TOXICITY OF CASSAVA EFFLUENT ON CATFISH (*Claris gariepinus)* AND SOME TARGET ORGANS

BY

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FAQ/11/0027

SUBMITTED TO

THE DEPARTMENT OF FISHERIES AND AQUACULTURE

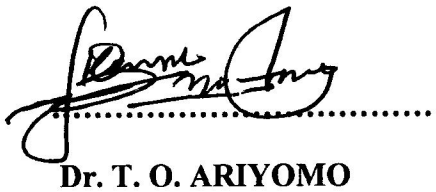
FEDERAL UNIVERSITY, OYE-EKITI, EKITI STATE, NIGERIA,

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR IN FISHERIES AND AQUACULTURE (B. FISHERIES AND AQUACULTURE) DEGREE IN FISHERIES AND AQUACULTURE

SEPTEMBER 2016.

CERTIFICATION

We certify that **this study was carried out by ADENIRAN ADEDOYIN FUNMILAYO, FAQ/11/0027 of Fisheries and Aquaculture Department,** Federal university, Oye-Ekiti.



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Dr. T. O. ARIYOMO

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DEDICATION

This research work is dedicated to Almighty God and my lovely parents.

ACKNOWLEDGEMENTS

To God be the glory for his love and kindness towards me before, during and after this project work.

I sincerely appreciate my supervisors, Dr T. O. Ariyomo and Dr T. Jegede for their effective and invaluable assistance, at every stage of this research work. My profound gratitude goes the lecturers of the Department of Fisheries and Aquaculture for their support towards this project: Dr T. Babalola (HOD), Dr F. George, Dr J.B. Olasunkanmi, Dr A. Akinsorotan, Dr. S. Okeke, Mr Bayode Omobepade, Mrs F.E Elesho, Mrs Joy and Mr T. Oyawoye.

I want to appreciate the good gesture of my colleagues: Omolounnu Omoyemi, Olaniyi Oluwafunmilayo, Adeniji Micheal, Akeredolu Treasure, Awolumate Oluwasanmi, Adesuyi Titilayo, Afolabi Victor, Ogunrinde Tobi and Oyelakin Saheed, it's my pleasure knowing you all and may our lights never go deem.

I sincerely appreciate the effort of my parents Mr David Adeniran and Mrs Funmilayo Adeniran for their love, support and prayers. I also appreciate the support of my brothers and sisters, Mr & Mrs Ayodele Adeniran, Mrs Yetunde Adeleke, Mr Olukayode Adeniran, Funke Adeniran, Mr & Mrs Adebayo Adeniran, Mr & Mrs Oluwafemi Adeniran, Mr & Mrs Babatunde Adeniran, Toosin & Pelumi Omotuyi, Oluwaferanmi Adeniran, Pelumi Adeniran, Oluwasegun Adeleke, Adeola Adeleke ,Boluwatife Adeniran, Adesewa Adeleke, Oluwanifemi Adeniran, Taiwo & Kehinde Adeniran, Adedamola Adeleke, Peace Adeniran, Adeseye Adeniran, Mercy Adeniran, Taiwo & Kehinde Adeniran, Inioluwa Adeniran and Bright Adeniran thanks so much for your concern and prayers.

I am indebted to my best friend in person of Raji Ololade , also to Ibiyemi Kehinde, Ibileye Tolulope, Oni Tolulope who stood by me throughout my five years of studies in this school, God will bless u all, many thanks to you.

I appreciate my lovely family of God "Chapel of Transformation" I thank you all for your love and support, I specially thank all chapel's staffs for all your love, food, advice, support and parental care. God Almighty will reward you abundantly, to all chapel members you people are just too wonderful, we shall meet at the top, and I love you all.

Finally, I want to specially thank my uncle, mentor and school father in person of Mr Bayode Omobepade for his assistance, tutor, inspiration, moral advice, invaluable support and unquantifiable contributions towards the completion of this programme, I really appreciate you, God bless you sir.

ABSTRACT

A 96 hour bioassay was conducted to determine the toxicity of cassava effluent to ***Claries gariepinus*** juveniles and its effects on the gills and liver as well as on some blood parameters such as packed cell volume (PCV), Red blood cells (RBC), White blood cells (WBC), Haemoglobin concentration (Hb) and their counts. Juveniles of *C. gariepinus* of the same age and size showed varying degrees of hyper-activity, mortality, stress and lesions to different concentration of cassava effluent. Static bioassay test revealed the 96 hour LC_{50} of cassava effluent as 4.365ml/L, exposure to sublethal concentration of cassava effluent resulted in the reduction of blood parameters such as PCV, RBC and Hb however, the value of white blood cells increased at the end of the experiment. Degenerative and erosive changes were observed on the tissues of the organs of *C. gariepinus*. The gills of fish in the varying concentrations particularly the higher concentrations (5.00ml/L and 5.50ml/L) showed signs of necrosis which means that at those concentrations cassava effluent is too toxic to *C. gariepinus* juvenile. The liver of fish in higher concentrations also showed hydropic degenerative changes such as space formation. Water quality parameters monitored during the experiment changed (temperature, Ph and Oxygen), the pH increased considerably throughout the course of the experiment, the dissolved oxygen concentration values recorded during the experiment decreased. Exposure to cassava effluent will prevent oxygen dissolution, cause destruction of breeding grounds as well as fish eggs, and ultimately, alteration of the entire aquatic environment leading to high mortality or total eradication of aquatic life.

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CHAPTER ONE

1.0 INTRODUCTION

Pollution can be defined as the introduction of contaminants into the natural environments that can cause adverse change (Merriam-Webster Online Dictionary: Merriam-webster.com. 2010-08-13. Retrieved 2010-08-26.). Pollution of the aquatic environment is a major challenge to man. Agrawal et al. (2010) stated that water pollution is the contamination of water bodies such as lakes, rivers, oceans and groundwater by human activities. All forms of water pollution affect organisms and plants that live in these water bodies and in almost all cases, the effect is damaging not only to the individual species and populations, but also to the natural biological communities (Agrawal et al., 2010). It occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful constituents (Agrawal et al., 2010). Man's constant quest to fully utilize the products of the environment has led to the production of wastes in such proportion as to threaten the very existence of certain strategic ecological habitats and directly or indirectly affect human population (Enujiugha & Nwanna, 2004).

Bryant, (2002) opined that pollution is an increasingly important fact determining the health and distribution of wildlife and biodiversity. Adverse effects of the activities of man upon the aquatic environment are of growing concern (Malins & Ostrander, 1991; Bucke, 1993). Changes in physical factors (water temperature, oxygen levels, pH and salinity), as well as in biological stressors (food availability and pathogens) can produce effects upon resident aquatic species.

Some agricultural products also serve as water pollutants e. g cassava effluent. Cassava (*Manihot esculenta Crantz)* is a woody shrub of Euphorbiaceae. It is extensively cultivated as annual crop in tropical and subtropical regions of the world for its edible starchy tuberous root, a major source of carbohydrates. It is the third largest source of carbohydrates for human food in the world with Africa being its largest Centre of production (Claude & Denis, 1990). Effluent from cassava is discharged as waste water. It contains cyanide (Arguedes & Cook, 2012), either in expressed juice or wash water spray (Cooke & Maduagwu, 1996). Environmental problems from cyanide may occur; for example, young stages of plants including vegetables may be negatively affected, and sensitive stages of fish may also be negatively affected (Bengtsson & Trient, 1994).

In Nigeria, there has been a sudden increase in agricultural practices, industrialization and food production arising as ways of alleviating food scarcity and poverty. In spite of these advantages, these practices have led to water and other forms of environmental pollution thus creating health hazards to man and other living organisms (Adeyemo, 2005). Annual cassava production in Africa is about 84 million tonnes with Nigeria having the highest production of 30million tonnes, Tanzania 5.7 million tonnes and Madagascar - 2.4 million (Adeyemo, 2005).

The upsurge in the production of cassava in Nigeria has led to the creation of cassava processing units where various cassava products are produced. Moreover, waste waters from the cassava processing units are discharged into the environment and the surrounding waters (Adeyemo, 2005). Cassava waste water containing most toxic chemicals such as cyanide is discharged from processing units into the nearby rivers and streams without prior proper treatment (Adeyemo, 2005). Cyanide, being the most toxic chemical in the cassava waste water to fish, induces some certain levels of alteration in

the naturally occurring chemical composition of aquatic phase which in turn alters the behavior, biochemistry, haematology and general physiology of aquatic faunas (International Cyanide Management Institute. 2006. Retrieved 4 August 2009). However, certain serum chemistry could be used to identify tissue damage (Patti & Kwkarini 1993). For example, Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) are normally found within the cells of the liver, heart, gills, kidneys muscles and other organs of aquatic faunas (Shalaby, 2009) but their increase in the plasma indicate tissue injury or organ dysfunction (Adewoye, 2010). Furthermore, changes in plasma glucose, total proteins and cholesterol concentrations can be indicative of a classical general adaptive response to stress in fishes exposed to pollutants (Martinez et al., 2004). Wepener (1997) also suggested that haematology, biochemistry changes, growth rate and oxygen consumption of fish can be used in determing the toxicity of pollutants.

In recent years, haematological variables were used more when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances as a result of environmental pollution (Wendelaar Bonga, 1997). Adewoye et al. (2005) stated that the effects of waste waters discharged into water bodies can be acute, which occurs rapidly and are clearly defined as fatal and rarely reversible or may be chronic which normally have lingering effects after long period of exposure and may ultimately cause death. The entry of toxicants into aquatic media may affect the water quality parameter which in turn leads to changes in the haematological variables of fish, due to its close association with the external environment (Kavitha et al., 2010. It has been reported that biological monitoring techniques like haematological and

biochemical variables are attractive and useful for monitoring environmental quality, water pollution, and the health conditions of aquatic organisms (Kavitha et al., 2011).

1.1 PROBLEM STATEMENT FOR THE STUDY

Relative to other agricultural products, the production and processing of cassava has increased drastically in Ikole. This has become particularly challenging given that the waste water is being released into the rivers directly or indirectly. The effluents from the cassava contains high quantity of cyanide which is toxic to aquatic lives and the end consumers, when it gets into the aquatic ecosystem, the impact and effect of cyanide on the fish and the ecosystem cannot be over emphasized. Aside from cyanide being acutely toxic to animals (especially fish) and humans, exposure of plants to cyanide can inhibit respiration and consequently lead to death. Furthermore, the presence of cyanide can leave aquatic ecosystems in a very bad condition leading to water pollution and cause detrimental effect on the fisheries resources. Moreover, water from all dug wells around the cassava processing factories may be unfit to drink because of the level of cyanide in it. The presence of cyanide may make the environments unfit for cultivation of any crop and cause detrimental effect on the fisheries resources and all this is a major concern in fisheries. (International Cyanide management Institute, 2006).

1.2 JUSTIFICATION FOR THE STUDY

Pollution is now the major cause of losses in fisheries resources and the improper managements of our water bodies. For more profitable yield from aquatic products, more attention should be paid to the effect of different pollutants and contaminants on the aquatic ecosystem and organism as a whole. Different research has been conducted on the effects of cassava on *Claris gariepinus* but control measures are not normally put into

place, therefore making all efforts to be less important. The research model will be a simulation of what may occur in the natural environment so as to effectively predict the effect of the pollutant on the fish.

The accelerating pace at which man-made changes are occurring in the aquatic environment seems to have channeled substantial interest into the search for immediate and efficacious solutions. The successful management of agricultural pollutants demands an ability to recognize and measure sub-lethal response, to interpret their impact upon fishes' rate of growth reproduction, disease and mortality, and to establish cause-effect relationships that enable appropriate remedial actions.

Introduction of cassava effluents into the aquatic environment may constitute a threat to fish production as the cyanide in the cassava can cause mortality, growth retardation, susceptibility to diseases and distorting the natural food production of fish.

Indicator organisms are needed to improve assessment programs on the ecological impacts of anthropogenic activities on the aquatic environment. Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution. It is therefore important to access the concentration of cassava effluent that will cause changes to the histological and the haematological characteristics of *Claris gariepinus*. *Claris gariepinus* was chosen as biological indicators because this fish has slim tolerant level to cyanide in cassava and will be able to ascertain the concentration at which each cassava effluent causes changes in the fish and to which level this fish will absorb cyanide in cassava without causing any damage to the fish.

This study is also important so as to be able to determine the cyanide content present in cassava effluents, their implications on fisheries resources as it affects both the ecosystem and the organisms in the sense that it can disrupt the genetic makeup of the aquatic organism. The growth of the fish as well and have impact on the food chain and food web thereby altering the processes within the aquatic ecosystem, in the same vain affecting fish quality and even reduce the population of fish that is available for capture from polluted water bodies, whereby it also affects fish processing and its export.

1.3 OBJECTIVES OF THE STUDY

The objectives of the study were to:

1. Determine the LC_{50} values (the concentration of cassava effluent which will kill 50% of the test organisms in 96hours) of *Claris gariepinus* juveniles exposed to varying concentrations of cassava effluents.
2. Determine the effects of sub-lethal concentration of cassava effluent on the histopathology of gills and liver cells of *Claris gariepinus*.
3. Determine the effects of sub-lethal concentration of cassava effluent on the hematological characteristics of *Claris gariepinus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy and biology of Clarias gariepinus

Clarias gariepinus belongs to the family Clariidae, its common name is African catfish, the air-breathing catfishes. They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems.

