TOXICITY OF "ABRO MOTOR FLUSH" TO *Clariasgariepinus*AND ITS EFFECTS ON SOME TARGET ORGANS

BY

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NOVEMBER, 2017.

Declaration

I, **OGUNMOLA, TEMILOLUWA PETER** hereby declare that this project titled; **"Toxicity of Abro Motor Flush on *Clarias gariepinus* and its effects on some target Organs"** has been performed by me in the Department of Fisheries and Aquaculture under the supervision of **Dr. (Mrs) ARIYOMO, Tolulope O.** The information derived from literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree at any University.

..... 11/04/2018

Ogunmola, Temiloluwa Peter DATE

FAQ/12/0467

CERTIFICATION

This is to certify that this work has not been presented elsewhere for the award of a degree or any other purpose and has been done by the student, OGUNMOLA TEMILOLUWA PETER., of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Oye-Ekiti, Ekiti State.

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Head of Department

DEDICATION

I dedicate this research work to the Almighty God who has given the strength that got me through. Also to my parents and kins who have shown their unrelenting support for me.

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Glory and honour be to the God of Heaven, the Creator of all for His unfailing and lingering love towards me. I sincerely appreciate my Supervisor; Dr. (Mrs) Tolulope O. ARIYOMO of the Department of Fisheries and Aquaculture, Federal university Oye Ekiti for her kindness, moral support and encouragement throughout the period of this project work. My prayer is that God continues to bless her and flourish her family with goodness in Jesus name. Amen.

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ABSTRACT

One of the major cases of pollution has resulted from man's need for improvement in lifestyle and technology i.e. improvement technologies such as motorized vehicles and vessels. Man's need to maintain and keep these technologies on the move has led them to introduce maintenance products such as brake oil, carburetor cleaner, STP Oil wash etc. Of these products is ABRO Motor Flush which serve as oil wash for motors. ABRO Motor Flush containing naphthalene is a combustible liquid with chronic health effects and carcinogenic characteristics. Naphthalene is a PAH [polycyclic aromatic hydrocarbon] which occurs naturally as a component of coal tar and crude oil and is manufactured for use principally as a chemical intermediate (e.g. phthalic anhydride). Emerging detrimental effects of pollution to the aquatic environment have called for immediate attention and action towards reducing pollution and its causes. *Clarias gariepinus* are tolerant of extreme environmental conditions, thereby making it adapt so readily to various environments, hence the choice for this study. ABRO Motor Flush was used for this study in four varying concentrations (1.00ml/L, 1.33ml/L, 1.67ml/L, and 2.00ml/L) and were duplicated; including the control treatment without a solution of ABRO Motor Flush were also prepared. All tests were conducted under standard bioassay procedures (American Public Health Association, 1977). Quality of water was checked at regular interval to observe effect of the chemical on the water, The LC_{50} of this product to *C. gariepinus juveniles* is 1.92ml/L. Results obtained from the haematological analysis indicated that value of blood indices decreased with increasing concentrations of ABRO Motor Flush compared to those of the control fish, likewise Histopathological results obtained from the experiments indicate that ABRO Motor Flush had a direct impact on the tissues of the gills and livers of *Clarias gariepinus* at the end of the 96hour exposure period.

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CHAPTER ONE

1.0. INTRODUCTION

Human activities are partly responsible for introducing contaminants into the environment with negative impacts on aquatic life (Clair *et al.*, 2003). Pollution is a major problem confronting the enviroment, News report recently equated the level of pollution in a city in India to the equivalent of smoking 40 sticks of cigarettes' per day for anyone one breathing 'normal' air in such a city. Pollution is thus a daily experience in most parts of the world. Environmental pollution is a a major issue militating against modern life. Globally, pollution has attained a frightening proportion and a leading cause of deaths, sicknesses and associated disasters.

Pollution is defined as the presence of one or more contaminants that persist and then alter the physical, chemical or biological features of the environment. (George *et al.*, 2014).

Exposure of aquatic organisms to fractions of pollutants has been shown to have significant impact on the organs and characteristics of aquatic organisms which may resort to mortality (Barron *et al.*, 2003; Couillard *et al.*, 2005, Liu *et al.*, 2006). It has been proposed that the haematological and histopathological changes in fish exposed to pollutants can be used as sensitive bio-markers for assessing the effects of several environmental contaminants on aquatic life (George *et al.*, 2014).

One of the major cases of pollution has resulted from man's need for improvement in lifestyle and technology i.e. civilization. Civilization has brought about improvement in technologies such as motorized vehicles and vessels. Man's need to maintain and keep these technologies on the move has led them to introduce maintenance products such as brake oil, carburetor cleaner, STP Oil wash etc. Of these products is ABRO Motor Flush which serve as oil wash for motors e.g. boat engines, powered canoes and ships.

The active ingredient in ABRO Motor Flush is naphthalene. Naphthalene may cause eye, skin and respiratory irritation. Inhalation of vapors may cause central nervous system effects including, dizziness, drowsiness and death. Small amounts aspirated during swallowing or vomiting may cause lung and intestinal damage. ABRO Motor Flush is a combustible liquid that may have chronic health effects and it is possibly cancerous or carcinogenic, given that it contains naphthalene, which may cause cancer based on animal data. The risk of cancer depends on the duration and level of exposure (MSDS ABRO Industries Inc., 2007).

Naphthalene is a non-polar polycyclic aromatic hydrocarbon (PAH) with two benzene rings. Naphthalene is a PAH which occurs naturally as a component of coal tar and crude oil and is manufactured for use principally as a chemical intermediate (e.g. phthalic anhydride). It may be found in a wide range of products, including petroleum products, mothballs, wood preservatives, solvents and dyes, and can be released into the aquatic environment by a variety of means (e.g. spillages from chemical and petroleum industries, coal gasification plants, atmospheric fallout). The main source of naphthalene in the environment is believed to be vehicle exhaust. Naphthalene is a combustion product of motor fuel (Gavin *et al.* 1996).

Brown *et al.* (1999) noted that polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants and are formed during the incomplete combustion of fossil fuels. Polycyclic Aromatic Hydrocarbons (PAHs) have toxic and carcinogenic characteristics. The distribution of PAHs into the environment have become so wide that it has motivated efforts to develop bio-remediation technologies to eliminate the sources of PAH exposure to environments (Samanta *et al.*, 2002; Xue & Warshawsky, 2005).

Eaton and Chapman (1992) stated that naphthalene has been proved to be biodegradable. In fact, the biodegradation of naphthalene was studied using gas chromatography and also by an indirect

impedance technique to measure the mineralization reaction. The production of carbon dioxide takes place after the mineralization of naphthalene. This mineralization requires few metabolic steps to produce salicylic acid through dihydroxylated intermediates (San Miguel *et al.*, 2009).

1.1. JUSTIFICATION FOR THE STUDY

The emerging detrimental effects of pollution to the aquatic environment have called for immediate attention and action towards reducing pollution and its causes. *Clarias gariepinus* is a very hardy species, it adapts easily to various environments, hence the choice for this study.

This study will serve as a control for other fish species that might be exposed to the same or similar conditions of exposure to Naphthalene. Naphthalene has been listed as one of the world's emerging contaminants and has been found to be contained in the widely used automobiles maintenance product, ABRO Motor Flush with 0.3% of its 443ml (NPIC, 2010).

1.2. Objectives of the study

The objectives of the study were to:

1. determine the LC_{50} values of *Clarias gariepinus* juveniles exposed to varying concentrations of "ABRO Motor Flush".
2. determine the effects of sub-lethal concentrations of ABRO Motor Flush on the histopathology of gills and liver cells of *Clarias gariepinus*.
3. determine the effects of sub-lethal concentration of ABRO Motor Flush on the hematological characteristics of *Clarias gariepinus*.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 Taxonomy and Biology of the African Catfish *Clarias gariepinus*.

Table 1: Taxonomy for African Catfish

Phylum	**Chordata**
Family	Clariidae
Class	Actinopterygii
Order	Siluriformes
Genus	Clarias
Species	*C. gariepinus*

C. gariepinus are tolerant of extreme environmental conditions. Water parameters appear to play only a very minor role (Teugels, 1986). The presence of an accessory breathing organ enables this species to breath air when very active or under very dry conditions. It prefers to remain in the muddy substrates of ponds and occasionally gulp air through the mouth (Teugels, 1986).. It is able leave the water at night using its strong pectoral fins and spines in search of land-based food and can move into the breeding areas through very shallow pathways. They are omnivorous bottom feeders which occasionally feed at the surface. Feed at night on a wide variety of prey like insects, plankton, invertebrates and fish but also take in young birds, rotting flesh and plants. It migrates to rivers and temporary streams to spawn (Teugels, 1986)..

Life cycle and mating behaviour

They are oviparous in nature and spawn during the rainy season in flooded deltas. They make a lateral migration towards the inundated plains to breed and return to the river or lake soon afterwards while the juveniles remain in the inundated area. Juveniles return to the lake or river when they are between 1.5 and 2.5 cm long. First sexual maturity occurs when females are between 40-45 cm and males between 35-40 cm. Eggs are greenish. Incubations lasts little - about 33 hours at 25°C (Teugels 1986).

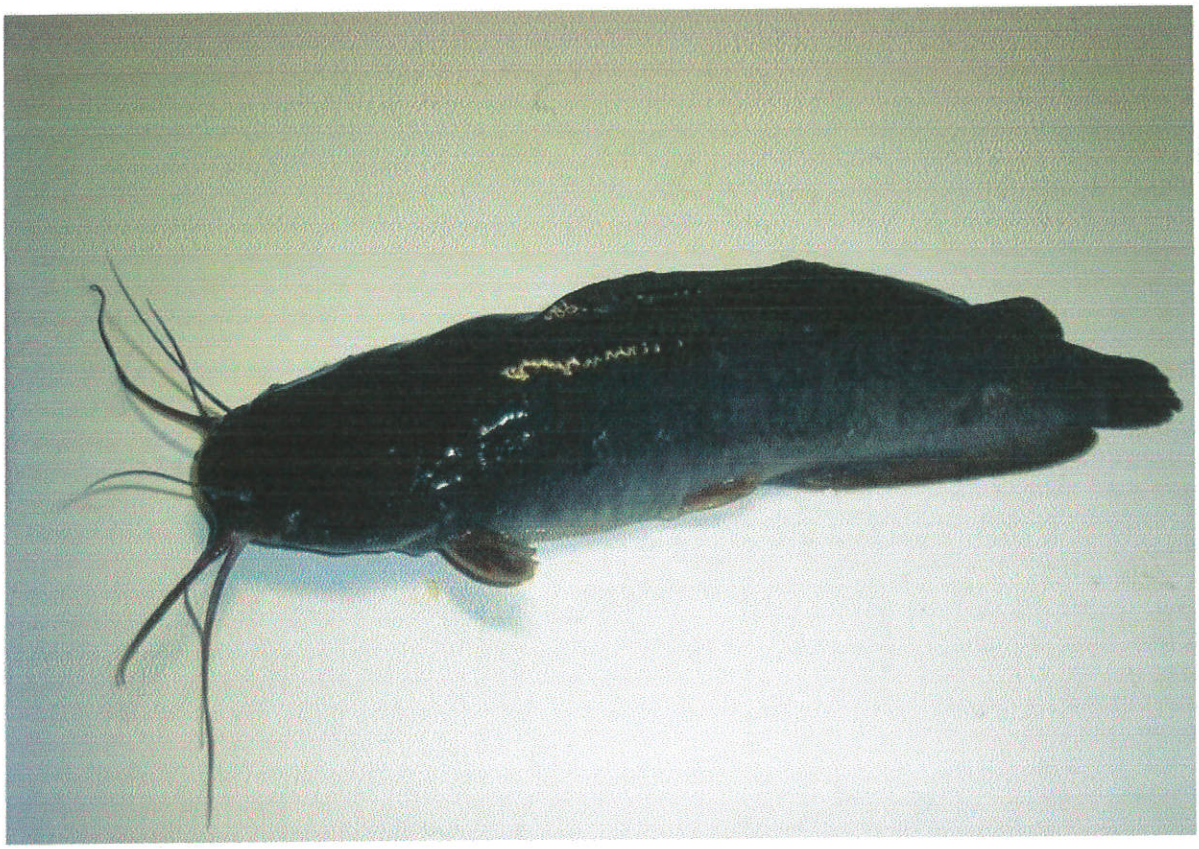

Plate 1: Clarias gariepinus **juvenile from Ishinla Fish Farm. Photographed by Temiloluwa Ogunmola, 2017.**

2.2. ABRO MOTOR FLUSH

ABRO Motor Flush, with the product code MF-390/MF-391, is an Auto-maintenance product by ABRO Industries Inc. It is a clear colourless liquid with a hydrocarbon odour. It is composed of Petroleum distillate (64741-42-0) of about 80 – 100%, 1, 2, 4 Trimethylbenzene (95-63-6) of about 1 – 5%, and naphthalene (91-20-3) less than 0.3%. (MSDS ABRO Inc.)

