TOXICITY EFFECT OF QUICKLIME (CaO) ON AFRICAN CATFISH *Clarias gariepinus* JUVENILES

BY

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FAQ/13/0994

DEPARTMENT OF FISHERIES AND AQUACULTURE

FEDERAL UNIVERSITY, OYE EKITI, EKITI STATE, NIGERIA.

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A PROJECT IN THE DEPARTMENT OF FISHERIES AND AQUACULTURE, FACULTY OF AGRICULTURE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR'S DEGREE IN FISHERIES AND AQUACULTURE IN THE DEPARTMENT OF FISHERIES AND AQUACULTURE.

FEDERAL UNIVERSITY, OYE EKITI, EKITI STATE, NIGERIA.

DECLARATION

I, **OLANIYAN BLESSING FUNMILOLA** hereby declare that this project titled "TOXICITY EFFECT OF QUICKLIME (CaO) ON AFRICAN CATFISH (CLARIAS GARIEPINUS) JUVENILES" has been performed by me in the Department of Fisheries and Aquaculture under the supervision of Professor L. C. Nwanna. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree at any University.

.... 22-03-2019

Olaniyan, Blessing Funmilola DATE

FAQ/13/0994

CERTIFICATION

This is to certify that this work has been carried out in the Department of Fisheries and Aquaculture of the Federal University of OYE EKITI by **OLANIYAN BLESSING FUNMILOLA** with Matriculation number **FAQ/13/0994** under the supervision of Pro. L.C Nwanna and has not been submitted in any form for any degree or diploma at any other institution.

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Pro L.C. Nwanna **DATE**

PROJECT SUPERVISOR

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Dr J.B OLASUNKANMI **DATE**

HEAD OF DEPARTMENT

DEDICATION

This project is dedicated to God Almighty, the maker and founder of my life, who has been my all in all and also to all who have contributed positively to my life.

ACKNOLEGMENTS

I give all glory to GOD ALMIGHTY for the success of this project. My profound gratitude also goes to my parents Dr and Mrs Olaniyan for immeasurable love, encouragement, support financially, morally, spiritually, and my amiable siblings Olaniyan Temitope, Olaniyan Tosin and Mrs Ilori-Faboro for their endless support.

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ABSTRACT

Apparently 100 (one hundred) healthy *Clarias gariepinus* juveniles which measurement of mean weight and length taken respectively was used for range finding test while 75 (seventy five) was used for definitive test. They were acclimatized in de-chlorinated water (tap water exposed to air for greater than 24 hours) in 100L capacity plastic bowl for 2 weeks. 5 fishes were kept per container. The result of this research shows the toxic nature of the quicklime on the behavioral, physicochemical parameters, hematological, histological and mortality of *Clarias gariepininus* juveniles. Physico-chemical Parameter of the research displays that there is significant effect ($p<0.05$) after exposure of quicklime to *Clarias gariepinus* on pH, temperature and dissolved oxygen. The data obtained from this investigation also show that quicklime causes a stress inducing effect on *Clarias gariepininus* during exposure period as indicated by the alternations in the blood parameters. In conclusion the values of RBC, NEU, and PCV decreased significantly ($p<0.05$) while WBC, LYM increased significantly ($p<0.05$) which was directly proportional to the quicklime concentrations. The morphological and physical changes in the gills, liver and kidney recorded in this study were erratic swimming, anorexia, loss of reflex and discoloration were observed. These have been reported in *Clarias gariepinus* juvenile after exposure to quicklime. In this study, brief exposure to toxicants for about 96 hours produced irreversible changes in the liver and kidney. The changes in the liver and kidney were adaptations by the fish to cope with the challenge of the toxicants.

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CHAPTER ONE

1.0 INTRODUCTION

Liming is an ancient agricultural practice to amend the pH of acidic soils and water. In aquaculture, the same principle is valid because acidic soils and waters can produce poor growth performance results (Cavalcante et al., 2012). Currently the standard liming product for aquaculture is agricultural limestone that is the commercial name of calcium carbonate ($CaCO_3$). In the water, calcium carbonate reacts with carbon dioxide forming soluble calcium and bicarbonate ions (Thunjai et al., 2004). Beside limestone, it is also used burned (CaO) or hydrated (Ca (OH_2)) lime to raise the pH of soil and water. However, lime has a better use as a disinfectant due to its explosive reaction with water (very fast pH rising) (Yuvanatemiya et al., 2011). Although calcium carbonate has a proven efficacy and safety for routinely use its effects in water are slow, requiring considerable time to be achieved (Yuvanatemiya et al., 2011). Lime is the first option when a rapid response is needed by the farmer. Lime, however as stated above is a dangerous product to be applied directly in the culture water because it can cause severe mortalities of fish (Ormerod and Durance, 2009). Some liming materials are caustic and can be hazardous to workers if proper precautions are not exercised.

Liming is usually necessary for fish ponds (Mayer, 2013). Liming is practiced widely in aquaculture for neutralizing acidity in pond bottom soils and water (Boyd and Tucker, 2014). Acidic bottom soil is a common problem in pond aquaculture, and fish farmers apply agricultural limestone to ponds as a remedy. Quicklime, in fact, reacts violently with water releasing a tremendous amount of heat in the process (Wright, 2001). Ita (2011) concluded that lime is to be applied when the pH and alkalinity of fish pond are too low and the pond is too muddy. Liming is recommended when hardness and alkalinity are below 20 mg/L (Boyd and Tucker, 2012).

Application of lime in highly hard water condition increases pH and it makes ammonia more toxic. Higher concentrations of the toxic form of ammonia (NH3) are formed in basic waters; while the less toxic form, ammonium (NH4+), is more prevalent in acidic waters. Since alkalinity increases pH, ammonia will be more toxic in waters with high total alkalinity.