2.1.1 Description

The African sharp-tooth catfish is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. *Clarias gariepinus* has an average adult length of 1m–1.5 m (3 ft 3 in–4 ft 11 in) (Froese., *et al* 2014). It reaches a maximum length of 1.7 m (5 ft 7 in) TL and can weigh up to and more than 60 kg (130 lb).These fish have slender bodies, flat bony heads, notably flatter than in the genus *Silurus*, and broad, terminal mouths with four pairs of barbels. They also have large accessory breathing organs composed of modified gill arches. Also, only the pectoral fins have spin. The African catfish is a dominant freshwater fish. It can also grow to between 1.4 and 2m long and can weigh anything from 8kgs to 59kgs. Its body coloration varies from olive green, to brown and black with the flanks often uniform grey to olive-yellow with dark slate or greenish brown back. Underparts are pale olive to white and are mottled irregularly with dark brownish green, or uniformly silvery olive. It is a heavy boned, flat headed fish with premaxilla and lower jaw pointed teeth arranged in several rows and four pairs of long trailing sensory organs known as 'barbels' around its mouth giving it a

similar appearance to a cat, hence the name catfish. It has a high number of gill rakers varying from 24 to 110, the number increasing with the size of the fish. The body is elongated with long, low dorsal and anal fins and a smoothly rounded tail fin. The skin is leathery and has no scales. It has a small but powerful pectoral fin set immediately in front of the anal fin which has a serrated spine. The eyes are small and set far forward in a flat and bony head. At the back of the head there is a subsidiary breathing organ above the gills which enables this animal to breathe air directly. Having a bi-loded swim bladder which is connected to the oesophagus via a narrow phneumatic duct all making the catfish negatively buoyant. The swim bladder is reduced to compensate for this buoyancy. Air is retained in the suprabranchial chamber when a vertical stationary position is required but the air is expelled when the fish need to plunge down suddenly to avoid predation (De Moor & Bruton, 2003).

2.1.2 Habits

It is a nocturnal fish like many catfish. It feeds on living, as well as dead, animal matter. Because of its wide mouth, it is able to swallow relatively large prey whole (Anoop KR et al., 2009). It is also able to crawl on dry ground to escape drying pools. Furthermore, it is able to survive in shallow mud for long periods of time, between rainy seasons.

2.1.3 Habitat and Biology

This species is found in lakes, streams, rivers, swamps and floodplains, many of which are subject to seasonal drying. The most common habitats are floodplain swamps and pools where they can survive during the dry season(s) due to their accessory air breathing organs. *Clarias gariepinus* undertake lateral migrations from the larger water bodies, in which they feed and

mature at about the age of 12 months, to temporarily flooded marginal areas in order to breed. These reproductive migrations typically take place shortly after the onset of the rainy season(s). The final gonadal maturation is associated with rising water levels. Under stable environmental conditions, adult *C. gariepinus* have mature gonads year-round. Under ideal conditions, a ripe female may lay about 60 000 eggs/kg. Prior to mating, males compete aggressively for females with which they mate in single pairs, the female swishing her tail vigorously to mix the eggs and sperm and distribute the fertilized eggs. The adhesive eggs stick to submerged vegetation and hatch in 20–60 hours, depending on temperature. The yolk sac is absorbed within 3–4 days and the stomach is fully functional within 5–6 days after onset of exogenous feeding. Sexual differentiation begins between 10 and 15 days after hatching. Larvae feed and grow rapidly in the warm (usually >24 °C) nutrient rich floodplains, reaching 3-7 g within 30 days. As flooded marginal areas dry up with the end of the rains, juveniles and adults make their way back to deeper water. In areas with two rainy seasons, there are usually two reproductive peaks during the year, corresponding in intensity to the magnitude of the rains.

2.2 CASSAVA

Cassava is one of the most important energy sources in the diet of people in the tropics (Presston, 2004). Recent estimates suggest that its storage roots provide eight percent or more of the minimum calorie requirement of more than 750 million people. Its starchy root produces more calories per unit of land than any other crop in the World (Presston, 2004). Cassava contains about 92.2 percent carbohydrates and 3.2 percent protein in its dry matter, and is said to have high energy content. According to Pedrosa (2002), cassava roots are generally rich in calcium and ascorbic acid and contain

significant amounts of thiamine, riboflavin and niacin. Cassava roots are rich source of carbohydrate. Most of the carbohydrate is present as starch (31% of the fresh weight) and with smaller amounts of free sugar (less than 1% of the fresh weight) (Pedrosa, 2002).

Cassava roots are low in protein (0.53 %), although higher concentration of 1.5 % protein and 0.17% fat have been reported by Ekpenyong (1984). Protein from other sources is therefore needed if cassava is to be used in fish diet. Cassava leaves in contrast to the root are high in protein (5.1 % on the fresh-weight basis, which exceeds 20 % on a dry matter basis). Cassava is generally considered to have a high content of dietary fibre, magnesium, sodium, riboflavin, thiamin, nicotinic acid and citrate (Bradbury & Holloway, 1988) with low iron and vitamins. However, some cassava varieties that are yellow in colour contain significant concentration of β-carotene, up to 1mg $100g^{-1}$ on a dry-weight basis (McDowell & Oduro, 1983). Its starchy, thickened, tuberous roots are valuable source of cheap calories and its use in animal feed is increasing because of its high energy content and low price (Salami, 2000). In recent times, the use of cassava as a substitute for cereals in livestock and fish feeds has been under investigation (Salami, 2000). Inclusion of cassava in the diet of white Fulani herds in Nigeria has been reported to increase milk production by 22% (IITA, 1990). Oke (2007) reported that cassava products are good energy feed ingredients for both monogastric and ruminant animals.

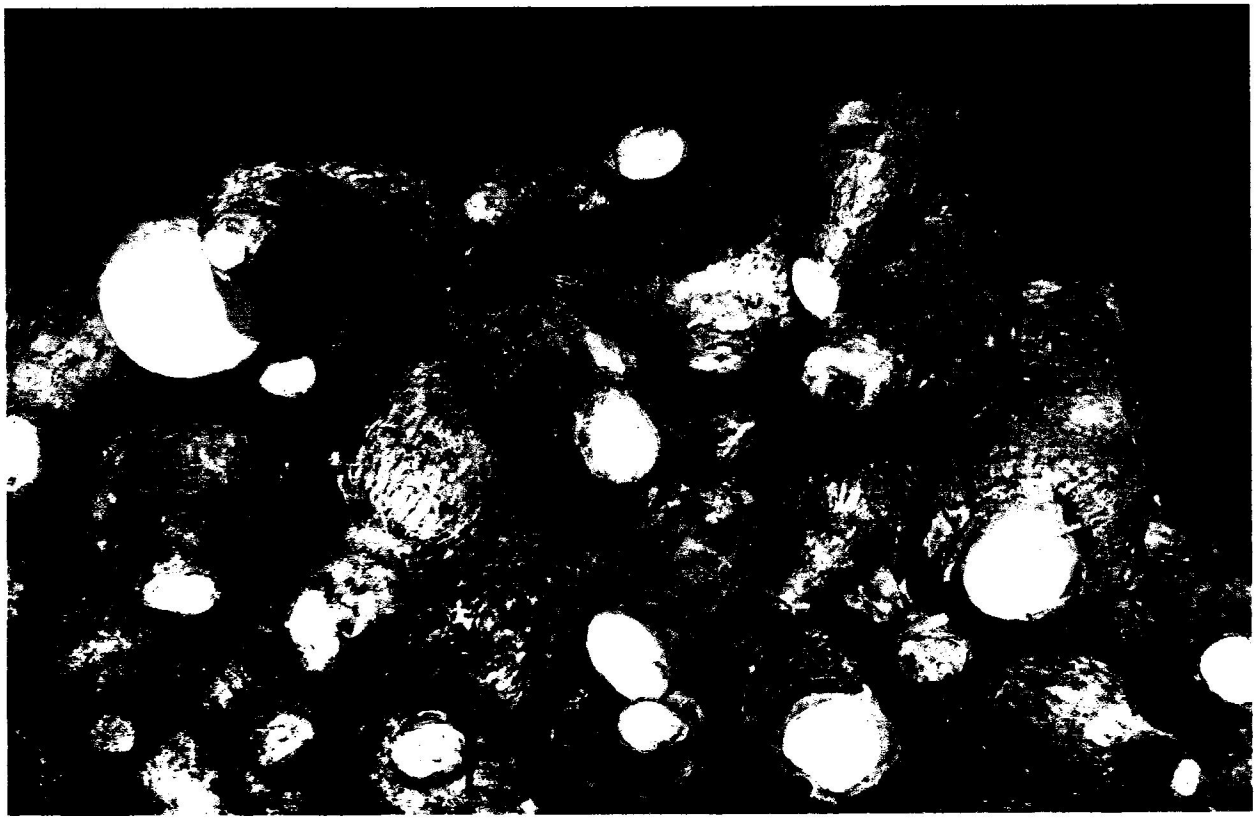


Fig 1: Cassava tubers

Talthawan et al. (2002) reported that the starch in cassava is highly digestible when compared to that of maize due to the high content of amylopectin. Cassava products are used in various forms for human consumption, livestock feed, and manufacturing of industrial products (Ene, 1992). According to IITA (1990), cassava products are also important feedstuff for livestock feed formulation. For example, cassava has a capacity of substituting up to 44 percent maize in pig feed without any reduction in the performance of pigs. Okeke (1998) also observed that in compounding feed for pigs, broilers, pullets and layers, cassava meal plays a significant role.

Cassava grows in a bushy form, up to 2.4 m tall, with greenish-yellow flowers, the roots are up to 8 cm thick and 91 cm long, the roots contain 20 to 32 % of starch at maturity and early maturing cultivars are ready for harvest after 8-12 months (Presston, 2004). Two

varieties of cassava are of economic value: the bitter, or poisonous; and the sweet, or non-poisonous, because the volatile poison can be destroyed by heat in the process of preparation, both varieties yield a wholesome food (Presston, 2004). Cassava is the chief source of tapioca and sauce and intoxicating beverage are prepared from the juice in South America. Cassavas belong to the family *Euphorbiaceae.* Both bitter and sweet cassava are classified as *Manihot esculenta* or *Manihot utilissima* or *Manihot Aipi* (Presston, 2004).

Cassava (*Manihot esculanta Crantz*) is a root tuber crop that is widely cultivated in the tropical regions of the world (Iyayi & Losel, 2001; Oboh and Akindahunsi, 2003). As a shrubby perennial that grow to a height of 6-8 ft, it is usually propagated by planting short section of the stem (O'Hair, 1995; Oboh, 2005). Suffix to say that different cultivars of cassava which abound worldwide mature at different rates. However, certain varieties contain large amount of cyanogenic glycosides (linamarin and lotaustralin) which can hydrolysed to hydrocyanic acid (HCN) by their endogenous enzyme (linamarase) when the plant tissue is damaged during harvesting, processing or other mechanical processing (Oboh & Akindahunsi, 2003). The protein content of cassava products can be increased by adding protein to the deficient food in a way that will not alter the organoleptic qualities of the original food (Oboh, 2005). Also, through controlled fermentation, microflora could be made into large numbers in the mash (Raimbault, 1998; Oboh et al., 2002; Oboh & Akindahunsi, 2003), thus increasing the protein content of cassava products.

Oboh (2005) identified two important wastes that are generated during the processing of cassava tubers to include cassava peels and the liquid squeezed out of the mash. The bioconversion of the cassava wastes have been documented (Antia & Mbongo, 1994; Okafor, 1998; Raimbault, 1998; Tweyongyere & Katongole, 2002; Oboh, 2005). The

waste water contains heavy loads of microorganisms, lactic acid, lysine (from L. coryneformis), amylase (from L. delbruckii) capable of hydrolyzing the glycosides (Raimbault, 1998; Akindahunsi et al., 1999). The industrial applications of amylases as additives in detergents for the removal of starch from textiles, liquefaction of starch and proper formation of dextrin in baking have been reported (Shaw et al., 1995).

In addition, amylases are used in high fructose corn syrup preparation, saccharification of starch for alcohol production and in brewing (Uzochukwu, et al., 2001; Aiyer, 2004). Cellulase is added to crush apples to increase juice yield and prevent contamination (Bhat, 2003). Cellulases are utilized in the textile industry for colour brightness (Csizer et al., 2001) and for stone wash look in jeans (Haki & Rakshik, 2003), paper processing, production of ethanol for fuel from the non-edible portion of corn and wheat (Iogen Corporation, 2003). Therefore, the industrial applications of these enzymes (amylases and cellulase) cannot be underscored.

2.2.1 CASSAVA PRODUCTION IN NIGERIA

Cassava *(Manihot esculenta)* production is vital to the economy of Nigeria as the country is the world's largest producer of the commodity (Daramola & Osanyinlusi, 2006). The crop is produced in 24 of the country's 36 states. In 1999, Nigeria produced 33 million tonnes, while a decade later, it produced approximately 45 million tonnes, which is almost 19% of production in the world. The average yield per hectare is 10.6 tonnes.

In Nigeria, cassava production is well-developed as an organized agricultural crop. It has well-established multiplication and processing techniques for food products and cattle feed. There are more than 40 cassava varieties in use. Cassava is processed in many

processing centres and fabricating enterprises set up in the country. Although the crop is produced in 24 of the country's 36 states, (Adeniyi et al., 2005), cassava production dominates the southern part of the country, both in terms of area covered and number of farmers growing the crop. Planting occurs during four planting seasons in the various geo-ecological zones. The major states of Nigeria which produce cassava are Anambra, Delta, Edo, Benue, Cross River, Imo, Oyo, and Rivers, and to a lesser extent Kwara and Ondo. (Adeniyi et al., 2005).

Cassava is grown throughout the year, making it more preferable than the seasonal crops such as yam, beans or peas. It displays an exceptional ability to adapt to climate change, (Adekanye et al., 2013) with a tolerance to low soil fertility, resistance to drought conditions, pests and diseases, and suitability to store its roots for long periods underground even after they mature. Use of fertilizers is limited, and it is also grown on fallow lands. (Adeniji, et al., 2005) Harvesting of the roots after planting varies from 6 months to 3 years.