2.3. NAPHTHALENE

Naphthalene is an organic compound with formula $C_{10}H_8$. It is the simplest polycyclic aromatic hydrocarbon, and is a white crystalline solid with a characteristic odour that is detectable at concentrations as low as 0.08 ppm by mass.

Molar mass: 128.1705 g/mol

Melting point: 176.5°F (80.26°C)

Formula: $C_{10}H_8$

Boiling point: 424.4°F (218°C)

Density: 1.14 g/cm³ (EPA, 2015)

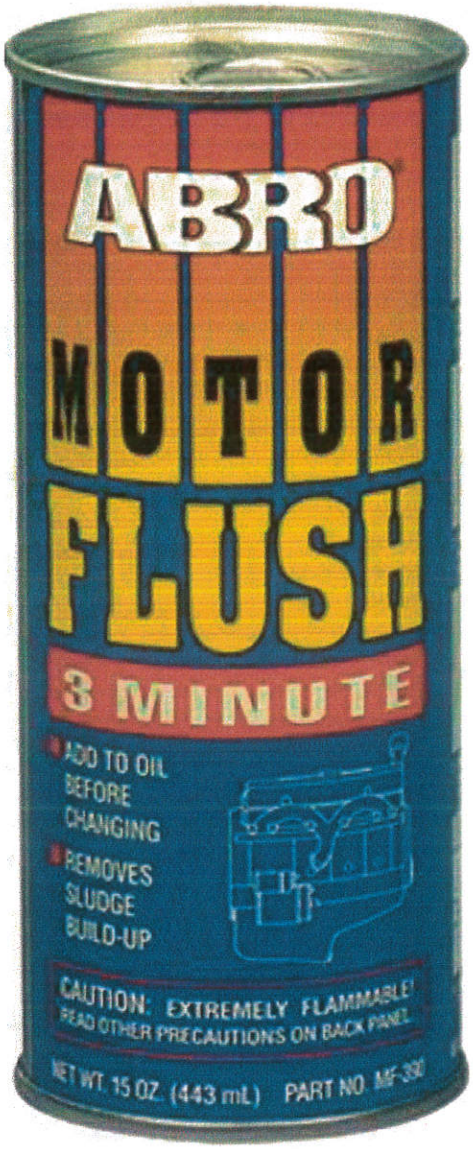

Plate 2: ABRO Motor Flush.

Naphthalene is made from crude oil or coal tar. It is also produced when things burn, like in cigarette smoke, car exhaust, and smoke from forest fires. It is used as an insecticide and pest repellent. Naphthalene was first registered as a pesticide in the United States in 1948 (NPIC, 2010)

Mothballs and other products containing naphthalene are solids that turn into toxic gas. The toxic gas kills insects and may repel animals. There are over a dozen products containing naphthalene registered for use by the U.S. Environmental Protection Agency (NPIC 2010).

Naphthalene is moderately soluble in water and only moderately adsorbs to soil, sediment or suspended solids. When released to the air, half-lives are generally in a few hours. In water volatilisation, adsorption, photolysis and aerobic biodegradation may be important fate processes, depending on local conditions. The half-lives for naphthalene in soil and water range from a few days to a few months (Bates *et al* 1997).

Bates *et al.* (1997) also stated the reports of the *octanol-water* partition coefficients (log Kow 3.01-3.45) suggesting that naphthalene is moderately hydrophobic and may thus have a tendency to adsorb to particulate matter (e.g. soil and sediment particles) and accumulate in biota – bio-accumulation.

Also that contamination of the aquatic environment with naphthalene is most frequently associated with discharges from the chemical and petroleum industries and accidental spillages or leakages of petroleum products to land or water. (NPIC, 2012)

2.3.1. EFFECTS OF NAPHTHALENE

Graham *et al.* (2007) stated that: (i) naphthalene is directly inhibitory or toxic when present at high concentrations; (ii) naphthalene's metabolism could lead to the accumulation of toxic or inhibitory metabolites when a high concentration of naphthalene is available to the cells; or (iii) both naphthalene and its metabolites play a role in growth inhibition.

In an experiment performed by Graham et al. (2007), it noted that not only naphthalene as mentioned, is toxic and growth-inhibitory but also its metabolites. The study continued in showing that while trying to metabolize naphthalene using *Polaromonas naphthalenivorans* CJ2, at inhibitory concentrations, resulted in the accumulation of toxic oxidation products derived from *1, 2-naphthoquinone*, which resulted in a complete loss of viability.Also, in a study done by Davies and Evans (1964), it was shown that 25μM solution of 1, 2-dihydroxynaphthalene is converted by non-enzymic oxidation to 1, 2-naphthoquinone at approximately 20% per minute at pH of 6.5.

Murphy and Stone, 1995 stated that 1, 2-Naphthoquinone has been known to accumulate and inhibit growth and naphthalene metabolism when ferrous and magnesium salts are omitted from the growth media.

2.3.2. MOLECULAR STRUCTURE OF NAPHTHALENE:

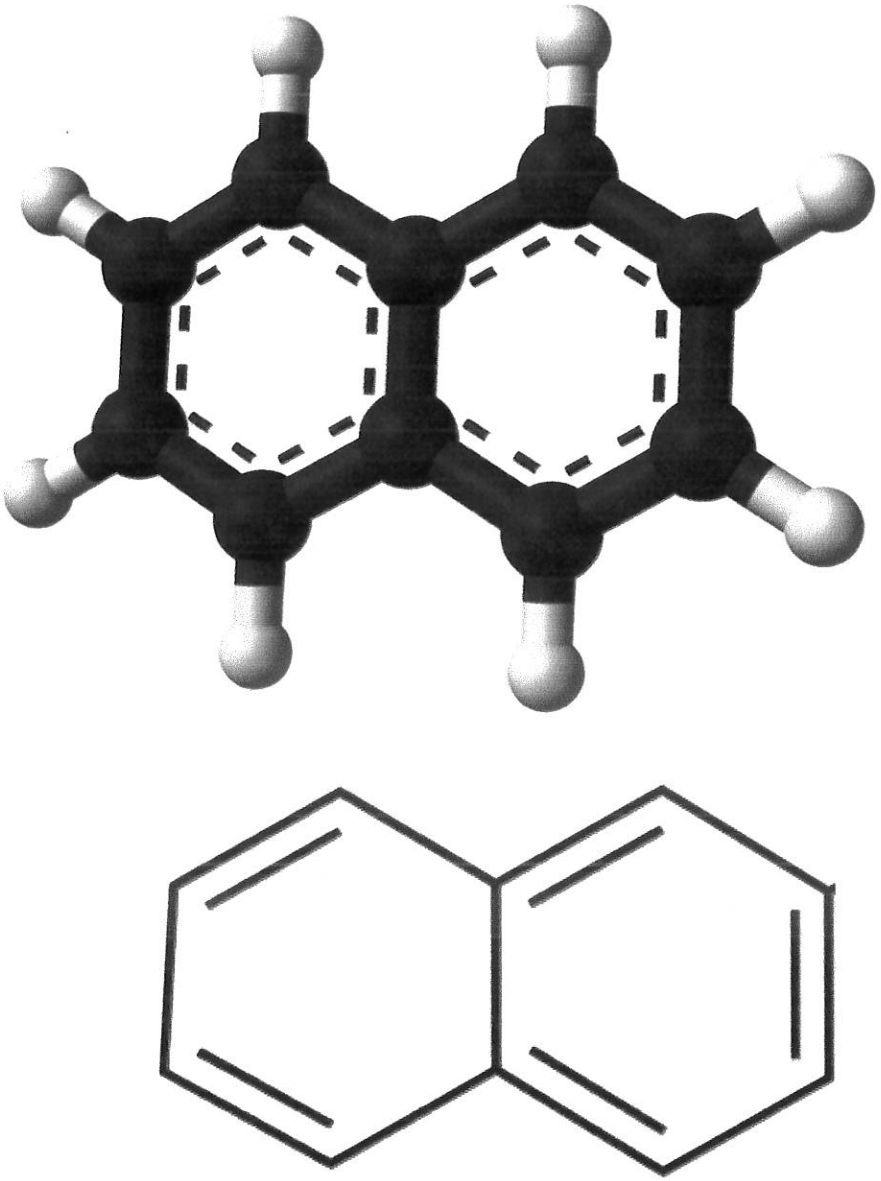

Plate **3: Bicyclo-Structure of Naphthalene**

2.4. WATER QUALITY MONITORING

All living organisms on earth needs water for survival and growth. Therefore, necessary it is that the quality of water be checked at regular interval for that which it is purposed (Basavaraja Simpi et al. 2011).

The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life. Natural water contains different types of impurities, and are introduced in to aquatic system by different ways such as weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from several human activities, including mining, processing and the use of metal based materials (Adeyeye 1994).

2.4.1. Temperature

In an established system, the water temperature controls the rate of all chemical reactions, and affects fish growth, reproduction and immunity. Drastic temperature changes can be fatal to fish.

Barnabe, (1994) stated that in the aquatic environment, temperature is the major dominating factor. In addition, Temperature affects physical, chemical and biological processes in water bodies and also, the concentration of many variables (Enujiugha and Nwanna, 2004). Usually, body systems will show a 50% increase in activity for every 5^0C rise in temperature (Branson, 1993). Chapman and Kimstach (1992) stated; increased temperature increases the rate of chemical reactions and decreases the solubility of gases (especially oxygen) in water.