Bears et al. (2016) indicate that fish hematology and histology studies can serve as vital indicators of toxicity as when fishes are continuously exposed to pollution through gill respiration and ingestion of contaminated food it results in changes in their blood properties and body organs. Although the toxicity studies and the determination of the lethal concentration for 50% (LC_{50}) of fishes have been worked out in different fish species (Roy et al., 2006; Ghosh et al., 2007), the effects of this lime toxicity on definite fish function systems are yet to be clarified (Datta et al., 2007). While several long-term experiments have been carried out (Ormerod and Durance, 2009), there has previously been no systematic review appraising whether liming can be toxic to fish. In this paper, we provide such a systematic review, aiming to source, analyze and summarize the best available data. Specifically, we posed the question: "can quicklime be toxic to *Clarias gariepinus*"?

1.1 STATEMENT OF RESEARCH PROBLEM

The main liming method is to add the lime directly to the water body. Liming of water directly, however, causes aluminum and other metals to come out of solution and fall to the bottom of the lake, causing toxicity problems for organisms living on the bottom of the water body. This is a major problem as it will affect the benthic fishes and the bottom feeding fishes. Furthermore, over-utilization of lime by local fish farmers is also a major problem that prompted this research, because high pH following large lime applications could be harmful to fish. Mitigation against acidification caused by acid rain on water bodies is also a problem that warrants the addition of lime in water bodies.

1.2 JUSTIFICATION

Lime is a widely used tool for disinfecting the pond during pond preparation because it is easy to use and as such can be misused by local farmers who use it without the recommended standard for quantity of usage, this justify the rationale for this study as to be able to investigate if quicklime could be toxic to fishes. Furthermore, quicklime is used in crop farming to boost the soil and when rain falls and erosion occurs, run-off from this farms find their way into the ponds which must have already being limed during pond preparation, rivers and lakes are also not left out as this run-offs get into them too causing changes in some physiochemical parameters of both water bodies, so it is paramount that the effect of this phenomenon be ascertain so has to be able to advice farmers and fisheries managers on the need to protect ponds, lakes and rivers from such agricultural run-offs.

1.3 RESEARCH OBJECTIVES

The general objective of this study is to investigate the toxicity of quicklime on African catfish, *Clarias gariepinus* while the specific objectives are to determine:

- Determine the changes that occur in some hematological parameters of *Clarias gariepinus* exposed to toxic levels of quicklime,
- determine some water quality parameters, and
- Determine some behavioral and morphological changes of *Clarias gariepinus* exposed to toxic levels of quicklime.
- Determine the changes that occur in histological properties of *Clarias gariepinus* exposed to toxic levels of quicklime.

1.4 RESEARCH HYPOTHESIS

- Ho_1: there is no significant changes in hematological parameters of *Clarias gariepinus* exposed to toxic levels of quicklime,
- Ha_1: there is significant changes in hematological parameters of *Clarias gariepinus* exposed to toxic levels of quicklime,
- Ho_2: there is no significant changes in the water quality parameters of *Clarias gariepinus* exposed to toxic levels of quicklime,
- Ha_2: there is significant changes in the water quality parameters of *Clarias gariepinus* exposed to toxic levels of quicklime,
- Ho_3: there is no significant changes in behavioral and morphological properties of *Clarias gariepinus* exposed to toxic levels of quicklime,
- Ha_3: there is significant changes in behavioral and morphological properties of *Clarias gariepinus* exposed to toxic levels of quicklime.
- Ho_4 - there is no significant difference in the histological properties of *Clarias gariepinus* exposed to varying concentration of quicklime lime;
- Ha_4 – there is significant difference in the histological properties of *Clarias gariepinus* exposed to varying concentration of quicklime;

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy and Biology of the African catfish, *Clarias gariepinus*

African catfish (*Clarias gariepinus*) is a species of catfish of the family Clariidae, the air-breathing catfishes. Catfish belongs to the kingdom Animalia, phylum Chordata, Superclass Osteichthyses, class Actinopterygii, subclass Neopterygii, infraclass Teleostei, superorder Ostariopysi and order Siluriformes. 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 (Froese *et al.*, 2014).

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 (Froese *et al.*, 2014).

Plate 1: a diagram of catfish

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 *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 (Froese *et al.*, 2014). *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 flood 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 (Anoop *et al.*, 2009).

2.2 Lime

Lime is a general term for calcium containing inorganic materials, in which carbonates, oxides and hydroxides predominates. Liming is practiced widely in aquaculture for neutralizing acidity in pond bottom soils and water (Boyd and Tucker 1998). The residual effect of liming, based on results from laboratory mud-water systems, should last for about 10 water exchanges. Hydrated lime (calcium hydroxide, Ca(OH) and quicklime (calcium oxide, CaO) generally are not recommended for treating surface waters because they are corrosive, difficult to control, and may not be legal to apply (Helfrich et aI., 2015). Quicklime reacts violently with water releasing a tremendous amount of heat in the process (Wright, 200 I). Ita (1980) concluded that lime is to be applied when the pH and alkalinity of fish pond are too low and when the pond is too muddy. Liming maintains pH in treated soils containing organic sludge, in accord with earlier observations (Martikainen et al., 2`017; Rudebeck and Persson, 1998; Bckman and Klemedtsson, 2003). Lime routinely applied corrects acidification (Golez, 2016) and is considered as one of the most important procedures for maintaining soil at a satisfactory pH (Charman and Roper, 2014). Pillay and Boyd (2013) revealed the simple methods for calculating liming rates for fish ponds. Lime application maybe a very promising technique for integrated control of both macrophyte and algal production in lake eutrophic hard water systems (James et al., 2017. Mckie et al. (2017) demonstrated that stream liming can alleviate the effects of anthropogenic acidification. Alkalinity, pH, conductivity and calcium concentrations got increased following liming. The potential deleterious impacts of liming need to be balanced against its desired outcomes in regions where acidity is largely attributable to natural causes. One of the most effective coagulants is alum, or aluminium sulphate, which has been used to clarify muddy waters since the time of the early Egyptians (2000 B.c). Alum makes water more acidic. In ponds with low alkalinity (less than 20 mg/l as CaC03) it can reduce water pH to levels that may affect fish growth and survival. In low alkalinity ponds, 1/2 part hydrated lime for every part of alum is applied in order to maintain proper pH (Hargreaves, 2016).

