# TOXICITY OF HYDRATED LIME $Ca(OH)_2$ ON AFRICAN CATFISH (*Clarius gariepinus)*


**BY**

**ADEKUNLE DELE 'SUNBO**

**FAQ/13/0987**


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## ABSTRACT

This study was conducted to determine the 96h LC50 of hydrated lime to African catfish juveniles, and to access the effect of the lime on haematological and histopathological profile of the fish. Results showed the 96h LC 50 to be . The blood parameters reduced with increasing concentration of the hydrated lime. There were various degrees of lesions and necrosis on the liver and gills of the fish and these effects increased with increasing concentration of the toxicant. Similarly, the lime stressed the fish as they showed erratic movement, hyperactivities.

**DECLARATION**

I, ADEKUNLE DELE 'SUNBO hereby declare that this project titled "TOXICITY OF HYDRATED LIME $Ca(OH)_2$ ON AFRICAN CATFISH, *Clarius gariepinus*" was carried out by me in the department of Fisheries and Aquaculture under the supervision of PROFESSOR LC. 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

21/03/2019.

Adekunle, Dele 'Sunbo

DATE

## DEDICATION

This research project is dedicated to Almighty God. The All-compassionate, All-beneficient and All-merciful. Who through his rare and unending magnanimities saw me through the course of the research project.

## ACKNOWLEDGEMENT

I return all glory and adoration to my father in heaven for his grace and guidance throughout the five years journey. I would like to express my thanks to the people who have helped me most throughout my project. I am grateful to my supervisor PROF.L.C NWANNA for nonstop support during the project. A special thanks of mine goes to my colleague who helped me out in completing the project, where they all exchanged their own interesting ideas, thoughts and made this possible to complete my project with all accurate information.

My unhindered gratitude to all my industrious lecturers in the department of Fisheries and Aquaculture.

I wish to thank my parents for their personal support and attention who inspired me to go my own way. MRS F.E ADEKUNLE, CHIEF MRS BABATOLA.

I want to thank my friends who treasured me for my hard work and encouraged me MISS.AJIBOYE IFEDOLAPO, MR AKIWOWO USMAN and finally to God who made all the things possible for me till the end.

My course mates: Akiwowo Usman, Olatoye Dolapo Salim, Mustapha Qazeem and others. I will not forget all the memories we have made together.

## CERTIFICATION

This is to verify that this project work was carried out by MR ADEKUNLE, DELE 'SUNBO with MATRIC NUMBER FAQ/13/0987 in the department of Fisheries and Aquaculture of Federal University, Oye-Ekiti.

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PROF. LC., NWANNA
PROJECT SUPERVISOR

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DATE

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DR. J. B. OLASUNKANMI
HEAD OF DEPARTMENT

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DATE

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# CHAPTER ONE

## 1.0 INTRODUCTION

Fish production through aquaculture has great potential in poverty eradication and alleviating malnutrition in Nigeria. As a cheap source of high quality animal protein (16 - 20%), it has comparative advantage over poultry eggs and milk. Fish industry therefore is promising in fulfilling the mandate of the Millennium Development goals towards 2020. With digestibility rating of 94 (NRC, 1989) and Biological Value of 75, fish proteins are comparable to red meat and indeed superior to plant proteins (McDonald, 2010). Application of lime to fish ponds has now become a cultural practice by farmers to get rid of external parasites and other pathogens and considered as excellent pond disinfectant (NIFFR, 2001). Quicklime, slaked or Hydrated lime and Agricultural lime if properly applied to fish ponds have advantages of increasing the alkalinity of acidic soil, increasing pond biological productivity and neutralizing the harmful effects of magnesium, sodium and potassium salts (FAO, 2010). Hydrated lime is a caustic agent that greatly elevates pH when dissolved in water. Though it is unstable in seawater (it readily combines with dissolved carbon dioxide to form harmless limestone, $CaCO_3$), the fact that relatively large amounts of it may be released into the environment in single locations within a bay (i.e., on aquaculture lease sites) raises concerns about its possible effects on other organisms in the environment. Field observations by divers have revealed that large clouds of lime particles can settle on the seafloor at sites where hydrated lime is released at the estuary surface (. MacNair, Locke et al. 2009). In the laboratory, injections of highly concentrated lime solutions in seawater aquaria can result in the formation of flocculent carpets on the substrate (Reebs et al., 2011). In an undisturbed aquarium such deposits are stable. In the field, where

currents are likely to be present, the persistence of flocculent material is less likely but remains undetermined.