2.2.2 Classification of Cassava

There are two varieties of cassava which are traditionally designated as "bitter" or "sweet" and have different levels of cyanogenic glucosides, the precursors of the highly toxic cyanic acid (HCN). Sweet cassava (*Manihot palmata or Manihot dulcis*) has lower content of HNC which is store in cassava as glucosides but higher in the bitter variety. They are concentrated in the skin and cortex and removed during peeling while in the sweet cassava, glucosides are distributed throughout the root. The sweet variety can be eaten directly. Over the years consumers have developed a way of processing cassava to remove these poison. (Sanni et al., 1998). Normally, the roots of the sweet variety are used for

human consumption and are boiled before being eaten. Sweet cassava is a major food or food ingredient in many countries. The composition of this tuber is 38% carbohydrate and 60% water (Charles & Huang, 2009). A few studies have indicated that the carbohydrates in cassava tubers contain monosaccharides (fructose, arabinose, and galactose) and polysaccharides (Charles et al., 2008). It has also been reported that the intake of high-carbohydrate foods increases muscle glycogen content, which can prolong exercise time and delay fatigue (Charles & Huang, 2009). Recently, several studies have indicated that extracted polysaccharides in sweet cassava provides the following benefits: enhancing muscle glycogen and sports performance, extending endurance times, resistance to fatigue, decreasing oxidative stress after strenuous exercise and detoxifying the body. Although sweet cassava is a staple food in many countries, and the literature indicates that it contains abundant carbohydrates and seems beneficial for sports performance (Shephard & Leatt, 2007). Another variety of sweet cassava is *Manihot utilissima or Manihot esculenta*. The variety is considered to require some additional form of processing, such as sun-drying, before it is used as animal feed. Cassava leaves have been claimed to be a very important component of integrated farming systems in tropical countries (Preston, 2001). Cassava varieties are classified according to the level of cyanogenic glucosides in the tubers and leaves, (IITA, 1990). Stem pigmentation is used by many farmers in Africa to differentiate varieties

2.3 CASSAVA EFFLUENT

2.3.1 SOURCES OF CASSAVA EFFLUENTS

Cassava is processed in many processing centres and fabricating enterprises set up in the country. During the processing of cassava tubers in various products, liquid waste water

generated has been reported to cause serious havoc to vegetation, houses and bring about infection. This no doubt has being causing serious environmental pollution as a result of the indiscriminate discharge. The liquid squeezed out can be dried and used as animal feeds (Okafor, 1998; Oboh & Akindahunsi, 2003).

2.3.2 CYANIDE IN CASSAVA

A cyanide is any chemical compound that contains monovalent combining group CN. This group, known as the cyano group, consists of a carbon atom triple-bonded to a nitrogen atom (International Cyanide Management Institute. 2006). In inorganic cyanides, such as sodium cyanide and potassium cyanide this group is present as the negatively charged polyatomic cyanide ion (CN^-); these compounds, which are regarded as salts of hydrocyanic acid, are highly toxic (Vetter, J. (2000). The cyanide ion is isoelectronic with carbon monoxide and with molecular nitrogen (Jones, D. A. (1998). Organic cyanides are usually called nitriles; in these, the CN group is linked by a covalent bond to a carbon-containing group, such as methyl (CH_3) in methyl cyanide (acetonitrile). Holleman, A. F.; Wiberg, E. (2001) Because they do not release cyanide ions, nitriles are generally less toxic, or in the case of insoluble polymers such as acrylic fiber, essentially nontoxic unless burned. Hydrocyanic acid, also known as hydrogen cyanide, or HCN, is a highly volatile liquid used to prepare acrylonitrile, which is used in the production of acrylic fibers, synthetic rubber, and plastics Holleman, A. F.; Wiberg, E. (2001). Cyanides are employed in a number of chemical processes, including fumigation, case hardening of iron and steel, electroplating, and the concentration of ores. In nature, substances yielding cyanide are present in certain seeds, such as the pit of the cherry and the seeds of apples (Wolfgang, 2006).

2.3.3 Cyanide and Aquatic Organisms

Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. Concentrations of free cyanide in the aquatic environment ranging from 5.0 to 7.2 micrograms per liter reduce swimming performance and inhibit reproduction in many species of fish. Other adverse effects include delayed mortality, pathology, and susceptibility to predation, disrupted respiration, osmoregulatory disturbances and altered growth patterns (Fish site, 2008). Concentrations of 20 to 76 micrograms per liter free cyanide cause the death of many species, and concentrations in excess of 200 micrograms per liter are rapidly toxic to most species of fish. Invertebrates experience adverse nonlethal effects at 18 to 43 micrograms per liter free cyanide, and lethal effects at 30 to 100 micrograms per liter (although concentrations in the range of 3 to 7 micrograms per liter caused death in the amphipod *Gammarus pulex*) (Fish site, 2008). Algae and macrophytes can tolerate much higher environmental concentrations of free cyanide than fish and invertebrates, and do not exhibit adverse effects at 160 micrograms per liter or more. Aquatic plants are unaffected by cyanide at concentrations that are lethal to most species of freshwater and marine fish and invertebrates (Fish site, 2008).

However, differing sensitivities to cyanide can result in changes to plant community structure, with cyanide exposures leaving a plant community dominated by less sensitive species (Wolfgang, 2006). The toxicity of cyanide to aquatic life is probably caused by hydrogen cyanide that has ionized, dissociated or photo chemically decomposed from compounds containing cyanide (Anon, 2004). Toxic effects of the cyanide ion itself on aquatic organisms are not believed to be significant, nor are the effects of photolysis of Ferro- and ferricyanides. It is therefore the hydrogen cyanide concentration of water that is

of greatest significance in determining toxicity to aquatic life rather than the total cyanide concentration. The sensitivity of aquatic organisms to cyanide is highly species specific, and is also affected by water pH, temperature and oxygen content, as well as the life stage and condition of the organism.

2.3.4 TOXICITY IN CYANIDE

Many cyanides are highly toxic. The cyanide anion is an inhibitor of the enzyme cytochrome oxidase (also known as aa3) in the fourth complex of the electron transport chain (found in the membrane of the mitochondria of eukaryotic cells). It attaches to the iron within this protein. The binding of cyanide to this enzyme prevents transport of electrons from cytochrome c to oxygen (International Cyanide Management Institute, 2006). As a result, the electron transport chain is disrupted, meaning that the cell can no longer aerobically produce ATP for energy (Biller, José 2007). Tissues that depend highly on aerobic respiration, such as the central nervous system and the heart, are particularly affected (Anon, 2004). This is an example of histotoxic hypoxia. The most hazardous compound is hydrogen cyanide, which is a gas at ambient temperatures and pressure and can therefore be inhaled. For this reason, an air respirator supplied by an external oxygen source must be worn when working with hydrogen cyanide. Hydrogen cyanide is produced when a solution containing labile cyanide is made acidic, because HCN is a weak acid. Alkaline solutions are safer to use because they do not evolve hydrogen cyanide gas. Hydrogen cyanide may be produced in the combustion of polyurethanes; for this reason, polyurethanes are not recommended for use in domestic and aircraft furniture (Biller, José et al., 2007). Oral ingestion of a small quantity of solid cyanide or a cyanide solution as little as 200 mg, or to airborne cyanide of 270 ppm is sufficient to cause death

within minutes. Organic nitriles do not readily release cyanide ions, and so have low toxicities. By contrast, compounds such as trimethylsilyl cyanide (CH3)3SiCN readily release HCN or the cyanide ion upon contact with water (Biller, José et al., 2007).

2.4 PHYSICO-CHEMICAL PARAMETERS OF WATER

Aquatic habitats are remarkably diverse with respect to the chemical and physical properties of the water, for example; salinity vary between full-strength seawater and near distilled water while pH may differ by as much as 6.0 pH units (Perry, 1993). Furthermore, aquatic environments especially the freshwater ecosystems, are typically unstable and characterized by marked natural fluctuations of temperature, pH, dissolved oxygen, dissolved carbon-oxide and dissolved ions (Perry & Laurent 1993).

2.4.1 Temperature

Temperature is the degree of hotness or coldness of a body (Merriam-Webster Online Dictionary: Merriam-webster.com. 2010-08-13. Retrieved 2010-08-26). In the aquatic environment, temperature is the major controlling factor (Barnabe, 1994). Temperature affects physical, chemical and biological processes in water bodies and also, the concentration of many variables (Enujiugha & Nwanna, 2004). Usually, body systems will show a 50% increase in activity for every 5^0C rise in temperature increases the rate of chemical reactions and decreases the solubility of gases (especially oxygen) in water.

2.4.2 Dissolved oxygen

Barnabe (1994) reported that oxygen is essential for living things/organisms. It is used in the oxidation of food, liberating the energy necessary for all vital activities such as swimming, hunting, reproduction, growth, etc. Dissolution of oxygen in water is dependent on temperature, at

5^0C for instance, water requires 12.7mg/l of oxygen to become saturated, whereas at 20^0C, 9.1mg/l of oxygen is needed (Templeton, 1995).

According to Barnabe (1994), the aquatic environment contains relatively little oxygen (less than $10cm^3/l$, contrasting $200cm^3/l$ of air), thus, concentration of oxygen is close to saturation (and sometimes super saturation) in the natural environment and varies from $8cm^3/l$ in cold water to $4.5cm^3/l$ in tropical waters, being less in marine waters and shallow fresh water because of the photosynthetic activities of plants. Enujiugha & Nwanna (2004) reported that high oxygen depletion can be so severe to fish life, oxygen content of natural waters varies with temperature, salinity, turbulence, the photosynthetic activity of algae and plants and atmospheric pressure.

Chapman & Kimstach (1992) noted that dissolved oxygen concentrations below 5mg/l adversely affect the functioning and survival of biological communities and below 2mg/l may lead to the death of most fish. Different fishes have different minimum requirements of dissolved oxygen below which they will die (Banarbe, 1994; Templeton, 1995). Ross & Ross (2002) reported that oxygen is the first limiting component of the aquatic environment and the minimum tolerated oxygen level varies with species. Tilapia, for instance are relatively hardy and will tolerate dissolved oxygen as low as 3mg/l whereas salmonids respond badly below 5mg/l or higher.

2.4.4 pH

pH is an expression of the hydrogen ion concentration of water ($pH= -\log_{10} [H^+]$. It is a measure of the acidity or alkalinity of water which is expressed on a scale between 0 and 14. Between 0 and 7, the water is acidic; it is neutral at 7 and basic above this value (Barnabe, 1994). Enujiugha & Nwanna (2004) stated that pH changes can drastically affect the structure and function of ecosystem, both directly and indirectly, pH of any

water body is dependent on temperature and temperature affects physical, chemical and biological processes in water bodies.

pH has a very important influence on the chemical environments, for example, the equilibrium between NH_4^+ and NH_3 in water is shifted towards the formation of NH_3 which is extremely toxic to fish as pH rises (Barnabe, 1994), while lower pH can adversely affect fishes' gills and can be detrimental to the growth of denitrifying bacteria (Cooper, 2004). The pH equilibrium depends on other interactions, mainly the carbondioxide carbonate system and pH governs the carbonate content of waters.

2.5 LIVER

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

2.6 GILLS

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

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

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Acclimation of Test fish

A total of 200 apparently healthy *Clarias garipienus* juveniles of mean weight and mean total length 9.73g and 12.5cm respectively, were bought live from Federal polytechnic Ado-Ekiti fish farm. The fish were transported live in oxygenated plastic bags to the Fisheries Laboratory of the Federal University Oye-Ekiti, Ekiti-state. Acclimation of fish was done in fresh water by gradually changing the water in the rectangular glass tanks (75 x 40 x 40cm) from 100% holding water to 100% dilution water for over five days. The fishes were fed daily to satiation during the first one week of acclimation, with a pelleted commercial feed (35% crude protein) in order to remove any problem that could arise as a result of starvation.

3.2 RANGE-FINDING TEST

One hundred (100) juveniles of *C. garipienus* of mean weight and mean total length 9.73g and 12.50cm respectively were used for the range finding test. Mettler top loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent cylindrical plastic containers for three days in order to adapt to laboratory conditions. The cassava effluent that was used as toxicant was collected from Ikole cassava processing factory Ikole- Ekiti. Feeding was discontinued during this period to reduce the production of waste in the transparent cylindrical containers thus minimizing the chances of ammonia production. Both the ranging finding and definitive tests were conducted under standard bioassay procedures (American Public Health Association, 1977). The range-finding test was carried out using ten transparent cylindrical plastic

containers of 21 litre capacity, each filled with 10 litres of water prior to the introduction of cassava effluent. Four varying concentration used were obtained as follows:

(i) $\frac{\text{30ml 0f cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **3.00ml/L**

(ii) $\frac{\text{40ml of cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **4.00ml/L**

(iii) $\frac{\text{50ml of cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **5.00ml/L**

(iv) $\frac{\text{60ml of cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **6.00ml/L**

Each of the four varying concentrations (4.00ml/L, 4.00ml/L, 5.00ml/L and 6.00ml/L) were duplicated; two replicates of the control treatment without a cassava effluent were also prepared.

3.3 DEFINITIVE TEST

A hundred (100) juveniles of *C. gariepinus* of mean weight and mean total length 9.73g and 12.50cm respectively were used for the definitive test. Mettler top loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent cylindrical plastic container. The cassava effluent that was used as toxicant was collected from Ikole cassava processing factory Ikole- Ekiti. Feeding was discontinued during this period to reduce the production of waste in the transparent cylindrical containers thus minimizing the chances of ammonia production. Ten transparent cylindrical plastic containers of 21 litre capacity, each filled with 10 litres of water were used for definitive test, prior to the introduction of cassava effluent. Four varying concentrations used in the Definitive experiment were obtained as follows:

(i) $\frac{\text{40ml 0f cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **4.00ml/L**

(ii) $\frac{\text{45ml of cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **4.50ml/L**

(iii) $\frac{\text{50ml of cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **5.00ml/L**

(iv) $\frac{\text{55ml of cassava effluent}}{\text{10litres of water in transparent cylindrical plastic container}}$ = **5.50ml/L**

Each of the four varying concentrations (4.00ml/L, 4.50ml/L, 5.00ml/L and 5.50ml/L) were duplicated and introduced into the transparent cylindrical plastic containers, two replicates of the control treatment without a cassava effluent were also prepared.

