide (Sodium Azide). The sample bottle was re-stoppered and a careful shaking of the bottle was done for proper mixing of the contents. This was allowed to settle for about five minutes then 2ml of conc. sulphuric acid was added. This was shaken carefully until a solution was formed. A quantity of 200ml of the solution was transferred into a conical flask and titrated to pale yellow using 0.025 of Sodium thiosulphate. 1ml of 1% starch solution was then added resulting in a blue-coloured solution instantly. Further titration was carried out until the blue colour disappeared. The value of the 0.025N Sodium thiosulphate used in the titration was recorded as the amount of oxygen in the water sample, American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Pollution Control Federation [WPCF] (2005).

3.3.3 Carbon Dioxide (CO₂)

Free Carbon dioxide was determined using titrimetric method. 100ml of water sample was collected in a graduated cylinder and was allowed to overflow. Ten drops of phenolphthalein indicator were then added to it. No colour change was observed, indicating the presence of free carbon dioxide: the appearance of a red colour at the addition of the indicator shows the absence of CO₂. The colourless solution was titrated rapidly with standard alkaline solution (0.4N NaOH). It was stirred gently with a

stirring rod until a pink colour persisted for more than 30 seconds. The quantity of dissolved CO₂ per liter of water was calculated using the formula:

$$\text{ml of CO}_2 = \frac{V \times M \times 4000}{\text{water sample (ml/L)}}$$

Where V = ml of Sodium hydroxide solution

M = molarity or concentration of NaOH used,

American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Pollution Control Federation [WPCF] (2005).

3.3.4 pH

This is a measure of the hydrogen ion concentration (H⁺) of water for each experimental group as determined weekly using a pH meter, Luthron Model-201. The measurements were calculated to give a single pH mean for each experimental group.

3.3.5 Total Alkalinity

To determine this parameter, two drops of Methyl orange indicator was added to 50 ml of the water sample in a conical flask. This was then titrated against 0.02N sulphuric acid until an orange colour was observed. Total alkalinity was calculated using the formula:

$$\text{Total alkalinity (ml/L)} = \frac{\text{titre value} \times 0.002 \text{ NH}_2\text{SO}_4 \times 1000}{\text{ml of water sample used}}$$

American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Pollution Control Federation [WPCF] (2005).

3.4 ANALYSIS OF THE ELEMENTAL COMPOSITION OF USED CRANKCASE OIL

The analysis of the used crankcase oil to detect the elemental components was carried out at the Centre for Energy Research, Ahmadu Bello University, Zaria. The process uses a nuclear reactor named Nigeria Research Reactor 1 (NIRR – I) which employs a multi – element analysis and the neutron activation method. NIRR – 1 is a Miniature Neutron Source Reactor (MNSR) and has a tank-in-pool structural configuration with a nominal thermal power rating of 31KW. Due to its neutron activation analysis (NAA), this nuclear reactor is capable of analyzing trace, minor and major elements in different sample matrices as described by Jonah, Umar, Oladipo, Balogun and Adeyemo (2006).

3.5 DETERMINATION OF THE PROXIMATE COMPOSITION OF FISH TISSUES EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

Analysis of proximate composition of tissues of the experimental fish was carried out using the method described by the Standard procedures of the Association of Official Analytical Chemists (AOAC, 2005).

3.5.1 Moisture Content

Fish tissue weighing 2.0g was taken from each experimental group/sublethal concentration. Each sample was weighed & oven-dried in a hot air oven to a constant weight at 105°C for 24 hours. Each sample was cooled in a desiccator and re-weighed. The loss in the weight of sample was recorded as the moisture content which was further expressed as a percentage of the original weight.

$$\% \text{ Moisture} = \frac{\text{loss in weight due to oven-drying (g)}}{\text{Fresh weight (g)}} \times 100$$

(AOAC, 2005)

3.5.2 Ash Content

Small amounts of dried fish samples (2.0g) were weighed and put in pre-dried crucible. Each sample was properly covered and the content burnt in a muffled furnace at a temperature of 600°C for 24 hours. The sample was cooled in a desiccator and reweighed. The final weight of each sample was taken as the ash content which was further expressed as a percentage of the original weight.

$$\% \text{ Ash} = \frac{G - A}{C} \times 100$$

where G = weight of crucible + ash

A = weight of crucible

C = weight of sample before ashing

(AOAC,2005).

3.5.3 Crude Protein Content

A pre-weighed sample of 2.0g dried fish powder was put in a Kjeldah flask and 2.0g of catalyst mixture was added. 10ml of conc. Sulphuric acid (H₂SO₄) was added, and the flask was heated for a few minutes until the mixture ceased to froth. Heat was then increased to digest the sample in two hours. The set-up was allowed to cool down after which it was diluted with distilled water to a known volume for each sample. Some 10ml of the diluted solution was pipeted into a micro-kjeldal distillation apparatus and 10ml of 45% Sodium hydroxide solution was added, steamed, and distilled into 10.0ml of 2% Boric acid containing mixed indicator before it was titrated with the standard 0.01N Hydrochloric acid grey end point.

$$\text{Crude protein (\%)} = \frac{(a - b) \times 0.01 \times 14.01 \times c \times 100}{d \times e} \times 6.25$$

Where: a = Titre value of sample

b = Titre value of blank

c = Volume to which digest is made up with distilled water

d = Aliquot taken for distillation

e = Weight of dry sample (mg), (AOAC, 2005).

3.5.4 Lipid Content

A sample of 2.0g of fish tissue was weighed and transferred into a fat-free extractor thimble plugged tightly with cotton wool. The thimble was placed in the extractor and petroleum ether (boiling point 80°C) was added until it distilled over once. More petroleum ether was added until the barrel of 300ml extractor was half full. The condenser was replaced; the joint was tightly fixed up and placed in a water bath. The source of heat was adjusted so that the ether boiled gently and had it distilled over. When the ether just stopped siphoning over, the flask was detached; the content of the barrel of extractor was siphoned into the petroleum ether stock bottle. The condenser and the flask were replaced and distillation continued until the flask was practically dry. The ether extract was determined by:

$$\% \text{ Ether extract} = \frac{\text{weight of oil}}{\text{weight of biological materials}} \times \frac{100}{1}$$

(AOAC,2005)

3.6 DETERMINATION OF CARBOHYDRATE RESERVES IN THE TISSUES OF *O. NILOTICUS* FINGERLING EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

Muscle and liver glycogen of the control and treatment of the fish groups were assayed by the Anthrone method as described by Wedemeyer and Yasutake (1977):

3.6.1 Muscle Glycogen

A weight of 100 mg of muscle was boiled in a test tube containing 3.0ml of 30% Potassium hydroxide (KOH) until it dissolved completely in about 20 minutes.

0.5ml of saturated Na₂SO₄ and 3.5ml of 95% Ethanol were added and heated to boiling point in hot water. The set-up was allowed to cool before centrifuging at 600 rpm and the supernatant discarded. The glycogen was dissolved in 2.0ml of distilled water and reprecipitated with 2.5ml of 95% Ethanol. The sample was again centrifuged and the supernatant decanted. The precipitated glycogen was hydrolyzed for 30 minutes in 2.0ml of 5M HCl in a boiling water bath.

The hydrolyzate was cooled and neutralized with 0.5M NaOH, a drop of Phenol red was added as indicator before titration according to the method of Wedemeyer and Yasutake (1977). The neutralized solution for the test was diluted to a known volume of 100ml depending on the expected glycogen content. 5.0ml of the diluted hydrolyzate was transferred into a test tube. A glucose standard of 5.0ml was transferred into another test tube and 5.0ml of distilled water was transferred into a third test tube. These test tubes were immersed in cold water while 10.0ml of Anthrone reagent was added to each test tube. The test tubes were capped with glass marbles for 10 minutes in boiling water. These were cooled and the absorbance of the samples was read at 620nm wavelength in a colorimeter. The muscle and liver glycogen were calculated in mg/L as follows:

$$\text{Liver or Muscle glycogen (mg/L)} = \frac{\text{Au (Cs)}}{\text{As}}$$

Where:

Au = Absorbance of Unknown, As = Absorbance of Standard

Cs = Concentration of Standard, Wedemeyer and Yasutake (1977).

3.6.2 Liver Glycogen

A weight of 100 mg of liver was boiled in a test tube containing 3.0ml of 30% Potassium hydroxide (KOH) until it dissolved completely in about 20 minutes. 0.5ml of

saturated Na₂SO₄ and 3.5ml of 95% Ethanol were added and heated to boiling point in hot water. The set-up was allowed to cool before centrifuging at 600 rpm and the supernatant discarded. The glycogen was dissolved in 2.0ml of distilled water and reprecipitated with 2.5ml of 95% Ethanol. The sample was again centrifuged and the supernatant decanted. The precipitated glycogen was hydrolyzed for 30 minutes in 2.0ml of 5M HCl in a boiling water bath.

The hydrolyzate were cooled and neutralized with 0.5M NaOH, a drop of Phenol red was added as indicator before titration according to the method of Wedemeyer and Yasutake (1977). The neutralized solution was diluted to a known volume of 100ml, 5.0ml of the diluted hydrolyzate was transferred into a test tube. A glucose standard of 5.0ml was transferred into another test tube and 5.0ml of distilled water was transferred into a third test tube. These test tubes were immersed in cold while 10.0ml of Anthrone reagent was added to each test tube. The test tubes were capped with glass marbles for 10 minutes in boiling water. These were then cooled and the absorbance of the samples was read at 620nm wavelength in a colorimeter. The liver glycogen was calculated in mg/L as follows:

$$\text{Liver glycogen (mg/L)} = \frac{\text{Au (Cs)}}{\text{As}}$$

Where:

Au = Absorbance of Unknown, As = Absorbance of Standard

Cs = Concentration of Standard, Wedemeyer and Yasutake (1977).

3.7 GROWTH AND FEED UTILIZATION OF *O. NILOTICUS* FINGERLING EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The growth parameters of the experimental fish were determined using standard methods. The weight and length were measured using a Mettler balance and a

centimeter rule respectively according to the method of Nehemiah *et al.* (2012) and Mortuza and Misned (2013) to ascertain any increase or decrease in the parameters during the period of the investigation.

3.7.1 Length-Weight Measurements of *O. niloticus* Fingerlings Exposed to the Sublethal Concentrations of Used Crankcase Oil

The total length of the fish groups was measured by placing the fish laterally on a dissecting board and using a centimeter rule to take the measurement. This was done fortnightly and averages were recorded. A total of twenty fingerlings were used in each experimental group for the morphometric measurements. The means were subjected to statistical analysis using one-way Analysis of Variance ANOVA and correlation coefficient.

3.7.2 Specific Growth Rate (SGR) is a term used in aquaculture to estimate the production of fish after a certain period. It is expressed in weight at harvest–weight at stocking/production period x 100.

SGR was computed as follows:

$$\frac{\log_e W_2 - \log_e W_1}{T - t} \times 100$$

Where W1 = Initial weight (g) at time t, W2 = Final weight (g) at time T.

Cook, McNiven, Richardson and Sutterlin (2000).

3.7.3 Food Conversion Ratio (FCR)

The FCR was calculated as:

$$\frac{\text{Weight gained per week (g)}}{\text{Feedstuff consumed per week (g)}} \text{USAID (2011).}$$

3.8 DETERMINATION OF GONADAL DEVELOPMENT OF *O. NILOTICUS* FINGERLINGSEXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

Sequel to size and age factors, the gonadosomatic index (GSI) of the experimental fish could not be determined. Under normal circumstances the effects of the use of used crankcase oil on *O. niloticus* fingerlings can be investigated by excising and weighing the gonads to the nearest milligramme (mg). The gonadosomatic index (GSI) can then be computed following the method described by Wahbeh and Ajiad

(1985) and Ikomi (1990) thus:
$$GSI = \frac{\text{Gonad wt (g)}}{\text{Fish wt (g)}} \times 100$$

3.9 MEAN CONDITION FACTOR OF *O. NILOTICUS* FINGERLINGS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The mean condition factor for the experimental fish was computed using the formula:

$$K = \frac{W}{L^3} \times 100 \text{ where } K = \text{condition factor}$$

W = weight, L = length, following the method described by Wahbeh and Ajiad (1985), Ikomi (1990).

3.10 BIOACCUMULATION OF METALS IN THE TISSUES OF *O. NILOTICUS* FINGERLINGSEXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL AFTER TEN WEEKS

Some 10 mg each of the gills, muscle and liver tissues of *O. niloticus* fingerlings exposed to sublethal concentrations of used crankcase oil was digested with nitric acid. 5ml of hydrogen peroxide was added to further digest it. The required volume of 100 ml was then made up by adding deionized water and then stored in a polypropylene container. The concentrations of heavy metals were determined by an AAS SOLAR 969 Unicam (ThermoFisher iCE3000 series) at the Sheda Science and Technology Complex (SHESTCO), Abuja Nigeria.

3.11 DETERMINATION OF HAEMATOLOGICAL INDICES OF *O.NILOTICUS* FINGERLINGS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL AFTER TEN WEEKS

The blood of *O. niloticus* was collected from each experimental tank for the evaluation of haematological parameters according to the method of Meyer *et al.*(1992). Haematological indices of *O. niloticus* were determined using an Auto-Haematology Analyzer, Model Kz Erma 2700. The analyses was carried out in the Haematology Laboratory of Asokoro, General Hospital FCT Abuja, Nigeria. Blood samples were collected from the caudal peduncle into heparinised tubes according to the method of Meyer, Coles and Rich (1992). The blood was drawn into EDTA bottles, and the whole blood sample was introduced into the probe needle of the analyser. The probe needle then injected the blood into the analyzing sensor directly. The ‘analyze key’ was pressed and the machine ran the test automatically, in less than 30 seconds the full blood counts were displayed on the bright screen.

3.12 DETERMINATION OF THE ACTIVITIES OF SOME SELECTED ENZYMES IN THE ORGANS OF *O.NILOTICUS* FINGERLINGS

A known weight of 1g each of tissues from the gills, liver and muscle of the fish were taken and used for the determination of the activities of Alkaline Phosphatase (ALP) and Alanine Aminotransaminase (ALAT) according to the method used by Aziz and Azmat (2011). The tissues were homogenized in a Polytron type homogenizer in ice cold conditions at 4⁰C. The homogenates strength was adjusted to 10 % with 0.25 sucrose solution and used as the source of enzymes. The biochemical analysis involved the use of Biosystem Kits in the determination of the activities of ALP and ALAT. The activities of these two enzymes were measured in an Autoanalyzer Biosystem A25 Model, Spain. The results are represented as mean \pm standard error of mean (S.E.M).

3.13 STATISTICAL ANALYSES

Statistical tests were carried out to help in the interpretation of data obtained during this investigation. One-way analysis of variance (ANOVA) was used to interpret the data on the mean concentration of metals in the experimental water and fish organs as well as the variations in mean values of liver and muscle glycogen and the growth performance and feed utilization of the experimental fish. This was used to check the data for replicate tanks to ascertain the significant differences between the different treatments at the 0.05 confidence level.

The degree of association between mean length and mean weight of *Oreochromis niloticus* was measured using the correlation coefficient (r). This is given by the formula:

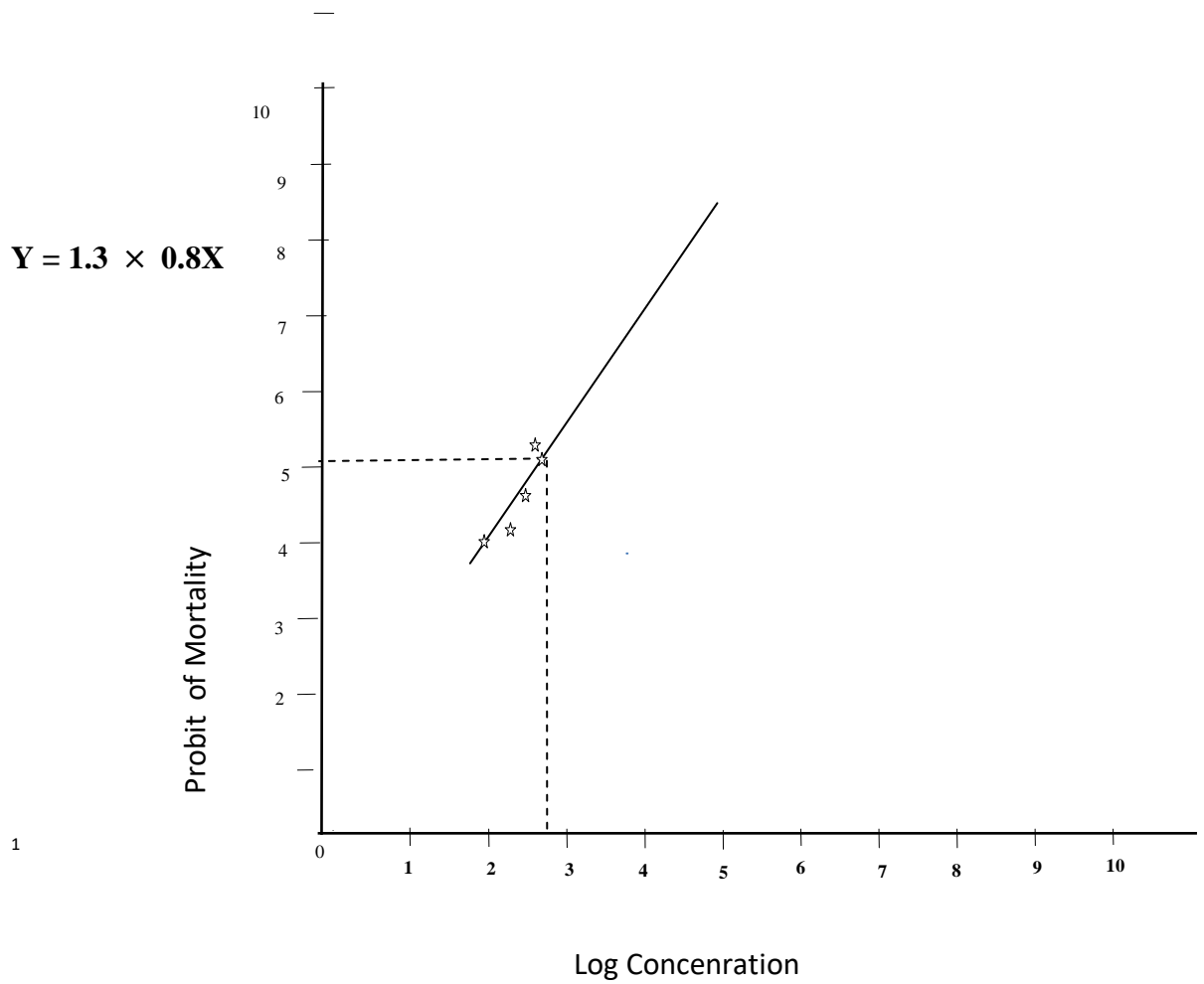
$$r = \frac{\Sigma xy - (\Sigma x \Sigma y / n)}{\sqrt{(\Sigma x^2 - (\Sigma x)^2 / n)(\Sigma y^2 - (\Sigma y)^2 / n)}} \quad \text{Statistics solutions (2008).}$$

Mean length was represented by x and mean weight by y and statistical analysis was performed using SPSS programme v.19 x 86 running on windows 7 x 86 (Developers: IBM Corporation, USA).

