TOXIC EFFECTS OF GLYPHOSATE ON THE BEHAVIOUR, HAEMATOLOGY, HISTOPATHOLOGY AND GROWTH OF THE AFRICAN CATFISH, *Clarias gariepinus* (Burchell, 1822) JUVENILES

BY

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ABSTRACT

Fish are particularly sensitive to a wide variety of agrochemicals including glyphosate herbicide that may arise from not only deliberate discharge of these chemicals into waterways but also from approved agricultural practices. The toxic effects of glyphosate on *Clarias gariepinus* juveniles of mean weight 10.05 ± 1.50 g and mean length of 10.75 ± 1.80 cm were investigated for a period of 12 weeks. The concentrations of the toxicant were prepared as 0.00 (control), 39.02, 78.04, 156.09, 312.19 and 624.39 mg/L with replicates each for the acute effects of glyphosate for mortality rate and behavioural responses for 96 period hours. Behavioural, haematological, histopatological, growth and physico-chemical analysis were carried out based on standard experimental procedures. Percentage treatments from LC_{50} were determined as follows: 00 (control), 23, 38, 53, 68 and 83 mg/L, respectively using the LC_{50} of the toxicant which was 151.36 mg/L. The physical and behavioural changes of the exposed fishes occurred as the concentration increased; restlessness, uncoordinated movement, air gasping, discolouration of the skin and eventual death. The haematological study indicated a significant reduction in White Blood Cells (WBC) and Packed cell Volume (PCV) counts with increased concentrations of the toxicant. In the liver, the enlargement of the hepatocytes was related to the concentration and duration of exposure resulting in large vacuoles in the hepatocytes with pyknotic nuclei. Focal necrosis was also observed in the hepatocytes. The skin showed few atrophying mucous cells, dense inflammatory cells, necrotic epidermis. The weight and length growth occur more in the lower concentrations. The physico-chemical parameters were dose and duration dependent. From this research it was evident that glyphosate had deleterious effects on the fish exposed. Therefore, their use near fish farm or in areas close to aquatic environment should be discouraged or minimized.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Herbicides are widely used all over the world to control the harmful effects of pests and weeds on agricultural productions and fish farm. However, despite the good results of using herbicides in agriculture, their use in the environment is usually accompanied by deleterious environmental and public health effects. The herbicide after being used, ultimately find their way into different aquatic ecosystems and have been found to be highly toxic to non-target organisms, especially aquatic life form and their environment (Nwani *et al.,* 2013).

Herbicides pollution severely affects aquatic organism strophic levels including human beings. The effects of herbicides on fishes are of great concern (Bagheri & Nezami, 2012). Herbicides at high concentration are known to reduce the survival, growth and reproduction of fish and can produce many visible effects on them (Rahman *et al.* 2002).

Toxicity testing of herbicides on animal has been used for a long time to detect the potential hazards posed by herbicides to man (Rahman *et al.,* 2002). These herbicides and pesticides when applied in restricted areas are washed and carried away by rains and floods to nearby aquatic system, thereby affecting aquatic biota, especially fish, which serves as a rich protein supplement for man (Ndimele *et al.,* 2010; Chattopadhay *et al.,* 2013). The herbicides affect not only the physiology and survival of aquatic organisms but also interact with their genetic make-up leading to mutations and/or carcinogenesis (Goksoyr, 2011; Nwani *et al.,* 2013).

Behaviour is an organism-level effect defined as the action, reaction, or functioning of a system under a set of specific circumstances. We rationalize that a greater understanding of behavioural responses in effect to chemical stress may increase. Therefore, in current scenario there is a need of developing newer and effective methods to study the behavioural responses. Behavioural changes in a fish form an efficient index to measure any alterations in the environmental conditions (Strentiford *et al.*, 2013).

Toxicant exposure often completely eliminates the performance of behaviours that are essential to fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality (Graham, 2004). Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on the aquatic organisms (Fagbenro, 2002). Pollutants in water significantly affect the ability of fish to detect and respond to chemical stimuli, feeding, growth, and reproductive performances could also be seriously affected by such polluted habitat. Pollution of aquatic habitat may result in mass fish mortality or their failure to breed in the polluted environment.

Glyphosate, a broad-spectrum weedicide is one of the most frequently applied pesticides in agriculture for the control of great variety of annual biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs. It is also used for aquatic weed control in fish ponds, lakes, canals and slow running water. Glyphosate is formulated as an isopropylamine salt and can be described as an organophosphorous compound. Glyphosate is described by the manufacturer as pesticide of low toxicity and environmental friendliness. But research has shown that higher concentrations of the product can be toxic, producing a number of physiological changes in organisms and in

some cases resulting to death depending on the level, duration and route of exposure. Various concentrations of glyphosate have been shown to be toxic to juveniles of fishes, producing mortality, low survival and various abnormal behavioural changes such as loss of equilibrium status, air gulping, hyper activity, decreased opercula movement, erratic swimming and jerky movements which have been shown to be deleterious to the survival rate of the affected species (Franz *et al.,* 1997).

Haematology is an indicator of immunological status and can provide definitive diagnosis of fish during toxicant exposure (Campbell and Ellis, 2007; Akinrotimi *et al.* 2010; Nte *et al.* 2011). Haematological indices are of different sensitivity to various environment factors and chemical (Akinrotimi *et al.,* 2012). Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in values of one or more haematological parameters of aquatic organisms (Akinrotimi *et al.,* 2010; Gabriel *et al.,* 2012). However, Ramesh *et al.* (2008) noted that there is a possibility that studies on fish blood might reveal conditions within the body of the fish long before there is any outward manifestation of disease. Blood reflects the patho-physiological state of the whole body; therefore, parameters of blood are useful in the diagnosis of the structural and functional status of body organs exposed to toxicants (Felix and Saradhamani, 2015).

Clarias gariepinus is a genus of clariid (order Siluriformes) of the family Clariidae, the air breathing catfish (Goksoyr *et al.,* 2011). It is a popular species in warm water aquaculture and it is indigenous to Africa. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size, low bone content, tolerance to poor water quality parameters, omnivorous in its feeding habit, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and fingerlings easily available (Osman *et al.,* 2006). For

sustainable fish production in Nigeria, the ecotoxicology monitoring programmes need to incorporate proper management programmes for herbicide use and disposal in aquatic habitat. This study was therefore aimed to determine if atrazine is toxic to *C. gariepinus* juveniles.

This fish species has been reported to be most sensitive to aquatic pollutants during their early life stages (Jiraungkoorskul *et al.,* 2003) and the biochemical parameters in fish liver are considered sensitive for detecting potential adverse effects of pollutant damage. Liver micromorphometry has been found to be a reliable biomarker of toxic damage because histological and ultrastructural changes in the cells can be used to predict pollutant stress in acute and chronic concentrations changes in the tissue of individual organisms (Stentiford *et al.,* 2003). The liver is generally regarded as central organ of xenobiotic metabolism in fish and is a target organ affected by toxicant exposure (Mohamed, 2009).

1.2 Statement of Research Problem

Nigerian water bodies suffer from neglect and abuse because of non-enforcement of laws regulating their use. Environmental Impact Assessment (EIA) is not carried out as a prerequisite for the establishment of industries, Ponds are dug indiscriminately even on dunghills that toxic materials has been dumped on for years without any restriction from government agencies. The constant flow of agricultural and industrial effluents into fresh water often leads to a variety of pollutant and accumulation of various metals, which becomes apparent when considering toxic pollution (Martinez, 1991).

These herbicides and pesticides when applied in restricted areas are washed and carried away by rains and floods to nearby aquatic system, thereby affecting aquatic biota, especially fish, which serves as a rich protein supplement for man (Ndimele *et al.,* 2010). The herbicides affect not only the physiology and survival of aquatic organisms

but also interact with their genetic make-up leading to mutations and/or carcinogenesis (Nwani *et al.,* 2010). Toxicants contaminate freshwater bodies and affect non-target organisms. Various researchers have reported the effects of chemicals on aquatic organisms (Jiraunghkoorskul *et al.,* 2003). Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on the aquatic organisms (Fagbenro, 2002).

According to a study by (Loper *et al.,* 2002), non-target effects of the herbicide are under increased attention, and injury to non-target plants may increase with the growing use of post emergence herbicides. Suspected physical drift, volatilization, sprayer contamination, over-spray, carry-over of agrochemicals can result in the need for selective use of these agrichemicals. Because most plants are susceptible to glyphosate, endangered plant species are also seriously impacted.

Most environmental problems of concern today are attributed to the production and release of toxic chemicals which are not only capable of interacting with the environment but also disrupting the ecosystem. Indiscriminate discharge of herbicides from agricultural run-off and other sources into aquatic media affects non target organisms such as fish and prawn which are of great economic importance to humans. Toxicants contaminate freshwater bodies and affect non-target organisms. Various researchers have reported on the effects of chemicals on aquatic organisms.

1.3 Justification for the Study

Fish happens to constitute the cheapest source of protein compared to that of livestock as it contains all biological value of all essential amino acids, which are not found in plant protein. Furthermore, fishes are ideal for toxicity test because they are sensitive

indicators of chemical pollutants, an important integral part of aquatic communities and can be used as subrogate species for other species acute toxicity.

Glyphosate is chosen for this study because it is used to kill weeds (herbicides), especially annual broad leaves and grasses that compete with commercial crops grown around the globe. As a broad-spectrum herbicide, glyphosate has potent acute effects on most plant species. These include effects on endangered species, reduction in the ability of the plant to fix nitrogen and increased susceptibility to plant diseases. Glyphosate has been described by the manufacturer as a pesticide of "low toxicity and environmental friendliness" (Franz *et al.*, 1997) and as such can seem like a silver bullet when dealing with unwanted vegetation. However, glyphosate has been shown from many studies to pose a variety of health and environmental hazards (Cox, 2000).

1.4 Aim of the Study

The aim of this study is to determine the acute and Sub-lethal toxicities of Glyphosate on the behavior, haematology, histopathology and growth as well as the physico-chemical parameter of tank water of African Catfish *Clarias gariepinus* juveniles

1.5 Objectives of the Study

The objectives of the study are to determine the:

i. Lethal concentration (LC_{50}) of Glyphosate on juvenile of *Clarias gariepinus* juveniles.
ii. Behavioral changes of the *Clarias gariepinus* juveniles exposed to glyphosate.
iii. Haematology of the exposed fishes (*Clarias gariepinus* juveniles); white blood cell (WBC), red blood cell (RBC), platelets (PTL), haemoglobin (HGB), packed cell volume (PVC), mean corpusular volume (MCV), mean cell haemoglobin

(MCH) and mean cell haemoglobin concentration (MCHC) compared with the control.

iv. Histopathology of the (skin and liver) of *Clarias gariepinus* juveniles exposed to glyphosate of various concentrations compared with the control.

v. Growth rate (standard length and weight) parameters of the *Clarias gariepinus* exposed to glyphosate compared with the control.

vi. Changes in water quality parameters of tank water of *C. gariepinus* juveniles exposed to glyphosate

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Glyphosate Herbicide

Glyphosate, with IUPAC name, N-(phosphonomethyl) -glycine is a non-selective, broad spectrum, post emergent systemic herbicide. Its herbicidal activity is expressed through direct contact with the leaves and subsequent translocation throughout the plant (Popoola *et al.,* 2005). In pure chemical terms, glyphosate is an organophosphate, however, it does not affect the nervous system in the same way as organophosphate insecticides, and it exploited for its anticholinesterase effect (Marrs, 1993). Glyphosate was first reported as herbicide in 1971 when it was developed and patented by Monsanto Inc. USA as isopropylamine salt of glyphosate. Now it is being formulated into dozens of products by many other pesticide's companies under various trade names. Glyphosate represents about 60 % of global non-selective herbicide sales (Aspelin, 1997).

Glyphosate use is currently (as at 1998) growing at the rate of 20 % annually, primarily because of recent introduction of crops genetically engineered to be tolerant of the herbicide (Bureau of National Affairs, 1998). Formulated glyphosate (like Roundup®) is highly soluble in water and could be mobile in aquatic systems. However, glyphosate binds strongly to cations and are adsorbed to soils (Carlisle and Trevors, 1998). The binding is unlike other organic compounds that primarily adsorb to organic matter in the soils. Glyphosate binding is similar to phosphate binding (Gimsing *et al.* 2004). There are conflicting reports of organic carbon partition coefficient (Koc) values for glyphosate (US Department of Environment, 2003; Monsanto, 2003). For many organic compounds, low Koc and Kow values imply that limited binding to soils is not directly

to carbon (Battaglin *et al.,* 2005). Glyphosate, having low vapour pressure, is suggestive that loss to the atmosphere from treated surfaces will be small.

2.2 Properties of Glyphosate

Glyphosate is a weak acid comprising a glycine moiety and a phosphonomethyl moiety (Solomon and Thompson, 2003). Chemically, glyphosate closely resembles naturally occurring substances and does not possess chemical groups that will confer great reactivity, atmospheric mobility or biological persistence. Its physical and chemical properties indicate that it will not bioaccumulate, nor bio magnify through the food chain to any appreciable extent (Giesy *et al.,* 2000). Pure glyphosate is a colourless, odourless, crystalline solid with a melting point of 180 ^{0}C and decomposes at 187 ^{0}C producing toxic fumes including nitrogen oxides and phosphorous oxides. Solutions of glyphosate are corrosive to iron or galvanized steel. Pure glyphosate is slightly soluble in water (12 g/L at 25 ^{0}C), and is practically insoluble in most organic solvents. The alkali-metal and amine salts are readily soluble in water. Glyphosate formulations are stable over extended periods below 60 ^{0}C. It has vapour pressure of less than 1 x 10-5 Pa at 25 ^{0}C (WHO/FAO, 1996).

Although it is apparent great solubility would lead one to expect glyphosate to be mobile in water. It is readily ionized and, as the anion, will be strongly adsorbed to sediments and soils of pH greater than 3.5. It thus has almost no mobility in soils and is rapidly removed from water to sediments and suspended particulate matter. Applied to soil, glyphosate shows low activity because the strong binding to soil organic matter makes the substance biologically unavailable to plants (Solomon and Thompson, 2003).

2.3 Mode of action

Glyphosate inhibits plant growth through interference with the production of essential amino acids by inhibition of the enzyme enolpyruvylshikimate phosphate synthase EC 2.5.1.19 which is responsible for the biosynthesis of chorismate, which is an intermediate in the phenylalanine, tyrosine and tryptophan biosynthesis (Williams *et al.,* 2011).

2.4 Glyphosate toxicity

Glyphosate has been described by the manufacturer as a pesticide of "low toxicity and environmental friendliness" (Franz *et al.,* 1997) and as such can seem like a silver bullet when dealing with unwanted vegetation. Virtually, every pesticide contains other ingredients other than the "active" ingredients. Such ingredients called surfactants are required at higher concentrations than necessary for maximum reduction of the spray solution surface tension indicating that their mode of action is not limited to increasing the spreading characteristics of the spray droplets (Rehulka *et al.,* 2004) but are also involved in increasing permeability of the cuticle, plasma membrane, or both in increasing foliar uptake of glyphosate and promoting phytotoxicity (Reichman *et al.,* 2006). Such surfactants are more toxic than glyphosate individually and may synergistically increase glyphosate toxicity as reports have demonstrated that the presence of multiple toxicants generally result in greater toxicity than any of the individual components (Belden *et al.,* 2007).

In as much as technical glyphosate may not be very toxic, commercial glyphosate which includes surfactants are acutely more toxic than glyphosate alone. The amount of Roundup required to kill rats is about a third the amount of glyphosate alone (Martinez *et al*. 1991). Such surfactants include polyoxyethyleneamine (POEA) (Mikula *et al.,* 2008), 3-iodo-2-propynyl butylcarbamate (US Environmental Protection Agency,

1997), isopropylamine (Sindel, 1994). Other surfactants include benzisothiazolone, isobutene, pelargonic acid, potassium hydroxide, sodium sulphite, sorbic acid, and others. Monsanto (2003). These surfactants have been implicated as the cause of the symptoms experienced after accidental or intentional exposure to glyphosate herbicide formulations. Such symptoms include eye irritations, blisters, chest pain, facial numbness, swollen eye, face and joints, increased heart beat and palpitations, elevated blood pressure, coughing, headache, nausea, itchy and tingling skin, recurrent eczema, e.t.c. (Temple and Smith, 1992).