3.4 WATER QUALITY DETERMINATION

During the exposure period of four days, water temperature, dissolved oxygen, and pH were determined at 24hrs intervals, using standard methods.

3.4.1 Temperature

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

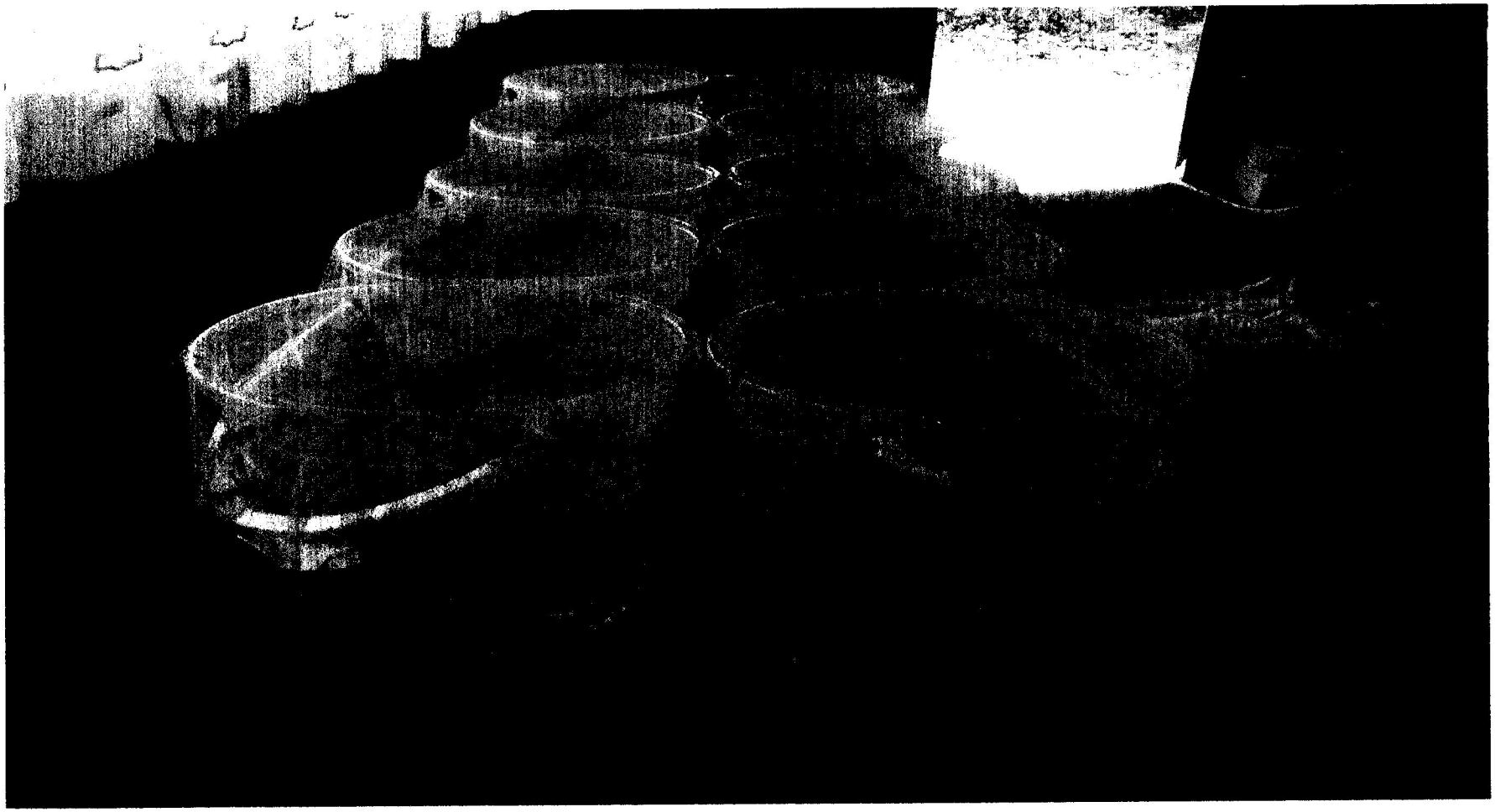


Fig 2: The Experimental Setup showing Clarias gariepinus with varying concentrations of cassava effluent

3.4.2 pH

pH was determined by using pH meter (model, METTLER TOLEDO 320). The probe was inserted into the sample bottles containing the different sample, and readings were taken and recorded.

3.4.3 Dissolved Oxygen (DO) Concentration

DO was determined using a dissolved oxygen meter (DO model 90.71). The probe was inserted into the sample bottles containing the different treatments. The unit of measurement is mg/l.

3.5 SURVIVAL AND OBSERVATIONS

The number of dead fish, in each transparent cylindrical plastic buckets was recorded every 24hrs. The percentage mortality in each concentration was estimated. General behaviour of the fish was monitored. Observations made include erratic swimming and discoloration.

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

The 96h – LC_{50} value is defined as the concentration of a toxic material which will kill 50% of test organisms in 96-hours (Aquatext, 2002). Data collected on mortality was subjected to Probit and Logit transformation method (Finney, 1982) and the LC_{50} value was determined accordingly.

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

Gills and liver of *C. gariepinus* were excised upon dissecting the fish. They were fixed in 10% formalin for three days to preserve the organs. The fixed organs were dehydrated in graded levels of alcohol (50%, 70%, 90%, and 100%) and cleared in 50/50 mixture of alcohol and xylene for three hours. The specimens were embedded in molten wax after fixing and later sectioned with

the aid of a microtone to thin sections using a microtone to 7μm sections and then stained in haematoxylin and eosin. The stained specimens were observed under a light microscope fitted with a camera. Photographs of the stained specimens were observed under a light microscope. Photographs of the stained specimens were finally taken and interpreted accordingly.

3.8 HAEMATOLOGICAL CHARACTERISTICS OF *C. gariepinus*

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

3.8.1 Packed Cell Volume (PCV)

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

3.8.2 Haemoglobin Concentration (Hb)

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

until the colour matched that of the standard sample. The standard sample is the colour produced by a known haemoglobin concentration and the blood was obtained by measuring the amount of oxygen or iron in the haemoglobin. The amount of solution in the graduated tube gives the haemoglobin concentration as a percentage, where the value obtained is multiplied by 17.2g/100ml and then divided by 100.

3.8.3 ERYTHROCYTE COUNTS

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

3.8.4 LEUCOCYTE COUNTS

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

3.9 STATISTICAL ANALYSIS

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

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 WATER QUALITY

The water parameters monitored were temperature, dissolved oxygen and pH. The values of the water parameters during the 96 hour period are shown in Table 1. The parameters were measured at 9h each day and at 24 hours internal. The temperature increased as the concentration increased. Similarly, pH increased as the concentration increased. However, dissolved oxygen increased greatly as the concentration increases.

4.2 OBSERVATIONS OF THE BEHAVIOUR OF *Clarias gariepinus*

The mortality of *C. gariepinus* was observed in all concentrations (4.00ml/L, 4.50ml/L, 5.00ml/L, 5.50ml/L) of cassava effluent used as seen in Table 2 and 3 below except in the control. Mortality was recorded at 24hours interval this varied significantly amongst the different concentrations (Table 2). Table 3 shows the behavioural responses of the test organisms during the toxicity test, *Clarias gariepinus* exhibited distress behavioural responses due to the effects of the cassava effluent. These behavioural responses were the sudden change in the organism's response to the environment such as erratic swimming, occasional gasping for breath and frequent surfacing which increased as the concentration increased. All these are indications that the concentrations had become hypoxic and as a result induced brain dysfunction in the test organisms due to low oxygen supply. As the experiment progressed, some of the test organisms got weaker as evident by the reduction in movement; their ventral surfaces were subsequently turned upward while those that couldn't tolerate the concentrations any longer became motionless. Normal behaviour was however observed in the control. Upon addition of cassava effluent (Table 3) fish showed

rapid opercula movements especially in higher concentrations (5.00ml/L and 5.50ml/L). Movement of fish in the control experiment (0ml/L) was observed to be generally in different directions, while movements of fish in the varying concentrations of cassava effluent were sporadic and vertical i.e. surfacing; this was particularly evident in plastic containers with higher concentrations. The LC_{50} obtained using Probit and Logit for fish exposed to cassava effluent was 4.365ml/L (Fig 3). The presence of cyanide on the gills could reduce the surface area where respiration could take place in the fish, resulting in mortality due to severe stress and discomfort.

4.3 HAEMATOLOGICAL PROPERTIES OF AFRICAN CATFISH EXPOSED TO VARYING CONCENTRATION OF CASSAVA EFFLUENT

The concentrations of cassava effluent used for the haematological test were 0ml/L, 4.00ml/L, 4.50 ml/L, 5.00 ml/L and 5.50 ml/L. The values of the haematological characteristics are presented in Table 4 .The value of the packed cell volume (PCV) in fish not exposed to cassava effluent was the highest (27.00 ± 0.58). However, PCV in African catfish exposed to 4.00ml/L and 5.50ml/L or 4.00ml/L and 5.50ml/L were not significantly different ($p>0.05$) from each other (22.00 ± 0.58 and 22.00 ± 0.58 respectively). The lowest PCV (10.00 ± 0.58) was recorded in test fish exposed to 4.50ml/L of cassava effluents and it was significantly different ($p>0.05$) from other concentrations. The value of Red blood cells (RBC) in the control fish (0.13 ± 0.04) was not significantly different ($p>0.05$) from fish treated with 5.00ml/L (0.2 ± 0.58) and 5.50ml/L (0.1 ± 0.58) of cassava effluent respectively. However, RBC increased in the blood sample analysed in fish exposed to 4.00ml/L (2.3 ± 0.58) and 4.50ml/L (1.2 ± 0.58) respectively. The highest RBC (2.3 ± 0.58) was recorded in fish exposed to 4.00ml/L while the lowest (0.1± 0.58) was recorded in the fish exposed to 5.50ml/L of cassava effluent.

The quantity of White blood cell (WBC) recorded in test organisms were significantly similar ($p>0.05$) in fish exposed to 4.50ml/L (11000 ± 577.35) of cassava effluent and the control (10000 ± 577.35). The lowest (2000 ± 577.35) was recorded in catfish exposed to 4.00ml/L of the effluent, this was however different ($p<0.05$) from WBC recorded in fish exposed to 5.50ml/L (5000 ± 577.35). The Haemoglobin concentration (Hb) of test organisms were not significantly different ($p>0.05$) in fish exposed to 4.00ml/L (7.00 ± 0.58) and 4.50ml/L (7.00 ± 0.58) of cassava effluent. Similarly, the quantity of Hb in African catfish exposed to 5.00ml/L (4.00 ± 0.58) and 5.50ml/L (3.00 ± 0.58) were not significantly different ($p>0.05$). However, Hb was highest in the control and lowest in 5.50ml/L (3.00 ± 0.58).

Blood count differentials showed that neutrophils were significantly different ($p<0.05$) in all the fish exposed to the varying concentrations of cassava effluent. The highest neutrophils was recorded in 5.00ml/L (63.00 ± 0.58) while the lowest was recorded in the control (40.00 ± 0.58). Lymphocytes equally showed significant differences ($p<0.05$) in blood samples collected from the varying concentrations with the lowest (12.00 ± 0.58) recorded in the control while blood Lymphocytes was high (31.00 ± 0.58) in *Clarias gariepinus* exposed to 5.00ml/L. Eosinophil was similar ($p>0.05$) in fish exposed to 4.50ml/L (0.30 ± 0.06), 5.00ml/L (0.20± 0.06) and 5.50ml/L (0.77 ± 0.33). However, eosinophil in the control (8.10 ± 0.55) and 4.00ml/L (4.20 ± 0.55) were different ($p<0.05$) from the ones recorded in 4.50ml/L, 5.00ml/L and 5.50ml/L.

Basophilis were similar ($p>0.05$) in fish treated with 5.00ml/L (0.20± 0.06) and 5.50ml/L (0.30± 0.06) respectively. This was however different ($p<0.05$) from the basophils value in the control (15.00 ± 0.58), 4.00ml/L and 4.50ml/L. The highest (25.00 ± 0.58) was recorded in fish treated with 4.50ml/L while the lowest was recorded in 5.00ml/L (0.20± 0.06). The results obtained from

the haematological analysis indicate that, the value of the blood indices decreased with increasing concentrations of cassava effluent.

