TOXICITY OF PARAQUAT TO NILE TILAPIA (*Oreochromis niloticus)* FINGERLINGS

By

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FAQ/12/0463

A PROJECT REPORT SUBMITTED TO THE

DEPARTMENT OF FISHERIES AND AQUACULTURE

IN PARTIAL FULFILLMENT OF REQUIREMENT FOR

THE AWARD OF BACHELOR OF FISHERIES AND AQUACULTURE

IN THE FACULTY OF AGRICULTURE

FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE,NIGERIA

NOVEMBER, 2017.

DECLARATION

I, AJISODUN, ABIIMBOLA FUNMILAYO hereby declare that this project titled "TOXICITY OF PARAQUAT TO NILE TILAPIA (*Oreochromis niloticus)* FINGERLINGS" has been performed by me in the Department of Fisheries and Aquaculture under the supervision of Dr. A.M. AKINSOROTAN. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree at any University.

.....pee..... .....18/12/2017.....

Ajisodun, Abimbola Funmilayo DATE

CERTIFICATION

This is to certify that the experiment reported here was conducted by :

MISS .AJISODUN, ABIMBOLA FUNMILAYO

The report has been read and approved by the department of fisheries and aquaculture having met the requirement of the department of fisheries and aquaculture, faculty of agriculture, Federal University Oye-Ekiti, for the award of Bachelor of Fisheries and Aquaculture.

.....

Dr. A.M. AKINSOROTAN

PROJECT SUPERVISOR

23-01-18

DATE

Dr. T.O. BABALOLA

HEAD OF DEPARTMENT

.....

DATE

DEDICATION

I dedicate this project to almighty God and to my late father Mr. Ajisodun Joseph and my mother Mrs. Ajisodun Florence and to the Ajisodun's.

ACKNOWLEDGEMENTS

I appreciate almighty God for the success of this project and for his support, guide, help during my study in the university.

My appreciation goes to my supervisor Dr. A.M. Akinsorotan for his advice and contribution towards the success of this project work, and I also appreciate the Head of the Department, Dr. T.O Babalola, and other lecturers, Prof. Nwanna, Prof. Araoye, Dr. J.B. Olasunkanmi, Dr. T.O Ariyomo, Dr. Okeke, Mr.B.P Omobepade and Mrs. Elesho.

I specially appreciate my parents Late Mr. Ajisodun Joseph and Mrs. Ajisodun Florence for their great support and love. I also appreciate my siblings Ajisodun Gbekeloluwa, Ajisodun Mojisola, and Ajisodun Akolade for their advice, support and love.

I also appreciate my course mates Adedapo Akinola, Ogunremi Ismail, Ogunmola Temiloluwa, Odeyemi Kemisola, Rabiu Halimat and Omisanmi Oluwatosin for their support.

ABSTRACT

The study evaluated the toxicological effects of paraquat on *Oreochromis niloticus* fingerlings. Two hundred and sixteen (216) fingerlings (Average weight:7.50g) were purchased from a local farmer in Ikole-Ekiti, Ekiti State, Nigeria and were transported to the Wet Laboratory of the Department of Fisheries and Aquaculture, Federal University Oye-Ekiti, Nigeria and acclimatized for two weeks. After range finding test, the fish were exposed to acute concentration of 2.96 mg/l ,3.31mg/L, 3.86mg/L ,4.4mg/L and 4.97mg/L of paraquat for 96 hours while the first treatment contained no paraquat (0.00mg/l). Fish were distributed in triplicate (12 fish per tank) into 20 litres plastic tanks. Physiochemical parameters of water were taken. Fish behavior and mortality were observed and recorded. Probit analysis was used to determine the lethal concentration of paraquat on Nile tilapia. Results of bioassay procedure showed that the LC_{50} after a 96 hours was 3.85mg//l. Restlessness, erratic swimming, loss of equilibrium, discolouration, 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 increased with an increase in concentration. The differences observed in the mortalities of *Oreochromis niloticus* at varying concentrations were significant ($p \leq 0.05$), an indication that mortality could be a factor of concentration and time of exposure. Therefore the usage of paraquat must be controlled and monitored.

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CHAPTER ONE

1.0 INTRODUCTION

Agriculture have greatly depend on using herbicides to control weeds, however they may endanger other organisms specially when they enter into aquatic environment. Floods and run off could dissolve herbicides and flow into the rivers or seas. Aquatic animals may uptake herbicide from surrounding water by different rout as non-target organisms. (Safahieh *et al.*, 2012).

The growth of human population and increasing activities associated with agriculture, urbanization and industrialization have resulted in a staggering release of anthropogenic sources, including pesticides. (Nwani *et al .,2014*) .

Water quality is rapidly declining due to the rapid growth of industries, various applications of chemical fertilizers and the use of pesticides. One of the main causes of pollution of aquatic ecosystems is agricultural pesticide, which is used to deal with pests, weeds and agricultural diseases and they have adverse effects on the environment (Hashemi *et al.* .,2017). Within a few weeks of using these pesticides in agricultural activities, they entered into aquatic ecosystems through surface runoff and subsurface drainage. 40% of the world's production of pesticides is related to herbicides (Hashemi *et al.*, 2017).

Herbicides are generally used by farmers to control weeds, remove aquatic plants in rivers, lakes and water reservoirs, which generally have harmful effects on aquatic animal health (Hashemi *et al.* .,2017). Most chemical pollutants find their way into the environment via various products and processes. Once in the environment, they can persist for long periods of time or break down into other more or less toxic chemicals with their associated environmental risks (UNEP, 2003). Pesticide-contaminated water may have undesirable effects in fish and other aquatic biota.

Pesticide-surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream (Toughill, 1999).

One of the important factors contaminating the natural habitat is agricultural herbicides. The increasing use of herbicides and pesticides in agriculture (including commercial and household production of vegetables) for the control of pest and herb causes chemical pollution of aquatic environment. The chemical pollution causes potential health hazards to live stock, especially to fish, frogs, birds and mammals. However, the introduction of herbicides to the natural environment has other negative effects, including unintentional intoxication of useful insects, fish, birds, mammals, and other inhabitants of aquatic and terrestrial biosensors (Senthil kumar, 2001).Toxic substances in aquatic environment can affect fish growth indirectly by reducing food availability, or directly by changing their metabolism.(Nwani *et al.*.,2014). Herbicides could hamper reproduction, food conversion efficiency, growth and mortality of inhabitants (Ateeq *et al.*, 2002).

Paraquat dichloride is the trade name for N,N- dimethyl – 4, 4- bipyridinium chloride, one of the most widely used herbicides in the world. It is poisonous to humans, having toxic effects on the liver, lungs and kidneys if ingested.