2.2.1 Lime and Water Quality

The tanks which are acidic in nature are less productive than alkaline ponds. Lime is used to bring the pH to the desired level. In addition lime also has the following effects:

a) Increases the pH.,

b) acts as buffer and avoids fluctuations of pH,

c) increases the resistance of soil to parasites,

d) toxic effect of lime kills the parasites; and

e) hastens organic decomposition.

The normal desired dose of lime ranges from 200 to 250 Kg/ha. However, the actual dose has to be calculated based on pH of the soil and water (Boyd, 2013). Indian carp culture in Andhra Pradesh (India) mainly focus on the local practices for fish culture and their effects on the environment, viz., issues on water quality, the use of fertilizers, liming, supplementary feeds, pond size, culture of new species, pond effluents, and pond additives (Edwards, 2008). The carp culture practice affecting water quality is liming, that increased alkalinity, pH and water transparency and decreased ammonia (Milstein et al., 2012). Suitable water quality parameters are maintained in ponds through intermittent liming, manuring and fertilization (Jena and Oas, 2011).

2.3 Quicklime

Quicklime is an odorless, white or grayish-white material that ranges from pebble to a granular powder. Contact can cause irritation to eyes, skin, respiratory system, and gastrointestinal tract. Quicklime reacts vigorously with water, releasing heat which may ignite combustible materials in specific instances. Quicklime, the product of calcination of limestone, consists of the oxides of calcium and magnesium. The primary forms of quicklime are; high calcium quicklime which is derived from limestone containing 0 to 5 percent magnesium carbonate and dolomitic quicklime which is derived from limestone containing 35 to 46 percent magnesium carbonate. Quicklime is a white to gray solid having a crystalline structure. Quicklime is highly reactive with water, generating considerable heat in the hydration process. This material will react with the moisture in the air, and as such, it can be used as a desiccant. In the presence of moisture, lime reacts slowly with carbon dioxide in the air, reforming calcium carbonate. As a chemically active material it is desirable to reduce atmospheric exposure during handling and storage to a minimum. Hydrated lime, though only slightly soluble in water, forms suspensions easily; the resulting solution and suspension is strongly alkaline, possessing a pH of 12.4. Quicklime is commercially available by pneumatic tanker truck, rail hopper car, or in bulk or in paper bags. It is available in a number of sizes as follows (definitions derived from ASTM standard C51):

- Large lump lime- the product with a maximum size of eight inches in diameter.
- Crushed or pebble lime- the product ranging in size from about 1/4 to 2 ½ in.
- Ground lime- the product of a size 1/4 inches and smaller.
- Pulverized lime- the product resulting from a more intense grinding than is used to produce ground lime. A typical size is substantially all passing a No. 20 sieve.

- Pelletized lime- one inch sized pellets or briquettes, molded from quicklime fines.