Table 1: Physio-Chemical Parameters of Water with Varying Concentrations of Cassava Effluent during 96 Hour Period

	24	7.07 ± 0.07[d]	7.79 ± 0.05[a]	26.34 ± 0.08[a]
	48	6.08 ± 0.07[c]	7.82 ± 0.05[a]	27.57 ± 0.08[b]
	72	5.34 ± 0.07[b]	7.91 ± 0.05[ab]	27.63 ± 0.08[b]
	96	4.86 ± 0.07[a]	8.04 ± 0.05[b]	27.72 ± 0.08[b]
	Control	6.58 ± 0.08[d]	7.18 ± 0.06[a]	25.87 ± 0.09[a]
	4.00ml/L	6.21 ± 0.08[c]	8.04 ± 0.06[b]	27.44 ± 0.09[b]
	4.50 ml/L	5.72 ± 0.08[b]	8.03 ± 0.06[b]	27.57 ± 0.09[b]
	5.00 ml/L	5.53 ± 0.08[b]	8.08 ± 0.06[b]	27.87 ± 0.09[c]
	5.50 ml/L	5.16 ± 0.08[a]	8.14 ± 0.06[b]	27.85 ± 0.09[c]

Mean ± S.E with Different Superscript along row is significant at $p < 0.05$

Table 2: Mortality of *C. Gariepinus* Exposed To Cassava Effleunt at Varying Concentrations

	[illegible]	[illegible]	0.00 ± 0.00[a]	0.50 ± 0.50[ab]	1.50 ± 0.50[b]
	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.50 ± 0.50[ab]	1.50 ± 0.50[b]	1.50 ± 0.50[b]
	[illegible]	2.50 ± 0.50[b]	2.00 ± 0.50[b]	[illegible]	2.00 ± 0.00[b]
	0.00 ± 0.00[a]	1.00 ± 0.00[ab]	2.00 ± 1.00[ab]	2.50 ± 0.50[b]	2.50 ± 0.50[b]

Mean ± S.E with Different Superscript along row is significant at $p < 0.05$

Table 3: Effect of Cassava Effluent on Body Features of *Clarias Gariepinus* Juveniles

Observations	24Hour					48Hour					72Hour					96Hour				
	4.00	4.50	5.00	5.50	(Control) 0.00	4.00	4.50	5.00	5.50	(Control) 0.00	4.00	4.50	5.00	5.50	(Control) 0.00	4.00	4.50	5.00	5.50	(Control) 0.00
Erratic Swimming	-	-	-	+	-	-	-	+	+	-	+	+	+	+	-	+	+	+	+	-
Loss of reflex	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	+	+	+	-
Changes in behaviour	-	-	-	+	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	-
Discoloration	-	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Excessive mucus	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-	+	+	+	+	-
Air gulping	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	-
Death	-	-	+	+	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-

Keys

+ = present

- = not present

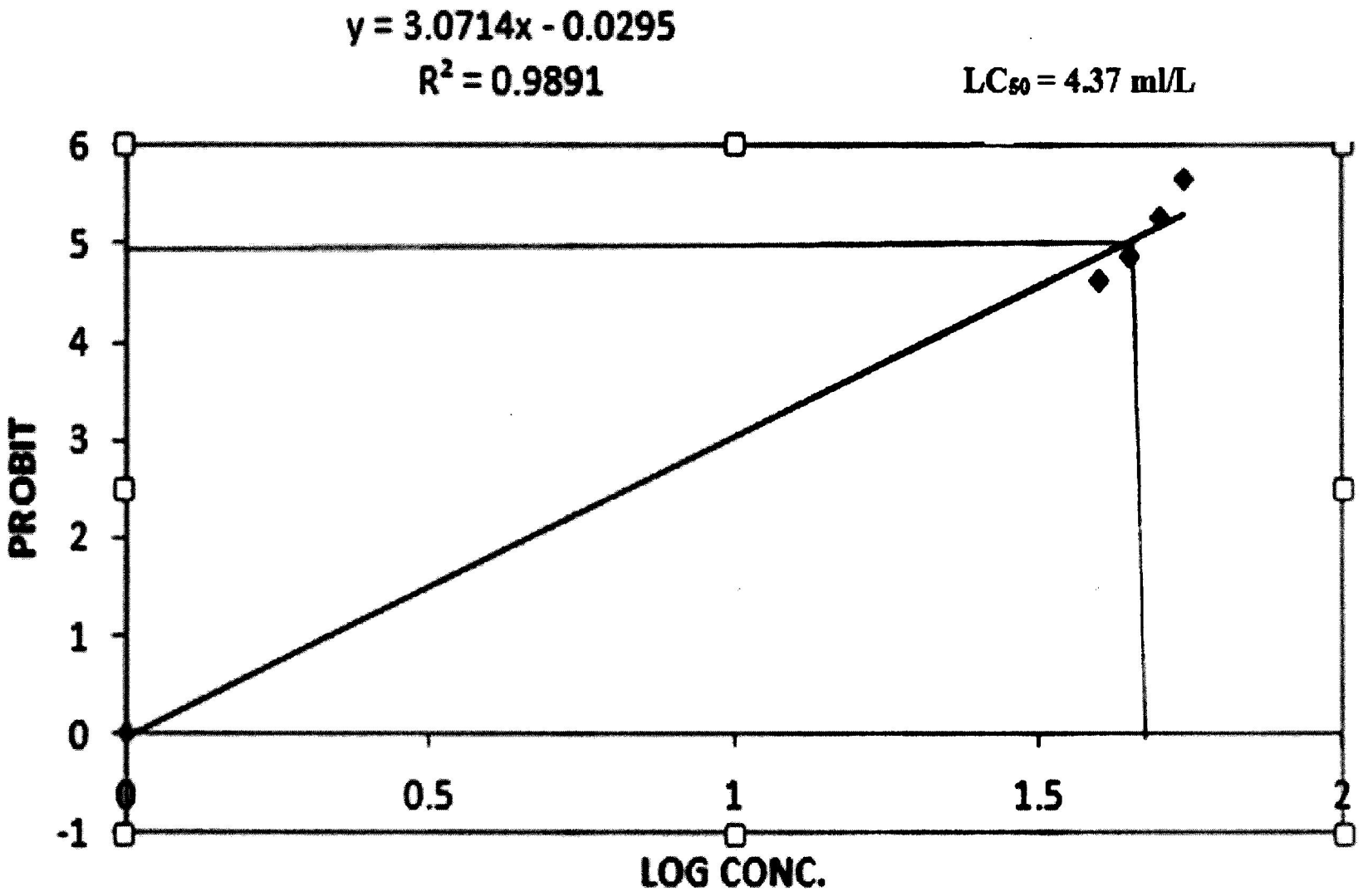


Fig. 3: Mortality (96h – LC_{50}) of *Claris gariepinus* in different concentrations of Cassava effluent

Table 4: Haematological Properties of African Catfish Exposed to Varying Concentrations of Cassava Effluent

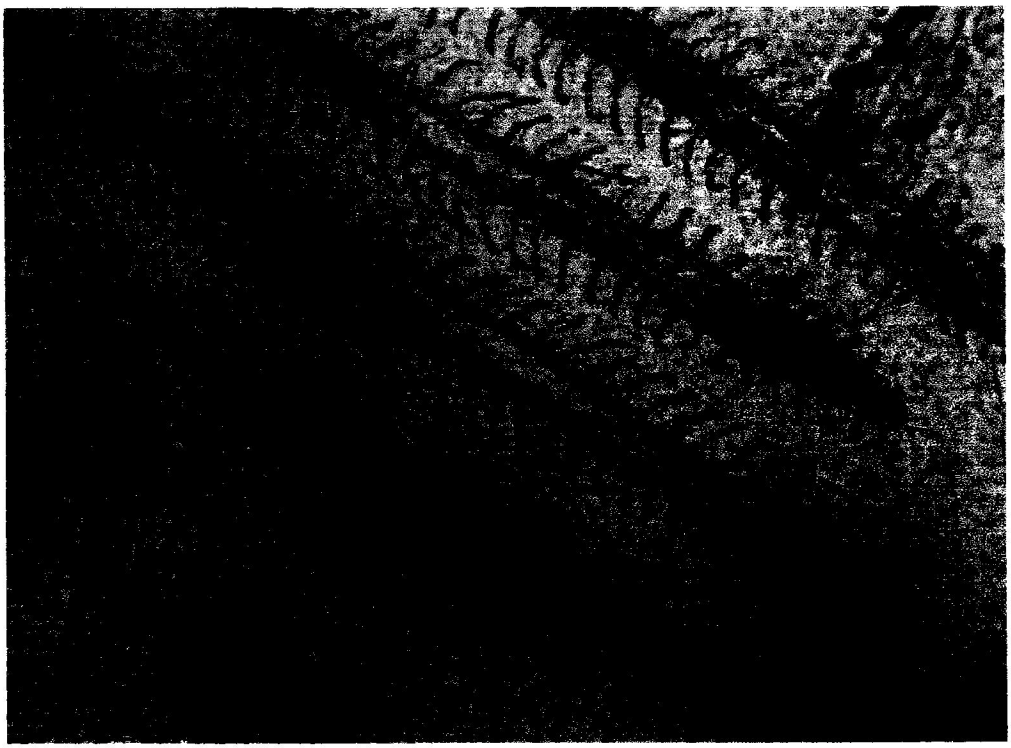
Parameters	Control (0 ml/L)	4.00 ml/L	4.50 ml/L	5.00 ml/L	5.50 ml/L
PCV (%)	27.00 ± 0.58^{d}	22.00 ± 0.58^{c}	10 ± 0.58^{a}	12 ± 0.58^{b}	22 ± 0.58^{c}
RBC($\times 10^{12}$)	0.13 ± 0.04^{a}	2.3 ± 0.58^{b}	1.2 ± 0.58^{ab}	0.2 ± 0.58^{a}	0.1 ± 0.58^{a}
WBC ($\times 10^{9}$)	10000 ± 577.35^{c}	2000 ± 577.35^{a}	$11000\ 577.35^{c}$	5000 ± 577.35^{b}	10000 ± 577.35^{c}
Hb (g/dl)	9.00 ± 0.58^{c}	7.00 ± 0.58^{b}	7.00 ± 0.58^{b}	4.00 ± 0.58^{a}	3.00 ± 0.58^{a}
Neutrophils	40.00 ± 0.58^{a}	48.00 ± 0.58^{b}	50.00 ± 0.58^{c}	63.00 ± 0.58^{e}	53.00 ± 0.58^{d}
Lymphocytes	12.00 ± 0.58^{a}	25.00 ± 0.58^{c}	27.00 ± 0.58^{d}	31.00 ± 0.58^{e}	20.00 ± 0.58^{b}
Eosinophils	8.10 ± 0.55^{c}	4.20 ± 0.55^{b}	0.30 ± 0.06^{a}	0.20 ± 0.06^{a}	0.77 ± 0.33^{a}
Basophilis	15.00 ± 0.58^{c}	12.00 ± 0.58^{b}	25.00 ± 0.58^{d}	0.20 ± 0.06^{a}	0.30 ± 0.06^{a}
Cyanide (mg/L)	ND	0.09 ± 0.05^{a}	0.13 ± 0.07^{b}	0.15 ± 0.06^{b}	0.18 ± 0.08^{b}

Mean ± S.E with different superscripts are significant at $P < 0.05$

4.4 HISTOLOGICAL CHANGES

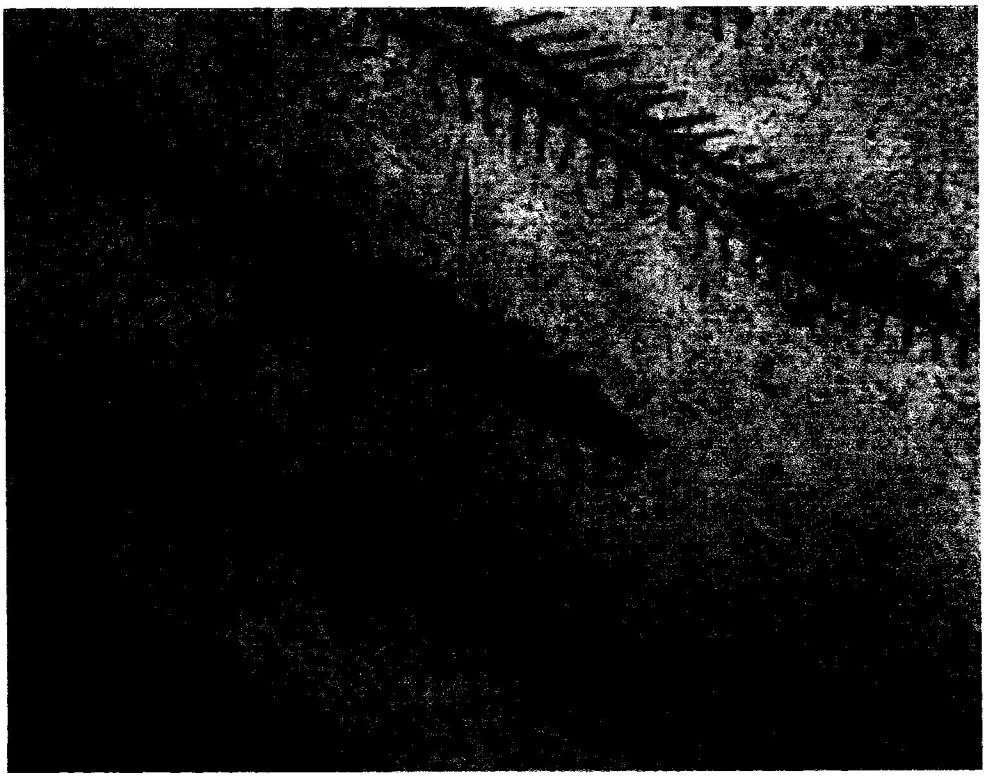
The gills and liver (plates 1-10) of the fish were examined to assess the histological effect of cassava effluent on them. Examination of gills and livers of fish in the varying concentrations showed varying degrees of damage to the tissues. Examination of the gills of fish in the control revealed a normal gill filament consisting of primary lamella with arrays of delicate secondary lamella, primary epithelium and secondary epithelium covering the primary and secondary lamella respectively, this was no vacuolation. Plate 2 of 4.00 ml/L cassava effluent concentration shows slight degeneration in the gill architecture and there was also slight congestion of the gills, with slight vacuole information in gills of the fish in that concentration. There was degeneration of the gills filament and the lamella of the fish in 4.50 ml/L concentration; it also shows erosion of the gills filament. However at higher concentrations (plates 4 and 5) 5.00 ml/L and 5.50 ml/L there was high level of degeneration in the filament, fragmentation of the lamella, vacuolation of the filaments, erosion of the gills and they showed the sign of necrosis (the cells were already dying because of too little oxygen reaching the cells via the blood).