Paraquat is not easily broken down and can persist in the environment when adsorbed into soil particles. In Nigeria, paraquat is extensively applied in agriculture for weed control.

However, it is pertinent to study its hazardous effects on the aquatic system. It is assumed that the residue might affect fish as a result of run-off into the water body (Ogamba *et al.*., 2014).

Paraquat is the most effective herbicide that exists and one of the world's worst poisons on the earth. It is a fast acting non-selective compound which destroys tissues of green plants on contact and by translocation within the plant (Suntres, 2002). Due to the high solubility and repeated use

of paraquat in agricultural and non-agricultural areas, such as horticulture, large quantities of paraquat could penetrate into surface water through runoff with damaging effects to the biota. Even at low concentrations, it still could elicit deleterious effects, such as cytogenetic damage, physiological effects, and even death, to exposed non-target species. (Nwani *et al.*., 2014).

Fish are most sensitive to the aquatic pollutants during their early life stages. Therefore, any type of aquatic pollution either by agricultural runoff or by discharge of untreated effluents from manufacturing industries into natural waterways might endanger the fish population and other forms of aquatic life, and may contribute long term effects in the environment by changing the water quality.

Worldwide, tilapias (including all species) are the second most important group of farmed fish after carps, and the most widely grown of any farmed fish. Nile tilapia (*Oreochromis niloticus*) is by far the most important species .However, Nile tilapia is an omnivorous fish which eats detritus, phytoplankton and zooplankton.(Alkobaby *et al.* 2017).

Tilapia, *Oreochromis niloticus* is a freshwater surface feeding omnivore fish which belong to the family Cichlidae. They grow fast, mature quickly, and breed easily without inducement (Palas *et al.*,2 016). Tilapia is commonly available and easy to manipulate as both capture and culture fisheries due to its qualities. Tilapinnes are commonly acclaimed as having qualities that make them highly cultivable species. Cichlids are native to Africa, but they have migrated to other parts of the world such that, they are now present in all the continents of the world (Fitzsimmons, 2002; Peterson *et al.*., 2004). Cichlids were originally fresh water fishes but some can adapt to brackish water and others capable of adapting to full strength salt water (Gomez-Marquez *et al.*., 2003; and Peterson *et al.*., 2004). Tilapinnes are good candidates for aquaculture especially in developing countries where there are high levels of animal protein deficiencies. The admirable

characteristics of these fishes as cultivable species include , resistance to diseases, the ease with which to reproduce in captivity, switching of diet and tolerance to poor water qualities such as low dissolved oxygen levels and 'over –crowding".(Fidelis *et al.* 2011).

There are five potential routes for a pollutant to be incorporated into fish, via the food, non-food particles, gills, oral consumption of water and the skin. Once the pollutant is absorbed, it is transported by the blood either to a storage point (fat) or to the liver for transformation (Heath, 1991). If the pollutant is transformed by the liver, it may be stored there, excreted in the bile or passed back into the blood for possible excretion through the gills, kidneys or stored in fat which is an extra-hepatic tissue (Heath, 1991).

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety. Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals. The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding, and for the evaluation of the effects of noxious compounds (Ladipo *et al.*.,2011)

Determination of the toxic compounds in aquatic environments and their effects on aquatic organisms are a fundamental issue in ecotoxicology science. Toxic substances present in the environment can be determined by chemical analysis, but its effects on aquatic organisms in aquatic ecosystems cannot be determined by chemical analysis. Hence, to assess the environmental impact of toxic compounds, the use of mortality or bioassay experiments is necessary. (Hashemi *et al.*., 2017). The toxicity of a chemical is totally dependent on the

concentration of the chemical in organisms or even the concentration at the target receptor in the organism (Ayoola, 2008).

It is therefore pertinent to determine the tolerance limit of some of these agrochemicals to fish in general and the most widely cultured species in our area in particular. This is because these chemicals are increasingly used in weed control in the area. (Daramola *et al.*, 2007).

1.1 Research Objectives

The general objective of this research was to determine the toxicity of paraquat on Nile tilapia fingerlings. The specific objectives are to:

a. Assess behavioral response of *Oreochromis niloticus* exposed to paraquat; and

b. Determine lethal concentration (LC_{50}) of paraquat on *Oreochromis niloticus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pesticide

Pesticides are chemical compounds that are used to eradicate pests, including insects, rodents, fungi and weed according to World Health Organization, (1990). Pesticides are categorized into four main substituent chemicals: herbicides; fungicides; insecticides and bactericides (Gilden *et al..*, 2010). Different pesticides have markedly different effects on aquatic life (Dhakal, 2012). The important point is that many of these effects are chronic (not lethal), are often not noticed by casual observers, yet have consequences for the entire food chain – ranging from death of the organism, cancers, tumors and lesions on fish and animals, reproductive inhibition or failure, suppression of immune system and disruption of endocrine (hormonal) system. Toxicity of the pesticide refers to how poisonous it is. Some pesticides are extremely toxic, whereas others are relatively nontoxic. On the aquatic ecosystem, pesticides can bioaccumulate and biomagnify in the tissue and organs of fisheries. Pesticides have the tendency to cause alteration in metabolism and other relevant parameters such as enzymes, electrolytes, haematology and growth response. (Inyang *et al.*.,2016).

2.2 Herbicides

A herbicide is a pesticide, described by The United States Environmental Protection Agency (USEPA) as an agent used to prevent, destroy, repel or mitigate any pest ranging from insects, animals and weeds to microorganisms such as fungi, molds, bacteria and viruses. A herbicide is

any compound capable of killing or severely injuring plants and may be used for the elimination of plant growth or the killing off of plant parts (Amdur *et al.*., 1991).

Herbicides can be classified in several ways such as usage, translocation patterns, mode of action, toxicology and chemical structure. Systemic herbicides are translocated to sites that use high amount of energy such as the root and shoot growing points and reproductive structures. Herbicides are translocated in the xylem (apoplast) or phloem (symplast). Apoplasm is the non-living portion of the plant cell walls and xylem. Herbicides applied to foliage can move into plants through cracks in the leaf, open stomata and leaf cuticle. (DiTomaso, 2000).

2.3 Toxicology

Toxicology is the study of the adverse effects of chemicals or physical agents on living organisms. Several toxicological tests can be conducted on each herbicide. From these tests, a variety of hazard indicator values are derived. The most commonly used of these include an Oral LD_{50}, Inhalation LC_{50} and Dermal LD_{50}. A LD_{50} or LC_{50} value is the lethal dose or concentration of a herbicide which will kill 50% of the test animals based on mg of chemical per kg of body weight. (DiTomaso, 2010).