2.4.2. Dissolved oxygen

DO is one of the most important parameter. Its correlation with water body gives direct and indirect information e.g. bacterial activity, photosynthesis, availability of nutrients,

stratification etc. (Premlata Vikal, 2009). In the progress of summer, dissolved oxygen decreased due to increase in temperature and also due to increased microbial activity (Moss 1972, Morrissette 1978, Sangu 1987, Kataria, 1996). During the summer, DO increases. This is because temperature and duration of bright sunlight has influence on the % of soluble gases (O^2 & CO^2). During this period, the long days and intense sunlight seem to accelerate photosynthesis by phytoplankton, utilizing CO_2 and giving off oxygen.

Krishnamurthy R, 1990 stated that this possibly accounts for the greater qualities of O_2 recorded during summer. DO in sample is measured titrimetrically by Winkler's method after 5 days incubation at 293K.

Barnabe (1994), DO abundance is supplemented by photosynthetic plants in tropical waters and shallow fresh waters. Enujiugha and Nwanna (2004) reported that high oxygen depletion can be so impinging to fish life, oxygen content of natural waters varies with temperature, salinity, turbulence, the photosynthetic activity of algae and plants and atmospheric pressure.

2.4.3. pH

pH is most important in determining the corrosive nature of water. The lower the pH value, the higher the corrosive nature of water (Guptaa 2009). Reduced rate of photosynthetic activity, the assimilation of carbon dioxide and bicarbonates are ultimately responsible for increase in pH. Various factors bring about changes in the pH of water.

Hydrogen ions (acidic) as well as hydroxyl ions (alkaline) are the result of the ionization of water. Any change in the concentration of any one of these ions bring about a change in the concentration of the other. Therefore, a single scale of numbers, called the pH scale (measured

on a scale of 0-14) is used to measure the acidity or alkalinity of water and the number expresses the concentration of hydrogen ions indirectly (Ramchandra and Solanki, 2007)

2.5. LIVER

Branson (1993) described the liver as a relatively large organ usually reddish-brown in carnivores and lighter brown in herbivores. In farmed fish when diets may be less than ideal, it may be lighter in colour than in equivalent wild fish. Lindgaard - Jorgensen and Bender (1994) reported that the liver serves a number of functions, related to the physiological activities – inter-conversion of foodstuff, metabolism of sex hormones, and biotransformation of organic xenobiotics and excretion of harmful trace metals. Branson (1993) also reported that the liver acts as a storage organ for carbohydrates (as glycogen) and fats. It is also involved with blood cell destruction and blood chemistry as well as metabolic functions such as the production of urea and other compounds concerned with nitrogen excretion. The liver is important when considering the action of a toxic chemical on aquatic animals. Aquatic animals encounter a wide range of anthropogenic chemicals in their environments. The concentration of these anthropogenic chemicals in water, sediments and organisms may often result in eco-toxicological effects (Lindgaard-Jorgensen and Bender, 1994).

2.6. GILLS

According to Perry and Laurent (1993), the ability of fish to inhabit diverse and oscillatory environments arises from a variety of adaptive physiological mechanisms. The gill is located between the external and internal environments; water contains only about 5% of the amount of oxygen that is available in air. This level falls further as temperature and ionic concentration increase. Branson (1993) reported that a consequence of this is that the respiratory apparatus of fish must be very efficient to take advantage of the available oxygen. This has been achieved by the development of gills over which there is constant flow of water from which oxygen is extracted. The gill surface consists of a thin epithelium carried on lamellae, providing an intimate interface for the uptake of oxygen and excretion of carbon dioxide.

Apart from gaseous exchange, the gill also plays crucial roles in acid-base balance, osmoregulation and excretion of nitrogenous waste products (Perry and Laurent, 1993; Branson, 1993). Thinness of the respiratory surface makes it vulnerable to damage and inversion by pathogens, which will lead to disruption of the entire gill's functions (Branson, 1993; Perry and Laurent, 1993)

2.7. EFFECTS OF TOXICANTS TO FISH

2.7.1 **Toxicity Studies**.

It is the study of adverse effects of chemical and physical agents and the degree to which a substance can harm human or animals. Toxicity studies can be of, acute and chronic toxicity. Acute toxicity: it involves harmful effects in an organism through a single or short term

exposure. Sub-chronic toxicity: it is the ability of a toxic substance to cause effects for more than one year but less than the life time of exposed organism. Chronic toxicity: it is the ability of the substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated and continuous exposures

Clark (1992) defined toxicity as a measure of how poisonous a substance is or how large a dose is required to kill or change an organism. The more toxic substances are, the smaller the dose.

Singh and Yadav (1978) reported that fish are likely to be killed due to direct effects of the herbicides and indirectly due to asphyxiation, following the disappearance of vegetation. In a test they conducted to test the toxicity of copper sulphate and 2, 4 – D amine to fingerlings at concentrations of 1 to 100 ppm showed that copper sulphate proved to be more toxic, causing 40% fish mortality at 1ppm and 2 4-D amine was less toxic, causing no mortality even at 100ppm concentration.

Chemicals usually used in the treatment of water and fish diseases cause fatal effects on the fish and other aquatic organisms when the doses of such chemicals are not accurately applied.

Their test results proved that *C .gariepinus* and *H. bidorsalis* fingerlings showed erratic swimming, loss of reflex, discolouration, peeling and eventual death. Examination of the gills showed some alterations as gills were swollen and the lamellae were fused. In addition, the cells of the liver were deformed while the kidney lost their colouration.

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Collection and Conditioning of Test Fish

Apparently healthy live *Clarias gariepinus* juveniles were collected from Ishinla Fish Farm, Ado Ekiti and transported in oxygenated plastic bags to the Fisheries Laboratory of the Federal University of Oye- Ekiti. After which acclimation of fish was done in fresh water by tempering – gradually changing the water in the rectangular glass tanks (75 x 40 x 40cm). Test fishes were fed daily to satiation during the one week acclimatization period, with a pelleted commercial feed (42.5 % crude protein).

3.2. RANGE FINDING TEST

One hundred (100) juveniles of *Clarias gariepinus* of mean weight 12.73g and mean total length 7.40cm were used for the range finding test. Mettler top precision loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent rectangular plastic containers for two days in order to adapt to laboratory conditions. Feeding was halted during this period to reduce the production of waste in the transparent cylindrical containers thus minimizing the chances of ammonia production.

Ten transparent cylindrical plastic tanks were used, each filled with 15litres of water, to hold test fish in denomination of ten test fish per holding tank. These were subjected to the introduction of "ABRO MOTOR FLUSH (liquid)" having in ratios of 0.3% to 5% of naphthalene and 1,2,4 Trimethylbenzene respectively in 80 – 100% of petrol distillates (solvent).

The range finding test was done by introducing varying amounts in concentration of ABRO Motor Flush into each container. For this study, the range finding test was done using 0mg/L, 2mg/L, 6mg/L, 12mg/L and 16mg/L respectively.

(i) 2ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **0.13ml/l**

(ii) 6ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **0.4ml/l**

(iii) 12ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **0.8ml/l**

(iv) 16ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **1.07ml/l**

Each of the four varying concentrations (0.13ml, 0.4ml, 0.8ml, and 1.07ml) were duplicated; two replicates of the control treatment without a solution of ABRO Motor Flush were also prepared.

Both the range finding and definitive tests were conducted under standard bioassay procedures (American Public Health Association, 1977).

3.3. DEFINITIVE TEST

The definitive test, after which the desired range has been determined, was carried out using One hundred juveniles of *C. gariepinus* of mean weight and mean total length 12.73g and 7.40cm respectively. Also, Mettler top loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent cylindrical plastic containers. The auto-product, ABRO Motor Flush used was obtained from Gbara Market, Lekki roundabout in Lagos state. Ten transparent cylindrical plastic containers of 25litre capacity, each filled with 15litres of water were used for the Definitive test. "ABRO Motor Flush" with the same constituents as was in the range finding test was also used. Four concentrations used in the Definitive experiment were obtained as follows:

(i) 15ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **1.00ml/l**

(ii) 20ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **1.33ml/l**

(iii) 25ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **1.67ml/l**

(iv) 30ml of ABRO Motor Flush

15litres of water in transparent cylindrical plastic container = **2.00ml/l**

Each of the four varying concentrations in ml per litre (1.00, 1.33, 1.67 and 2.00) were duplicated and introduced into the transparent cylindrical plastic containers, two replicates of the control treatment without "ABRO Motor Flush" were also prepared.

3.4. PHYSIOCHEMICAL OR WATER QUALITY PARAMETERS

George et al. (2014) stated that "Water quality parameters should be monitored before start of experiment, and also specified on daily basis according to standard method (APHA, 1989). Parameters to be monitored include; Dissolved Oxygen (DO), pH, temperature (^{0}C), Nitrite (NO2) and Ammonia (NH3).

This was adhered to for the 96 hours (four days) period of experimentation. Water quality parameters as follows: temperature, dissolved oxygen, pH, total dissolved solid and conductivity were determined at 24hrs intervals, using standard methods.

3.4.1. Conductivity

Conductivity or electrical conductivity is the measure **of ions in a solution and was measured** in $\mu S/m^2$, with conductivity meter model **SX751**. The probe of the conductivity meter was inserted into each of the transparent cylindrical plastic containers, containing the different treatments and the readings were taken.

3.4.2. Temperature

Temperature was determined using digital thermometer calibrated in degree centigrade (^{O}C). It was inserted into the water in each of the transparent cylindrical plastic containers, containing the different treatments for more than two minutes till readings stabilizes, then were taken.

3.4.3. Dissolved Oxygen (DO) Concentration

DO was determined using a dissolved oxygen meter (DO model **SX751**). The probe was inserted into the sample bottles containing the different treatments. The unit of measurement is mg/l.

3.5. OBSERVATIONS ON BIOLOGICAL DATA

The amount and rate of fish mortality were recorded for each day. Also, behavioural changes such as flashing while swimming, piling up on each other and gulping for air at the water tip due to reduction in dissolved oxygen level, response to external disturbances and intrusion were observed and noted.

3.6. DETERMINATION OF THE LETHAL CONCENTRATION (LC_{50})

Here, the lethal concentration of the toxicant, ABRO Motor Flush on *C. gariepinus* mortality is determined. In other words, also known as the 96h – LC_{50} value and is defined as the concentration of a toxic material which will kill 50% of test organisms in 96-hours (Aquatext, 2002). Mortality data were subjected to Probit and Logit transformation method (Finney, 1982) and the LC_{50} value was determined accordingly.

3.7. HISTOLOGICAL EXAMINATION OF *C. gariepinus*' TARGET ORGANS

Gills and liver of *C. gariepinus* were collected upon dissecting the fish. They were kept in 10% forma-saline [10% formaline – 90% Normal saline] for two days to preserve the organs. The fixed organs were dehydrated in graded levels of alcohol (50%, 70%, 90%, and 100%) and cleared in 50/50 mixture of alcohol and xylene for three hours. The specimens were embedded in molten wax after fixing and later sectioned with the aid of a microtone to thin sections using a microtone to 7µm sections and then stained in haematoxylin and eosin. The stained specimens were observed under a light microscope fitted with a camera. Photographs of the stained specimens were observed under a light microscope. Photographs of the stained specimens were finally taken and interpreted accordingly. Fig 4 shows the Experimental set up for the 96-hours tests.

Plate 4: The Experimental set up for *Clarias gariepinus* and ABRO Motor Flush, of five in duplicates. *Photographed by* Temiloluwa Ogunmola, *2017.*

3.8. DETERMINATION OF HAEMATOLOGICAL CHARACTERISTICS

Collection of blood samples was done on fishes in both tested and control treatments by caudal puncture into the 2.5ml heparinised syringes already treated with Ethylene diamine tetra acetic acid (EDTA) to prevent coagulation. Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells, white blood cells and the counts were estimated using various methods described by Svobodova *et al.* (1991).