2.5 Glyphosate and plants

As a broad-spectrum herbicide, glyphosate has potent acute effects on most plant species. These include effects on endangered species, reduction in the ability of the plant to fix nitrogen, increased susceptibility to plant diseases and reduction in the activity of mycorrhizal fungi.

2.5.1 Risks to endangered and other non-target plants

According to a study by Loper *et al.,* (2002) non-target effects of the herbicide are under increased attention, and injury to non-target plants may increase with the growing use of post emergence herbicides. Suspected physical drift, volatilization, sprayer contamination, over-spray, carry-over of agrochemicals can result in the need for selective use of these agrichemicals. Because most plants are susceptible to glyphosate, endangered plant species are also seriously impacted.

2.5.2 Glyphosate and plant diseases

Glyphosate treatment increases susceptibility of crop plants to a number of diseases. For example, glyphosate increased susceptibility of tomatoes to crown and root diseases (Lydy *et al.,* 1988), and reduced the ability of bean plants to defend themselves against

anthracnose (Johal and Rahe, 1988). Another study also linked glyphosate with the ability of offering additional benefit apart from its herbicidal activities by controlling leaf rust disease in genetically modified wheat and soy farms. (Franz *et al.*, 1997).

2.5.3 Glyphosate and weed resistance.

A common perspective in the late 1990s was that weed resistance to the herbicide, glyphosate, was not probable (Bradshaw *et al,*. 1997). This was accepted because the complex manipulations required for the development of glyphosate-resistant crops were not expected to be duplicated in nature to evolve glyphosate resistant weeds. A study carried out in the University of Tennessee and Mississippi State University by Mueller *et al.* (2003), was able to demonstrate the duplication of such resistance in horse weed Conyza Canadensis L.Cronq. Other studies have been able to implicate transgene flow by pollen transport as one of the avenues of successful escape of transgenes into wild populations. Dispersal of crop seeds is another (Mallory-Smith *et al.,* 2005; Reichman *et al.,* 2006). Also reported in Australia is a population of ryegrass which had developed resistance to glyphosate and tolerated five times the recommended field application rates (Sindel, 1996).

2.6 Glyphosate and animals

Glyphosate can impact many organisms especially animals not intended as targets of the herbicide. This impact could be by direct mortality to the organism of concern or indirectly through destruction of food and/or shelter.

2.6.1 Effect of Glyphosate on Fish

Some studies, in contradiction to the already stated benign nature of glyphosate, have shown that the herbicide is acutely toxic to fish. In general, pure glyphosate alone is less toxic than the common glyphosate product, Roundup, and other glyphosate products

have intermediate toxicities. Part of these differences can be explained by the toxicity of the surfactant in Roundup. It is 20 to 70 times more toxic to fish than glyphosate itself (Folmar *et al.,* 1979). Acute toxicities of glyphosate vary widely with median lethal concentrations, LC_{50}, ranging from10ppm to 200 ppm being reported depending on the species of fish and test conditions (WHO/UNEP/ILO, 1994). Acute toxicities to the commercial glyphosate, Roundup to fish range from 2 ppm to 55 ppm (WHO, UNEP, ILO, 1994). Part of this variability is due to age: young fish are more sensitive to Roundup than older fish (Gabriel *et al.,* 1979).

In soft water, there is little difference between toxicities of glyphosate and Roundup (Fidelis *et al.,* 1989) Roundup toxicity increases with temperature. In both rainbow trout and blue gills, toxicity about doubled between 7 ^{0}C and 17 ^{0}C (Gabriel *et al.,* 1979). Because the herbicide kills shading vegetation, treatment of riparian areas with glyphosate causes water temperature increases several years after herbicidal treatment (Jiraungkoorskul *et al*., 2003) and hence could increase toxicity to fish. Sub-lethal effects of glyphosate occur at low concentrations in rainbow trout and tilapia. Concentration of about ½ and 3 1 of the LC_{50} caused erratic swimming, laboured breathing as well as affected feeding, migration and reproduction (Madua *et al.,* 1989). Sub-lethal doses also caused damage and changes in the liver and kidney structure in Nile tilapia, *Oreochromis niloticus* (Jiraungkoorskul *et al*., 2003).

2.7 Biology and Morphology of *Clarias gariepinus*

Clarias gariepinus is a genus of clariid (order Siluriformes) of the family Clariidae, the air breathing catfish (Froese *et al.* 2011). The name was derived from the Greek chlaros, which means lively, in reference to the ability of the fish to live for a long time out of water (Froese and Pauly 2011).

It is a popular species in warm water aquaculture and it is indigenous to Africa. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size, low bone content, tolerance to poor water quality parameters, omnivorous in its feeding habit, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and fingerlings easily available (Osman *et al.,* 2006). *Clarias gariepinus* are readily recognized by their cylindrical body with scaleless skin, flattened bony head, small eyes, elongated spineless dorsal fin and four pairs of barbels around a broad mouth. The upper surface of the head is coarsely granulated in adult fishes but smooth in young fish. These species of fish are the only ones having pectoral fins armed with spines Ezemmuye *et al.,* 2010).

2.8 LC_{50} Lethal Concentration of Glyphosate

Ali and Muhammad (2012) undertook a study to show the constant discharge of agricultural waste into aquatic environment has led to accumulation of heavy chemicals and other variety of pollutants. Herbicides present in these wastes are washed down, carried by rains and flood to nearby aquatic environment. Glyphosate is one of the most popular herbicides used by farmers in Kano because of its active reaction on killing weeds without affecting the crops. A toxicity test of glyphosate was conducted using concentrations of 0, 0.004, 0.005, 0.006, 0.007 ml/l. The mortality rate of each concentration was determined and the physicochemical parameters (Dissolved oxygen and pH) were also determined. The result showed that high mortality occurs at 0.007 ml/l and less mortality was found at 0.004 ml/l. Hence, mortality is dose dependent. DO and pH decreases with increase in glyphosate concentration. Furthermore, the juveniles showed abnormal behaviour. The LC_{50} value at 96 h was 0.0072 ml/l. There was significant difference between the initial and final pH value ($P<0.05$). On the other

hand, the initial and final DO values showed no significant difference (P>0.05). However, correlation between DO and pH showed no significant difference (P>0.05). The findings of this study established that glyphosate has some level of toxicity on *Clarias gariepinus* juveniles. In addition, it was found that mortality, changes in behaviour, D.O and pH are dose dependent. Therefore, it was suggested that an appropriate concentration that will not be detrimental to non-target organisms should be used by farmers. Alternatively, Biological method should be used as a substitute for chemical method of controlling weeds.

Ekpo and Chude, (2002) worked on the fingerlings of both *Clarias gariepinus* and *Oreochromis niloticus* fish species exposed to various concentrations of glyphosate herbicide in the laboratory were found to have both suffered toxic effects. *O. niloticus* fingerlings were found to be more susceptible to death from the herbicide. For both species immediate reaction to toxicity was hyperactivity and monitored frequencies of opercular plate and pectoral fin of both fish species showed ascending and descending phases. Behavioral patterns exhibited by both fish species could be classified into: (i) the pattern common to both fish species like restlessness, loss of balance, attempts at jumping out and haemorrhaged gills (ii) the pattern peculiar to either fish species; for example, *C. gariepinus* was more prone to swimming in erect posture with snout popping out of water but *O. niloticus* was more prone to swimming on either of its sides. Death occurred in all concentrations used on a daily basis. Furthermore, all concentrations killed off 50 % of test fish population within 60 hours for *O. niloticus* but for *C. gariepinus* except for lowest concentration (20 ppm) which achieved this feat in 73 hours all other concentrations killed off 50% of test fish population within 60 hours. For *C. gariepinus* the 96hr LC_{50}, threshold (safe level), lower limit and upper limit values were determined to be 20.89 ppm, 13.18 ppm, 17.26 ppm and 25.28 ppm

respectively. Whereas, for *O. niloticus* 13.49 ppm, 10.47 ppm, 10.94 ppm and 16.63 ppm were respectively obtained. Observed water quality parameters showed similar trend with both fish species.

An experiment was carried out by Orikpono *et al.* (2011). Ninety (90) hatchery bred fingerlings of *Clarias gariepinus* (mean weight: 0.96 ± 0.1 g) were randomly placed in 15 plastic baths (25 litres each) at the Research laboratory and were exposed to different concentrations of oil products to determine their effects on the fish, to facilitate inferential deductions that will enhance effective aquatic environmental management. Three (3) replicate basins of 5 experimental treatments (crude oil, petrol oil, kerosene oil, engine oil and control) were used at a concentration of 1.25 ml/L. The control experiment was devoid of oil treatment. Six (6) fingerlings were placed in each replicate basin, flooded with 20 litres of clean tap water and fed with nutrafincichilid food, 2 times daily at 3 % body weight. The results showed that the feeding behavior and swimming performances of fish were reduced after 24 hours of the addition of the various oil pollutants. Mortality of fingerlings in the oiled basins increased as the hours of exposure increased (i.e. 24, 48, 72 and 96 hours). Recovery was not immediate in the treated basin while surviving fingerlings in the control basins grew up to post-fingerlings after 90 days (3 months). There were significant differences ($P<0.01$ and $P<0.05$) in the effect of crude oil and the petroleum products on the mortality rate of *C. gariepinus* when exposed to oil pollutants at 1.25 ml. L^{-1} concentration.

It was also put forward by Gharedaeshi *et al.* (2012) that the acute toxicity of lead nitrate to grass carp (*Ctenopharyngodon idella*) juveniles was assessed in a static renewal bioassay for 96 h. In addition, an experiment was conducted to determine the growth performance during 60-day sublethal ($Pb(NO3)_2$) exposure. The results indicated that median lethal concentration (LC_{50}) of lead nitrate to Grass carp for 96 h

of exposure was 246.455 µ. The chronic exposure to sublethal concentration of lead nitrate to the studied fish showed a significant decrease in final body weight in comparison to control group. The lead nitrate also had significantly decreased effect on body weight in comparison to the control. Also, the food conversion ratio (FCR) was significantly increased in comparison to control ($P<0.05$). The lead nitrate also caused a significant decrease in the survival rate ($P<0.05$).

It was also published by Omoregie (1998) that fingerlings of *Oreochromis niloticus* (mean weight 11.8 g) were exposed to various sublethal concentration of cypermethrin (0.032, 0.034, 0.036, 0.038 and 0.040 mil/l), a toxicant, and the histopathological effect were recorded. The 96 hours bioassays were also conducted to determine the lethal concentration (LC_{50}) of cypermethrin of the test fish. The 96h LC_{50} of *H. bidorsalis* exposed was 0.036 ml/l. the physical reaction observed in the fishes were; discoloration of the skin, loss of reflex, hyperactivities, surfacing and these effects increase with increase in concentration of the toxicant and duration of exposure. The pH and dissolved oxygen of the test media showed slight decrease from lower concentration (0.032 ml/l) to the highest concentration (0.040 ml/l) while the temperature increases slightly with increasing concentration (0.032, 0.034. 0.036, 0.038, 0.040 ml/l). The histopathological examination of the gills, kidney and liver of the fish after 96houres showed pathological changes and alteration such as gill infiltration, inflammation of the liver, vacuolation and necrosis. There is excessive necrosis degeneration in higher concentration and there was increase in mortality as the concentrations increases.

Rose *et al.* (2013) experimented acute toxicity of crude *Euphorbia tirucalli* latex extracts to *Oreochromis niloticus* juveniles was investigated in the college of agriculture, Lafia. A four-day static acute toxicity test was performed to determine the LC_{50} value for crude of *Euphorbia tirucalli* latex extracts for the fresh water fish, *O.*

niloticus. Various concentration of the toxicant was prepared as 0.00 ml/L (control), 10, 15, 20, 25 and 30 ml/L respectively. The LC_{50} values, their upper and lower confidence and slope were calculated. The acute toxicity of *Euphorbia tirucalli* latex extracts exhibit a positive correlation between fish mortality and exposure periods. It was found that *Euphorbia tirucalli* latex had higher piscicidal activity as compared with other synthetic pesticides, organophosphates and pyrethroid for *O. nilitichus*, hence, adequate precaution must be exercised when *Euphorbia tirucalli* latex is being used near fish inhabiting water reservoirs and/ or as ornamental plant.

Also, Ogueji and Auta (2007) used Lambda-cyhalothrin in the laboratory studies to determine the 96 h acute toxicity, using juvenile of *Clarias gariepinus*. The fish was exposed to glass aquaria to 0.008, 0.009, and 0.012 ml/l. the pesticide was found to have lethal effect on fish as they change their behavior which results to death. The unique behavioral symptoms elicited by the toxicant included gill failing, excessive lateral flexure and spasms. The 96 hours LC_{50} value for *C. gariepinus* was estimated at 0.008 ml/l. the behavioral toxicology bioassay may be valuable in comparing and predicting the mode of action of new or unknown toxicant in this species of fish.

2.9 Behavioural Changes of the Fishes

Graham and Katherine (2012) carried out a study on environmental pollutants such as metals, pesticides, and other organics pose serious risks to many aquatic organisms. Accordingly, a great deal of previous research has characterized physiological mechanisms of toxicity in animals exposed to contaminants. In contrast, effects of contaminants on fish behaviour are less frequently studied. Because behaviour links physiological function with ecological processes, behavioural indicators of toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations. Here we

consider the many toxicants that disrupt complex fish behaviours, such as predator avoidance, reproductive, and social behaviours. Toxicant exposure often completely eliminates the performance of behaviors that are essential to fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioural toxicity of many xenobiotics is still unknown, warranting their future study.

Sloman and McNeil (2012) studied the use of early life stages of fishes (embryos and larvae) in toxicity testing has been in existence for a long time, generally utilizing endpoints such as morphological defects and mortality. Behavioural endpoints, however, may represent a more insightful evaluation of the ecological effects of toxicants. Indeed, recent years have seen a considerable increase in the use of behavioural measurements in early life stages reflecting a substantial rise in zebra fish *Danio rerio* early life-stage toxicity testing and the development of automated behavioural monitoring systems. Current behavioural endpoints identified for early life stages in response to toxicant exposure include spontaneous activity, predator avoidance, capture of live food, shoaling ability and interaction with other individuals. Less frequently used endpoints include measurement of anxiogenic behaviours and cognitive ability, both of which are suggested here as future indicators of toxicant disruption. For many simple behavioural endpoints, there is still a need to link behavioural effects with ecological relevance; currently, only a limited number of studies have addressed this issue. Understanding the physiological mechanisms that underlie toxicant effects on behaviour so early in life has received far less attention, perhaps because physiological measurements can be difficult to carry out on individuals of this size. The most commonly established physiological links with behavioural disruption in early life stages are similar to those seen in juveniles and adults including

sensory deprivation (olfaction, lateral line and vision), altered neurogenesis and neurotransmitter concentrations. This review highlights the importance of understanding the integrated behavioural and physiological response of early life stages to toxicants and identifies knowledge gaps which present exciting areas for future research.

Madu *et al.* (2019) carried out a study due to industrialization and urbanization many pollutants are being introduced directly and indirectly into aquatic ecosystem. Behavioural bioassay have been widely used in toxicity assessment. Bioassay based on behavior is faster, more sensitive and ecologically more relevant as assessing growth and reproduction need longer bioassay. Behavioural bioassay is more promising alternatives than lethality evaluating bioassay which are currently used for the risk assessment of toxicant. Behavioural changes provide early warning signals about the health of exposed population which other standard tests do not take in to consideration. These endpoints may be 10–100 times more sensitive than those derived from acute or chronic tests because chemicals can induce rapid behavioural responses in organisms even at very low concentrations. Behaviour is an organism-level effect defined as the action, reaction, or functioning of a system under a set of specific circumstances. We rationalize that a greater understanding of behavioural responses in effect to chemical stress may increase. Therefore, in current scenario there is a need of developing newer and effective methods to study the behavioural responses. Behavioural changes in a fish form an efficient index to measure any alterations in the environmental conditions.