2.3.1 Aquatic Toxicology

Aquatic toxicology is the study of the effects of environmental contaminants on aquatic organisms, such as the effect of pesticides on the health of fish or other aquatic organisms. A pesticide's capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, and persistence in the environment.(Helfrich, 2009).

2.4 Paraquat

Paraquat (1,1'-dimethyl-4,4'-dipyridylium) is a quaternary-nitrogen, non-selective, contact herbicide belonging to the bipyridinium class of compounds. Paraquat's mode of action involves the inhibition of photosynthesis (specifically photosystem I) which generates superoxide, leading to lipid peroxidation and membrane damage. Plants die rapidly after treatment and exposure to light. (OCS,2016). Paraquat was introduced in 1962 for agricultural use, but was originally synthesized by Weidel and Russo in 1882 (WHO, 1984).

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is one of the most widely used herbicidal chemicals in the world and is now available in more than 130 countries Its chemical structure was first described in 1882, its oxidizing and reducing properties in 1933, and its herbicidal properties in 1955. Paraquat was marketed commercially in the United Kingdom in 1962 and registered for use in the United States in 1964.(Eisler,1990).

Paraquat dichloride is one of the most widely used herbicides in the world (Suntres, 2002). It is quick-acting and non-selective, killing green plant tissue on contact and becomes biologically inactive when it comes in contact with soil and rain within minutes of application. Paraquat is moderately to highly toxic to many species of aquatic life including rainbow trout, bluegill, and channel catfish. Its toxicity to fish varies with the species, size, and the softness or hardness of water (Madeley, 2002). At high levels, paraquat inhibits the photosynthesis of some algae in stream water (Kenneth, 1990). It is also toxic to human beings when swallowed. Paraquat applied to water for aquatic weed control purposes quickly disappears due to uptake by weeds and absorption by soil, silt and particulate suspended matter. However, some quantities of this herbicide end up in the body of fish and other aquatic life.

Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioral changes in fish that reduce populations, such as abandonment of nests and broods, decreased immunity to disease, and increased failure to avoid predators (Helfrich *et al.*., 1996).

Paraquat is the most highly acutely toxic herbicide to be marketed over the last 60 years. Yet it is one of the most widely used herbicides in the world, and in most countries where it is registered it can be used without restriction. It is used on more than 100 crops in about 100 countries. Gramoxone is manufactured by Syngenta, It is the most common trade name for paraquat, but the herbicide is also sold under many different names by many different manufacturers. China is now the world's largest manufacturer of paraquat, producing more than 100,000 tonnes per year.(Watts 2011)

2.4.1 Chemical profile of paraquat

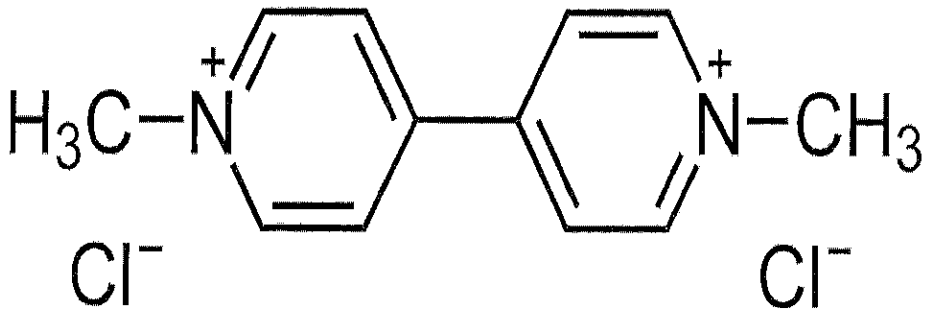

Figure2.1: Chemical structure of paraquat (Watts, 2011).

Common name -Paraquat, paraquat dichloride

Common trade name - Gramoxone

Chemical names -1,1'-dimethyl-4,4'-bipyridinium

Chemical form-White crystalline solid, aqueous solution or granules; typically available as 10-30%concentrated solutions coloured a dark blue..

Molecular formula and structure-Paraquat: $C_{12}H_{14}N_2$,Paraquat dichloride: $C_{12}H_{14}N_2C_{l2}$.It is a quaternary nitrogen compound.

Chemical group-Bipyridyl

Other related chemicals-paraquat dichloride trihydrate, paraquat bis(methyl sulfate)paraquat bistribromide..

CAS numbers

Paraquat 4685-14-7

Paraquat dichloride 1910-42-5

Paraquat dimethyl sulphate 2074

Synonym

Methyl viologen (Watts 2011)

2.4.2 Properties of paraquat

Physical and chemical properties of paraquat

Form: Liquid

Colour: Dark-blue

Odor: Characteristic of pyridine bases

Boiling point: Approximately. 100 °C aqueous solution

Melting point: Not available

Flash point: Does not flash

Explosive properties: Non – explosive

Vapor pressure: Not available

Density: 1.08 g/ml

Solubility: Soluble in/with water

PH-Value: 6.5-7.5

Oxidizing properties: non-oxidizing (Arivu *et al.*.,2016).

2.5 Uses of paraquat

Paraquat is used as a herbicide, desiccant, defoliant, and plant growth regulator (US EPA1997). It is used for controlling broadleaf weeds and grasses in more than 100 different crops, including plantations (Paraquat Information Centre 2010a).

Other major crop uses are for maize, orchards, soybeans, vegetables, potatoes, rice, and cotton. It is used for wheat, apples, oranges, coffee, cocoa, and rubber. It is used as a pre-harvest defoliant or desiccant on crops such as cereals, cotton, beans, hops, sugar cane, pineapple soy, potatoes, and as a post-harvest desiccant to speed up removal of spent plants such as tomato plants. It is applied to pine trees to induce turpentine production. Paraquat is employed in no-till agriculture, killing grasses and weeds to minimize ploughing and help prevent soil erosion. It is used for

weed control in non-agricultural areas such as roadsides, airports, around commercial buildings, drains, irrigation ditches, and waterways. It has been employed for killing illegal marijuana crops in the U.S. and in Mexico. It has also been reported as used in shrimp and prawn farming (PAN UK 2003).

2.6 Mode of action of paraquat

Paraquat reacts as an electron acceptor in radical reactions. As a herbicide, paraquat acts by inhibiting photosynthesis. In light-exposed plants, it accepts electrons from photosystem I and transfers them to molecular oxygen. In this manner, destructive reactive oxygen species are produced. In forming these reactive oxygen species, an oxidized form of paraquat is regenerated, and is again available to shunt electrons from photosystem I to start the cycle again (Summers, 1980).Paraquat is a non-selective, contact herbicide that is absorbed by the foliage. It destroys plant tissue by disrupting photosynthesis and rupturing cell membranes, which allows water to escape leading to rapid desiccation of foliage (Dinis-Olivera *et al.*., 2006).