3.8.1. Packed Cell Volume (PCV)

Capillary tubes of 100mm, already treated with EDTA, were used to collect blood samples from test organisms. The blood was drawn by capillary tube until 4/5 full, the dry end of the capillary tubes were then sealed immediately with plasticine. The capillary tubes were placed in microhaematocrit centrifuge, they were then placed in the microhaematocrit reader already calibrated in percentage and the readings were taken.

3.8.2 Haemoglobin Concentration (Hb)

This was determined by the indirect acid haematin (Salilic) method using special haemoglobin meter and a pipette. The reagents and the process were done according to Kelly (1979). The haemoglobin is converted to acid haematin by using salinometer N/10Hcl and 0.02ml pipette. The salinometer was filled to the 20ml mark with N/10 HCl and 0.02ml blood added and mixed thoroughly. This was kept for five minutes with the distilled water being added in drops, until the colour matched that of the standard sample. The standard sample is the colour produced by a known haemoglobin concentration and the blood was obtained by measuring the amount of

oxygen or iron in the haemoglobin. The amount of solution in the graduated tube gives the haemoglobin concentration as a percentage, where the value obtained is multiplied by 17.2g/100ml and then divided by 100.

3.8.3. ERYTHROCYTE COUNTS

The blood samples for the erythrocyte count was diluted with Hayem's fluid, comprising of 1g NaCl, 5g Na_2SO_4, 0.5g $HgCl_2$ and 200ml diluted water. A haemocytometer placed on a compound microscope was used to estimate the erythrocyte number. The number of cells counted was multiplied by a diluting factor 200 and volume factor (VF) of 50 as described by Svobodova *et al.* (1991).

3.8.4. NEUTROPHIL COUNTS

The haemocytometer was used as in the erythrocyte count. The dilution fluid was 10% glacial acetic acid; 100ml distilled water and pinch of crystal violet. The dilution factor of 20 was multiplied by the volume factor (VF) of 2.5 (Svobodova *et al*, 1991).

3.9. STATISTICAL ANALYSIS

Statistical analysis as relating to the s was determined using Analytical software, SPSS, v.16.

Data collected on haematological characters of *C. gariepinus* were subjected to the one-way analysis of variance (AN0VA) test. Some of the data collected during the course of the experiment were further subjected to Statistical analysis using the regression routine of SPSS (Statistical Package for Social Sciences) version 16.

CHAPTER FOUR

4.0 RESULTS

4.1. WATER QUALITY

Water quality parameters were measured during the 96-hours (four days) tests. The physiochemical parameters measured are Temperature, dissolved oxygen, pH and Electrical conductivity. The results for the analysis is shown on table 3 below. These parameters were measured at 09h each day and at 24hours interval.

4.2. BEHAVIOURAL OBSERVATIONS OF *Clarias gariepinus*

The mortality of *C. gariepinus* was observed in all concentrations (1ml/L, 1.33ml/L, 1.67ml/L, 2.00ml/L) of "ABRO Motor Flush" used as seen in Table 4. Mortality was recorded at 24hours interval. Fish exhibited restlessness in movements and at points tried to escape the test container especially in the higher concentrations (1.67ml/L and 2.00ml/L) upon addition of "ABRO Motor Flush" (Table 2). In the control experiment containers (0ml/L), fish showed steady movements in various directions as they would to secure space and evade shootouts. Movements were observed of fish in the varying concentrations of "ABRO Motor Flush" after a while (about 6 hours after) and were found to be piling up on each other as there were some areas in the container where there could have been shortage of resources like DO. The LC_{50} obtained using Probit and Logit for fish exposed to "ABRO Motor Flush" was 1.92 ml/L (Fig 4). It is hereby deduced that the presence of ABRO Motor Flush, having an oily nature that spreads on water surface before homogenizing, could reduce the surface area where respiration could take place in the container, resulting in mortality due to severe stress and discomfort that the treated water caused the fish.

4.3. HAEMATOLOGICAL EXAMINATION

The concentration of "ABRO Motor Flush" used for the haematological test were 0ml/L, 1ml/L, 1.33 ml/L,19.67ml/L and 2.00ml/L, the results obtained from the haematological analysis indicate that, the value of the blood indices decreased with increasing concentrations of ABRO Motor Flush. The value of the packed cell volume (PCV), Red blood cells (RBC) and White Blood Cells (WBC) of *C. gariepinus* exposed to the varying concentrations of ABRO Motor Flush, reduced significantly, compared to those of the control fish while values of the white blood cells increased at the end of the 96hour exposure period (Table 4). The haematological result showed that the "ABRO Motor Flush" had a significant effect on the Packed cell Volume (PCV), Red Blood Cell (RBC), and White Blood Cell (WBC) counts of the fish blood with control having the highest value of 13.50±0.00%, 21.55±0.00, 1.25±1.00 in PCV, WBC, and RBC levels respectively, while 2.00ml/L had the lowest PCV of 4.50 ± 0.00% and RBC of 0.45±0.00 as recorded in Table.

4.4. HISTOLOGICAL EXAMINATION

The gills and liver (plates 1-10) of the fish were examined to assess the histological effect of "ABRO Motor Flush" on them. Examination of gills and livers of fish in the varying concentrations showed varying degrees of damage to the tissues.

The gills and liver (plates 1-10) of the fish were examined to assess the histological effect of ABRO Motor Flush on them. Examination of gills and livers of fish in the varying concentrations showed varying degrees of damage to the tissues. Examination of the gills of fish in the control revealed a normal gill filament consisting of primary lamella with arrays of delicate secondary lamella, primary epithelium and secondary epithelium covering the primary

and secondary lamella respectively, there was no vacuolation. Fig 6 of 1.00 ml/L ABRO Motor Flush concentration shows slight degeneration in the gill architecture (in filaments), with slight vacuole information in gills. There was degeneration of the gills filament and the lamella of the fish in 1.33 ml/L concentration; it also shows erosion of the gills filament. However at higher concentrations (Figures 8 and 9) 1.67 ml/L and 2.00 ml/L there was high level of degeneration in the filament, fragmentation of the lamella, erosion of the filaments and abject sign of necrosis (the cells were peeled off and dying because of the stresus placed over fish oxygen level and immune system).

Histological studies on the liver revealed that the control (Fig 10) had normal liver architecture (normal hepatocellular architecture). Fig 11 (T2) showed a slight/hydropic degeneration (evidence of leaching/vacuolation), also Figures 12 & 13 of 1.33ml/L & 1.67ml/L concentrations showed hydropic degeneration of the liver and at high concentration (2.00ml/L), the liver were dying (severe signs of necrosis). The cellular arrangement of liver cells were distorted, lesions were also present on the tissues of the liver. The results obtained from the experiments indicate that ABRO Motor Flush had a direct impact on the tissues of the gills and livers of *Clarias gariepinus*.

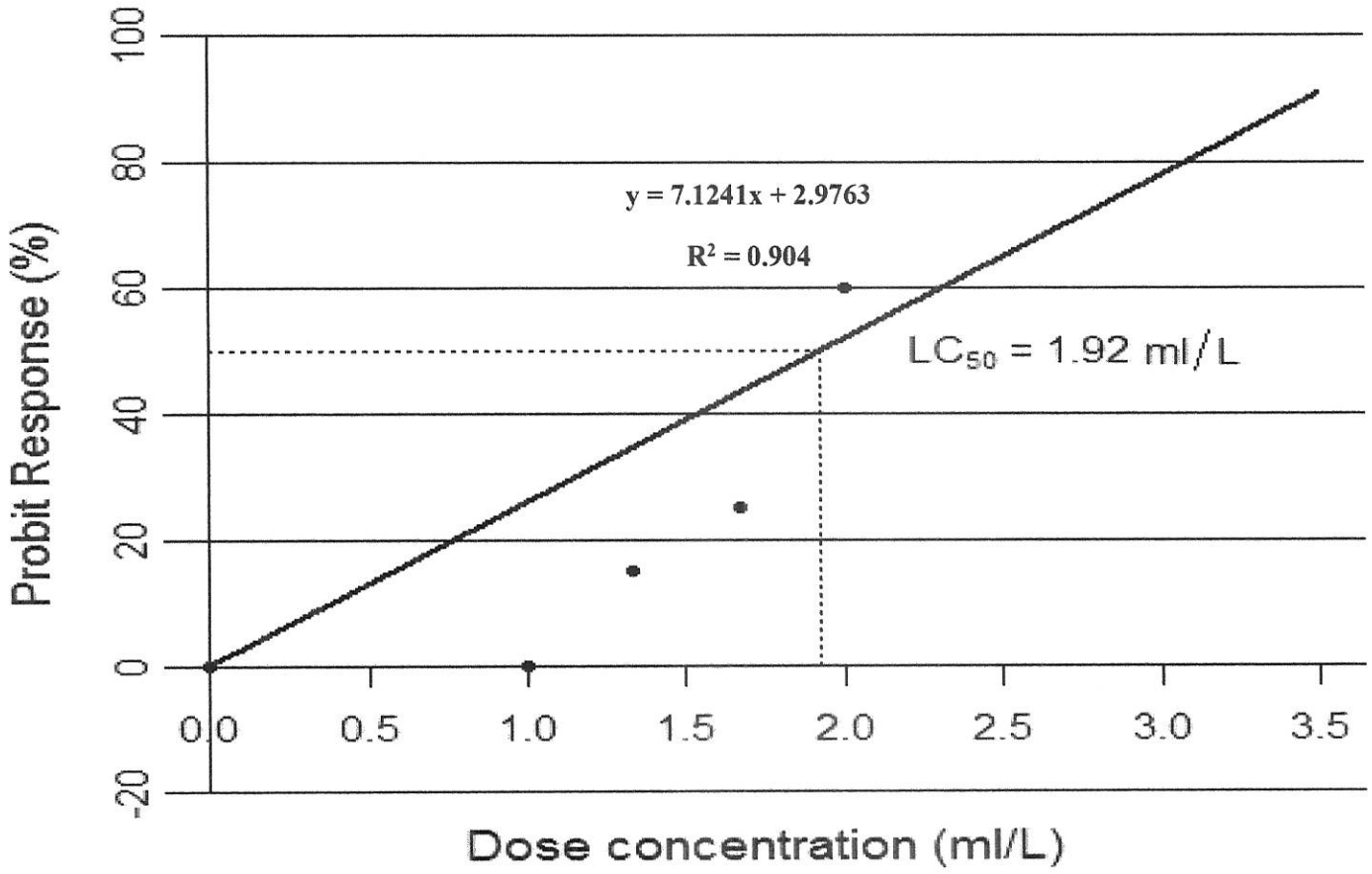

Fig.1: Mortality (96h – LC_{50}) of *Clarias gariepinus* juveniles in different concentrations of ABRO Motor Flush

TABLE 2: EFFECT OF "ABRO MOTOR FLUSH" ON FEATURES OF *Clarias gariepinus* JUVENILES