2.10 Haematological Test of the Exposed *Clarias gariepinus* juvenile

Okwara *et al.* (2016) studied that juveniles of *Clarias gariepinus* exposed to 0.00, (control) 20, 40 and 60 mg/l of glyphosate solution for 96 hours. Clinical signs were observed and blood was analyzed for hematological parameters such as haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC) and its

indices: mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). The results from the study indicated a significant ($P<0.05$) reduction which is concentration dependent on the value of PCV, Hb, RBC, MCV, MCH and MCHC. The value of WBC increased significantly. These alterations were more pronounced in the fish exposed to 60mg/l of the toxicant. Respiratory stress, erratic swimming, piping and darts were observed in the exposed fish which varied with the concentration of the toxicant. From this study, it can be concluded that glyphosate is toxic to *C. gariepinus* juveniles; therefore, its use should be controlled so as to prevent its entrance into water bodies to minimize aquatic pollution.

Olaifa and Onwude (2003) carried out a study to determine the Lethal and sub-lethal effects of copper on *Clarias gariepinus* juveniles were studied using a 96-hour static bioassay. Copper (as copper chloride, $CuCl_2$. H_2O) was used to prepare the stock solution from which five standard concentrations 0.0, 1.8, 3.2, 5.6, and 10.0 mg/L were prepared (coded A – E). Fifteen (15) juvenile *C. gariepinus* fish having a mean weight and length 5.8g and 18 cm respectively were used. The 96 hour LC_{50} estimated using the logarithm method were 0.6, 0.71 and 0.7 mg/L for replicates 1, 2 and 3 respectively with mean as 0.67 mg/l. Haematological changes were generally not significant ($P>0.05$). Copper concentrations in bone and muscle-tissues were also determined. The mean copper concentration in bone ranged from 1.86 (treatment A) to 17.04 ppm (Treatment E) and muscle 1.29 (treatment A) to 55.5 ppm (treatment E). There were significant differences ($p<0.05$) in mortality among treatments.

Akinrotimi *et al.* (2017) carried out a study on Seventy-Two (72) male and female African catfish *(Clarias gariepinus)* juveniles of mean length (10.74 ± 1.81 g) were exposed to different concentrations of atrazine and metolachlor (0.00 mg/L -control,

0.01, 0.02, 0.03, 0.04 and 0.05 mg/1) for 14 days to determine its effect on haematological parameters of the fish. The results obtained indicated significant ($P<0.05$) reductions with increased concentrations of the chemical in haemoglobin (Hb), Red blood Cell (RBC), packed cell volume (PCV), lymphocytes, platelets and mean corpuscular volume (MCV). The white blood cell (WBC), neutrophils, monocytes, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) in fish exposed to the pesticides were significantly ($P<0.05$) higher than that of the control. The data obtained from this work will contribute to the base line haematological parameters for use in monitoring health status of *Clarias gariepinus* in the wild and culture medium

Popoola *et al.* (2018) studied the impact of short-term exposure to herbicides, atrazine on *Clarias gariepinus* juveniles was evaluated using standard methods that assessed fish histology, haematological and biochemical. Histology analysis of the fish organs examined revealed varying degrees of pathological alterations to the gill and liver in the study. The gill of fish showed alterations like thickening of lamella and sloughing off. The liver exhibited changes such as vacuolar degeneration of hepatocytes, hepatocyte swelling and necrosis amongst other were observed. The assessment of biochemical parameters revealed significant increase ($p<0.05$) in the activities of enzymes, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogense (LDH) in all experimental groups compared with control exposures. There was also a significant increase ($p<0.05$) in the value of blood glucose of the fishes. All experimental groups showed significant different ($p<0.05$) in the values across the treatments for PCV, HB, WBC, MCHC, MCV and MCH. The study therefore revealed that atrazine exposure had a toxic effect on *C. gariepinus* juvenile.

2.11 Histopatological (Liver and Skin) Test of *Clarias gariepinus*

Erhunmwunse *et al.* (2014) carried out a study on one hundred and twenty normal post juvenile catfish *(Clarias gariepinus)* of both sexes with a mean weight of 135.44±1.99 g and mean length of 28.32±0.844 cm were purchased from Osayi farm. They were kept in 60 l aquaria at 27.5±0.4 ^{0}C, pH 7.3, with 12:12 h photoperiod, well aerated, provided with external filtration and a layer of gravel on the bottom. Histopathological alteration in the brain tissues when the fish were exposed to various concentrations (18 mg/L, 32 mg/L and 75 mg/L) of glyphosate for a period of 7 – 28 days revealed that glyphosate herbicide may be neurotozic to post juvenile African catfish *C. gariepinus* as characterized by severe degeneration of dark-stained purkinje neurons, oedema, vacuolar changes with empty spaces which appeared as moth eaten area and showed proliferation of glial cells.

Bagheri *et al.* (2017) undertook an investigation to evaluate the biochemical and histopathological effects of glyphosate on the liver of freshwater fish, Cyprinus carpio (Linn.) after calculating the 96 h LC_{50} of glyphosate (Roundup® 41 % SL) which was 3.260 ppm. The fish fingerlings having mean wt. 3g ±0.5 and mean length 5.5 cm ±0.35 were exposed to two sublethal concentrations of glyphosate i.e. 25% of LC_{50} (T_1) and 50% of LC_{50} (T_2) for a period of 28 days. Total soluble proteins, lipids and enzymatic activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) were recorded at weekly intervals and significant ($p>0.05$) decrease in protein and lipid content of the liver was continually observed till the termination of the experiment. However, the enzymatic activities of AST and ALT in liver showed a significant ($p<0.05$) increase with increasing concentrations of glyphosate and duration of exposure. The histomorphology of liver in fish exposed to glyphosate exhibited vacuolation of hepatocytes, pyknotic nuclei, degeneration of cytoplasm, and infiltration

of leukocytes, necrosis and severe vasodilation in the treatments. The severity of biochemical and histological alterations was more pronounced in T2 after 28 days of exposure. The increase in activities of AST and ALT and the decrease in protein and lipid content of the liver following exposure of fish to the herbicide suggest enhanced protein catabolism, hepatocellular damage and increased utilization of energy stores to compensate for higher energy demands during stress. This indicates that the above said herbicide causes potential harm to the aquatic life.

Olurin *et al.* (2006) examined African catfish, *Clarias gariepinus* fingerlings exposed to sub lethal concentrations of herbicide, glyphosate (0, 0.05, 0.1 %, v/v) over 42 days period. The gills showed marked alterations in the epithelia in response to glyphosate treatment. There was fusion in adjacent secondary lamellae resulting in hyperplasia, with profound oedematous changes, characterized by epithelial detachment. In the liver, the enlargement of the hepatocytes was related to the concentration and duration of exposure to glyphosate. There were also large vacuoles in the hepatocytes, with pyknotic nuclei, and cytolysis that increased with concentration. Focal necrosis was also observed in the hepatocytes. It was concluded that glyphosate has a deleterious effect on the organs of *C. gariepinus.*

Ezemmue *et al.* (2010) carried out a study on *Clarias gariepinus* fingerlings exposed to lethal and sublethal concentrations of Gammalin 20 were investigated in a renewal static bioassay with particular reference to behaviour, survival, and histopathological changes. Early symptoms of gammalin 20 lethal poisoning were, respiratory distress, increased physical activity, convulsions, erratic swimming, loss of equilibrium, and increased breathing activity. Behavioural response was dose dependent and decreased with decreased concentration. The 96-hour lethal concentration (LC_{50}) value was 30 ppb. Histopathological changes of the gill, liver, and intestinal tissues of fish treated with

sublethal concentration of gammalin 20 for twelve weeks showed gill distortion and fusion of adjacent secondary lamella as a result of hyperplasia and excessive mucus accumulation. The liver showed swelling of hepatocytes with mild necrosis, pyknosis, and vacuolation, while the intestine showed yellow bodies of the laminapropria at the tip of the mucosal fold.

2.12 Growth Rate (Standard Length and Weight) Parameters

Chukwuemeka *et al.* (2014) carried out a study to elucidate the morphometrics of three fish species namely, *Tilapia galilaea, Tilapia aurea and Auchenoglanis occidentalis* from Tagwai Lake, Minna, using standard procedures. The results indicated the following ranges for parameters investigated: Body Girth (BG) (3.78±0.76 to 5.48±0.84 cm), and Total Gut weight (TGW) (2.10±0.84 to 3.12±1.73 g); values that did not vary significantly ($P>0.05$) among the fish species. On the other hand, standard length (SL) (range = 10 .94±1.34 to 20.46±2. 98 cm), Gut Length (GL) (range = 25.92± 6.67 to 139.77±30.56 cm) and Total Body Weight (TBW) (range = 49 .99±18.34 to 175.31± 66.96) varied significantly ($P<0.05$). Cross correlation amongst certain morphometric variables, i.e., Standard Length and Total Body Weight, Body Girth and Total Body weight, Total Gut Weight and Standard Length were strong, while Standard Length and Gut Content Weight, Gut Length and Standard Length, Gut Content Weight and Standard Length were weakly correlated. These findings no doubt support close evolutionary ties among the species, and should provide baseline information for sustainable exploitation of the fish species.

Puvaneswai and Karuppasamy (2007) conducted a study on cadmium (Cd) which pollution continues to be a global problem. Early life stages of fish appear to be especially susceptible to this form of pollution. The fish *Heteropneustes fossilis* is a useful indicator species for freshwater pollution. Investigations were carried out on

acute (96 h) and Chronic (21 day) mortality rate, accumulation of toxicant and growth of fish larvae under Cd exposure. Ten-day old *H. fossilis* larvae were exposed to graded series of concentrations of Cd under static-renewal test conditions. Present results indicated that *H. fossilis* larvae were very sensitive even to low concentration of Cd. The No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) and LC_{50} values for survival were 500, 750 and 1921 μg L-1 for acute (96 h) and 90, 125 and 382 μg L-1 for chronic (21 day) exposure, respectively, while the mean NOEC, LOEC and LC_{25} values for growth were 60, 90 and 125 μg L-1 after 21 days of exposure, respectively. *H. fossilis* larvae rapidly bioaccumulated with Cd during prolonged period (21 days) of exposure. Cd accumulation in the larvae reached the level of 62.53 μg g-1 after 21 day of exposure, representing a two-fold increase over the use of lowest test concentration of 30 μg L-1 in their exposure media. Also, the Cd accumulation in larvae was time and concentration dependent.

Ki *et al.* (2016) conducted a study on Rockfish, *Sebastes schlegelii* (mean length 14.53 ± 1.14 cm and mean weight 38.36 ± 3.45 g), were exposed for 4 weeks with the different levels of ammonia in the concentrations of 0, 0.1, 0.5, and 1.0 mg/L at 19 and 24 ^{0}C. The indicators of growth performance such as daily length gain, daily weight gain, condition factor, and hematosomatic index were significantly reduced by the ammonia exposure and high temperature. The ammonia exposure induced a significant decrease in hematological parameters, such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), and hematocrit (Ht), whose trend was more remarkable at 24 ^{0}C. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also notably decreased by the ammonia exposure. Blood ammonia concentration was considerably increased by the ammonia concentration exposure. In the serum components, the

glucose, Glutamic Oxalate Transaminase (GOT), and Glutamic Pyruvate Transaminase (GPT) were substantially increased by the ammonia exposure, whereas total protein was significantly decreased. But the calcium and magnesium were not considerably changed.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Classification of test animal (*Clarias gariepinus*)

Kingdom: Animalia

Subkingdom: Metazoa

Phylum: Chordata

Subphylum: Vertebrata

Superclass: Pisces

Class: Osteichythes

Sub-class: Actinopterygii

Superorder: Teleostei

Order: Siluriformes

Family: Clariidae

Genus: Clarias

Species: *Clarias gariepinus*

Plate 1: Photograph of Experimental Animal; African Catfish (*Clarias gariepinus*) juvenile.

3.2 Description of Study Area

The study was carried out in Minna, the capital of Niger State, North Central Nigeria. Minna is located within longitude $6^0$33 'E and latitude $9^0$37 'N, covering a land area of 88km, with an estimated human population of 1.2 million. The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20 ^{0}C, 61.00 % and 1334.00mm, respectively. The climate presents two distinct seasons, a rainy season (May to October) and a dry season (November to April). The vegetation in the area is typically grass-dominated with scattered tree species.

3.3 Specimen Collection

Clarias gariepinus fingerlings of average length (7.8 ± 0.20 cm) and weight (12.0±2.00 g) were purchased from Kidan Kifi, Niger state, Nigeria. The fishes were acclimatized for 14 days in the laboratory in a plastic tank. Ten fishes were randomly distributed into each aquarium and were aerated throughout the acclimation period. Water quality parameters such as temperature, dissolved oxygen and pH of the experimental setup were monitored using standard methods, APHA (2005).

3.4 Acclimation and Feeding of Fish

The fish were held in 90 cm by 45cm by 30 cm, aquarium containing non-chlorinated water. The fish were allowed to acclimatize for two weeks under laboratory conditions to allow they adapt to experimental conditions. The fish were inspected for disease conditions and general fitness. The fish were fed during the period of acclimatization and the water was changed once in every four days in order to remove faecal and unconsumed feeds. Feeding was discontinued 24 hours prior to the bioassay media to avoid interface of faeces. The fishes were accepted as well as adapted to laboratory condition after less than 5 % of the fish died for the 14 days period (Nwani *et al.*, 2013).

3.5 Range Finding and definitive Test for Glyphosate on *Clarias gariepinus*

Series of preliminary investigation were conducted to obtain LC_{50} (Median lethal concentration that cause 50 % mortality) of the exposed animals within 24-hour period (Adebayo *et al.,* 2016). The test was conducted on a single exposure system by using several concentrations of the toxicant. The predetermined glyphosate concentrations were of the glyphosate concentrations were 0 (as control), 19.51, 39.02, 78.04, 156.09, 312.19, 624.39, 1248.78 and 2497.56 mg/L. Ten (10) fishes of *Clarias gariepinus* juveniles were exposed to these various concentrations to observe the rate of mortality (50%) within 24 hours of exposure (OECD Guideline for Testing of Chemicals, 1984; Hinchman *et al.,* 1995). No mortality occurred in the lowest concentration 19.51 mg/L within the 24-hour period but the concentrations of 1248.78 to 2497.56 mg/L killed the fishes two (2) hours into exposure to the toxicant.

3.6 Procedure for Conduction of Acute Toxicity

Acute toxicity of glyphosate was carried out according to the methods described by EPA (2002). Juvenile of *C. grariepinus* (mean length 9.5 ± 0.2 cm) determined by use of mettle weighing balance and mean weight of 14.0±2.0 g were used for the experiment.

After a range – finding test, the concentrations prepared for the experiment were 39.02, 78.04, 156.09, 312.19, 624.39 and 0.00 mg/L with two replicates as described by Rahman *et al.,* (2002). The LC_{50} value of the *Clarias gariepinus* for 96 hours was calculated using the probit analysis. Behavioral responses and changes were observed hourly for the first 6hour and recorded at 12, 24, 48, 72 and 96^{th} hour intervals. Dead fish were identified by an absolute lack of movement or failure to respond to stimuli using a glass rod. They were removed as soon as this was noticed.

Mortalities observed were transformed into percentage probit kill using a probit table. The probit kill was plotted against a log concentration and a straight-line equation is used to fix the regression line between the points plotted on the graph. The 96 hours LC_{50} was determined on the graph of which the lower and upper confidence limits were calculated using the method of Lichfield and Wilcoxon (1948). The 96 hour LC_{50} of glyphosate was estimated to be 151.36 mg/L base on probit analysis. From the 96 hour LC_{50} obtained, five sublethal concentrations of 23 (15 % of LC_{50}), 38 (25 % of LC_{50}), 53 (35 % of LC_{50}), 68 (45 % 0f LC_{50}) and 83 (55 % of LC_{50}) and control were prepared in duplicate (OECD, 1992; Audu *et al.,* 2015).

