

**TOXICITY OF THE HERBICIDE GLYPHOSATE (ROUND-UP) ON  
FINGERLINGS OF HETEROCLARIAS (HYBRID)**

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**TOXICITY OF THE HERBICIDE GLYPHOSATE (ROUND-UP) ON  
FINGERLINGS OF HETEROCLARIAS (HYBRID)**

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**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU  
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**DEPARTMENT OF BIOLOGICAL SCIENCES,  
FACULTY OF SCIENCE,  
AHMADU BELLO UNIVERSITY, ZARIA**

**NIGERIA**

**MAY, 2015**

## **DEDICATION**

I sincerely dedicate this work to my late father, my mother, brother, sisters, uncles and friends for their immeasurable contributions to the success of this work.

## DECLARATION

I declare that the work in this thesis report entitled, “*Toxicity of the herbicide glyphosate (Round-up) on fingerlings of Heteroclarias (hybrid)*” has been performed by me in the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project thesis was previously presented for another degree at this or any other institution.

Adekunle David MICAH

\_\_\_\_\_

Signature

\_\_\_\_\_

Date

## CERTIFICATION

This thesis entitled “**TOXICITY OF THE HERBICIDE GLYPHOSATE (ROUND-UP) ON FINGERLINGS OF HETEROCLARIAS (HYBRID)**” by Adekunle David MICAH, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## ABSTRACT

The toxicity effect of glyphosate on fingerlings of *Heteroclaris* after series of range finding tests, the fishes were exposed to lethal concentration of 0.00mg/l, 5.40mg/l, 7.20mg/l, 9.00mg/l, 10.80mg/l and 12.60mg/l for 96 hours as well as sub-lethal concentrations of 0.00mg/l, 0.30mg/l, 0.70mg/l and 1.40mg/l of glyphosate for 8 weeks in a renewal bioassay procedure showed that the 96 hours LC<sub>50</sub> was 6.838mg/l with upper and lower limits of toxicities were 8.237 and 5.674mg/l respectively. Respiratory disturbance, erratic swimming, loss of equilibrium, lethargies and sudden fish death were observed in the exposed fish and these varied greatly with differences in concentration of the toxicant and this shows that mortality increases with an increase in concentration. Also, as the concentration of glyphosate increased the beats of the tail and operculum increased in 12 and 24 hours. The differences observed in the mortalities of *Heteroclaris* at varying concentrations were significant ( $p < 0.05$ ), an indication that mortality could be a factor of concentration and time of exposure. The physico-chemical parameters obtained before and after the experiment were within the tolerated limit. Also the toxicant led to significant changes ( $p < 0.05$ ) in haematological parameters as the toxicant concentration increased. Mean Red Blood Cells (RBC), Haemoglobin content (Hb), Packed Cell Volume (PCV), reduced as the concentration of toxicant increased while other parameters increased proportional with the toxicant concentration. The changes in gills were characterized by mononuclear infiltration, inflammation of primary and secondary lamella, cellular infiltration, vacuolation. The liver showed vascular congestion, kupffer cell hyperplasia, necrosis, adipocyte infiltration, lymphocyte hyperplasia in the liver of fish treated with 5.40mg/l, 9.00mg/l, 10.80mg/l, 12.60mg/l

concentrations of glyphosate. Significant alteration in oxidative stress parameters in the treated fish were dose dependent. The growth rates were significantly reduced in fish exposed to sub-lethal concentration of the herbicide. Glyphosate showed high significant ( $p < 0.05$ ) inhibition in all nutrient utilization parameters i.e specific growth rate, feed conversion rate, gross feed conversion efficiency, feed efficiency, live weight gain and nutrient metabolism (SGR, FCR, GFCE, FE, LWG and NM).

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## CHAPTER ONE

### 1.0

### INTRODUCTION

#### 1.1 Background

Glyphosate a unique global herbicide under the trade name Round-up® was introduced to the market by Monsanto Company during the 1970s. The tonnage of glyphosate herbicide application has been constantly increasing since the introduction of this group of chemicals in 1971 (Dill *et al.*, 2010). The 2008 global production was estimated to be 620,000 tonnes, representing a value of 8.3 billion US\$, making glyphosate the most widely used herbicide ingredient worldwide (Pollak, 2011). The most common herbicide formulations such as the brands of Round-up contain various salts of glyphosate that ensure high water solubility, mainly isopropylamine salt (IPA salt of glyphosate) (Woodburn, 2000). It was initially registered as a broad-spectrum, non-selective, systemic herbicide for certain non-crop and plantation crop uses (fallowed fields, orchards, vineyards and timber plantations) and for the control of annual and perennial weeds before the emergence of agronomic crops (Folmar *et al.*, 1979; Woodburn, 2000; Çavas, and Könen, 2007). The major formulation is Round-up, in which glyphosate is formulated as isopropylamine salt and a surfactant, polyethoxylene amine (POEA), is added to improve the quality of the herbicide (Tsui and Chu, 2004; Releya, 2005). Many commercial herbicides have been formulated using glyphosate as active ingredient such as Rodeo, Round-up and Aquamaster (Mozdzer *et al.*, 2008; Papchenkova *et al.*, 2009). Due to its low persistence, repeated applications of this herbicide are practiced for the control of weeds in agricultural fields and thereby, large quantities find their way into the water bodies (Ayoola, 2008b). The indiscriminate use of herbicides, careless handling,

accidental spillage, or discharge of untreated effluents into natural water-ways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment (Ayoola, 2008b).

Human acute toxicity is dose related. Acute fatal toxicity has been reported in deliberate overdose (Bradberry *et al.*, 2004; Sribanditmongkol *et al.*, 2012). Japanese researchers analyzing suicides have found that drinking 3/4 of a cup (200 millilitre) of commercial glyphosate products is fatal. Survivors (those who consumed less than 3/4 of a cup) suffered a range of severe problems (Caroline, 1998). Those problems included intestinal pain, vomiting, erosion of the gastrointestinal tract, excess fluid in the lungs, pneumonia, lung dysfunction, clouding of consciousness, destruction of red blood cells, abnormal electrocardiograms, low blood pressure, kidney damage and damage to the larynx. It is important to note that damage to the kidneys and the lungs is usually permanent. These body tissues do not repair themselves, instead forming scar tissue that does not function to help filter toxins from the blood or breathe oxygen (Hardell and Eriksson, 1999).

The major pathway for glyphosate uptake in plants is through the foliage; however, some root uptake may occur. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants (Anthelme and Marigo, 1998; Filizadeh and Rajabi, 2011). The glyphosate degradation is mostly determined by microbial metabolism with amino-methylphosphonic acid (AMPA) as the main degradation product (Kolpin *et al.*, 2006; Cribner *et al.*, 2007). The half-life ranges from a few days to several weeks and even years, but the average is two months (Giesy *et al.*,

2000; Gluszczak *et al.*, 2007) and its water solubility is around 157µg/L (Rodrigues and Almeida, 1998).

Most aquatic herbicides have undergone some toxicity testing to evaluate effects on non-target organisms (Urban and Cook, 1986). Ayoola (2008a) conducted a research on histopathological effects of glyphosate on juvenile African catfish (*Clarias gariepinus*). This is because tests are rarely conducted on the early life stages of fish commonly found in water bodies in Nigeria being treated for 'weed control'. The continuous use of these herbicides has brought about some concern of the effects of these chemicals on the early life stages of fish (Skea *et al.*, 1987). The herbicide could bring about a decline in the Fishery of water, if a herbicide that is toxic to the early life stages of fish are used annually (Ayoola, 2008b). Hence, the toxicity study of these chemicals to early life stages is essential to understand the environmental impacts of herbicides. An effect of sub-lethal concentration of glyphosate on fish has been reviewed by several authors. Neskovic *et al.* (1996) conducted sub-acute toxicity test (14 days) of sub-lethal glyphosate concentrations on histopathological changes of Carp organs such as gills, livers and kidneys. Ayanda and Egbamuno (2012) also reported the sub-lethal effect of glyphosate on juvenile of *C. gariepinus* which showed histological changes in the liver. Histological alterations were also observed in liver, gills and kidneys of Nile tilapia (*Oreochromis niloticus*) after acute and chronic exposure to sub-lethal concentrations of Round-up (Jiraungkoorskul *et al.*, 2002, 2003).

## **1.2 Statement of Research Problems**

Contamination of surface waters by herbicides derived from agricultural premises has become a serious problem Worldwide (Oruc and Uner, 1999; Grzegorz *et al.*, 2012). Carp fish exposed to 5mg/L and 10mg/L of glyphosate for two weeks had gill and liver damage respectively (Neskovic *et al.*, 1996). Herbicides disrupt ecological balance affecting non-target organisms including fish (Bretand *et al.*, 2000; Oruc and Uner, 1999).

Toxicants produce several biochemical and physiological responses when they enter an organism which may be an acclimation to the organism or may lead to toxicity (Fatima *et al.*, 2006; Lissandra *et al.*, 2006). Many water contaminants can stimulate the production of Reactive Oxygen Species (ROS) and result in oxidative damage to aquatic organisms (Sturve *et al.*, 2008).

## **1.3 Justification**

Heteroclaris is an important fish for aquaculture and essential source of protein in Nigeria. It is very hardy and is fast grower of which this experiment is aimed at determining such conditions. However, studies conducted by Szarek *et al.* (2000), Elahee and Bhagwant, (2007) on *Cyprinus carpio* indicated that the sub-lethal concentrations of glyphosate, corresponding to less than 2% of the LC<sub>50</sub> caused ultra structural damage in the liver. Also, histological alterations have been observed in liver and gills on juvenile of *C. gariepinus* after acute and chronic exposure of glyphosate (Ayoola, 2008a; Ayanda and Egbamuno, 2012).

The indiscriminate use of herbicides such as Round-up through careless handling, accidental spillage, or surface run-off into natural water-ways has harmful effects on the fish population and other forms of aquatic life. Thus, Round-up may contribute to long term effects in the environment even in small concentration. This is because they do not degrade easily into the environment; therefore, the need to subject glyphosate to varying concentration to determine their toxicity on *Heteroclaris*.

Since gill is the first site where these toxins gain access and the liver which is a detoxifying organ, there is need to check the histopathological indices of the fish. Oxidative stress develops as a consequence of disturbance between generation and elimination of Reactive Oxygen Species (ROS) with certain physiological consequences (Luschak, 2011b) and some of this antioxidant enzymes catalase (CAT) and peroxidase (POD) acts to eliminate these ROS produced within the cell. Thus, the levels of these enzymes that were determined during this investigation reflect the stress level caused by glyphosate.

Therefore, this work was aimed at presenting information which is lacking in the environment.

#### **1.4 Aim of the Study**

The aim of the study is to establish the acute and sub-lethal effects of glyphosate on the freshwater fish *Heteroclaris*.

#### **1.5 Objectives of the Study**

To determine the effects of:

1. short term (acute) exposure 96 hr LC<sub>50</sub> on Heteroclarias.
2. glyphosate on behavior and physico-chemical parameters on Heteroclarias.
3. acute and sub-lethal toxicity of glyphosate on haematology of Heteroclarias such as RBC, WBC, PCV, Hb, MCH, MCHC, MCV, neutrophils, lymphocytes.
4. acute and sub-lethal toxicity on histopathology of Heteroclarias.
5. glyphosate on some antioxidant response of Heteroclarias.
6. glyphosate on growth and nutrient utilization.

### **1.6 Statement of Research Hypotheses**

1. There is no significant effect on short term (acute) exposure 96 hr LC<sub>50</sub> on Heteroclarias.
2. There is no significant effect on behavior and physico-chemical parameters on Heteroclarias.
3. The effects of acute and sub-lethal toxicity of glyphosate on haematology of Heteroclarias such as RBC, WBC, PCV, Hb, MCH, MCHC, MCV, neutrophils, lymphocytes do not significantly differ.
4. There is no significant effect on acute and sub-lethal toxicity on histopathology of Heteroclarias.
5. There is no significant effect on some antioxidant response to Heteroclarias to glyphosate exposure.
6. There is no significant effect on growth and nutrient utilization of the fish.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Pesticides Toxicity and Fish Behaviour

The first site of toxicant action in fish might be the gills, being the primary site of respiration and osmoregulation. Damage of the gill architecture may trigger physiological responses in the fish as it tries to off the balances by breathing faster causing more toxicant to be passing over the gills (Balinth *et al.*, 1997). Surprisingly, only a few studies have shown that fish inhabiting natural freshwater ecosystems may be affected by unintentional spreading of pesticides (Balinth *et al.*, 1997; Csillik *et al.*, 2000). Pesticides toxicity to fish has been reported in several studies (Balinth *et al.*, 1997; Csillik *et al.*, 2000; Aguiwo, 2002 and Ogundele *et al.*, 2004). However, fish are not usually target organism for pesticides and knowledge about effects of pesticides in the field is still sparse.

Behavioural abnormalities have been reported by Swarup *et al.* (1981) when studying the toxicity of endosulfan to the freshwater fish, *Cirrhinus mrigala*. Influences of pesticides on behavioural responses of fishes have been reported in *Oreochromis mossambicus* and *Labeo rohita* (Baskaran *et al.*, 1989; Malla Reddy and Bashamohideen, 1989; Baskaran, 1991 and Vineet Kumar and David, 2008). These authors reported that the fish exposed to pesticide media showed erratic jumping movements, occasional somersaults and swim laterally on one side of the body. The body of the fish has been covered with secretion of mucus. The colour of the gill of the pesticide treated fish changed from red to brown and pale red. The caudal region of the fish lost regular shape and twisted that greatly affected normal swimming pattern.

## **2.2 Mechanism of Glyphosate Toxicity**

Glyphosate (*N*-[phosphonomethyl] glycine) is a weak organic acid comprising a glycine moiety and a phosphonomethyl moiety. Glycine the smallest amino acid found in proteins and in the glyphosate molecule, one of the amino hydrogen atoms of glycine is replaced with a phosphonomethyl group. Glyphosate prevents the synthesis of aromatic amino acids in plants and some microorganisms by inhibiting the enzyme 5-enolpyruvyl shikimate-3-P synthetase (Devine *et al.*, 1993). Many animals do not possess this pathway of synthesis and obtain the necessary aromatic amino acids from plants and other sources. Because of this, glyphosate is relatively non-toxic to animals while it is very effective as a herbicide. There are many different commercial formulations of glyphosate herbicide that, in addition to the active ingredient, contain a number of additives to increase efficacy. Most of these additives are inert and their identities are protected as trade secrets. Of these additives, the available information shows that certain surfactants pose the greatest ecotoxicological risk. Surfactants reduce surface tension and increase wetting of hydrophobic plant cuticles, increasing penetration of glyphosate into plant tissues. A common surfactant in glyphosate herbicides is polyoxyethylene tallow amine (also called polyoxyethyleneamine, POEA).

In addition to POEA, several other surfactants are used in commercial formulations of glyphosate (Solomon and Thompson, 2003). The surfactants are not single substances and may contain a mixture of ingredients blended to provide desirable properties to each commercial formulation of glyphosate herbicide. Some commercial formulations of glyphosate herbicide, such as Rodeo and Accent (other trade names), are sold without surfactants, but they need surfactants blended in before use (NCASI, 2004). Even



whether POEA also accounts for the toxicity of Round-up to microorganisms such as bacteria, microalgae and protozoa which were very different from invertebrates and vertebrates (e.g. fishes and amphibians) in terms of morphology, cytogenetics and physiology. Furthermore, the lack of toxicity data of many kinds of pesticides on aquatic bacteria and protozoa was highlighted in a recent review (DeLorenzo *et al.*, 2001), and this is also the case for glyphosate based herbicides.

#### **2.4 Effects of Herbicides Toxic Aquatic Environment on Physico-chemical Parameters**

Several environmental factors such as pH and temperature have been identified to modify the toxicity of pesticides (Sprague, 1985; Fisher, 1990; Howe *et al.*, 1994). For glyphosate-based formulations, Wan *et al.* (1989) examined the effects of different dilution waters on the toxicity of Round-up, while Folmar *et al.* (1979) investigated the effects of pH and temperature on Round-up toxicity. Nevertheless, these studies employed different organisms and test conditions and therefore could not easily generalize the individual effects of different factors on Round-up (glyphosate) toxicity.

#### **2.5 Effect of Glyphosate and other Herbicides on Organisms**

##### **2.5.1 Effects of herbicides on fish haematology**

The exposure of fish to several types of chemical agents may induce changes in several haematological variables (Heath, 1995), which are frequently used to evaluate fish health (Martinez and Souza, 2002). The study of blood parameters in fishes has been widely used for the detection of physiopathological alterations in different conditions of stress (Nussey *et al.*, 1995). Hematological parameters such as hematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for

application in environmental monitoring and toxicity studies in aquatic animals (Sancho *et al.*, 2000; Barcellos *et al.*, 2003).

Haematological tests are important diagnostic tools and recent findings have suggested that they may be equally valuable as indicators of disease or stress due to pollutants and environmental fluctuations in fishes (Bhatkar and Dhande, 2000). The blood plays an integrated and inevitable part in all immune systems. Haematological parameters are related to responses of the organisms to the changing environmental conditions and therefore can be used to screen the healthy state of fish experimented to the exposed toxicant. Further, haematological observations have greater contribution to the pathological changes obtained during toxicological studies. Attempts have been made to report the effect of various water pollutants on haematology of fishes. Thus, the altered haematological parameters are noticed due to the exposure of textile-mill effluents in the fresh water fish, *O. mossambicus* (Haniffa and Arulselvam, 1991), pesticides in *Channa punctatus* (Pandey *et al.*, 1970), Rainbow trout (Juelich, 1978), and of tannery effluent in *M. keletius* (Subramanian *et al.*, 1988).

Many fish physiologists have turned to the field of haematology because it has proven as a valuable diagnostic tool in evaluating human health (Clark *et al.*, 1979). In toxicological experiments the blood often shows pathological changes before external signs of toxicity and so, haematological studies in fish form a promising tool for investigating physiological changes caused by environmental pollution. Studies on the effect of pesticides in the haematology of fishes have been made by many workers (Dalela *et al.*, 1981; Shekar and Christy, 1996; Anandkumar *et al.*, 2001; Joshi *et al.*, 2002; Bhatia *et al.*, 2002; Johal and Grewal, 2004).

### 2.5.2 Histopathological effects of herbicide on fish liver and gills

Histopathological studies have been conducted to help establish causal relationship between contaminated exposure and various biological responses. It should also be given equal importance to bioassay and ethological studies, in determining the causes of death and predicting the mode of action of toxicants. Bioassays are important tools to evaluate toxic effects and impact of volatile organic compounds on freshwater fish and estuarine organisms (Hansen, 1978). The advantage of histopathology as a biomarker lies in its intermediate location with regard to the level of biological organization (Adams *et al.*, 1989). Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs (Johnson *et al.*, 1993). The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Sindermann, 1979; Bucke *et al.*, 1996). Gills (Mallatt, 1985; Poleksic and Mitrovic-Tutundzic, 1994), kidney (Oronsaye, 1989; Bucher and Hofer, 1993), liver (Hinton and Lauren, 1990; Myers *et al.*, 1993; ICES, 1997) and skin (Vethaak *et al.* 1994) are suitable organs for histological examination in order to determine the effect of pollution. Jiraungkoorskul *et al.* (2002) reported histopathological changes in the liver and gills of Nile tilapia, *Oreochromis niloticus*, exposed to glyphosate herbicide. In the gills, filamentous cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm were observed. In liver, there were vacuolations of hepatocytes and nuclear pyknosis. There are several investigations dealing with the negative impact of Round-up and glyphosate on aquatic organisms and fish, among others. For example histopathological studies on *Clarias gariepinus* showed that glyphosate in concentration

of 1.9 to 45 mg/L caused mononuclear infiltration, mononuclear degeneration, and spongiosis in the brain (Ayoola, 2008a). In other hand Round-up affected acetylcholine esterase activity in the brain and muscle of fish (Gluszczak *et al.*, 2011; Modesto and Martinez, 2010). Studies on alkaline phosphatase activity in liver and heart of carp showed that Roundup increases the activity of this enzyme at concentrations of 2.5 to 10 mg/L (Neškovic *et al.*, 1996). Also in gills from Round-up treated fish it was registered histological alterations such as epithelial hyperplasia and subepithelial edema (Neškovic *et al.*, 1996; Olurin *et al.*, 2006).

### 2.5.3 Effect on locomotion

Most non-sedentary aquatic organisms move from one place to another to obtain food, escape from danger, to reach spawning areas and to maintain positions against water current, however toxicants has far reaching effects by interfering with fish. The signs of toxicity of glyphosate in fish *Clarias gariepinus* include jumping, imbalance and death (Ayoola, 2008a). *H. fossilis* showed behavioural changes to dimethoate intoxicification, there was increased opercular ventilation movement, sluggish, lethargic and abnormal swimming. Auta *et al.* (2000) found out acute concentration of cypermethrin causes behavioural abnormalities which include irregular and erratic movement, air gulping, loss of balance, increased secretion of body mucous and eventually death. Rice *et al.* (1997) reported abnormal swimming response in juveniles of *O. latipes* which include hyperactivity with excessive lateral flexure in caudal region, hypoactive, under reactive to startle response exposed to cypermethrin.

#### 2.5.4 Effect on respiration

Fish exhibited several behavioural changes as a result of respiratory distress such as restlessness, rapid swimming, loss of balance and haemorrhaging of the gill filaments (Ogundele *et al.*, 2004). Most fish breath in the water in which they live, changes in the chemical properties of water due to pesticide toxication reflected in the animal's ventilatory activity, particularly affect respiratory gas exchange. A change in respiration rate is common physiological responses to toxicants. It is easily detectable through changes in oxygen consumption rate, which frequently used to evaluate the changes in metabolism under environment deterioration. Many authors investigated that pesticide toxicity induced respiratory distress in fishes. David (2003) showed disturbances in oxidative metabolism in *Tilapia mossambica* under cypermethrin toxicity. Nataranjan (1981) showed reduction in oxygen consumption in *Channa punctatus* exposed to organophosphate pesticide. Boradbury *et al.*, (1986) stated that the greater decrease in the rate of oxygen consumption in the fish *Cirrhinus mrigala* may be due to internal action of pesticide, as toxicant altering the metabolic cycle at subcellular level. Nagarathanamma and Ramamurty (1982) showed variation in the oxygen consumption of *Cyprinus carpio* under methyl parathion stress. Khillare and Wagh (1987) observed reduced rate of oxygen consumption in the fish *Barbus stigma* when exposed to malathion and nuvan. Ali (1982) studied effect of pesticide, demecron on fresh water fish, *Channa punctatus* and reported that respiration rate reduced in the fish. Lomte and Jadhav (1982) reported that decrease in oxygen consumption after pesticidal stress and observed in *Corbicula regularis*. Verma and Dalela (1975) showed that reduction in oxygen consumption of fish might be due to suspended solids present in the effluents which cause mechanical injuries

to fish and disturb the osmotic regulation. Malla Reddy (1988) showed effect of fenvalerate and cypermethrin on the oxygen consumption of fish, *Cyprinus carpio* and reported significant drop in rate of oxygen consumption. Janardhan Rao (1991) studied oxygen consumption rate of *Macronus salki* exposed to endosulfan. He reported that oxygen consumption rates increased at 24 hours and decreased gradually at 48, 72 and 96 hours. Several authors reported that the disturbance in oxidative metabolism leads alteration in whole animal oxygen consumption in different species of fish exposed to pesticides (Holden, 1962; Wilkinson and Dension, 1982; David, 2003; Vatukuru, 2005; Vinnatkumar 2008; Anneta Susan, 2010).