Concentrations of "ABRO Motor Flush" in ml/L

Observations	24Hour					48Hour					72Hour					96Hour				
	1.00	1.33	1.67	2.00	(Control) 0.00	1.00	1.33	1.67	2.00	(Control) 0.00	1.00	1.33	1.67	2.00	(Control) 0.00	1.00	1.33	1.67	2.00	(Control) 0.00
Erratic Swimming	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Reduced reflex	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-
Changes in behaviour	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-
Rapid opercula movement	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rapid release of bubbles	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-
Death	-	-	-	+	-	+	+	+	+	-	-	-	+	+	-	-	-	+	+	-

Keys

+ = present

- = not present

TABLE 3: RESULTING PHYSIOCHEMICAL PARAMETERS OF WATER MEASURED DURING THE 96-hour (Four Days) TEST PERIOD OF ABRO MOTOR FLUSH ON *Clarias gariepinus* JUVENILES

Treatments (ml/L)	Temperature (°C)	pH	Total Dissolved Solids (mg/L)	Conductivity (µS)	DO (mg/L)
0.00	25.00 ± 0.33^{c}	7.60 ± 0.08^{b}	15.73 ± 0.51^{a}	23.70 ± 0.67^{d}	8.92 ± 0.10^{d}
1.00	25.10 ± 0.64^{b}	7.60 ± 0.22^{a}	16.15 ± 0.58^{b}	25.43 ± 0.50^{c}	9.14 ± 0.10^{ab}
1.33	25.08 ± 0.17^{a}	7.60 ± 0.19^{a}	16.53 ± 0.67^{bc}	25.88 ± 0.68^{b}	9.09 ± 0.03^{c}
1.67	25.08 ± 0.17^{a}	7.60 ± 0.19^{a}	16.53 ± 0.67^{bc}	25.88 ± 0.68^{b}	9.27 ± 0.36^{a}
2.00	24.90 ± 0.26^{d}	7.60 ± 0.10^{a}	19.68 ± 3.66^{d}	27.80 ± 1.28^{a}	9.06 ± 0.02^{bc}

(Mean±Standard Deviation), ($P>0.05$)

TABLE 4: DESCRIPTIVE STATISTICS OF MORTALITY OF *C. gariepinus* EXPOSED TO "ABRO MOTOR FLUSH" AT VARYING CONCENTRATIONS

Treatments (ml/L)	**24hours (%)**	**48hours (%)**	**72hours (%)**	**96hours (%)**
0.00 (Control)	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
1.00	0.0 ± 0.00^{c}	0.0 ± 0.00^{c}	0.1 ± 0.00^{b}	0.0 ± 0.00^{a}
1.33	0.0 ± 0.00^{c}	0.2 ± 0.00^{a}	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
1.67	0.3 ± 0.00^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}	0.0 ± 0.00^{a}
2.00	0.8 ± 0.00^{a}	0.2 ± 0.00^{a}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}

(% mortality$\pm$Standard Deviation), ($P>0.05$)

TABLE 5: HAEMATOLOGICAL ANALYSIS OF *Clarias gariepinus* EXPOSED TO VARYING CONCENTRATIONS OF "ABRO MOTOR FLUSH"

TREATMENTS (ml/L)	Parked Cell Volume (%)	White Blood Cell ($X10^9$)	Red Blood Cell (10^{12})	NEUTROPHIL (%)	LYMPHOCYTE (%)
0.00 (Control)	13.50 ± 0.00^{a}	21.55 ± 0.00^{a}	1.25 ± 0.00^{a}	30 ± 0.00^{a}	70 ± 0.00^{a}
1.00	10.50 ± 0.00^{b}	18.75 ± 0.00^{b}	1.05 ± 0.00^{ab}	21 ± 0.00^{b}	79 ± 0.00^{b}
1.33	9.50 ± 0.00^{c}	16.95 ± 0.00^{c}	0.85 ± 0.00^{c}	18 ± 0.00^{c}	82 ± 0.00^{c}
1.67	7.00 ± 0.00^{d}	14.5 ± 0.00^{d}	0.65 ± 0.00^{cd}	16 ± 0.00^{d}	84 ± 0.00^{d}
2.00	4.50 ± 0.00^{e}	11.5 ± 0.00^{e}	0.45 ± 0.00^{de}	14 ± 0.00^{e}	86 ± 0.00^{e}

(Mean±Standard Deviation)

Values in a column followed by similar letters are not significantly different (P>0.05)

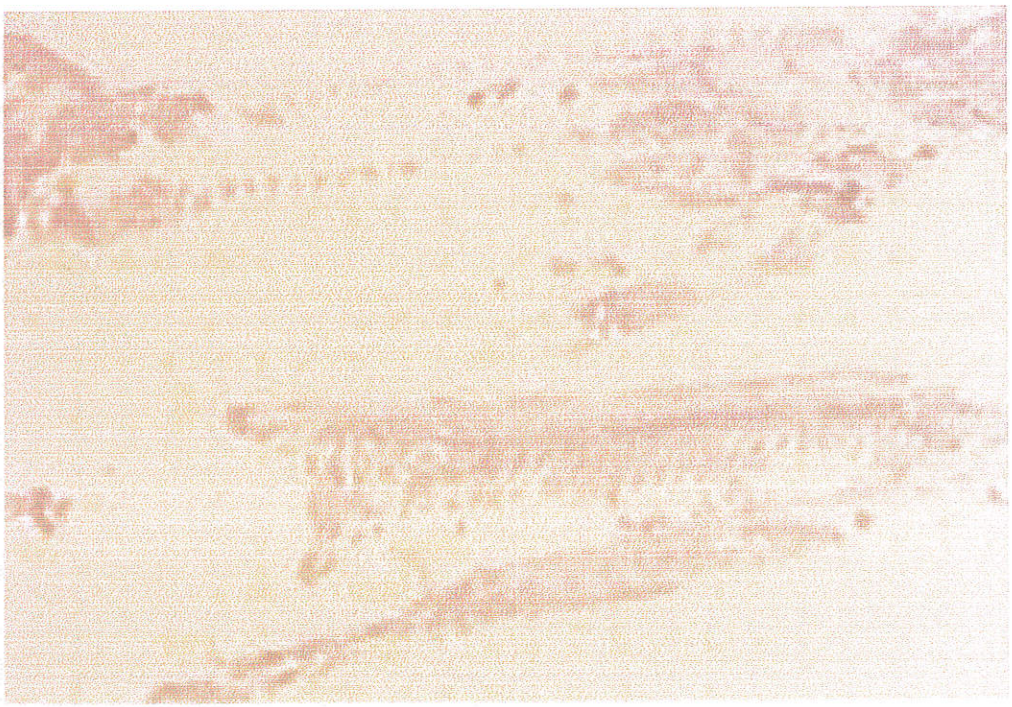

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Plate 5: Gill sample of the T1 (control) showing normal array of filaments

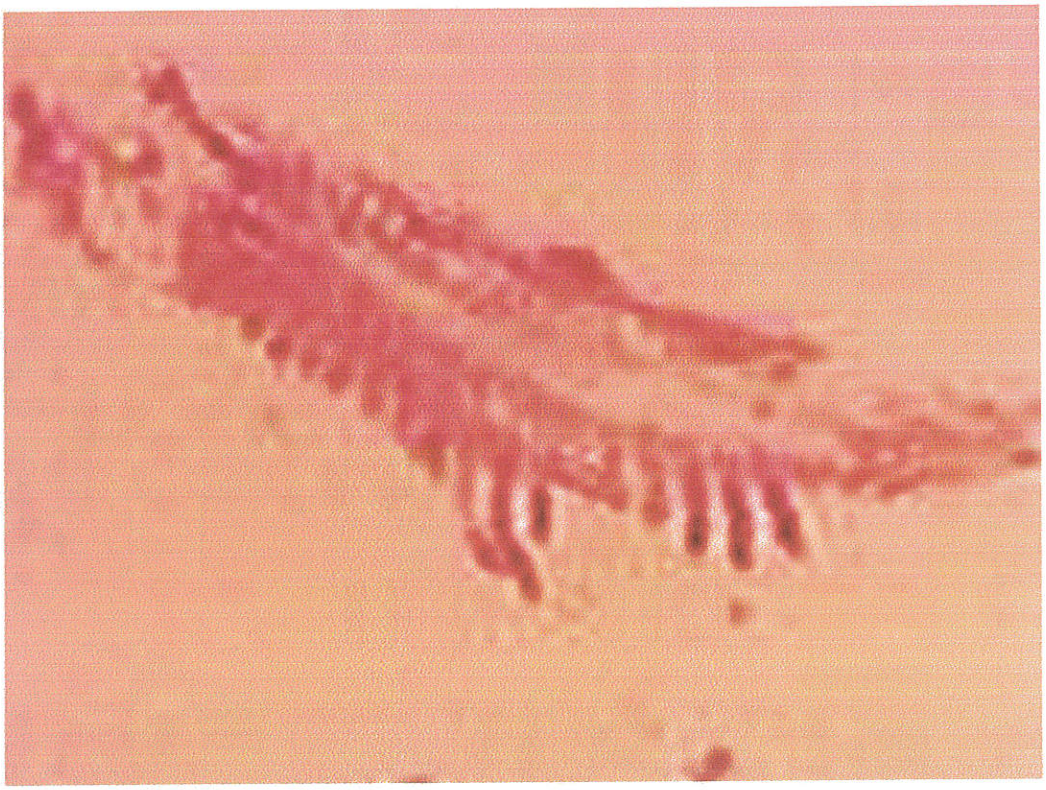

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Plate 6: Gill sample of T2 (1.00ml/L of ABRO Motor Flush used) showing disarray and degradation of filaments towards the tip.

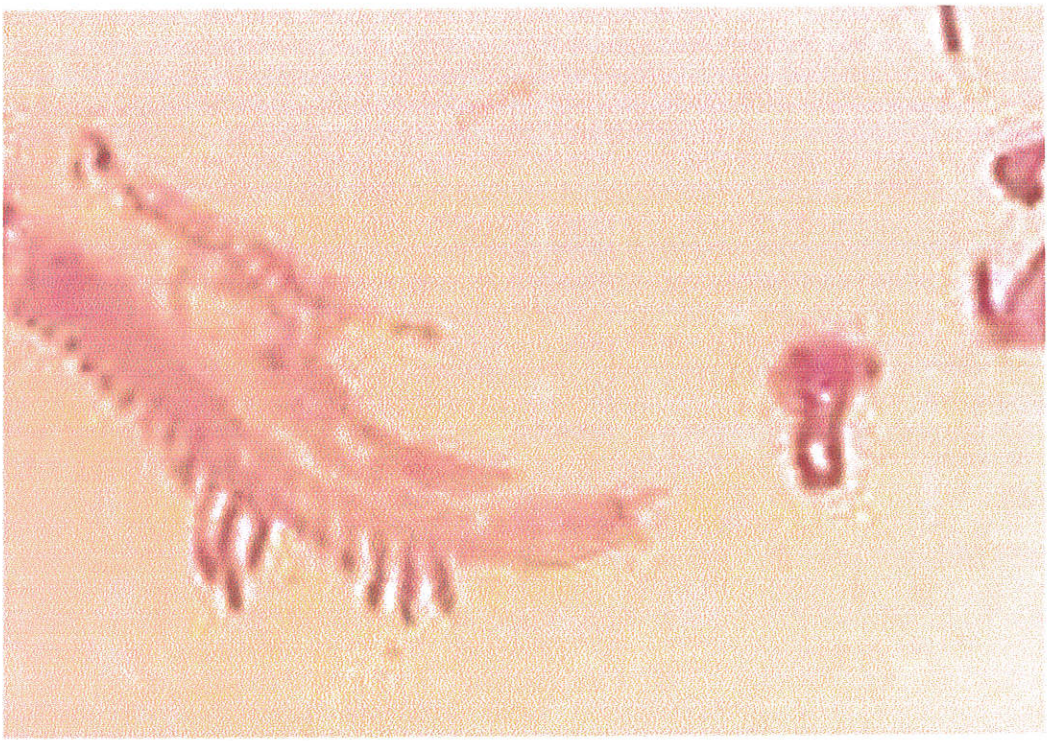

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Plate 7: Gill sample of T3 (1.33ml/L of ABRO Motor Flush used) showing disarray and degradation of filaments at both the anterior(s) and posterior(s).