CHAPTER FOUR RESULTS

4.1 ACUTE TOXICITY/SUBLETHAL TESTS OF WSF OF USED CRANKCASE OIL ON *O. NILOTICUS* FINGERLINGS

The acute and sublethal tests were performed following the Organization for economic Cooperation and Development (OECD) guideline number 203 [OECD] (1992) for fish acute bioassays. The range-finding values for the preliminary assay in the 796hrs acute toxicity test were 0.00, 150.00, 300.00, 450.00, 600.00 and 750.00 ml/L while the definitive values for the sublethal concentrations were 0.00 (control), 8.75, 17.50, 35.00, 70.00 and 140.00 ml/L. Susceptibility of *O. niloticus* fingerlings to the impact of the wsf of used crankcase oil was found to increase in mortality with increase in the concentration of the toxicant. In the control, which had dechlorinated tap water, mortality was virtually absent (Fig.1, Appendix B1). Results based on probit analysis gave the median lethal concentration (LC50) of the used crankcase oil to *O. niloticus* fingerlings for the 96hr exposure as 630ml/L. Linear relationship between the probit mortality and the concentration of used crankcase oil indicated a positive correlation and showed a significant difference ($P < 0.05$) indicating that mortality rate of exposed fish increased as the concentration of used crankcase oil also increased. No adverse behavioural changes or any mortality were recorded in the control fish throughout the period of the bioassay. But symptoms of toxicosis observed in fish behaviour in the sublethal concentrations include erratic swimming, restlessness, gulping of air, lack of balance, frequent bottom to surface movement and excessive secretion of mucus. Some of the exposed fish became weak, settled at the bottom of the tank and died.



1

Figure 1 Linear Relationship Between Probit Mortality and Log Concentration of *O. niloticus* Fingerlings Exposed to Used Crankcase Oil After 96 Hrs.

4.2 DETERMINATION OF WATER QUALITY PARAMETERS

Water quality parameters during the period of investigation can be seen in Table 2. Water temperature was measured two minutes after dipping an ordinary mercury-in-glass thermometer to a depth of about 5.0cm below the water surface. Temperature values ranged between 24.01 – 24.40°C with a mean of 24.17°C. Dissolved oxygen (DO) had a range of 2.05 – 6.75ml/L, with a mean of 5.05ml/L; pH ranged between 6.00 – 8.40 with a mean at 6.84. Alkalinity values ranged between 3.25 – 6.50 ml/L with a mean of 4.70 ml/L. Conductivity ranged from 6.01 – 6.03 µmhos with a mean of 6.02 µmhos. Free carbon – dioxide was in the range of 4.30 – 4.32 ml/L and a mean of 4.31 ml/L. Ammonia had a value range of 0.12 – 0.29mg/L with a mean of 0.17 mg/L while the range of values for phosphate was 0.27 – 0.83 mg/L and the mean was 0.57mg/L. The values for nitrate ranged between 0.01 – 0.15 mg/L with a mean at 0.04 mg/L. Water hardness ranged between 35.00 – 106 mg/L with a mean of 62.00mg/L. The analysis of water quality parameters presented in Table 2 showed no significant difference ($P > 0.05$) between the means of the various concentrations of the used crankcase oil.

4.3 VARIATIONS IN MEAN VALUES OF METALS IN THE EXPERIMENTAL WATER AFTER TEN WEEKS

Fig. 2, Appendix B4 show the trends in the elemental composition of the water in the experimental medium. Some of these values are high compared to the World Health Organization (WHO) values allowed in drinking water (Appendix B2). Calcium had the highest value of 4.81mg/L in the sublethal concentrations followed by Magnesium with 2.86mg/L, Iron, 2.84mg/L; Copper and Chromium both had the least value of 0.01mg/L each. The highest and the lowest values of metals were obtained in the highest sublethal concentration and the control tanks respectively.

Table 2
Mean Values of Water Quality Parameters Analysed During the Ten Weeks Experimental Period

Water Parameters	Concentrations of Used Crankcase Oil					
	0.00	8.75	17.50	35.00	70.00	140.00
Temperature (°C)	24.40 ±0.03	24.20±0.03	24.20±0.03	24.10±0.03	24.00±0.03	24.10±0.03
Ph	6.00 ±0.01	6.20±0.01	6.40±0.01	6.60±0.01	7.45 ±0.01	8.40±0.01
DO (m/gL)	6.50±0.01	6.35±0.01	6.75 ±0.02	5.40±0.01	3.26±0.02	2.05±0.02
CO ₂ (mg/L)	4.32 ±0.01	4.31±0.01	4.32±0.01	4.31±0.01	4.30±0.01	4.30±0.01
Alkalinity (mg/L)	3.25±0.01	3.45±0.01	4.21±0.01	5.33±0.01	5.48±0.01	6.50±0.01
Conductivity (µm hos)	6.01	6.01	6.03	6.02	6.02	6.03
Ammonia (mg/L)	0.12 ±0.01	0.12±0.01	0.12±0.01	0.18±0.02	0.29±0.02	0.17±0.01
Phosphate (mg/L)	0.27± 0.01	0.46± 0.02	0.48± 0.02	0.57± 0.02	0.81± 0.03	0.83± 0.03
Nitrate (mg/L)	0.01 ±0.003	0.01 ±0.003	0.15 ±0.006	0.01 ±0.003	0.04 ±0.001	0.01 ±0.003
Hardness (CaCO ₃)	35.00	50.00	50.00	56.00	75.00	106.00

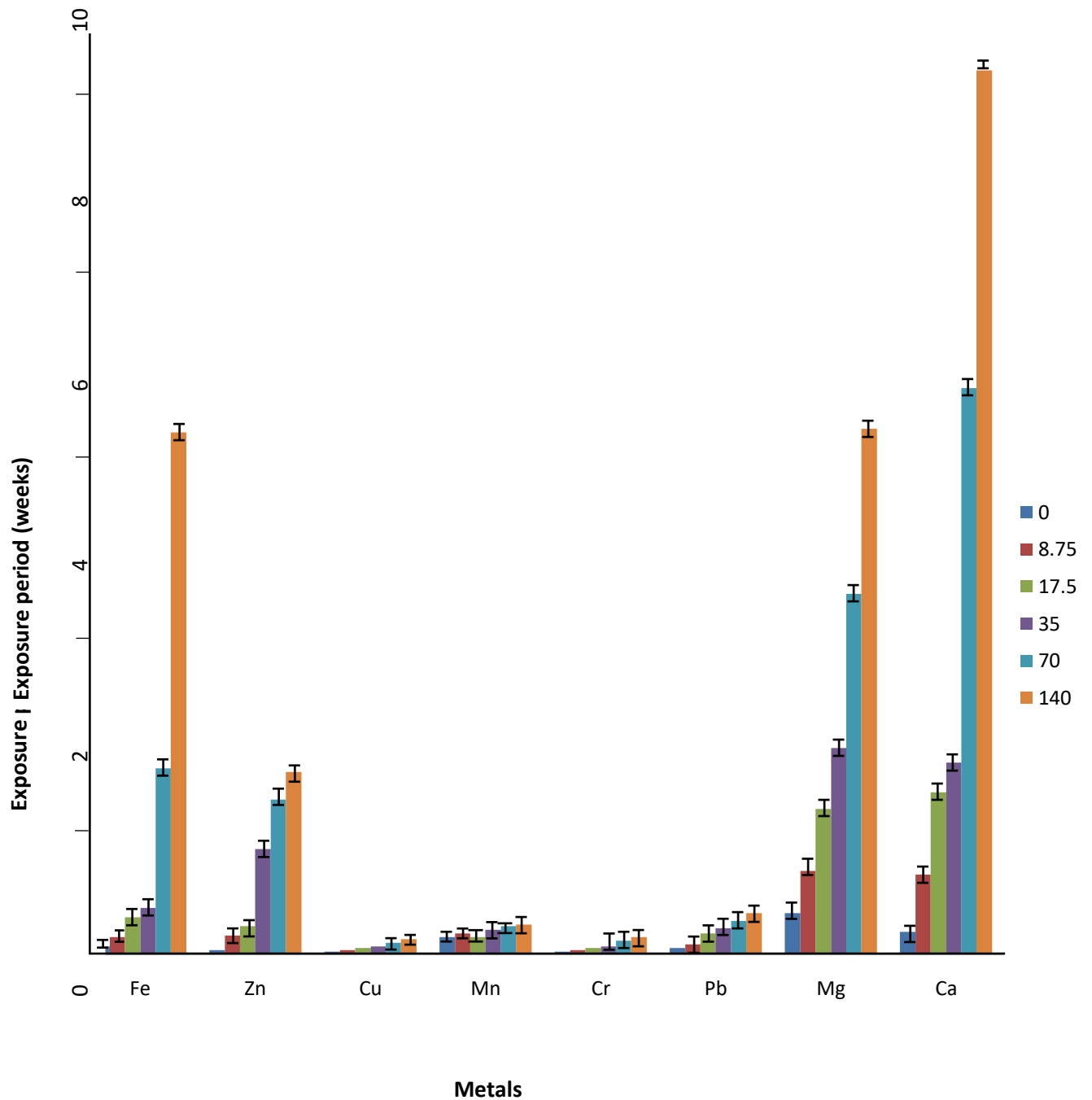


Figure 2 Variations in Mean Values of Metals in the Experimental Water after Ten Weeks

4.3.1 Analysis of the Elemental Composition of Used Crankcase Oil

Analysis of Used crankcase oil using the Nigeria Research Reactor 1 (NIRR-1) and the Atomic Absorption Spectrophotometer (AAS) revealed a variety of elements and compounds. Calcium had the highest value of 1467 ± 374 mg/L followed by Zinc with 384.2 ± 61.7 mg/L, then Chlorine came next with a concentration of 228.7 ± 38.7 mg/L. Sodium had a concentration of 116.6 ± 2.5 mg/L, Iron measured 98.95 mg/L while Silicon had a concentration of 85.00 mg/L and Aluminum was 59.9 ± 5.5 mg/L followed by Copper with 29.09 mg/L then Manganese, Bromine and Lead with the least concentrations of 3.84 ± 0.28 , 2.1 ± 0.1 , and 1.27 mg/L respectively. Metals such as Magnesium (Mg), Tin (Sn) and Potassium (K) were below detection level (BDL) in both NIRR-1 and AAS analysis, while Iron (Fe), Copper (Cu), Lead (Pb) and Silicon (Si) were below detection level in NIRR-1, Aluminium (Al), Calcium (Ca), Sodium (Na), Manganese (Mn), and Zinc (Zn) were not detected by the AAS machine (Table 3).

4.4 PROXIMATE COMPOSITION OF THE TISSUES OF *O. NILOTICUS* FINGERLINGS EXPOSED TO WSF OF USED CRANKCASE OIL

At the end of the exposure period, it was observed that *O. niloticus* fingerlings had significant decreases ($P < 0.05$), in protein and lipid contents and proportionately to the sublethal concentrations of the used crankcase oil as the exposure period progressed as can be seen in Tables 4 and 5. The protein and lipid contents were however the same at the beginning of the bioassay. Decreases in protein content were at 28.06, 19.70, 11.95, 9.46 and 6.72, % dry weight (DW) in descending order of the sublethal concentrations of used crankcase oil. The fish in the control tank increased in protein content by 9.93 % DW from the initial 58.08 to 68.01% DW. Lipids reduced in content from 16.12 at the beginning of the bioassay to 9.02, 10.34, 11.64, 14.02, and 15.15, (%
Table 3

Analyses of the Elemental Composition of Used Crankcase Oil

Elements	*NIRR-1 Values (mg/L)	Atomic Absorption Spectrophotometer (AAS Values) (mg/L)
Aluminium, (Al)	59.9 ± 5.5	BDL
Tin, (Sn)	*BDL	BDL
Calcium, (Ca)	1467 ± 374	BDL
Magnesium,(Mg)	*BDL	BDL
Potassium, (K)	*BDL	BDL
Iron, (Fe)	*BDL	98.95
Sodium, (Na)	116.6 ± 2.5	BDL
Manganese (Mn)	3.84 ± 0.28	BDL
Chlorine, (Cl)	228.7 ± 38.7	BDL
Zinc, (Zn)	348.2 ± 61.7	BDL
Bromine, (Br)	2.1 ± 0.1	BDL
Copper, (Cu)	BDL	29.09
Lead, (Pb)	BDL	1.27
Silicon, (Si)	BDL	85

*BDL = Below Detection Level *NIRR-1 =Nigeria Research Reactor-1 values

*AAS = Atomic Absorption Spectrophotometer

Table 4

Variations in Mean Values of Carcass Protein Content of *O. niloticus* Exposed to Sublethal Concentrations of Used Crankcase Oil (%)

Conc. (ml/L)	Start	Exposure Period (weeks)				
		2	4	6	8	10
140.00	58.08±0.02	50.16±0.03	46.27±0.03	39.05±0.04	35.23±0.04	30.02±0.01
70.00	58.10±0.01	52.26±0.01	48.31±0.02	49.06±0.03	47.85±0.03	44.35±0.01
35.00	58.07±0.02	54.65±0.01	51.02±0.02	51.31±0.02	51.59±0.01	46.12±0.03
17.50	58.09±0.03	54.92±0.01	52.21±0.02	53.76±0.01	53.06±0.02	48.63±0.02
8.75	58.08±0.02	56.91±0.02	52.23±0.01	55.20±0.02	55.05±0.01	51.36±0.02
0.00	58.08±0.02	58.23±0.03	59.92±0.02	61.23±0.03	64.49±0.01	68.01±0.02

Table 5

Variations in Mean Values of Carcass Lipid Content of *O. niloticus* Exposed to Sublethal Concentrations of Used Crankcase Oil (%)

Conc. (ml/L)	Exposure Period (weeks)					
	Start	2	4	6	8	10
140.00	16.02±0.02	13.08±0.01	13.06±0.01	13.00±0.02	12.24±0.01	9.02±0.01
70.00	16.±0.03	13.09±0.04	13.07±0.01	13.05±0.03	13.00±0.03	10.34±0.02
35.00	16.12±0.01	13.25±0.01	13.65±0.02	13.07±0.03	13.03±0.03	11.64±0.03
17.50	16.13±0.01	13.25±0.03	13.25±0.03	13.20±0.04	13.15±0.01	14.02±0.03
8.75	16.12±0.02	13.25±0.02	13.50±0.03	13.25±0.03	13.20±0.03	15.15±0.04
0.00	16.12±0.03	13.65±0.04	13.55±0.03	13.96±0.01	13.96±0.01	20.07±0.02

DW) from the lowest to the highest sublethal concentration of the used crankcase oil at the end of the exposure period as recorded in Tables 4 and 5, pages 53 and 54

respectively. The *O. niloticus* fingerlings group in the control tank had significant increases in lipid content also throughout the exposure period. Lipid content increased from 16.12 at the start of the experiment to 20.07 at the end of the investigation in the control tank.

4.5 EFFECTS OF THE WSF OF USED CRANKCASE OIL ON THE GROWTH PERFORMANCE AND FEED UTILIZATION OF *O. NILOTICUS* FINGERLINGS EXPOSED TO THE WSF OF USED CRANKCASE OIL AFTER TEN WEEKS

The result of the mean length and weight of fish exposed to the various concentrations of used crankcase oil during the period of 10 weeks can be seen in Table 7. Statistical analysis showed significant differences ($P < 0.05$) in the weight of the different fish groups in the sublethal concentrations investigated. After ten 10 weeks exposure, it was observed that the fish groups exposed to the concentrations of 140.00, 70.00, 35.00 and 17.50 ml/L of toxicant significantly ($P < 0.05$) reduced in weight with values of 1.10, 1.50, 1.60 and 1.80g respectively. The fish groups exposed to the control of 0.00 concentration and 8.75ml/L of toxicant significantly increased in weight during the exposure period from 6.30g to 20.10g and 16.81g in that order (Fig. 3, Appendix B3). The percentage weight-gain for fish in the control tank was 68.66 % while the lowest concentration had 41.67 % weight-gain (Table 6).

Mean weight increases over the 10 weeks period of the bioassay were 13.80 and 10.53g for control (0.00 ml/L) and the least concentration of 8.75 ml/L respectively. Fish in the control tank and the lower toxicant concentration tanks of 8.75 mg/L and 17.50 mg/L had percentage weight gains of 68.66, 41.67 and 40.00 respectively. Analysis of variance indicated a significant difference ($P < 0.05$) in the weight gain of

Table 6
Growth Performance and Feed Utilization of *Oreochromis niloticus* Fingerlings during the Exposure Period

	Concentration of used crankcase oil (ml/L)					
	0.00	8.75	17.50	35.00	70.00	140.00
Growth parameters						
Initial Weight (g)	6.30 ± 0.1	6.30 ± 0.1	6.30 ± 0.1	6.30 ± 0.1	6.30 ± 0.1	6.30 ± 0.1
Final Weight (g)	20.10 ± 0.54	10.80 ± 1.20	4.50 ± 0.65	4.70 ± 0.60	4.80 ± 0.44	5.20 ± 1.22
Weight Gain (g)	13.80 ± 0.90	4.50 ± 0.65	1.80 ± 0.84	1.60 ± 0.20	1.50 ± 0.22	1.10 ± 0.75
% Weight-Gain	68.66	41.67	40.00	34.04	31.25	21.15
FCR	1.33 ± 0.06	2.42 ± 0.60	2.99 ± 0.06	3.08 ± 0.24	3.20 ± 0.05	3.20 ± 0.01
PER	1.33 ± 0.01	1.03 ± 0.02	1.01 ± 0.02	0.90 ± 0.01	0.60 ± 0.01	0.24 ± 0.01
SGR	0.91 ± 0.01	0.75 ± 0.04	0.74 ± 0.02	0.35 ± 0.01	0.01 ± 0.01	0.01 ± 0.01

± = standard error of the means

fish in the control and the least concentration of toxicant. This is irrespective of the fact that both fish groups increased in weight. The feed conversion ratio (FCR) for the control and the lower sublethal concentrations of 0.00, 8.75, and 17.5 ml/L were 1.33, 2.42 and 2.99, respectively. While the higher sublethal concentrations of 35.00, 70.00 and 140 ml/L had FCR values of 3.08, 3.20 and 3.20 in the ascending order of the concentrations (Table 6).