3.7 Procedure for the conduction of Sublethal toxicity

After determining the sublethal concentrations of the toxicants, fourteen (14) juveniles of the test organism (*Clarias gariepinus*) were randomly distributed into each of the 6 aquaria and their replicates containing 23, 38, 53, 68 and 83 mg/L concentration of glyphosate and 0.00 mg/L which served as the control were devoid of the toxicant. The test was performed under a renewal static system while the fish were fed throughout the experimental period of 12 weeks. Test solutions were renewed regularly to maintain the concentration of the toxicant (Audu *et al.,* 2015). After the exposure period, the blood, liver and skin were obtained from both the exposed and control of the experimental fish for hematological, histopathological parameters using appropriate procedures outlined.

Haematological, Histopathological and growth examination of the experimental fish were examined using appropriate procedures outlined (Blaxhall and Daisley, 1973; Avwioro, 2011; Audu *et al.,* 2014).

3.8 Determination of physicochemical parameter of the water

3.8.1 Water temperature (T ^{0}C)

Water temperature was determined with common mercury-in-glass thermometer (-10-110 ^{0}C range). Water temperature was determined by lowering the thermometer into the tank in an inclined position for about 5 minutes to allow equilibrium before taking the reading.

3.8.2 Dissolved oxygen (D.O)

Dissolved oxygen was determined by using Winkler Azide method (American Public Health Association (APHA, 2000). Water samples from each, the control and treatment tanks was collected in 250ml dissolved oxygen bottles and was fixed right in the laboratory with 1ml of reagent (A) (Manganous sulphate), 1ml of reagent (B) Alkaline iodide solution (KOH + KI) and 2 ml of concentrated sulphuric acid (H_2SO_4) was added to each sample. 10ml of the sample was titrated with 0.025N sodium thiosulphate using starch as indicator until it turns colorless. Calculation was based on the formula below (Boyd, 1979).

Dissolved oxygen (mg/L) $= \frac{volume\ (Na2SO3)\ \times\ 8\ \times\ (Normality)\ 0.025x\ 1000}{\text{Sample volume (ml)}}$

Where:

Normality =0.025

8= Molar mass of oxygen

1000= Mole

3.8.3 Hydrogen ion concentration (pH)

The pH of the water sample from the control and treatment aquaria was determined with Jenway 3305-meter model, Tecpet China). The pH meter probe was inserted into the

sampled water for about 5minutes until it stabilizes before taken the reading. The meter was standardized with a buffer solution of pH 4.0 and 7.0 before the reading.

3.8.4 Free carbon dioxide

Free carbon (iv) oxide was measured using the method described by AOAC (2012). 100ml of water from each test tank was put into a conical flask. Ten (10) drops of phenolphthalein indicator was added and the clear solution was titrated with N/44 sodium hydroxide (NaOH) until a weak pink colour was observed free carbon dioxide was calculated in mg/L CO_2 = 10 x ml of N/44 NaOH used (APHA, 1980).

3.8.5 Total alkalinity

Total alkalinity was measured using the method described in APHA (1980). 100ml of water sample was drawn and qualitatively transferred into a conical flask. A drops of 4 phenolphthalein indicator were added. If water sample remained clear, the reading for phenolphthalein was recorded as 0.0 mg/L. If the sample turned pink, it was titrated with 0.2N sulphuric acid until the water sample becomes clear and the titre valve recorded – 100 ml of the water sample was again drawn and qualitatively transferred into a conical flask. To it, 4 drops of methyl orange indicator was added. The solution turned green or yellow. The titre valve was recorded. The total alkalinity was then calculated as 10 times the concentration of the titre valve of methyl orange in mg/L as calcium trioxocarbonate (iv), $CaCO_3$. Total alkalinity (mg/L) $CaCO_3$ = $phenolphthalein\ alkalinity + methyl\ orange\ alkalinity$ (APHA, 1980).

3.9 Haematological Examination

Blood sampling was conducted at the expiration of 14 days. Blood samples were collected from a total of 24 fishes (four fishes/ treatment) with heparinized plastic syringe, fitted with 21-gauge hypodermic needle and preserved in disodium salt of

ethylenediamine tetra acetic acid (EDTA) bottles for analysis. The Blaxhall and Daisley (1973), & Wedemeyer *et al.* (1983) haematological methods were adopted for this study. The cyano-haemoglobin method was used to determine haemoglobin (Hb) using diagnostic kits from Sigma diagnostics USA, and packed cell volume (PCV) was determined by the micro haematocrit method. Red blood cell (RBC), leucocrit (LCT) and thrombocyte count were determined with the improved Neubauer haemocytometer according to (Dacie and Lewis, 1991). White blood cell (WBC) was determined with the improved Neubauer counter, while differential counts such as neutrophils, lymphocytes and monocytes were determined on blood film stained with May-Grunwald-Giemsa stain (Mirale, 1982). The values of haematological indices were calculated (Brown, 1980).

3.10 Histopatological Examination

Histopatological sampling was conducted at the expiration of 14 days and at the end of the experiment, two live fishes per concentration were sampled for histological analysis. The skins and livers were examined after dissecting the fish. They were fixed in 10 % formalin for two days to preserve the organs. Fixed organs were dehydrated in graded levels of alcohol (50, 70, 90, 100 %) after which they were immersed in 50/50 mixture of alcohol and xylene for three hours followed by cleaning in 100 % xylene for three hours after which they were embedded in petri dishes with wax. The specimen was later mounted on wooden blocks and sectioned with the aid of a microtome to 7 μm sections before staining in haematoxylin and eosin.

3.11 Determination of Growth of *Clarias gariepinus* juvenile

Determination of the effect of glyphosate on the growth (total length and total weight) of *Clarias gariepinus* juveniles was carried out for the period of twelve (12) weeks. Fishes (*Clarias gariepinus* juveniles) being exposed to the various concentrations of

these toxicants throughout the process of the research had their total length and total weight measured. At the end of every week starting from (0 -12th) week, four fishes were randomly picked and their average weight and length determined. Total length (cm) of each fish was taken from the tip of snout (mouth closed) to the extended tip of caudal fin using a measuring board. Body weight was measured to the nearest gram using a digital balance after removing adhered water and other particles from the surface of the body.

3.12 Experimental design and procedure

The experimental design was a completely randomized design (CRD) with five treatments levels and a control with each level having two replicates. Ten (10) *C. gariepinus* juveniles were introduced individually into 12 aquaria tanks of 12 cm × 10 cm × 12 cm dimension, containing 0.00 (control), 39.02, 78.04, 156.09, 312.19 and 624.39 mg/L of Glyphosate. Each treatment and control had two replicates and lasted for 96-hour period. The tanks were covered with netted materials and supported with pegs to prevent the fish from escaping.

After 96-hour bioassay, the sublethal phase started and was carried out in rectangular glass aquaria labeled (T- Treatment and R- Replicate) as T_1R_1, T_2R_2, T_3R_3, T_4R_4, T_5R_5 and Control tanks. The top was covered with mesh net aided by a peg to prevent the fish from escaping. Each aquarium size of 25 L capacity contained ten (14) fishes of mix sexes. The solution for each concentration was renewed weekly, with freshly prepared solution of Glyphosate.

3.13 Data Analysis

The dose response of mortality were analyzed by probit analysis (Finney, 1971) based on a computer program by Ge Le PaHoure, Imperial College, London and adopted by Otitoloju (2001). This was used to derive the LC_{50}. LC_{50} = Median lethal concentration that causes 50 % mortality of exposed animals.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Mean water Quality parameters in tanks for *Clarias gariepinus* juveniles exposed to various concentrations of glyphosate for 96 hours.

Result from mean water quality parameters measured in the tanks during the 3 months (90 days) experimental period are represented in Table 4.1. The mean physicochemical parameters of the test concentrations (glyphosate) on fish shows Dissolved Oxygen changed steadily with as the concentration of the toxicant increases in each of the aquarium. Dissolved oxygen ranged from 5.4±0.2 mg/L (83.0 mg/L toxicant concentration) to 6.2±0.1 mg/L (control). Dissolved oxygen concentration declined continuously leading to stressful condition on the fishes exposed to the aquaria containing different concentration of the toxicant. The Hydrogen Ion Concentration (pH) reduces as the concentration of the toxicant increases with the lowest concentration having a pH of 7.0 and the highest concentration having a pH of 6.7. The concentration of the water gradually turns acidic as the toxicant increases.

It was observed that the temperature (T ^{0}C) decreased with increase in toxicant concentration. The temperature increases slightly from the control 0.00 to 38.0 mg/L and remains slightly steady till the highest concentration (83.0 mg/l). Free carbon (iv) oxide (CO_2) content increased with increase in concentration of the toxicant. The highest free carbon (IV) oxide value of 10.50 mg/L was obtained in the tank with concentration of 83.0 mg/L while the least value of 6.0 mg/L was recorded in the control (0.00 mg/L).

Total alkalinity was also observed to increase in toxicant concentrations. The lowest toxicant concentration (0.00 mg/L) recorded the lowest alkalinity value of 18.60 g/L while the highest value of 22.30 g/L was recorded from the highest toxicant concentration.

Table 4. 1 Mean water Quality parameters in tanks for *Clarias gariepinus* juveniles exposed to various concentrations of glyphosate for 96hours.

Glyphosate con. (mg/L)	Dissolved oxygen (mg/L)	Ph	Temperature(^{0}C)	Free CO_2 (ppm)	Alkalinity (/L)
Control	6.0±0.2	7.10±0.2	27.5±0.5	6.00±0.2	18.60±1.40
39.02	5.9±0.3	7.0±0.2	27.9±0.6	7.25±0.4	18.45±1.58
78.04	5.8±0.2	6.85±0.2	29.6±0.3	7.55±0.4	19.55±1.52
156.09	5.7±0.2	6.8±0.3	29.7±0.4	8.50±0.3	20.50±1.50
312.19	5.5±0.4	6.7±0.4	28.7±0.5	9.15±0.5	22.85±1.20
624.39	5.4±0.1	6.6±0.3	29.8±0.7	10.50±0.3	22.30±1.40

Values in ± are Standard Mean Error.
Source: Field Survey 2019

4.1.2 Mortality rate of *C. gariepinus* juveniles exposed to lethal concentrations of glyphosate for a period of 96 hours

The percentage of survival of juveniles of *C. gariepinus* juveniles exposed to glyphosate of the control was 100 % all through the exposure period. The survival rate decreased with increase in duration of exposure period as well as the concentration increased until they all died at 624.19 mg/l (the highest lethal concentration) (Table 4.2).

Table 4.2: Mean mortality, log concentration and probit kill of juveniles of *Clarias gariepinus* juveniles exposed to various concentrations of glyphosate for 96 hours.

Conc. (mg/L)	Log. Conc.	No. of Fish	24	42	72	96	Mean Mortality	Percentage mortality (%)	Probit kill
Control	0	10	0	0	0	0	0	0	0
39.02	1.591	10	0	0	0	1	1	10	3.72
78.04	1.892	10	0	0	1.5	0	1.5	15	3.96
156.09	2.193	10	0	0.5	1	2	2	20	4.33
312.19	2.494	10	1.5	2	3.5	6	6	60	5.52
624.39	2.795	10	10	10	10	10	10	100	8.09

4.1.3 Behavioral patterns of *Clarias gariepinus* juvenile during the 96 hours Acute Toxicity Exposure to Glyphosate.

Upon introduction of the toxicant, the fish were seen gathering at one end of the experimental tanks with the highest concentrations (312.19 mg/L and 624.39 mg/L) of the toxicant. After about five hours, behavioural patterns such as jerky movement, air gulping, restlessness, erratic movement and loss of stability were observed. Fishes became inactive and death resulted after a prolonged exposure (Table 4.3).

At 156.09 mg/L concentration of the toxicant, fish were observed to also gather at one end of the tanks, swimming to the bottom of the tank and remained for a short while before coming to the surface intermittently to gulp for air. Fishes were then observed to swim rather faster than normal (compared with the control) and began to lose stability and sank to the bottom of the tank. They became inactive and death resulted after prolonged (>12 hrs and < 36 hrs) period of exposure to the toxicant. At the lower concentrations of 39.02 and 78.04 mg/L, there was no noticeable difference in terms of behavior when compared with the control, however death was observed to occur after a prolong exposure of about (>80 hrs) (Table 4.3).

Table 4.3: Behavioral patterns of *Clarias gariepinus* juvenile exposed to glyphosate for Acute Biossay.

Exposure time	24						48					
Behaviour/Conc.(mg/L)	0	39.02	78.04	156.09	312.19	624.39	0	39.02	78.04	156.09	312.19	624.39
Erratic swimming	-	+	+	+	+	+	-	+	+	+	+	+
Jerky movement	-	-	-	+	+	+	-	-	-	+	+	+
Air gulping	-	-	+	+	+	+	-	+	+	+	+	+
Skin discoloration	-	-	-	-	-	-	-	+	+	+	+	+
Attempts to jump out	-	+	+	+	+	+	-	+	+	+	+	+

			72						96		
0	39.02	78.04	156.09	312.19	624.39	0	39.02	78.04	156.09	312.19	624.39
-	+	+	-	-	-	-	-	-	-	-	-
-	+	+	+	+	+	-	-	-	-	-	-
-	+	+	+	-	-	-	-	-	-	-	-
-	+	+	+	+	+	-	-	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-

Continuation:

Key: + = present
- = Not present
Source: field survey 2019

4.1.4 Haematology of *C. gariepinus* juveniles exposed to sublethal concentrations of glyphosate.

The summary of the mean values of haematological parameters carried out on the blood of *C. gariepinus* juvenile exposed to various concentrations of glyphosate showed that the mean values of blood indices are significantly different ($P<0.05$) as compared with the control.

The result of the study is presented in table 5. The result showed that the glyphosate had effect on the Packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), Mean cell volume (MCV), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC) and blood film pictures of *C. gariepinus.*

Table 4.4: Mean Hematological parameters of *C. gariepinus* juveniles exposed to Sublethal concentration of Glyphosate during the 120 days period.

Conc.	**WBC (mm3 µL)**	**RBC (mm3/µL)**	**PLT (µL)**	**Hb (g/dL)**	**PCV (%)**	MCV	MCH	MCHC
0	178 X 10^3	2.03 X 10^6	930 X 10^3	6.8	30.33	493	86.7	29.5
23	170 X 10^3	1.92 X 10^6	819 X 10^3	6.6	25.67	130.1	42.9	32.9
38	168 X 10^3	1.85 X 10^6	708 X 10^3	5.6	23.33	105.8	51.2	48.4
53	165 X 10^3	1.80 X 10^6	761 X 10^3	5.4	19.01	109.3	69.1	63.2
68	163 X 10^3	1.65 X 10^6	750 X 10^3	5.1	18.33	100.8	70.5	56.8
83	160 X 10^3	1.50 X 10^6	720 X 10^3	4.6	17.67	90.8	68.8	73.8

WBC- White Blood cells, RBC- Red Blood cells, PTL- Platelets, Hb-Hemoglobin, PCV-Packed Cell volume, MCV- Mean Corpusular volume, MCH- Mean cell hemoglobin, MCHC- Mean cell hemoglobin concentration.

4.1.5 Histopatological screening of the skin of *C. gariepinus* juveniles exposed to sublethal concentrations of glyphosate.