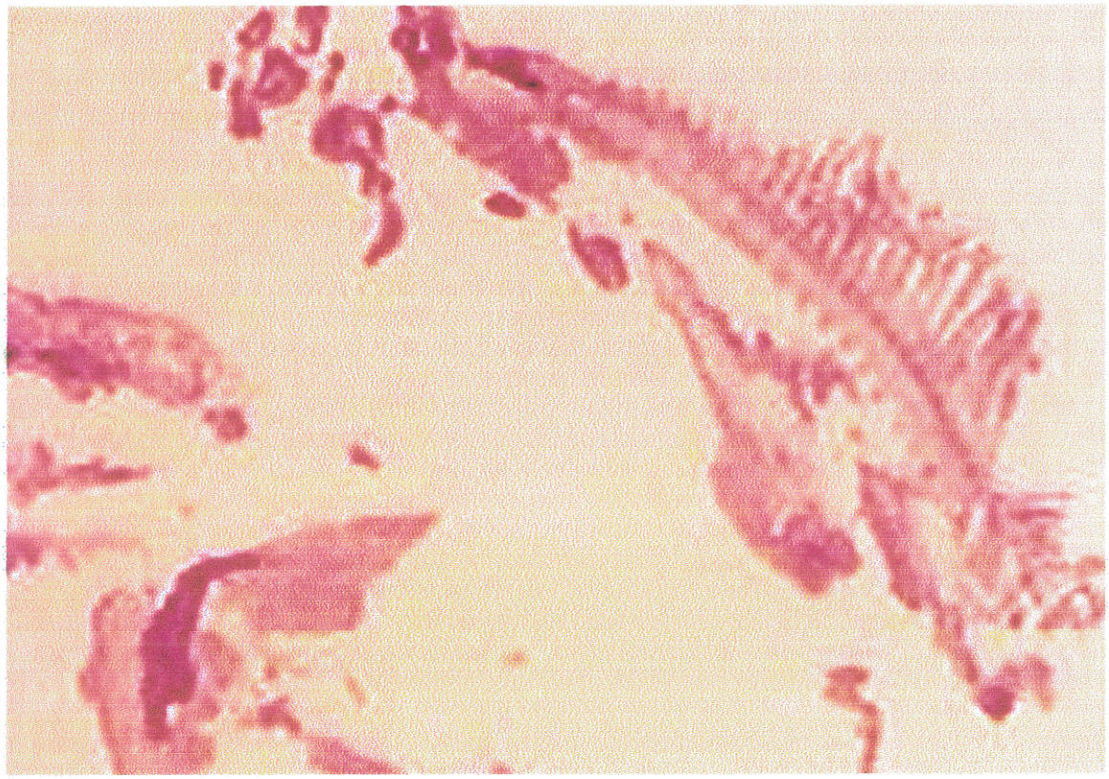

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Plate 8: Gill sample of T4 (1.67ml/L of ABRO Motor Flush used) showing signs of necrosis in filaments and gill cells at both the anterior(s) and posterior(s).

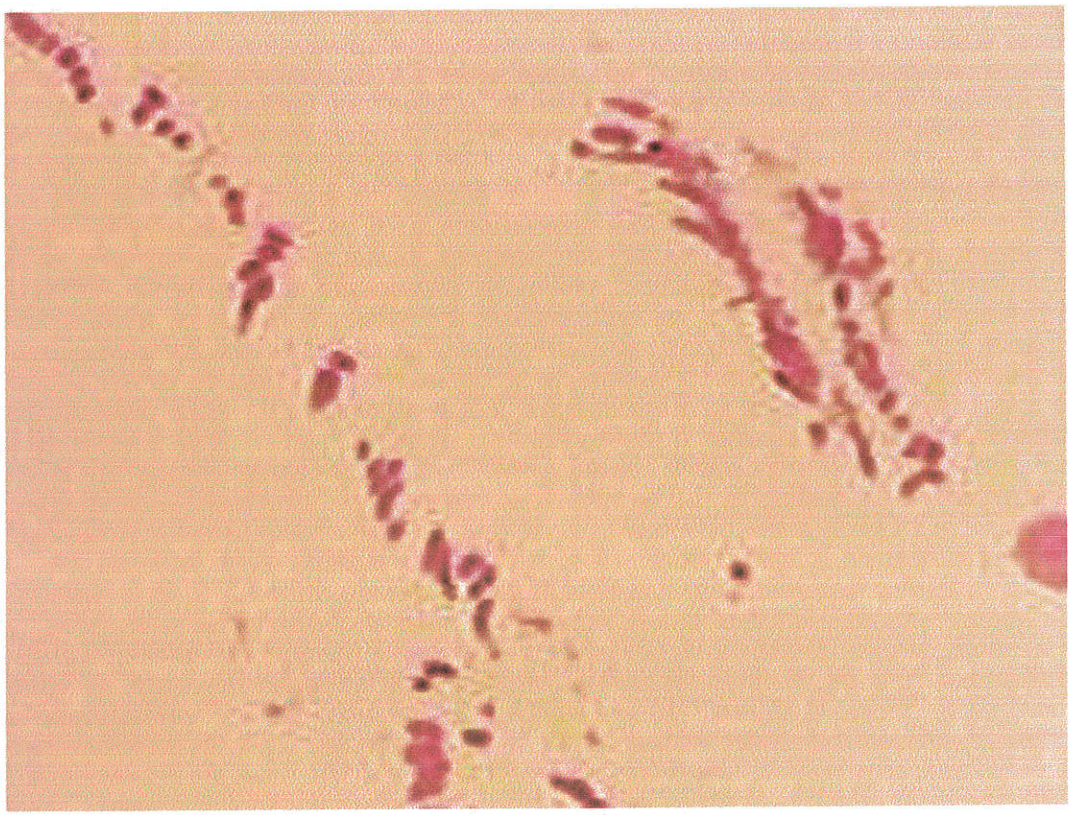

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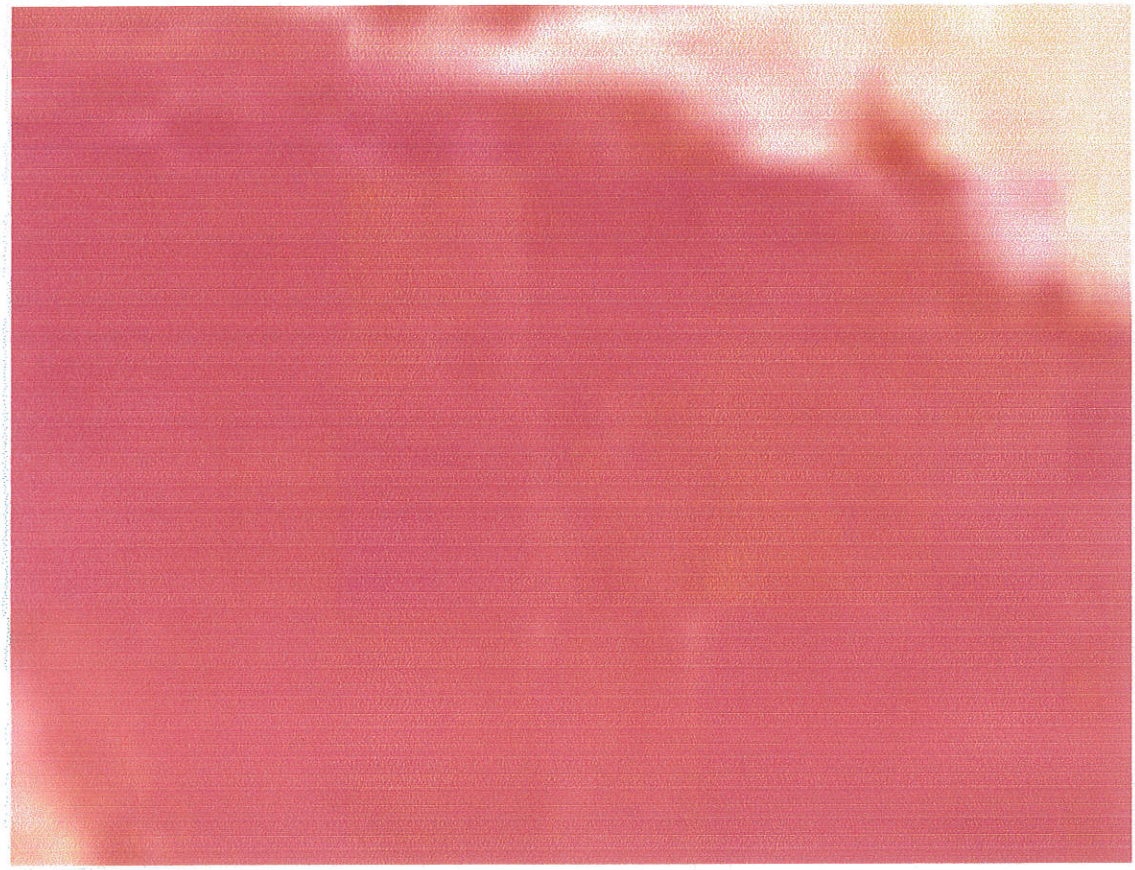
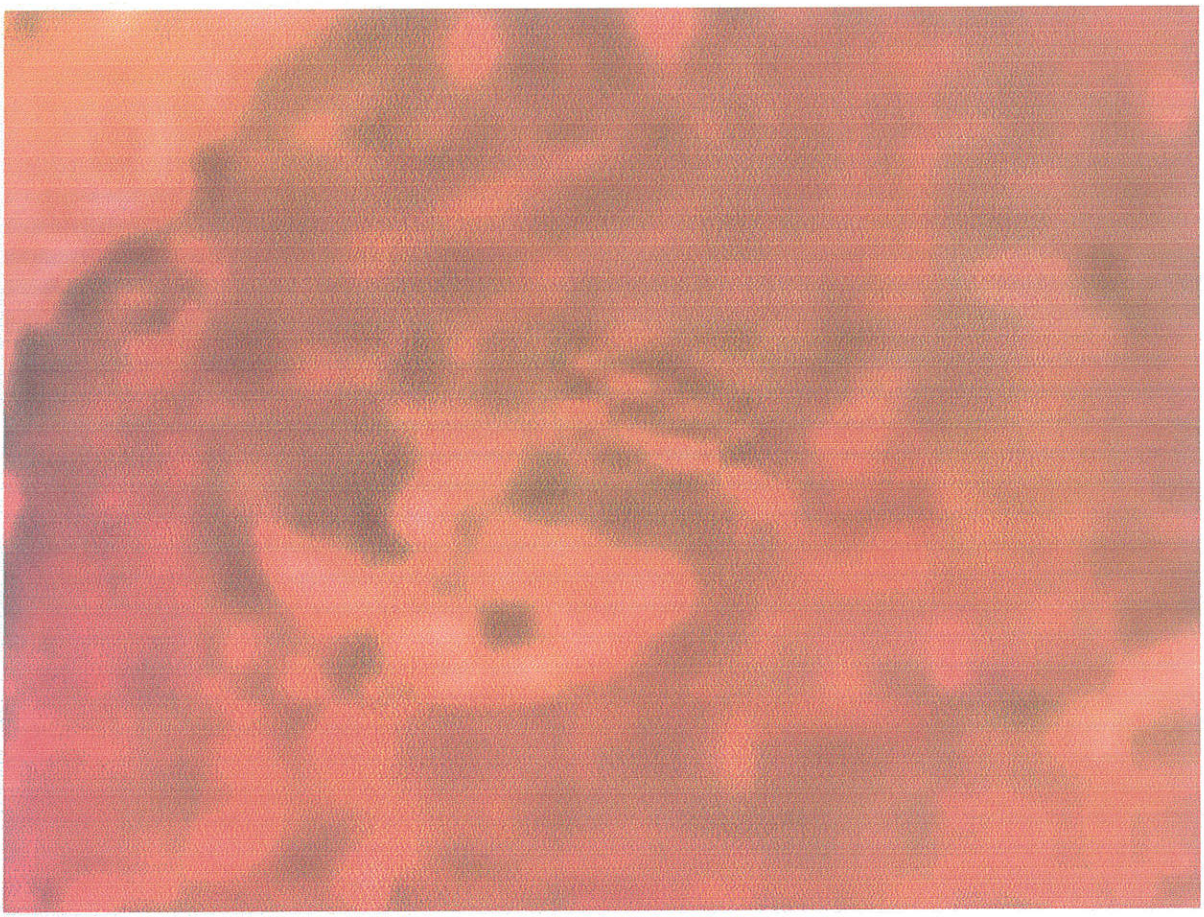
Plate 9: Gill sample of T5 (2.00ml/L of ABRO Motor Flush used) showing severe necrosis in filaments and gill cells.