2.7 Absorption and environment distribution of paraquat

Paraquat can be rapidly absorbed by inhalation and through the intestine after ingestion and absorption after oral intake is about 10% (EC, 2003).Absorption through intact skin is generally low, 0.5% according to EC (2003), but is substantially increased if the skin is damaged.

Paraquat deposits on plant surfaces undergo photochemical degradation into compounds that have a lower order of toxicity than the parent compound. On reaching the soil, paraquat becomes rapidly and strongly adsorbed to the clay minerals present. This process inactivates the herbicidal activity of the compound (Zain, 2007). While free paraquat is degraded by a range of soil microorganisms, degradation of strongly-adsorbed paraquat is relatively slow. In long-term studies, degradation rates were 5-10% per year (Zain, 2007).

Strongly bound paraquat has no adverse effects on soil micro fauna or soil microbial process. Paraquat residues disappear rapidly from water by adsorption on aquatic weeds and by strong adsorption to the bottom mud (Zain, 2007).

2.8 Paraquat toxicity

Paraquat is highly acutely toxic and enters the body mainly by swallowing, or through damaged skin, but may also be inhaled. Thousands of deaths have occurred from ingestion or dermal exposure to paraquat. Paraquat is corrosive to the skin and once the skin is damaged it is easily absorbed into the body. As little as a teaspoon of concentrated paraquat can result in death. Death is by respiratory failure and may occur within a few days after poisoning or as long as a month later. There is no antidote. Paraquat damages the lungs, heart, kidneys, adrenal glands, central nervous system, liver, muscles and spleen, causing multi-organ failure, as well as damaging the skin and eyes.(Watts 2011).

2.8.1 Acute toxicity

The European Commission has described the acute hazard of paraquat as very toxic by inhalation, toxic in contact with skin and if swallowed danger of serious damage to health by prolonged exposure if swallowed,, irritant to the eyes, respiratory system and skin.

The World Health Organization classifies paraquat as Class 2, moderately toxic; but PAN believes it should be reclassified as Class I because of its acute toxicity, delayed effects and lack of antidote. Paraquat causes extensive damage to the mitochondria of cells through the production of free radicals and oxidative stress, resulting in the interruption of important biochemical processes and causing cell death. WHO (2010) Recommended Classification of Pesticides by Hazard and is classified as Class II Moderately toxic. However, it is argued that

paraquat, because of its acute toxicity, delayed effects, and absence of an antidote should be in WHO Class 1a or 1b(Isenring 2006).

2.8.2 Chronic toxicity

Paraquat has been shown to inhibit insulin action in laboratory tests on rat liver cells (it impaired the suppressive effect of insulin on insulin-like growth factor-binding protein-1gene expression) (Kimura *et al.* 2007, 2010).Another study showed that paraquat inhibits insulin-dependent glucose uptake through oxidative stress (Shibata *et al.* 2010). Paraquat has also been shown to cause hyperglycaemia in sheep (Webb 1982).

2.8.3 Acute effect and sublethal effect of paraquat

Acute effects of paraquat on fish include abnormal stress behavior such as excessive gulping of air, erratic swimming, restlessness, loss of movement, loss of equilibrium, increased beating of the gill operculum, excessive secretion of mucus, swimming on the back, and paralysis 23 (Omitoyin *et al.*., 2006). Sublethal effects on fish include adverse effects on the immune system, with the effect enhanced by elevated temperatures within the environment (Salazar-Lugo *et al.*., 2009); alterations to gonads likely to affect reproductive activity particularly in males (Figueiredo-Fernandes *et al.*., 2006); and oxidative stress (Stephensen *et al.*., 2002).

2.8.4 Lethal dose

A lethal dose is the amount of pesticide necessary to cause death. Because not all animals of a species die at the same dose (some are more tolerant than others), a standard toxicity dose measurement, called a Lethal Concentration $_{50}$ (LC_{50}), is used. This is the concentration of a pesticide that kills $_{50}$% of a test population of animals within a set period of time, usually 24hr to 96hr(Helfrich 2009).

2.9. Physiochemical Parameters Of Water

Assessment of water quality can be defined as the analysis of physical, chemical and biological characteristics of water. The physiochemical parameters include the following

2.9.1. Temperature

Temperature of aquatic environment is important for ensuring survival, distribution and normal metabolism of fish. Failure to adapt to temperature fluctuations is generally ascribed to the inability of fish to respond physiologically with resultant mortality, which is related to changes in the metabolic pathways (Adeyemo *et al.*., 2003). When water is heated, much energy, oxygen and vapor is released into the air, leaving behind a high concentration of carbon dioxide which makes the water more acidic. The result is a collapse in osmoregulatory functions during temperature extremes (Gubbins *et al.*., 2000). The normal range of temperature in the tropics to which fish are adapted is 22-35°C (Howerton, 2001).

2.9.2. pH

pH can be viewed as an abbreviation for power of hydrogen or more completely, power of the concentration of hydrogen ion. Most natural water is alkaline in nature due to presence of bicarbonates and carbonates formed due to dissolution of atmospheric Carbon dioxide. pH can be drastically change due to prevailing biochemical activities undergoing in water. Photosynthetic activity increases the pH due to consumption of free CO2 and dissociation of bicarbonates into carbonates. The carbonate are much stronger alkalise than the bicarbonates4. There is now abundant evidence that acidification, particularly of soft water has a damaging effect on fish and a water body that feature (naturally or artificially) episodic or chronic pH levels of about 4.0-4.2 or less are devoid of fish (Odunze, 2004).

2.9.3. Conductivity

The electrical conductivity is the capacity of waters to conduct current, and is caused by the present of salt, acids and bases, called electrolytes, capable of producing cations and anions. (Abineet *et al.* 2014) 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 100-2,000μSi/cm and the acceptable range is 30-5,000μSi/cm (Stone and Thomforde, 2006).

2.9.4. Total Dissolved Solids

Total dissolved solids, 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).

2.9.5 Total Hardness

Hardness is caused by divalent metallic ions that are capable of reacting with sops to form ppt. And with certain anions present in the water to form scale. There are two types of hardness-temporary hardness is also known as carbonate hardness and it is mainly due to presence of carbonate and bicarbonates of Ca and Mg which is removed by boiling or by adding $Ca(OH)_2$ to it. The permanent hardness is also known as non-carbonate hardness and is due to the sulphate, chlorides and nitrates of calcium and magnesium.