After three months of exposure, a section of the skin seen in the light microscope (LM) at magnification of X100 reveals the general histology of the organ. The fishes exposed to 0.00mg/L of glyphosate at plate 1 showed Preserved epithelium composed of epidermis made up of stratified squamous cells, few mucous cells and numerous enlarged alarm cells. Mild to moderate inflammatory cells at the basal layer. The dermis shows no abnormality. On fishes exposed to glyphosate concentration of 23 mg/L, Preserved epithelium, atrophic epidermis with little to no alarm and mucous cells. (Mucous cell atrophy/ hypoptrophy), with Pigment cells preserved were seen (Plate II). The skin at 38 mg/L also showed Preserved epithelium composed of epidermis made up of stratified squamous cells, few mucous cells and numerous enlarged alarm cells. Mild to moderate inflammatory cells at the basal layer (Plate III). On concentration 53 mg/L, the histology of the fish also shows Preserved epithelium, enlarged alarm cells. The dermis shows no abnormality (Plate IV). At 63 mg/L concentration, Preserved epithelium, enlarged alarm cells and mucos cells were seen. The dermis shows no abnormality (Plate V). Finally, at concentration 83mg/L, shows focal necrotic epidermis, increased number of pigment cells and oedematous dermis. Enlarged alarm cells and few mucous cells (mucous cell atrophy) are also seen. (Plate VI)

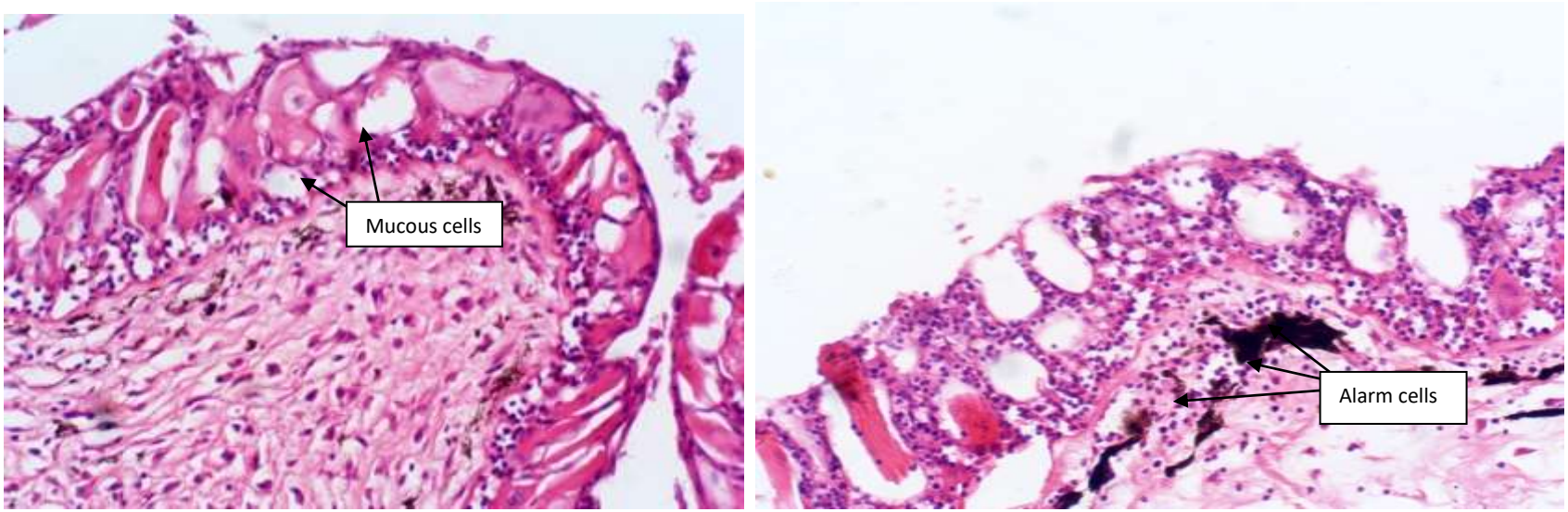

Plate II: Skin of *Clarias gariepinus* juveniles exposed to 0.00mg/L of glyphosate for 12 weeks showing Preserved epithelium; few mucous cells and numerous enlarged alarm cells. The dermis shows no abnormality. Mag. X100

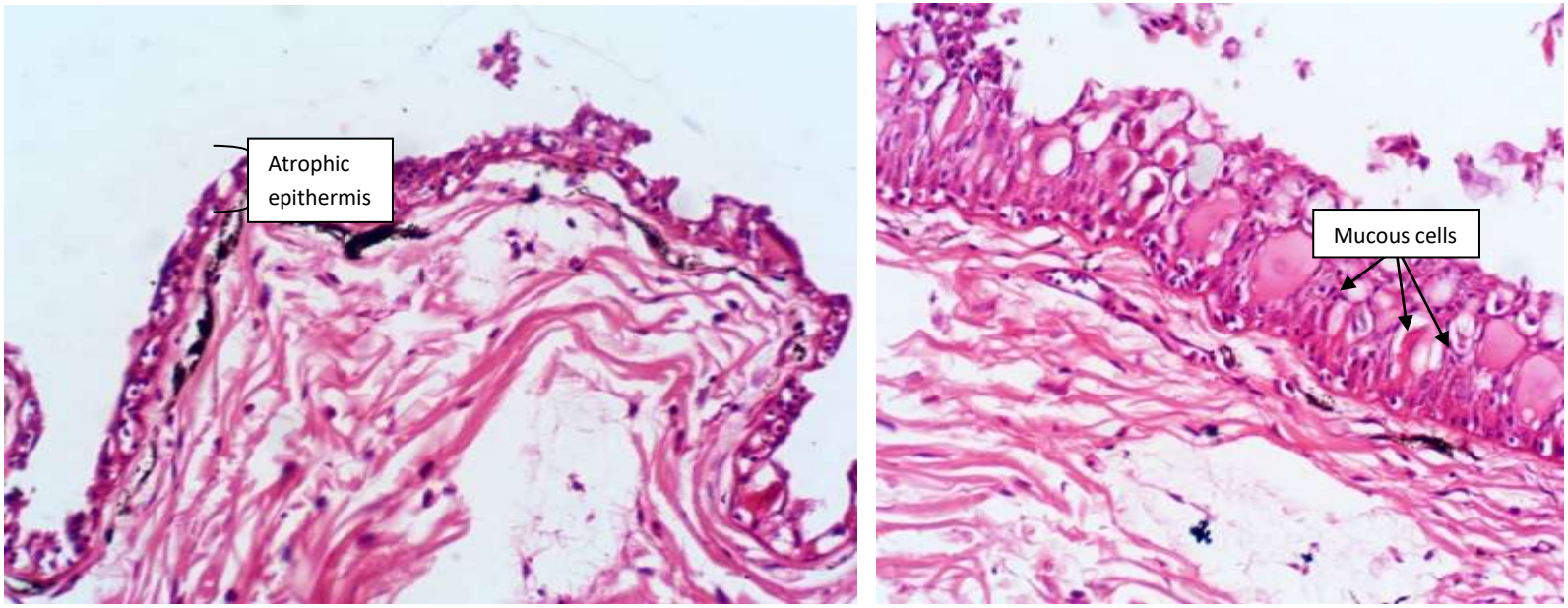

Plate III: Skin of *Clarias gariepinus* juveniles exposed to 23.0mg/L of glyphosate for 12 weeks showing Preserved epithelium, atrophic epidermis with little to no alarm and mucous cells. (Mucous cell atrophy/hypoptrophy) Pigment cells are preserved. Mag. X100

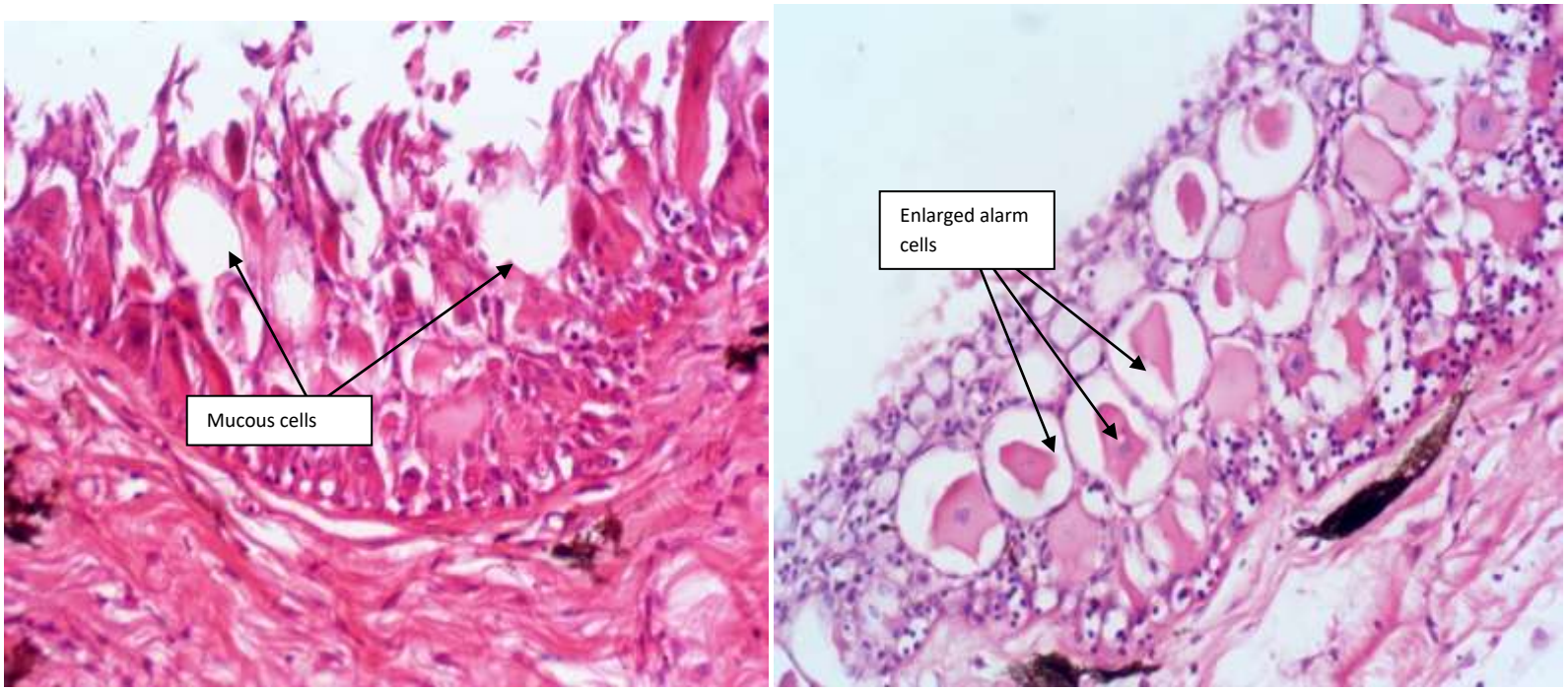

Plate IV: Skin of *Clarias gariepinus* juveniles exposed to 38.0mg/L of glyphosate for 12 weeks showing Preserved epithelium composed of epidermis made up of stratified squamous cells, few mucous cells and numerous enlarged alarm cells. Mild to moderate inflammatory cells at the basal layer. Mag. X100

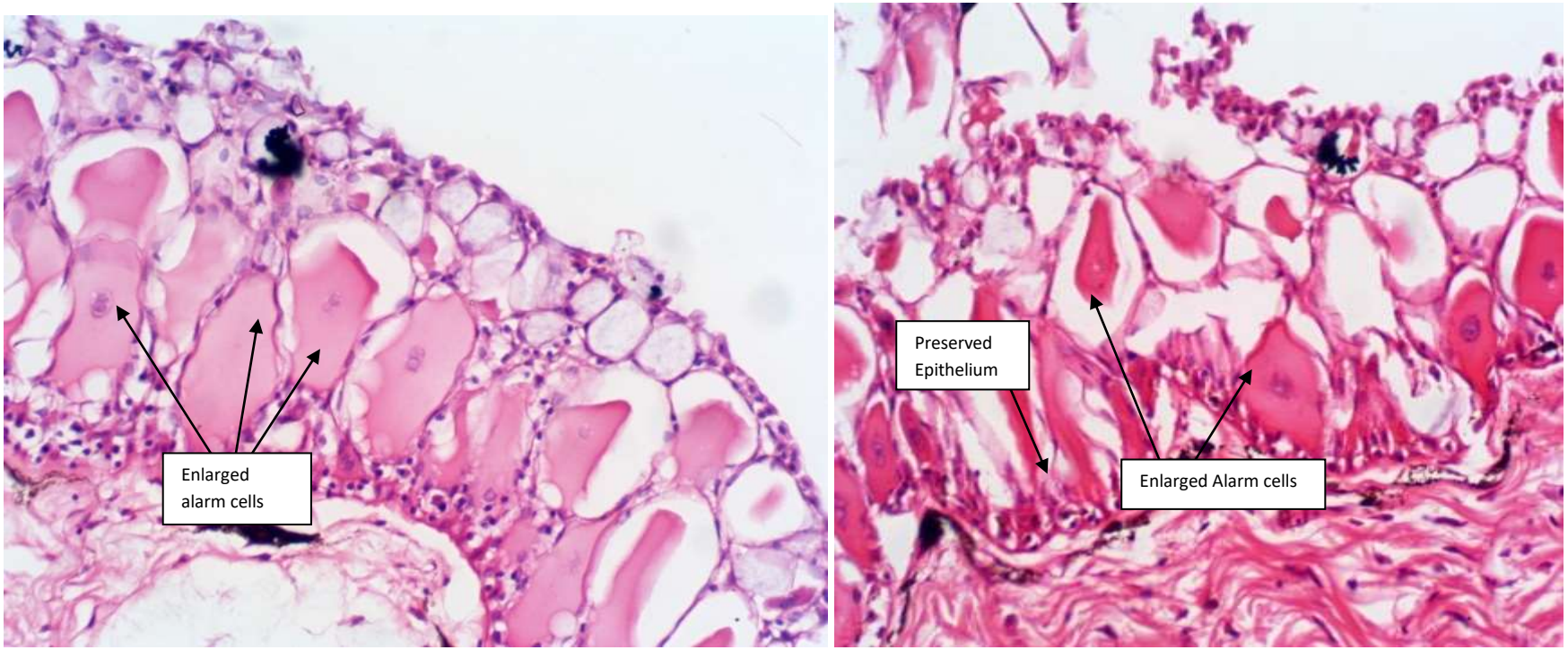

Plate V: Skin of *Clarias gariepinus* juveniles exposed to 53.0mg/L of glyphosate for 12 weeks showing Preserved epithelium, enlarged alarm cells. The dermis shows no abnormality. Mag. X100

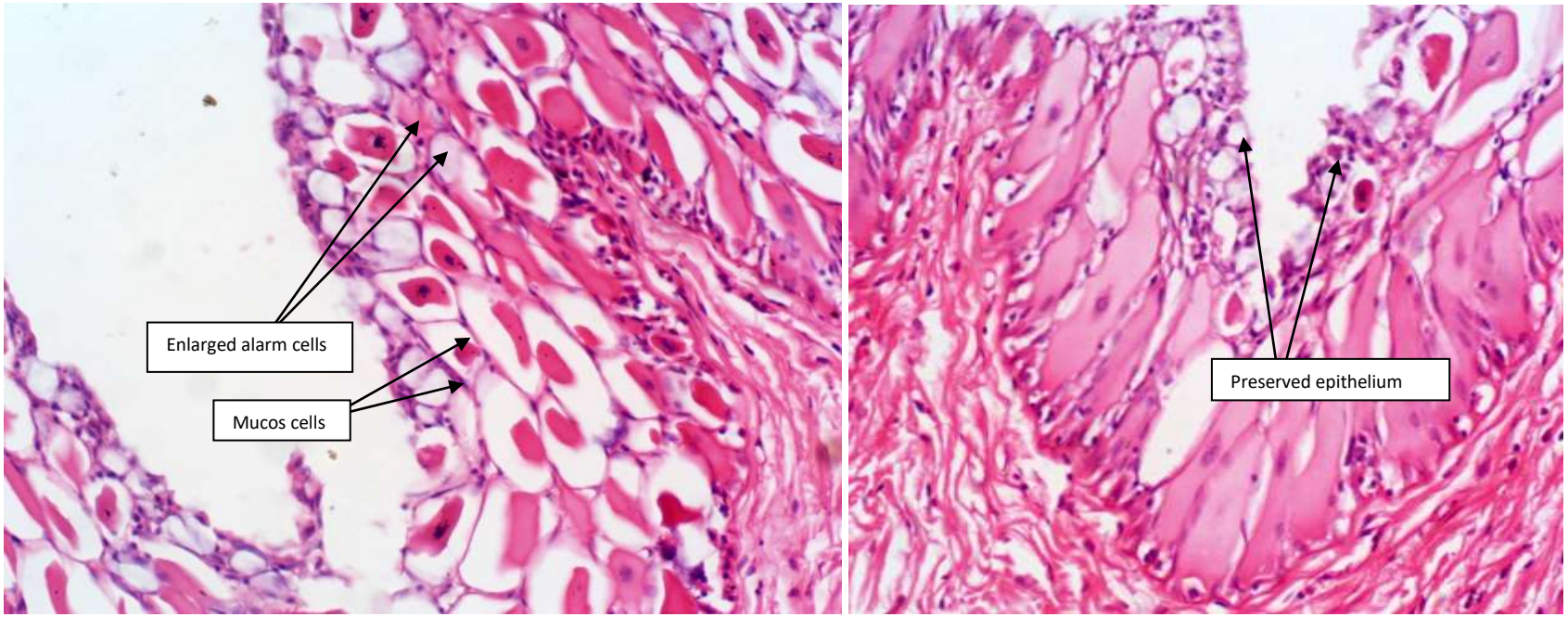

Plate VI: Skin of *Clarias gariepinus* juveniles exposed to 68.0mg/L of glyphosate for 12 weeks showing Preserved epithelium, enlarged alarm cells and mucous cells. The dermis shows no abnormality. Mag. X100