Histological studies on the liver revealed that the control (Plate 6) had normal liver architecture (normal hepatocellular architecture). Plate 7 of 4.00ml/L concentration showed a slight/ hydropic degeneration (evidence of leaching/vacuolation), also plates 8 & 9 of 4.50ml/L & 5.00ml/L concentrations showed hydropic degeneration of the liver and at high concentration (5.50ml/L), the liver were dying (signs of necrosis). The cellular arrangement of liver cells were distorted, lesions were also present on the tissues of the liver. The results obtained from the experiments indicate that cassava effluent had a direct impact on the tissues of the gills and livers of *Clarias gariepinus.*



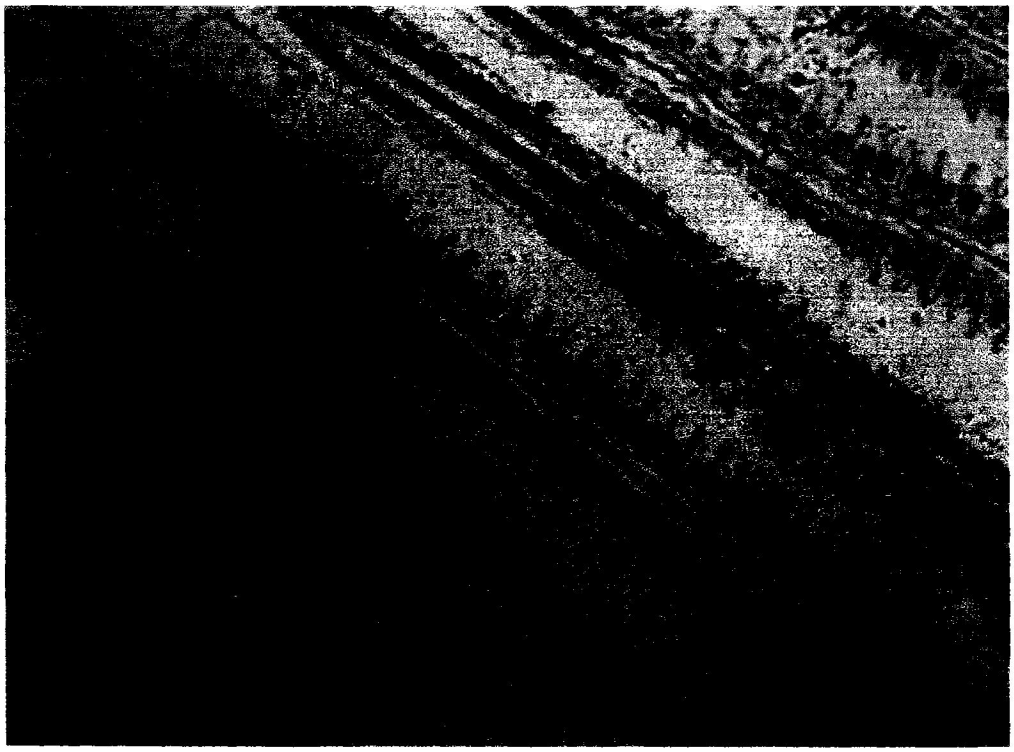
x 200

Plate 1: Gills of *C. gariepinus* in the control treatment showing normal gill architecture



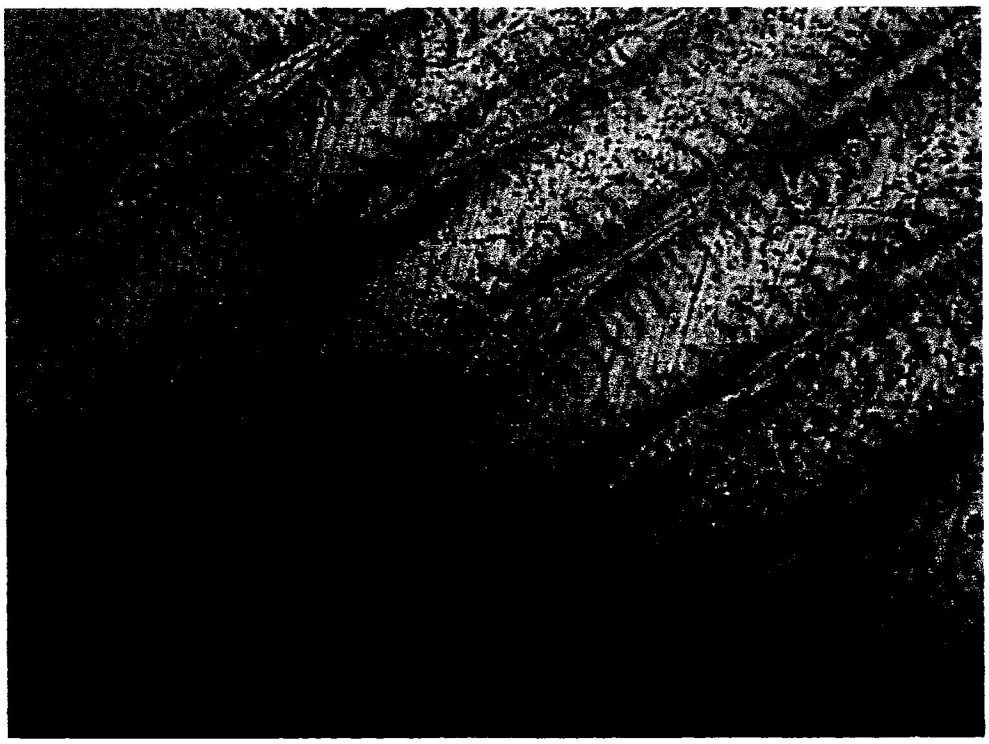
x 200

Plate 2: Gills of *C. gariepinus* exposed to 4.00 ml/L cassava effluent showing slight degeneration in the gill.



x 200

Plate 3: Gills of *C. gariepinus* exposed to 4.50 ml/L cassava effluent showing erosion of the gill filaments.



x 200

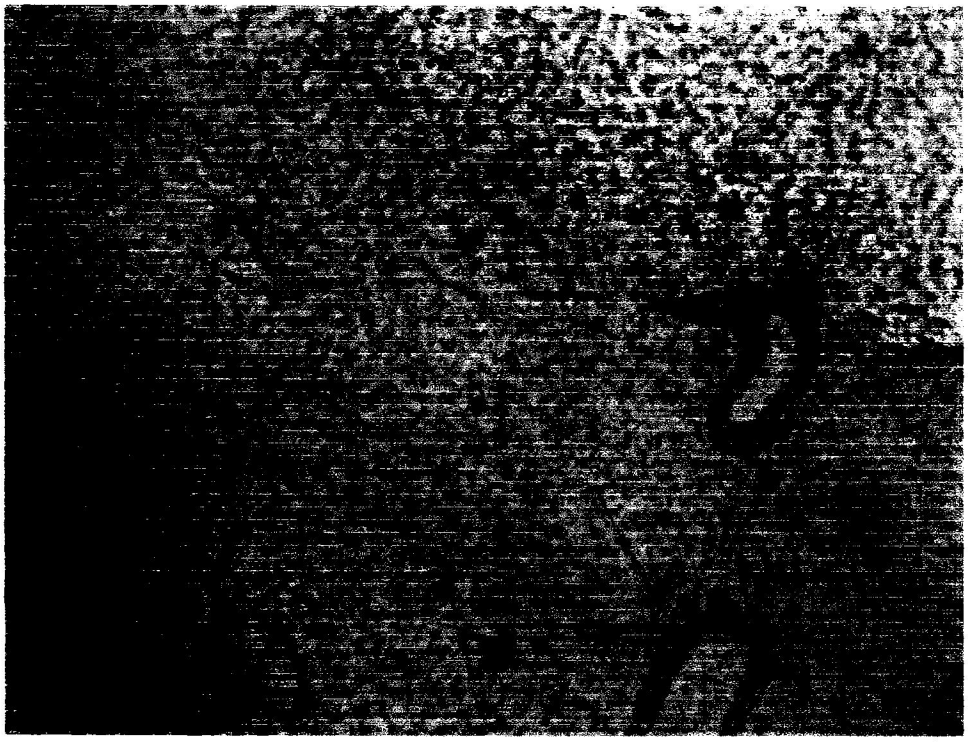
Plate 4: Gills of *C. gariepinus* exposed to 5.00ml/L cassava effluent showing high level of Degeneration in the filaments and gills erosion

x 200

Plate 5: Gills of *C. gariepinu* exposed to 5.50 ml/L cassava effluent showing the signs of necrosis.

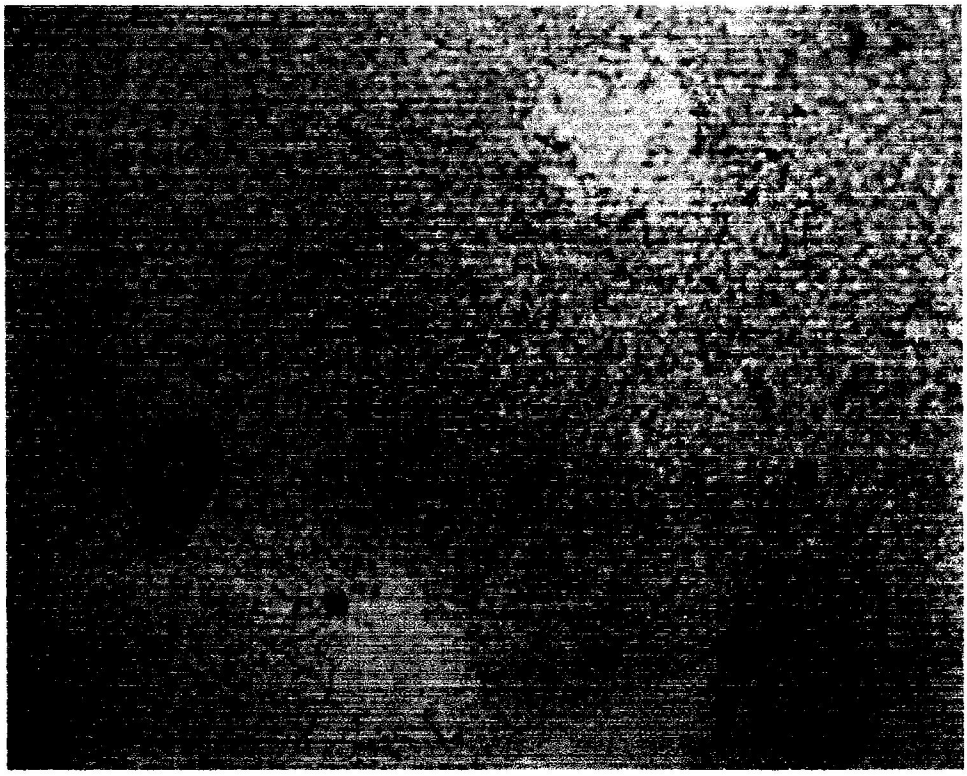
x 200

Plate 6: Liver of *C. gariepinus* in the control treatment with normal hepatocellular architecture.



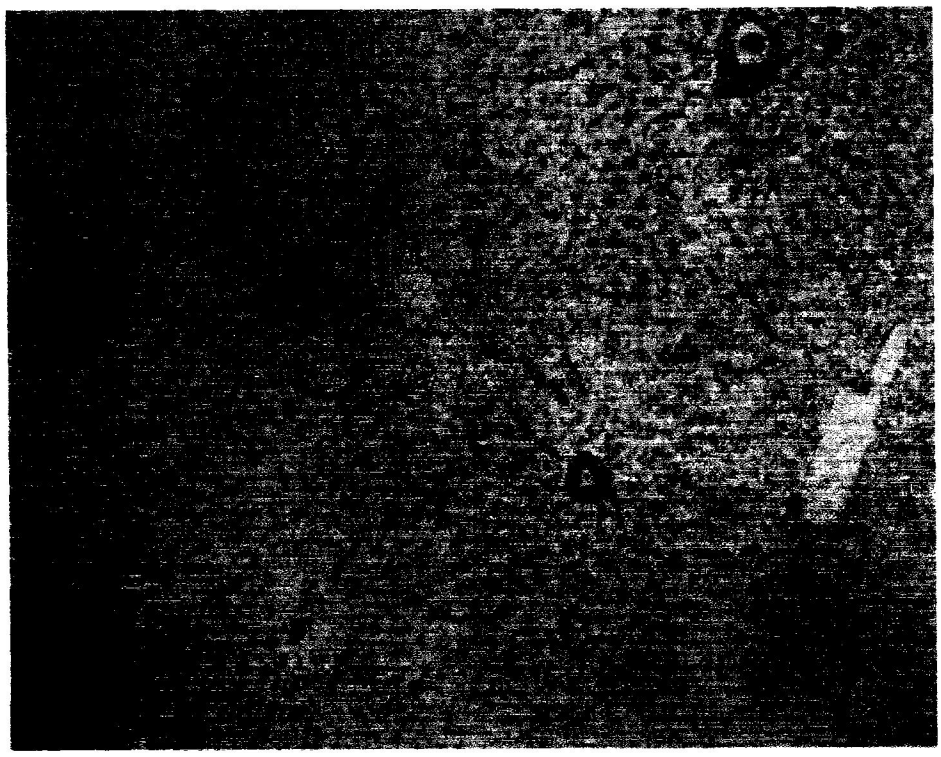
x 200

Plate 7: Liver of *C. gariepinus* exposed to 4.00ml/L showing slight/hydropic degeneration.



x 200

Plate 8: Liver of *C. gariepinus* exposed to 4.50ml/L cassava effluent showing hydropic degeneration.



x 200

Plate 9: Liver of *C. gariepinus* exposed to 5.00ml/L showing hydropic degeneration.

x 200

Plate 10: Liver of *C. gariepinus* exposed to 5.50ml/L showing the signs of necrosis

4.5 DISCUSSION

The mortalities recorded in the course of this study are an indication that the toxic effect of cyanide in the cassava effluent had disrupted the physiological system of the fishes to the extent that death is inevitable. The fish in the higher concentrations were affected the most and this shows that, the higher the concentration the higher the effect of the cyanide on the fish behavior. This corresponds with the finding reported by Adewoye et al. (2009) when they exposed fish X to effluent Y, it was observed that lethal effect occurred quickly and death was inevitable. Catfish juveniles exposed to varying concentration of cassava effluent were stressed progressively with time before death. The stress signs included erratic swimming, increased opercular ventilation, air gulping, and increased mucus secretion on the skin and gills. Studies have revealed that fish exposed to toxicants usually exhibits some behavioral changes such as increased opercular rate, erratic swimming, mucus secretion and gulping for air before death (Davis, 1973; Nwanna et al., 2000; Ariyomo et al., 2009). The excessive mucous secretion was a protective response of the exposed fish to coating the absorptive surfaces and preventing the continuous entry of the toxicant. Omoniyi et al. (2002) reported similar signs in *C gariepinus* exposed to the lethal and sub-lethal concentrations of *Nicotiana tobaccum* leaf dust extract. The respiratory distress may have also been due to gill epithelial damage as earlier reported by Fafioye et al. (2004) in *C. gariepinus* exposed to extracts of *Parkia biglobosa* stem bark. Air gulping and surfacing observed during the study were attempts by the exposed fish to cope with the increasing demand for oxygen (Schmidt et al., 2005; Ariyomo et al., 2009). The irregular, erratic and darting movements coupled with the observed loss of balance and the adoption of different postures by the exposed fish might be due to the stress caused by the cyanide content of the cassava effluent. Similar signs

were reported in *C. gariepinus* exposed to the aqueous extract of *N. tobaccum* leaf dust (Kori-Siakpere & Oviroh, 2011).