The correlation coefficient (r) of mean weight and length of the experimental fish showed a strong positive correlation between the weight and length with $r = 0.861$, $n = 36$ (Appendix B15). The analysis of variance of growth performance between groups and within groups was significantly different at the 0.05 confidence level during the ten weeks exposure period.

4.6 EFFECTS OF SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL ON THE CARBOHYDRATE RESERVES OF *NILOTICUS* FINGERLINGS

Statistical analysis showed significant differences ($P < 0.05$) between the means of the fish groups observed. After the 10 weeks investigation period, observations showed that fish groups exposed to 140.00, 70.00, 35.00, 17.50, and 8.75 ml/L of the used crankcase oil had significant reduction in quantity of liver glycogen, with values at 0.86, 0.91, 0.66, 0.78 and 0.64 ($P < 0.05$) respectively (Figs. 9-14).

4.6.1 Muscle Glycogen

Results showed that the mean values of muscle glycogen of the fish groups in the various sublethal concentrations decreased significantly ($P < 0.05$) as the weeks of exposure progressed. This decrease is proportional to the sublethal concentrations as could be seen in tanks with concentrations of 140.00, 70.00, 35.00 and 17.50 ml/L of toxicant. The mean values showing reduction were 0.06, 0.07, 0.04 and 0.06 respectively. These fish groups had the same values of liver and muscle glycogen at the beginning of the investigation. However, the mean values of the liver glycogen in the

control group (0.00 ml/L) increased significantly ($P < 0.05$), throughout the period of the experiment (Figs. 9-14).

The mean value of muscle glycogen in the least concentration tank of 8.75 ml/L showed no significant decrease in the first two weeks of the exposure period. At the start of the exposure period, the mean values of the muscle glycogen were the same. However, as the investigation progressed, the mean values of the muscle glycogen increased significantly ($P < 0.05$) in the control group.

4.6.2 Liver Glycogen

The mean values of liver glycogen of *O. niloticus* fingerlings in the various sub-lethal concentrations had a significant decrease ($P < 0.05$) as the weeks of exposure progressed. The decrease in liver glycogen corresponded to the sublethal concentrations as could be seen in tanks with concentrations of 140.00, 70.00, 35.00, and 17.50 ml/L of toxicant. The mean values showing reduction were 0.93, 0.65, 0.47, and 0.41, respectively. These fingerlings had the same values of liver glycogen at the beginning of the investigation. However, the mean value of the liver glycogen in the control group (0.00) ml/L increased significantly ($P < 0.05$), throughout the period of the experiment. The mean value of liver glycogen in the least concentration tank of 8.75 ml/L showed no significant decrease in the first two weeks of the exposure period. At the start of the exposure period, the mean values of the liver glycogen were the same. However, as the investigation progressed, the mean values of the liver glycogen increased significantly ($P < 0.05$) in the control group. At the highest sublethal concentration of this investigation, liver glycogen decreased to a mere 0.30mg/L. This may be attributed to poor feed conversion ratio as well as increased metabolism due to detoxification reaction and impaired health. Mean value of liver glycogen maintained almost the same level of 1.2mg/L to the fourth week, then increased to 2.12mg/L in the final week of exposure in the control tank. It is only in this control group that the glycogen level

increased appreciably during the experimental period. The value of liver glycogen and muscle glycogen showed a significant decrease ($P < 0.05$) in the higher sub-lethal concentrations of the used crankcase oil when compared with fish in the control tank. Figures 3 - 8 above show a significant decrease in the value of mean muscle glycogen starting from the beginning to the last three weeks of the investigation. A gradual and progressive decrease in values of muscle glycogen continues as the weeks of investigation progressed. The sublethal concentrations of the used crankcase oil of 17.50 ml/L showed a very low value of muscle glycogen of 0.01 mg/L at the beginning of the experiment, which later rose to 0.09 mg/L and finally decreased to a value of 0.05 mg/L. The same general trend of values decreasing with increasing time can be observed in Figs. 3-8 with the values of liver glycogen during the exposure period.

4.7 EFFECTS OF USED CRANKCASE OIL ON THE TOTAL LENGTH AND WEIGHT OF *OREOCHROMIS NILOTICUS* FINGERLINGS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

There was a strong positive correlation ($r = 0.861$) between length and weight of fish groups in the control (0.00 mg/L). As the weight of fish increased so also did the length in the control group. But in the highest sublethal concentration, while the weight of fish decreased, the length remained constant. Fig. 15, Appendix B3 show the mean total length of the fish groups exposed to various concentrations of toxicant for the period of experiment. At the end of the exposure period, it was observed that the fish groups exposed to 140.00, 70.00, 35.00 and 17.50 ml/L concentrations of toxicant increased significantly ($P < 0.05$) in total length during the first four weeks of exposure. However, the total length of the same fish groups remained the same during the last six weeks of exposure. The increase in total length of these fish groups were 0.8, 0.8, 0.8 and 0.9 cm respectively with the mean values ranging between 5.7 to 6.5; 5.7 to 6.5; 5.7 to 6.5 and 5.7 to 6.6 cm in the descending order of the

concentrations. The groups of fish in the control tank and in the 8.75 ml/L concentration had significant increases ($P <$

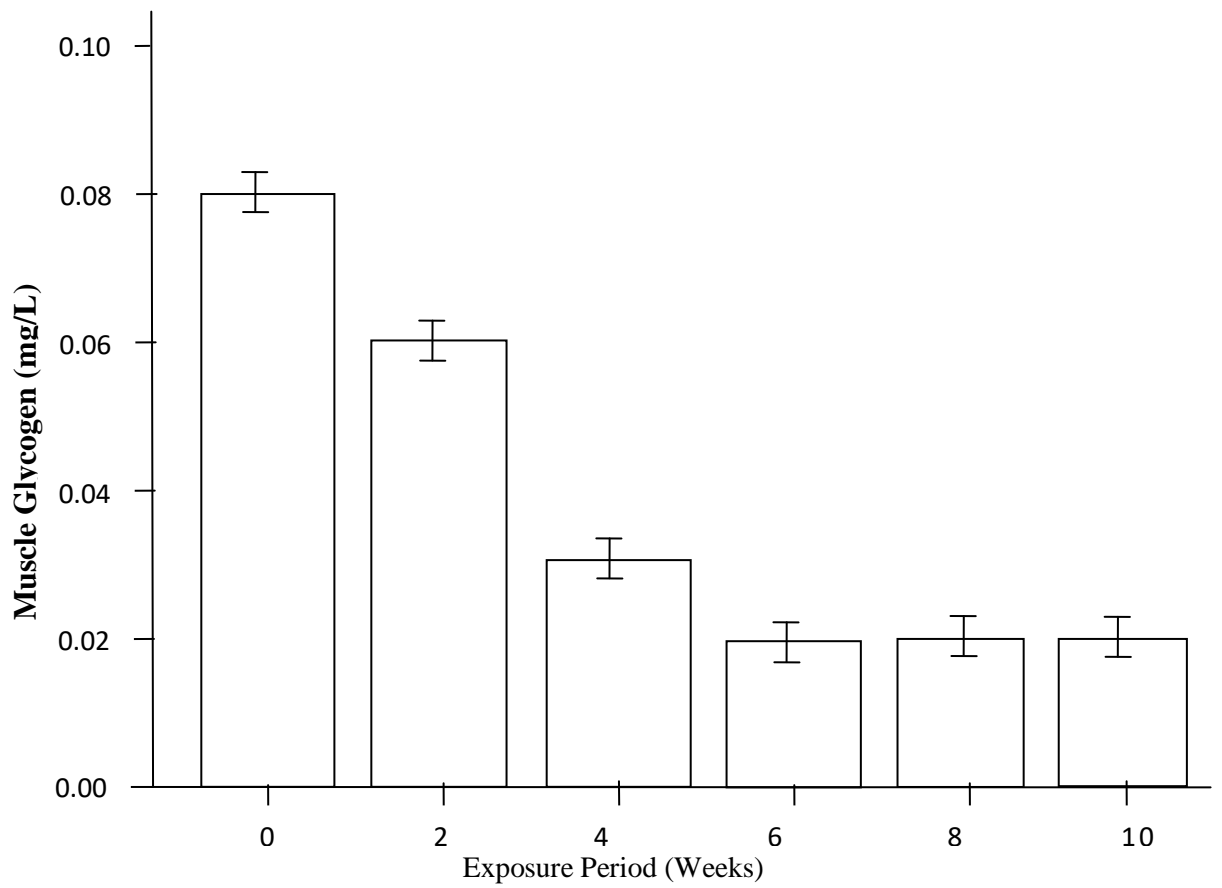


Figure 3 Variations in Mean Values of Muscle Glycogen for Fish Group at 140 ml/L of the Sublethal Concentration

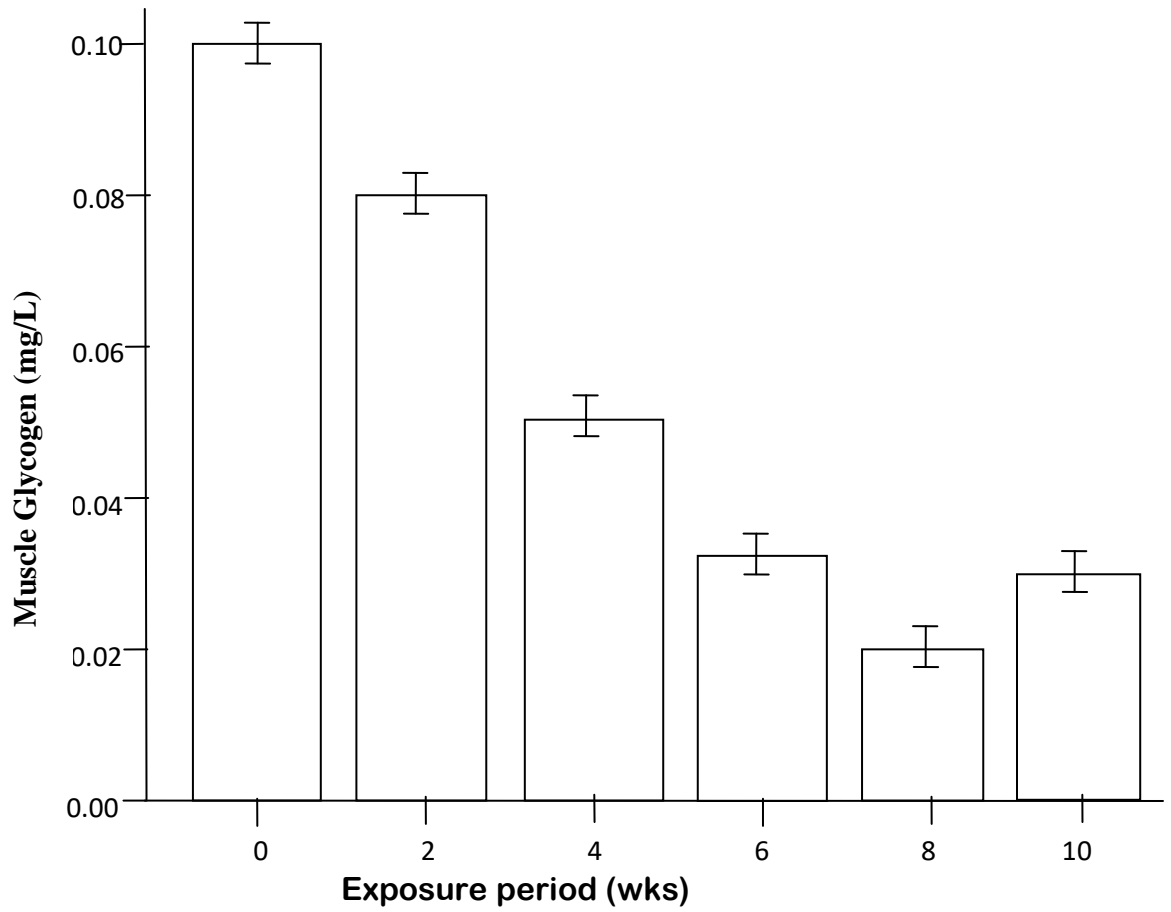


Figure 4 Variations in Mean Values of Muscle Glycogen for Fish Group at 70ml/L of the Sublethal Concentration

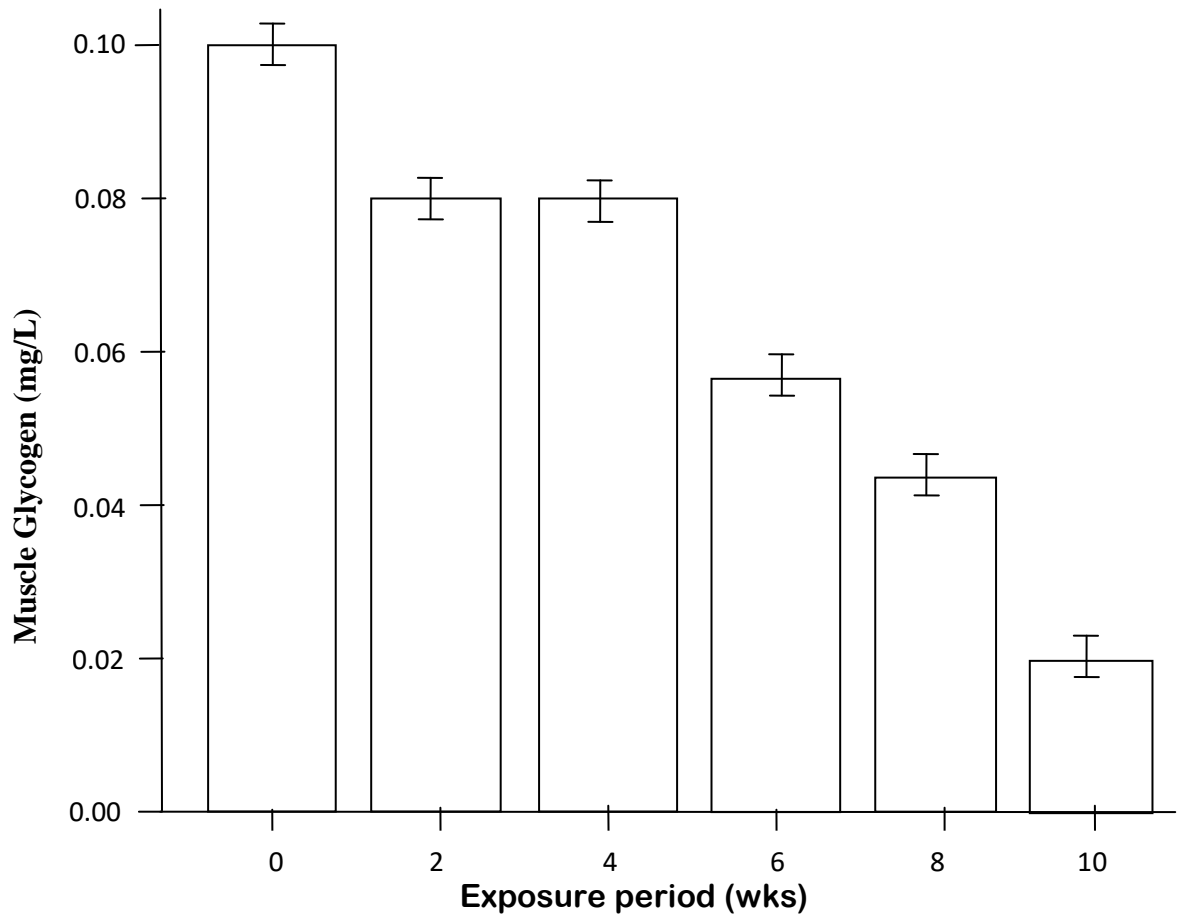


Figure 5 Variations in Mean Values of Muscle Glycogen for Fish Group at 35ml/L of theSublethal Concentration

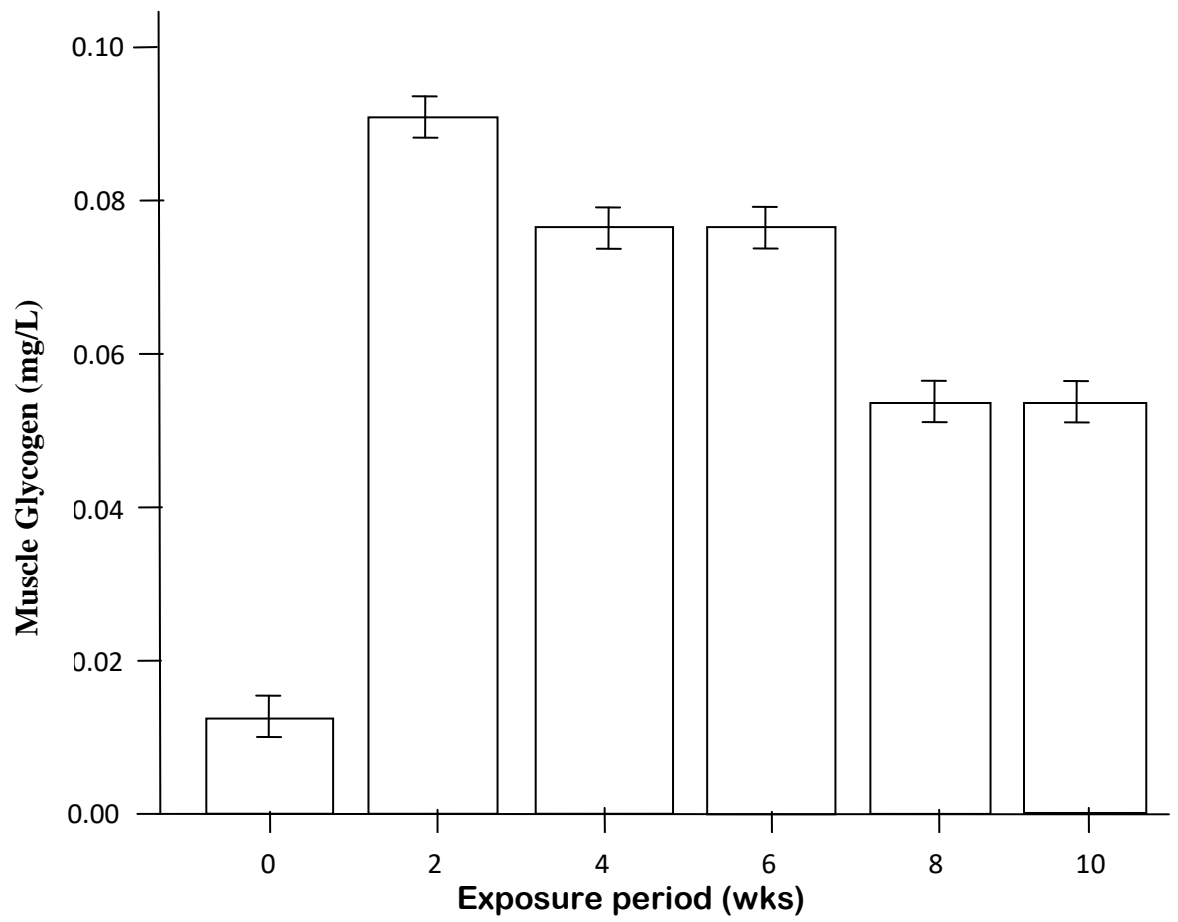


Figure 6 Variations in Mean Values of Muscle Glycogen for Fish Group at 17.50ml/L of the Sublethal Concentration