2.9.6. Dissolved oxygen Concentration

Adequate dissolve oxygen is necessary for the life of fish and other aquatic organism. The dissolved oxygen concentration may also be associated with corrosives of water, photosynthetic activity simplicity. The Dissolved oxygen test used in the biological oxygen demand determination as carried out by the dilution method. The amount of oxygen required to carry out biological decomposition solids in sewage under aerobic condition at standard temperature is known as B.O.D. The test for B.O.D is very important. As the river water under normal condition will not contain much amount of dissolve solids. Direct method can safely be used. .(Abineet *et al.*.,2014)

2.9.7. Alkalinity

Alkalinity is measure of solution's capacity to react with a strong acid to a predetermined pH. Alkalinity of water is due to the presence of hydroxides, carbonates and bicarbonates.(Abineet *et al.*.,2014)

2.9.8. Nile Tilapia (*Orechromis niloticus*)

The Nile tilapia (*Oreochromis niloticus*) is a species of tilapia, a cichlid fish native to Africa from Egypt south to east and central Africa, and as far west as Gambia. (Wikipedia 2017 .)*Oreochromis niloticus,* like other tilapias has deep laterally compressed body (Pinnoy, 2006). Nile tilapia is a tropical species that prefers to live in shallow water. The lower and upper lethal temperatures for Nile tilapia are 11-12 °C and 42 °C, respectively, while the preferred temperature ranges from 31 to 36 °C. It is an omnivorous grazer that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films

associated with detritus. Nile tilapia can filter feed by entrapping suspended particles, including phytoplankton and bacteria, on mucous in the buccal cavity, although its main source of nutrition is obtained by surface grazing on periphyton mats. (FAO, 2017).

Physical features of Nile Tilapia

Nile Tilapia body is compressed with caudal peduncle depth equal to length and cycloid scales. A knob-like protuberance absent on dorsal surface of snout. It lateral line is interrupted with Spinous and soft ray parts of dorsal fin continuous. It has Dorsal fin with 16 - 17 spines and 11 to 15 soft ray.It has anal fin with 3 spines and 10-11 rays. Caudal fin truncate. (FAO,2017).

CHAPTER THREE

3.0: METHODOLOGY

3.1 Experimental Fish and System

The experimental fish, *Oreochromis niloticus* fingerlings were purchased at a fish farm in Ikole, Ekiti state and transported to laboratory with an open keg. The initial average weight of the fish was 7.50g, and the initial average length was 5.66cm .The fingerlings were acclimatize in a 25litres rectangular plastic tank for 2weeks and feed on a pelleted feed. The water were changed during acclimatization and fed twice daily.

3.2 Experiment design

216 *Oreochromis niloticus* fingerlings were used. The fingerlings is divided into six treatment with three replicates.. 12 fingerlings were stocked into each tank with replicate treatment which are labeled as 0T, 1T, 2T, 3T, 4T and 5T. 0T serves as the control. A range finding test (trial test) was carried out using the paraquat as a toxicant. Six concentrations of the paraquat were prepared from the original (276g/l).

3.3 Toxicity test

Preparation of the test solution

The definitive concentration of 1.5ml,1.8ml,2.1ml,2.4ml,and 2.7ml were converted into 2.96mg/l, 3.31mg/L, 3.86mg/L ,4.4mg/L and 4.97mg/L respectively and were measured with syringe from paraquat solution of 276g/l and were introduced into experimental tank of 15litres of water in each tank.

3.3.1. Bioassay Test

The range finding test were carried out to determine the definitive concentration range for testing. The bioassay test was carried out in the 18 plastic tanks and the paraquat solution was introduced into each tank: 1T, 2T, 3T, 4T, and 5T.The fish was starved for 24hrs before the experiment.

3.3.2 Behavioral response observation

The behavioral and morphological responses of the fish is observed after exposure of the fish to various concentration of the toxicant. The control tank was monitored with the tanks of various concentration of the toxicant, assessing any behavioral or morphological changes. The responses which are air gulping index, opercular ventilation count, tail fin movement, restlessness, erratic swimming,and loss of reflex were recorded from 24hrs-96hrs. The air gulping index, opercula 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. Two fish were used for the counting per tank and values were computed.

3.4 Mortality rates observation.

Mortality were also recorded from 24hrs -96hrs, The dead fish were removed after 24hours

3.5 Water Quality Analysis

The water quality parameters, temperature, dissolved oxygen ,conductivity pH were determined using Model SX751pH ORP/Conductivity/DO water analysis kit.

3.5.1 Determination of pH and Temperature

The pH mode on the instrument was selected with the set/Hold button. The pH meter was calibrated, the pH electrode was washed in purified water and shake off the water, and then the pH electrode was immersed in a 7.00 buffer solution. The pH electrode was submerged in the sample water for 1minute. 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.

3.5.2 Determination of Conductivity

The conductivity mode on the instrument was selected with Set/Hold button. The conductivity electrode was plugged into the meter's corresponding socket, the conductivity meter was calibrated, the conductivity electrode was rinsed with purified water and shake off water, then insert it into the 1413μS/cm calibration solution .The electrode was submerged in the water sample. The measurement was taken when the stability symbol on the top left of the liquid crystal display (LCD) disappeared.

3.5.3 Determination of dissolved oxygen

The DO probe was rinse with distilled water to calibrate, after which it was dipped into the treatments to read the dissolved oxygen of the treatment medium.

3.6 Statistical Analysis

Probit analysis (Finney, 1971) was used to determine LC_{50}. Data was subjected to one-way analysis of variance (ANOVA) using SPSS software version 16.0 to test for the significant differences between means . graph analysis were plotted using Microsoft excel window 2010.

CHAPTER FOUR

4.0 RESULTS

4.1 Physio-Chemical Parameters of Water

Results of the physico-chemical analysis of the water used for exposure concentrations of the administered Paraquat concentrations in the laboratory are presented in Tables 4.1. The ranges were within the recommended values for culture fisheries.

Parameters such as pH, Electrical Conductivity, temperature and dissolved oxygen before exposure have their range values as 6.53-6.59, 53.53-56.42 μS/m, 24.13-24.24^{0}C, and 5.38-5.40 respectively. However, after exposure of the toxicant for 96hr, there was increase in pH (6.47-7.10), , Electrical Conductivity (75.83-117.6μS/m), and dissolved oxygen (5.35-5.63mg/L,.but decrease in dissolved oxygen at 24hrs and 48hrs. There was however decrease in the range value for temperature (24.13-22.47^{0}C).