#### 2.5.5 Effect on growth of fish

Decline in growth of *Cyprinus carpio* exposed to sub-lethal concentration dipterex, fatty acid degradation in the liver, oedema of secondary gill lamella and respiratory impairment of *Oreochromis niloticus* have been reported by several workers (Chindah *et al.*, 2004; Omoregie and Ufodike, 1991). Omoregie and Ufodike (1995) observed lower weight gain in *O. niloticus* exposed to various concentrations of the water soluble fractions of crude oil. In sub-lethal concentration of cymbush, growth parameters such as specific growth rate, food conversion efficiency and protein efficiency ratio decreased as the concentration of cymbush increased to *C. gariepinus* (Aguigwo, 2002).

## **2.6 Antioxidant Defenses against Toxicity of Xenobiotics and Oxidative Stress by Free Radicals**

Often times, xenobiotics entering into the environment exert their effects through their ability to redox cycle (one electron reduction and oxidation reactions). This may be intended in order to gain maximum effect for the xenobiotics purposes or may just be a

byproduct of their chemical structure. Negative side effects include the formation of reactive oxygen species with the ability to damage cellular molecules. These polluting compounds will often retain these qualities in the aquatic environment as well, with the ability to cause oxidative stress in organisms. The physiological systems of many aquatic organisms, which are in place to metabolise these compounds and resulting by products, are evolutionarily similar to those in humans.

### 2.6.1 Biomarkers in aquatic environment

Biomarkers can be defined as a necessary changes of cellular or biochemical compounds, structures or functions caused by xenobiotics, namely after exposure to environmental contaminants. Biological response can occur on molecular, cellular, tissue or organ level and can be measurable in biological systems such as tissues, cells and biological fluids (Kimmenade and Januzzi, 2012). These changes are related to the exposure or to the effects of toxicants and have been successfully applied to monitor the presence and the effects of contaminants in various toxicological and ecotoxicological studies (Adonis *et al.*, 2003; Almroth *et al.*, 2005; Risom *et al.*, 2005). Monitoring the parameters of an initial change caused by the interaction of organism and xenobiotic compound can characterize the level of exposure or toxic effect (Smith and Warner, 1992). Biomarkers can provide information on the health of organisms, and can be used as early warning signals for general or particular stress (Korte *et al.*, 2000). High sensitivity of biomarkers enables their applications for detection of the early changes in pathogenesis or physiological adaptation mechanisms. Moreover, molecular and biochemical markers of biological response to chemical compounds can be used as diagnostic or prognostic tools for assessing the effects of pollutants in the environment (Saint-Denis *et al.*, 1999).

The exposure and effects of chemicals in living organisms can be studied by biomarkers, specific changes (responses) in biological parameters reflecting specific toxicity mechanism such as overproduction of ROS and oxidative stress (Boelsterli, 2003). Several oxidative stress biomarkers were validated, and they included: (i) direct assessment of ROS overproduction after exposure to tested chemicals (due to short lifetime of ROS, the approach is suitable mostly for in vitro studies (Li *et al.*, 2003), (ii) assessment of oxidation products of biological molecules as measurement of lipid peroxidation product malondialdehyde, MDA, (iii) determination of changes in antioxidant mechanisms and detoxification parameters such as concentrations of glutathione (GSH) – critical cellular antioxidant and conjugation substrate of detoxification enzymes. Monitoring of the biomarkers in living organisms including fish is a validated approach and serves as early warning of adverse changes and damage resulting from chemical exposure (Van der Oost *et al.*, 2003).

Proteins are considered to be important targets of free radical attack in cells (Eustace and Jay 2004; Almroth *et al.*, 2008b; Lushchak, 2011b) and thus compromise antioxidant defense, cellular function, and survival (Padmini, 2010). Therefore, protein oxidation, often under investigation in proteomic studies, has been recently proposed as a biomarker of oxidative stress (Sheehan, 2006; Lushchak, 2011a). In flounders, living in contaminated waters with xenobiotics, increased levels of oxidised proteins were reported (Fessard and Livingstone, 1998). Studies on dynamics showed that proteins can be oxidized before lipids or DNA in ROS exposed cells (Du and Gebicki, 2004). At the same time, many other factors can influence cell cycle and correspondingly, injury of proteins, related particularly to their oxidative damage. In any case, protein carbonyls

(PC), so successfully explored in the studies of model oxidative stress in short-term laboratory experiments (Parvez and Raisuddin, 2005; Kubrak *et al.*, 2010; Lushchak, 2011b), are very seldomly used in the field studies for the assessment of environmental effects on fish. In the set of studies devoted to the consequences of a dredging campaign in Göteborg harbor, Sweden, to fish on the example of eelpout (*Z.viviparous*), as a sentinel species, monitor the impact of these events, the formation of additional carbonyl groups in proteins was studied (Almroth *et al.*, 2005).

#### 2.6.2 Overview on antioxidant defences

The removal of xenobiotics, and even some endogenous substances, from the cell is catalyzed by a number of different enzymes, so called phase I and II enzymes. Phase I enzymes are involved in xenobiotic biotransformation via the introduction of a polar moiety which renders a lipophilic contaminant more hydrophilic. The cytochrome P450 (CYP) family is the most well studied in fish, especially CYP1A. CYP1A serves to increase the solubility of hydrophobic molecules through a reduction reaction involving an oxygen molecule. It is inducible by a number of xenobiotics, for example PAHs and planar PCBs, via binding to the aryl hydrocarbon receptor (AhR) which acts as a transcription factor. Phase II enzymes are involved in conjugating metabolised xenobiotics to endogenous molecules, thereby easing excretion. Examples of phase II enzymes commonly used in biomonitoring programs involving fish include glutathione S-transferase (GST) and UDP glucuronyl transferase (UDPGT) (Halliwell and Gutteridge, 1999). The activity of phase I enzymes can lead to an increase in ROS production or the generation of reactive, redox cycling intermediates. Antioxidant enzymes facilitate the

removal of these reactive chemical intermediates and resulting ROS molecules. The action of CYP1A can result in the production of  $O_2^{\bullet-}$  which in turn can be metabolized by superoxide dismutase (SOD) to  $H_2O_2$ . This hydrogen peroxide molecule can then be reduced to  $H_2O$  and  $O_2$  by catalase (CAT).

Hydroxyl radicals ( $OH^{\bullet}$  ions) can form from both  $H_2O_2$  and  $O_2^{\bullet-}$  via reactions with redox cycling metal ions, for example iron and copper. This highly potent hydroxyl radical can attack both protein and lipid molecules to form oxidative damage products. Glutathione peroxidase (GPx) can reduce lipid peroxides to their respective alcohols and water. DT diaphorase (DTD) can react directly with redox cycling xenobiotics, i.e. quinones, thereby reducing them in a two electron reaction which stops the redox cycle and facilitates removal (Dinkova-Kostova and Talalay, 2000).

## **2.7 Growth Studies**

### **2.7.1 Growth and nutritional performance of fish in a toxic environment**

Decline in growth of *Cyprinus carpio* exposed to sub-lethal concentrations of dipterex, affects fatty degradation in the liver, oedema of secondary gill lamella and respiratory impairment of *Oreochromis niloticus* (Chinabut *et al.*, 1978; Omeregie and Ufodike, 1991). Contamination of aquatic environment by pesticides may cause several physiological behaviours, respiration, nutrition and reproductive functions leading to fish kills and low fish productivity (Babatunde, 1997).

The rate of feeding in “Heteroclaris” reduced considerably with increasing concentrations of pesticides; thiodon, malathon and carbaryl (Avoaja and Oti, 1997). Ponmani *et al.* (1997) observed a significant reduction in feeding rates, consumption

absorption, metabolism and growth as well as conversion efficiencies of *Cyprinus carpio* exposed to sub-lethal concentration of monocrotophos. Pal and Konar (1987) reported that methyl parathion inhibited the appetite of fish through inhibition of various digestive enzymes. A reduced growth rate may also be attributed to an increased activity associated with attempts to avoid the contaminated water, or an increased expenditure of energy on chemical detoxification and tissue repair.

In sub-lethal tests, growth parameters such as specific growth rate, food conversion efficiency and protein efficiency ratio conversion decreased as the concentration of cymbush pesticides increase. Growth effects in rohu were accompanied by impaired fuel conversion efficiency for lipids, proteins and carbohydrates (Ramaneswari and Rao, 2002). The toxicity of aquatic contaminants depends considerably on the route by which the animal is exposed. For example, the toxicity of the endocrine distributing chemical 4-tert-nonylphenol to fat head minnows was 10 fold greater when exposure occurred by water borne pathway than via the diet (Pickford *et al.*, 2003). Fishes are noted to increase their metabolic activities for the excretion of toxicants, hence, making more energy available for homeostatic maintenance than storage, which could be used for growth (Gbem *et al.*, 2003). Coimbra *et al.* (2005) showed impaired plasma levels of thyroxine and inactivated thyronine, associated with decrease hepatic deiodinase enzyme activity in diet exposed tilapia, suggesting disrupted thyroid metabolism. Salmonids are significantly less sensitive to endosulfan through the dietary route than via water. Dietary levels of 500µ/kg resulted in only minor sub-lethal effects, such as reduction in condition factor and transient changes in haematological parameters (Dietrich *et al.*, 2006). Prolonged exposure of *Oreochromis niloticus* in water induces a variety of abnormalities

in the feeding, behavior, food utilization, and growth. The authors reported a significant reduction in the following growth parameters; specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (APP NPU), and productive protein value (PPV) when compared with control (Auta and Ugueji, 2006). Shaw and Handy (2006) reported a reduction in growth associated with reduced food intake after feeding Nile tilapia with 2000 CU mg/kg food. The effects of sub-lethal doses of dimethoate (20, 10 and 5mg/L) and malathion (2.0, 1.0 and 0.5mg/L) on growth parameters of Nile tilapia (12.0cm and 40.0g weight) was investigated by Sweilum (2006). The author reported that sub-lethal toxicity of the pesticide decreased plankton abundance and water quality in fish ponds. In comparison with controls, sub-lethal levels of these pesticides led to a significant decrease in final body weight, specific growth rate and normalized biomass index of fish. The survival rate of Nile tilapia decreased with increasing concentrations of pesticides. Feed utilization (total food consumed, feed conversion ratio, and protein efficiency ratio) varied with pesticide treatments. The author also opined that total production, net returns and profitability of reared fish decreased with increasing concentrations of pesticides. *Clarias gariepinus* fed excessive dietary copper (Cu) *in vivo* showed only marginal reduction in body weight during dietary copper exposure, but overall, the exposure caused a significant decrease in specific growth rate (SGR) in the copper fed fish compared to the control over 30 days period. The author further opined that poor absorption of the major nutrients were likely given that FCR, condition factor, proximate composition and intestinal morphology were similar in both control and treated groups (Hoyle *et al.*, 2007).

Ogueji and Auta (2008) investigated biochemical parameters of *Clarias gariepinus* due exposure of the fish to sub-lethal concentrations of chlorpyrifos ethyl showed that in the exposed group, toxicant elicited highly significant dose dependent inhibition protein, cholesterol, triglyceride, GOT, ALP and highly significant dose dependent increases in glucose and GTP. The authors opines that increase in glucose (hyperglycaemia) which was dose and duration dependent in the sub-lethal exposure to both chemicals may be considered to be manifestation of stress induced by toxicants. The toxicants and their metabolites may have stimulated the adrenal tissue, resulting in increased level of circulatory catecholamine and glucocorticoids. These in turn stimulated the release of amino acids, glycerol and fatty acids, present in the blood and increased the synthesis enzymes in the liver, which converted amino acids and glycerol into glucose (gluconeogenesis) (Ogueji and Auta, 2008).

Shallangwa and Auta (2008) reported a reduction in growth of *Clarias gariepinus* exposed to sub-lethal effects of 2, 4-Dichlorophenoxy – acetic acid. The authors attributed this to lower feeding rate and or the toxicant made the feed unsuitable for consumption. They further opined that it could be due to an increased expenditure of energy on chemical detoxification and tissue repair.

## **2.8 Water Quality Parameter**

Fish and other aquatic organisms such as shrimps and crayfish are known to be very rich in protein and the need to cultivate these in clean water in the locality is highly needed. The need for clean water to raise this protein rich and most needed aquatic commodity cannot be over emphasized. The productivity of a given body of water is determined by

its physical, chemical and biological properties. The environmental properties of water need to be conducive for fish to grow well; therefore an ideal water condition is a necessity for the growth and survival of fish. The population density of organisms of any water system such as inland freshwater and lakes always vary according to the physico-chemical factors such as hydrogen-ion concentration (pH), dissolved oxygen (DO), conductivity, nutrients and temperature (Abolude, 2007).

### 2.8.1 Temperature (T°C)

Water temperature is one of the major environmental factors that affect and control food utilization at all levels and stages of fish growth. The suggested temperature ranges 20°C to 30°C while the lethal levels are from 2°C to 42°C. Fishes are poikilothermic and water plays an important role in their feeding as it affects their metabolic activities, feeding potential, growth, reproduction in all species of fishes (Dupree and Hunner, 1993) and efficiency of food conversion (Martinez-placious *et al.*, 1993). Temperature has a pronounced effect on the rate of chemical and biological processes in water; for instance, fish require twice as much as oxygen at 30°C than 20°C (Adeniji and Ovie, 1990). It is recommended that fish in the tropics be kept in water whose temperature range is between 25°C and 30°C (Auta, 1993). Sudden increase in temperature will stress or kill fish and this has formed the basis for the acclimation of fish (Adeniji and Ovie, 1990). Temperature has been found to affect the dominance and distribution of phytoplankton in water as it influences the growth rate and mortality of zooplankton and other organisms (Orchutt and Porter, 1983). Temperature is known to influence other factors such as Dissolved Oxygen and may affect organisms to varying degrees, depending on their

sensitivity; thus fish survival in lakes depend on temperature and dissolved oxygen (Countant, 1987).

### 2.8.2 Dissolved oxygen

Dissolved oxygen in water is very essential to life in the aquatic environment as it affects the physiology and distribution of the aquatic organisms. Nearly all aquatic organisms, with the exception of some bacteria must have oxygen to survive and most of these organisms must extract their oxygen from liquid water. The two main sources of oxygen into the aquatic environment are the atmosphere and photosynthetic activities of aquatic plants. The ideal range of dissolved oxygen in the water must be at least 5mg/l, is required to sustain fish and other aquatic life in water bodies (Adakole, 2000). Insufficient dissolved oxygen (DO) in a water system tend to cause anaerobic decomposition of organic material in water thereby leading to the production of obnoxious gases such as carbon dioxide, hydrogen sulphide and methane which bubbles to the surface. The physiology of aquatic organisms is such that they can tolerate only narrow ranges of temperatures, outside which they cannot function normally (Willoughby, 1976).

Kutty (1968) and Sanders (1973) reported that Atlantic salmon stopped swimming when dissolved oxygen concentration remained below 5ppm but goldfish, tilapia and carp swim at oxygen level of 1-2ppm. Inadequate dissolved oxygen has many effects on fish like reduced feeding; impaired growth and leading to fish becoming stressed thereby becoming more susceptible to disease. Cold water fish requires large amounts of

dissolved oxygen with temperature ranged of 5°C-15°C while, warm water fish with a temperature range of 20°C-40°C are able to survive with low oxygen content.

### 2.8.3 Hydrogen-ion concentration (pH)

The hydrogen-ion concentration (pH) of any water is the measurement of the acidity or alkalinity of that water body. It is usually measured on a scale of 0-14 with 7 being neutral. The effects of pH on the chemical, biological and physical properties of a water system, makes its study very crucial to the lives of the organisms in the medium. Freshwater with a pH ranging from 6.5-9.0 have been known to be productive and recommended as suitable for fish culture (Adeniji, 1986; Auta, 1993). Increase in acidity and alkalinity of any water body may increase or decrease the toxicity of poison in that water; solar radiation and temperature accelerates photosynthesis, which in turn increase carbon dioxide absorption altering the Bicarbonate equilibrium and producing OH thus raising the pH (Branco and Senna, 1996).

Hynes (1974) observed that pH values of below 5 or above 9 are harmful to most animals. pH has more range, according to Wahramann and Woker (1982) and Krenkel (1974), pH has more influence on some poison. Chronic pH levels may reduce fish reproduction and are associated with fish die-offs (Stone and Thomforde, 2006). Adeniji and Ovie (1990) reported that acid and alkaline deaths points are approximately at pH 4 and 11 respectively.

#### 2.8.4 Total dissolved solids (TDS)

Total dissolved solids (TDS), refers to the inorganic substances that are dissolved in water while dissolved solids are finely divided materials in suspension (organic or inorganic) particles that tend to screen out light from the bottom of a water body (Abolude, 2007). These substances include carbonate, bicarbonate, chloride, sulphate, nitrate, calcium, sodium etc. When some of these substances are in suspension, they cause turbidity in the water, reducing photosynthesis, decreasing the amount of dissolved oxygen and affect the feeding habits of the organisms that depend on sight to catch its prey. A certain level of these ions in water is necessary for aquatic life (Stone and Thomforde, 2006). TDS are parameters used in measuring the fitness factor and as a general measure of edaphic relationship that contributes to productivity within a water body (Ryder, 1965; Welcomme, 1979).

Changes in TDS concentration can be harmful to the animal due to the density of the flow of water in and out of the organism's cells. In fish, concentration too high or low, may affect the growth of fish and death may result. Reduction in water clarity can contribute to a decrease in photosynthetic activity combined with toxic compounds and heavy metals; may lead to an increase in water temperature (Murphy *et al.*, 2005).

#### 2.8.5 Electrical conductivity

Electrical conductivity is the ability of a water body to receive and conduct electrical current in correlation to its salt content. It is an indicator of the type and number of ions present or dissolved in water or in solution, which are almost proportional to the amount of dissolved matter. Freshwater fish thrive over a wide range of electrical conductivity.

The desirable range is 100-2,000 $\mu$ Si/cm and the acceptable range is 30-5,000 $\mu$ Si/cm (Stone and Thomforde, 2006). Lind (1979) reported that estimation of total ion in matter in a solution or water bodies is related to its fertility while, Ryder *et al.* (1974), reported that electrical conductivity and mean depth of a reservoir could be used to calculate the potential fish yield of a lake. High conductivity is an indication of the presence of large amount of dissolved salts, while at low level major ions may be determined by the nature of the fauna (Moss, 1993).

#### 2.8.6 Total alkalinity

The alkalinity of water is a measure of its capacity to neutralise acids. The alkalinity of natural waters is due to the salts of carbonates, bicarbonates, borates, silicates and phosphates along with the hydroxyl ions in Free State. However, the major portion of the alkalinity in natural waters is caused by hydroxide, carbonate and bicarbonate, which may be ranked in order of their association with high pH values. Alkalinity values provide guidance in applying proper doses of chemicals in wastewater treatment processes, particularly in coagulation, softening and operational control of anaerobic digestion. Alkalinity is expressed as mg/L.

#### 2.8.7 Total hardness

Water hardness is the traditional measure of the capacity of water to react with soap, hard water requiring a considerable amount of soap to lather. Hardness is generally caused by the calcium and magnesium ions (bivalent cations) present in water. Polyvalent ions of some other metals like strontium, iron, aluminium, zinc and manganese, etc. are also capable of precipitating the soap thus contributing to hardness. However, the

concentration of these ions is very low in natural waters; therefore hardness is generally measured as concentration of only calcium and magnesium, which are far higher in quantities over other hardness producing ions. Hardness is measured in terms of mg/L as CaCO<sub>3</sub> using standard methods involving reagents.

## **2.9 Heteroclarias (hybrid)**

Heteroclarias is a species of the catfish family with remarkable fast growth rate (Ending and Kamstra, 2001). The genus Heteroclarias belongs to the family clariidae. It is highly esteemed in Nigeria and commands very high commercial value in our markets due to its ability to adapt readily to pond condition, fast growth rate, acceptability of artificial feed, high conservation of artificial feeds, tolerance to crowded condition and high quality of its flesh. Heteroclarias belongs to the Kingdom- Animalia, Phylum- Chordata, Subphylum- Vertebrata, Class- Actinopterygii, Order- Siliriformes, Family- Clariidae and Genus- Heteroclarias.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Collection and Acclimation of Experimental Fish

Fingerlings of *Heteroclarias* of mixed sexes and fairly uniform size ( $2.2\pm 0.7$ g weight and  $6.7\pm 0.7$ cm standard length) were obtained from National Open University Nigeria (NOUN) Fisheries Unit Kaduna-Zaria express road, Kaduna and transported in plastic container to the laboratory in the Department of Biological Sciences, Ahmadu Bello University Zaria. They were acclimatized for two weeks in four oval/rectangular shaped bath tubs, separately containing water of about 150L. The fish were been fed twice daily on at 35% crude protein diet. The tubs were cleaned daily and the water was changed every 24 hours. Glyphosate (Round-up) was purchased from local market of Samaru, Zaria, Kaduna State.

#### 3.2 Description of Experimental Fish

*Heteroclarias* is a hybrid of the African catfish *Clarias gariepinus* and *Heterobranchus bidorsalis*. They are omnivores which are desirable as food valuable species world-wide. They are one of the commercially important species of fish for rapid aquaculture expansion in Nigeria and elsewhere in the developing World. Thus, the objective of this study is to investigate the acute, sub-lethal toxic effects of glyphosate on *Heteroclarias* with emphasis in the haematology, histopathology and oxidative stress in the gill and liver.