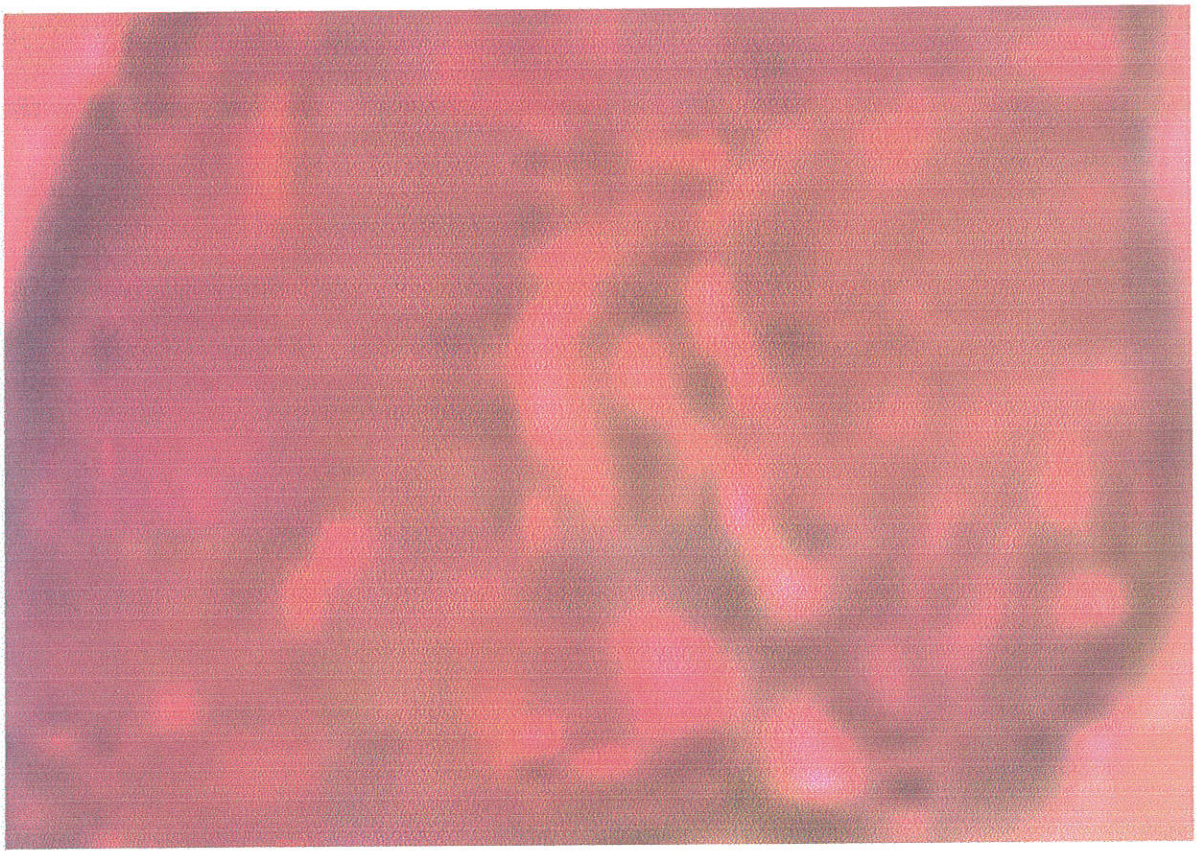
X200

Plate 10: Liver sample of T1 (Control - 0.00ml/L of ABRO Motor Flush used) showing normal arrangement of liver cells (hepatocytes), closely packed.



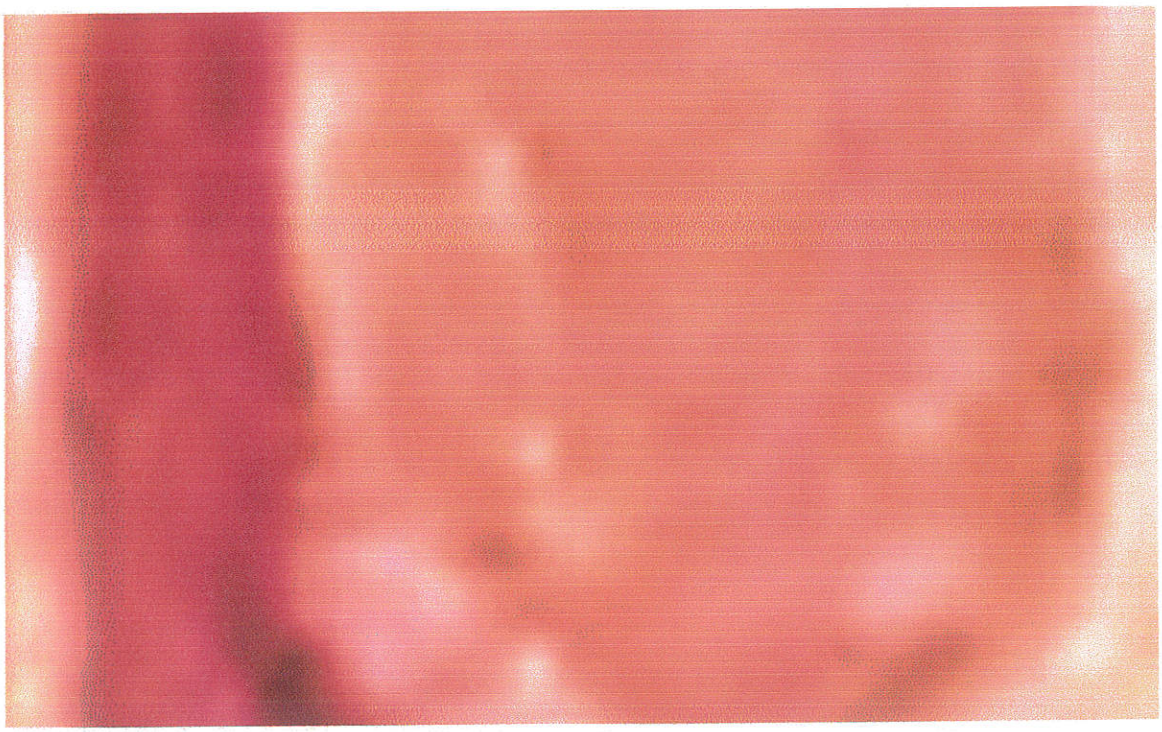
X200

Plate 11: Liver sample of T2 (1.00ml/L of ABRO Motor Flush used) showing slight signs of necrosis liver cells (hepatocytes), towards the center.



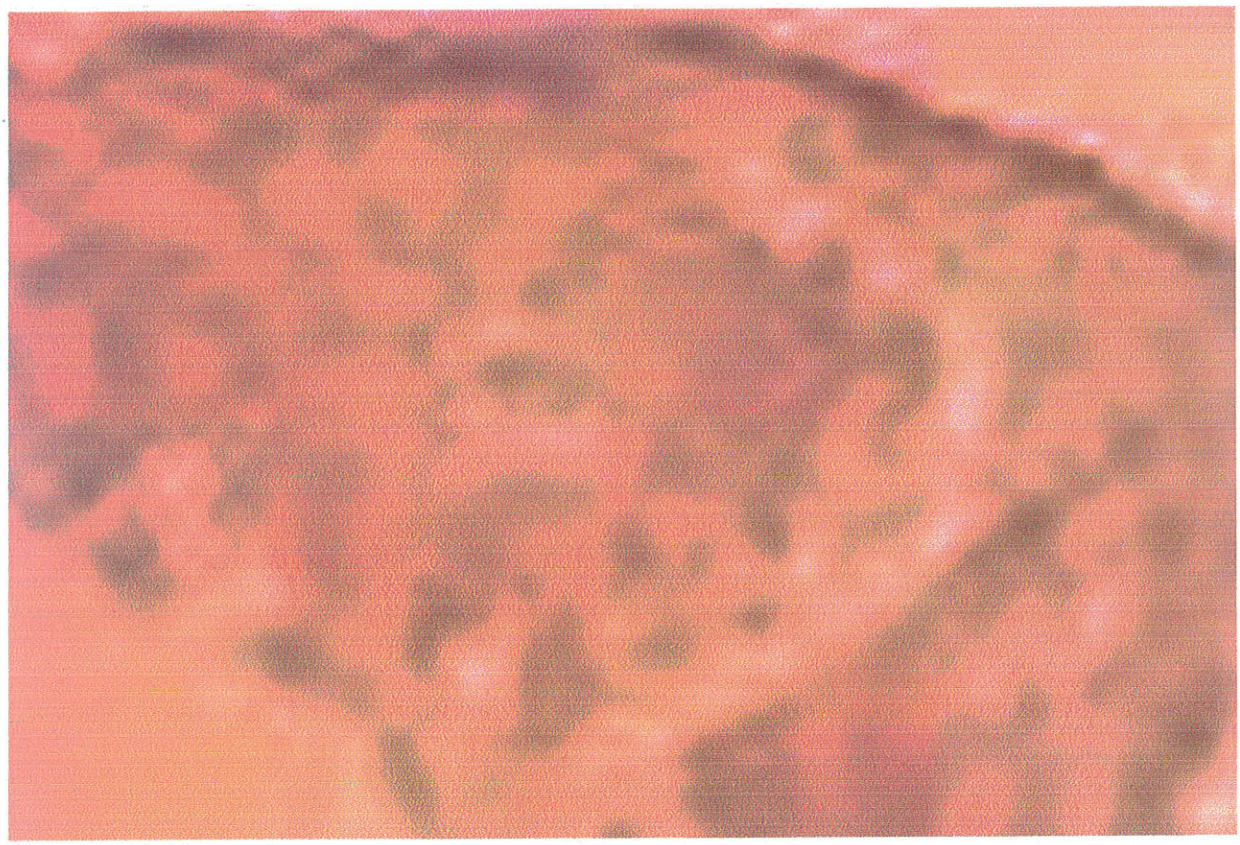
X200

Plate 12: Liver sample of T3 (1.33ml/L of ABRO Motor Flush used) showing disintegration of liver cells (hepatocytes), and expanding necrosis.