Table 4.1: Water quality parameter of paraquat herbicide on fingerlings of *Oreochromis niloticus*.(range finding test)

Parameter	Exposure Period (hours)	CONCENTRATION OF PARAQUAT (mg/L) 0	2.76	3.31	3.86	4.42	4.97
Temperature	24	24.13 ± 0.11^{b}	24.03 ± 0.29^{ab}	24.07 ± 0.06^{b}	23.93 ± 0.06^{ab}	23.97 ± 0.06^{ab}	23.76 ± 0.10^{a}
	48	23.53 ± 0.06^{c}	23.20 ± 0.17^{a}	23.40 ± 0.10^{abc}	23.60 ± 0.10^{c}	$23.50\pm0.10b^{c}$	23.3 ± 0.10^{ab}
	72	22.87 ± 0.06^{a}	22.77 ± 0.23^{a}	22.70 ± 0.10^{a}	22.80 ± 0.44^{a}	22.77 ± 0.29^{a}	22.67 ± 0.10^{a}
	96	22.73 ± 0.32^{a}	22.60 ± 0.10^{a}	22.57 ± 0.07^{a}	22.47 ± 0.16^{a}	22.53 ± 0.06^{a}	22.56 ± 0.11^{a}
DO	24	5.38 ± 0.02^{a}	5.39 ± 0.03^{a}	5.40 ± 0.01^{a}	5.40 ± 0.01^{a}	5.39 ± 0.03^{a}	5.46 ± 0.02^{b}
	48	5.52 ± 0.02^{a}	5.55 ± 0.01^{a}	5.54 ± 0.01^{a}	5.50 ± 0.03^{a}	5.35 ± 0.30^{a}	5.55 ± 0.01^{a}
	72	5.59 ± 0.06^{abc}	5.59 ± 0.00^{ab}	$5.6\pm0.06b^{cd}$	5.58 ± 0.02^{a}	5.62 ± 0.00^{d}	5.61 ± 0.01^{cd}
	96	5.61 ± 0.03^{a}	5.62 ± 0.01^{a}	5.63 ± 0.06^{a}	5.63 ± 0.06^{a}	5.62 ± 0.01^{a}	5.62 ± 0.11^{a}
pH	24	6.53 ± 0.06^{a}	6.57 ± 0.06^{a}	6.53 ± 0.12^{a}	6.53 ± 0.06^{a}	6.47 ± 0.06^{a}	6.53 ± 0.15^{a}
	48	6.70 ± 0.10^{a}	6.73 ± 0.06^{ab}	6.87 ± 0.06^{c}	6.77 ± 0.06^{abc}	6.87 ± 0.06^{c}	6.87 ± 0.06^{bc}

	72	7.10±0.10^{b}	7.07±0.06^{ab}	7.0±0.06^{ab}	7.1±0.06^{ab}	7.03±0.01^{ab}	6.93±0.00^{a}
	96	6.97±0.06^{b}	7.03±0.12^{b}	6.97±0.06^{b}	6.97±0.06^{b}	6.93±0.06^{ab}	6.80±0.10^{a}
Conductivity	24	53.53±2.89^{a}	75.00±1.14^{b}	139.60±16.39^{c}	132.50±4.30^{c}	139.83±11.8^{c}	137.43±19.00^{c}
	48	95.07±5.66^{a}	128.33±3.7^{b}	188.4±23.98^{d}	134.17±3.93^{b}	149.43±16.07^{bc}	173. ±23.7^{cd}
	72	75.83±3.94^{a}	82.43±2.05^{a}	159.53±21.00^{b}	153.23±11.6^{b}	160.73±19.60^{b}	177.6±20.88^{b}
	96	117.67±4.40^{ab}	102.8±32.50^{ab}	141.53±17.55^{b}	89.5±11.26^{a}	96.03±24.30^{a}	112.7±19.00^{ab}

Mean values with the same superscript along same columns are not significantly different ($p \geq 0.05$). (Mean values ± S.E)

4.2 Behavioral response

Oreochromis niloticus showed behavioral changes on exposure to paraquat herbicide. Immediately the fish were introduced into the tank containing paraquat at concentrations 2.76 mg/L ,3.3 mg/L, 3.86 mg/L, 4.42mg/L and 4.97mg/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. The fish showed abnormal swimming movements including loss of orientation, loss of buoyancy and spasms before death.

4.2.1 Air gulping index

Oreochromis niloticus exposed to paraquat showed increased air gulping index)with increase in the concentration of the toxicant 2.76 mg/L ,3.31 mg/L, 3.86 mg/L ,4.42mg/L and 4.97mg/L respectively,according to(Table 4.2).

The air gulping were observed and counted per 60seconds,of air gulped. The result of air gulping as presented in Table (4.2),showed that the air gulping of *Oreochromis niloticus* fingerlings to the toxicant increases especially during 24 hrs and 48hrs of exposure than the control.At 24 hrs the airgulping index are (74.30±3.36,75.25 ±9.38,77.33±5.21,70.67±1.45 and 75.67±0.88) while at 48hrs the air gulping index are (54.00±9.45,70.67±8.57,87.33±0.33,91.33±1.20and84.00±4.00) at concentration 2.76 mg/L ,3.31 mg/L, 3.86 mg/L,4.42mg/L and 4.97mg/L respectively and the air gulping index of control at 24hrs and 48hrs are(26.00±1.52 and 33.00± 0.58) respectively. The air gulping index reduces during 72hrs and 96hrs.

Table 4.2 Effect of acute concentration of paraquat with time on *Oreochromis niloticus* air gulping index movement

	EXPOSURE TIME			
Conc(mg/L)	**24hrs**	**48hrs**	**72hrs**	**96hrs**
0	26.00 ± 1.52^{a}	33.00 ± 0.58^{b}	25.33 ± 0.67^{a}	34.33 ± 1.20^{b}
2.76	74.30 ± 3.36^{b}	54.00 ± 9.45^{a}	51.33 ± 0.88^{a}	53.33 ± 0.33^{a}
3.31	75.23 ± 9.38^{c}	70.67 ± 8.57^{d}	50.00 ± 3.79^{a}	45.00 ± 1.15^{b}
3.86	77.33 ± 5.21^{c}	87.33 ± 0.33^{d}	36.00 ± 1.00^{a}	53.33 ± 0.88^{b}
4.42	70.67 ± 1.45^{c}	91.33 ± 1.20^{d}	60.33 ± 2.33^{b}	43.00 ± 2.00^{a}
4.97	75.67 ± 0.88^{b}	84.00 ± 4.00^{b}	53.00 ± 4.04^{a}	50.00 ± 1.00^{a}

Means with the same superscript along same columns are not significantly different ($p \geq 0.05$). (Mean values ± S.E).