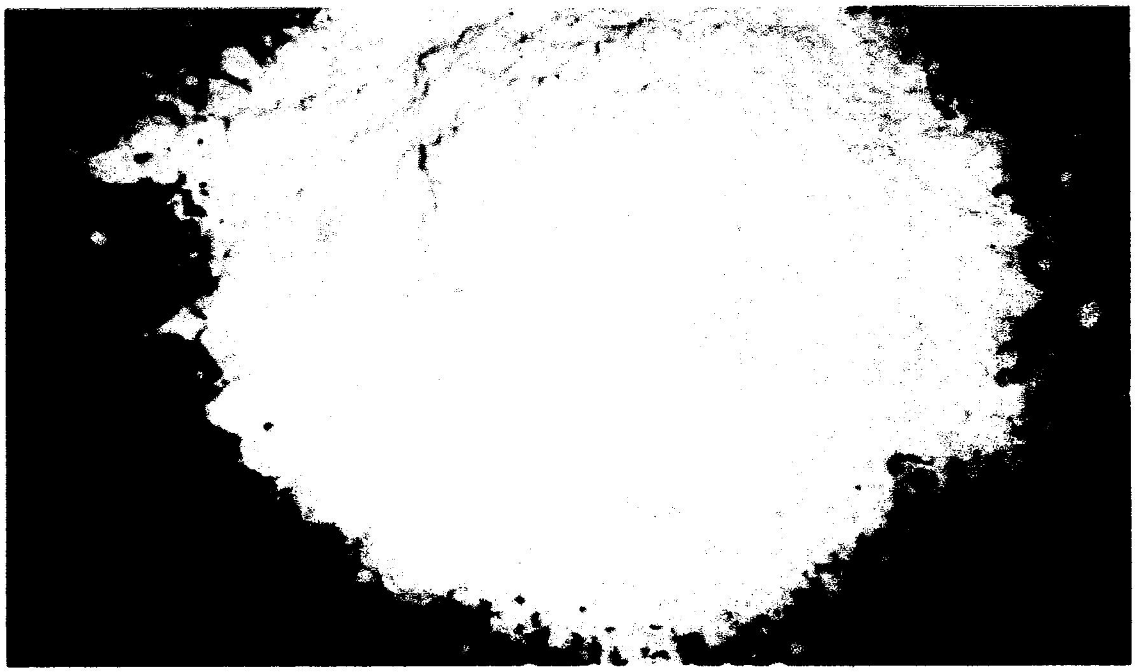

Plate 2: diagram of powdered quicklime

2.3.1 PROPERTIES OF TYPICAL COMMERCIAL LIME PRODUCTS

QUICKLIME	HIGH CALCIUM	DOLOMITE

Primary constituents	CaO	CaO* MgO
Specific Gravity	3.2-3.4	3.2-3.4
Bull Density (Pebble Lime), lb./cu. ft.	55-60*	55-60*
Specific Heat at 100° F., Btu/lb	0.19	0.21
Angle of Repose	55°**	55°**

2.3.2 SIZE CLASSIFICATION

There are many different sizes of lime available from the quicklime manufacturers. The most common sizes are:

•Pulverized quicklime (100% passes 200 mesh)

•0 x 1/8" (crushed lime)

•1/8" x ¼" (rice lime)

•¾" x ¼" (pebble lime)

Pulverized quicklime will slake very quickly and produce a very reactive hydrate. Due to its fine particle size, pulverized quicklime causes dusting as well as build-up within the slaker, which results in additional maintenance. Due to its fineness, it is also more susceptible to "air slaking" prior to its introduction into the lime slaking equipment. If pulverized quicklime is to be used, it is best to pulverize the quicklime just before slaking so the quicklime does not have time to absorb moisture from the atmosphere. The ideal size for quicklime, for slaking is 1/8" x ¼", (rice lime) with the next best size range being ¼" x ¾" (pebble lime). This size range minimizes air slaking and reduces slaker maintenance and housekeeping. Large size limestone (1 ½" to 2") is difficult to slake. The difficulty is due to the large size since a great deal of steam is generated at the moment the 2" piece of lime comes into contact with the hot slurry. The reaction, in the case of a reactive lime is like a mini-explosion with a sudden release of a large amount of steam. Splashing and build-up occur within the slaking chamber, resulting in extra build up and maintenance.

2.3.3 USES OF LIMESTONE

In the early 16th century it was discovered that quicklime could be used to 'improve' acid peaty ground to allow grass to be grown for sheep grazing. Slaked lime was widely used and many small clamp kilns were built, many of them producing a batch of quicklime only a few times a year. The fuels used included wood, bracken and peat, so the quicklime quality was probably poor. Limestone could be used instead of quicklime but only if ground very finely. Ground limestone is very much slower acting but is used today as it is cheaper than quicklime. In modern the uses includes:-

•Purification of drinking water,

•Removal of silica, sulfur and carbon in the basic oxygen process, which is used to treat molten iron discharged from a blast furnace,

•Manufacture of aerated concrete blocks,

•Treatment of sewage,

•Neutralization of acid mine drainage, and

•Flue gas desulfurization to mention but a few.

2.3.4 METHOD OF TRANSPORTATION

Based on the above comments, it is obvious that quicklime must be transported in airtight containers to isolate it from moisture in the atmosphere. Typically, this is done by tankers specially designed for this type of dry chemical. Loading is done mechanically, and unloading is done pneumatically. When pneumatically unloading, the transfer velocity must be kept to a minimum to reduce degradation in the case of pebble lime. For this reason, mechanical unloading is the preferred choice, but it requires a higher investment. Pneumatic unloading where fresh air is used for transport should be avoided in high humidity or rainy circumstances.

2.3.5 METHOD OF STORAGE AT THE SITE

Typically, lime is stored at the site either in super sacks or in silos. Industrial plants require a storage capacity equal to two weeks consumption. In some remote areas, the storage capacity may be as much as two months. If the lime is going to be stored for more than two weeks in the silo, some precautions are necessary to prevent air slaking:

1. Dry the air above the material in the silo by using a desiccant or refrigerant air dryer and recirculate this air periodically.

2. Purge the air above the lime in the silo with dry instrument air periodically.

3. Avoid pneumatic loading of the silo during rainy days.

4. Store the quicklime in silos painted white in areas where humidity is high and day/night temperatures are substantially different. This causes condensation inside the silo and build-up on the silo sidewall, where the sun hits during the afternoon.

2.4 WATER QUALITY PARAMETERS

Water quality refers to the chemical, physical, biological and radiological characteristics of water. It is a measure of the condition of water relative to the requirements of one or more biotic species and or to any human need or purpose. It is most frequently used by reference to a set of standards against which compliance, generally achieved through treatment of the water, can be assessed. The most common standards used to assess water quality relate to health of ecosystems, safety of human contact, and drinking water.

Temperature

Water temperature is affected by air temperature, storm water runoff, groundwater inflows, turbidity, and exposure to sunlight. In considering the health of organisms, it is necessary to consider their maximum temperature and optimum temperature. The maximum temperature is the highest water temperature at which the organism will live for a few hours. The optimum temperature is the temperature at which it will thrive.

pH

pH is a measure of a solution's acidity. In water, small numbers of water molecules (H_2O) will break apart or disassociate into hydrogen ions ($H+$) and hydroxide ions ($OH-$). Other compounds entering the water may react with these, leaving an imbalance in the numbers of hydrogen and hydroxide ions. When more hydrogen ions react, more hydroxide ions are left in solution and the water is basic; when more hydroxide ions react, more hydrogen ions are left and the water is acidic. pH is a measure of the number of hydrogen ions and thus a measure of acidity.

pH is measured on a logarithmic scale between 1 and 14 with 1 being extremely acid, 7 neutral, and 14 extremely basic. Because it is a logarithmic scale there is a tenfold increase in acidity for a change of one unit of pH, e.g. 5 is 100 times more acid than 7 on the pH scale. The largest variety of freshwater aquatic organisms prefer a pH range between 6.5- 8.0.