X200

Plate 13: Liver sample of T4 (1.67ml/L of ABRO Motor Flush used) showing more disintegration of liver cells (hepatocytes), and expanding necrosis.



X200

Plate 14: Liver sample of T5 (1.33ml/L of ABRO Motor Flush used) showing disintegration of liver cells (hepatocytes), and expanding necrosis.

4.5. DISCUSSION

The result obtained from this study indicated that juveniles of *Clarias gariepinus* of the same age and size showed varying degrees of hyper-activity, mortality, stress and lesions to different concentrations of "ABRO Motor Flush". These observations agree with the findings of Ariyomo (2002), that climbing perch of the same age exhibited differences in their tolerance to "ABRO Motor Flush" concentrations.

Upon the addition of the ABRO Motor Flush, behavioural changes were observed particularly in the higher concentrations, these include erratic movement and surfacing behaviours. Towards the end of the 96-hours test period, there was rapid vertical movement and fish released bubbles at each surfacing, however the rate at which bubbles were released in the higher concentrations (1.67ml/L & 2.00ml/L) was high when compared with the control experiment, Hervey and Hems (1973) stated that under normal conditions, *Clarias gariepinus* takes about four gulps of air every minute. The gulp of air is automatically pressed into the labyrinth (i.e. the accessory breathing organs on each side of the head) and the oxygen absorbed directly into the blood steam and a deoxygenated air is expelled as a single bubble. The values of the physiochemical parameters of both the control and the other concentration used during the experiment are shown on Table 3. The values revealed that there are no significant differences between the control and varying concentrations unless in the higher concentrations (1.67ml/L and 2.00ml/L). Death/mortality of fish could have resulted from changes in the water quality particularly the pH values. During the 96-hours test, ABRO Motor Flush was tested to have a pH value of 7.5 while the water used in each transparent, cylindrical, plastic test containers was measured to be 6.60. The rise in the pH of the water in respective test containers during the 96-hours (four days) test is sure to have resulted from the introduction of ABRO Motor Flush.

Reduction in reflex and gradual release of bubbles indicated stress, which could have been a result of dissolved oxygen reduction, during the 96hour exposure period, this conforms with the report of Enujiugha and Nwanna (2004) that reduction of oxygen levels can severely affect fish life, when dissolved oxygen values fall below minimum oxygen requirement for a particular species of fish, they are subjected to stress, which can result in mortality, pH values increased during the exposure period.

The temperature and acid pH were statistically different between the concentrations possibly because of the effluent toxicity. However, oxygen concentration reduced with increase concentration of effluent. The heightened activities of the fish due to poison can also remove oxygen from the water body (Shallangwa & Auta, 2008; Ariyomo et al., 2009).

CHAPTER FIVE

5.1. CONCLUSION

Improper waste disposals where there are surrounding waters, as seen in Automobile shops and industries will alter the physicochemical parameters of the water and result in irreversible damage to the ecosystem and the organisms therein, damages such as death of eggs, aquatic organisms and water pollution and consequently affect the end users i.e. people who eat the organisms and use the surrounding waters for drinking and people that fished on the water bodies. Naphthalene in ABRO Motor Flush poses a threat to aquatic environment if it is discharged into water body. Through biodegradation and bioaccumulation, causes death of the fishes or impairment of fishes' health and also reduction in the fitness of the legitimate use of the water environment, hence there must be an effective awareness to all about the effect of naphthalene *(in most auto-products)* to the environment at large. It's evident from this study that increasing concentrations of ABRO Motor Flush, when present in any water body, could lead to abnormal bahavioural responses in fish health. Hence, adequate preventive measures must be taken to prevent the indiscriminate channelling of this effluent into water bodies as man is the ultimate target of any negative consequence resulting from such acts. Effective hazard analysis and critical control point (HACCP) monitoring is therefore advocated. Modern technologies in effluent treatment should be embraced by all stakeholders in order to maintain friendly environment and good usage of our water resources.

The above scenario (simulated during the experiment) typifies the real life situation to be expected in any water body having automobile shops and companies, even constant users of this product situated close to it. Extensive and prolonged exposure to ABRO Motor Flush will prevent oxygen dissolution, destruction of breeding grounds as well as fish eggs, and ultimately,

alteration of the entire aquatic environment leading to high mortality or total eradication of aquatic life in the area because of the naphthalene present in it and its other petroleum distillates that are released into those water bodies. This could seriously affect the economic life of the inhabitants of the area as most of them are presently chiefly engaged in fishing and fish-related activities for sustenance.

5.2. RECOMMENDATIONS

Some steps to be taken to preserve the ecosystem and strike an acceptable balance between commercial interest and the wellbeing of the people includes:

1. There is a critical need for an Environmental Impact Assessment (EIA) studies to be sanctioned for the entire populace. In the alternative, there should be a significant appraisal of the Environmental Impact Assessment earlier conducted (if any) on the effect of naphthalene and its metabolites as well as a thorough evaluation of various methods and technologies to be deployed in wastes disposal, making it environmentally friendly and conservative.

2. Development of a CASHEs policy to enforce environmental preservation objectives. CASHES is Community Awareness Safety Health Environment strategy. In order to prevent the ugly experience resulting from naphthalene in auto-products, government must enforce compliance with such a policy document from the very start.

3. A Disaster Response Initiative (DRI) must be made a regular feature of the activities of the processing factories to ensure that they can respond within record time to accidents and unforeseen negative occurrences especially if perchance there is pollution or ecosystem damages.

4. Eventual relocation of the Automobile Firms to a site or area away from dwellings and water bodies.

5. Knowledge: The people need to know. There is need for sensitization and massive awareness drive to activate the consciousness of the people to the economic

advantages/disadvantages of ABRO Motor Flush as well as the price or opportunity cost that this may impose on them.

Unhealthy dumping of wastes, most of which are detrimental to aquatic life should be prohibited. Law enforcement Agencies on Environmental Protection should take seriously every act that endangers the environment, Aqua and Terrestrial.

Automobile products, insects and pest repellent are almost all carcinogenic. Avoid long – term exposures to them.

Consumptions of exposed fishes should be avoided. Better, Quality assessment should not only be based on the freshness but also the health condition of the source and tributaries.

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APPENDICES

Mortality of *C. gariepinus* with solution of ABRO Motor Flush in water at different concentration on the definitive test.

Descriptives

Mortality in 24 hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	1	.0000	.	.	.	.	.00	.00
2	1	.0000	.	.	.	.	.00	.00
3	1	.0000	.	.	.	.	.00	.00
4	1	3.0000	.	.	.	.	3.00	3.00
5	1	8.0000	.	.	.	.	8.00	8.00
Total	5	2.2000	3.49285	1.56205	-2.1369	6.5369	.00	8.00

Descriptives

Mortality(48h

ours)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	1	.0000	.	.	.	.	.00	.00
2	1	.0000	.	.	.	.	.00	.00
3	1	2.0000	.	.	.	.	2.00	2.00
4	1	1.0000	.	.	.	.	1.00	1.00
5	1	2.0000	.	.	.	.	2.00	2.00
Total	5	1.0000	1.00000	.44721	-.2417	2.2417	.00	2.00

Descriptives

Mortality in

72hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	1	.0000	.	.	.	.	.00	.00
2	1	1.0000	.	.	.	.	1.00	1.00

Descriptives

3	1	.0000	.	.	.	.	.00	.00
4	1	1.0000	.	.	.	.	1.00	1.00
5	1	1.0000	.	.	.	.	1.00	1.00
Total	5	.6000	.54772	.24495	-.0801	1.2801	.00	1.00

Descriptives

Mortality in 96

hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	1	.0000	.	.	.	.	.00	.00
2	1	.0000	.	.	.	.	.00	.00
3	1	.0000	.	.	.	.	.00	.00
4	1	.0000	.	.	.	.	.00	.00
5	1	1.0000	.	.	.	.	1.00	1.00
Total	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Temperatur	1	4	24.9750	.33040	.16520	24.4493	25.5007	24.60	25.40
e	2	4	25.1000	.63770	.31885	24.0853	26.1147	24.50	26.00
	3	4	25.0750	.17078	.08539	24.8032	25.3468	24.90	25.30
	4	4	25.0750	.17078	.08539	24.8032	25.3468	24.90	25.30
	5	4	24.9000	.25820	.12910	24.4891	25.3109	24.60	25.20
	Total	20	25.0250	.32747	.07322	24.8717	25.1783	24.50	26.00
pH	1	4	7.6000	.08165	.04082	7.4701	7.7299	7.50	7.70
	2	4	7.5750	.22174	.11087	7.2222	7.9278	7.30	7.80
	3	4	7.3500	.19149	.09574	7.0453	7.6547	7.10	7.50
	4	4	7.3500	.19149	.09574	7.0453	7.6547	7.10	7.50
	5	4	7.6250	.09574	.04787	7.4727	7.7773	7.50	7.70
	Total	20	7.5000	.19467	.04353	7.4089	7.5911	7.10	7.80
TDS	1	4	15.7250	.51235	.25617	14.9097	16.5403	15.20	16.30
	2	4	16.1500	.58023	.29011	15.2267	17.0733	15.70	17.00
	3	4	16.5250	.66521	.33260	15.4665	17.5835	15.70	17.20
	4	4	16.5250	.66521	.33260	15.4665	17.5835	15.70	17.20

	5	4	19.6750	3.66003	1.83002	13.8511	25.4989	16.80	24.80
	Total	20	16.9200	2.10678	.47109	15.9340	17.9060	15.20	24.80
Conductivit	1	4	23.7000	.66833	.33417	22.6365	24.7635	22.90	24.30
y	2	4	25.4250	.49917	.24958	24.6307	26.2193	24.90	26.10
	3	4	25.8750	.68496	.34248	24.7851	26.9649	24.90	26.50
	4	4	25.8750	.68496	.34248	24.7851	26.9649	24.90	26.50
	5	4	27.8000	1.28323	.64161	25.7581	29.8419	26.90	29.70
	Total	20	25.7350	1.52187	.34030	25.0227	26.4473	22.90	29.70
DO	1	4	8.9225	.09674	.04837	8.7686	9.0764	8.80	9.00
	2	4	9.1425	.00957	.00479	9.1273	9.1577	9.13	9.15
	3	4	9.0925	.03304	.01652	9.0399	9.1451	9.05	9.13
	4	4	9.2675	.35612	.17806	8.7008	9.8342	9.05	9.80
	5	4	9.0625	.01500	.00750	9.0386	9.0864	9.05	9.08
	Total	20	9.0975	.18694	.04180	9.0100	9.1850	8.80	9.80