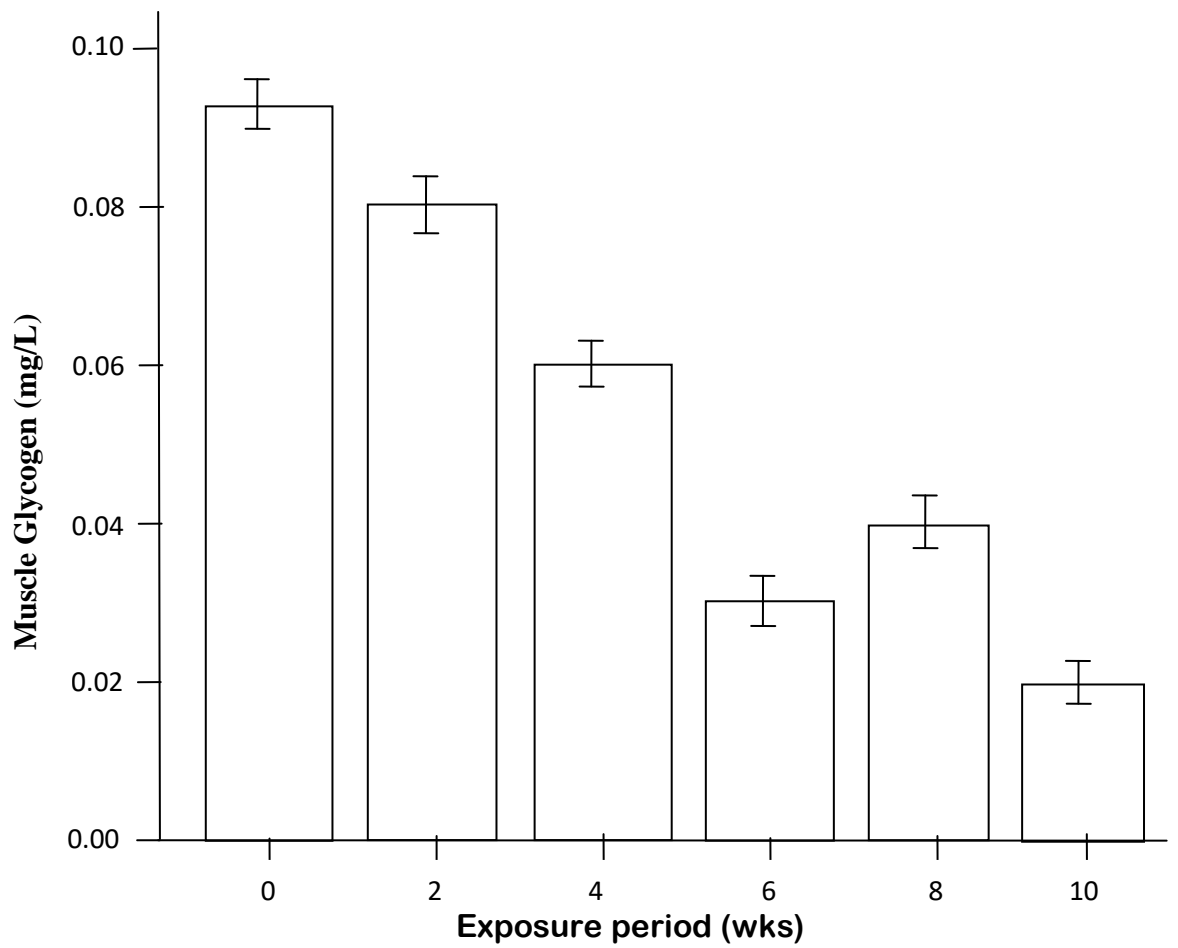


Figure. 7 Variations in Mean Values of Muscle Glycogen for Fish Group at 8.75ml/L of the Sublethal Concentration

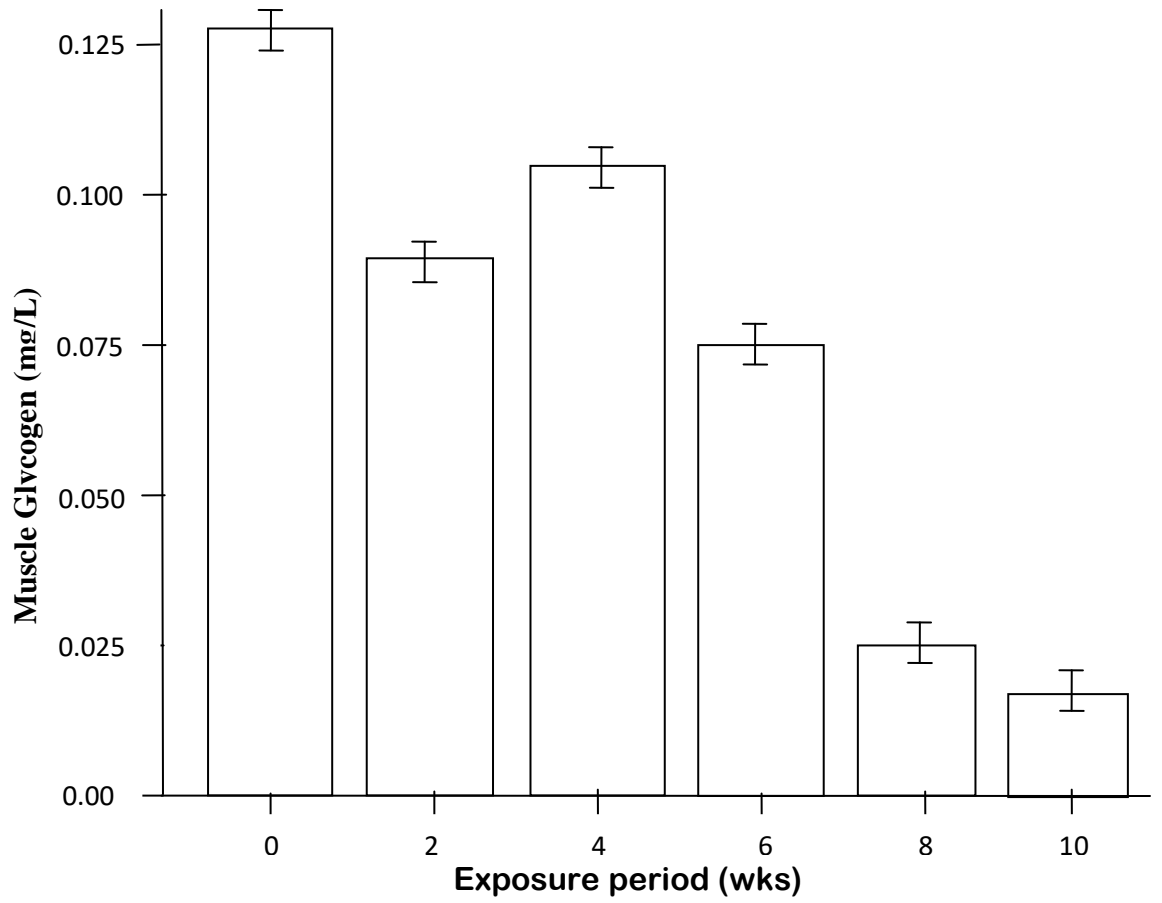


Figure 8 Variations in Mean Values of Muscle Glycogen for Fish Group at 0.00ml/L of the Sublethal Concentration

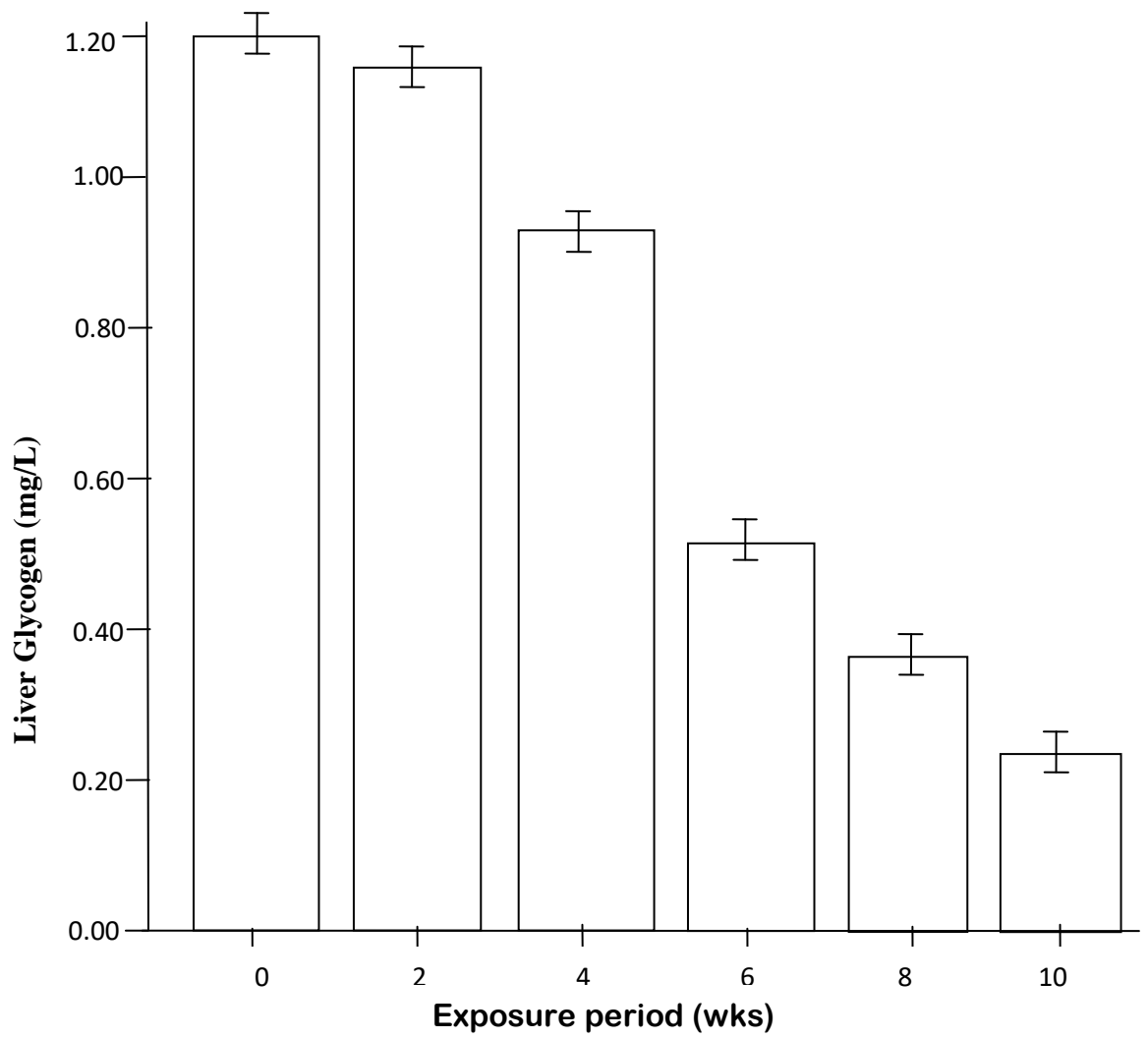


Figure 9 Variations in Mean Values of Liver Glycogen for Ten Weeks at 140ml/L Sublethal Concentration

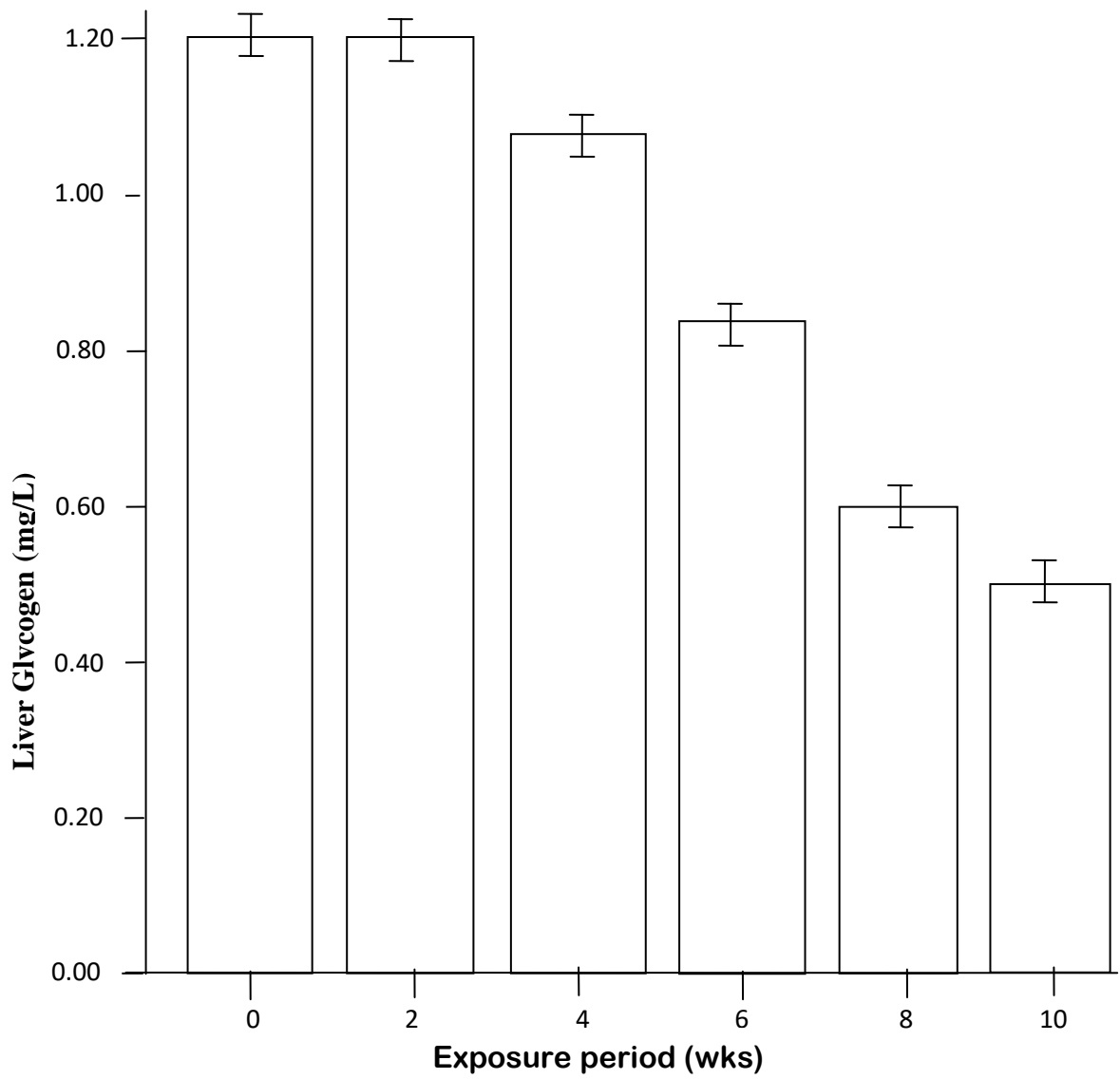


Fig. 10 Variations in Mean Values of Liver Glycogen for Ten Weeks at 70ml/L of the Sublethal Concentration

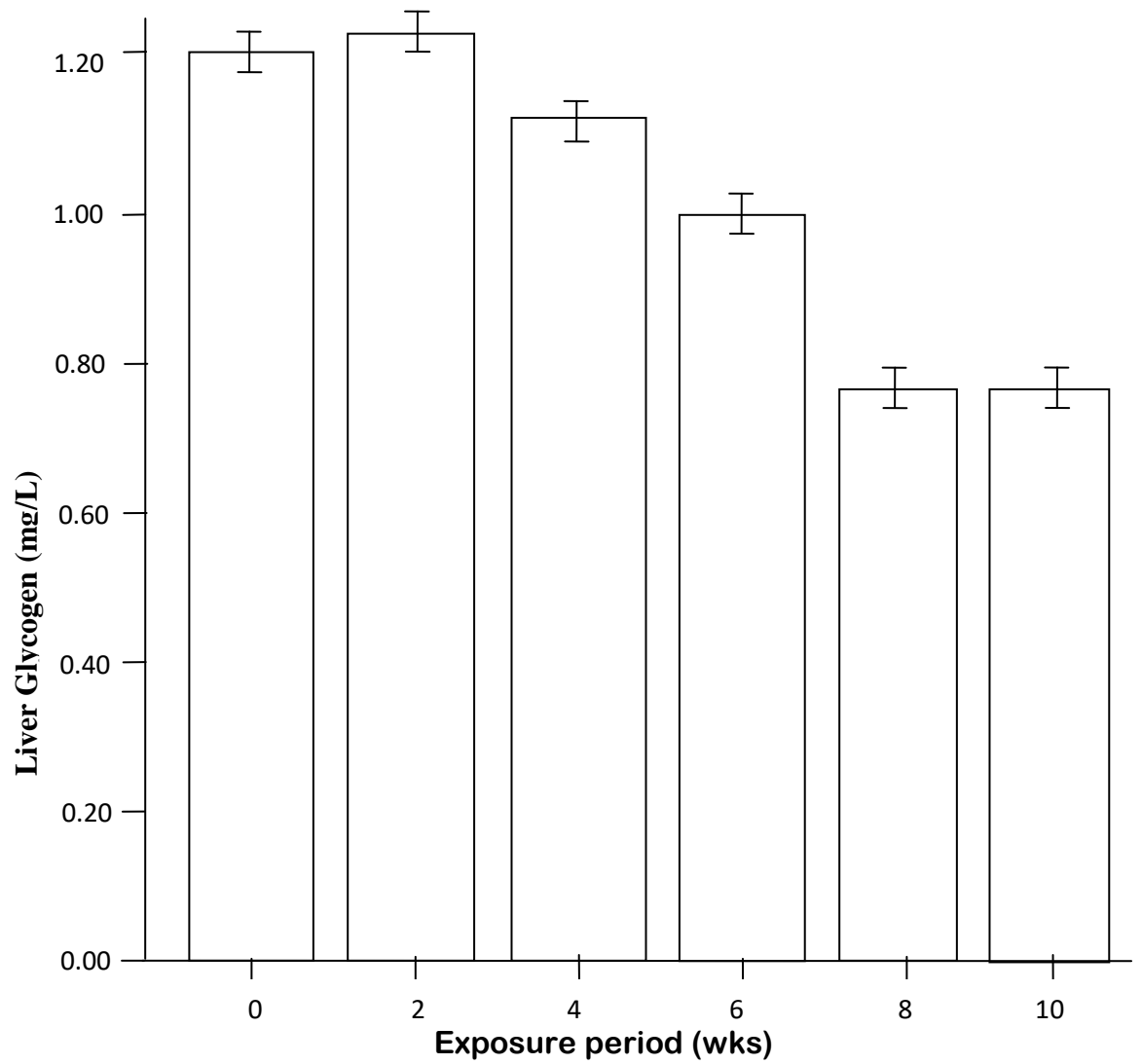


Fig. 11 Variations in Mean Values of Liver Glycogen for Ten Weeks at 35ml/L of the Sublethal Concentration