Dissolved Oxygen

Dissolved oxygen is oxygen gas molecules ($O2$) present in the water. Plants and animals cannot directly use the oxygen that is part of the water molecule ($H2O$), instead depending on dissolved oxygen for respiration. Oxygen enters streams from the surrounding air and as a product of photosynthesis from aquatic plants. Consistently high levels of dissolved oxygen are best for a healthy ecosystem.

Levels of dissolved oxygen vary depending on factors including water temperature, time of day, season, depth, altitude, and rate of flow. Water at higher temperatures and altitudes will have less dissolved oxygen. Dissolved oxygen reaches its peak during the day. At night, it decreases as photosynthesis has stopped while oxygen consuming processes such as respiration, oxidation, and respiration continue, until shortly before dawn.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area, Collection and Acclimatization of fishes

Apparently healthy *Clarias gariepinus* juveniles which measurement of mean weight and length taken respectively was purchased from a reputable fish farm. They were acclimatized in de-chlorinated water (tap water exposed to air for greater than 24 hours) in 7L capacity plastic bowl for 2 weeks. Fishes were kept per container and the water in each container was changed daily. The fish were fed twice daily at 3% of their body weight with a feed containing 40% crude protein for the catfish. The fishes in poor health condition were replaced.

3.2 Collection of lime

Quicklime was purchased from a reputable farm.

3.3 Experimental Design

The experiment was conducted in the wet laboratory of the Department of Fisheries and Aquaculture, Federal University of Oye Ekiti, Ekiti State, Nigeria. The fish was stocked in 15 transparent plastic containers with five treatments containing different concentrations of quicklime, with the same amount of litre (7L) was used for this assay. Each treatment had three replicates with 10 fishes in each aquarium and the experiment was conducted for 96hours.

3.4 Range Finding Test

One hundred (100) juveniles of *Clarias gariepinus* of a measured mean weight and mean total length respectively was used for the range finding test. Mettler top precision loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent plastic containers for two days in order to adapt to laboratory conditions. Feeding was discontinued during this period to reduce the production of waste in the transparent plastic containers thus minimizing the chances of ammonia production. The range finding was conducted under standard bioassay procedures (American Public Health Association, 1977). The range-finding test was carried out using ten transparent plastic containers filled with 7litres of water prior to the introduction of quick lime. Four varying concentration used were (0, 2, 4, 6, 10 g/l of water (0 ml). The five different concentrations were carried out in triplicate; triplicate of the control was also prepared

3.4.1 Definitive Test

Seventy-five (75) juveniles of *Clarias gariepinus* was used for the definitive test. Mean weight and mean total length was measured for each species of fish. Mettler top precision loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into separate transparent plastic containers for 96 hours in order to adapt to laboratory conditions. Feeding was discontinued during this period to reduce the production of waste in the transparent containers thus minimizing the chances of ammonia production. Definitive test was conducted under standard bioassay procedures (American Public Health Association, 1977). The concentration of quicklime used in each treatment was determined from the range finding test and the experiment was conducted in triplicate to reduce bias.

3.5 Fish behavior

The behavioral pattern of *Clarias gariepinus* juveniles before, during and immediately after the introduction of quick lime was observed and recorded.

3.5.1 Physiochemical parameters of the water

The physiochemical parameters of the water that was measured were; dissolved oxygen concentration, temperature, and pH. The temperature of the water was measured using a Gold Cross mercury- in- glass thermometer. This was dipped into the water for two minutes with the mercury bulb fully immersed and the reading recorded. The dissolved oxygen concentration of the water was measured with a Dissolve oxygen meter (model 40) while the pH concentration was measure using a pH meter. The total hardness of the water was calculated using the formula: Total hardness (mg/L) = 20 X titre value / 96.

3.6 Haematological evaluation

At the termination of the experiment, the fish was captured and sampled after being anesthetized according to Horváth et al. (1984) (20 mgl-1MS-222, Finquel®; Argent TR2905, Redmond, WA, USA). Then, approximately 2 ml of blood was collected from the caudal vein using a 2 ml heparinized syringe. Half of the blood was used for separating plasma (blood chemistry measurements) by centrifuging (3000 rpm for 5 min) and the remaining blood was used for hematological analysis. The numbers of white blood cells (WBC) (×103µl), red blood cells (RBC)(×106µl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular volume (MCV) (fl), and meancorpuscular hemoglobin concentration (MCHC) (g dl-1) was calculated according to Ranzani-Paiva etal. (2014). the hematocrit value (%) was determined with the standard microhematocrit method and expressed in percentages. Hemoglobin (Hb) (g dl-1) was measured with a spectrophotometer at 540 nm absorbance using the cyanmethemoglobin procedure with a commercial kit (Pars Azmoon) according to Hayatbakhsh et al. (2014). The differential leukocyte count was performed with blood smears stained with Giemsa solution. The smears was examined with light microscopy (Olympus, Tokyo, Japan) in oil immersion at 100 × magnification. All biochemical and hematological parameters was measured in triplicate.