### **3.3 Preparation of Concentrations used for the Bioassay Tests**

The stock solution of the Round-up contain 360g/L of glyphosate and the definitive concentration for the bioassay were 0.3, 0.4, 0.5, 0.6 and 0.7ml/L measured with syringe which were converted to 5.40, 7.20, 9.00, 10.80 and 12.60mg/L respectively using dilution formular ( $C_1V_1=C_2V_2$ ). About 20 litres of dechlorinated water was poured into each glass tanks while 5.40, 7.20, 9.00, 10.80 and 12.60mg/L of water were removed and replaced with glyphosate from the stock solution in each of the respective glass tanks to topped it to 20 litres mark. This was done same for sub-lethal bioassay.

### **3.4 Bioassay Test**

#### **3.4.1 Range finding test**

Pilot studies were carried out to determine the definitive concentration range for testing Round-up following the methods of Solbe (1995). This was done by introducing three nominal concentrations into three separate test tanks (using pipette) containing 20 litres of dechlorinated water in triplicate. Five fish per concentration of toxicant was used with 3 replicates each for 96 hours. When the fish died in all the test tanks, lower range of concentrations of the toxicants were prepared until when 80 to 90% of fish died in the highest concentration test tank and 20 to 30% of fish died in the lowest concentration test tank. The five nominal concentrations were then range between the highest and the lowest concentrations geometrically (5.40, 7.20, 9.00, 10.80 and 12.60mg/l).

#### **3.4.2 Acute toxicity test**

The methods of acute toxicity tests as described by Sprague (1973) and APHA (1995) was employed. The range of concentrations of glyphosate (5.40, 7.20, 9.00, 10.80 and

12.60mg/L) obtained in the pilot tests were dispensed with a pipette into 20 litres of each test tank in duplicate. Ten fingerlings fish were exposed to five different concentrations of the toxicant in each test glass tank in duplicate and the control.

Fingerlings of fairly equal weight ( $2.2 \pm 0.7\text{g}$ ), total length ( $6.7 \pm 0.7\text{cm}$ ) and standard length ( $5.9 \pm 0.6\text{cm}$ ) was selected randomly, weighed and distributed into 10 glass aquaria containing definitive concentration of the glyphosate and 2 controls with only distilled water without glyphosate. The bioassay test was carried out in 12 glass tanks each of size  $30.5 \times 30.5 \times 92.5\text{cm}$  into which approximate quantity of glyphosate were taken and to give a final volume of 20.0L. The fish were starved for 24 hours before commencement of the experiment. The solutions was stirred for homogenous mixing before each aquarium were randomly stocked in duplicates with 10 fingerlings of fish while the test solution and control were renewed daily.

### **3.5 Behavioural Studies**

After exposure of the fish to various concentrations of the toxicant, observations were carried out on the behavioural and morphological responses of the fish at 12, 24, 48, 72 and 96 hours (Drummond *et al.*, 1986). Control fish were also monitored along with the toxicant concentrations to provide a reference for assessing any behavioural or morphological changes. Responses were recorded if they differed from the control and occurred in 10% of the fish in each test tank. The behavioural and morphological characteristics that were monitored are erratic swimming, loss of equilibrium, general activity, increased excitability, mortality, vertical suspension, mucous secretion, startle response, deformities and haemorrhage. Each test tank was observed for 10 to 15 minutes

which allowed sufficient time for an accurate evaluation of each fish. Startle responses were monitored by the following procedures sequentially: passing hand over the test tank (overhead moving visual stimulus), rapping on the tank (vibration stimulus), and lightly touching the fish with a wooden applicator stick (tactile stimulus).

### 3.5.1 Opercular ventilation/tail fin movement rates

The investigation of opercular ventilation count and tail fin movement rate was carried out for 96 hours which were counted using stop watch at 12, 24, 48, 72 and 96 hours per minutes. Three fish were used for the counting per tank and the average.

### 3.5.2 Mortality rate of LC<sub>50</sub> and threshold

Observations to determine the mortality rate of *Heteroclaris* were made at 12, 24, 48, 72 and 96 hours. Fingerlings of *Heteroclaris* were considered dead when there was no sign of opercular movement or no response to gentle prodding. The number of dead fish in each group was recorded against the time of their death in a tabular form (Sprague, 1973). The dead fish were removed immediately to avoid fouling and buried.

## **3.6 Sub-lethal Test**

From the results that were obtained from the acute toxicity, sub-lethal concentration of one-fifth, one-tenth, one-twentieth of 96 hours LC<sub>50</sub> was used to determine sub-lethal concentration range following Oladimeji and Ologunmeta (1987) and Mohammed (1995). This experiment lasted for 8 weeks. Twelve glass aquaria were used with three replicates per treatment. Ten groups of fish were exposed to 3 sub-lethal concentration of glyphosate. As at the time of exposure, fresh solution was added in every 48 hours to maintain the concentration level before the wastes was siphoned out using rubber tube.

The fish were fed on pelleted feeds containing 35% crude protein. Two fish per each replicate was sacrificed biweekly to isolate gill and liver which were stained using haematoxylin and Eosin for pathological studies.

### **3.7 Haematological Test**

#### **3.7.1 Blood sampling**

The blood was sampled as described by Blaxhall and Diasely (1973) and was collected by severance of caudal peduncle from the caudal artery at 2cm away from the caudal peduncle. This process was done on the surviving fish tanks.

##### *3.7.1.1 Total erythrocyte count*

Hendricks solution was used for the erythrocyte count. Blood was drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool and adjusted the volume to exactly 0.5 mark. The pipette was filled to 101 mark with the diluting fluid and shaken for 30 minutes to ensure thorough mixing. The diluted suspension of cells was thereafter drawn in to the Neubauer's chamber haemocytometer. The haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer (Hesser, 1960) were counted with 4mm objectives and X 40 eyepiece of the microscope. The number of cells was multiplied by X10 and this gave the total number of cells per cubic millimeter ( $\text{mm}^3$ ) of blood (Hesser, 1960).

##### *3.7.1.2 Total leucocytes count*

Leucocytes were counted by using Shaw's solution A (neutral red (25mg), sodium chloride (0.9g), distilled water (100mls) and B (crystal violet (12mg), sodium citrate (3.8g), distilled water (100mls)). The blood was drawn up to the 0.5 mark on the stem of

a white cell pipette. Solution A was drawn to shake the bulb of the pipette half way and then filled to 101 mark with solution B. A few drops were dispensed in to the haemocytometer. The cells in the four large squares of the chamber (Hesser, 1960) were counted with 4mm objective and X40 eyepiece microscope. The number of cells was multiplied by 500 to obtain the total number of leucocytes per cubic millimeter ( $\text{mm}^3$ ) of blood (Hesser, 1960).

#### *3.7.1.3 Haematocrit (Packed Cell Volume)*

Determination of packed cells volume (PCV) was carried out by micro-westegren method as described by Blaxhall and Daisely (1973). The well mixed sampled blood from the severed caudal penducle was drawn in to micro-haematocrit tube (75mm long and 1.1-1.2mm internal diameter). The tubes were then centrifuged for five minutes. The reading was taken with the aid of a micro-haematocrit reader and expressed as the volume of the erythrocytes per  $100\text{cm}^3$ .

#### *3.7.1.4 Leucocytes differential count*

Two drops of blood was placed on a slide, made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol, then stained with giemsa's stain and 170 buffered distilled water. It was allowed to stand for about 20-30 minutes after which the slide was rinsed again with buffered distilled water and allowed to air dried. Counting was made by the use of microscope and the parameters counted include neutrophils, lymphocytes, basophils, eosinophils, monocytes. This was applicable for both acute and sub-lethal bioassay.

### 3.7.1.5 Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration

The 'absolute values' made up of Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated from the results obtained for RBCC, haemoglobin and PCV/Ht (Dacie and Lewis, 1968) as:

$$\text{MCV } (\mu\text{m}^3) = \frac{\text{Ht\%} \times 10}{\text{RBCC (cells mm}^3)} \quad (3.1)$$

$$\text{MCH (Pg cell)} = \frac{\text{Hb (g/100ml)} \times 10}{\text{RBCC (cells mm}^3)} \quad (3.2)$$

$$\text{MCHC (g/100ml)} = \frac{\text{Hb (g/100ml)} \times 100}{\text{Ht\%}} \quad (3.3)$$

### 3.8 Methods for the Preparation of the Slides of Examined Tissues

Two fish per replicate were carefully dissected to isolate gill and liver and fix in formal saline solution (Thompson and Hunt 1966; Alan *et al.*, 1983). The tissues were washed in running water to remove traces of formalin, followed by dehydration using successive percentages of graded alcohol (30, 50, 70, 90 and 100%). This was cleared through gradually increasing the concentration of chloro-alcohol (50, 75 and 100%) and then embedded in paraffin wax at 58<sup>0</sup>C to 60<sup>0</sup>C melting point. Sections were cut at 5 $\mu$ m thickness and were produced by using Bright rotary microtome.

Harris's haematoxylin and acetic eosin was used as general stain at the Department of Anatomy, Ahmadu Bello University (ABU), Zaria. All the slides were examined under a light microscope (NILCON TE 3000) and photomicrographs were taken at X10, X20, or X40 with a digital camera at its highest resolution. Photomicrographs of the various

sections showing the effects of toxicants on the cell structure and morphology of the organ were made at X400 magnification. These were compared with tissues obtained from fish of the control experiment. This process was done on surviving fish tanks.

### **3.9 Determination of Water Quality Parameter**

Water quality parameters were carried out in the Hydrobiology/Fisheries Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The water temperature, dissolved oxygen (DO), hydrogen ion concentration (pH), conductivity, and total dissolved solids were monitored throughout the duration of the experiment.

#### **3.9.1 Determination of pH, temperature, conductivity and total dissolved solids**

pH, temperature, conductivity and total dissolved solids were measured with Hanna instrument (Model- HI 98129. HI 98130).

*pH and Temperature measurement:* The pH mode on the instrument was selected with the set/hold button. The electrode was submerged in the sample water for 60 seconds. The measurement was then taken when the stability symbol on the top left of the liquid crystal display (LCD) disappeared. The pH value automatically compensated for temperature was shown on the primary LCD while the secondary LCD shows the temperature of the sample.

*Conductivity/Total Dissolved Solids measurement:* The Electrical Conductivity (EC) or TDS mode was selected with Set/hold button. The probe was submerged in the sample. The measurement was taken when the stability symbol on the top left of the liquid crystal display (LCD) disappeared. The electrical conductivity (EC) or total dissolved solid

(TDS) value automatically compensated for temperature was shown on the primary LCD while the secondary LCD shows the temperature of the sample.

#### *3.9.1.1 Dissolved oxygen (DO)*

The DO was determined by modified Winkler Azide method, Lind (1979). Water samples were collected in 1litre glass bottles (Mayer flask). They were fixed with 2ml manganese sulphate ( $MnSO_4$ ) solution using a graduated syringe and inserted its tip below the water surface in the bottle; 2ml alkaline iodide azide reagent was added next in a similar manner as the first reagent followed by the addition of 2ml concentrated sulphuric acid ( $H_2SO_4$ ). 100ml of the fixed sample was poured into a conical flask with addition of 2 drops of starch solution and titrated with 0.0125N sodium thiosulphate ( $Na_2SO_3$ ) until it turns colorless as the end-point. The reading was then taken and recorded in mg/L.

#### *3.9.1.2 Total alkalinity*

About 100ml of the water sample was poured into a conical flask after which 2 drops of methyl red solution and 2 drops of Bromo Creasol green were added, a faint greenish color was observed then Shake well. This was then titrated against 0.02N standard sulphuric acid solution until a pink colour was observed which served as the end point APHA (1989).

$$\text{Total alkalinity} = \text{mg/L} = \text{ml of titrant} \times 10. \quad (3.4)$$

### 3.9.1.3 Total hardness

About 25ml of the water sample and 25ml of distilled water was poured into a beaker after which 2ml of buffer solution of pH 10.4 was added and chips of Errochrome black T dye were added, this was then titrated against ethylenediamine tetracetic acid (EDTA) titrant(0.01M) until it changed to blue. The titrant value was multiplied by 40, as  $\text{CaCO}_3/\text{L}$  APHA (1989).

### 3.9.2 Antioxidants enzyme determination

The enzymic antioxidants that were analysed are catalase and peroxidase

#### 3.9.2.1 Assay of catalase

Catalase activity was determined by the method of Luck (1974).

#### Principle

The UV light absorption of hydrogen peroxide can be easily measured between the range of 230-250 nm. On decomposition of hydrogen peroxide by Catalase, the absorption decreases with time. The enzyme activity can be estimated by this decrease in absorption.

#### Reagents

1. Phosphate buffer 0.067 M (pH 7.0)
2. Hydrogen peroxide in phosphate buffer (2mM)

#### Procedure

The tissue homogenate employed for the assay was prepared in phosphate buffer (0.067 M, pH 7.0). The samples were read against a control without homogenate, but containing the  $\text{H}_2\text{O}_2$  phosphate buffer (2mM). To the experimental cuvette, 2.9 ml of  $\text{H}_2\text{O}_2$  phosphate buffer was added, followed by the rapid addition of 0.1 ml enzyme extract and mixed thoroughly. The samples were read against a control containing 3ml  $\text{H}_2\text{O}_2$

phosphate buffer without enzyme extract. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme solution containing H<sub>2</sub>O<sub>2</sub> free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

The Catalase activity was calculated as follows:

$$\text{Catalase activity (units/ml enzyme)} = (3.45) / (\text{Min})(0.1) \quad (3.5)$$

Where,

3.45 correspond to the decomposition of 3.45 micromoles of hydrogen peroxide in a 2.0 ml of reaction mixture.

0.1= Volume of enzyme used (in milliliters)

### 3.9.2.2 Assay of peroxidase

The activity of peroxidase was measured by the method of Reddy *et al.* (1995).

#### Principle

Peroxidase catalyses the conversion of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> in the presence of the hydrogen donor pyrogallol. The oxidation of pyrogallol to a coloured product called purpurogalli can be measured spectrophotometrically at 430 nm with the specified time interval. The intensity of the product is proportional to the activity of the enzyme

#### Reagents

1. Pyrogallol (0.05M in 0.1M phosphate buffer, pH 6.5)
2. H<sub>2</sub>O<sub>2</sub> (1% in 0.1M phosphate buffer, pH 6.5)

#### Procedure

The tissue samples used for the assay were prepared as homogenate in 0.1M phosphate buffer (pH 6.5). 2.9 ml of pyrogallol solution (0.05 M in 0.1 M phosphate buffer, pH 6.5)

and 0.1 ml enzyme extract was placed in a cuvette using a pipette. The spectrophotometer was adjusted to read zero at 430 nm followed by the addition of 0.5 ml of H<sub>2</sub>O<sub>2</sub> (1% in 0.1M phosphate buffer, pH 6.5) after which it was mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes. One unit of peroxidase activity was then defined as the change in absorbance per minute at 430 nm.

$$\text{Peroxidase activity (units/mg)} = \frac{\Delta A_{510}/\text{mins}}{6.58 \times \text{ml enzyme/ml rxn mixture}} \quad (3.6)$$

Where,

A = Absorbance

### 3.9.3 Growth parameters

Fingerlings of *Heteroclaris* were acclimatized in tanks for two weeks, during which they were fed with commercial feeds. The fish were fed at 3% body weight because feeding was observed to be reduced. The average weight of the fish at commencement of the experiment was taken, and same at the end of the experiment (Shalaby *et al.*, 2006).

#### 3.9.3.1 Fish weight measurement

The weight gain of fish in each tank and treatment was obtained every two weeks. All the fish in each test tank were removed by using aquarium net, water drained off and weight of fish taken by using the electric sorties metler balance. The average weight of fish in each tank was determined by dividing the total weight by the number of fish. Mean weight and percentage cumulative weight was calculated at the end of the test (Auta *et al.*, 2000; Gbem *et al.*, 2003).

### 3.9.3.2 Analysis of fish growth and nutrient utilization

The data collected during weight increment measurements are analysed for fish growth, feed conversion protein utilization as shown below

#### (A) Specific growth rate (SGR)

SGR was calculated as described by Hephher (1988) and Shalaby *et al.* (2006).

$$\text{SGR} = \frac{\log W_t - \log W_o}{t - t_o} \quad (3.7)$$

Where

$W_t$  = weight at the time of observation (g)

$W_o$  = initial weight (g)

$t - t_o$  = the period under study (days)

#### (B) Feed conversion rate (FCR)

FCR was computed as the dry weight of feed offer divided by the wet weight gain of fish (Hephher, 1988).

$$\text{FCR} = \frac{\text{Feed supplied (g)}}{\text{Weight gain}} \quad (3.8)$$

#### (C) Feeding rate and practice

Fish were fed at 3% body weight two times (7.00am and 7.00pm)

#### (D) Fish weight measurement

The average weight of the fish was determined by dividing the total number of fish.

(E) *Weight gain (WG)*

WG was calculated by as the difference between the final and initial weight in grams.

(F) *Percentage life weight gain (LWG %)*

LWG % was computed as the difference between the initial weight and the final weight expressed as percentage (Wannigamma *et al.*, 1985) and (Shalaby *et al.*, 2006) methods.

(G) *Gross feed conversion efficiency (GFCE)*

GFCE is the reciprocal of FCR express as percentage (Stickney, 1979; Shalaby *et al.*, 2006).

$$\text{GFCE} = \frac{1 \times 100}{\text{FCR}} \quad (3.9)$$

(H) *Feed efficiency (FE)*

FE was computed as a ratio that expresses the fish weight gain to the quantity of feed.

$$\text{FE} = \frac{\text{Weight gain (g)}}{\text{Feed fed}} \quad (3.10)$$

(I) *Protein efficiency ratio (PER)*

PER was computed as the ratio of fish weight gain (g) to the crude protein consumed (Wilson, 1989) and (Shalaby *et al.*; 2006).

$$\text{PER} = \frac{\text{Fish wet weight gain (g)}}{\text{Crude protein fed (g)}} \quad (3.11)$$

(J) *Nitrogen metabolism (NM)*

NM was computed as described by Dabrowski (1977) and (Shalaby *et al.*; 2006).

$$\text{NM} = \frac{0.549 (a-b)h}{2} \quad (3.12)$$

Where

a = initial weight of fish (g)

b = final weight of fish (g)

c = experimental period (days)

#### 3.9.4 Data analysis

Data was subjected to one-way analysis of variance (ANOVA) using SPSS software to test for the significant differences between means and where significant differences are found, the Duncan's Multiple Range Test (DMRT) was used to separate the significantly different means. Correlation of length-weight relationship was determined and  $LC_{50}$  was determined using the Minitab version 17.0 software for windows.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Acute Toxicity Test

The result for acute toxicity test is based on the behaviour and respiratory mortality rates of the fish for 96 hr as explained below:

##### 4.1.1 Behaviour

Heteroclarias showed behavioural changes on exposure to glyphosate herbicide. Immediately the fish were introduced into the tank containing glyphosate at concentrations of 5.40, 7.20, 9.00, 10.80 and 12.60mg/L, they became restless and agitated. Fishes came to the surface of water much more frequently. They occasionally tried to jump out of the water. Treated fish exhibited increased mucous secretion and progressively became sluggish and lethargic. As the time of exposure increased fish stood in vertical position with their heads above the water surface. The fish showed abnormal swimming movements including loss of orientation, loss of buoyancy and spasms before death occurred.