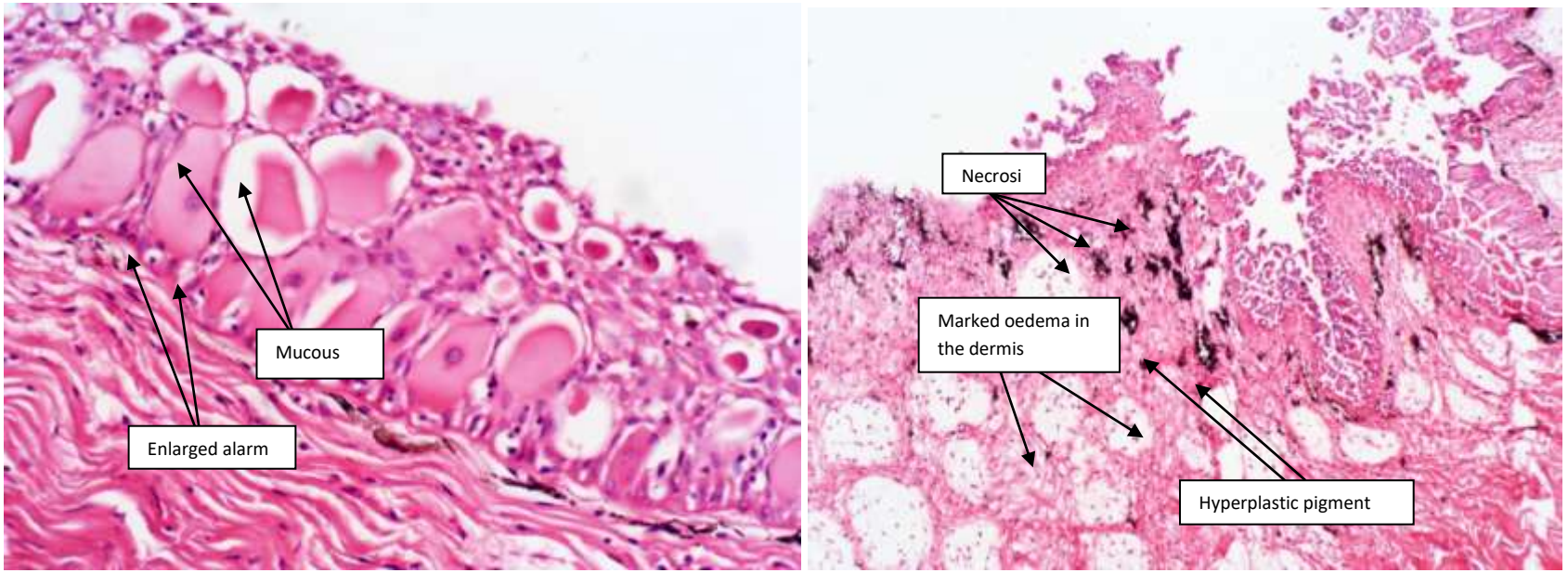

Plate VII: Skin of *Clarias gariepinus* juveniles exposed to 83.0mg/L of glyphosate for 12 weeks shows focal necrotic epidermis, increased number of pigment cells and oedematous dermis. Enlarged alarm cells and few mucous cells (mucous cell atrophy) are also seen. Mag. X100

4.1.6 Histopatological Screening of the liver of *C. gariepinus* juveniles exposed to sublethal concentrations of glyphosate

Liver of *Clarias gariepinus* exposed to no toxicant of glyphosate (0.00) for 12 weeks showed hepatic tissue with preserved architecture, the sinusoids were not obvious (Normal). There were no abnormalities in the cytoplasm, no fatty infiltration or cytoplasmic vacuolations seen. No significant inflammation, no acute or chronic damage seen. In plate VII of concentration 23.0mg/L, the micrograph showing hepatic tissue with preserved architecture, the sinusoids are not obvious (Normal), with mild congestion around the cytoplasm (not significant). The liver at plate VIII of concentration 53.0mg/L showed congestions surrounding the hepatic vessels with enlarged hepatocytes. At 68.0mg/L, the liver showed enlarged cytoplasm with some pyknotic nuclei (Plate VI). Finally, at 83.0 mg/L, the liver shows presence of Hemorrhage, enlarged nuclei with normal hepatocytes (Plate VII).

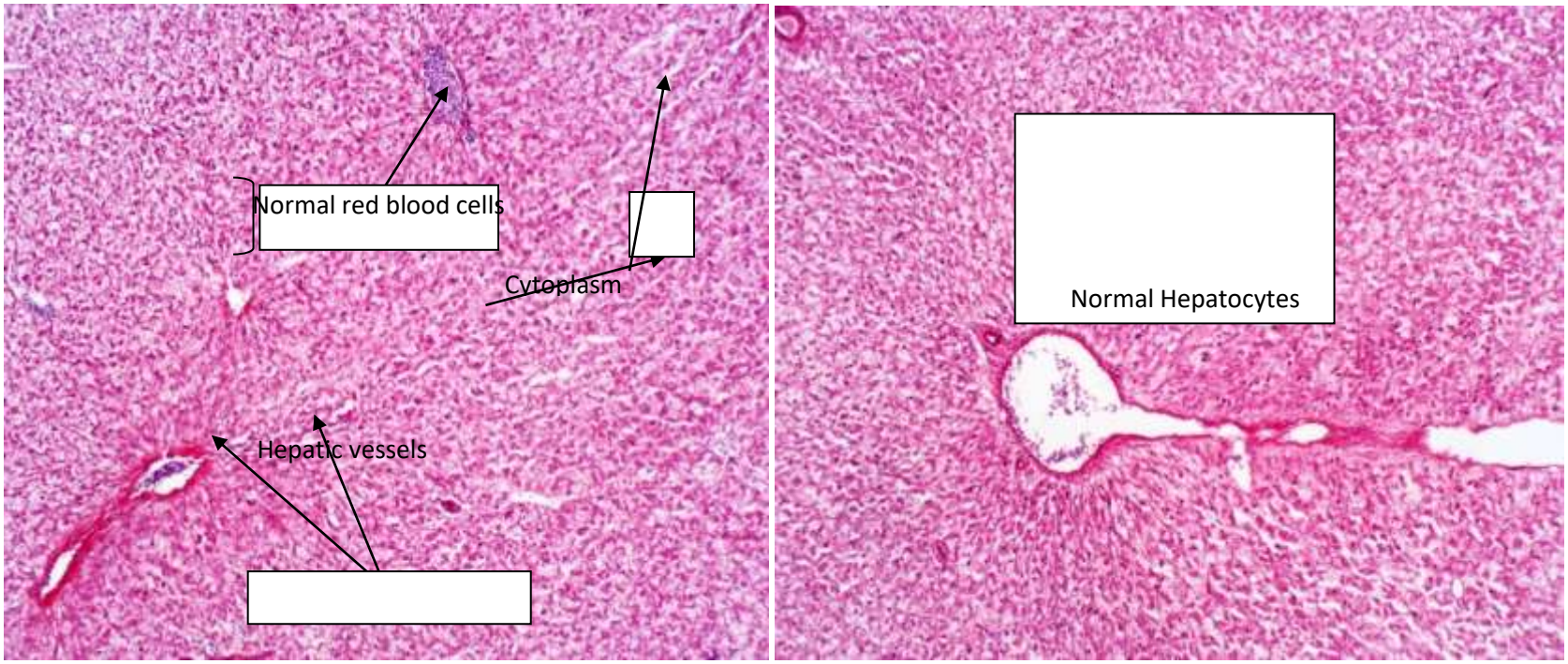

Plate VIII: Liver of *Clarias gariepinus* juveniles exposed to 0.00mg/L of glyphosate for 12 weeks showing hepatic tissue with preserved architecture, the sinusoids are not obvious (Normal). There are no abnormalities in the cytoplasm, no fatty infiltration or cytoplasmic vacuolations seen. No significant inflammation, no acute or chronic damage seen. Mag. X100

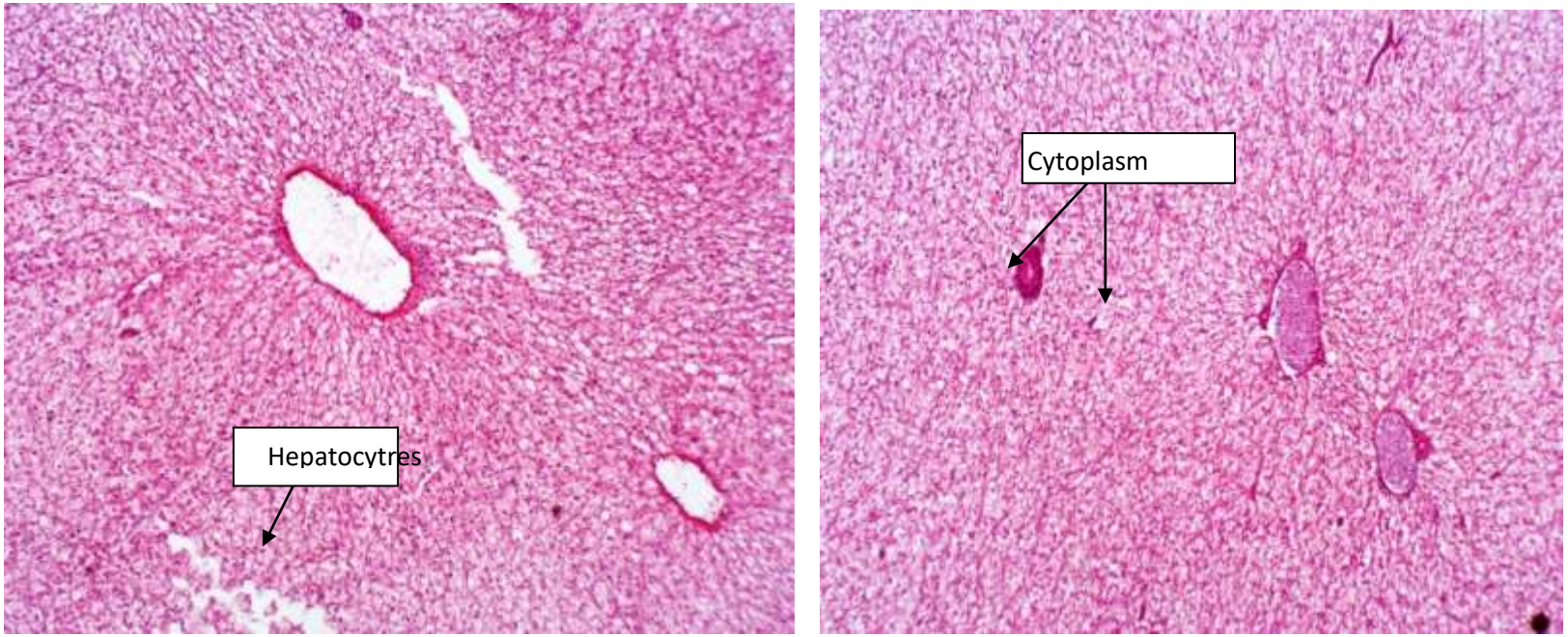

Plate IX: Liver of *Clarias gariepinus* juveniles exposed to 23.0 mg/L of glyphosate for 12 weeks showing hepatic tissue with preserved architecture, the sinusoids are not obvious (Normal), with mild congestion around the cytoplasm (not significant). Mag. X100

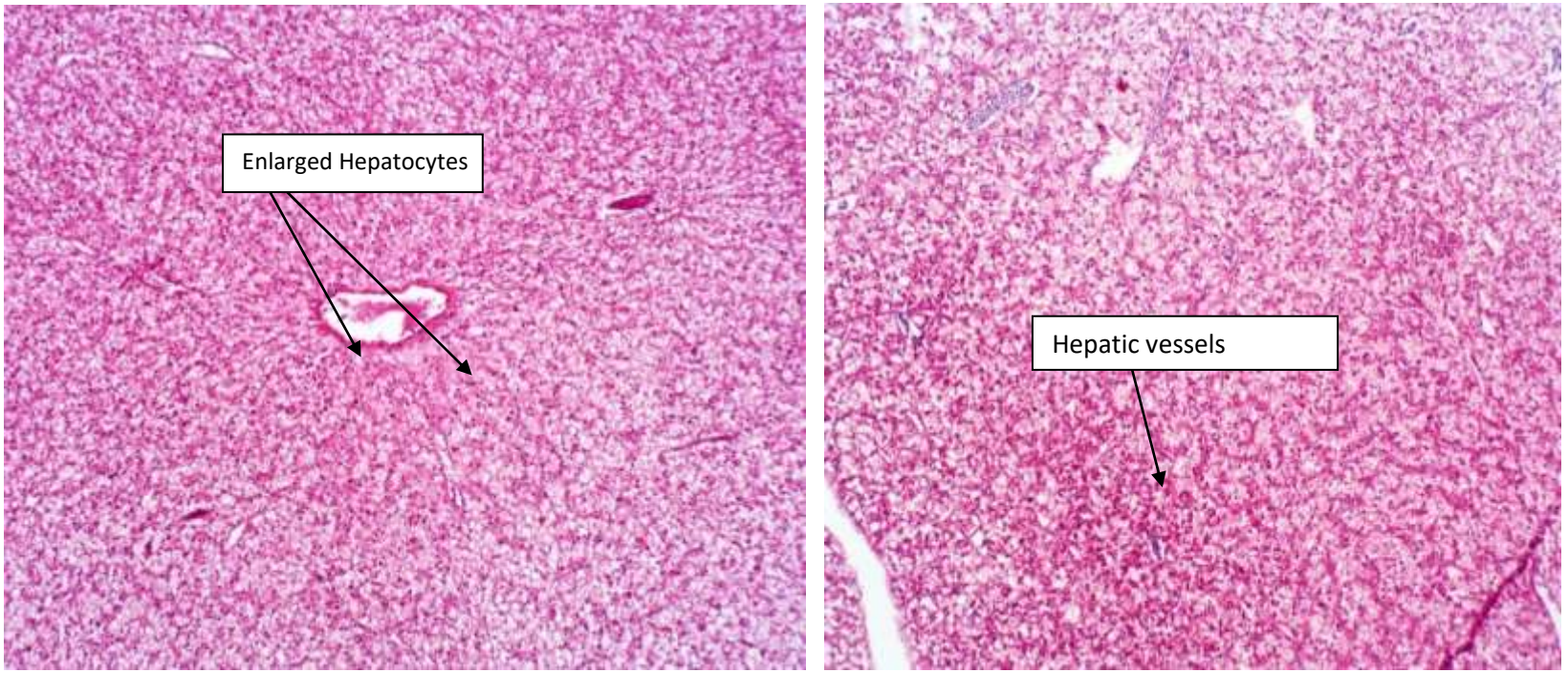

Plate X: Liver of *Clarias gariepinus* juveniles exposed to 38.0mg/L of glyphosate for 12 weeks showing congestions surrounding the hepatic vessels. Mag. X100