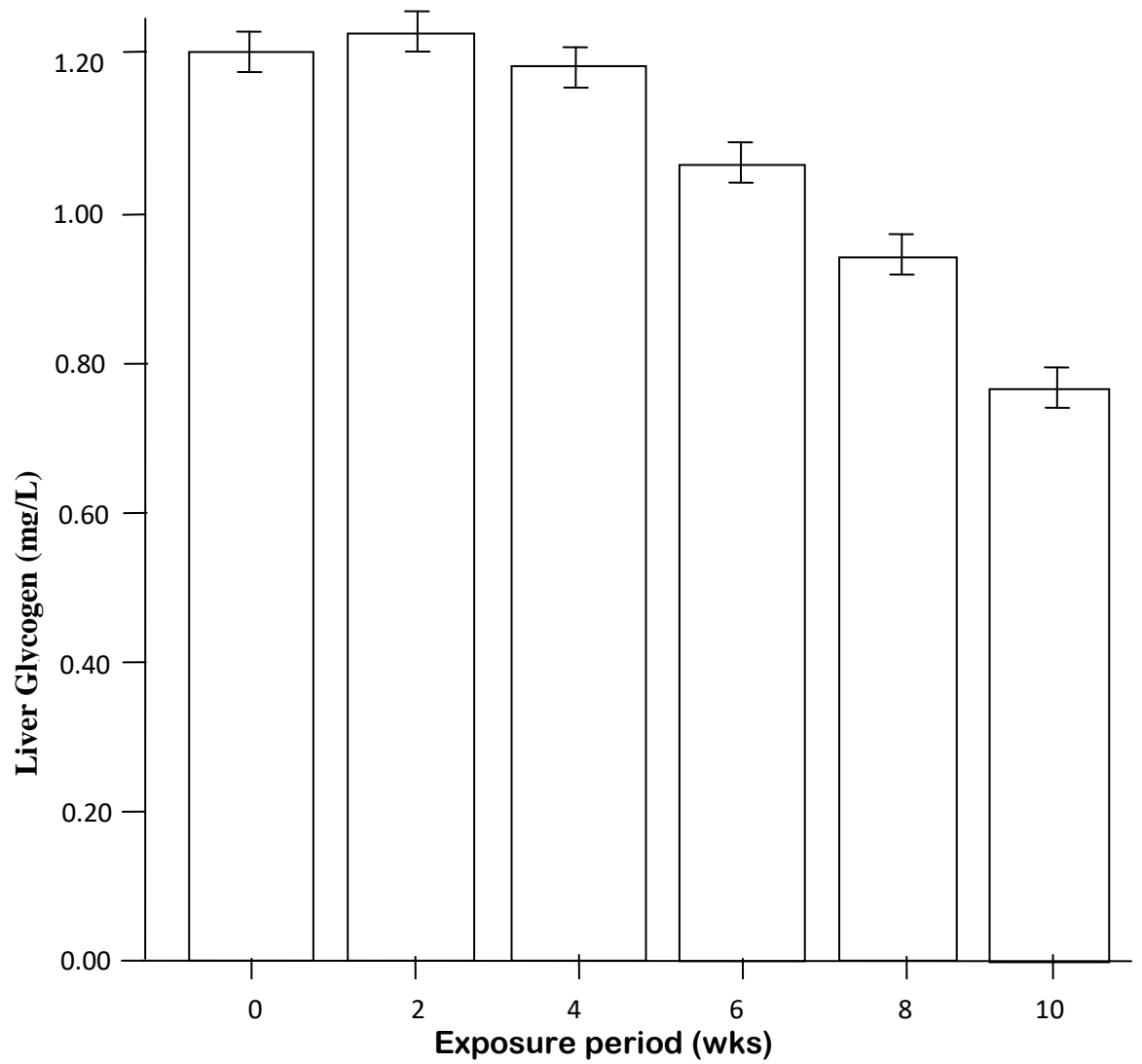


Fig. 12 Variations in Mean Values of Liver Glycogen for Ten Weeks at 17.5ml/L of the Sublethal Concentration

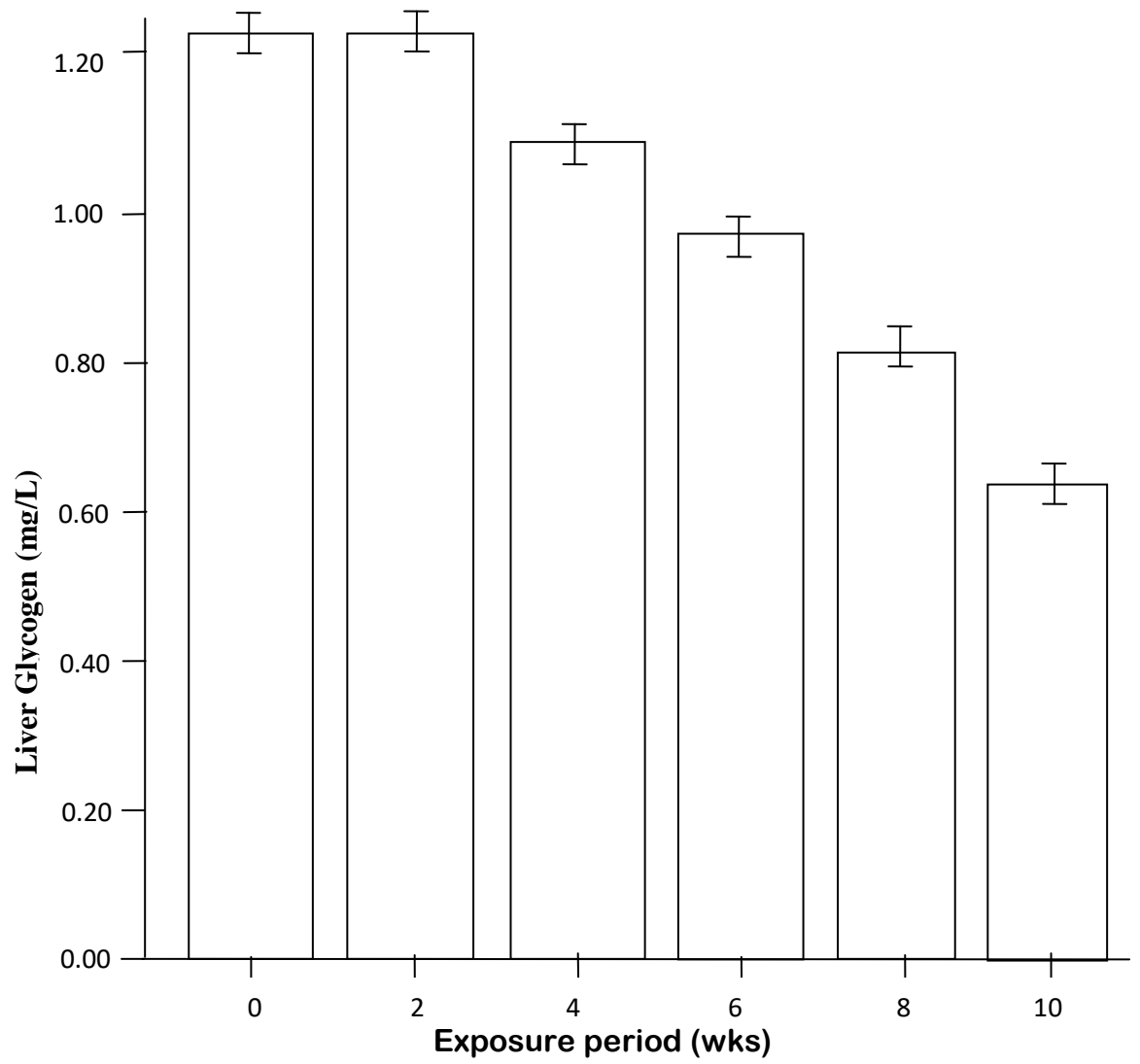


Fig. 13 Variations in Mean Values of Liver Glycogen after Ten Weeks at 8.75 ml/L of the Sublethal Concentration

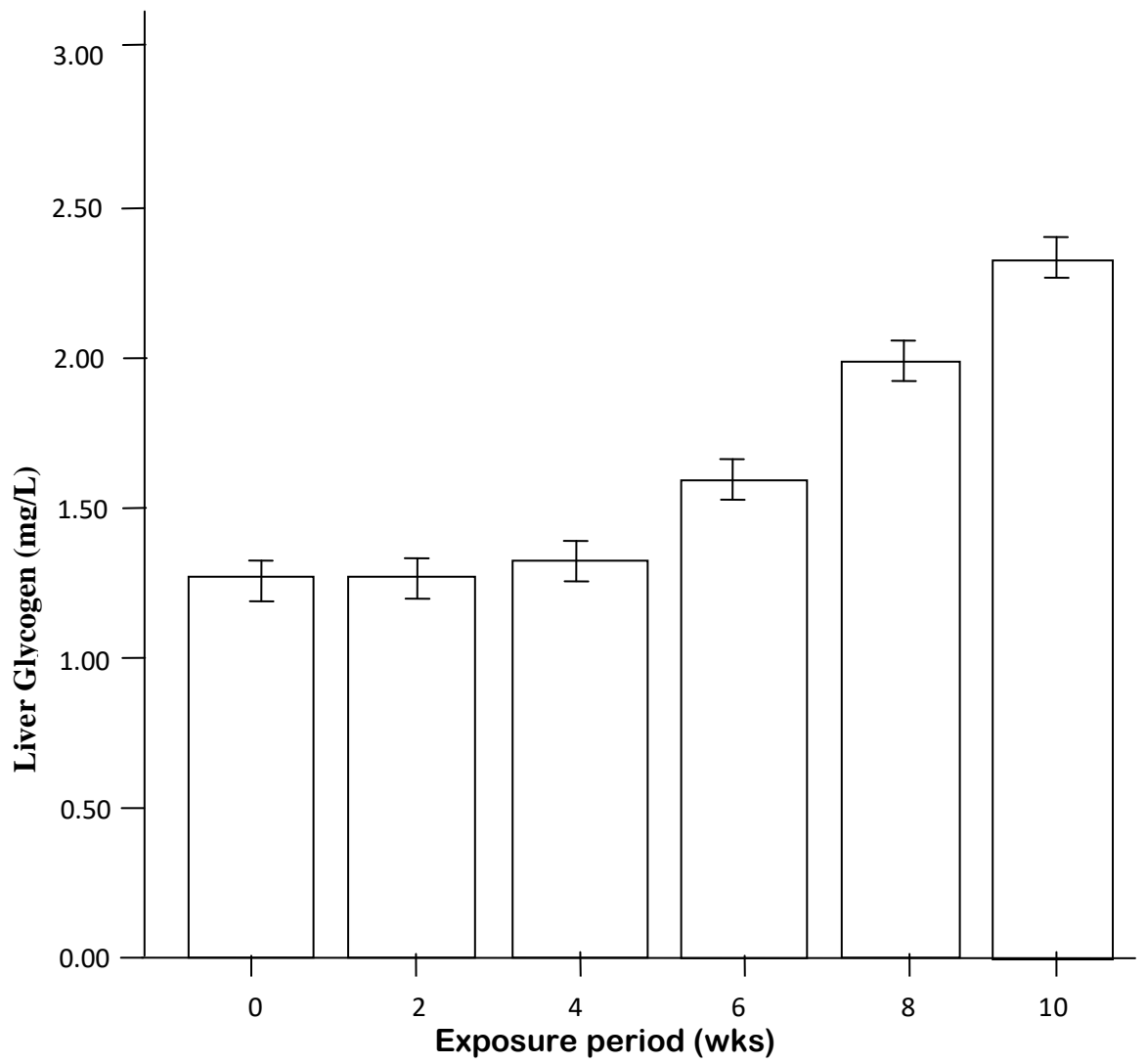


Fig. 14 Variations in Mean Values of Liver Glycogen after Ten Weeks at 0.00 ml/L at the Control Tank

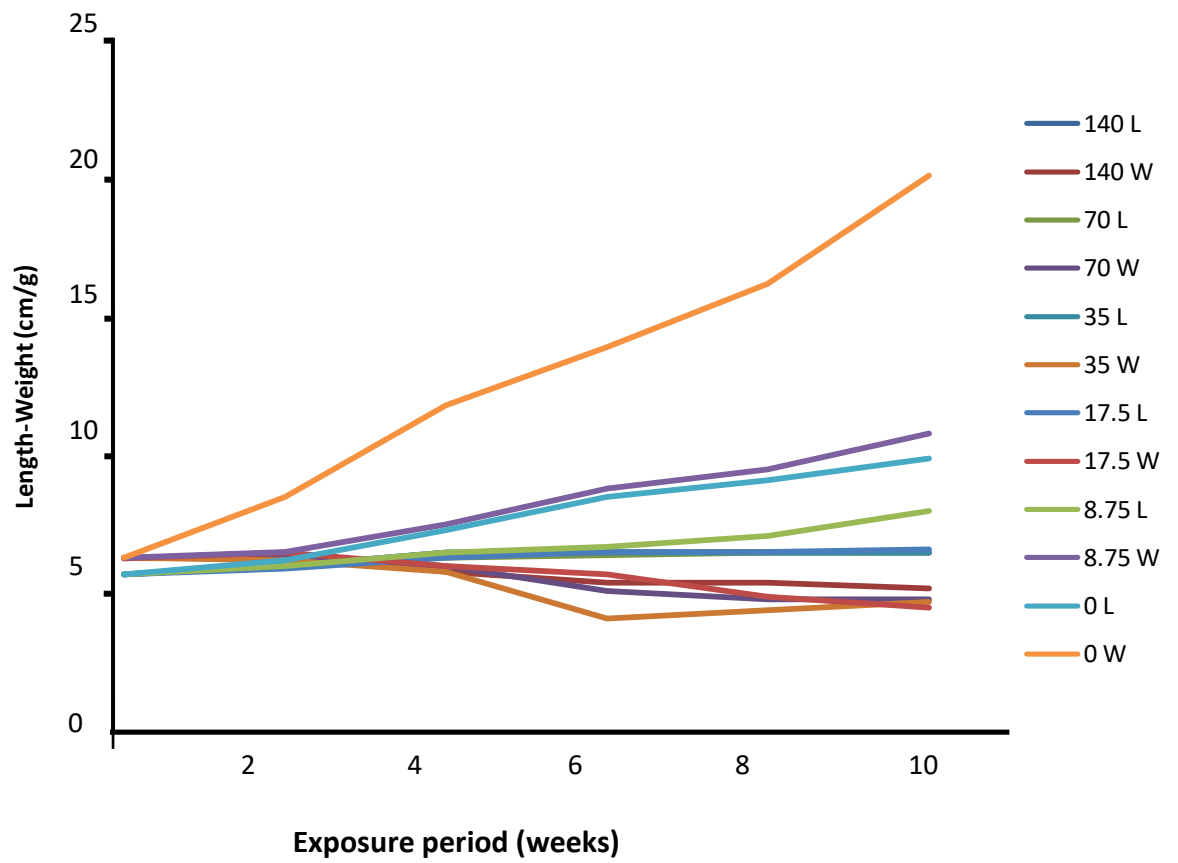


Figure 15 Variations in Mean Length-Weight Relationship of *Oreochromis niloticus* Fingerlings Exposed to Sublethal Concentrations of Used Crankcase Oil

0.05) in total length throughout the period of exposure. The increase in the total length of these fish groups were 4.2 and 2.3 cm respectively during the 10 weeks investigation period. The range of values was 5.7 to 9.9 for the former and 5.7 to 8.0 for the latter. The higher concentrations of 70.00 ml/L and 140 ml/L lost weight with values of 31.25% and 21.15% respectively (Table 6). Appendix B2 and Fig. 3 shows a mean weight of 20.10g for the fish group in the control with the mean length at 9.9cm after ten weeks. But the highest sub-lethal concentration had a mean weight of 5.20g and a mean length of 6.5cm. The growth performance between and within the fish in the sub-lethal concentrations was statistically significant ($P < 0.05$) at the end of the investigation period.

4.8 MEAN CONDITION FACTOR OF *O. NILOTICUS* EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The study revealed that each of the experimental groups of *O. niloticus* fingerlings had a mean condition value of less than one (< 1) showing a condition below mean average (Table 7). This is obvious from the fact that the laboratory condition under which this investigation was carried out cannot equate mean natural conditions. Consequently, the K-values did not show a definite pattern of decrease or increase between the control and the experimental tanks with respect to the feeding index of the fish. However, the fish groups showed allometric growth pattern during the exposure period.

4.9 BIOACCUMULATION OF METALS IN THE TISSUES OF *O. NILOTICUS* FINGERLING EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL AFTER TEN WEEKS

The bioconcentration of metals in the fingerlings increased with increasing concentration of toxicant and exposure time. Among the metals in the fish organs, iron in the liver had the highest concentration of 253.00 μ g/L followed by Iron in the gills with a value of 209.18 μ g/L. Iron (Fe) in the muscles was at a concentration of

75.19 μ g/L, while lead (Pb) had the least concentration of 0.04 μ g/L in the liver, 0.59 μ g/L in the muscles and 1.23 μ g/L in the gills (Figure 16 and Appendix B5).