3.6.1 Histological examination

Histological examination of the liver and kidney was carried out by fixing the dead fish after 96 hours into 10% formalin and preserved for 3 days. The fixed tissues was dehydrated in graded levels of alcohol and cleared in 50/50 mixture of alcohol and xylene for consecutive 6 h. This was followed with the impregnating of the specimens in molten wax placed in an oven for 6 h and embedded in petri dishes covered with paraffin wax. The specimens was later mounted in wooden blocks and sectioned properly into thin sizes with microtome and then stained later. The sectioned organs was mounted on a glass slide and observed under the light microscope (model Olympus 399817, Japan). Photomicrographs of stained section was taken and interpreted accordingly.

3.7 Determination of LC_{50}

The 96-h median tolerance limit (96-h LC_{50}) was determined (at a static condition) by exposing the fishes to five ascending concentrations of quick lime. Cumulative mortality was determined after 96-h; the dead fish was removed once it is observed. The 96- h LC_{50} was determined by graph analysis of the percentage mortality versus the quicklime concentrations.

3.8 Statistical Analysis

The statistical analysis of this work was carried out using SPSS software (Version 16.0). A one -way analysis of variance (ANOVA) was used to determine the significance of the toxicity of quicklime on the fish species. The data of this work was presented as mean ± standard deviation. Pair wise comparison was done between control and experimental groups by employing paired T-test to resolve the statistical significance of the difference between the groups (Pipkin, 1984).

CHAPTER FOUR

4.0 Results

4.1 HEMATOLOGICAL EXAMINATION RESULT

Table 1 shows some haematological parameters compared between the different levels of quicklime toxicity and the apparently normal control. There were significant differences as determined by one-way ANOVA between the Red Blood Cell group ($F = 12.469$, $p = 0.000$), White Blood Cell group ($F = 78.391$, $p = 0.000$), Lymphocyte group ($F = 31.692$, $p = 0.000$) and Neutrophil group ($F = 32.701$, $p = 0.000$). However, no statistically significant difference was recorded between the Packed Cell Volume group ($F = 2.733$, $p = 0.071$).

Table 1: Some hematological parameters compared between the different levels of quicklime toxicity and the apparently normal control

Variable	Treatment					
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Parameter	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
RBC	1.10±0.17	1.03±0.20	0.95±0.02	0.88±0.80	0.85±0.047	0.67±0.15
Stat. test	F =12.469 , p-value =0.000					
WBC	40.63±1.50	53.83±2.64	58.26±1.53	71.13±2.06	103.70±3.70	110.20±12.34
Stat. test	F = 78.391 , p-value = 0.000					
PCV	13.86±1.76	13.17±5.07	10.46±3.15	9.53±0.25	8.90±1.08	7.53±0.66
Stat. test	F = 2.733 , p-value =0.071					
LYM	89.46±1.02	92.78±1.99	96.16±1.42	97.45±0.49	98.26±0.48	98.58±0.27
Stat. test	F = 31.692 , p-value = 0.000					
NEU	10.64±0.87	7.22±1.99	3.83±1.42	2.54±0.49	1.73±0.48	1.41±0.27

Stat. test	F = 32.701 , p-value =0.000

Table 2 depicts the tukey post-hoc test for the multiple comparison of mean haematological parameters under this study between the different levels of quicklime toxicity and the apparently normal control. It was revealed that the mean Red Blood Cell count in the *Clarias gariepinus* got significantly lower from treatment 3 (when 0.9g was added)

Table 2: Tukey post-hoc test for the multiple comparison of mean haematological parameters between levels of quicklime toxicity and the apparently normal control

Variable	**Mean Difference (Control – Treatment|)**				
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
Parameter					
RBC	0.07	0.15	0.22	0.25	0.43
p-value	0.844	0.198	0.030	0.014	0.000
WBC	-13.20	-17.63	-30.50	-63.07	-69.57
p-value	0.100	0.019	0.000	0.000	0.000
PCV	0.69	3.40	4.33	4.96	6.33
p-value	0.999	0.612	0.375	0.250	0.093
LYM	-3.32	-6.70	-7.99	-8.80	-9.12
p-value	0.030	0.000	0.000	0.000	0.000

NEU	3.42	6.80	8.09	8.09	9.22
p-value	0.024	0.000	0.000	0.000	0.000

4.2 PHYSICO- CHEMICAL PARAMETERS OF WATER

Results of the physico-chemical analysis of the water used for exposure concentrations of the quicklime concentrations in the laboratory are presented in Tables 4

The result display some water quality parameters compared between the different levels of quicklime toxicity and the apparently normal control. There were significant differences as determined by one-way ANOVA between the potential of Hydrogen ($F = 104.360$, $p = 0.000$), Dissolved Oxygen ($F = 65.983$, $p = 0.000$), Temperature ($F = 121.002$, $p = 0.000$).

Table 3: Some water quality parameters compared between the different levels of quicklime toxicity and the apparently normal control

Variable	Treatment					
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
Parameter	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
pH	7.13±0.057	7.63±0.03	7.72±0.04	7.60±0.02	7.69±0.02	7.64±0.03
Stat. test	F =104.360	, p-value =0.000				
DO	6.43±0.09	5.57±0.10	5.52±0.02	5.32±0.20	5.15±0.06	5.03±0.02
Stat. test	F =65.983 , p-value =0.000					
Temp.	22.63±0.17	23.70±0.14	24.14±0.10	24.26±0.11	24.53±0.12	24.84±0.061
Stat. test	F =121.002 , p-value =0.000					

Table 3 shows the tukey post-hoc test for the multiple comparison of some water quality parameters under this study between the different levels of quicklime toxicity and the apparently normal control. It was revealed that the pH, Dissolved Oxygen, Temperature in the water got significantly higher from treatment 1 to treatment 5

*Table 4: **Tukey post-hoc test for the multiple comparison of water quality parameters between levels of quicklime toxicity and the apparently normal control***

Variable	**Mean Difference (Control – Treatment)**				
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
Parameter					
pH	-0.49	-0.59	-0.47	-0.56	-0.51
p-value	0.000	0.000	0.000	0.000	0.000
DO	0.85	0.90	1.10	1.27	1.39
p-value	0.000	0.000	0.000	0.000	0.000
Temp.	-1.07	-1.51	-1.63	-1.90	-2.21
p-value	0.000	0.000	0.000	0.000	0.000

4.3 LC50 DETERMINATION

Chart 1: mortality (96 hour- LC50) of *clarias gariepinus* juvenile exposed to different concentration of quicklime.