##### 4.1.2 Respiration rates

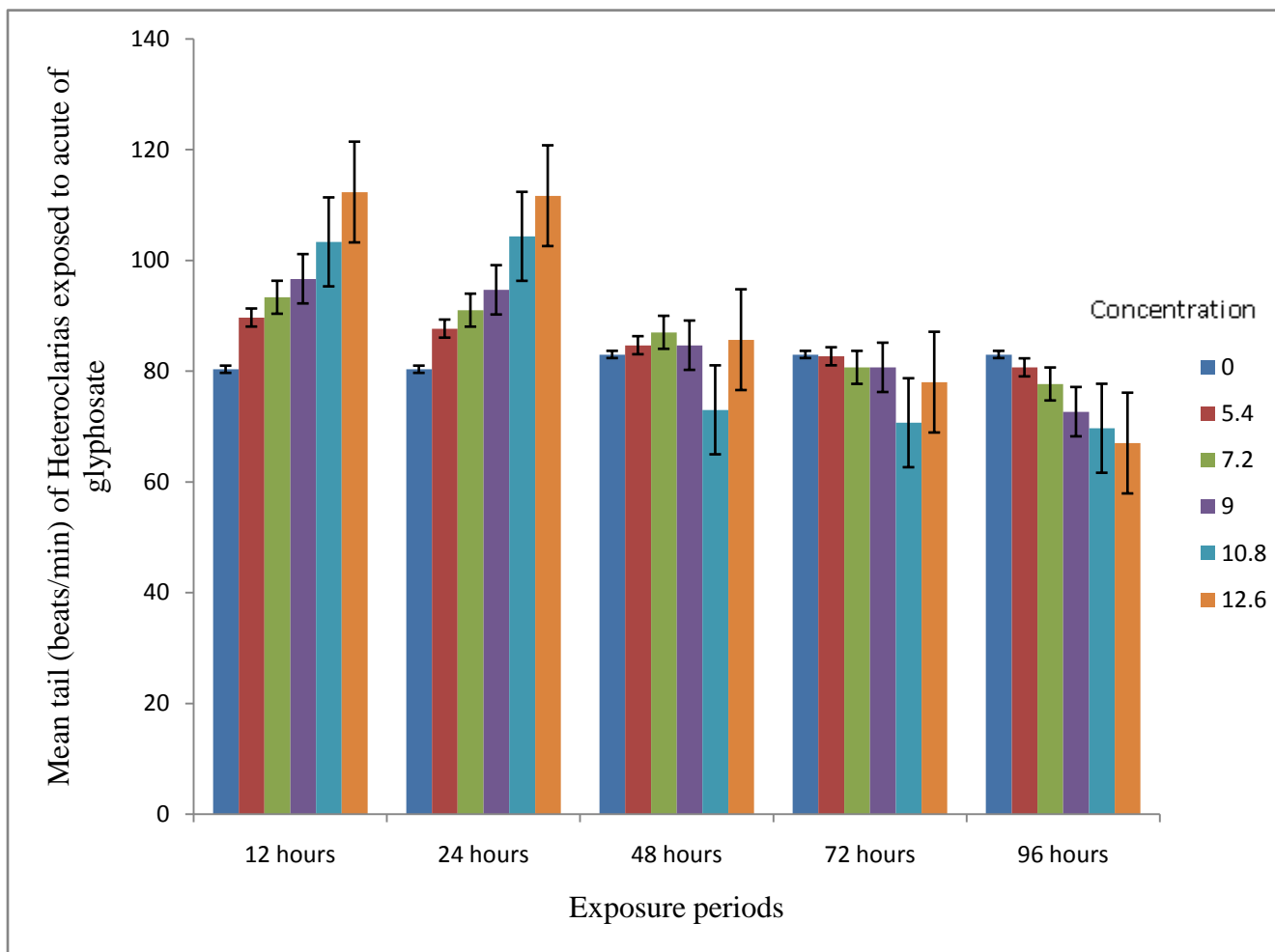
###### 4.1.2.1 Opercular ventilation beat

Heteroclarias exposed to glyphosate showed increased opercular ventilation and tail fin beat with increase in the concentration of the toxicant for 5.40, 7.20, 9.00, 10.80, and 12.60mg/L respectively (figure 4.1). The activity of the opercular were observed and counted, especially during the first 48 hrs of exposure with glyphosate to Heteroclarias fingerlings. The result of opercular ventilation as presented in figure 4.1 showed that the

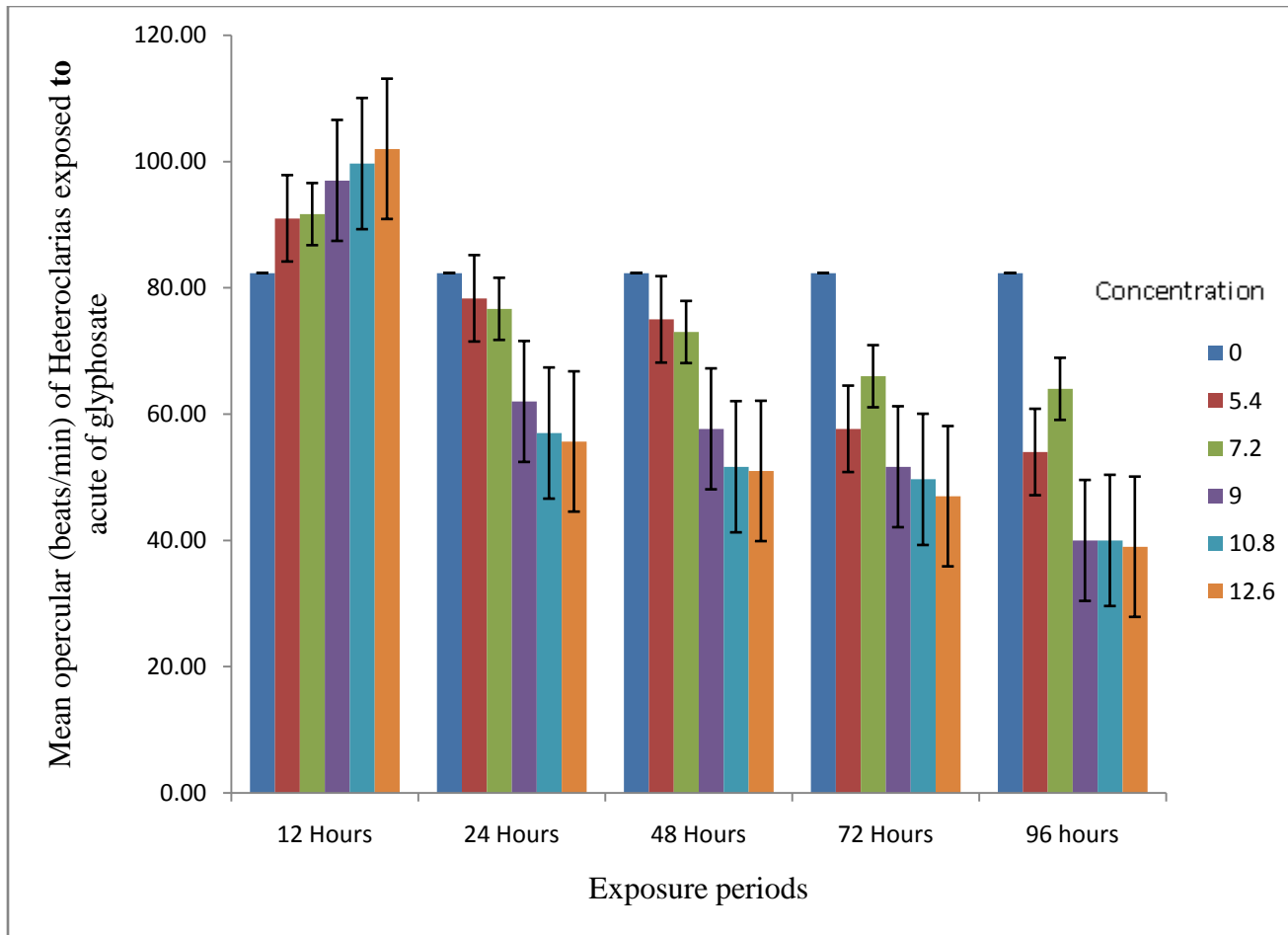
opercular beats of the exposed fish to the toxicant at 12 and 24 hours were higher than the one in the control fish. As the concentration of glyphosate increased the beats of the operculum increases except at the 48<sup>th</sup> hours when the beats started to decrease. Further duration of exposure led to more decrease in the opercular ventilation beat of the fish. By the 96<sup>th</sup> hour the opercular ventilation rates of the exposed fish were significantly ( $P < 0.05$ ) lower than those of the control group.

#### *4.1.2.2 Tail fin beat*

However, *Heteroclaris* exposed to glyphosate also showed increased tail fin beat with increase in the concentration of the toxicant for 5.40, 7.20, 9.00, 10.80 and 12.60mg/L. The activity of tail fin beat were observed and counted, in particular during the first 48 hours of exposure with glyphosate to *Heteroclaris* fingerlings. The result of tail fin beat is presented in figure 4.2. The graph showed that the tail fin beat of the exposed fish to the toxicant at 12 and 24 hours were higher than the one in the control fish. Even though the concentration of glyphosate increased the beats of tail also increases except at the 48<sup>th</sup> hours when the beat started to decrease. As the duration of exposure progresses led to more decrease in tail fin beat of the fish. There was significant difference between the tail fin beat of the treated fish as seen in the figure. The values were dose dependent.



**Figure 4.1:** Mean( $\pm$ SE) mean tail rate of Heteroclaris exposed to acute concentration of glyphosate



**Figure 4.2:** Mean( $\pm$ SE) opercular beat rate of Heteroclaris exposed to acute concentration of glyphosate

#### 4.1.3 Physico-chemical parameters of diluting water used in acute toxicity test for glyphosate.

Results of the physico-chemical analysis of the water used for dilution of the administered pesticide concentrations before and after 96hr of exposure in the laboratory are presented in Table 4.1. The parameters such as pH, Total Dissolved Solids, Electrical Conductivity, and Hardness before exposure have their range values as 5.95-6.28, 53-68mg/L, 96-141 $\mu$ S/m, and 136-164CaCO<sub>3</sub> respectively. However, after exposure of the toxicant for 96hr, there was a sharp increase in pH (6.30-6.67), Total Dissolved Solids (60-83mg/L), Electrical Conductivity (128-163 $\mu$ S/m), and Hardness as CaCO<sub>3</sub> (116-180) with their range values in parentheses.

For the parameters such as Temperature, Dissolved Oxygen, Alkalinity before exposure have their range values as 25.90-26.20<sup>□</sup>C, 7.40-8.40mg/L, and 36-59mg/L.CaCO<sub>3</sub> respectively. There was however increase in the range value after exposure for 96hr such as Temperature (26.70-28.60<sup>□</sup>C) and decrease in Dissolved Oxygen (5.40-6.80mg/L), and Alkalinity (31-36mg/L.CaCO<sub>3</sub>) with their range values in parenthesis.

#### 4.1.4 Mortality rates and Log of concentration in Heteroclaris exposed to acute concentration of glyphosate.

Fish mortality was observed in all the aquaria tanks except in the control tanks. The result of the acute toxicity test showing mean mortality of each at various concentration of glyphosate is represented in Table 4.2. The first mortality was observed at 12 hours at 5.40mg/L, 9.00mg/L, 10.80mg/L and 12.60mg/L of glyphosate.

By the 24<sup>th</sup> hour more mortality was observed at the various concentrations except in the control tank. But at 48, 72 and 96 hours, mortality was observed in all concentrations except the control. The highest mortality value of 9 was observed at 9.00mg/L and 12.60mg/L concentrations while lowest value of 4 for the lowest concentration of 5.40mg/L.

Similarly, mortality rates and Log of concentration in *Heteroclaris* exposed to acute concentration of glyphosate as shown in Table 4.3 indicates that the concentration 12.60mg/L as having the highest number of mortality rate (17) and Log of concentration value (1.10037) while concentration 5.40mg/L as having the lowest number of mortality rate (6) and Log of concentration value (0.73239) with the control having the value 0.0000 respectively.

#### 4.1.5 Mortality rate of LC<sub>50</sub> and threshold

The mortality rate of LC<sub>50</sub> as shown in figure 4.3 was determined by the antilog of 0.8349mg/L. The result of the acute toxicity showed that glyphosate was toxic to *Heteroclaris* (hybrid) with LC<sub>50</sub> value of 6.838mg/L. The threshold mortality was 0.9000mg/L as shown in figure 4.4.

**Table 4.1:** Mean( $\pm$ SE) of physico-chemical parameters before and after 96hr exposure.

Parameters	Before Exposure		After Exposure	
	Range	Mean	Range	Mean $\pm$ SE
pH	5.95-6.28	6.09 $\pm$ 0.08	6.30-6.67	6.47 $\pm$ 0.04
Temperature ( $^{\circ}$ C)	25.90-26.20	26.15 $\pm$ 0.09	26.70-28.60	27.65 $\pm$ 0.19
Total Dissolved Solids (mg/L)	53-68	57.34 $\pm$ 2.21	60-83	71.67 $\pm$ 1.18
Electrical Conductivity ( $\mu$ S/m)	96-141	115.67 $\pm$ 7.59	128-163	145 $\pm$ 1.58
Dissolved Oxygen (mg/L)	7.40-8.40	7.85 $\pm$ 0.05	5.40-6.80	6.12 $\pm$ 0.05
Alkalinity (mg/L.CaCO <sub>3</sub> )	36-59	48.34 $\pm$ 4.70	31-36	36.33 $\pm$ 1.64
Hardness (CaCO <sub>3</sub> )	136-164	153.34 $\pm$ 6.83	116-180	154.67 $\pm$ 12.25

**Table 4.2:** Mortality of Heteroclaris exposed to acute concentration of glyphosate.

Exposure Period	Concentration (mg/L)											
	0.00	0.00	5.40	5.40	7.20	7.20	9.00	9.00	10.80	10.80	12.60	12.60
	C1	C2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
12	-	-	1	1	-	-	1	1	-	1	-	1
24	-	-	2	-	2	3	3	4	4	2	3	1
48	-	-	1	2	3	2	2	3	2	2	1	6
72	-	-	1	-	1	1	-	-	1	2	3	1
96	-	-	-	1	2	2	3	1	1	-	1	-
	$\frac{0}{10}$	$\frac{0}{10}$	$\frac{5}{10}$	$\frac{4}{10}$	$\frac{8}{10}$	$\frac{8}{10}$	$\frac{9}{10}$	$\frac{9}{10}$	$\frac{8}{10}$	$\frac{7}{10}$	$\frac{8}{10}$	$\frac{9}{10}$

Key; C= Control, T= Treatment, No mortality (-)

**Table 4.3:** Mortality rates and Log of concentration in Heteroclaris exposed to acute concentration of glyphosate.

Concentration in mg/l	Log. of Conc.	No. exposed	Mortality rates	% mortality	Probit kill
0.00	0.00000	20	0	0	3.0
5.40	0.73239	20	6	30	4.4
7.20	0.85733	20	12	60	5.3
9.00	0.95424	20	14	70	5.4
10.80	1.03342	20	15	75	5.5
12.60	1.10037	20	17	85	6.0

Values representing mortality rates and values of log conc. at different concentration.

# Probability Plot for Mortality

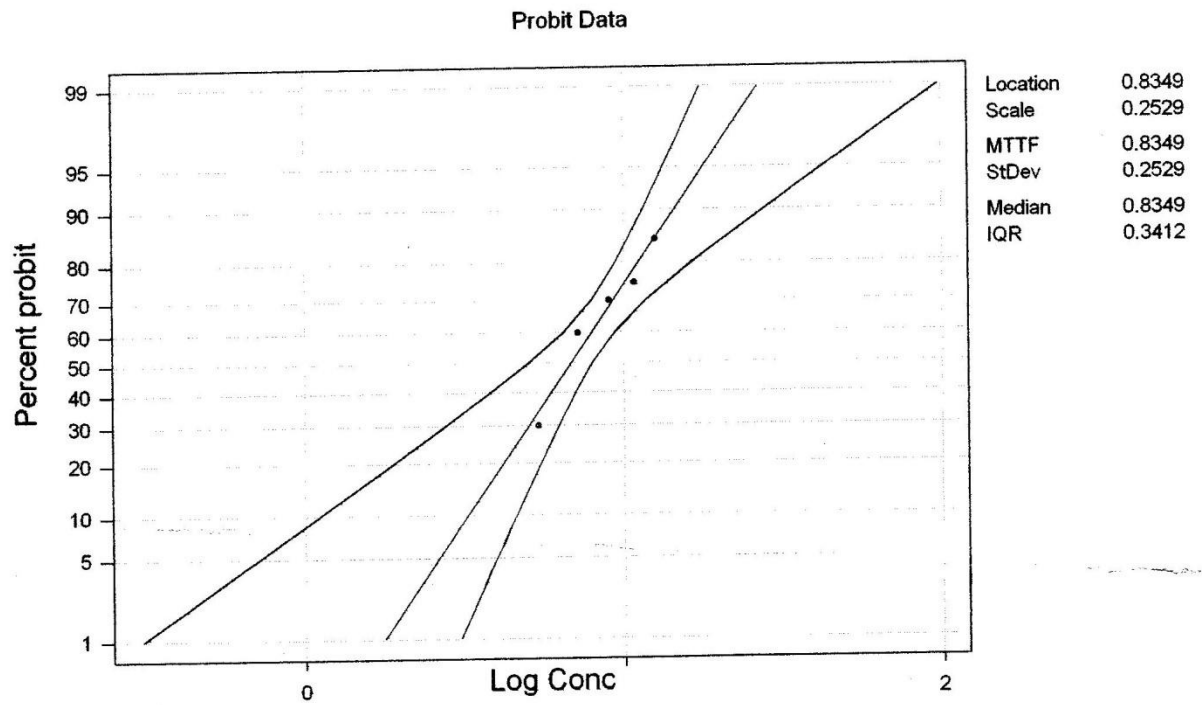
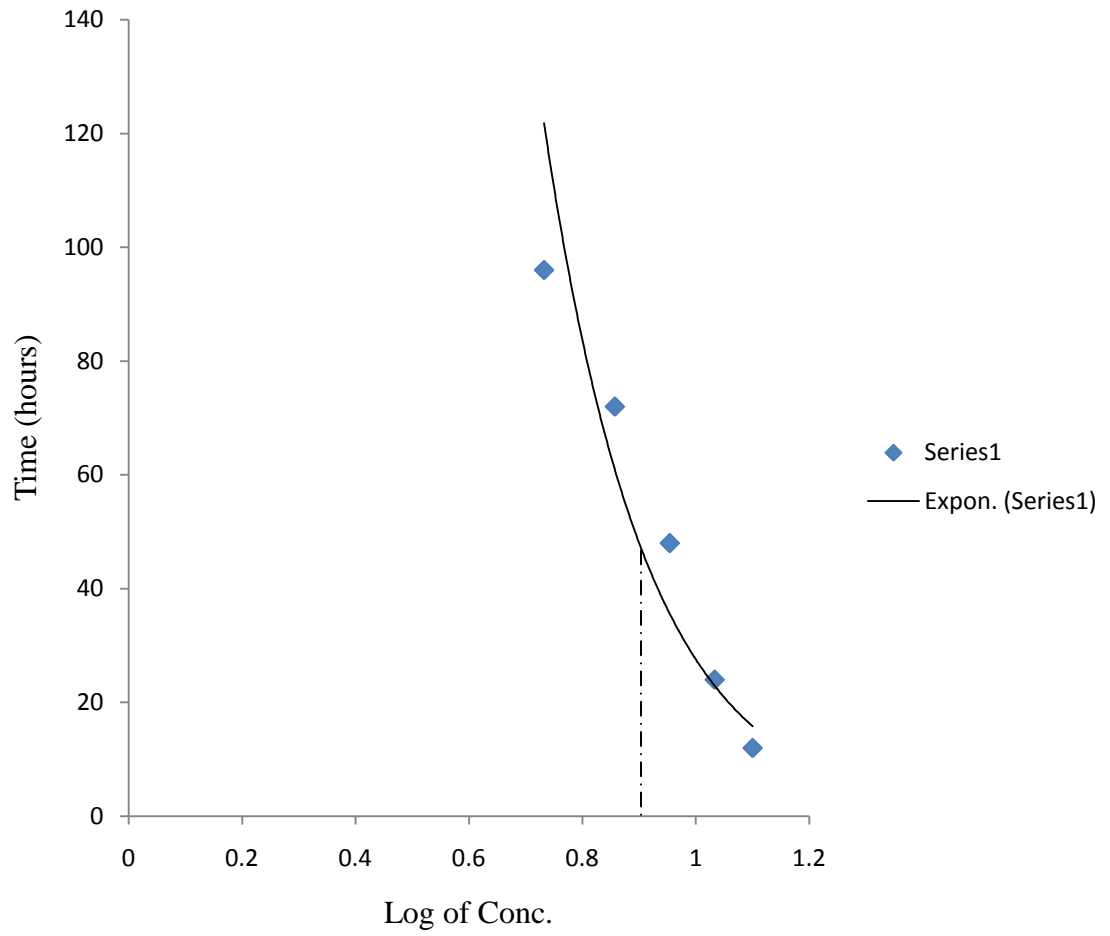


Figure.. Glysophate Normal  
Distribution - ML Estimates - 95.0% CI

**Figure 4.3:** 96 hrs of glyphosate herbicide on fingerlings of Heteroclarias.



**Figure 4.4:** Graph of mortality threshold of glyphosate herbicide on fingerlings of *Heteroclaris*.

#### 4.1.6 Haematological parameters of Heteroclaris exposed to acute toxicity of glyphosate.

Results of the acute bioassay on haematological parameters of fish exposed to acute nominal doses of glyphosate are presented in Table 4.4.

##### *4.1.6.1 Red blood cell count (RBCC)*

There was significant difference in RBCC ( $P < 0.05$ ) as the values have different superscript with the control (0.00) having the highest value followed by 5.40, 7.20, 9.00, 10.80 and 12.60mg/L concentrations having lower values respectively. This indicates that the RBCC of the exposed fish decreased with increase in concentration.

##### *4.1.6.2 Packed cell volume (PCV)*

The values shows a significant difference in PCV ( $P < 0.05$ ) as they have different superscript with the control (0.00) having the highest followed by 5.40, 7.20, 9.00, 10.80 and 12.60mg/L concentrations having lower values respectively as shown in Table 4.4. This also indicates that the PCV of the exposed fish decreased with increase in concentration.

##### *4.1.6.3 Haemoglobin (Hb)*

This indicates that there was significant difference in Hb ( $P < 0.05$ ) as the values have different superscript with the control (0.00) having the highest value followed by 5.40, 7.20, 9.00, 10.80 and 12.60mg/L concentrations having lower values respectively. This indicates also that the Hb of the exposed fish decreased with increase in concentration.

#### *4.1.6.4 White blood cell count (WBCC)*

The recorded values shows significant difference in WBCC as they have different superscript with the highest concentration (12.60mg/L) having the highest value followed by 10.80, 9.00, 7.20, 0.00 and 5.40mg/L as shown in Table 4.5. The increase in WBCC for 12.60mg/L of the exposed fish is a form of defense against foreign intrusion.

#### *4.1.6.5 Mean corpuscular volume (MCV)*

There was significant difference in MCV as the values have different superscript with 9.00mg/L having the highest value followed by 7.20 and 12.60mg/L respectively. However, there was no significant difference in the MCV ( $P>0.05$ ) of 0.00, 5.40 and 10.80mg/L of glyphosate.

#### *4.1.6.6 Mean corpuscular haemoglobin (MCH)*

There was however significant difference in MCH as the values have different superscript with 9.00mg/L having the highest value followed by 7.20 and 12.60mg/L. However, there was no significant difference in the MCH ( $P>0.05$ ) of 0.00, 5.40 and 10.80mg/L.

#### *4.1.6.7 Mean corpuscular haemoglobin concentration (MCHC)*

Finally, there was significant difference in MCHC as the values have different superscript with 12.60mg/L having the highest value followed by 0.00. However, there was no significant difference in the MCHC ( $P>0.05$ ) of 5.40, 7.20, 9.00, 10.80mg/L of glyphosate.

**Table 4.4:** Effect of differential concentration of glyphosate on haematological parameters of Heteroclaris after 96 hours of exposure.