Table 7

Variations in Mean Condition Factor for *Oreochromis niloticus* Fingerlings Exposed to Sublethal Concentrations of Used Crankcase Oil

Concs. of Used Crankcase Oil	Mean Weight (g)	Mean Length (cm)	K-Value
140	34.6 \pm 0.64	37.7 \pm 0.07	0.06
70	39.4 \pm 0.50	37.4 \pm 0.09	0.08
35	37.5 \pm 0.63	37.7 \pm 0.07	0.07
17.50	33.9 \pm 0.70	37.5 \pm 0.08	0.04
8.75	49.4 \pm 0.55	40.0 \pm 0.06	0.08
0.00	76.8 \pm 0.43	46.7 \pm 0.04	0.08

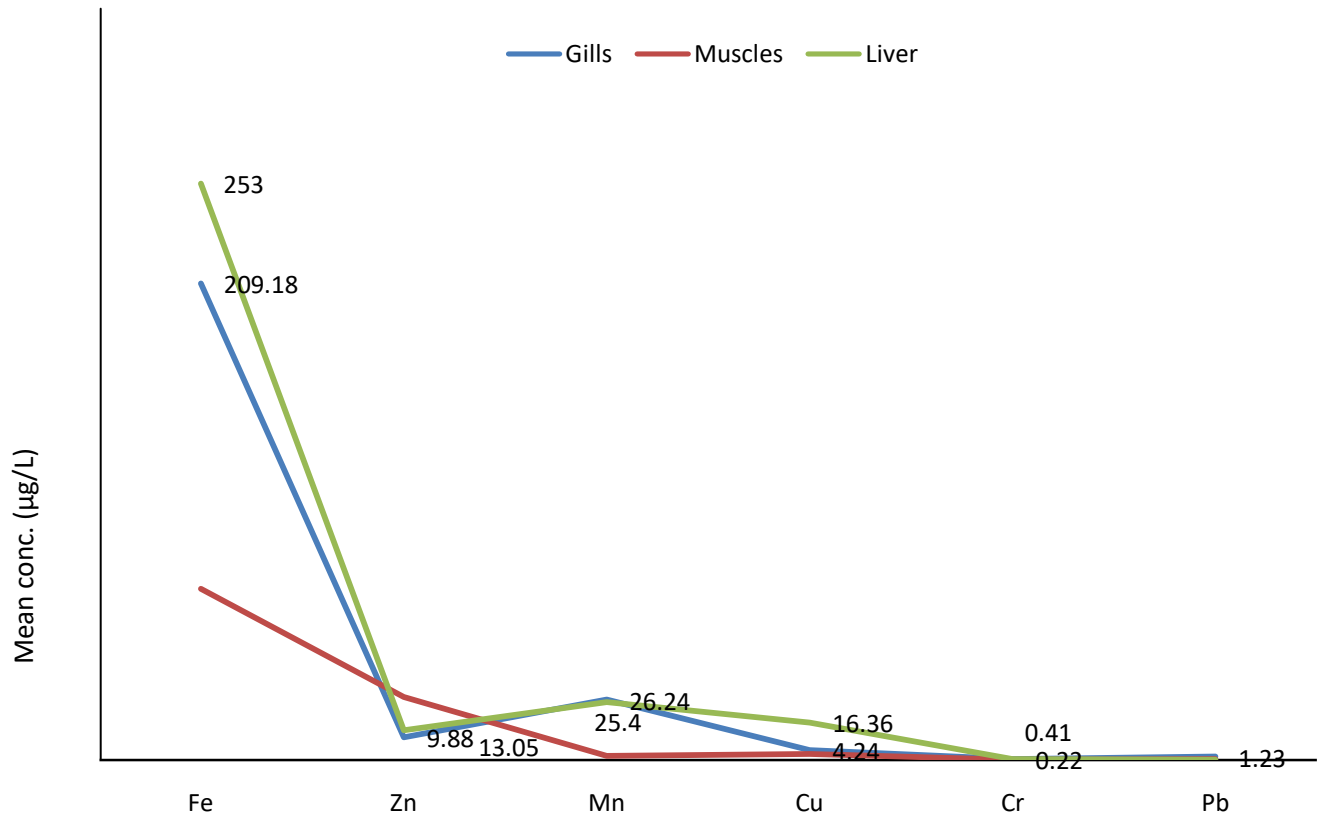


Fig. 16 Variations in Mean Concentration of Metals in the Gills, Muscle and Liver of *O. niloticus* Exposed to Sublethal Concentrations of Used Crankcase Oil

4.10 EFFECTS OF USED CRANKCASE OIL ON THE HAEMATOLOGICAL INDICES OF *O. NILOTICUS* EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The blood parameters of *O. niloticus* analysed after the ten weeks exposure period include: white blood cells (WBC), red blood cells (RBC), haemoglobin (Hgb), platelets (PLT), lymphocytes (LY), monocytes (MO), Granulocytes (GR), Haematocrite (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The result is presented in Table 9. There was a decrease in circulating erythrocytes, from 1.16 to 0.62 μl attributable to possible decreases in synthesis and haemolysis as well as decreases in MCV from 87.0 to 86.8 μl and blood platelets from 274.0 to 0.0 μl respectively. Conversely, the result showed increases in WBC, LY, MO, GR, and MCHC from 6.5 to 27.8, 4.1 to 20.9, 0.7 to 2.8, 1.8 to 5.9 and 125.5 to 198.9 μl in that order. A moderate increase in Hgb from 2.8 to 3.0 μl was observed (Table 8).

There was a significant difference ($P < 0.05$), between WBC and RBC of fish in the control tank and those in the sublethal concentrations. A strong positive correlation was observed between WBC and PLT with $r = 0.979$. The analysis of variance (ANOVA) showed that there is a significant difference ($P < 0.05$) between the blood indices of fish in the control and those exposed to used crankcase oil. The correlation between WBC and RBC showed a strong negative correlation with $r = -0.716$. The relationship between RBC and PLT also showed a strong negative correlation with $r = -0.720$.

4.11 EFFECTS OF USED CRANKCASE OIL ON THE ACTIVITIES OF SOME ENZYMES IN *O. NILOTICUS* FINGERLINGS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

Significant differences ($P < 0.05$) between the control and the fish groups in the sublethal concentrations were observed in both enzymes analysed.

Table 8

Values of Haematological Indices of *O. niloticus* Fingerlings Exposed to Sublethal Concentrations of Used Crankcase Oil

	Haematological Indices ($10^3 \mu\text{l}$)										
	WBC	LY	MO	GR	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Sublethal Concs. (ml/L)											
0.00	6.5	4.1	0.7	1.8	1.16	2.8	2.8	87.0	109.3	125.5	274
8.75	7.5	4.0	0.8	2.9	1.07	2.6	1.3	8.9	21.3	240.0	221
17.50	9.8	4.4	0.9	4.6	1.03	2.8	1.1	8.3	22.2	427.1	171
35.00	21.4	12.8	1.6	4.6	1.01	2.9	1.3	58.7	53.3	198.5	145.5
70.00	13.5	7.3	2.7	4.15	0.70	2.9	0.8	30.2	52.3	227.8	00.5
140.00	27.8	20.9	2.8	5.9	0.62	3.0	10.1	86.8	34.2	198.9	0.0

Key:

WBC = White Blood Cells (Leucocytes)

LY = Lymphocytes

HGB = Haemoglobin

MO = Monocytes

GR = Granulocytes

RBC = Red Blood Cells (Erythrocytes)

HCT = Haematocrit

MCV = Mean Corpuscular Volume

MCH = Mean Corpuscular Haemoglobin

MCHC = Mean Corpuscular Haemoglobin Concentration

PLT = Platelets (Thrombocytes)

ALP and ALAT concentrations were elevated correspondingly with the increasing concentrations of the wsf of used crankcase oil (Tables 9 and 10). The activities noted were higher in the gills, liver and muscle of the fish in the sublethal concentrations when compared to the control. The activities of the enzymes ALP and ALAT reveal a significant increase ($P < 0.05$) in both cases when compared to the control. The concentration levels for ALP in the highest sublethal concentration of used crankcase oil (140ml/L) were 256.06 ± 0.441 , 200.12 ± 0.831 , and 100.00 ± 0.762 iu in the fish muscle, liver and gills respectively. While in the control, the levels were 35.00, 16.65 and 13.35 iu in the muscle, liver and gills in the same order (Table 10). ALAT concentration levels were 462.41 ± 0.098 , 430.425 ± 0.126 and 398.00 ± 0.056 iu in the fish muscle, liver and gills respectively at the highest sublethal concentration of used crankcase oil (140ml/L). Concentration levels at the control tank were 5.712 ± 0.031 , 48.137 ± 0.005 and 5.195 ± 0.038 iu in the fish muscle, liver and gills in that order (Table 10).

Table 9

Effectsof Used Crankcase Oil on Alkaline Phosphatase(ALP)in the Gills, Liver and Muscle of *O.niloticus* Exposed to Sublethal Concentrations of Used Crankcase Oil

Concentration of Used Crankcase oil (ml/L)	ALP Values in the Fish Organs (iu/L)		
	Gills	Liver	Muscle
140.00	100.00±0.762	200.12±0.831	256.06±0.441
70.00	118.35 ±170.4	90.00 ± 10.00	73.35 ± 36.85
35.00	106.65 ± 23.1	88.35 ± 20.20	61.65 ± 39.50
17.50	80.00 ± 30.40	38.30 ± 7.65	50.00 ± 0.00
8.75	41.65 ± 795	21.65 ± 7.65	46.65 ± 2.90
0.00	13.35±75.90	16.65±7.75	35.00 ± 5.00

Table 10
 Effect of Used Crankcase oil on Alanine Aminotransaminase (ALAT) in the Gills, Liver and Muscle of *O. niloticus* Fingerlings Exposed to Sublethal Concentrations of Used Crankcase Oil

Concentration of Used Crankcase oil (ml/L)	<u>ALP Values in the Fish Organs (iu/L)</u>		
	Gills	Liver	Muscle
140.00	398±0.056	430.425±0.126	462.412±0.098
70.00	2.98±0.026	41.763±0.056	4.204±0.015
35.00	3.108±0.046	44.323±0.010	5.350±0.025
17.50	3.414±0.003	46.013±0.005	5.601±0.006
8.75	4.321±0.005	47.343±0.006	5.570±0.032
0.00	5.195±0.038	48.137±0.005	5.712±0.031

5.1 DETERMINATION OF WATER QUALITY PARAMETERS OF THE EXPERIMENTAL AQUARIA

A large amount of used crankcase oil is generated each year when engine oil is changed. This is usually discarded into the environment. Contamination of the environment with heavy metals is a very important problem in contemporary time as observed by Miretzky, Saralegui and Cirelli (2004), Ugwu, Mgbenka and Ugwuaka (2008), Madu, Tagwoi and Babalola (2008), Eissa, Gado, Laida, Mona and Noor-Eldeen (2011) and Nwani *et al.* (2015). The physio-chemical parameters reported in this investigation did not vary too significantly when compared to the recommended WHO values. For example, total hardness (which is a general index of water type, buffering capacity and productivity) with a value of between 75-150 mg/L is conducive for fish production.

Total hardness varied between 35-106 mg/L in the control and the highest sublethal concentrations respectively during the period of this investigation. Thurston, Russo, Fatterolf, Edsall and Narner (1979) as reported in Annune and Bako (1998) and WHO (2011) reported moderately hard waters of 159 mg/L and above which are preferred for fish culture. Water temperature is one of the most influencing environmental factors affecting water dynamics and the growth and metabolism of fish (Boyd, 1990; El-nemaki, Ali, Zeinhom & Radwan 2008). The water temperature which ranged between 24.10 °C and 24.40 °C was not significantly different ($P < 0.05$) between the means of the various concentrations of the used crankcase oil in the experimental tanks and the control, therefore the use of the used crankcase oil had no influence on temperature.

Dissolved oxygen (DO) concentration which showed a range of 2.05 – 6.50 mg/L with a mean of 5.50 mg/L was within the range for tropical fish culture as

reported by Azim and Little (2008). It is the most critical parameter that influences the survival of fish. The pH values which ranged between 6.00 - 8.40 agrees with the values of 6.60 - 9.30 reported by Boyd (1990) and 6.70 - 8.50 recorded by Azim and Little (2008) considered suitable for aquatic life. Wade (1985) also reported that the pH of tropical disused Tin mine lake ecosystems is generally alkaline which corroborates the observation in this investigation. The free carbon dioxide of water serves as an alternative source of carbon for photosynthesis in aquatic habitats. The values obtained in the present study could be said to be normal for the survival of fish species (Lagler, 1952 and BioWorldSupport, 2013).

The values for total alkalinity were in the range of 3.25 – 6.50 mg/L with a mean of 4.5mg/L. Boyd and Lichtoppler (1979) stated that the hydrolysis of bicarbonate ions at higher pH values may lead to reduced total alkalinity. Autotrophic activity increases pH through CO₂ absorption while heterotrophic activity decreases pH through respiration since the two processes affect both alkalinity and pH. Total alkalinity values were stable in all the experimental tanks. The concentration of divalent metals (metal ions Ca⁺⁺, Mg⁺⁺) in water contributes to its hardness. Nonetheless, total hardness of water comprises both the total alkalinity (the level of CO₂ and HCO₃ present) and the permanent hardness due to the presence of Ca⁺⁺ and Mg⁺⁺ (Ugwu *et al.*, 2008).

5.2 ACUTE TOXICITY/SUBLETHAL TESTS OF USED CRANKCASE OIL ON *O. NILOTICUS* FINGERLINGS

The median lethal concentration (LC₅₀) is the most widely accepted basis for acute toxicity test, it is the concentration of a test chemical which kills 50% of the test organism in a particular length of exposure, usually 96hrs (Nwani *et al.*, 2015). In aquatic ecosystems, fish have become indicators for the evaluation of the effects of

noxious compounds. The Nile Tilapia, *O. niloticus* is one of the commercially most important freshwater fish species cultured world-wide. Due to this reason, the investigation of its susceptibility to toxins in the environment is very important. The effects of the use of used crankcase oil on *O. niloticus* fingerlings was found to increase with increasing concentration of the oil. Results obtained showed a gradual decrease in the slope function corresponding to the increase in exposure period. This observation is similar to that of Gharedaashi and Imanpour (2013) when *O. niloticus* was exposed to copper sulphate and lead nitrate. They reported that increase in exposure time and in concentration of test substance resulted in increase in mortality.

The acute/sublethal test conducted in this research showed a median lethal concentration (LC50) of the use of used crankcase oil of *O. niloticus* fingerlings to be 630ml/L. Ayoola and Alajabo (2012) reported a median lethal concentration (LC50) of the Tilapia, *Sarotherodon melanothon* exposed to engine oil to be 460ml/L. Gharedaashi and Imanpour (2013) also stated that different test organisms have different sensitivity and that age and size of the organism as well as water quality such as water hardness can affect toxicity. Physiological responses like rapid opercular movement and frequent gulping of air were observed during the initial stages of exposure after which it became occasional.

5.3 VARIATIONS IN MEAN VALUES OF METALS IN THE USED CRANKCASE OIL AFTER TEN WEEKS

The present study showed concentrations of metals and other elements in the order Ca > Zn > Cl > Na > Fe > Si > Al > Cu > Mn > Br > Pb in the used crankcase oil. Some of the metals for example Ti, Mg, K etc, were below detection level. More elements were detected by the NIRR-1 than the AAS (Table 4). This result gives a picture of the two analysers and their sensitivity in tests such as this. In recent years, there has been a

growing interest about the effect of these metals on the health of fish and attention has been drawn to the measurement of contamination levels in public food supplies particularly fish (Sikiric, Brajenovic, Pavlovic, Havranek, & Plavljanic, 2003, Atef 2005, Awofolu *et al.*, 2005, & Etonihu, Ikhiuwu, Etonihu, & Nweze (2011). Heavy metals which are of particular interest, are dispersed in water and consequently in human beings through food chain biomagnification or bioaccumulation causing chronic ailments (Rai & Pal, 2002). Most metals found in used crankcase oil stay in the environment for a long time. Thus, they can build up in animals, plants, soils, sediments and non-flowing surface water, (Osman, 2012).

Vazquez-Duhalt (1989) reported that used motor oil contains high concentration of lead, zinc, calcium, barium and magnesium with lower concentrations of iron, sodium, copper, aluminum, chromium, manganese, potassium, nickel, tin, silicon, boron, and molybdenum. He also added that many of these metals are harmful to humans and other living organisms. The high concentrations of some heavy metals obtained from the analysis of the used crankcase oil corroborates the observations of Vazquez-Duhalt (1989), Irwin *et al.* (1997), Rai and Pal (2002), Peterson *et al.* (2003), Farid, Emani, and Wajid (2004), Vidal, Mireles and Solis (2004), Pavlovic, Sikiric, Havranek, Plavljanic and Brajenovic (2004), Dobrzanski *et al.* (2005), Atef (2005), Atolaye, Aremu, Shaye and Pennap (2006), Kester *et al.* (2007), Ubaluwa and Ezeonye (2007), Samir and Shaker (2008), Abowei and Hart (2009) on the toxic potentials of oil and heavy metals in view of the grave health risks they pose to man. A potential threat for aquatic organisms is contamination arising from being exposed to significant amounts of heavy metals which at high concentrations can cause harmful effects on metabolic, physiological and biochemical systems of fishes together with long term

ecotoxicological effects (Abedi *et al.*,2012). Heath (1987) and Al-Nagawaay (2008) reported that the accumulation levels vary considerably among metals and species.

The most likely explanation for growth reductions in this study is a combination of reductions in feeding and feed conversion, coupled with increased metabolism due to detoxification and impaired health. The present study supports the hypothesis that the above named alterations are associated with reduced growth following exposure of the fish to used crankcase oil. Moles and Norcross (1994) stated that the juveniles of flatfishes exposed to oil in sediments had reduced growth along with a variety of physical abnormalities as they could not avoid the oil.

Short *et al.*, (2003) reported that there is a mounting evidence that hydrocarbon exposure during embryogenesis can lead to reduced growth and survival as well as morphological abnormalities in those species of fish that incubate their eggs in near shore environment. Moles (2001) observed that the early life stages of salmon and herring are particularly vulnerable to oil, probably because the oil was sequestered into lipid rich tissues that are then incorporated into the developing fish tissue as the lipid reserves were metabolized.

5.4 PROXIMATE COMPOSITION OF THE TISSUES OF *O.NILOTICUS* FINGERLINGS EXPOSED TO WSFOF USED CRANKCASE OIL

There are a number of variables that can affect the proximate composition of fish tissues. Protein decreased correspondingly to the sublethal concentrations from the initial percentage dry weight to 30.02, 44.35, 46.12, 48.63 and 51.36% respectively at the end of the exposure period. However, an increase in protein from 58.08 ± 0.02 to 68.01 ± 0.02 was observed in the control fish group. The difference between the control and the highest sublethal concentration was statistically significant ($P < 0.05$).