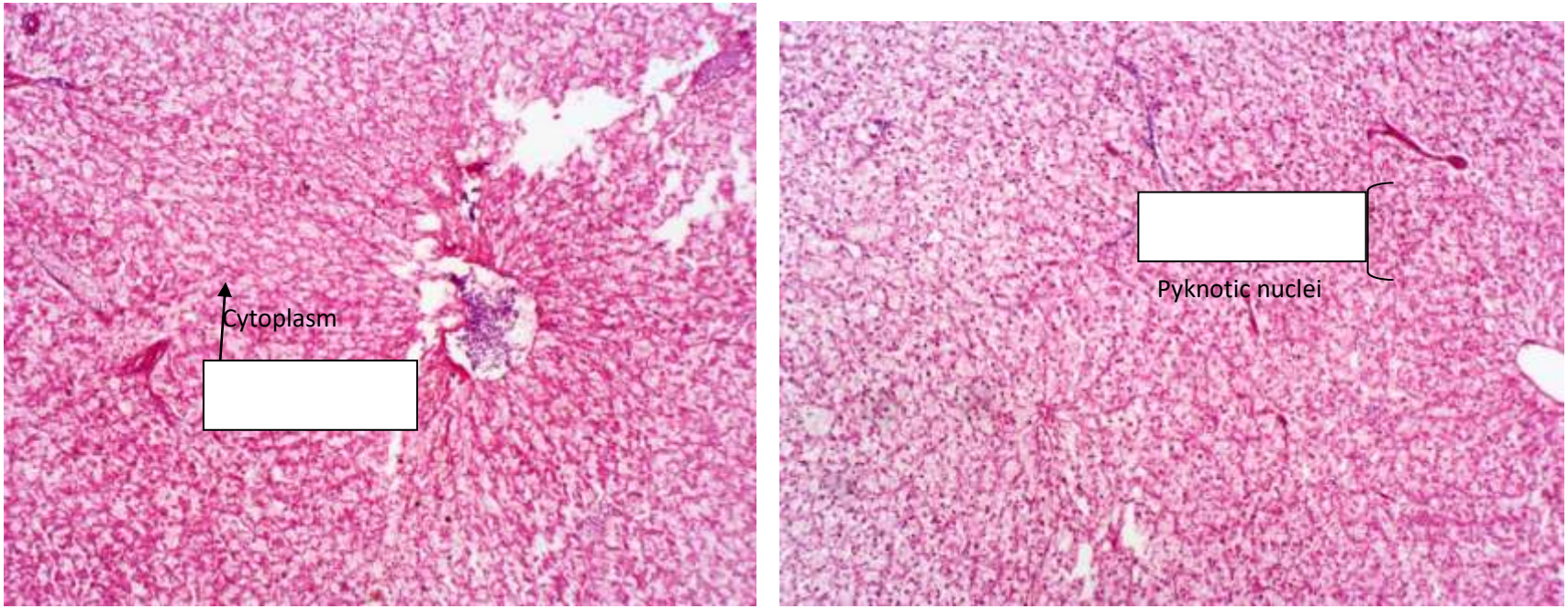

Plate XI: Liver of *Clarias gariepinus* juveniles exposed to 53.0mg/L of glyphosate for 12 weeks showing enlarged cyplasm with some pyknotic nuclei. Mag. X100

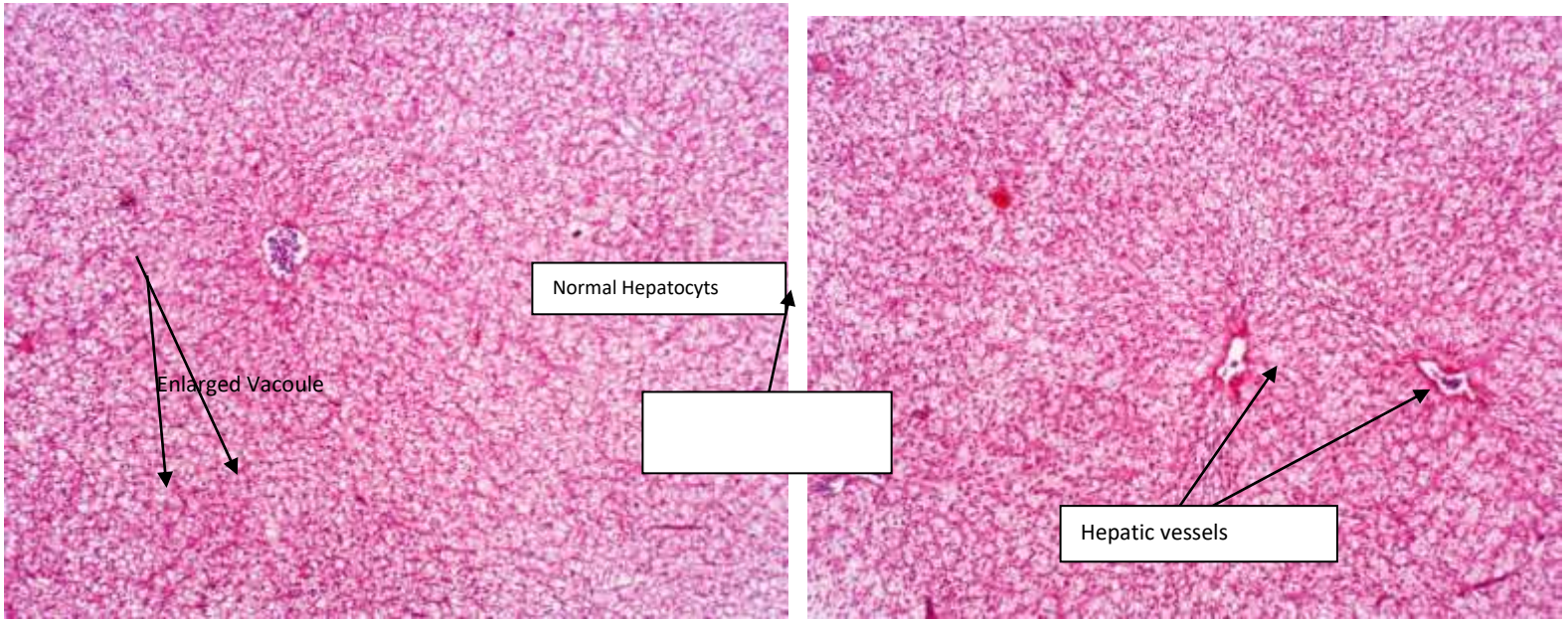

Plate XII: Liver of *Clarias gariepinus* juveniles exposed to 68.0mg/L of glyphosate for 12 weeks showing presence of Hemorrhage, enlarged nuclei with normal hepatocytes. Mag. X100

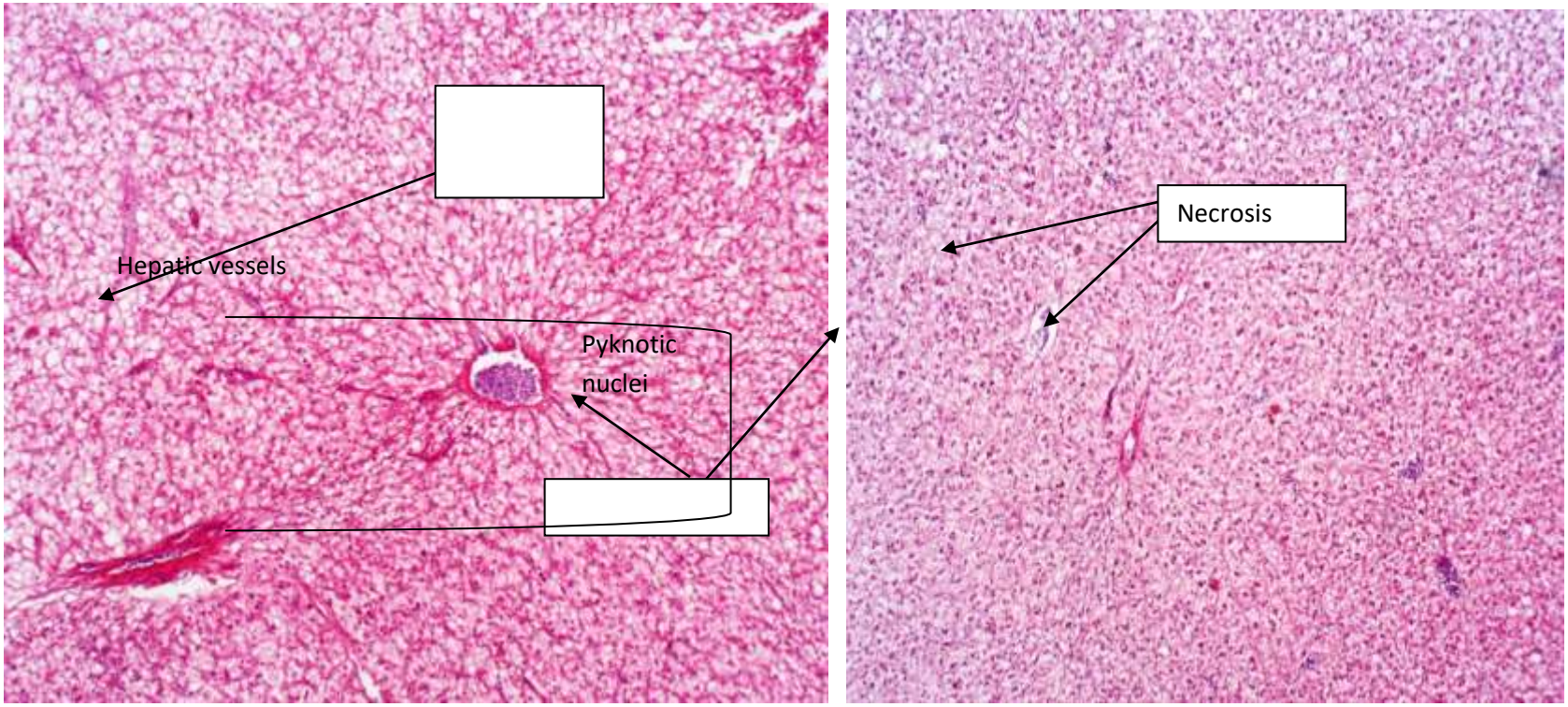

Plate XIII: Liver of *Clarias gariepinus* juveniles exposed to 83.0mg/L of glyphosate for 12 weeks shows hepatic evidence surrounded by few necrosis, enlarged pyknotic nuclei and Hemorrhage. Mag.: X100.

4.1.7 Growth (total length and total weight) of *Clarias gariepinus* exposed to sublethal concentrations.

The length and weight of the juveniles of *Clarias gariepinus* exposed to the various concentrations of toxicant were normal from the first week but at the second week it was observed that only the fishes at the control increased in length and weight. The other aquaria containing sublethal concentrations (23, 38, 53, 63 and 83 mg/L) experienced a slow growth compared to the control because of the change in the environmental conditions of the medium. At the fifth week of the exposure, the growths (length and weight) were very low compared to the initial week (week 1-4). At the end of experiment at the 12^{th} week, the results of the total weight and total length analysis are presented in Figure 4.1 and 4.2. The weight and length gained by the *Clarias gariepinus* juvenile exposed to the toxicant were very slow compared to the control.

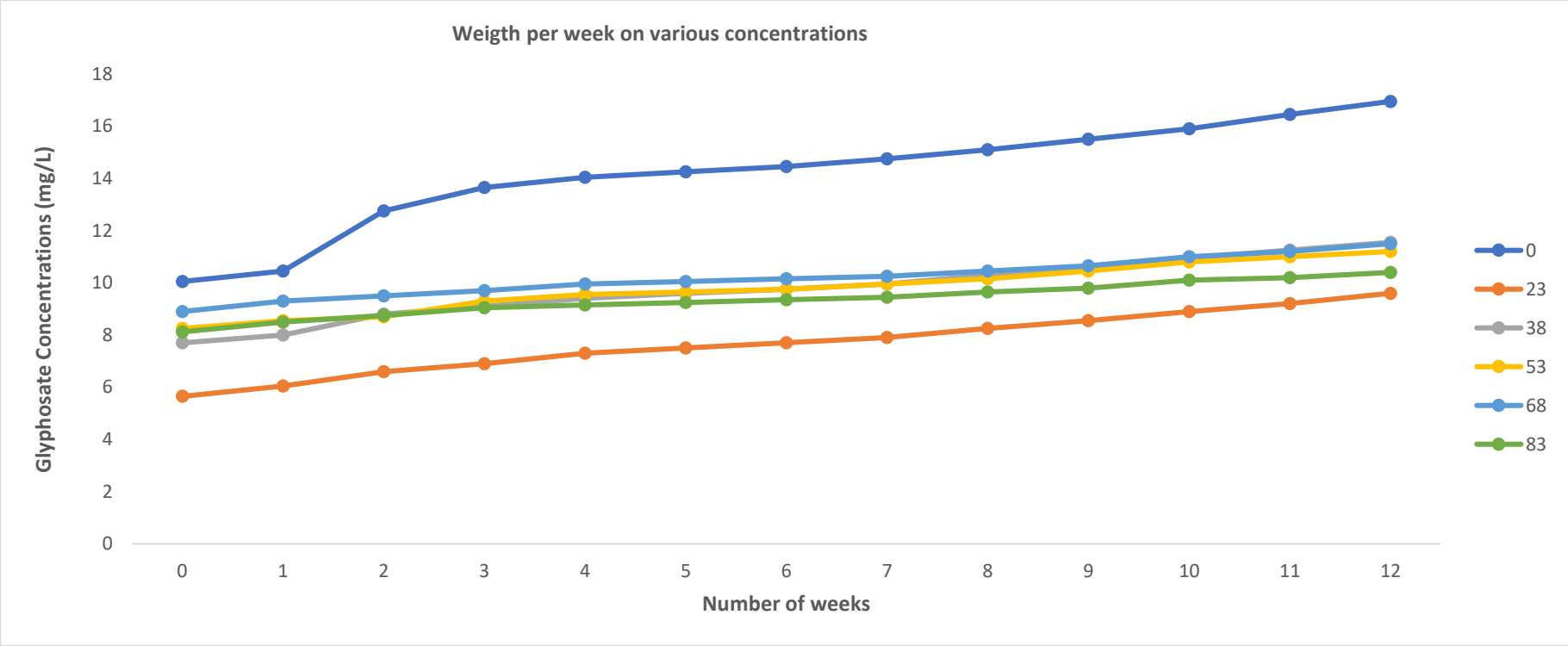

Fig. 4:1 Effect of Sublethal concentrations of Glyphosate on the weight of *Clarias gariepinus* juveniles