Conc. (mg/L)	RBCC ( $\times 10^6 \text{mm}^3$ )	WBCC ( $\times 500 \text{mm}^3$ )	Hb (g/100ml)	PCV (%)	MCV ( $\times 10^6 \text{Pgcell}$ )	MCH ( $\times 10^6 \text{Pgcell}$ )	MCHC (g/100ml)
0.00	227.67 $\pm$ 1.45 <sup>a</sup>	7552.00 $\pm$ 73.90 <sup>e</sup>	10.50 $\pm$ 0.12 <sup>a</sup>	31.67 $\pm$ 0.33 <sup>a</sup>	1.39 $\pm$ 0.01 <sup>c</sup>	0.46 $\pm$ 0.03 <sup>c</sup>	32.49 $\pm$ 0.66 <sup>b</sup>
5.40	222.67 $\pm$ 1.45 <sup>b</sup>	6272.00 $\pm$ 73.90 <sup>f</sup>	9.87 $\pm$ 0.09 <sup>b</sup>	29.67 $\pm$ 0.33 <sup>b</sup>	1.33 $\pm$ 0.01 <sup>c</sup>	0.44 $\pm$ 0.00 <sup>c</sup>	33.26 $\pm$ 0.13 <sup>ab</sup>
7.20	187.67 $\pm$ 1.45 <sup>c</sup>	8064.00 $\pm$ 73.90 <sup>d</sup>	9.17 $\pm$ 0.09 <sup>c</sup>	27.67 $\pm$ 0.33 <sup>c</sup>	1.47 $\pm$ 0.01 <sup>b</sup>	0.49 $\pm$ 0.00 <sup>b</sup>	33.13 $\pm$ 0.14 <sup>ab</sup>
9.00	137.67 $\pm$ 1.45 <sup>d</sup>	9856.00 $\pm$ 73.90 <sup>c</sup>	7.50 $\pm$ 0.12 <sup>d</sup>	22.67 $\pm$ 0.33 <sup>d</sup>	1.65 $\pm$ 0.01 <sup>a</sup>	0.54 $\pm$ 0.00 <sup>a</sup>	33.09 $\pm$ 0.26 <sup>ab</sup>
10.80	122.67 $\pm$ 1.45 <sup>e</sup>	10363.67 $\pm$ 74.03 <sup>b</sup>	5.67 $\pm$ 0.20 <sup>e</sup>	17.00 $\pm$ 0.58 <sup>e</sup>	1.38 $\pm$ 0.03 <sup>c</sup>	0.46 $\pm$ 0.01 <sup>c</sup>	33.33 $\pm$ 0.12 <sup>ab</sup>
12.60	112.67 $\pm$ 1.45 <sup>f</sup>	10880.00 $\pm$ 73.90 <sup>a</sup>	4.37 $\pm$ 0.20 <sup>f</sup>	13.00 $\pm$ 0.58 <sup>f</sup>	1.15 $\pm$ 0.04 <sup>d</sup>	0.39 $\pm$ 0.01 <sup>d</sup>	33.58 $\pm$ 0.15 <sup>a</sup>

Means with the same superscript along the columns are not significantly different (P>0.05).

#### 4.1.7 Leucocytes differential counts of Heteroclaris exposed to acute concentrations of glyphosate.

Leucocytes Differential Counts of fish exposed to acute concentrations of glyphosate are shown in Table 4.5. The parameters tested include neutrophils, lymphocytes, basophils, eosinophils and monocytes as explained below.

##### *4.1.7.1 Neutrophils*

There was significant difference in Neutrophils ( $P < 0.05$ ) as the values have different superscript with 7.20mg/L having the highest value followed by 10.80mg/L. However, there was no significant difference in the Neutrophils ( $P > 0.05$ ) of 0.00, 5.40, 9.00 and 12.60mg/L of glyphosate.

##### *4.1.7.2 Lymphocytes*

The values shows a significant difference in Lymphocytes ( $P < 0.05$ ) as they have different superscript with 12.60mg/L having the highest value followed by 10.80, 9.00, 7.20, 5.40 and 0.00 respectively.

Others, such as Basophils, Eosinophils and Monocytes were tested but not detected.

**Table 4.5:** Mean( $\pm$ SE) of Heteroclaris exposed to acute concentration of glyphosate after 96 hours on some leucocytes differential count.

Conc.(mg/L)	Neutrophils(%)	Lymphocytes(%)	Basophils(%)	Eosinophils(%)	Monocytes(%)
0.00	19.00 $\pm$ 0.58 <sup>b</sup>	45.00 $\pm$ 0.58 <sup>f</sup>	nd	nd	nd
5.40	15.00 $\pm$ 0.58 <sup>d</sup>	51.00 $\pm$ 0.58 <sup>e</sup>	nd	nd	nd
7.20	22.00 $\pm$ 0.58 <sup>a</sup>	61.67 $\pm$ 0.88 <sup>d</sup>	nd	nd	nd
9.00	20.00 $\pm$ 0.58 <sup>b</sup>	66.00 $\pm$ 0.58 <sup>c</sup>	nd	nd	nd
10.80	17.00 $\pm$ 0.58 <sup>c</sup>	70.00 $\pm$ 0.58 <sup>b</sup>	nd	nd	nd
12.60	13.67 $\pm$ 0.58 <sup>d</sup>	75.00 $\pm$ 0.58 <sup>a</sup>	nd	nd	nd

Means with the same superscript along the columns are not significantly different (P>0.05).

nd = not detected

#### 4.1.8 Catalase and peroxidase activity for acute exposure of glyphosate concentration on *Heteroclaris* gills.

Catalase and peroxidase of fish exposed to acute concentration of glyphosate are shown in Table 4.6.

There was significant difference in gill catalase activity ( $P < 0.05$ ) as the values have different superscript along the column with 5.40mg/L having the highest activity followed by 7.20, 9.00, 10.80, 12.60 and 0.00 respectively. This indicates that as the concentration increases the activity increases i.e. the activity is dose dependent.

However, there was also significant difference in gill peroxidase activity ( $P < 0.05$ ) as values have different superscript along the column with 9.00mg/L having the highest followed by 7.20, 12.60, 10.80, 5.40 and 0.00.

#### 4.1.9 Catalase and peroxidase activity for acute exposure of glyphosate concentration on *Heteroclaris* liver.

Catalase and Peroxidase of fish exposed to acute concentration of glyphosate are shown in Table 4.7.

There was significant difference in liver catalase activity ( $P < 0.05$ ) as the values have different superscript along the column with 12.60mg/L having the highest activity followed by 5.40, 7.20, 9.00, 10.80 and 0.00 respectively. This indicates that as the concentration increases the activity also increases.

However, there was also significant difference in liver peroxidase activity ( $P < 0.05$ ) as values have different superscript along the column with 12.60mg/L having the highest activity followed by 7.20, 9.00, 5.40, 10.80 and 0.00 as shown in Table 4.7.

**Table 4.6:** Mean( $\pm$ SE) of catalase and peroxidase activity (U/mg protein) for exposure of glyphosate concentration on *Heteroclaris* gills.

Conc.(mg/L)	GCAT	GPOD
0.00	0.003 $\pm$ 0.000 <sup>f</sup>	0.005 $\pm$ 0.001 <sup>f</sup>
5.40	1.323 $\pm$ 0.000 <sup>a</sup>	0.007 $\pm$ 0.001 <sup>e</sup>
7.20	1.213 $\pm$ 0.001 <sup>b</sup>	0.123 $\pm$ 0.001 <sup>b</sup>
9.00	1.006 $\pm$ 0.000 <sup>c</sup>	0.141 $\pm$ 0.000 <sup>a</sup>
10.80	0.855 $\pm$ 0.000 <sup>d</sup>	0.027 $\pm$ 0.000 <sup>d</sup>
12.60	0.750 $\pm$ 0.001 <sup>e</sup>	0.037 $\pm$ 0.001 <sup>c</sup>

Means with different superscript along the columns are significantly different ( $P < 0.05$ ).

GCAT = Gill Catalase Activity

GPOD = Gill Peroxidase Activity

**Table 4.7:** Mean( $\pm$ SE) of catalase and peroxidase activity (U/mg protein) for acute exposure of glyphosate concentration on *Heteroclaris* liver.

Conc.(mg/L)	LCAT	LPOD
0.00	0.013 $\pm$ 0.002 <sup>f</sup>	0.027 $\pm$ 0.001 <sup>f</sup>
5.40	1.261 $\pm$ 0.000 <sup>b</sup>	0.033 $\pm$ 0.001 <sup>d</sup>
7.20	1.233 $\pm$ 0.000 <sup>c</sup>	0.145 $\pm$ 0.003 <sup>b</sup>
9.00	1.162 $\pm$ 0.001 <sup>d</sup>	0.042 $\pm$ 0.003 <sup>c</sup>
10.80	0.995 $\pm$ 0.001 <sup>e</sup>	0.025 $\pm$ 0.000 <sup>c</sup>
12.60	7.768 $\pm$ 4.307 <sup>a</sup>	24.867 $\pm$ 12.519 <sup>a</sup>

Means with different superscript along the columns are significantly different (P<0.05).

LCAT = Liver catalase activity

LPOD = Liver peroxidase activity

## **4.2 Sub-lethal Effect Concentrations of Glyphosate on Heteroclaris**

### 4.2.1 Behavioural responses

Sub-lethal concentration of glyphosate causes some physiological malfunction such as erratic swimming, nesting at the bottom and skin lesion. Hyperactive response was observed in the first week particularly in the highest concentration and this response reduced to the state of hypoactivity as the exposure period increased.

### 4.2.2 Haematological parameters of Heteroclaris exposed to sub-lethal concentration of glyphosate.

Results of the sub-lethal bioassay on haematological parameters of fish exposed to acute nominal doses of glyphosate are presented in Table 4.8.

#### *4.2.2.1 Red blood cell count (RBCC)*

From week 2 to 8, there was significant difference in RBCC as the values have different superscript with 0.00 having the highest values followed by 0.30, 0.70 and 1.40mg/L respectively.

#### *4.2.2.2 White blood cell count (WBCC)*

The values for week 2 to 8 shows there was significant difference ( $P < 0.05$ ) in WBCC as they have different superscript with 1.40mg/L having the highest values followed by 0.70, 0.30 and 0.00 respectively.

#### *4.2.2.3 Packed cell volume (PCV)*

The values for week 2 – 8 however shows there was significant difference ( $P<0.05$ ) in PCV as the values have different superscript with 0.00 having the highest values followed by 0.30, 0.70 and 1.40mg/L respectively.

#### *4.2.2.4 Haemoglobin (Hb)*

For week 2 to 8, there was significant difference ( $P<0.05$ ) in Hb as the values have different superscript with 0.00 having the highest values followed by 0.30, 0.70 and 1.40mg/L.

#### *4.2.2.5 Mean corpuscular volume (MCV)*

Week 2 to 4 shows there was significant difference ( $P<0.05$ ) in MCV as the values have different superscript with 0.00 having the highest values followed by 0.30 while for 0.70 and 1.40mg/L, there was significant difference ( $P<0.05$ ) in MCV as the values have different superscript with 0.00 having the highest values followed by 0.30, 0.70 and 1.40mg/L respectively.

#### *4.2.2.6 Mean corpuscular haemoglobin (MCH)*

For week 2, there was significant difference in MCH as the values have different superscript with 0.40 having the highest value followed by 0.30 and 1.40 while there was highly significant difference in MCH for 0.70mg/L. For week 4-8, there was significant

difference in MCH as the values have different superscript with 0.00 having the highest value followed by 0.30, 0.70 and 1.40mg/L.

#### *4.2.2.7 Mean corpuscular haemoglobin concentration (MCHC)*

Finally, for week 2-6, there was no significant difference in MCHC as the values have the same superscript for 0.00, 0.30, 0.70 and 1.40mg/L respectively. For week 8, there was significant difference in MCHC as the values have different superscript with 0.00 having the highest value followed by 0.30, 0.70 and 1.40mg/L.

#### *4.2.3 Leucocytes differential counts of Heteroclaris exposed to sub-lethal concentrations of glyphosate.*

Leucocytes Differential Counts of fish exposed to sub-lethal concentrations of glyphosate are shown in Table 4.9.

##### *4.2.3.1 Neutrophils*

For week 2, there was significant difference in Neutrophils as the values have different superscript with 1.40 having the highest value followed by 0.00 and 0.30 which do not show significant difference. Week 4 do not show significant difference in Neutrophils as the values have same superscript. 0.00 and 1.40 have same superscript while 0.30 and 0.70 also have same superscript. Week 6 shows significant difference in Neutrophils as the values have different superscript with 1.40 having the highest value followed by 0.70, 0.30 and 0.00 respectively. Finally for week 8, there was significant difference in

Neutrophils as the values have different superscript with 1.40 having the highest value followed by 0.70 and 0.00 which do not show any significant difference while 0.40mg/L show significant difference from the rest of the treatment.

#### *4.2.3.2 Lymphocytes*

Finally, week 2 shows there was significant difference in Lymphocytes as the values have different superscript with 0.30 having the highest value followed by 0.70 while 0.00 and 1.40 shows no significant difference for Lymphocytes. For week, there was significant difference in Lymphocytes as the values have different superscript with 0.70 having the highest followed by 0.30, 0.00 and 1.40. For week 6 to 8, there was also significant difference in Lymphocytes as the values have different superscript with 0.30 having the highest followed by 0.70, 1.40 and 0.00 respectively.

#### *4.2.4 Catalase activity for eight weeks exposed to sub-lethal concentration of glyphosate for Heteroclaris gills.*

Catalase activity per two weeks for eight weeks exposed to sub-lethal concentration of glyphosate for Heteroclaris gills are shown in Table 4.10.

There was significant difference in gill catalase activity ( $P < 0.05$ ) for 2 to 8 weeks as the values have different superscript along the column with 0.30mg/L having the highest activity followed by 0.70, 1.40 and 0.00 respectively. This is also presented in Figure 4.3.

#### 4.2.5 Catalase activity for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on liver.

Catalase activity per two weeks for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* liver is shown in Table 4.11.

There was significant difference in liver catalase activity ( $P < 0.05$ ) for 2 to 8 weeks as the values have different superscript along the column with 0.30mg/L having the highest activity followed by 0.70, 1.40 and 0.00 respectively. However, it is also presented in figure 4.4.

**Table 4.8:** Mean( $\pm$ SE) of sub-lethal concentration of glyphosate on some haematological parameters of *Heteroclaris*.

Parameter	Conc.(mg/L)	Weeks			
		2	4	6	8
RBCC( $\times 10^6 \text{mm}^3$ )	0.00	237.67 $\pm$ 1.45 <sup>a</sup>	242.33 $\pm$ 0.88 <sup>a</sup>	232.00 $\pm$ 1.15 <sup>a</sup>	246.33 $\pm$ 0.88 <sup>a</sup>
	0.30	217.67 $\pm$ 1.45 <sup>b</sup>	205.33 $\pm$ 2.60 <sup>b</sup>	192.00 $\pm$ 1.15 <sup>b</sup>	163.33 $\pm$ 0.88 <sup>b</sup>
	0.70	207.67 $\pm$ 1.45 <sup>c</sup>	191.00 $\pm$ 2.08 <sup>c</sup>	181.33 $\pm$ 1.86 <sup>c</sup>	153.33 $\pm$ 0.88 <sup>c</sup>
	1.40	197.67 $\pm$ 1.45 <sup>d</sup>	182.33 $\pm$ 1.45 <sup>d</sup>	175.33 $\pm$ 0.88 <sup>d</sup>	147.67 $\pm$ 0.88 <sup>d</sup>
WBCC( $\times 500 \text{mm}^3$ )	0.00	6016.00 $\pm$ 73.90 <sup>d</sup>	4152.33 $\pm$ 1.45 <sup>a</sup>	3963.00 $\pm$ 0.58 <sup>d</sup>	4675.00 $\pm$ 0.58 <sup>d</sup>
	0.30	6528.00 $\pm$ 73.90 <sup>c</sup>	5256.67 $\pm$ 0.88 <sup>c</sup>	4153.67 $\pm$ 1.20 <sup>c</sup>	5251.00 $\pm$ 0.58 <sup>c</sup>
	0.70	7040.00 $\pm$ 73.90 <sup>b</sup>	5870.00 $\pm$ 1.15 <sup>b</sup>	4867.00 $\pm$ 0.58 <sup>b</sup>	5876.33 $\pm$ 0.88 <sup>b</sup>
	1.40	7552.00 $\pm$ 73.90 <sup>a</sup>	6119.00 $\pm$ 2.08 <sup>a</sup>	5101.67 $\pm$ 1.20 <sup>a</sup>	6121.00 $\pm$ 0.58 <sup>a</sup>
PCV(%)	0.00	33.67 $\pm$ 0.33 <sup>a</sup>	35.00 $\pm$ 0.58 <sup>a</sup>	30.00 $\pm$ 0.58 <sup>a</sup>	29.00 $\pm$ 0.58 <sup>a</sup>
	0.30	28.67 $\pm$ 0.33 <sup>b</sup>	22.33 $\pm$ 1.45 <sup>b</sup>	20.00 $\pm$ 0.58 <sup>b</sup>	15.00 $\pm$ 0.58 <sup>b</sup>
	0.70	26.67 $\pm$ 0.33 <sup>c</sup>	18.00 $\pm$ 0.58 <sup>c</sup>	16.00 $\pm$ 0.58 <sup>c</sup>	12.00 $\pm$ 0.58 <sup>c</sup>
	1.40	24.67 $\pm$ 0.33 <sup>d</sup>	15.00 $\pm$ 0.58 <sup>d</sup>	13.00 $\pm$ 0.58 <sup>d</sup>	10.00 $\pm$ 0.58 <sup>d</sup>
Hb(g/100ml)	0.00	11.17 $\pm$ 0.10 <sup>a</sup>	11.67 $\pm$ 0.20 <sup>a</sup>	10.00 $\pm$ 0.17 <sup>a</sup>	9.67 $\pm$ 0.20 <sup>a</sup>
	0.30	9.50 $\pm$ 0.11 <sup>b</sup>	7.40 $\pm$ 0.49 <sup>b</sup>	6.67 $\pm$ 0.20 <sup>b</sup>	5.00 $\pm$ 0.17 <sup>b</sup>
	0.70	8.87 $\pm$ 0.09 <sup>c</sup>	6.00 $\pm$ 0.17 <sup>c</sup>	5.33 $\pm$ 0.20 <sup>c</sup>	4.00 $\pm$ 0.17 <sup>c</sup>
	1.40	8.17 $\pm$ 0.09 <sup>d</sup>	5.00 $\pm$ 0.17 <sup>d</sup>	4.33 $\pm$ 0.20 <sup>d</sup>	3.33 $\pm$ 0.20 <sup>d</sup>
MCV( $\times 10^6 \text{Pgcell}$ )	0.00	1.42 $\pm$ 0.01 <sup>a</sup>	1.45 $\pm$ 0.02 <sup>a</sup>	1.29 $\pm$ 0.03 <sup>a</sup>	1.18 $\pm$ 0.02 <sup>a</sup>
	0.30	1.32 $\pm$ 0.01 <sup>b</sup>	0.36 $\pm$ 0.02 <sup>b</sup>	1.04 $\pm$ 0.02 <sup>b</sup>	0.92 $\pm$ 0.03 <sup>b</sup>
	0.70	1.29 $\pm$ 0.01 <sup>b</sup> <sup>c</sup>	0.31 $\pm$ 0.01 <sup>b</sup> <sup>c</sup>	0.88 $\pm$ 0.02 <sup>c</sup>	0.78 $\pm$ 0.03 <sup>c</sup>
	1.40	1.25 $\pm$ 0.02 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>c</sup>	0.74 $\pm$ 0.03 <sup>d</sup>	0.68 $\pm$ 0.03 <sup>d</sup>
MCH( $\times 10^6 \text{Pgcell}$ )	0.00	0.47 $\pm$ 0.00 <sup>a</sup>	0.48 $\pm$ 0.01 <sup>a</sup>	0.43 $\pm$ 0.01 <sup>a</sup>	0.39 $\pm$ 0.01 <sup>a</sup>
	0.30	0.44 $\pm$ 0.00 <sup>b</sup>	0.36 $\pm$ 0.02 <sup>b</sup>	0.35 $\pm$ 0.01 <sup>b</sup>	0.31 $\pm$ 0.01 <sup>b</sup>
	0.70	0.43 $\pm$ 0.00 <sup>b</sup> <sup>c</sup>	0.31 $\pm$ 0.01 <sup>c</sup>	0.29 $\pm$ 0.01 <sup>c</sup>	0.26 $\pm$ 0.01 <sup>c</sup>
	1.40	0.41 $\pm$ 0.01 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>d</sup>	0.25 $\pm$ 0.01 <sup>d</sup>	0.23 $\pm$ 0.01 <sup>d</sup>
MCHC(g/100ml)	0.00	33.17 $\pm$ 0.16 <sup>a</sup>	33.33 $\pm$ 0.05 <sup>a</sup>	33.33 $\pm$ 0.05 <sup>a</sup>	3.90 $\pm$ 0.10 <sup>a</sup>
	0.30	33.14 $\pm$ 0.20 <sup>a</sup>	33.13 $\pm$ 0.06 <sup>a</sup>	33.13 $\pm$ 0.06 <sup>a</sup>	3.06 $\pm$ 0.10 <sup>b</sup>
	0.70	33.25 $\pm$ 0.15 <sup>a</sup>	33.34 $\pm$ 0.11 <sup>a</sup>	33.34 $\pm$ 0.11 <sup>a</sup>	2.60 $\pm$ 0.10 <sup>c</sup>
	1.40	33.11 $\pm$ 0.16 <sup>a</sup>	33.34 $\pm$ 0.12 <sup>a</sup>	33.34 $\pm$ 0.13 <sup>a</sup>	2.25 $\pm$ 0.12 <sup>d</sup>

Means with the same superscript along the columns are not significantly different (P>0.05).

**Table 4.9:** Mean( $\pm$ SE) of sub-lethal concentrations of glyphosate on some leucocytes differential count of *Heteroclaris*.