The pattern of behavioural changes observed in this study compared favorably with the records of Fafioye et al. (2004) when African catfish (*C. gariepinus*) was exposed to *P. biglobossa* and *Raphia vinefera* extracts and catfish hybrid fingerlings were treated with cassava mill effluents Oti (2002). Increased concentrations of cassava effluent led to increased behavioural changes and mortality as was also similarly observed in *C. gariepinus* exposed to aqueous extracts of *Blighia sapida* and *Kigelia africana* (Onusiriuka& Ufodike, 1994), catfish hybrid exposed to *Thevetia peruviana* (Oti & Ukpabi, 2000), and 2,4ichlorophenyloxyacetic acid (Koffi, 2005) and mudskippers (*Periophthalmus papillio*) exposed to *L. alopecuriodes* (Obomunu et al., 2007). The marked deviation in the rate of behavioral changes from reference (control) suggests an adjustment in physical fitness as a result of the stress condition (Edwards & Fushur, 1991; Leight & Van Dolah, 1999).

The value of 96hrs LC_{50} of 0.4365ml/L reported in this study is much higher than those earlier reported by Oti & Ukpabi (2000) and Fafioye et al. (2004) for some clariid species exposed to some plant extracts. This observation implies that cassava effluent is more toxic to African catfish than T. peruviana, P. biglobossa and R. vinifera. Mucus production and accumulation on the gills may have contributed immensely to the increase in the opercular ventilation and mortalities recorded in this study. Konar (1970) reported that accumulation of mucus on the gills reduces respiratory activity in fishes. This might be due to inability of the gills surface to actively carry out gaseous exchange. The observed restlessness and mortalities of the test fish might be due to the effect of cyanide present in the cassava effluent (Obomanu et al., 2005). Olaifa et al. (2004)

and Omitoyin et al. (2006) reported a 96-h LC_{50} of copper as 0.67 mg/l and lindane as 0.38mg/l for *C. gariepinus* respectively stating that they are highly toxic. The results of these studies were similar to those of several workers (Kulakkattolickal, 1987, 1989; Van Andel, 2000; Singh and Singh, 2009) on various aquatic organisms to different pesticides. The estimated 96- h LC_{50} (0.4365ml/L) of cassava effluent for the juvenile *C. gariepinus* in the present study is lower than 2.44 mgl^{-1} reported by Fafioye (2012) when white Tilapia fingerlings were exposed to water extract of Almond *Terminalia catappa.* The calculated LC_{50} value of 0.4365mg/L over the 96-h exposure period was different from the LC_{50} value of 2.8 mg/L reported by Fafioye et al. (2004) or the LC_{50} value of 105.83 mg/l reported by Abalaka & Auta (2010). This could be due to differences in fish age, treatment, the nature of the toxicity bioassay and methods of the LC_{50} determination as well as differences in the types and concentrations of the treatment used (Olaifa *et al.*, 2008), which is normally a function of the age and parts of the plants used as well as a function of the differences in the genetic make-up between species of the plant (cassava), including those of the climatic condition and the soil profile upon which the plants were grown (Norton, 1975; Botes et al., 2008; Borokini & Ayodele, 2012).

The behavioural changes associated with the different concentrations of cassava effluent indicated that the survival rate for the fish declined with decrease in the concentration of the toxicant. The agitated behaviours were attempts to escape from the toxic aquatic environment. Similar signs were reported in *C gariepinus* exposed to the aqueous extract of *Carica papaya* seed powder (Ayotunde *et al.*, 2011).

This study revealed that cyanide in cassava effluent is highly toxic to the catfish. It acts as respiratory poison possibly affecting the gills, impairing respiration and the various abnormal

behaviours and eventually death. These effects on fish are directly proportional to the toxicant concentrations. Fish mortality increased with increasing concentration, but later decreased with time. This shows that mortality is dose-dependent. No mortality was recorded in the control experiment during the toxicity test. This is also an indication that toxicity is dose-dependent and varies within the time of exposure of aquatic organisms to toxicants (Akinwande et al., 2007; Ayoola et al., 2011; Fafioye, 2012). Hence, the use of this toxicant in aquatic environment needs proper control to avoid reduction in fish production and non-target aquatic fauna. The abnormal behaviour in relation to fish stress included erratic swimming, mucus secretion, gasping for air, discoloration; which are indications of effect of cassava effluent. The abnormality observed could also be due to nervous disorder to impaired metabolism, but could in addition be due to nervous disorder as earlier reported by Agbon et al. (2002) and Aguigwo (2002).

In this study, rapid opercula movement and rapid release of bubbles indicate stress; this will further lead to the reduction of available oxygen which could result in fish mortality (Ariyomo et al. 2009). The values of the physico-chemical parameters of both the control and the other concentration used during the experiment are shown in Table 1. The values revealed that there is significant difference between the control and varying concentrations. Death/mortality of fish could have resulted from changes in the water quality particularly the pH values because the treatment is acidic, the pH increases based on the concentration and also the DO decreases accordingly as the temperature increases. Rapid opercula movement and rapid release of bubbles indicate stress, which could have resulted in the reduction of oxygen, during the 96hour exposure period, this conforms with the report of Enujiugha and Nwanna (2004) that reduction of oxygen levels can severely affect fish life, when dissolved oxygen values fall below minimum oxygen

requirement for a particular species of fish, they are subjected to stress, which can result in mortality. pH values increased during the exposure period and layers of mucus were seen on gills, particularly on gills of fish in the higher concentration (5.00ml/L and 5.50ml/L), this could have caused reduction in oxygen intake and resulted in the death of fish. This is in line with the findings reported by Korwin-Kossakowksi (1992), that reduction in fish respiration is mainly due to mucus coagulation in the gills which was caused by high pH to which *Cyprinus carpio* larvae were subjected, which led to lowered oxygen consumption of carp fry at high pH. However, (Adesina et al., 2013) indicated that dissolved oxygen, pH and temperature during their acute-lethal toxicity test were 3.3-6.2mgl^{-1}, 7.05-7.75, and 23.0 ^{0}C respectively. These values are within acceptable ranges for culturing Tilapia fish in the tropics (Ayoola et al., 2011; Fafioye, 2012). Adesina et al. (2013) stated that mortality was highest (77%) at 200.0 mgl-1 and lowest, (20%) in 19 mgl-1 after 96-h of exposure but this does not apply to catfish as seen in the present study. Fish mortality increased with increasing concentration, but later decreased with time. This study showed that the cassava effluent used caused the pH to increase and lowered the dissolved oxygen (DO) content of the water. This indicates that the effluent is toxic and should not be discharged indiscriminately into our immediate environment. Furthermore, Adewoye et al. (2005) stated that the observed characteristics features may have resulted from the organic loads in the wastewater. The abnormalities (gasping for breath and frequent surfacing) observed prior to mortality are indications of depleted oxygen content (hyposia) due to higher demand for oxygen. There was an observed positive correlation between concentration and response of the test organisms. The concentration-dependent nature of fish mortality in this study agrees with the work of Fafioye et al. (2004) who exposed C. gariepinus to extracts of *P. biglobosa* bark. Oxidative bio-degradation

of the extract over time as suggested by Kela et al. (1989) might be responsible for the subsequent decrease in the toxicity of the effluent with increasing exposure period. However, the findings in this study did not agree with the work of Fafioye et al. (2004) who reported an increase in toxicity with increasing exposure period. This may be due to the static nature of the present toxicity bioassay compared to the non-static nature of the previous work by Fafioye et al. (2004).

The investigation further showed that fishes can tolerate low concentrations of pollutants with reduced mortality as suggested by Oyedapo et al. (2011), who found that the abnormal behaviour observed in fish subjected to *Morinda lucida* increased with increasing concentration of the pollutant used. The 96-h LC_{50} value for the acute test in this study was 0.4365mg/L which mean that at this concentration of the effluent in the aquatic environment, half of the entire natural population will become dead and the fitness of the natural population of an aquatic environment would be relatively impaired and as the concentration increases, the mortality rate also increases (Adewoye et al., 2005). The temperature and acid pH were statistically different between the concentrations possibly because of the effluent toxicity. However, oxygen concentration reduced with increase concentration of effluent. The heightened activities of the fish due to poison can also remove oxygen from the water body (Shallangwa & Auta, 2008; Ariyomo et al., 2009).

The high WBC count recorded in this study could be due to an attempt by the fishes to fight against the antigens (pollutants) and this led to the production of more antibodies (WBC) to improve the health status of the organism. Similar findings were reported by Ates B et al. (2008) that the increase in WBC during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defence mechanism of the fish to tolerate the toxicity. The increase in lymphocytes count indicates the stimulatory effects of the toxicant on the immune system. The

gradual reduction in the values of WBC at 40ml/l and 50ml/l concentrations may be due to the breakdown of vital metabolic activities as a result of possible blockage in the metabolic pathway which then lowered the toxic production of WBC.

The observed reduction in haematocrit (PCV) percentage and haemoglobin concentration of the fish exposure to the effluent was as a result of uncontrolled hemolysis of the RBC due to the toxicity level of the effluent; while the decrease in haematocrit compared to the haemoglobin standards may be attributed to shrinkage of the erythrocytes (Abdel-Hadi YM et al., 2011, Atamanalp M et al., 2003; Martinez CBR et al., 2002)

There was a significant decrease in both the white and red blood cell counts with increasing concentration of the toxicant to a point. They then increased as the concentration increased but the red blood cell decreased as concentration of the effluent increased. Similar results were reported by Ayotunde et al. (2010) and Ayoola (2006). This may have accounted for the significant fall in packed cell volume, which was lower in the exposed groups compared to the control group. This is because the packed cell volume is influenced by both the number (population) of cells and their size. In this experiment, the cell volume changed significantly in the exposed groups compared with the control. It could therefore be assumed that the decrease in packed cell volume was due to decrease in cell population. In this study, the cell haemoglobin was reducing with increasing concentration of effluent, this may have triggered the multiplication of the blood cells to compensate for the low load of haemoglobin per cell , given that haemoglobin is an oxygen carrier, its availability in tissues of vertebrate is important. Its reduction invariably means anoxic internal environment that confers stress on the organism (Ayotunde et al., 2010). Tissue respiration and metabolism induces morbidity and mortality. Increase in blood cell count is seen

as an adaptation of organism to fight cell poisons. Baker et al. (2001) explained that the population of blood cells exposed is usually made of younger and smaller cells because the older ones are killed. The surviving ones have to multiply rapidly to fight their destroyers. The increase in the number of younger and smaller cells may have been responsible for the slight reduction but statistically similar cell volume observed between exposed and controlled groups.

Exposure of *C. gariepinus* to sub lethal concentration of cassava effluent caused a decrease in packed cell volume (PCV), and haemoglobin concentration (Hb). Similar reductions were reported by Sampath et al. (1993), Omoregie et al. (1994) and EkpoJide (2005), when fish were exposed to polluted environment under laboratory conditions. The significant reduction in these parameters is an indication of severe anaemia, (Musa & Omoregie 1999; Nwanna et al., 2003). Increase in number of White blood cells was observed at the end of the exposure period; however, Musa & Omoregie (1999) reported that no significant changes were observed in the lymphocytes of *Clarias gariepinus* exposed to Malachite green but Sampath et al., (1993), recorded an increase in the lymphocytes of *Oreochromis niloticus* exposed to toxic environment, this they attributed to the stimulation of the immune mechanism of the fish to eliminate the effects of the pollutants which correlate with this present study.

Histological examination of the gills as presented on plates 4 and 5, from fish in 5.00 ml/L and 5.50 ml/L of cassava effluent showed the gills were covered with a layer of slime (mucus), degeneration of the gills tissue, gill enlargement at the end of the experiment, and signs of necrosis were obvious. Similar observations were made by Aderiye (1998), that the gill structure of the fish *O. niloticus* treated with petrol and engine oil mixture was fused together and that there was extensive hyperplasia and separation of the epithelial layer from the supportive tissues.

Onwumere (1986) also reported that the histology of the organs (livers and gills) of *O. niloticus* fingerlings exposed to 30, 40, and 50% effluent from the NNPC Refinery at Kaduna showed that the gills were swollen and this bulged the opercula. Ekpojide (2005) expressed that gills of *O. niloticus* fingerlings exposed to African locust bean processing industry effluent were swollen and the lamellae were extensively fused and congested with blood at the end of the 96 hours exposure period. Marcano et al. (1991) and Svecevicius et al. (2003) noted that gills of rainbow trout exposed to the dispersed and water soluble fractions of orimulsion (a stable emulsion of natural Venezuelan bitumen-70% and water-30%, used as fuel) were blocked in parts by insoluble components of orimulsion and in parts by small particles of dispersed crude.