4.2.2. Operculum ventilation count

Oreochromis niloticus exposed to paraquat showed increased Operculum ventilationcount)with increase in the concentration of the toxicant 2.76 mg/L ,3.31 mg/L ,3.86 mg/L ,4.42mg/L and 4.97mg/L respectively as shown in(Table 4.3). Operculum ventilation count were observed and counted per 60seconds,. The result of Operculum ventilation count as presented in (Table 4.3) showed that the exposed fish to the toxicant increases especially during 24 hrs and 48hrs of exposure of paraquat to *Oreochromis niloticus* fingerlings than the control.At 24hrs the opercular ventilation count are(78.71 ±4.76,78.75 ±6.42,77.33 ±5.20,71.67 ±3.28 and 75.67 ±0.33) while at 48hrs the opercular ventilation count are(72.00 ±3.21,78.33 ±6.64,87.33 ±0.33,90.67 ±1.86 and89.00 ±1.73), at concentration 2.76 mg/L ,3.31 mg/L, 3.86 mg/L,4.42mg/L and 4.97mg/L respectively.The opercular ventilation count of control at 24hrs and 48hrs are (29.00±0.00and 36.33±0.33)respectively. The opercular ventilation count reduces during 72hrs and 96hrs as presented in (Table4. 3).

Table 4.3. Effects of Acute Concentrations of Paraquat with Time on *Oreochromis niloticus* opercular ventilation count.

Conc (mg/L)	EXPOSURE TIME			
	24hrs	48hrs	72hrs	96hrs
0	29.00 ± 0.00^{a}	36.33 ± 0.33^{c}	32.33 ± 1.20^{b}	35.00 ± 0.58^{c}
2.76	78.71 ± 4.76^{b}	72.00 ± 3.21^{b}	52.67 ± 2.19^{a}	45.00 ± 0.00^{a}
3.31	78.75 ± 6.42^{b}	78.33 ± 6.64^{b}	57.00 ± 5.77^{a}	47.00 ± 0.00^{a}
3.86	77.33 ± 5.20^{c}	87.33 ± 0.33^{d}	36.00 ± 1.00^{a}	54.67 ± 0.33^{b}
4.42	71.67 ± 3.28^{c}	90.67 ± 1.86^{d}	56.67 ± 1.20^{b}	43.00 ± 2.31^{a}
4.97	75.67 ± 0.33^{b}	89.00 ± 1.73^{c}	55.33 ± 3.28^{a}	50.00 ± 1.00^{a}

Means with the same superscript along same columns are not significantly different ($p \geq 0.05$). (Mean values ± SE)

4.2.3 Tail fin movement

Oreochromis niloticus exposed to paraquat also showed increased tailfinmovementwith increase in the concentration of the toxicant2.76 mg/L ,3.31 mg/L 3.86 mg,/L 4.42mg/L and 4.97mg/L respectively as shown in (Table4.4)The activity of tail fin movement were observed and counted per 60seconds . The result of tail fin movement presented in(Table 4.4), showed that the tail fin movement of *Oreochromis niloticus* increases at 48hrs and decreases at 72hrs at concentration of paraquat 2.76 mg/L ,3.31 mg/L 3.86 mg,/L 4.42mg/L and 4.97mg/L respectively

Table 4.4: Effect of acute concentration of paraquat on *Oreochromis niloticus* on tail fin movement

	EXPOSURE TIME			
Conc(mg/l)	**24hrs**	**48hrs**	**72hrs**	**96hrs**
0	30.00 ± 6.08^{b}	47.00 ± 1.00^{c}	32.67 ± 0.58^{b}	22.0 ± 1.00^{a}
2.76	52.33 ± 5.51^{a}	52.67 ± 6.66^{a}	59.33 ± 7.37^{a}	$44.61\pm10._{50}{}^{a}$
3.31	65.00 ± 5.29^{b}	97.67 ± 1.53^{d}	53.67 ± 4.04^{a}	85.33 ± 5.51^{c}
3.86	66.67 ± 10.11^{ab}	63.00 ± 7.81^{a}	70.00 ± 0.00^{ab}	76.33 ± 1.53^{b}
4.42	66.00 ± 1.00^{a}	67.00 ± 1.00^{a}	66.00 ± 1.00^{a}	68.00 ± 1.73^{a}
4.97	64.00 ± 3.61^{a}	65.67 ± 2.08^{a}	74.67 ± 0.00^{b}	74.00 ± 2.00^{a}

Means with the same superscript along same columns are not significantly different ($p\geq0.05$).

(Mean values ± SE)

4.3: Mortality rates and Log of concentration in *Oreochromis niloticus* exposed to acute concentration of paraquat.

Fish mortality was observed in all the tanks except in the control tanks. . The first mortality was observed at24 hours at, 3.31mg/L, 3.86mg/L ,4.4mg/L and 4.97mg/L of paraquat.

The highest mortality value of 22 and 23 was observed at 4.42mg/L and 4.97mg/L concentrations respectively while lowest value of 11 for the lowest concentration of 2.76mg/L. Similarly, mortality rates and Log of concentration in *Oreochromis niloticus* exposed to acute concentration of paraquat as shown in (Table 4.5) indicates that the concentration 4.97mg/L have the highest number of mortality rate (23) and Log of concentration value (0.696) while concentration 2.76mg/L have the lowest number of mortality rate (11) and Log of concentration value (0.441)with the control having the value 0.000 respectively. The mortality rate of LC_{50} as shown in(figure 4.1) was determined by the antilog of 0.5853 mg/L. The result of the acute toxicity showed that paraquat was toxic to *Oreochromis niloticus* with LC_{50} value of 3.849 mg/l .