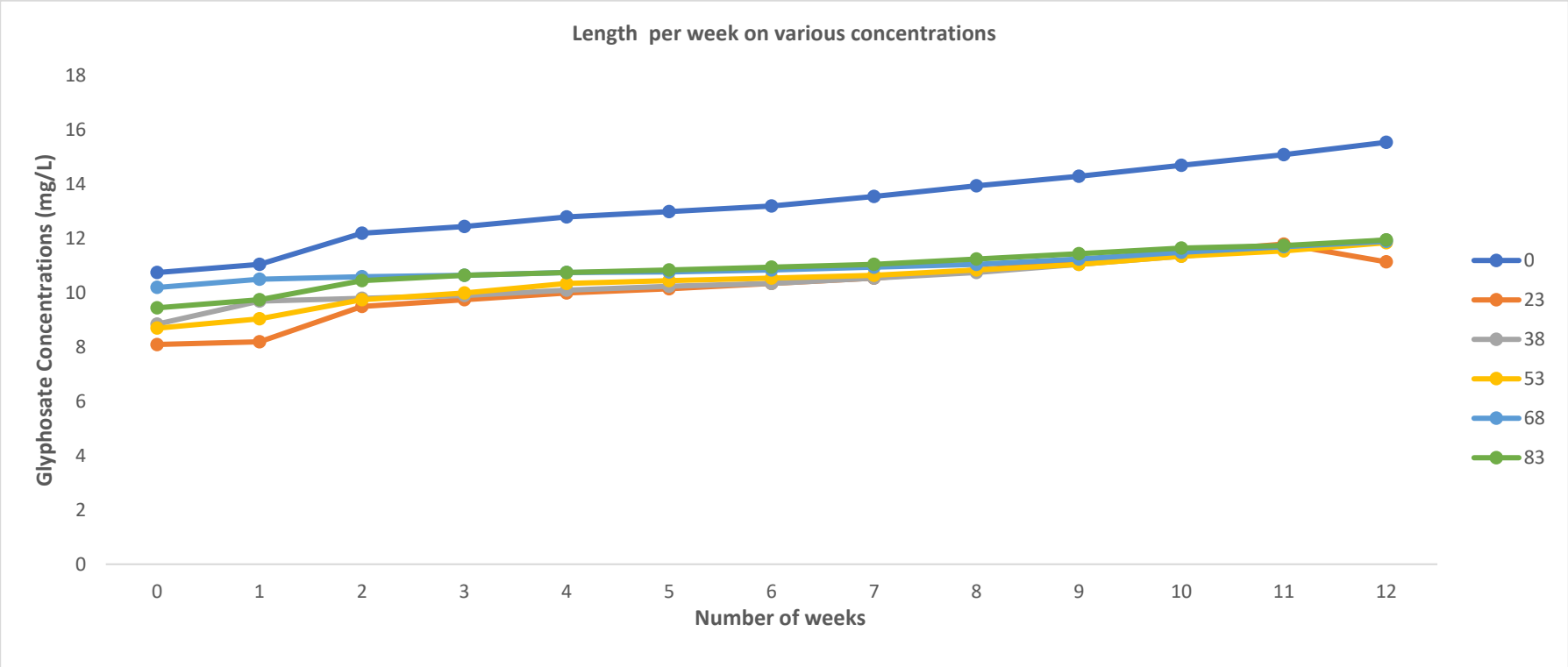

Fig. 4.2: Effect of Sublethal concentrations of Glyphosate on the length of *Clarias gariepinus* juveniles

4.2 Discussion

Glyphosate is most widely used as herbicides. It is said to be persistent and mobile in soil and water constituting most common terrestrial and aquatic contaminant (Cox 1998). They can be detected in most aquatic system with the value exceeding the required load (Jiraungkoorskul *et al.,* 2003). At control, the survival rate was 100 % all through the exposure period. The survival in 39.02 mg/L was 100 % until 96hr where it dropped to 90 %. At 78.04 mg/L, survival was 100 % until 72 hr where it dropped to 85 %. Also, survival was 100 % in concentration 156.09 mg/L at 24 hr of exposure but survival dropped to 95 %, 85 % and 65 % in the subsequent time of exposure. There was no complete survival at 312.19 mg/L concentration where it dropped from 85 %, 65 % and 30 % as the hours of exposure increases. Finally, at 624.19 mg/L there was no survival after 24 hours of exposure (0 % survival). Mortality increased with increase in concentrations of the toxicant which was similar with the finding of (Audu *et al.,* 2017) who reported 100, 70 and 50 % mortality of *C. gariepinus* after 96 hours of exposure to acute concentration of *Veronica amygdalina* leaf extract.

The mean physico-chemical parameters of the test concentrations (glyphosate) on fish shows dissolved oxygen changed steadily as the concentration of the toxicant increased in each of the aquarium. Dissolved oxygen concentration declined continuously leading to stressful condition on the fishes exposed to the aquaria containing different concentration of the toxicants, this agreed with the study of (Alabaster and Lloyd, 1982) which states that toxicant can exacerbate the effects of dissolved oxygen by damaging gills and their ability to extract oxygen from water. The hydrogen ion Concentration (pH) reduces as the concentration of the toxicant increases from 7.0 ± 0.2 to 6.7 ± 0.2. This slight reduction occurred as a result of the acidic nature of this toxicant (glyphosate). There was no significant difference ($P>0.05$) in pH values between the

treatment tanks and the control throughout the 12 weeks. It was observed that the temperature increased with increase in toxicant concentrations. The temperature increased slightly from the control 0.00 to 78.04 mg/L and remains slightly steady till the highest concentration (624.39 mg/L). Since the temperature was within the accepted limit and did not differ significantly (P>0.05) in all tanks which did not affect survival of the fishes (Ayotunde *et al.,* 2011). The total alkalinity in the experiment was observed to increase with increase in the amount of toxicant concentration. The total alkalinity recorded no significant difference (P>0.05) between the test tank and the control, this result agreed with Karahan (2006) who reported that alkalinity ($CaCO_2$) levels above 20 mg/L can significantly affect the survival of fishes as a result of increase in alkaline level. It was observed that the temperature decreased with increase in toxicant concentration. The temperature increased slightly from the control 0.00 to 78.04 mg/L and remains slightly steady till the highest concentration (624.39 mg/L). Temperature was within the accepted limits as such did not differ significantly with the (P>0.05) in all the test tanks including the control, did not affect the survival of the fish.

There was also an increase in mortality of the fishes as the concentrations of the toxicant increased with duration of time. This observation was related to report by Adebayo and Fapohunda (2016) who showed that the mortality for *C. gariepinus* was directly proportional to the concentration of Premium Motor Spirit (PMS). This finding was also in agreement with the finding of Audu *et al.* (2017) who reported that the mortality rate of *C. gariepinus* juvenile increases with increase in concentration of *Veronica amygdalina* leaf extract. The acute bioassay of glyphosate on *C. gariepinus* shows it is highly toxic to *C. gariepinus* which agreed with the finding of Patel (2016) which showed that bottle guard contains toxic tetracyclic triperpenoid compounds called cuberbitacins which are responsible for the bitter taste and toxicity.

Several abnormal behaviours were observed in the fish during the exposure period in the course of the study. Their severities were dependent on dose and time. Air gulping and sudden quick movements observed were similar to the observation of Omoniyi *et al.* (2002) and Okomoda *et al.* (2011). At higher concentrations, the juveniles showed eratic swimming and jerky movements which was similar to the report by Akinsorotan *et al.* (2013) when same herbicide was applied to *Clarias gariepinus* fingerlings. In this study, jerky movements which increased with concentration were observed and were also reported by Nwani *et al.* (2013) in *Clarias gariepinus* juveniles exposed to paraquat herbicide. The abnormal rapid movements of the fish in higher concentrations of the herbicide suggest the action of the herbicide on the nervous system of the fish which was also reported by Temple *et al.* (1992). Air gulping and increased opercular movements as shown by the fish signify impaired respiration which is due to the introduction of a toxicant (glyphosate) which has been reported by Warren (1997) to decrease the dissolved oxygen concentration in an aquatic system when introduced to it. These symptoms were also due to the effects of the toxicant on the gills as reported by Okomoda and Ataguba (2011). Skin discoloration was noticed on the fish skin when exposed to the higher herbicide concentrations. It was also observed that the discoloration disappeared after the exposure period when the fish were returned to fresh water devoid of the toxicant.

The toxicity test carried out showed a significant change ($P<0.05$) in WBC, RBC, PLT, Hb, PCV, MCV, MCH and MCHC of *C. gariepinus juveniles.* This signified that glyphosate had toxic effects on *C. gariepinus* juveniles. The is in line with study of Audu *et al.* (2014) who reported that the sub-lethal concentration of *Agave americana* leaf dust caused deleterous effects on the haemotological indices of *C. gariepinus*. The WBC in concentrations of 53 mg/L and 83 mg/L significantly reduced in a dependent

manner compared to the control group ($p<0.05$). The reduction in values as reported was in agreement with the findings of Fidelis *et al.* (2012) in *Orechromis niloticus* exposed to paraquat herbicide but contrary to that of Okomoda *et al.* (2013) in which there was steady increase in the WBC with increasing level of the toxicant. This might be due to degree of the potency of the toxicant. No definite pattern was observed in the values of RBC indices (MCV, MCH and MCHC) relative to the herbicide concentrations. This was in agreement with the findings of Akinrotimi *et al.* (2012) in *Clarias gariepinus* exposed to Cypermetrin. The PCV, Hb and RBC are good indicators of oxygen transportation capacity of the fish (Lamas *et al*., 1994). The significant reduction in PCV, HGB and RBC as compared with the control could be an indication of severe anaemia in the test fish which probably is caused by destruction of the erythrocytes or hemo-dilution resulting from osmoregulation across the gill epithelium as there was significant reduction in oxygen level of the exposed fish compared to the control. The result agrees with the finding of Omoniyi *et al.* (2002) and Adeyemo (2005). White blood cells provide protection against infectious agent caused by microbial and chemical factors (Akinfunmi *et al.*, 2007). The decrease in WBC counts with increase in concentration of glyphosate observed in this study was in line with the finding of Osman and AbouelFadi (2018) who reported a significant decrease in the leucocytes count collected from Nile Tilapia and African Catfish from contaminated sites as compared to other sites. Also, the white blood cells count was decreased in Nile tilapia and African Catfish after exposure to nickel (Ololade and Ogunbjimi, 2010).

Section of the skin of *C. gariepinus* juveniles exposed to sublethal concentrations of glyphosate revealed continuous and gradual striking histological alterations such as enlarged alarm cell, numerous mucous cells, stratified squamous cells, inflammatory epithermal cells, focal necrotic epidermis, oedematous dermis, e.t.c. These changes are

concentration and duration dependent. Therefore, glyphosate have effect on the skin of *C. gariepinus.* The observation was similar to the report by Audu, *at al.* (2017) who reported a progressive moderate to severe histo-architectural changes observed in the gills of *C. gariepinus* exposed to concentrated grades of *V. amydalina* which depicted a dose- dependent distortion especially with marked severity in those given the high concentration of the extract.

The histopathological examination of the liver, gill, and intestinal tissues of the exposed fish indicated that the liver and gills were the organs most affected. In fish, gills are critical organs for their respiratory, osmoregulatory, and excretory functions. Roberts (1978). The liver is the main organ for detoxification (Dutta *et al.,* 1993) that suffers serious morphological alterations in fish exposed to pesticides (Rodrigues and Fanta 1998). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, and this is similar to the observation of Rahmn *et al.* (2013). This study was also similar with the work of Audu *et al.* (2017) who reported that the histo-architectural changes in the liver tissues of *C. gariepinus* exposed to grades of *V. amygdalima* of low concentration of the extract appear to be toxic and run a concentration dependent histological disruption.

The toxicant exposure can induce the inhibition of growth performance in aquatic animals. Erickson *et al.* (2010) reported a significant reduction of growth performance of rainbow trout, *Oncorhynchus mykiss*, exposed to arsenic. In this study, the results indicated that fish exposed to sublethal concentrations of glyphosate had reduced weight gain when compared to the control, although this difference was not statistically

significant. And, the reduction of growth performance may result from the demand for energy to detoxicate the glyphosate which cause the drop in the energy. Also, the growth performance of *Clarias gariepinus* juveniles was affected by the water temperature change in higher concentration of the toxicant. Carvalho and Fernandes (2006) reported that the high temperature causes the severe accumulation in fish by the toxicant exposure which may need more energy for detoxification. This is also in accordance with report of Chinabut *et al.* (1988) who observed that exposure to formalin at therapeutic levels for eight weeks reduced the growth of common carp fry. Similarly, Omoregie *et al.* (1998) had observed depressed weight in Nile Tilapia (*Oreochromis nitlotcus*) exposed to sublethal concentrations of formalin.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The result of this study indicates that despite the usefulness of glyphosate in the control of weeds, it is harmful to fish when it finds its way into aquatic bodies over a period of time. The physico-chemical parameters were within the accepted limits as such did not differ significantly ($P>0.05$) in all the test tanks including the control, and as such did not affect the survival of the fish.

In determining the LC50, there were some abnormal behaviour with the increase being directly proportional ($p<0.05$) to the glyphosate concentration. Several abnormal behaviours were observed in the fish during the exposure period in the course of the study. Their severities were dependent on dose and time.

The toxicant exposure inhibits the growth performance (length and weight gained/loss) in the fish when compared to the control, although this difference was not statistically significant ($P>0.05$).

The toxicity test carried out showed a significant change ($P<0.05$) in RBC, PCV, Hb, PL of *C. gariepinus juveniles.* This signified that glyphosate had toxic effects on *C. gariepinus* juveniles. At higher concentrations of the toxicant, there were effects on the skin and liver of the exposed fish but at lower concentrations the effect was gradual as it effect were seen at the later phase of the experiment.

The physiological changes shown in the behavioral, haematological, histological and growth parameters of the exposed *C. gariepinus* juveniles to the glyphosate might have affected the general health and status of the fish resulting in death of fish. This may also

not result in fish kill immediately but definitely represents a health hazard to human consumers.

5.2 Recommendations

There is evidence that indiscriminate use of glyphosate by farmers for agricultural purpose to control weed which might be washed into streams and rivers by rain water, can have severe harmful effect on the ecosystem leading to the destruction of our fish stock and perhaps other forms of life. If the government's goal of self-reliance in food production particularly protein production is to be achieved, attempt should be made to control and monitor the use of glyphosate and reduce its use to the barest minimum. Herbicide producing industries should also be encouraged to look into the possibility of reducing the potency to non-target organisms yet still maintaining its effectiveness. Alternatively, research into the discovery of new effective but less potent agro-chemical or herbicides should be encouraged.

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APPENDICES

APPENDIX A: RESULT OF ACUTE BIOASSAY OF JUVENILE OF CLARIAS GARIEPINUS EXPOSED TO VARIOUS CONCENTRATIONS OF GLYPHOSATE.

Conc. (mg/L)	No. of fish	Mortalities/time (hours) 24	48	72	96	Total mortality
Control	10	-	-	-	-	-
39.02	10	-	-	-	1	1
78.04	10	-	-	1	-	1
156.09	10	-	1	1	-	2
312.19	10	1	2	2	1	6
624.39	10	5	5	-	-	10

APPENDIX B: RESULT OF SUBLETHAL EFFECTS OF VARIOUS CONCENTRATIONS OF GLYPHOSATE ON GROWTH OF CLARIAS GARIEPINUS JUVENILE

WEIGHT

	WEEK												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	10.05	10.45	12.75	13.65	14.05	14.25	14.45	14.75	15.1	15.5	15.9	16.45	16.95
23	5.65	6.05	6.6	6.9	7.3	7.5	7.7	7.9	8.25	8.55	8.9	9.2	9.6
38	7.7	8	8.8	9.1	9.4	9.6	9.75	9.95	10.3	10.6	10.95	11.25	11.55
53	8.25	8.55	8.7	9.3	9.55	9.65	9.75	9.95	10.15	10.45	10.8	11	11.2
68	8.9	9.3	9.5	9.7	9.95	10.05	10.15	10.25	10.45	10.65	11	11.2	11.5
83	8.1	8.5	8.75	9.05	9.15	9.25	9.35	9.45	9.65	9.8	10.1	10.2	10.4

LENGTH

	WEEK												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	10.75	11.05	12.2	12.45	12.8	13	13.2	13.55	13.95	14.3	14.7	15.1	15.55
23	8.1	8.2	9.5	9.75	10	10.15	10.35	10.55	10.85	11.15	11.5	11.8	11.15
38	8.85	9.7	9.8	9.9	10.1	10.25	10.35	10.55	10.75	11.05	11.35	11.65	11.95
53	8.7	9.05	9.75	10	10.35	10.45	10.55	10.65	10.85	11.05	11.35	11.55	11.85
68	10.2	10.5	10.6	10.65	10.75	10.77	10.85	10.95	11.05	11.25	11.5	11.7	11.9
83	9.45	9.75	10.45	10.65	10.75	10.85	10.95	11.05	11.25	11.45	11.65	11.75	11.95