This decrease in protein with increasing wsf concentration agrees with the findings of Parthipan and Muniyan (2013) when they exposed *Cirrhinus mrigalato* sublethal concentrations of nickel. They reported a statistically significant ($P < 0.05$) decrease in protein level in the muscle, gill, liver and kidney of the fish while there was an increase in protein in the control fish. They attributed the reduction in protein to a failure in the synthesizing capacity of the endoplasmic reticulum in the cell which is considered the primary biochemical parameter for early indication of stress. Dahunsi and Oranusi (2011) observed a decrease in protein in the liver of *Clarias gariepinus* exposed to sublethal concentration of chemical additives. Nwani *et al.*, (2015) also observed a decrease in protein in the liver and kidney of *Clarias gariepinus* juveniles attributed it to damage of these organs by paraquat stress and the consequent utilization of available protein for metabolic activities. The drop in protein level may be due to proteolysis and a decrease in RNA in the various organs of the fish.

Lipids reduced from 16.12% at the start of the investigation to 9.02, 10.34, 11.64, 14.02 and 15.15 % in the descending order of the sublethal concentrations. *O. niloticus* fingerlings in the control tank increased in their lipid content from the initial 16.12 % to 20.07 %. Dahunsi and Oranusi (2011) observed a decrease in lipids when *Clarias gariepinus* was exposed to sublethal concentrations of chemical additives and an increase in lipids in the unexposed fish in the control. In all these research findings, the test organisms exposed to sublethal concentrations of toxicants had decreases in both proteins and lipids while test fish in the control showed an increasing trend.

5.5 GROWTH PERFORMANCE AND FEED UTILIZATION OF O.NILOTICUS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The growth performance and feed utilization of *O. niloticus* fingerlings during the period of exposure to the sublethal concentrations of the used crankcase oil showed a specific growth rate (SGR) of 0.91 in the control and 0.01 in the highest sublethal concentration. Food conversion ratio (FCR) was 1.33 in the control and 3.20 in the highest sublethal concentration. The same trend was observed for the protein efficiency ratio (PER). Fish groups in the control and lower sublethal concentrations performed better in terms of the feed conversion ratio, protein efficiency ratio and the specific growth rate. The lower value of FCR and PER in the control indicates a better feed utilization in the absence of the use of used crankcase oil.

Statistical analysis of the results showed a significant increase ($P < 0.05$) in the values of FCR and PER in the higher concentrations of the water soluble fractions of used crankcase oil whereas the SGR decreased as the sublethal concentrations of used crankcase oil increased. A further corroboration of the results obtained in this investigation can be seen from the report of Moles and Norcross (1994) which stated that "as the concentration of oil increased, specific growth rate decreased." The effect of used crankcase oil on growth rate was significant ($P < 0.05$) for the high sublethal concentrations.

Growth rates in most of the used crankcase oil treatment groups were significantly lower than rates of the unexposed *O. niloticus* fingerlings in the control. During the ten weeks exposure period, used crankcase oil concentrations significantly reduced the growth rates in all fish. In the higher concentrations of 35.00, 70.00 and 140.00 ml/L, growth rates remained at the same reduced level during the tenth week of exposure. Abdel-Tawab and Wafeek (2008) observed that fish growth, survival and feed utilization reduced when *O. niloticus* was exposed to cadmium. They reported that fish survival decreased significantly with increasing Cd concentration the same way as

growth and feed utilization were affected with increasing sublethal concentrations of used crankcase oil in this study.

Moles(2001),Kumari and Abraham (2012),Onwurah, Okejim and Ajie (2013) and Nwani *et al.*,(2015)noted that the threat of oil pollution to fish and invertebrates is seldom just from acutely toxic concentrations that result in immediate fish and zooplankton kills, but also from the more subtle effects of heavilyweathered oil to sensitive life stages. Aquatic organisms exposed to heavily weathered oil exhibit a number of sublethal effects that have the potential to be just as ecologically lethal as any outright kill. Moles and Norcross (1998) also reported that exposure to sediments containing heavily weathered crude oil reduced growth in three species of flatfishes, namely:*Pleuronectes bilineatus*, *P. asper* and *Hippoglossus stenolepis* by 34% as well as inducing fin erosion, an increase in gill parasites and alterations in liver and gill tissues.

Autu and Ogueji (2006) observed a significant dose-dependent reduction in growth and food utilization in *O. niloticus* exposed to dimethoate indicating a severe stress. They reported that the suppressive effects of the sublethal concentrations on the specific growth rate, food conversion efficiency and protein efficiency ratio could be attributed to the toxicant. Omoregie and Ufodike (2000) and Eissa *et al.*(2011) stated that the suppression in weight gain was observed to be directly proportional to the concentration of the water soluble fractions of the used motor oil. They further stated that the inhibition of growth may be due to a disturbance of the normal metabolism by the toxic components of the crude oil. Fish increased their metabolic rate to metabolize and excrete aromatic hydrocarbons and to allocate more energy to homeostatic maintenance than storage leading to a reduction in the SGR, FCR and PER as seen in this study.

5.6 EFFECTS OF THE SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL ON THE CARBOHYDRATE RESERVES OF *O. NILOTICUS* FINGERLINGS

The least FCR values were obtained from the control and the lower sublethal concentrations where higher growth was recorded indicating a more efficient conversion of feed to body mass when compared to the other sublethal concentrations. In fishes, it is known that the carbohydrate reserve is in general utilized under unfavourable conditions (Parthipan and Muniyan, 2013). The significant reduction in glycogen level in liver and muscle of the fish could be attributed to the hypoglycaemic response of the fish to the toxic environment. Oladimeji and Ologunmeta (1987) noted by Omoregie *et al.*, (2000) stated that the above observation may be due to inefficient absorption of soluble glucose from the intestine as a result of the constant swallowing of sublethal levels of the broken-down products of petroleum effluent from the surrounding. Parthipan and Muniyan (2013) further observed that a depletion in liver glycogen shows an extensive utilization of energy stores under toxic stress. Reduction in glycogen in the liver and muscle indicates the utilization of carbohydrate as the principal and immediate precursor of energy production under stress.

Mean glycogen values in the muscles generally presented a lower picture than in the liver. As Martos (2011) observed, the liver has a greater capacity for glycogen storage than the muscle. Liver cells are said to have the ability to store up to 8% of their weight as glycogen while muscle cells can only store up to 3%. Omoregie *et al.* (2000) further reported that the significant reduction in muscle and liver glycogen confirms that the fish were severely stressed and metabolism of carbohydrate was impaired. These statistical trends in the results show evidence of an impairment of

carbohydrate metabolism in the *O. niloticus* fingerlings as well as a decreased capacity of the fish to efficiently utilize protein.

5.7 EFFECTS OF USED CRANKCASE OIL ON LENGTH AND WEIGHT OF *OREOCHROMIS NILOTICUS* FINGERLINGSEXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The use of length – weight relationship in assessing the maturity, growth and production of fish in water bodies is a normal bioassay (Mshelia *et al.*, 2009). Analysis of variance (ANOVA) showed significant differences ($P < 0.05$) in the weight of the experimental fish groups. This is in spite of the fact that the fish in the control and lower toxicant concentration tanks increased relatively in weight during the period of investigation. At the end of the exposure period, it was observed that fish groups in tanks with concentrations of 140.00, 70.00, 35.00 and 17.50 mg/L had significant increases ($P < 0.05$) in total length during the period of the experiment. The highest mean length for the highest concentration of toxicant was 6.5 cm, while the control had 9.9 cm as the highest mean length after 10 weeks of exposure from an initial mean length of 5.7 cm in both tanks. The values obtained showed that the control fish group had a statistically significant increase ($P < 0.05$) in length during the period of study when compared with the fish in the highest concentration of the used engine oil. It portrayed that the fish group in the control exhibited positive allometric growth throughout the period of the experiment.

There was a negative allometric growth in the groups exposed to the high sublethal concentrations. This can be seen from the loss in the weight of the fish groups in the tanks with concentrations of 70.00 and 140.00 mg/L. As the weight decreased in these groups of fish, the length remained constant, in some cases. This observation agrees with the findings of Torres (1991) and Mshelia *et al.* (2009) that aquatic ecosystems exert different impacts on fish populations. Milda and Androne (2006) and

Eissa *et al.*(2011) reported that low concentrations of heavy metals can cause a chronic stress which may not kill individual fish but may lead to a lower body weight, smaller size and by implication, low food and commercial values.

5.8 MEAN CONDITION FACTOR OF *O. NILOTICUS* EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The condition factor of fish in the experimental groups in both the control and the sublethal concentrations represented by K-value was less than 1. This reflects the laboratory condition under which the bioassay was carried out being insufficient in simulating true natural conditions. The K-values in the descending order of the sublethal concentrations were 0.06, 0.08, 0.07, 0.04, 0.08 and 0.08 for the control. This finding is similar to that of Ndimele and Owodeinde (2012) who stated that *Clarias gariepinus* generally had condition factor (K) of less than 1 adding that fishes with allometric growth patterns often have K values less than 1.

5.9 BIOACCUMULATION OF METALS IN THE TISSUES OF *O. NILOTICUS* FINGERLINGS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL AFTER TEN WEEKS

A potential threat to aquatic organisms is contamination of the environment with significant amounts of heavy metals which can cause harmful effects on metabolic, physiological and biochemical systems of fishes at high concentrations. The concentration of the metals in the organs of *O. niloticus* fingerlings was in the order $e > Zn > Mn > Cu > Pb > Cr$. Metals are said to accumulate in the tissues of aquatic animals and may become toxic when accumulation reach substantially high levels (Heath 1987, Awofolu *et al.*, 2005 and Ihuahi, Egila *et al.*(2009). The concentration of heavy metals in fish is related to several factors such as physicochemical properties of the water (like hardness) and the presence of ions in the environment (Awofolu *et al.*, 2005).

In this study, Iron had the highest concentration in the three organs investigated with the liver leading in the concentration. This agrees with the report of Samir and Shaker(2008) that gills and liver of *O. niloticus* contained the highest concentration of most heavy metals detected in their study while the muscles appeared to be the least preferred site for the bioaccumulation of the metals. This high metal concentration in the liver is consequent on its being the organ responsible for the detoxification of metals physiologically as reported by Benson, Etesin, Essien, Umoren, and Umoh (2006) and El-Nemakiet *al.* (2008).

Lead, the most toxic metal, is detectable in practically all phases of the inert environment and all biological systems, and it is toxic to most living things at high exposure levels. Sorensen (1991) stated that lead is processed along with calcium because of its chemical resemblance to calcium. However, tissues other than bone are considered the storage sites in fish. Lead had second to the least concentration after chromium in the organs of the fish. This level (1.23 μ g/g dry wt.) poses no risk to the health of the fish when compared with the level of 0.01 mg/L permitted by WHO (1993) except when concentration reaches chronic levels. Das *et al.*(2004) observed that sublethal concentrations may reduce growth, damage various organs and predispose the fish to disease. Ugwu *et al.*(2008) obtained calcium ion concentrations of about 5.00 mg/L while the mean value recorded in this study was 4.81mg/L. Magnesium ions concentration was 2.86 mg/L. But these two metals were below detection level in the fish organs. These values are similar to those of Balarabe and Abubakar (2007) at the Nguru Lake. Abdel-Tawab and Wafeek (2008) observed that exposure of *O. niloticus* to cadmium (Cd) which is a heavy metal, affected fish performance, survival and feed utilization. Al-Nagaawy (2008) reported that the presence of copper ions causes serious toxicological concerns, because it is usually

known to deposit in the brain, skin, liver, pancreas, and myocardium. Davis, Volesky and Vierra (2000) also reported that the occurrence of copper in large amounts is extremely toxic to living organisms.

5.10 EFFECTS OF USED CRANKCASE OIL ON THE HAEMATOLOGICAL INDICES OF *O. NILOTICUS* FINGERLINGS EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The effects of used crankcase oil on the haematological indices of *O. niloticus* fingerlings showed variations when fish in the sublethal concentrations are compared to those in the control tank. The analysis of variance (ANOVA) indicated a significant difference ($P < 0.05$) between fish in the control and those exposed to used crankcase oil. The correlation between WBC and RBC showed a strong negative correlation with $r = -0.716$. This implies that as the number of WBC increased, the number of RBC decreased and vice versa. There was a strong positive correlation between WBC and PLT with $r = 0.979$. This result also indicates that as the number of WBC increased, the number of PLT also increased and vice versa. On the other hand, the relationship between RBC and PLT showed a strong negative correlation with $r = -0.720$. This result means that as the number of PLT decreased, the number of RBC also decreased and vice versa.

In a stress situation, RBC count is one of the first parameters to be affected. It showed a decrease in fish exposed to the sublethal concentrations of used crankcase oil in this study. Al-Attar (2005) also observed significant decreases in RBCs and haemoglobin content of *O. niloticus* when exposed to Cadmium. Decreases in the circulating erythrocytes could be attributed to a decrease in the synthesis or their release into circulation or an increase in the destruction of the erythrocytes. Fazio *et al.*, (2013) observed that a decrease in RBC and an increase in Hct, MCV and Hgb is a compensatory mechanism to balance the deficit in RBC. While Dahunsi and Oranusi

(2013) explained that a decrease in haemoglobin content during stress conditions may indicate a decrease in the rate of haemoglobin synthesis which leads to impaired oxygen supply to various tissues resulting in decrease in the RBCs through haemolysis. On the other hand, Obasohan and Oronsaye (2011) reported the direct effect of the pollutant Copper on circulating blood cells to include the disintegration of erythrocytes. Kayode and Shamsudeen (2010), Fazio *et al.*(2013), Dahunsi and Oranusi (2013) and Nwani *et al.*(2015) observed significant ($P < 0.05$) decreases in RBC, Hct, MCV, MCHC and Hgb when fish was exposed to different toxicants.

There was a high WBC count possibly due to attempts by the fish to fight against the polluting substance in the test medium leading to the production of more antibodies to improve its health status. This may be because WBCs function as a defense to the body system against infections by producing antibodies which serve as body soldiers. The WBCs of fish in the control were low compared to those exposed to the sublethal concentrations because the antigens attached to the WBCs in the former were few while they were more numerous in the latter.

Reddy and Reddy (2013) reported that the increase they observed in leucocytes in the fish group exposed to toxicant compared to the control could be attributed to immunological responses of the fish to the toxicant. While Dahunsi and Oranusi (2013) explained that the increase in leucocyte count when *Clarias gariepinus* was exposed to rubber processing effluent indicated the stimulatory effect of the toxicant on the immune system.

A significant decrease ($P < 0.05$) in haemoglobin was observed in the blood of fish exposed to used crankcase oil at the end of the exposure period. Ashade and Kumoyi (2013) observed a similar trend in *Clarias lazera* when exposed to spent engine oil. They reported that the decrease they observed in haematocrit when compared to the

haemoglobin standard could be attributed to shrinkage of the erythrocytes. Etim *et al.* (2014) also reported similar findings in *Tilapia* species showing that a decrease in haemoglobin content during stress conditions may indicate a decrease in the rate of haemoglobin synthesis which leads to impaired oxygen supply to various tissues. The lysis of erythrocytes leads to a reduction in haematocrit value.

Kayode and Shamsudeen (2010) stated that prolonged reduction in haemoglobin content is deleterious to oxygen transport and a degeneration of the erythrocytes could be ascribed to pathological conditions in fishes exposed to toxicant. These decreases in haemoglobin and haematocrit may be attributed to uncontrolled lysis of the RBC due to the toxicity level of the pollutant. This agrees with the findings of Al –Attar (2005) who observed a decrease in the concentrations of haemoglobin and haematocrit in *O. niloticus* exposed to sublethal concentrations of Cadmium. Reddy and Reddy (2013) also reported decreases in haematocrit values when compared to the control due to the exposure of the fish *Catla catlato* heavy metals. A rise in the value of haematocrit is a danger sign of an increased rise in dengue shock syndrome, but low haematocrit with low MCV suggest a chronic iron-deficiency (erythropoiesis). When this persists, it may result in anaemia. Platelets help to clump together the red cells to form a clot for the prevention of bleeding.

There was a decreasing trend in values of mean corpuscular haemoglobin concentration (MCHC). This trend was observed by Dahunsi and Oranusi (2013) who attributed their result to an indication that young erythrocytes containing less haemoglobin were released into circulation. The result of this investigation not only follows the trends mentioned above but also agrees with the findings of Onwurah, Okejim and Ajie (2013) who reported significant decreases in MCV and MCHC when *O. niloticus* was exposed to crude oil pollution.

Lymphocytes and monocytes were found to be significantly reduced ($P < 0.05$) due probably to the effect of hydrocarbon in lymphoid tissues which have been found to cause antibody depression and impaired migration of phagocytic cells. This could be attributed to a stress stimulus eliciting a defence response. Ovuru and Ekweozor (2004) observed a similar result in rabbits exposed to crude oil.

5.11 EFFECTS OF USED CRANKCASE OIL ON SOME ENZYME ACTIVITIES: ALKALINE PHOSPHATASE (ALP) AND ALANINE TRANSAMINASE, (ALT) IN *O. niloticus* FINGERLING EXPOSED TO SUBLETHAL CONCENTRATIONS OF USED CRANKCASE OIL

The effects of used crankcase oil on the activities of the enzyme, Alkaline Phosphatase (ALP) in *O. niloticus* fingerlings showed a general elevation in the gills, liver and muscle tissues. The effects of the sublethal concentrations of used crankcase oil on the activities of ALP was altered during the weeks of exposure. There was a very significant ($P < 0.05$) elevation in the concentration level of ALP in the muscle at the highest toxicant concentration of 140ml/L with the value at 256 ± 0.441 while the same value in the control was as low as 13.35 ± 75.90 iu in the gills of the fish. The same trend was recorded in the liver with an elevated value of 200.12 ± 0.831 and gills with a value of 100.00 ± 0.762 at the highest sublethal concentration of 140ml/L. On the other hand, the control fish had ALP concentration values as low as 16.65 ± 7.75 in the liver and 35.00 ± 5.00 in the muscle when compared with the values in these organs at the highest concentration of used crankcase oil. Nalini, Ellaiah, Prabhakar and Girijasankar (2015) reported that ALP plays an indispensable role in phosphate metabolism and that it hydrolyses phosphate from many types of molecules like nucleotides, proteins, alkaloids esters and anhydrides of phosphoric acid. The activities of ALP recorded in this study agrees with the report of Vasanth et al. (2012) who observed increases in *L. rohita*. They posited that the significant increases in the ALP activities in the

liver, kidney, gill and muscle of *L. rohita* could either be due to leakage from the cytosol across damaged plasma membrane into the general blood circulation or increase in their synthesis as a result of organ dysfunction. This observation is similar to the present investigation where the elevated enzyme activities were concentration dependent and this could be considered to be manifestations of oxidative stress caused by the toxicant.