Parameter	Conc.(mg/L)	Weeks			
		2	4	6	8
Neutrophils (%)	0.00	31.67 $\pm$ 0.88 <sup>b</sup>	26.33 $\pm$ 0.88 <sup>a</sup>	19.00 $\pm$ 0.58 <sup>c</sup>	35.00 $\pm$ 0.58 <sup>b</sup>
	0.30	27.00 $\pm$ 0.58 <sup>c</sup>	21.00 $\pm$ 0.58 <sup>b</sup>	14.00 $\pm$ 0.58 <sup>d</sup>	28.00 $\pm$ 0.58 <sup>c</sup>
	0.70	30.00 $\pm$ 0.58 <sup>b</sup>	23.00 $\pm$ 0.58 <sup>b</sup>	23.00 $\pm$ 0.58 <sup>b</sup>	37.00 $\pm$ 0.58 <sup>b</sup>
	1.40	35.33 $\pm$ 0.33 <sup>a</sup>	28.00 $\pm$ 0.58 <sup>a</sup>	28.00 $\pm$ 0.58 <sup>a</sup>	41.33 $\pm$ 0.88 <sup>a</sup>
Lymphocytes (%)	0.00	53.67 $\pm$ 0.88 <sup>c</sup>	49.00 $\pm$ 0.58 <sup>c</sup>	46.00 $\pm$ 0.58 <sup>d</sup>	55.00 $\pm$ 0.58 <sup>d</sup>
	0.30	73.33 $\pm$ 2.08 <sup>a</sup>	52.00 $\pm$ 0.58 <sup>b</sup>	73.67 $\pm$ 0.88 <sup>a</sup>	75.00 $\pm$ 0.58 <sup>a</sup>
	0.70	62.00 $\pm$ 1.15 <sup>b</sup>	57.00 $\pm$ 0.58 <sup>a</sup>	67.00 $\pm$ 0.58 <sup>b</sup>	64.00 $\pm$ 0.58 <sup>b</sup>
	1.40	54.67 $\pm$ 0.88 <sup>c</sup>	46.00 $\pm$ 0.58 <sup>d</sup>	49.00 $\pm$ 0.58 <sup>c</sup>	57.00 $\pm$ 0.58 <sup>c</sup>
Basophils (%)	0.00	nd	nd	nd	nd
	0.30	nd	nd	nd	nd
	0.70	nd	nd	nd	nd
	1.40	nd	nd	nd	nd
Eosinophils (%)	0.00	nd	nd	nd	nd
	0.30	nd	nd	nd	nd
	0.70	nd	nd	nd	nd
	1.40	nd	nd	nd	nd
Monocytes (%)	0.00	nd	nd	nd	nd
	0.30	nd	nd	nd	nd
	0.70	nd	nd	nd	nd
	1.40	nd	nd	nd	nd

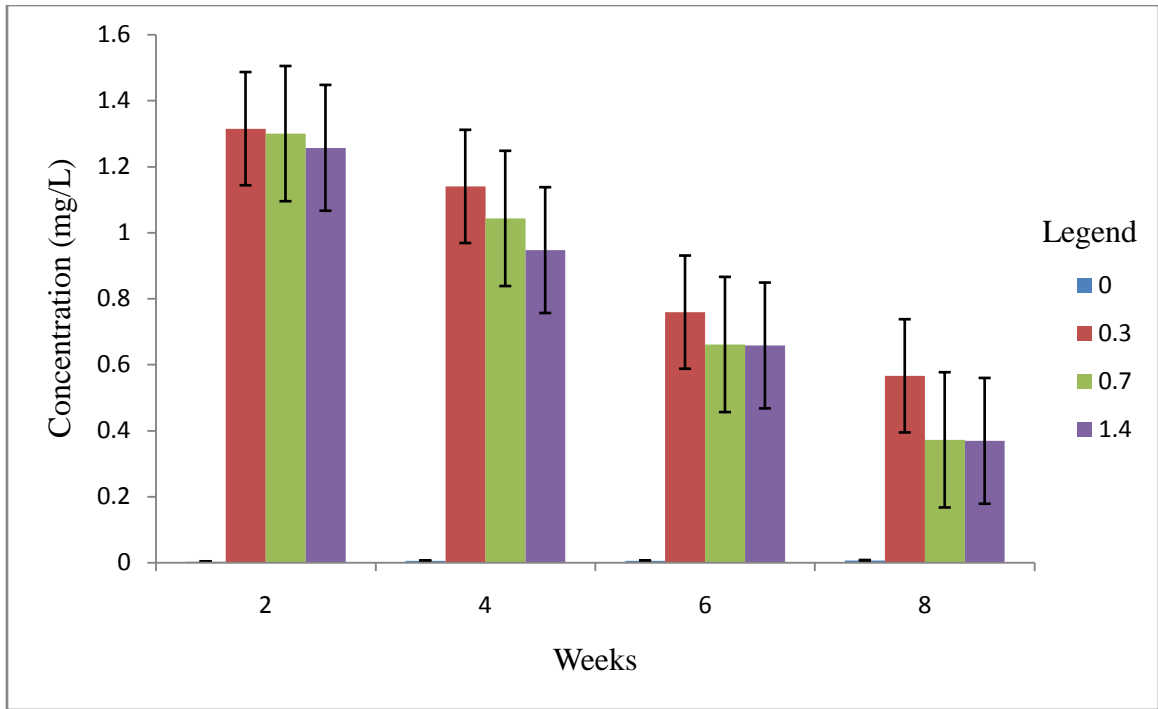
Means with the same superscript along the columns are not significantly different (P>0.05).

nd = not detected

**Table 4.10:** Mean( $\pm$ SE) of catalase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on gills.

Conc. (mg/L)	2 weeks	4 weeks	6 weeks	8 weeks
0.00	0.003 $\pm$ 0.000 <sup>d</sup>	0.006 $\pm$ 0.000 <sup>d</sup>	0.006 $\pm$ 0.000 <sup>d</sup>	0.007 $\pm$ 0.000 <sup>d</sup>
0.30	1.315 $\pm$ 0.000 <sup>a</sup>	1.140 $\pm$ 0.000 <sup>a</sup>	0.759 $\pm$ 0.000 <sup>a</sup>	0.566 $\pm$ 0.000 <sup>a</sup>
0.70	1.300 $\pm$ 0.001 <sup>b</sup>	1.043 $\pm$ 0.000 <sup>b</sup>	0.661 $\pm$ 0.000 <sup>b</sup>	0.372 $\pm$ 0.000 <sup>b</sup>
1.40	1.257 $\pm$ 0.001 <sup>c</sup>	0.947 $\pm$ 0.000 <sup>c</sup>	0.658 $\pm$ 0.000 <sup>c</sup>	0.369 $\pm$ 0.000 <sup>c</sup>

Means with different superscript along the columns are significantly different (P<0.05).

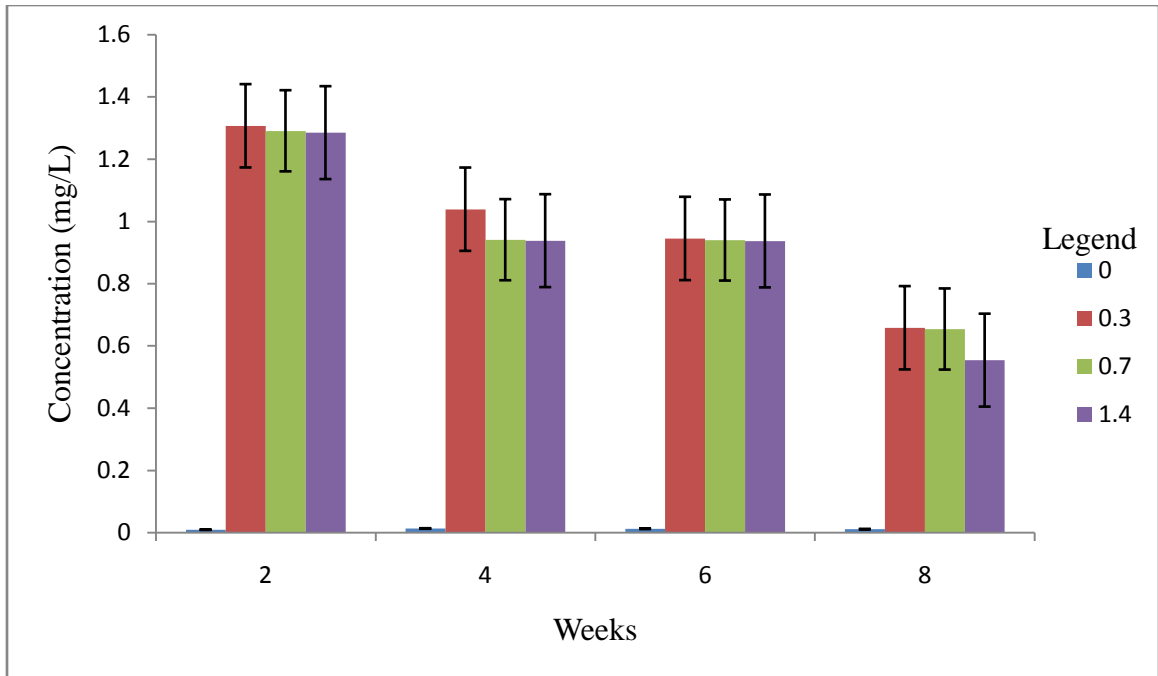


**Figure 4.5:** Graph of Mean±SE catalase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on gills.

**Table 4.11:** Mean( $\pm$ SE) of catalase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* liver.

Conc. (mg/L)	2 weeks	4 weeks	6 weeks	8 weeks
0.00	0.009 $\pm$ 0.000 <sup>d</sup>	0.013 $\pm$ 0.000 <sup>d</sup>	0.012 $\pm$ 0.000 <sup>d</sup>	0.011 $\pm$ 0.000 <sup>d</sup>
0.30	1.307 $\pm$ 0.000 <sup>a</sup>	1.039 $\pm$ 0.000 <sup>a</sup>	0.945 $\pm$ 0.000 <sup>a</sup>	0.658 $\pm$ 0.000 <sup>a</sup>
0.70	1.291 $\pm$ 0.001 <sup>b</sup>	0.941 $\pm$ 0.000 <sup>b</sup>	0.940 $\pm$ 0.000 <sup>b</sup>	0.654 $\pm$ 0.000 <sup>b</sup>
1.40	1.285 $\pm$ 0.000 <sup>c</sup>	0.938 $\pm$ 0.000 <sup>c</sup>	0.937 $\pm$ 0.000 <sup>c</sup>	0.554 $\pm$ 0.000 <sup>c</sup>

Means with different superscript along the columns are significantly different ( $P < 0.05$ ).



**Figure 4.6:** Graph of Mean $\pm$ SE catalase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on liver.

#### 4.2.6 Peroxidase activity for eight weeks exposed to sub-lethal concentration of glyphosate for Heteroclaris on gill.

Peroxidase activity per two weeks for eight weeks exposed to sub-lethal concentration of glyphosate for Heteroclaris gill is shown in Table 4.12.

There was significant difference in gill peroxidase activity ( $P < 0.05$ ) for 2 to 8 weeks as the values have different superscript along the column with 1.40mg/L having the highest activity followed by 0.70, 0.30 and 0.00 respectively. It is however presented in figure 4.5.

#### 4.2.7 Peroxidase activity for eight weeks exposed to sub-lethal concentration of glyphosate for Heteroclaris on liver.

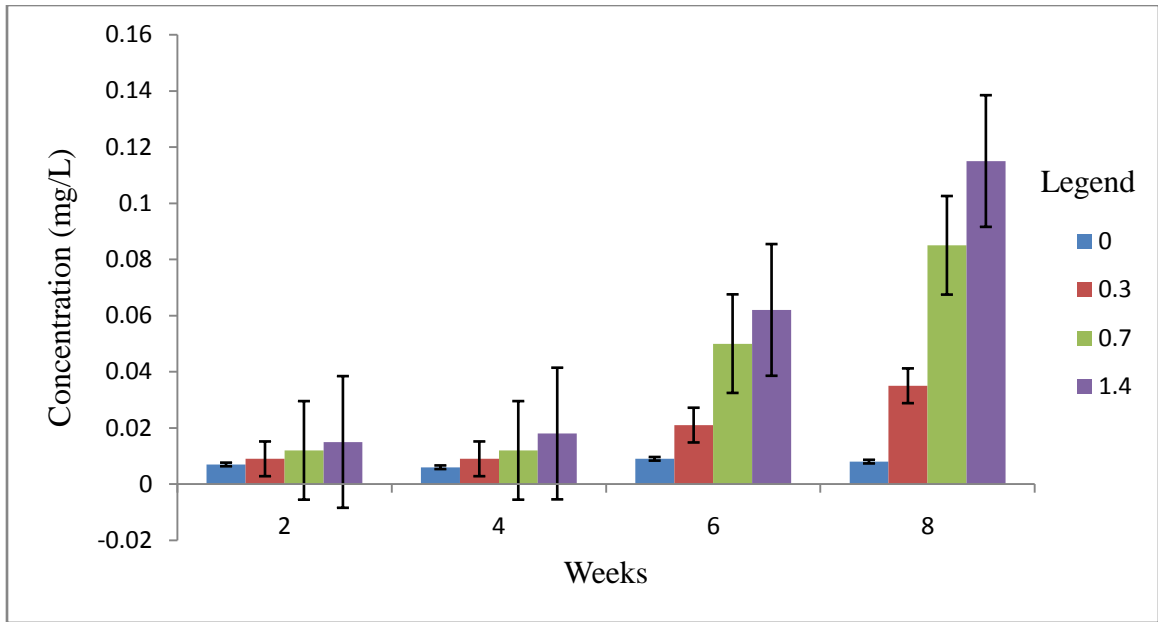
Peroxidase activity per two weeks for eight weeks exposed to sub-lethal concentration of glyphosate for Heteroclaris liver is shown in Table 4.13.

There was significant difference in liver peroxidase activity ( $P < 0.05$ ) for 2 to 8 weeks as the values have different superscript along the column with 1.40mg/L having the highest activity followed by 0.70, 0.30 and 0.00 respectively. It is also presented in figure 4.6.

**Table 4.12:** Mean( $\pm$ SE) peroxidase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on gill.

Conc. (mg/L)	2 weeks	4 weeks	6 weeks	8 weeks
0.00	0.007 $\pm$ 0.001 <sup>d</sup>	0.006 $\pm$ 0.000 <sup>d</sup>	0.009 $\pm$ 0.000 <sup>d</sup>	0.008 $\pm$ 0.000 <sup>d</sup>
0.30	0.009 $\pm$ 0.000 <sup>c</sup>	0.009 $\pm$ 0.000 <sup>c</sup>	0.021 $\pm$ 0.000 <sup>c</sup>	0.035 $\pm$ 0.000 <sup>c</sup>
0.70	0.012 $\pm$ 0.000 <sup>b</sup>	0.012 $\pm$ 0.000 <sup>b</sup>	0.050 $\pm$ 0.000 <sup>b</sup>	0.085 $\pm$ 0.000 <sup>b</sup>
1.40	0.015 $\pm$ 0.000 <sup>a</sup>	0.018 $\pm$ 0.000 <sup>a</sup>	0.062 $\pm$ 0.000 <sup>a</sup>	0.115 $\pm$ 0.000 <sup>a</sup>

Means with different superscript along the columns are significantly different ( $P < 0.05$ ).

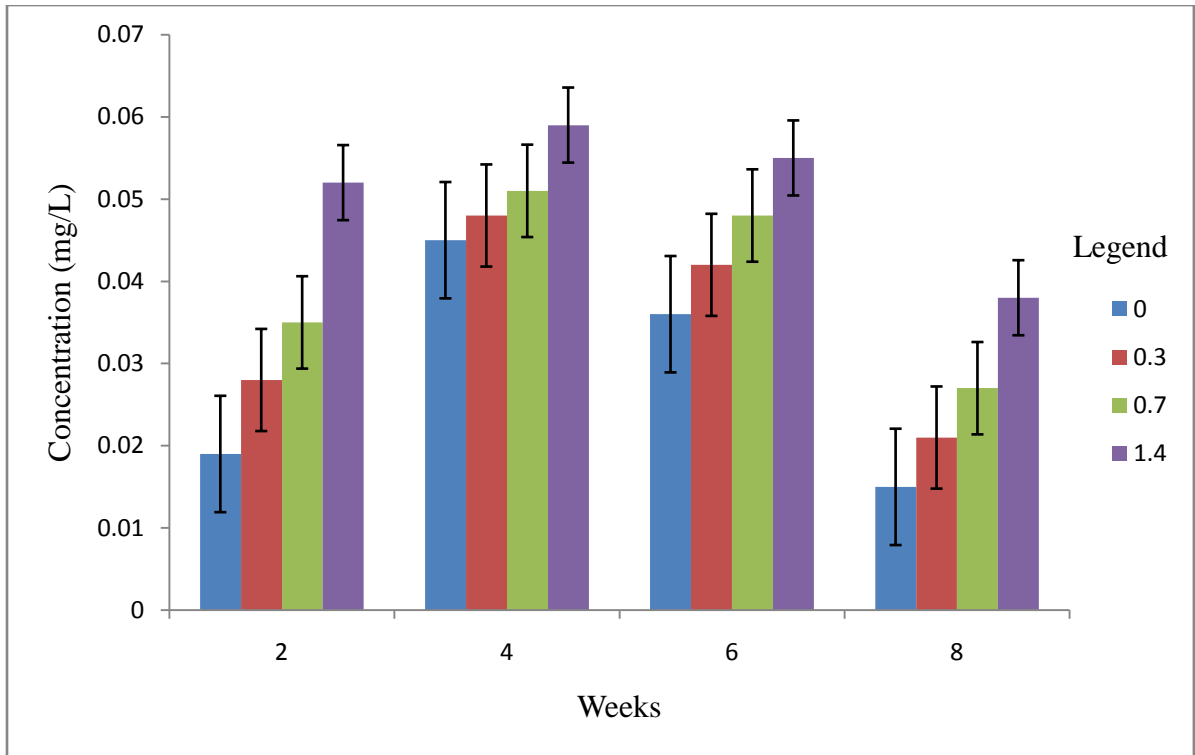


**Fig. 4.7:** Graph of Mean $\pm$ SE peroxidase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on gill.

**Table 4.13:** Mean( $\pm$ SE) peroxidase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heteroclaris* on liver.

Conc. (mg/L)	2 weeks	4 weeks	6 weeks	8 weeks
0.00	0.019 $\pm$ 0.001 <sup>d</sup>	0.045 $\pm$ 0.000 <sup>d</sup>	0.036 $\pm$ 0.000 <sup>d</sup>	0.015 $\pm$ 0.000 <sup>d</sup>
0.30	0.028 $\pm$ 0.001 <sup>c</sup>	0.048 $\pm$ 0.000 <sup>c</sup>	0.042 $\pm$ 0.000 <sup>c</sup>	0.021 $\pm$ 0.000 <sup>c</sup>
0.70	0.035 $\pm$ 0.001 <sup>b</sup>	0.051 $\pm$ 0.000 <sup>b</sup>	0.048 $\pm$ 0.000 <sup>b</sup>	0.027 $\pm$ 0.000 <sup>b</sup>
1.40	0.052 $\pm$ 0.000 <sup>a</sup>	0.059 $\pm$ 0.000 <sup>a</sup>	0.055 $\pm$ 0.000 <sup>a</sup>	0.038 $\pm$ 0.000 <sup>a</sup>

Means with different superscript along the columns are significantly different ( $P < 0.05$ ).



**Figure 4.8:** Graph of Mean±SE peroxidase activity (U/mg protein) for eight weeks exposed to sub-lethal concentration of glyphosate for *Heterocalrias* on liver.

### **4.3 Effect of sub-lethal Concentration of Glyphosate on Growth of Heteroclarias.**

The effect of sub-lethal concentration of glyphosate on the body mean weight of Heteroclarias is shown in Table 4.14.

The initial mean body weight was 21.30g for the control, while 20.80g, 24.60g and 24.80g for concentrations 0.30mg/L, 0.70mg/L and 1.40mg/L respectively. Growth was determined as the difference between the sum of initial individual wet gain and the final weight divided by the initial weight expressed as percentage weight gain as seen in Table 4.14. The percentage weight gain was lower in the treated fish compared to the control.

There was a reduction of growth in the fish exposed to higher concentrations compared to the control groups as seen in specific growth rate (SGR) table. Statistically, there was a significant difference ( $P < 0.05$ ) in percentage weight gain in fish exposed to the different concentrations of the toxicant compared to the control group.

#### **4.3.1 Nutrient utilization**

The effect of sub-lethal concentration of glyphosate on the nutrient utilization parameters of Heteroclarias is shown in Table 4.15.

There was significant reduction in GFCE, FE, PER and NM in the treated fish compared to the untreated fish. The result obtained showed that FCR was higher in treated group than the untreated. The best food utilization from the treated fish was obtained in 0.30mg/L. Statistical analysis shows no significant difference at  $P < 0.05$  in FE between the treated and untreated group.

**Table 4.14:** Growth of Heteroclarias exposed to sub-lethal concentration of glyphosate for eight weeks.

		Concentration (mg/L)			
		0.00	0.30	0.70	1.40
Parameters					
No. of fish		10	10	10	10
Mortality (%)		0	7.50	15.00	22.50
Av.	Initial	21.30	20.80	24.60	24.80
Weight (g)					
Average	Final	32.76	31.23	30.50	29.62
Weight (g)					
Weight Gain (%)		52.54	50.16	33.56	20.68
Weight Gain (g)		11.46 <sup>a</sup>	10.43 <sup>b</sup>	5.90 <sup>c</sup>	4.82 <sup>d</sup>
SGR		0.024 <sup>a</sup>	0.005 <sup>b</sup>	0.004 <sup>c</sup>	0.002 <sup>d</sup>

Means with the same superscript along the rows are not significantly different ( $P>0.05$ ).

**Table 4.15:** Nutrient utilization of *Heteroclaris* exposed to sub-lethal concentration of glyphosate for eight weeks.

Parameters	0.00	0.30	0.70	1.40
FCR	5.98 <sup>d</sup>	6.45 <sup>c</sup>	9.89 <sup>b</sup>	12.64 <sup>a</sup>
GFCE	15.76 <sup>a</sup>	14.86 <sup>b</sup>	8.98 <sup>c</sup>	7.10 <sup>d</sup>
FE	0.14 <sup>a</sup>	0.13 <sup>a</sup>	0.06 <sup>b</sup>	0.04 <sup>b</sup>
PER	0.31 <sup>a</sup>	0.22 <sup>b</sup>	0.17 <sup>c</sup>	0.10 <sup>d</sup>
NM	216.12 <sup>a</sup>	162.45 <sup>b</sup>	110.56 <sup>c</sup>	70.97 <sup>d</sup>

Means with the same superscript along the rows are not significantly different ( $P>0.05$ ).