Histological examination of the liver as presented on plates 9 and 10 (i.e. 5.00ml/L and 5.50ml/L) showed vacuolation, hydro degeneration, large space formation on the tissues of the liver, and necrosis. These agree with the report by Wong et al. (1977) and Kothari & Suneeta (1990) when disintegration and necrosis occurred in the liver of *Cyprinus carpio* due to zinc toxicity. John et al. (2007) also stated that livers of channel catfish (*Ictalurus punctatus*) exposed to chlorinated effluents from a wastewater treatment plant were enlarged and showed histological lesions.

Death in fish is the end product of the various effects caused in the various tissues and organs. When these tissues collectively stop functioning due to the toxin, the fish dies. For instance, the degeneration of gills causes a dysfunction of its gas exchange ability causing an anoxic internal environment (Ajani et al., 2007). The blood is a homeostatic organ in fish. Any attack made on it, if intense, may cause a damage that will result to outright death of the organisms. Its reduction of the hepatosomatic index is in line with destruction of the liver tissue. The liver being the target point as it is used as organ of poison modification or detoxification (Taylor et al., 1988) so that

when the liver is overwhelmed, it becomes degenerated and consequently gives way to become open for the poison to attack other tissues more freely and intensively.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, the continuous release of cassava waste water into the surrounding waters, as seen in almost all cassava processing industries will alter the physicochemical parameters of the water and result in irreversible damage to the ecosystem and the organisms therein, damages such as death of eggs, aquatic organisms and water pollution and consequently affect the end users i.e. people who eat the organisms and use the surrounding waters for drinking and people that fished on the water bodies. Since cyanide in cassava waste water poses a threat to aquatic environment if it is discharged into water body. Undetoxified or partly detoxified by causing death of the fishes or impairment of fishes' health and also reduction in the fitness of the legitimate use of the water environment, hence awareness must be brought to the hearing of cassava processing industries about the effect of untreated cassava waste water discharge to the environment. It's evident from this study that increasing concentrations of the cassava effluent when present in any water body could lead to abnormal bahavioural responses and dysfunction in fish health and general condition. Hence, adequate preventive measures must be taken to prevent the indiscriminate channeling of this effluent into our water bodies having in mind that man is the ultimate target of any negative consequences resulting from such acts. Effective hazard analysis and critical control point (HACCP) monitoring is therefore advocated. Modern technologies in effluent treatment should be embraced by all stakeholders in order to maintain friendly environment and good usage of our water resources.

The above scenario (simulated during the experiment) typifies the real life situation to be

expected in any water bodies very close to commercially cassava processing factories with high deposits of cassava effluent discharge in such water bodies. Extensive and prolonged exposure to cassava effluent will prevent oxygen dissolution, destruction of breeding grounds as well as fish eggs, and ultimately, alteration of the entire aquatic environment leading to high mortality or total eradication of aquatic life in the area because of the high loads of hydrogen cyanide in the effluent released into those water bodies. This could seriously affect the economic life of the inhabitants of the area as most of them are presently chiefly engaged in fishing and fish-related activities for sustenance.

5.2 RECOMMENDATIONS

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

1. Firstly, the cassava effluent must allowed to ferment and loose it's toxicity before been released into the environment.

There is a critical need for an Environmental Impact Assessment (EIA) studies to be sanctioned for the entire cassava processing factories. In the alternative, there should be a significant appraisal of the Environmental Impact Assessment earlier conducted (if any) on the effect of hydrogen cyanide in cassava effluent as well as a thorough evaluation of various methods to be deployed by the processing factories for compliance with environmental friendliness and conservation.

2. Development of a CASHEs policy to enforce environmental preservation objectives. CASHES is Community Awareness Safety Health Environment strategy. In order to prevent the ugly experience resulting from cassava effluent released, government must enforce compliance with such a policy document from the very start to every cassava processing factories.

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

5. Laws should be enforced on all cassava processor that their effluent should not be released directly without proper treatment and new processors should site their factories far away from water areas and from the household.

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APPENDICES

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
twtyfour	Between Groups	3.400	4	.850	4.250	.072
	Within Groups	1.000	5	.200		
	Total	4.400	9			
fourtyeight	Between Groups	4.600	4	1.150	3.833	.087
	Within Groups	1.500	5	.300		
	Total	6.100	9			
seventytwo	Between Groups	7.400	4	1.850	9.250	.016
	Within Groups	1.000	5	.200		
	Total	8.400	9			
ninetysix	Between Groups	9.400	4	2.350	3.917	.083
	Within Groups	3.000	5	.600		
	Total	12.400	9			

Post Hoc Tests

Homogeneous Subsets

Twtyfour

Duncan

treatment	N	Subset for alpha = 0.05	
		1	2
control	2	.0000	
4.00mg/L	2	.0000	
4.50mg/L	2	.0000	
5.00mg/L	2	.5000	.5000
5.50mg/L	2		1.5000
Sig.		.327	.076

Means for groups in homogeneous subsets are displayed.

Fourtyeight

Duncan

treatment	N	Subset for alpha = 0.05	
		1	2
control	2	.0000	
4.00mg/L	2	.0000	
4.50mg/L	2	.5000	.5000
5.00mg/L	2		1.5000
5.50mg/L	2		1.5000
Sig.		.414	.136

Means for groups in homogeneous subsets are displayed.

Seventytwo

Duncan

treatment	N	Subset for alpha = 0.05	
		1	2
control	2	.0000	
5.00mg/L	2		1.5000
4.50mg/L	2		2.0000
5.50mg/L	2		2.0000
4.00mg/L	2		2.5000
Sig.		1.000	.085

Means for groups in homogeneous subsets are displayed.

Ninetysix

Duncan

treatment	N	Subset for alpha = 0.05	
		1	2
control	2	.0000	
4.00mg/L	2	1.0000	1.0000
4.50mg/L	2	2.0000	2.0000
5.00mg/L	2		2.5000
5.50mg/L	2		2.5000
Sig.		.054	.121

Means for groups in homogeneous subsets are displayed.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PCV	control	3	27.0000	1.00000	.57735	24.5159	29.4841	26.00	28.00
	40 mg/l	3	22.0000	1.00000	.57735	19.5159	24.4841	21.00	23.00
	45mg/l	3	10.0000	1.00000	.57735	7.5159	12.4841	9.00	11.00
	50mg/l	3	12.0000	1.00000	.57735	9.5159	14.4841	11.00	13.00
	55mg/l	3	22.0000	1.00000	.57735	19.5159	24.4841	21.00	23.00
	Total	15	18.6000	6.78022	1.75065	14.8452	22.3548	9.00	28.00
RBC	control	3	.1300	.06083	.03512	-.0211	.2811	.09	.20
	40 mg/l	3	2.3000	1.00000	.57735	-.1841	4.7841	1.30	3.30
	45mg/l	3	1.2000	1.00000	.57735	-1.2841	3.6841	.20	2.20
	50mg/l	3	.2000	.10000	.05774	-.0484	.4484	.10	.30
	55mg/l	3	.1000	.10000	.05774	-.1484	.3484	.00	.20
	Total	15	.7860	1.04099	.26878	.2095	1.3625	.00	3.30
WBC	control	3	1.0000E4	1000.00000	5.77350E2	7515.8623	12484.1377	9000.00	11000.00
	40 mg/l	3	2.0000E3	1000.00000	5.77350E2	-484.1377	4484.1377	1000.00	3000.00
	45mg/l	3	1.1000E4	1000.00000	5.77350E2	8515.8623	13484.1377	10000.00	12000.00
	50mg/l	3	5.0000E3	1000.00000	5.77350E2	2515.8623	7484.1377	4000.00	6000.00
	55mg/l	3	1.0000E4	1000.00000	5.77350E2	7515.8623	12484.1377	9000.00	11000.00
	Total	15	7.6000E3	3718.67872	9.60159E2	5540.6644	9659.3356	1000.00	12000.00
Hb	control	3	9.0000	1.00000	.57735	6.5159	11.4841	8.00	10.00
	40 mg/l	3	7.0000	1.00000	.57735	4.5159	9.4841	6.00	8.00
	45mg/l	3	3.0000	1.00000	.57735	.5159	5.4841	2.00	4.00
	50mg/l	3	4.0000	1.00000	.57735	1.5159	6.4841	3.00	5.00
	55mg/l	3	7.0000	1.00000	.57735	4.5159	9.4841	6.00	8.00
	Total	15	6.0000	2.42015	.62488	4.6598	7.3402	2.00	10.00
Neu	control	3	40.0000	1.00000	.57735	37.5159	42.4841	39.00	41.00
	40 mg/l	3	48.0000	1.00000	.57735	45.5159	50.4841	47.00	49.00
	45mg/l	3	50.0000	1.00000	.57735	47.5159	52.4841	49.00	51.00
	50mg/l	3	63.0000	1.00000	.57735	60.5159	65.4841	62.00	64.00
	55mg/l	3	53.0000	1.00000	.57735	50.5159	55.4841	52.00	54.00

	Total	15	50.8000	7.77542	2.00760	46.4941	55.1059	39.00	64.00
Lymph	control	3	12.0000	1.00000	.57735	9.5159	14.4841	11.00	13.00
	40 mg/l	3	25.0000	1.00000	.57735	22.5159	27.4841	24.00	26.00
	45mg/l	3	27.0000	1.00000	.57735	24.5159	29.4841	26.00	28.00
	50mg/l	3	31.0000	1.00000	.57735	28.5159	33.4841	30.00	32.00
	55mg/l	3	20.0000	1.00000	.57735	17.5159	22.4841	19.00	21.00
	Total	15	23.0000	6.82433	1.76203	19.2208	26.7792	11.00	32.00
Eos	control	3	8.1000	.95394	.55076	5.7303	10.4697	7.10	9.00
	40 mg/l	3	4.2000	.95394	.55076	1.8303	6.5697	3.20	5.10
	45mg/l	3	.3000	.10000	.05774	.0516	.5484	.20	.40
	50mg/l	3	.2000	.10000	.05774	-.0484	.4484	.10	.30
	55mg/l	3	.7667	.05774	.03333	.6232	.9101	.70	.80
	Total	15	2.7133	3.22000	.83140	.9302	4.4965	.10	9.00
Ban	control	3	15.0000	1.00000	.57735	12.5159	17.4841	14.00	16.00
	40 mg/l	3	12.0000	1.00000	.57735	9.5159	14.4841	11.00	13.00
	45mg/l	3	25.0000	1.00000	.57735	22.5159	27.4841	24.00	26.00
	50mg/l	3	.2000	.10000	.05774	-.0484	.4484	.10	.30
	55mg/l	3	.3000	.10000	.05774	.0516	.5484	.20	.40
	Total	15	10.5000	9.76393	2.52104	5.0929	15.9071	.10	26.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PCV	Between Groups	633.600	4	158.400	158.400	.000
	Within Groups	10.000	10	1.000		
	Total	643.600	14			
RBC	Between Groups	11.124	4	2.781	6.871	.006
	Within Groups	4.047	10	.405		
	Total	15.171	14			
WBC	Between Groups	1.836E8	4	4.590E7	45.900	.000
	Within Groups	1.000E7	10	1000000.000		
	Total	1.936E8	14			
Hb	Between Groups	72.000	4	18.000	18.000	.000
	Within Groups	10.000	10	1.000		
	Total	82.000	14			
Neu	Between Groups	836.400	4	209.100	209.100	.000
	Within Groups	10.000	10	1.000		
	Total	846.400	14			
Lymph	Between Groups	642.000	4	160.500	160.500	.000
	Within Groups	10.000	10	1.000		
	Total	652.000	14			
Eos	Between Groups	141.471	4	35.368	95.934	.000
	Within Groups	3.687	10	.369		
	Total	145.157	14			
Ban	Between Groups	1328.640	4	332.160	549.934	.000
	Within Groups	6.040	10	.604		
	Total	1334.680	14			

Post Hoc Tests

Homogeneous Subsets

PCV

Duncan

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
45mg/l	3	10.0000			
50mg/l	3		12.0000		
40 mg/l	3			22.0000	
55mg/l	3			22.0000	
control	3				27.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

RBC

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
55mg/l	3	.1000	
control	3	.1300	
50mg/l	3	.2000	
45mg/l	3	1.2000	1.2000
40 mg/l	3		2.3000
Sig.		.076	.060

Means for groups in homogeneous subsets are displayed.

WBC

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
40 mg/l	3	2.0000E3		
50mg/l	3		5.0000E3	
control	3			1.0000E4
55mg/l	3			1.0000E4
45mg/l	3			1.1000E4
Sig.		1.000	1.000	.269

Means for groups in homogeneous subsets are displayed.

Hb

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
45mg/l	3	3.0000		
50mg/l	3	4.0000		
40 mg/l	3		7.0000	
55mg/l	3		7.0000	
control	3			9.0000
Sig.		.249	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Neu

Duncan

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
control	3	40.0000				
40 mg/l	3		48.0000			
45mg/l	3			50.0000		
55mg/l	3				53.0000	
50mg/l	3					63.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Lymph

Duncan

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
control	3	12.0000				
55mg/l	3		20.0000			
40 mg/l	3			25.0000		
45mg/l	3				27.0000	
50mg/l	3					31.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Eos

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
50mg/l	3	.2000		
45mg/l	3	.3000		
55mg/l	3	.7667		
40 mg/l	3		4.2000	
control	3			8.1000
Sig.		.301	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Ban

Duncan

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
50mg/l	3	.2000			
55mg/l	3	.3000			
40 mg/l	3		12.0000		
control	3			15.0000	
45mg/l	3				25.0000
Sig.		.878	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.