Table 4.5: Mortality rate and log of concentration in *Oreochromis niloticus* exposed to paraquat

Concentration of paraquat in (mg/L	Log of concentration	Number of fish exposed	Number of mortality	%Mortality	Probit value
0	0	36	0	0	0
2.76	0.441	36	11	30.56	4
3.31	0.51	36	13	36.11	4.64
3.86	0.587	36	20	55.56	5.15
4.42	0.645	36	22	61.11	5.28
4.97	0.696	36	23	63.89	5.36

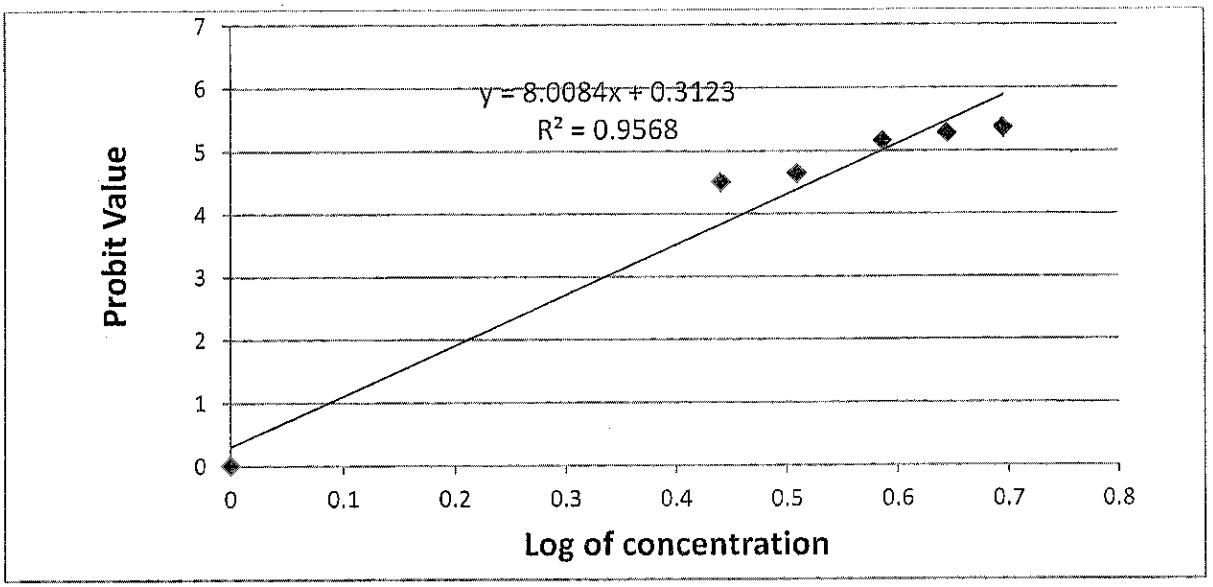

Figure 4.1: Graph of log of concentration and probit value

4.4 DISCUSSION

The result of this study indicates the toxic nature of the paraquat on the behavioural and physico-chemicalparameters *Oreochromis niloticus* fingerlings.

4.5 Physico-chemical Parameters of Diluting Water

The results of the study indicate that paraquat application does not result in significant changes in the physicochemical parameter to a point that is capable of causing deleterious effects in fish, the physico-chemical parameters shows there is significant effect ($p<0.05$) after exposure of paraquat to *Oreochromis niloticus* on pH, temperature,and dissolved oxygen ,and electric conductivity in contrast to the report of Olaifa, *et al.*, 2003. With the result on physio-chemical parameters, the water quality parameters were within the recommended range for the culture of *Oreochromis niloticus* similar to the report of Omitoyin *et al.*, 2006.

4.6. Effects of Acute Concentrations of paraquat on *Oreochromis niloticus* Behavioral Parameters

Behavioral reports of fish to most toxicant and differences in reaction times have been observed as due to the effects of chemicals ,their concentration ,species size and specific environmental conditions according to (Adakole 2006, Bob manuel *et al.*.,2006 and Babatunde *et al.*.,2001)researchs.

The result showed that exposure of *Oreochromis niloticus* to paraquat cause 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 concentration. *Oreochromis niloticus* showed

behavioural response such as loss of equilibrium, restlessness ,discolouration , frequent surfacing, agitated and erratic swimming, loss activity and finally death. Several abnormal behaviour such as incessant jumping and gulping of air, restlessness, surface tobottom movement, sudden quick movement, resting at the bottom were similar to the observations of (Omoniyi *et al.*. 2002),

The results of the effects of acute concentrations of paraquat on fish behavioural parameters showed that both herbicides have significant effects on the opercular ventilation count of fish, tail fin movement rate and air gulping index.

The opercular ventilation count ,air gulping index and tail fin movement increase during 24hrs,48 hrs . 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.,reported by (Chindah *et al.*., 2004) .

Opercular ventilation decreases gradually during 72hrs and 96hrs which may likely be decrease in oxygen and reduction and metabolic rates due to their attempt to escape from the tank..

Opercular ventilation rate increased from 24hrs and 48 hrs then reduced from 96hrs this indicates hyperventilation,according to (Babatunde and Oladimeji 2014) . The opercular movement in the Teleost,and *Tilapia guineensis* has been reported to decline after an initial increase on chlorpyrifos (Chindah *et al.*., 2004)

Tail fin movement changed significantly with duration of exposure, The tail fin movement increased at 24hrs and 48hrs.. The intial increase in tail fin beat may be associated with sudden response of the fish to the shock of exposure to the agro-chemical according to (Chindah *et al.*, 2004). The increase tail movement rate is due to avoidance syndrome and increased escape reflex and general restlessness.according to (Adeogun, 2012).

The Air gulping index was also observed to increased significantly at 24hrs and 48hrs. The observed respiratory distress may have been due to decrease in dissolved oxygen twith decreasing ability of the exposed fish to respire (Ojutiku *et al.*., 2013).

4.7 Mortality

The Mortality was observed to be concentration and time dependent in this experiment. These were similarly observed by (Ateeq *et al.*. 2005) and (Olurin *et al.*. 2006).

The LC_{50} values depend on fish species and the test conditions as well as herbicide formulations (WHO, 1994). The 96hr LC_{50} value of paraquat as observed in this study was 3.85mg/L.which is in contrast with (Babatunde *et al.*, 2014) who reported 96hr LC_{50} as 12.25mg/l after exposing *Oreochromis niloticus* fingerlings to paraquat .The 96hr LC_{50} value of paraquat as observed in this study was 3.85mg/Lwhich . is also in contrast with (Fidelis *et al.* 2012)which reported LC $_{50}$ at 96 hr as 7.00mg/l after exposing *Oreochromis niloticus* juvenile .

CHAPTER FIVE

5.0 Conclusion

The results of the acute toxicity showed that paraquat was toxic to *Oreochromis niloticus* ,with LC_{50} value of 3.85mg/L. Paraquat was toxic to fingerlings of *Oreochromis niloticus*. The LC_{50} of the paraquat to fingerlings of *Oreochromis niloticus*. was determined and paraquat was found to be more toxic.,therefore the usage must be controlled and monitored .

5.1 Recommendation

Further research should be carried out to investigate and establish the effects of this toxicant (paraquat) in rivers, ponds, lakes etc.

Further research should be carried out to investigate and establish the effects of paraquat on tissue muscle, skin and brain.of *Oreochromis niloticus*.

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