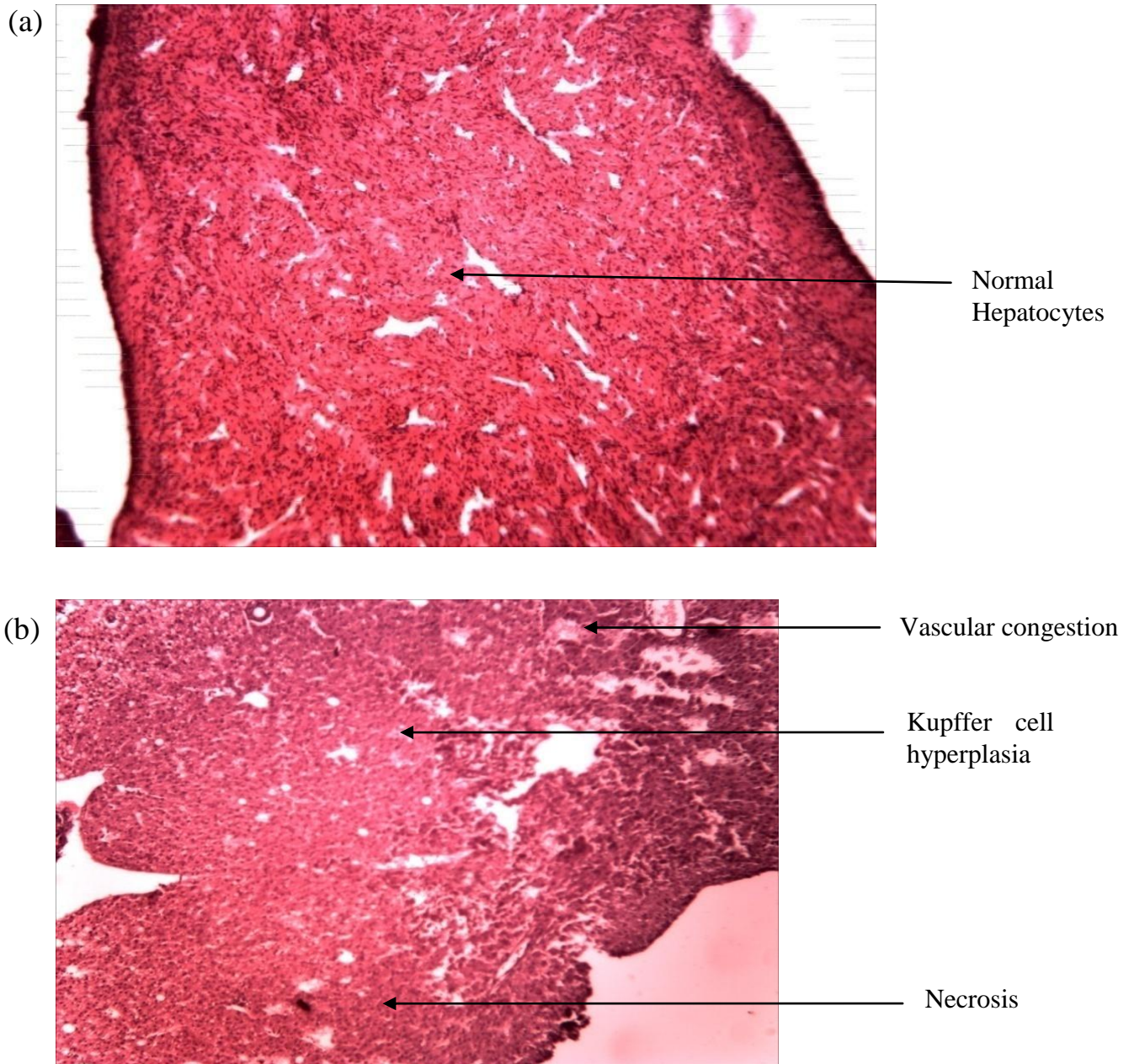
## **4.4 Histopathology of Acute and Sub-lethal Bioassay**

### **4.4.1 Liver**

Section of the liver exposed to the highest concentration (12.60mg/L) of glyphosate were observed to have adipocyte infiltration i.e a group of cells specialized for the storage of fat, found in connective tissue (plate II). Treatment with 10.80mg/L shows kupffer cell hyperplasia as seen in plate (II). The liver from the control group (plate I (a)) showed no sign of hepatic cells damage and no irregularities were observed.

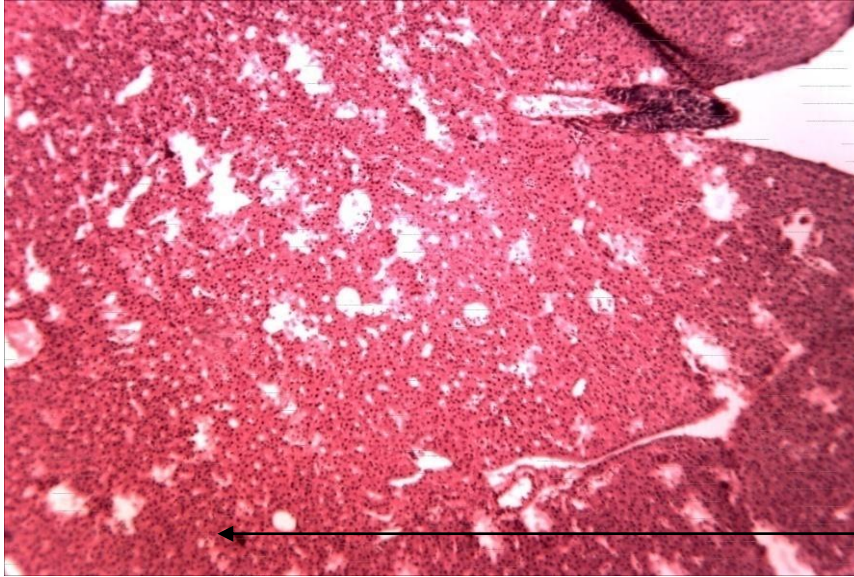
### **4.4.2 Gills**

The gills of the control fish and those of the treated fish exposed to different concentration of glyphosate showed marked difference. The gills of the control fish had normal structure of the filament and lamellae (plate IV (a)) consisting of filament attached to cartilaginous gill bar with finger like projection (secondary lamellae) on each side. The lamellae of treated fish were at various stages of distortion (plates IV (b), V (a, b) and VI (a)). The gill of the fish with concentration 1.40mg/L had marked distortion of the architecture concentrations showed atrophy (reduction of size) to both the filament and their lamellae (plate VI (b)).



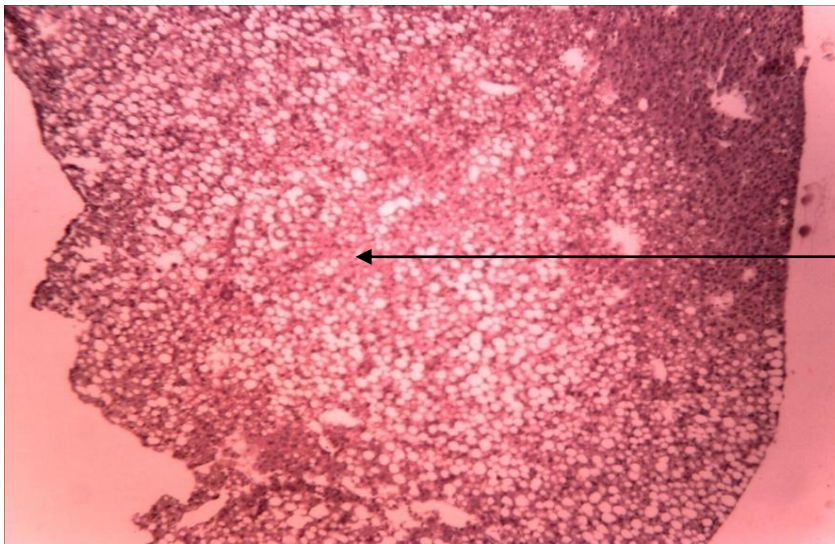
**Plate I:** (a) T.S of liver of *Heteroclaris* with no treatment showing normal hepatocytes (NH). (b) T.S of liver of *Heteroclaris* exposed to 9.0mg/L of glyphosate showing slight vascular congestion (VC), kupffer cell hyperplasia and necrosis (N) X100.

(a)



Kupffer cell  
hyperplasia

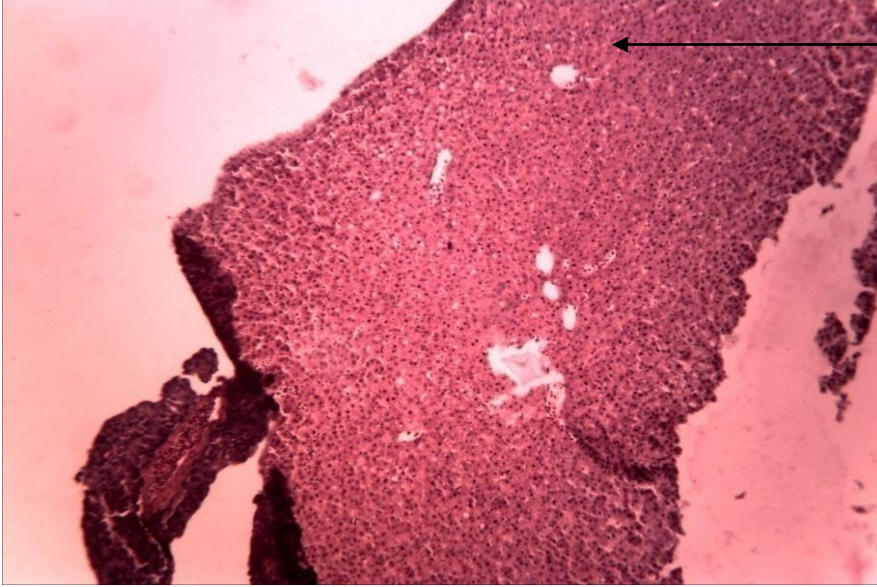
(b)



Adipocyte infiltration

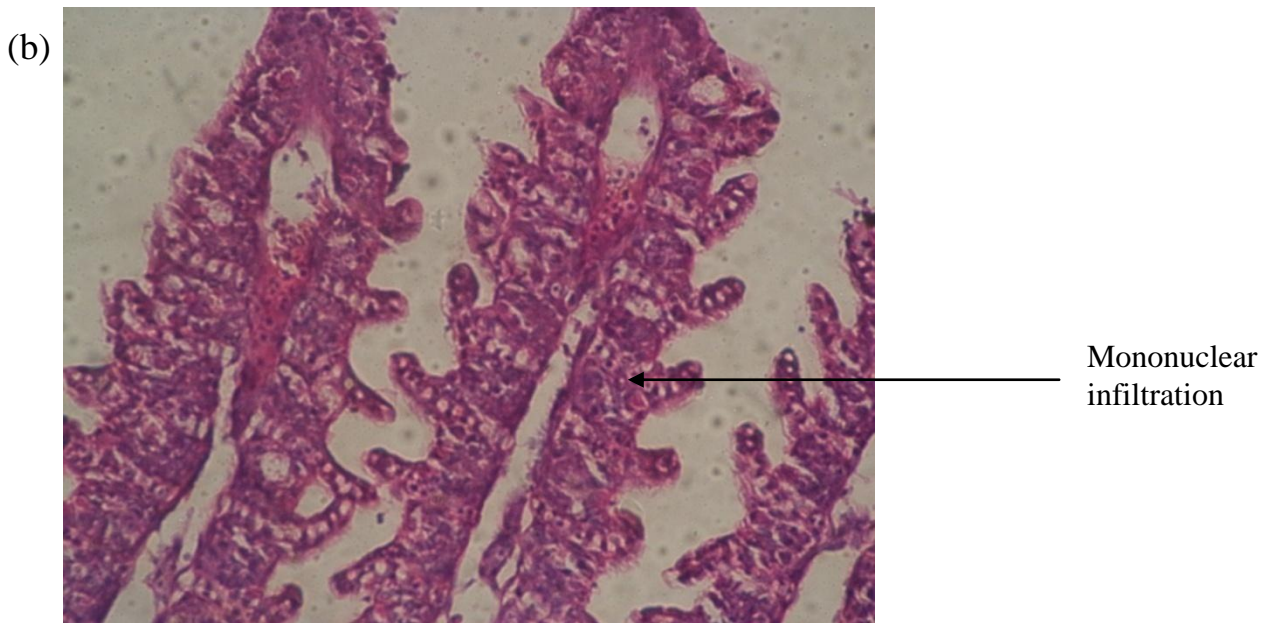
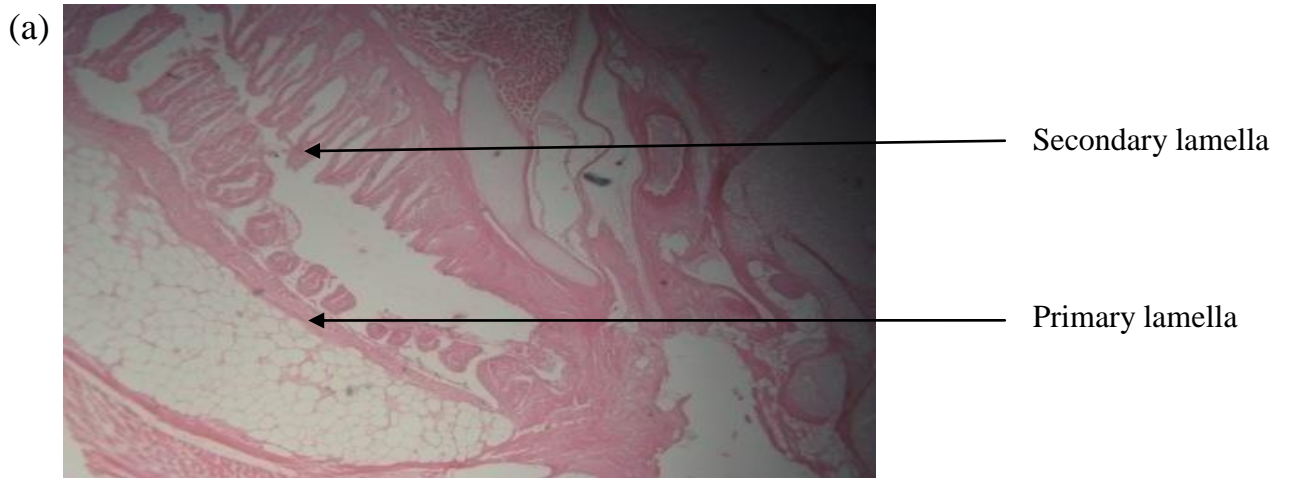
**Plate II:** (a) T.S of liver of *Heteroclaris* exposed to 10.80mg/L of glyphosate showing kupffer cell hyperplasia (KCH) X100. (b) T.S of liver of *Heteroclaris* exposed to 12.60mg/L of glyphosate showing intense adipocyte infiltration (AI) X100.

(a)



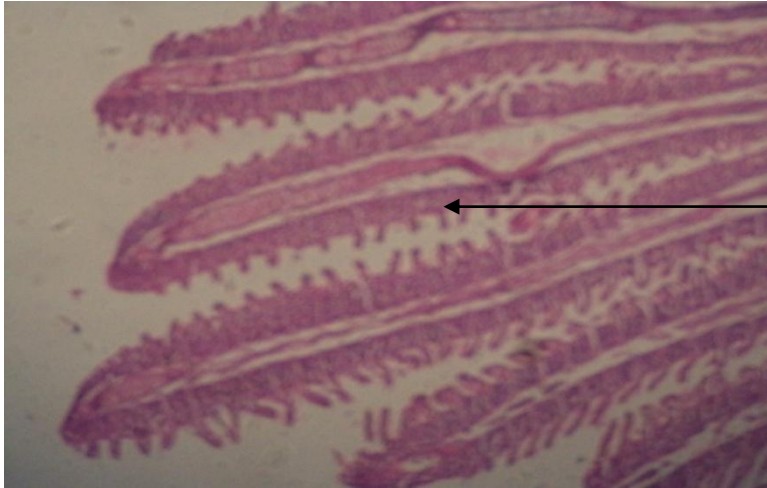
Lymphocyte  
hyperplasia

**Plate III:** (a) T. S of liver of *Heterocladia* exposed to 5.40mg/L of glyphosate showing lymphocyte hyperplasia (LH) X100.



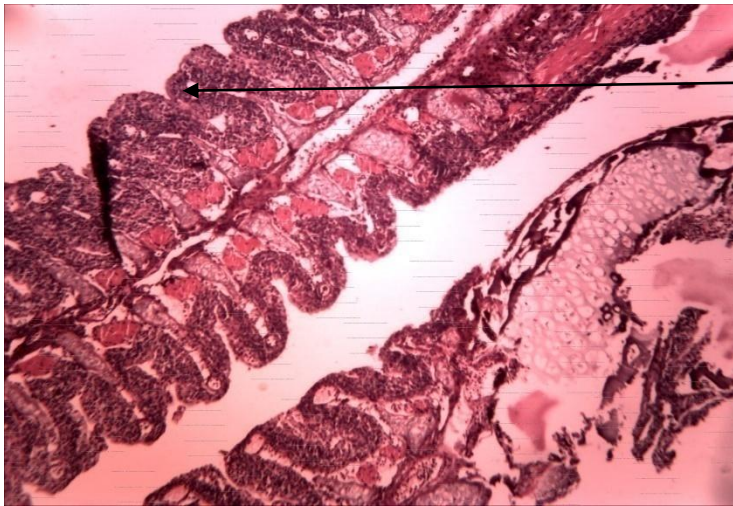
**Plate IV:** (a) Photomicrograph of the gill of the fish in the control group (b) T. S of gill filament of *Heteroclinus* exposed to 10.80mg/L of glyphosate showing mononuclear infiltration (MI) X100.

(a)



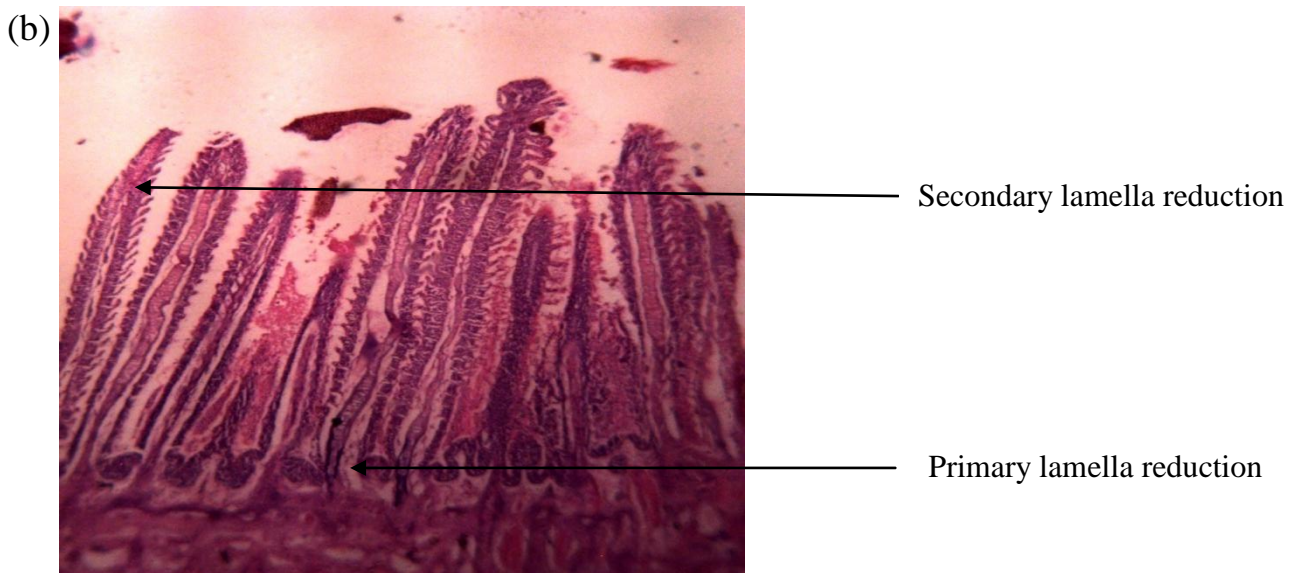
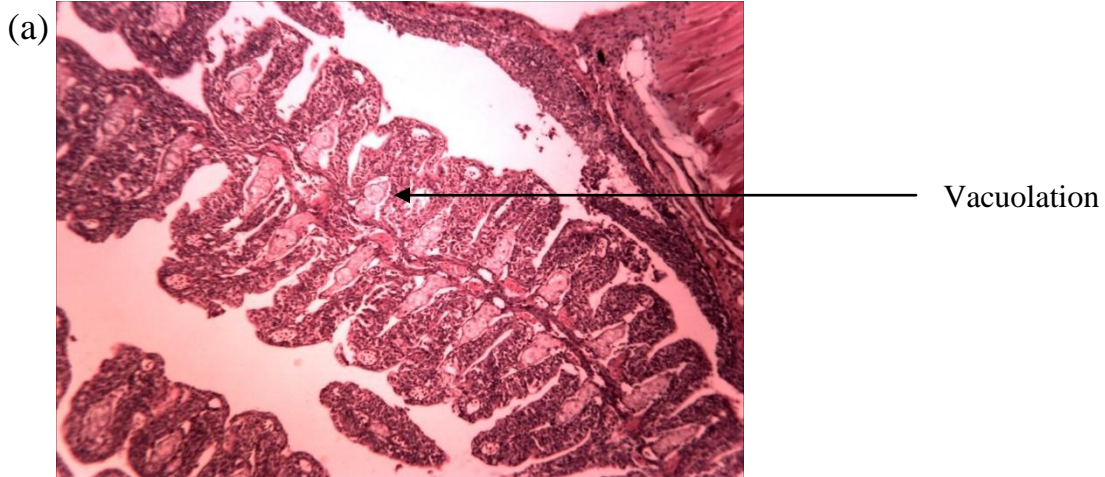
Inflammation of secondary lamella

(b)



Cellular infiltration

**Plate V:** Plate (a) T. S of gill filament of *Heteroclaris* exposed to 12.60mg/L of glyphosate showing inflammation of secondary lamellae (SL) X100. (b) T.S of gill filament of *Heteroclaris* for week 4 exposed to 0.30mg/L of glyphosate showing cellular infiltration (CI) X100.



**Plate VI:** (a) T.S of gill filament of *Heteroclarias* for week 6 exposed to 0.70mg/L of glyphosate showing vacuolation (V) X100. (b) T.S of gill filament of *Heteroclarias* for week 8 exposed to 1.40mg/L of glyphosate showing reduction in size of the primary and secondary lamella X100.

## CHAPTER FIVE

### 5.0 DISCUSSION

The result of this present study indicates the toxic nature of the glyphosate on the haematological, histopathological, oxidative stress, behavioural, physico-chemical parameters and growth rate of *Heteroclinas* fingerlings.

#### 5.1 Haematological Parameters

Poisonous substances exert their harmful effects by interfering with normal chemical reaction within the body; the primary site of action is usually within the cell (Lloyd, 1992). Cellular effects on this study were measured through haematological parameter. Changes in haematological values occur in relation to physiological stress, diseases and toxic environmental conditions (Wedemeyer and Yasutake, 1977). Fish blood reflects the pathophysiological status exposed toxicants (Sampath *et al.*, 1993). Anees (1978) reported from his work on haematological abnormalities in *Channa punctatus* exposed to sub-lethal and chronic effect of the organophosphorus insecticides that the blood was affected prior to any other visible change.

Haematological parameters observed during the acute and sub-lethal studies included; red blood cell counts (RBCC), white blood cell counts (WBCC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophils, lymphocytes, eosinophils, basophils and monocytes. Haematological parameters were of

importance in this study because of the role blood played in respiration (RBCC, PCV and Hb) and defense mechanism (WBCC, neutrophils, lymphocytes, eosinophils, basophils and monocytes).