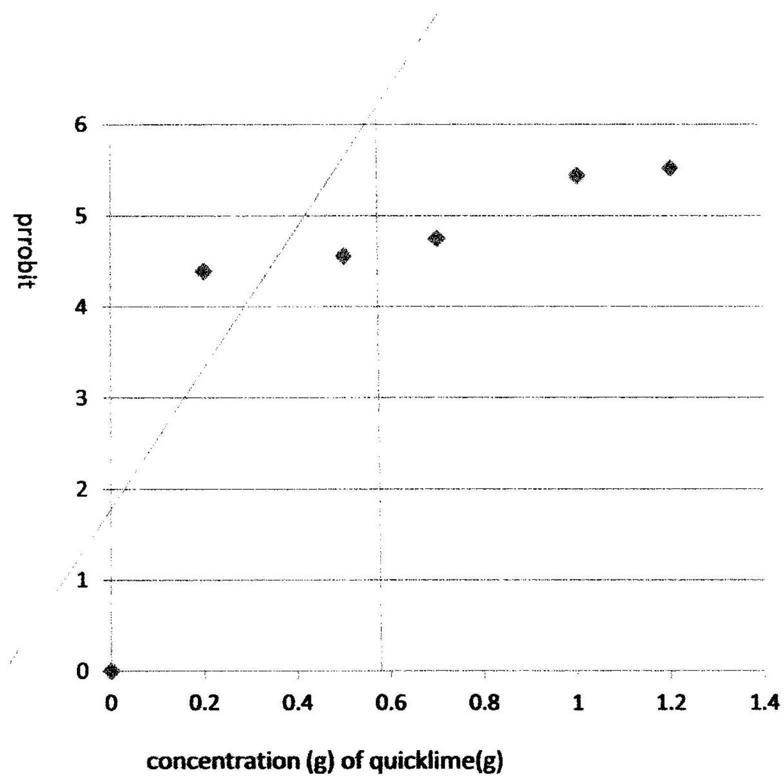

Chart 1: LC50 of quicklime on African catfish juvenile exposed to different concentrations of quicklime (LC50 =1g/8L)

4.4 Histological result

The results of the histological examinations are shown in plate 2-6. Kidney and liver histology of Clarias gariepinus exposed to different concentrations of quicklime is shown in plate 2-6 However the control (0ml/L) has no pathological lesion (plate 2).

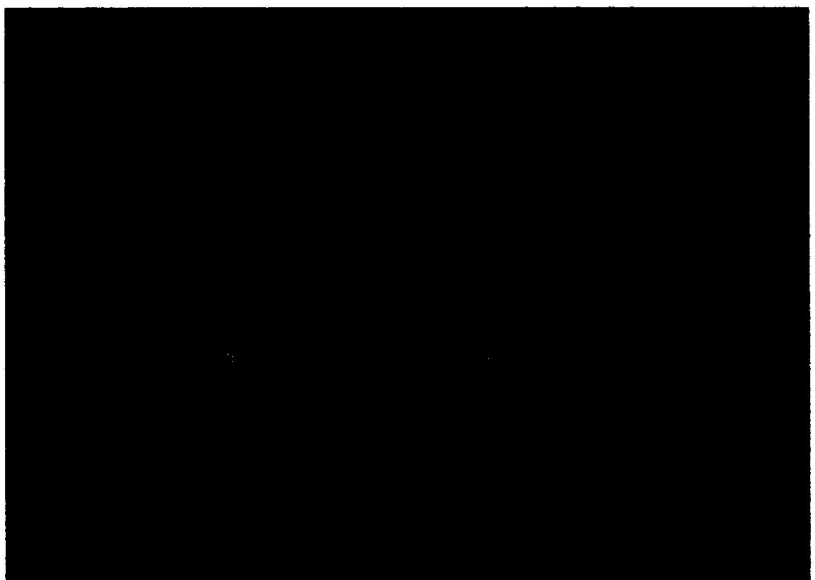

Plate 3: kidney of *clarias gariepinus* juvenile in the control treatment (0g/7L) showing no pathological lesion, (*400)

Plate 4: the photomicrograph of *clarias gariepinus* juvenile exposed to 0.2g/7L of quicklime, showing no visible changes