The concentration of ALT followed a similar trend with significantly elevated enzyme concentration of 462.412 ± 0.098 , 430.425 ± 126 and 398.00 ± 0.056 in the muscle, liver and gills respectively at the highest sublethal concentration of used crankcase oil. Vasanth et al. (2012), Abedi et al. (2013), Nalini et al. (2015) and Adeyemi et al. (2015) reported the same findings of significantly increased ALT activities in the organs of fish at high sublethal concentrations of toxicants. The accumulation of toxicants in the liver, gills and muscle of the test organism led to the functional damage of these organs as reflected in the increased activities of the enzymes. This biochemical dysfunction may interfere with homeostatic processes. The increase in the activities of the enzymes reinforces their important role in the detoxification of the pollutant. ALP and ALAT have through this study been shown as potential biomarkers that can be used for the study of used crankcase oil induced toxicity. Overall, the changes in the concentration and activities of the enzymes occurred either due to leakage of these enzymes from hepatic cells thus raising the levels through increased synthesis or by induction of the enzymes. Such changes in biochemical levels under the effect of used crankcase oil might result in the impairment of vital physiological processes consequently affecting the health status of the exposed fish.

CHAPTER SIX SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY OF FINDINGS

This study of the effects of used crankcase oil on some physiological parameters of *O. niloticus* fingerlings exposed to the wsf of used crankcase oil over a ten weeks period showed that:

1. The wsf of used crankcase oil significantly altered the values of water quality parameters when compared with WHO values for drinking water (Table 2 Appendix B2).
2. The toxicity of the wsf of used crankcase oil on the test fish is dose dependent.
3. The concentrations of metals and other elements in the used crankcase oil and the fish organs were significantly different compared to the control.
4. Proteins and lipids in *O. niloticus* fingerlings decreased in value due to exposure to used crankcase oil.
5. Exposure of the test fish to the wsf of used crankcase oil resulted in retarded growth as seen in the reduction in weight and constancy of length of fish in the higher sublethal concentrations (Table 6 and Appendix B4).
6. *O. niloticus* fingerlings had a poor feed conversion capability in the presence of used crankcase oil evidenced by decreases in muscle and liver glycogen at the higher sublethal concentrations (Figs. 4-15). FCR for the control was 1.33 while the same value for the highest sublethal concentration of toxicant was 3.20 showing a better growth and feed conversion in the control. This is close to the 1.6 to 1.8 general FCR range for Tilapia species under natural conditions.
7. Metals are capable of accumulating in the tissues of fish when concentration levels are high (Fig. 16, Appendix B5).

8. Gonadal development is rudimentary when *O. niloticus* is about 9cm in length and 20.10g in weight.
9. The condition factor of experimental fish was < 1 indicating a generally stressful condition (Table 7).
10. High concentrations of used crankcase oil significantly altered the haematological indices of *O. niloticus* fingerlings. RBC decreased significantly while WBC increased significantly (Table 8).
11. The enzymatic activities of ALP & ALAT in the test fish became significantly altered as a result of the exposure to the wsf of used crankcase oil (Tables 9 and 10).

6.2 CONCLUSION

This study has revealed that the wsf of used crankcase oil have very harmful effects in the aquatic environment when concentration levels are high. The components of this toxicant bioconcentrate in fish tissues as exemplified by the accumulation of metals in the gills, liver and muscles of *O. niloticus* fingerlings. The alteration of some physiological processes like carbohydrate, protein and lipid reserves as well as haematological indices and some activities of selected enzymes were recorded as some of the parameters affected by the presence of the test substance. This investigation has therefore achieved an overall prediction success.

6.3 RECOMMENDATIONS

Based on the results recorded in this research, the following recommendations are made:

1. The unregulated disposal of used crankcase oil poses a great threat to the health of the environment. Consequently, government at all levels should control this indiscriminate practice through legislation and by creating collection centres for used crankcase oil.
2. Bioremediation, recycling and other processes should be put in place to ensure proper disposal and to prevent the pollution of the environment.
3. The mandatory use of the right amount of Benzene and its derivatives in diesel, petrol and other fuels should be enforced by the government to minimize the carcinogenic effects on humans.
4. Environmental Impact Assessments should be carried out in oil spill areas to determine the level of oil concentration in the soil before projects are sited.

6.4 LIMITATIONS OF THE STUDY

This study had the following limitations:

1. The separation of the components of PAH is a very difficult process and the equipment is not easy to come by consequently, this exercise was not carried out.
2. Many of the reagents for enzyme analysis are very expensive and scarce therefore only two (ALP and ALAT) were analysed.
3. At a length of 9.90cm and a weight of 20.10g, gonadal development in *O. niloticus* was still quite rudimentary, consequently eggs could not be produced.

6.5 SUGGESTIONS FOR FURTHER STUDY

In view of the importance of this study to Public Health, further research needs to be carried out in the following areas:

1. A comparative study of the toxicity of the Used Crankcase Oil and the Virgin Oil should be carried out.

2. Haematological indices, skin and other possible test samples of motor mechanics should be assessed using unexposed human subjects as the control.
3. The carcinogenic effects of Polycyclic Aromatic Hydrocarbon (PAH) derivatives like Benzene and the permissible doze levels in petrol, diesel, wood sprays, paints, etc should be investigated.

6.6 CONTRIBUTION TO KNOWLEDGE

1. The values of carbohydrate reserves (muscle and liver glycogen) as well as proteins and lipids in *O. niloticus* fingerlings revealed decreases in these parameters at the end of the experimental period. But unexposed fish gained weight with FCR of 1.33 while fish in the highest sublethal concentration of used crankcase oil lost weight with FCR of 3.20 showing the negative effects of the toxicant on the growth and feed utilization of *O. niloticus* fingerlings.
2. Circulating erythrocytes, MCV and blood platelets decreased with increasing time while leucocytes, LY, MO, GR, and MCHC increased indicating the negative effects of used crankcase oil on the haemtological indices of the test fish.
3. The bioconcentration of Fe, Zn, Mn, Cu, Cr, Pb in the tissues of this fish is a practical demonstration of the danger PAHs in the aquatic environment poses to the human consumers of fish. The Nuclear Reactor (Nigeria Resrarch Reactor 1) detected more elements namely Al, Ca, Na, Mn, Cl, Zn, Brin the used crankcase oil than the Atomic Absorption Spectrophotometer (AAS). This reveals the sensitivity of the two machines in analysing the elemental contents of the oil.

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APPENDICES
APPENDIX A1: INSTRUMENTS CHEMICALS, AND REAGENTS USED

Instruments

Hot plate

Gallows

Maonetic stirrer

Jericans

Measuring Cylinder

Mercury cebntigrade dry bulb thermometer

Oxygenated plastic bags

Glass rod

Miniature Neutron Source Nuclear Reactor

Hot air oven

Desicator

Micro – Kjeldal distillation apparatus

Fat-free extractor thimble

Centrifuge

Test tubes

pH Meter (Luthron Model-201)

Conical flask

Crucible

Kjeldal flask

Metler balance

Dissecting board

Centimeter rule

Atomic Absorption Spectrophotometer SOLAR 969 Unicam (Thermo Fisher iCE3000 series).

Auto Haematology Analyzer (Model KZ Erma 2700)

Polytron type Homogenizer

Autoanalyzer Biosystem A25 Model Spain

EDTA bottles

Colorimeter

Polypropylene container

Chemicals and Reagents

Crankcase Oil

Dechlorinated Tap Water

Manganese sulphate

Alkaline Iodide

Sulphuric acid (Concentrated)

0.025 Sodium Thiosulphate

1% Starch solution

Phenolphthalein indicator

0.4N Sodium Hydroxide

Methyl Orange indicator

0.02N Sulphuric acid

45% Sodium Hydroxide

2% Boric Acid

0.01N Hydrochloric acid

30% Potassium hydroxide

Sodium Tetrasulphate VI (Saturated)

95% Ethanol

5M Hydrochloric acid

0.5M Sodium Hydroxide

Phenol red Indicator

Glucose Standard

Distilled water

Cold water

Anthrone reagent

Boiling water

Nitric acid

Hydrogen peroxide

0.25 Sucrose solution

APPENDIX B₁

96Hr — LC 50 of *O. niloticus* Exposed to Water—Soluble Fractions of Used Crankcase Oil.

Conc. (ml/L)	Log Conc.	Total No. of Fish	No. Dead after 96 hrs	Mean Mortality	% Mortality	Probit
00	00	20	00	00	00	00
150	2.1761	20	3	1.5	15	3.96
300	2.4771	20	4	2.0	20	4.16
450	2.6532	20	7	3.5	35	4.61
600	2.7782	20	10	5	50	5.00
750	2.8751	20	12	6	60	5.25

APPENDIX B₂**World Health Organization (WHO) Guideline Values for Drinking Water Quality**

pH	6.5-9.0
Total Dissolved Solids(mg/L)	500
Total Alkalinity as CaCO ₃ (mg/L)	30 – 500
Total Hardness as CaCO ₃ (mg/L)	100 – 200
Chloride (Cl ⁻)(mg/L)	250
Cyanide (CN ⁻) (mg/L)	0.07
Nitrate (NO ₃ ⁻) (mg/L)	50.0
Nitrite (NO ₂ ⁻) (mg/L)	3.0
Sulphate (SO ₄ ²⁻) (mg/L)	250
Arsenic (As) (mg/L)	0.01
Barium (Ba) (mg/L)	0.7
Cadmium (Cd) (mg/L)	0.003
Chromium (Cr) (mg/L)	0.05
Copper (Cu) (mg/L)	2.0
Iron (Fe) (mg/L)	0.3
Lead (Pb) (mg/L)	0.01
Manganese (Mn) (mg/L)	0.1
Mercury (Hg) (mg/L)	0.001
Nickel (Ni) (mg/L)	0.02
Zinc (Zn) (mg/L)	3.0

Source:WHO Guideline Values for Drinking Water Quality,WHO, Geneva; (1993)

Appendix B₃

Variations in Length – Weight Relationship of *Oreochromis niloticus* Fingerlings Exposed to Sublethal Concentrations of Used Crankcase Oil

Concs. (ml/L)		Exposure Period					
		0	2	4	6	8	10
140.00	*L	5.7	6.0	6.5	6.5	6.5	6.5
	*W	6.3	6.5	5.8	5.4	5.4	5.2
70.00	L	5.7	6.0	6.3	6.4	6.5	6.5
	W	6.3	6.4	6.0	5.1	4.8	4.8
35.00	L	5.7	6.0	6.5	6.5	6.5	6.5
	W	6.3	6.2	5.8	4.1	4.4	4.7
17.50	L	5.7	5.9	6.3	6.5	6.5	6.6
	W	6.3	6.5	6.0	5.7	4.9	4.5
8.75	L	5.7	6.0	6.5	6.7	7.1	8.0
	W	6.3	6.5	7.5	8.8	9.5	10.8
0.00	L	5.7	6.2	7.3	8.5	9.1	9.9
	W	6.3	8.5	11.8	13.9	16.2	20.1

*L = length *W = weight (r = 0.861, n = 36).

APPENDIX B₄

Variations in Mean Values of Metals in the Experimental Water Treated with Various Concentrations of used crankcase oil

	Used CrankcaseOil Concentration (ml/L)					
Metals	0.00	8.75	17.50	35.00	70.00	140.00
Fe	0.04	0.09	0.20	0.25	1.01	2.84
Zn	0.02	0.10	0.15	0.57	0.84	0.99
Cu	0.01	0.02	0.03	0.04	0.06	0.08
Mn	0.09	0.11	0.09	0.13	0.15	0.16
Cr	0.01	0.02	0.03	0.04	0.07	0.09
Pb	0.03	0.05	0.11	0.14	0.18	0.22
Mg	0.22	0.45	0.79	1.12	1.96	2.86
Ca	0.12	0.43	0.88	1.04	3.08	4.81

APPENDIX B₅**Variations in Mean Concentration of Metals in *O. niloticus* Gills, Muscles and Liver ($\mu\text{g/g}$ dry wt.)**

Organs	Metals					
	Fe	Zn	Mn	Cu	Cr	Pb
Gills	09.18	9.88	26.24	4.24	0.22	1.23
Muscles	75.19	27.60	1.98	2.80	0.19	0.59
Liver	253.00	13.05	25.4	16.36	0.41	0.04
Mean ()	179.12	16.84	17.54	7.80	0.27	0.62
SD*	75.64	7.72	11.25	6.08	0.09	0.49
COV(%)*	42.23	45.82	64.13	77.96	36.10	78.43

***SD = Standard Deviation**

***COV = Coefficient of Variation**

APPENDIX B₆
Variations in Mean Values of Liver Glycogen of *O. niloticus* for Ten Weeks

Exposure Period (wks)	
Conc. of toxicant (ml/L)	0 2 4 6 8 10
140	1.23 1.20 0.91 0.55 0.45 0.30 ±0.02 ±0.02 ±0.01 ±0.01 ±0.02 ±0.01
70	1.20 1.21 1.05 0.88 0.60 0.55 ±0.02 ±0.01 ±0.02 ±0.01 ±0.01 ±0.02
35	1.22 1.25 1.11 0.98 0.76 0.75 ±0.02 ±0.01 ±0.01 ±0.20 ±0.02 ±0.01
17.50	1.21 1.24 1.15 1.06 0.97 0.80 ±0.03 ±0.01 ±0.01 ±0.01 ±0.02 ±0.02
8.75	1.23 1.24 1.10 1.00 0.88 0.68 ±0.03 ±0.00 ±0.01 ±0.00 ±0.02 ±0.01
0.00	1.22 1.25 1.28 1.50 2.01 2.12 ±0.02 ±0.01 ±0.01 ±0.01 ±0.02 ±0.02

± = standard error of the means

APPENDIX B₇Variations in Mean Values of Muscle Glycogen for *O. niloticus* Fingerlings

Con. (mg/L)	Exposure Period (wks)					
	0	2	4	6	8	10
140	0.09	0.06	0.03	0.02	0.02	0.02
	±0.02	0.02	0.01	0.01	0.01	0.01
70	0.09	0.08	0.05	0.03	0.02	0.03
	±0.02	±0.02	±0.03	±0.02	±0.01	±0.02
35	0.09	0.07	0.07	0.05	0.04	0.03
	±0.02	±0.02	±0.02	±0.01	±0.01	±0.01
17.50	0.09	0.09	0.08	0.08	0.05	0.04
	±0.02	±0.02	±0.02	±0.02	±0.03	±0.03
8.75	0.09	0.08	0.06	0.03	0.04	0.04
	±0.02	±0.02	±0.02	±0.01	±0.01	±0.01
0.00	0.09	0.09	0.11	0.10	0.13	0.16
	±0.01	±0.02	±0.02	±0.02	±0.02	±0.01

APPENDIX B₈

**Analysis of Variance (ANOVA) Values of Liver Glycogen at 140.00ml/L Used
Crankcase Oil Concentration**

	N	Mean	Std. Deviation	
	N	Mean	Std. Deviation	
Control	6	1.5633	.40243	.16429
Conc 140	6	.7733	.39692	.16204

	Test Value = 0					
	T	Df	Sig. tailed)	(2- Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Control	9.516	5	.000	1.56333	1.1410	1.9857
conc140	4.772	5	.005	.77333	.3568	1.1899

APPENDIX B₉ANOVA Values of Liver Glycogen at 70.00ml/L Used Crankcase Oil
Concentration One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Control	6	1.5633	.40243	.16429
conc70	6	.9150	.28988	.11834

One-Sample Test

	Test Value = 0					
	T	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Control	9.516	5	.000	1.56333	1.1410	1.9857
conc70	7.732	5	.001	.91500	.6108	1.2192

APPENDIX B₁₀**ANOVA Values of Liver Glycogen at 35.00 ml/L Used Crankcase Oil Concentration**

	N	Mean	Std. Deviation	Std. Error Mean
Control	6	1.5633	.40243	.16429
con35	6	1.0117	.22031	.08994

One-Sample Test

	Test Value = 0					
	T	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Control	9.516	5	.000	1.56333	1.1410	1.9857
con35	11.248	5	.000	1.01167	.7805	1.2429

APPENDIX B₁₁
**ANOVA Values Liver Glycogen at 17.50ml/L Used Crankase Oil
 Concentration**

	N	Mean	Std. Deviation	Std. Error Mean
Control	6	1.5633	.40243	.16429
Conc 17.5	6	1.0717	.16606	.06779

One-Sample Test

	Test Value = 0					
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Control	9.516	5	.000	1.56333	1.1410	1.9857
Conc 17.5	15.808	5	.000	1.07167	.8974	1.2459

APPENDIX B₁₂**ANOVA Values of Liver Glycogen at 8.75ml/L Sublethal Concentration of Used Crankcase Oil**

	N	Mean	Std. Deviation	Std. Error Mean
Control	6	1.5633	.40243	.16429
Conc 8.75	6	1.0217	.21656	.08841

One-Sample Test

	Test Value = 0					
	T	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Control	9.516	5	.000	1.56333	1.1410	1.9857
Conc 8.75	11.556	5	.000	1.02167	.7944	1.2489

APPENDIX B₁₃ANOVA Values of Liver Glycogen of *O. niloticus* at the Control (0.00ml/L) of Used Crankcase Oil

	Test Value = 0					
	T	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Control	9.516	5	.000	1.56333	1.1410	1.9857
Conc 8.75	11.556	5	.000	1.02167	.7944	1.2489

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Conca	.04	6	.026	.010
	Control	.07	6	.051	.021

APPENDIX B₁₄ANOVA of Growth Performance and Feed Utilization of the *O. niloticus* During the Exposure to Used Crankcase Oil

		Sum of		Mean Square	F	Sig.
		Squares	df			
Weight	Between Groups	.000	5	.000	.000	1.000
	Within Groups	168.000	36	4.667		
	Total	168.000	41			
Observations	Between Groups	3158.614	5	631.723	1.885	.121
	Within Groups	12065.806	36	335.161		
	Total	15224.419	41			

APPENDIX B₁₅

**Correlation Between Weight & Length of *O. niloticus*
Fingerlings after Ten Weeks Exposure**

		Length	Weight
Length	Pearson Correlation	1	.861**
	Sig. (2-tailed)		.000
	N	36	36
Weight	Pearson Correlation	.861**	1
	Sig. (2-tailed)	.000	
	N	36	36

The above Table shows Pearson correlation between the weight and length of fish groups with $r = 0.861$ showing a very strong positive relationship between the weight and the length of the experimental fish.