The result of this study revealed that Heteroclaris exposed to various concentrations of glyphosate recorded decrease packed cell volume ( $\text{mm}^3$ ), total red blood cell (RBC) and haemoglobin (Hb) but an increase in total white blood cells (WBC). Similarly, the sub-lethal toxicity bioassay also showed dose dependent reduction in packed cell volume (PCV), red blood cells (RBC) and haemoglobin (Hb). Both the acute and sub-lethal toxicity tests showed haematological changes which is an indication of severity in the treated fish. The anaemia effect could be due to an inhibition in erythrocyte production and haemodilution. Erythropenia (deficiency in the number of red blood cells) was reflected by the reduced haemoglobin content and haematocrit value as well as erythrocyte sedimentation rate (ESR) (Eisler, 1967). Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen carrying capacity in the blood. The significant decrease in the packed cell volume (PCV) in this study could be attributed to gill damage and or impaired osmoregulation causing anaemia and haemodilution. The findings were similar with anaemia associated with erythropenia that was reported by Srivatava and Mishra (1979) in *Colisa fasciatus* after acute exposure to lead. Similar results have been reported for several freshwater fishes (Khalaf Allah, 1999; Balathakur and Bais, 2000; Rehulka, 2000; Gbem *et al.*, 2003; Aderolu *et al.*, 2010). Similar findings were reported by Maheswaran *et al.* (2008) where they observed a reduction in RBC and Hb in *Clarias batrachus* exposed to sub-lethal concentration of

mercuric chloride. Thargavel *et al.* (1994) reported similar results when exposing the fish *Sarotherodon mossambicus* to dimecron. While Singh and Srivastava (1994) showed another organophosphate insecticide formothion gave a significant increase in the total erythrocyte count and haemoglobin content in fish. *Oreochromis niloticus* exposed to cadmium also showed significant reduction in RBC, Hb and PCV (Kaoud *et al.*, 2011).

The increase in white blood cell in acute and sub-lethal bioassay studies could be associated with an increase in antibody production which help in survival and recovery in the fish exposed to sub-lethal concentration of glyphosate. Similar trend was also reported by Joshi *et al.* (2002) and Ekrem *et al.* (2013). The present findings suggest hypersensitivity of leucocytes to glyphosate. Similar result was obtained by Singh (1982) and Maheswaran *et al.* (2008) who showed increase in total leucocyte count of *Channa punctatus* and *Clarias batrachus* after exposure to derma orange and mercuric chloride respectively. Decreased erythrocyte count and haemoglobin content in freshwater fish *Channa punctatus* after acute exposure to diazonin was also reported by Anees (1978). Other effective substance of organophosphorus pesticide also induced changes which give evidence of decreased haematopoeisis followed by anaemia in fish. Further examples and changes in the erythrocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (Bernarji and Rajendranath, 1991), trichlorphon in *Patractus mesopotamicus* (Tavares *et al.*, 1999) malathion in *Cyprinus wabsoni* (Khattak and Hafeez, 1996), formothion in *Heteropneustes fossilis* (Singh and Srivastava, 1994) and Ekolux organophosphorus preparation in *Oreochromis mossambicus* (Sampath *et al.*, 1993).

The fluctuation in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the present study, clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, depicting anemic condition. Bhagwant and Bhikajee (2000) observed similar fluctuations. The Mean Corpuscular Haemoglobin Concentration is a good indicator of red blood cell swelling (Wepener *et al.*, 1992), the Mean Corpuscular Haemoglobin Concentration, which is the ratio of haemoglobin concentration as opposed to the haematocrit, is not influenced by the blood volume for the number of cells in the blood, but can be interpreted incorrectly only when the new cells, with a different haemoglobin concentration, are released into blood circulation (Savio and Nikinma 1981).

Neutrophils increases at 7.20mg/L, 9.00mg/L and decreased at 10.80mg/L and 12.60mg/L while lymphocytes increases with concentration for acute studies. For sub-lethal studies, neutrophils decreased from 5.40, 7.20, 9.00, and 10.80mg/L respectively and increased at 12.60mg/L while lymphocytes increased in all the treatments. This fluctuation could possibly be as result of antibodies released in the circulatory system of the fish as a form of defense. Babatunde (1997) stated that lymphocytes consist the majority of white blood cell present in peripheral blood of *O. niloticus*, this study also showed neutrophils and lymphocytes increased as the concentration of the toxicant increased compared with basophils, eosinophils and monocytes. Ghosh and Banerje (1993) reported lymphopenia on both neutrophils and eosinophils and also granulocytosis in *Heteropneustes fossilis* after exposure to 96h LC<sub>50</sub>. These changes in differential

leucocytes count also give evidence for decreased level non-specific immunity in fish after the exposure to toxic substances (Svoboda *et al.*, 2003).

## **5.2 Histopathology**

### 5.2.1 Gills

Gills are generally considered good indicator of water quality (Rankin *et al.*, 1982) being models for studies of environmental impact (Mckim and Erickson, 1991; Wendelaar Bonga and Lock, 1992) since they are the primary route for pesticides. In the present investigation, the gills of the fish exposed to glyphosate exhibited marked histopathological changes. The main features observed in exposed fish to acute concentration of the toxicant include degeneration of epithelium of secondary gill lamellae, shrunken filaments and secondary lamella, fusion of secondary gill lamellae. This suggests that the effect of the pesticide is damaging to the respiratory and osmoregulatory function of the fish and also cause vacuolization. Damage to the gills led to hyperventilation, fatigue and finally death. The finding was similar to that of Omoregie and Ufodike (1991), Omoregie *et al.* (1990), Omitoyin *et al.* (2006) and Ogundiran *et al.* (2010).

During the sub-lethal bioassay fish exposed to the highest concentration showed damage to the gill indicating hypertrophy condition. This is similar to Srivastava and Singh (1994) who studied the effect of sub-lethal concentration of malthion chloride on the histopathology of the gills of *Channa gachua* and observed hyperplasia, hypertrophy vacuolation in primary gill lamellae. Rana and Eragi (2004) worked on histopathological

alteration induced by pesticides on gills of mudskipper and reported that the exposure of fish for two weeks to cypermethrin resulted in the bulging and fusion of secondary lamellae, lifting of epithelial cells. Fish exposed to the lowest concentration of dimethoate had no histopathological changes. Similar observations were reported by Chinabut *et al.* (1987) and Auta (2001).

### 5.2.2 Liver

The liver is the main organ for detoxification (Dutta *et al.*, 1993) that suffers serious morphological alteration in fish exposed to pesticides (Rodrigues and Fanta, 1998). The liver of the exposed fish had vacuolated cells showing evidence of fatty degeneration as noted by Thiyyajah and Grizzle (1985). Liver of the treated fish also showed necrosis, which resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification and is similar to the observation of Rahman *et al.* (2002). Omoregie and Ufodike (1991) reported liver vacuolation in *O niloticus* exposed to Actellic 25EC.

### 5.3 Oxidative Stress

The antioxidant activity for acute toxicity on gill shows that catalase and peroxidase activities increased at 5.40mg/L and 7.20mg/L but decreased at 9.00mg/L, 10.80mg/L and 12.60mg/L while peroxidase activities increased from 5.40mg/L to 7.20mg/L and decreased from 9.00mg/L to 10.80mg/L and increased at 12.60mg/L. The increase in activities indicates that the fish is stressed as a result of the toxicant levels. Different

authors like Bindu and Philip (2001), Farombi *et al.* (2008), Rajamanickam and Muthuswamy (2009), Radovanovic *et al.* (2010), Kandemir *et al.* (2010), Doherty *et al.* (2010), Aysel *et al.* (2010), Nogueira *et al.* (2010), Obaiah and Usha (2012) variously supported the present result. For sub-lethal studies, catalase activities for gill shows increase in activity from 2-4 weeks and 6-8 weeks increased from 0.30mg/L which increased again from 0.70-1.40mg/L. The catalase activities for liver decreased with increase in concentration. This fluctuation could probably be that the fish was converting the hypoxic level to usable form. The catalase activities for the liver decreased with increase in concentration. However, the peroxidase activities for gill and liver shows increase in activity as the concentration increased from 0.30-1.40mg/L for 2-8 weeks. This trend is similar to the findings of Obaiah and Usha (2012).

#### **5.4 Behavioural**

The present investigation showed that exposure of *Heteroclinus* to glyphosate in water induces a variety of abnormalities in the behavior of the fish. To test the whole body response, observation was made on what happened to the fish immediately after been exposed to the acute and sub-lethal concentration. *Heteroclinus* showed behavioural changes against glyphosate intoxication. These were loss of equilibrium, frequent surfacing, agitated and erratic swimming, loss activity and finally death.

The result of both acute and sub-lethal concentration studies shows hyper-active to hypo-active to an initial agitated swimming response to increase exposure time when severely intoxicated. The behavioural alteration may occur as a result of nervous impairment due to blockade of nervous transmission among the system and various effector sites (Fryday

*et al.*, 1996). Similar observations were made by Matsumura (1975) who reported that hyperactivity of fish on introduction to an unfavourable environment as the primary and principal sign of system failure due to pesticide poisoning which affected physiological and enzyme activities. Pal and Konar (1981) also observed disruption of the functioning of the nervous system of fish, resulting in slow and lethargic swimming, erratic movement and loss of equilibrium. Heteroclaris were also found to secrete mucous on the gill filament and body surface prior to death following glyphosate poisoning. This phenomenon is evidently a symptom of the inflammatory reaction of the gill and body surface to the toxicant. Similar findings were made by many authors (Auta *et al.*, 2000; Hossain *et al.*, 1987; Svoboda *et al.*, 2003), who studied the effect of pesticides on different species. There was an increase in mortality as the concentration of glyphosate increases. The result showed that the mortality of fish were dose-dependent. In acute toxicity bioassay, the highest mortality of 9 fish was recorded in the highest concentration of 12.60mg/L, while the lowest of 5 was seen in the lowest concentration of 5.40mg/L, the 96hr LC<sub>50</sub> value in the acute toxicity test (Svoboda *et al.*, 2002). The 96hr LC<sub>50</sub> (the medium lethal concentration) of glyphosate to Heteroclaris in this study was 7.06mg/L. The 96hr LC<sub>50</sub> values ranges in tenths to several tens of mg/L (Seikai, 1982; Giddings *et al.*, 1996 and Tsuda *et al.*, 1997).

The beat frequency of Heteroclaris exposed to the various concentrations of glyphosate was used to measure the respiratory rate.

#### 5.4.1 Tail fin beat

The tail fin beat of *Heteroclinarias* to the toxicant (5.40, 7.20, 9.00, 10.80 and 12.60mg/L) at 12<sup>th</sup> and 24<sup>th</sup> hours were higher than in control fish at the 48<sup>th</sup> and 96<sup>th</sup> exposure time. The rates tend to stabilize at 48<sup>th</sup> hour but thereafter 72<sup>nd</sup> and 96<sup>th</sup> hour; there was steady decline with increase in exposure time and toxicant concentration. The increase in tail fin beat may be associated with sudden response of the fish to the shock of exposure to the agro-chemical (Chindah *et al.*, 2004). This also indicates hyperventilation to cope at the initial period but with increase in exposure, the fish become weak and finally die due to suffocation. Similar findings were reported by Babtunde (1997), Auta *et al.* (2000) and Yaji (2012).

#### 5.4.2 Operculum ventilation rate

The opercular ventilation rate of *Heteroclinarias* to the toxicant (5.40, 7.20, 9.00, 10.80 and 12.60mg/L) at 12<sup>th</sup> and 24<sup>th</sup> hours were higher than in control fish at the 48<sup>th</sup> and 96<sup>th</sup> exposure time. The rates tend to stabilize at 48<sup>th</sup> hour but thereafter 72<sup>nd</sup> and 96<sup>th</sup> hour; there was steady decline with increase in exposure time and toxicant concentration. The increase in opercular ventilation rate may also be associated with sudden response of the fish to the shock of exposure to the agro-chemical (Chindah *et al.*, 2004). This also indicates hyperventilation to cope at the initial period but with increase in exposure, the fish become weak and finally die due to suffocation. Similar findings were reported by Ufodike and Omoregie (1990), Babtunde (1997) and Auta *et al.* (2000). The opercular movement in the teleost, *Tilapia guineensis* has been reported to decline after an initial increase on chlorpyrifos (Chindah *et al.*, 2004). Pal and Konar (1987) observed a

decreased in respiratory rates and suggested decreased oxygen consumption because the fish has become fatigued due to several attempts to escape from the toxic medium or frequently surfacing to facilitate oxygen intake. These behaviour patterns suggest respiratory impairment, probably due to the effect of the toxicant on the gills and general metabolism.

### **5.5 Physico-chemical Parameters**

The results of the physicochemical parameters shows a significant increase after exposure for pH, temperature, total dissolved solids (TDS), electrical conductivity (EC), and hardness while dissolved oxygen and alkalinity decreased. Studies have shown that when water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they live in close association with their environment and are sensitive to slight fluctuation that may occur within their internal environment (Cassilas and Smith, 1977).

### **5.6 Growth Rate**

This study revealed a significant decrease ( $P < 0.05$ ) in weight gain of the treated group compared to the untreated group. The study also showed that growth rate was reduced both as the concentration of glyphosate and exposure period increased. The reduction in growth rate could be due to lower feeding rate due to the effect of the toxicant, or an increased activity associated with an attempt to avoid the intoxication, or more energy

was used up on chemical detoxification and tissue repair. O'Brien (1976) reported that the acetyl cholinesterase inhibition decreased the feeding rate due to impairment of impulse transmission. Pal and Konar (1987) reported that the growth of *Oreochromis niloticus* was considerably reduced by sub-lethal concentration of methyl parathion. Several workers reported a reduction of growth in fish to various pollutants (Babatunde, 1997; Lloyd, 1992; Omoregie *et al.*, 1995; Shammgavel *et al.*, 1988). Fishes are noted to increase their metabolic activities and excretion of toxicant, hence making more energy available for homeostatic maintenance than storage which could be used for growth (Gbem *et al.*, 2003). The fish from the untreated group gave the best weight gain, specific growth rate (SGR), feed conversion rate (FCR), protein efficiency ratio (PER). The result of this study showed a dose-dependent reduction in gross feed conversion efficiency (GFCE).

## CHAPTER SIX

### 6.0 SUMMARY

The results of the acute toxicity showed that glyphosate was toxic to *Heteroclaris* (hybrid), with  $LC_{50}$  value of 6.838mg/L. The mortality threshold indicates it is very toxic because it is below 1mg/L (Cairns and Dickson, 1975). The behavioural and morphological symptoms observed during acute study included loss of equilibrium, hyperactive to hypoactive, overreactive to underreactive to startle response and fish hold out their pectoral and pelvic fins. The acute and sub-lethal exposure of *Heteroclaris* to glyphosate elicited significant changes in histopathology of fish gill and liver, and some haematological and enzymatic parameters. The toxicant elicited acute and haemolytic anaemia due to exposure to acute and sub-lethal concentrations. The result of the study on enzymatic parameters showed that, acute and sub-lethal concentrations of the toxicants induced the oxidative stress of the fish. The acute and sub-lethal histopathological degenerations observed in the gills of fish exposed to the toxicant include; attenuated primary lamellae, loss of epithelial and mucous cells, marked loss of secondary lamellae and necrosis of secondary lamellae while the distortions are characterized in the liver as mild fatty change, mild inflammatory infiltrate, extensive areas of necrosis and moderate steatosis. It was also observed that the chemical adversely affected the growth of *Heteroclaris* due to exposure to sub-lethal concentration of toxicant. Glyphosate elicited high significant ( $P < 0.05$ ) inhibitions in all nutrient utilization parameters (LWG, SGR, FCR, GFCE, FE, and NM). The findings on the investigated parameters on this fish might be helpful for possible early diagnosis of a

disease before it becomes an epidemic and also in the assessment of possible toxic effects of various environments at both acute and sub-lethal concentration exposures.

## **6.1 Conclusions**

In conclusion, both acute and sub-lethal concentrations of glyphosate are harmful and posed toxic metabolic stress to *Heteroclaris* which include the following:

1. The results of the acute toxicity shows that glyphosate was toxic to *Heteroclaris* with LC<sub>50</sub> value of 6.838mg/L.
2. Changes in haematological parameters such as haemoglobin, erythrocyte count and PCV in both acute and sub-lethal bioassay were dose dependent while behavioural responses such as agitated and erratic swimming, increased gulping of air (ventilation rates) to cope with the respiratory distress.
3. The enzyme activity estimation in *Heteroclaris* intoxicated with glyphosate indicates that the exposed fish are faced with serious metabolic crisis.
4. A reduction in weight could be either due to lower feeding rate or an increased activity associated with attempts to avoid the polluted medium or even an increased expenditure of energy on chemical detoxification and tissue repair.

## **6.2 Recommendations**

Based on the major findings from this study, the following recommendations were made;

1. Further research should be carried out to investigate and establish the effects of this toxicant insitu i.e. rivers, ponds, lakes etc.
2. Further research should be carried out to investigate and establish the effects of this toxicant on kidney, muscle, skin and brain.
3. Finally, effects of other antioxidant enzymes like Superoxide Dismutase (SOD) and Glutathione-s-transferase (GST) can be further investigated.

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## APPENDICES

### Appendix I

Calculation of reagents for oxidative stress

Phosphate buffer

1. Catalase                    50mM                    pH 7.0
2. Peroxidase                100mM                   pH 6.5
3. Protein extraction    50mM                    pH7.8

### PROTEIN EXTRACTION

1. Add 1.5ml of phosphate buffer, squash on ice and centrifuge for 15 minutes at 10,000rpm.
2. Use the supernatant for protein and enzyme assay.

### CATALASE DETERMINATION

Phosphate buffer 50mM pH 7.0

Hydrogen peroxide 0.036%

50Mm of potassium phosphate buffer using  $\text{KH}_2\text{P}_0_4$ -MW- 36.9

$$\frac{x}{136} = 50\text{mM}$$

$$x = \frac{136 \times 50}{1000} = \frac{6845}{1000\text{ml}}$$

$$= 6.8\text{g}$$

$$6.8\text{g} = 1 \text{ litre}$$

$$500\text{ml}$$

$$\frac{6.8\text{g} \times 500}{1000} = \frac{3400}{1000}$$

$$1000 \quad 1000$$

$$= 3.4\text{g} - 500\text{ml}$$

$$100\text{ml}$$

$$\frac{3.4\text{g} \times 100}{500} = 0.68$$

$$0.68\text{g} - 100\text{ml}$$

$$1.36\text{g} - 200\text{ml}$$

Using  $\text{H}_2\text{O}_2$ —30%

$$C_1 = 30$$

$$V_1 = X$$

$$C_2 = 0.036$$

$$V_2 = 100\text{ml}$$

$$C_1V_1 = C_2V_2$$

$$30 \times X = 0.036 \times 100$$

$$X = \frac{0.036 \times 100}{30} = 120\mu\text{l}/1.2\text{ml}$$

1.2ml of  $\text{H}_2\text{O}_2$

98.8ml of distilled/Phosphate buffer

## PEROXIDASE DETERMINATION

1. Pyrogallol – 0.05M in 0.1M phosphate buffer, pH 6.5

0.1M Phosphate buffer  $\text{KH}_2\text{PO}_4$ —13.61g

13.61g—1000ml

17.42g—1000ml

Prepare 0.05M pyrogallol

MW of pyrogallol—126.11  $\text{C}_6\text{H}_6\text{O}_3$

$$\frac{x}{126.11} = 0.05$$

$$= \frac{0.05 \times 126.11}{1000}$$

$$= \frac{6.3055}{1000}$$

0.006g--- 1 litre

0.006g--- 1000ml      0.003--- 500ml

$x$       100ml

$$\frac{0.006 \times 100}{1000} = \frac{x}{1000}$$

$$1000 \quad 1000$$

$$= 0.0006 \longrightarrow 100\text{ml}$$

Should be freshly prepared.

0.0006 in 100ml of phosphate buffer 6.5

2. Preparation of 1% Hydrogen Peroxide

$\frac{30}{100} \times 1$

100

30

300

= 3.3ml of H<sub>2</sub>O<sub>2</sub>

96.7ml of buffer

3.3ml of hydrogen peroxides in 96.7ml of phosphate buffer freshly prepared.

## Appendix II

II. Conversation from ml/L to mg/L for 96hrs

1.  $C_1=360\text{g/L}$

$$V_1=0.3\text{ml/L}$$

$$C_2 = ?$$

$$V_2 = 20,000\text{ml}$$

$$C_2 = \frac{360 \times 0.3}{20,000} = \frac{108}{20000}$$

$$= 0.0054\text{g/L}$$

Conversation to mg/L

$$1\text{g}—1000\text{mg}$$

$$0.0054\text{g}-- \chi$$

$$\chi = 0.0054 \times 1000$$

$$= 5.4\text{mg/L}$$

2.  $C_1= 360\text{g/L}$

$$V_1= 0.4\text{ml/L}$$

$$C_2 = ?$$

$$V_2 = 20,000$$

$$C_2 = \frac{360 \times 0.4}{20000}$$

$$20000$$

$$= \frac{144}{20000} = 0.0072$$

$$20000$$

$$= 7.2 \text{mg/L}$$

3.  $C_1 = 360 \text{g/L}$

$$V_1 = 0.5 \text{mL}$$

$$C_2 = ?$$

$$V_2 = 20,000 \text{ml}$$

$$C_2 = \frac{360 \times 0.5}{20,000} = \frac{180}{20,000} = 0.009$$

$$20,000 \quad 20,000$$

$$= 9.0 \text{mg/L}$$

4.  $C_1 = 360 \text{g/L}$

$$V_1 = 0.6 \text{ml/L}$$

$$C_2 = ?$$

$$V_2 = 20,000$$

$$C_2 = \frac{360 \times 0.6}{20,000}$$

$$20,000$$

$$= \frac{216}{20,000} = 0.0108$$

$$20,000$$

$$= 10.8 \text{mg/L}$$

5.  $C_1 = 360 \text{g/L}$

$$V_1 = 0.7 \text{ml/L}$$

$$C_2 = ?$$

$$V_2 = 20,000$$

$$C_2 = \frac{360 \times 0.7}{20,000} = \frac{252}{20,000} = 0.0126 \text{g/L}$$

$$20,000 \quad 20,000$$

$$= 12.6 \text{mg/L}$$

## Appendix III

### III. Calculation for the sub-lethal bioassay value

$$LC_{50} = 6.838\text{mg/L}$$

$$1. \frac{1}{5} \times \frac{6.838}{1} = 1.3676$$
$$= 1.4\text{mg/L}$$

$$2. \frac{1}{10} \times \frac{6.838}{1} = 0.6838$$
$$= 0.7\text{mg/L}$$

$$3. \frac{1}{20} \times \frac{6.838}{1} = 0.341$$
$$= 0.3\text{mg/L}$$