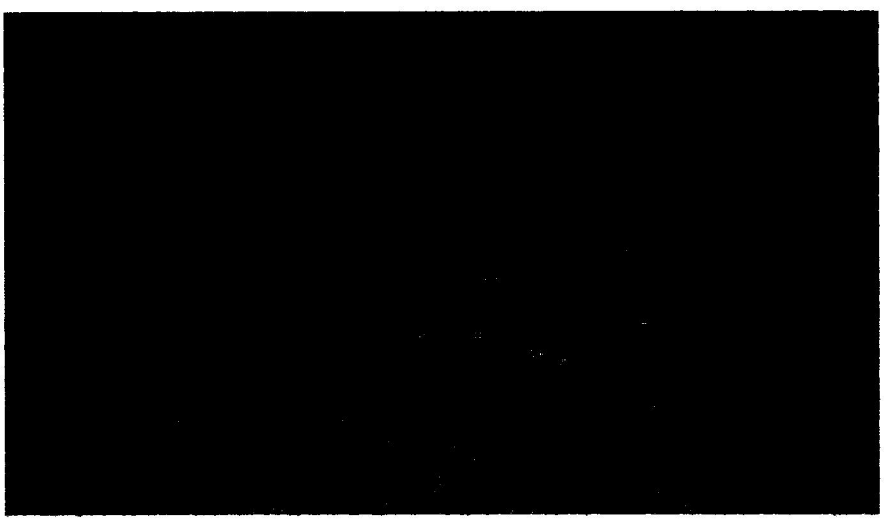

Plate 5: the kidney of *Clarias gariepinus* juvenile exposed to 0.7g/7L of quicklime showing degeneration in the renal cells.

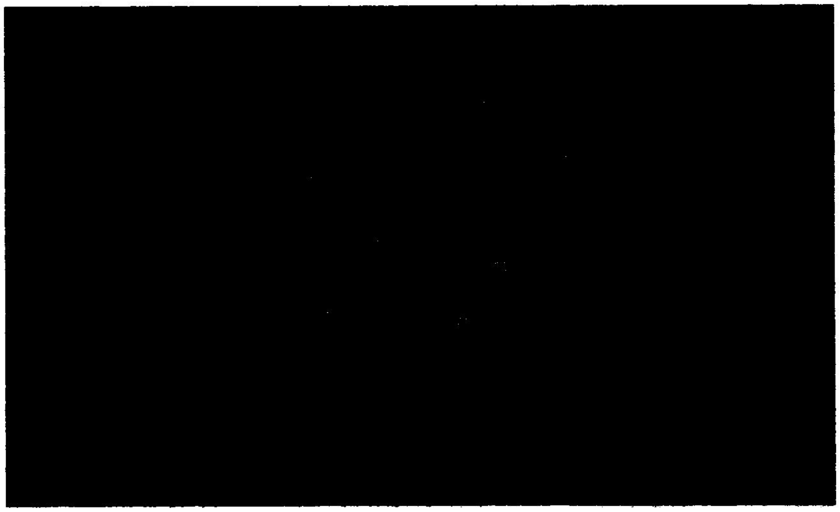

Plate 6: kidney of Clarias gariepinus juvenile exposed to 1.5g/7L of quicklime showing the hydropic degeneration in the renal cell

Plate 7: the photomicrograph of Clarias gariepinus juvenile's liver exposed to 1.5g/7L of quicklime showing increase in interstitial cell and vacuolation

4.5 Fish behaviour

Results shows that *Clarias gariepinus* exposed to quicklime showed some behavioural changes

Immediately the fish were introduced into the tank containing quicklime at concentrations 0.2mg/L ,0.5 mg/L, 0.7mg/L, 1.0mg/L and 1.2mg/L, they became restless and agitated. Fishes came to the surface of water much more frequently. They occasionally tried to jump out of the water. The fish showed abnormal swimming movements including loss of orientation, loss of buoyancy and spasms before death.

Table 5: shows the reaction of C.gariepinus after the introduction of quicklime

	Treatment					
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
Erratic swimming	Absent	Present	Present	Present	Present	Present
Petechial and Ecchymotic hemorrhages	Absent	Present	Present	Present	Present	Present

Aerophagia	Absent	Present	Present	Present	Present	Present
Loss of reflex	absent	Present	Present	present	present	Present

CHAPTER FIVE

5.0 DISCUSSION

The result of this research shows the toxic nature of the quicklime on the behavioral, physicochemical parameters, hematological, histological and mortality of *Clarias gariepininus* juveniles. Physico-chemical Parameters of the research displays that there is significant effect ($p<0.05$) after exposure of quicklime to *Clarias gariepinus* on pH, temperature and dissolved oxygen. Effects of Concentrations of quicklime on *Clarias gariepinus* Behavioral Parameters of fish to most toxicant and differences in reaction times have been observed as due to the effects of different concentrations ,species size and specific environmental conditions according to (Adakole 2006, Bob manuel et al..,2006 and Babatunde et al..,2001)research. The result showed that exposure of *Clarias gariepinus* to quicklime cause a variety of abnormalities in the behavior of the fish. Observation was made on what happened to the fish immediately after been exposed to the acute concentration *Clarias gariepinus* showed.

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

Effect on heamatology data obtained from this investigation show that quicklime causes a stress inducing effect on *Clarias gariepininus* during exposure period as indicated by the alternations in the blood parameters. The values of RBC, NEU, and PCV decreased significantly ($p<0.05$) while WBC, LYM increased significantly ($p<0.05$) which was directly proportional to the quicklime concentrations. Effect on histology, the morphological and physical changes in the liver and kidney recorded in this study were erratic swimming, anorexia, loss of reflex and discoloration were observed. These have been reported in *Clarias gariepinus* juvenile after exposure to quicklime. In this study, brief exposure to toxicants for about 96 hours produced irreversible changes in the gills (Ferdandez and Mazon, 2003). The changes in the gills, liver and kidney were adaptations by the fish to cope with the challenge of the toxicants.

5.1 CONCLUSION

The results of showed that quicklime was toxic to *Clarias gariepinus*. The LC_{50} of the quicklime to juveniles of *Clarias gariepinus* was determined (1g/8L) and quicklime was found to be poisonous therefore the usage must be controlled and monitored.

5.2 RECOMMENDATION

Further research should be carried out to investigate the effects of quicklime in rivers, ponds, lakes etc.

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