Fish are usually considered as organism of choice for assessing the effects of environmental pollution on aquatic ecosystem (Farombi*et al.*, 2007). In the 1990s, the concept of biomarkers with fish as a major biomarker was established (Achuba and Osakwe, 2003; Monterio*et al.*, 2007; Miller *et al.*, 2007;; Pavlović*et al.*, 2010).The use of *Clarias gariepinus* for this study is basically because the specie has a high tolerance level towards pollution in the aquatic ecosystem thus it can be used for toxicity assay and because it is the most farmed fish in Nigeria at the moment.

## 1.1 STATEMENT OF RESEARCH PROBLEM

On the course of sourcing for materials for the purpose of this research, it was observed that local farmers do not know or choose to ignore the amount of hydrated lime that should be administered to a pond based on pond size or volume of water in the pond.Most farmers actually think that since liming has positive effects of disinfecting the pond and correcting the pH of the water, it should be administered in excess because it is felt that the more the amount administered the more effective it becomes.This poses a problem because it can harm aquatic organism and even humans and that is one of the problems this research intended to solve by calculating the LC50 of hydrated lime to African catfish.

## 1.2 JUSTIFICATION

Due to the fact that liming is an important activity during fish pond preparation and it is widely accepted by fish farmers as a tool to disinfect and treat a pond coupled with the fact that hydrated lime is common and easy to use, it is paramount that the effect or impact of the lime on

the fishis investigated and documented so as a bridge the gap in knowledge [illegible] hydrated lime on fish. The choice of *Clarias gariepinus* juvenile for this study is [illegible] is the most cultured in Nigeria at the moment and due to that, farmers will [illegible] information that will be generated from this study. The notion that there can't [illegible] liming which makes some local fish farmers not to even check the pH of the water [illegible] little or too much lime to the water during pond preparation is also a rationale [illegible] study.Therefore, theresults of this study will help to educate fish folks on the toxicity of hydrated lime on aquatic organism.

## 1.3 RESEARCH OBJECTIVES

The objectives of this study are to;

- Examine the behavioral changes of *Clarias gariepinus* exposed to toxic levels of hydrated lime;
- Examine the changes that occur in the morphological parameter of *Clarias gariepinus* exposed to toxic levels of hydrated lime;
- Determine the LC50 of *Clarias gariepinus* exposed to toxic levels of hydrated lime;
- Examine the changes that occur in haematological parameters of *Clarias gariepinus* exposed to toxic levels of hydrated lime;
- Examine the changes that occur in histological properties of *Clarias gariepinus* exposed to toxic levels of hydrated lime.

## 1.4 RESEARCH HYPOTHESIS

The research hypotheses used for this study were as follows;

$Ho_1$ – there is no significant difference in the behavior of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ha_1$ – there is significant difference in the behavior of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ho_2$ – there is no significant difference in the mortality rate of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ha_2$– there is significant difference in the mortality rate of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ho_3$ — there is no significant difference in the hematological properties of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ha_3$ – there is significant difference in the hematological properties of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ho_4$- there is no significant difference in the histological properties of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

$Ha_4$ – there is significant difference in the histological properties of *Clarias gariepinus* exposed to varying concentration of hydrated lime;

# 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 Biology

Adults occur mainly in quiet waters, lakes and pools and prefer rather shallow and swampy areas with a soft muddy substrate and calmer water. They may also occur in fast flowing rivers and in rapids and are widely tolerant of extreme environmental conditions. Water parameters appear to play only a very minor role. The presence of an accessory breathing organ enables this species to breath air when very active or under very dry conditions. They remain in the muddy substrates of ponds and occasionally gulp air through the mouth. Can leave the water at night using its strong pectoral fins and spines in search of land-based food or can move into the breeding areas through very shallow pathways. They are omnivorous bottom feeders which occasionally feed at the surface. Feed at night on a wide variety of prey like insects, plankton, invertebrates and fish but also take in young birds, rotting flesh and plants. Migrate to rivers and temporary streams to spawn. During intra-specific aggressive interactions, this species was noted to generate electric organ discharges that were monophasic, head-positive and lasting from 5-260

ms. Known as sharptooth catfish in aquaculture, a highly recommended food fish in Africa. Marketed fresh and frozen; eaten broiled, fried and baked (Van de Nieuwegiessen et al., 2009).

### 2.1.2 Description

The catfish genus can be defined as displaying an eel shape, having an elongated cylindrical body with dorsal and anal fins being extremely long (nearly reaching or reaching the caudal fin) with both fins containing only soft fin rays. The outer pectoral ray is in the form of a spine and the pelvic fin normally has six soft trays. The head is flattened, highly ossified, the skull bones (above and on the sides) forming a casqued and the body is covered with a smooth, scale less skin. The skin is generally darkly pigmented on the dorsal and lateral parts of the body. The color is uniformly marbled and changes from greyish olive to blackish according to the substrate. On exposure to light the skin colour generally becomes lighter.

They have four pairs of unbranched barbels, one nasal, one maxillar (longest and most mobile) on the vomer and two mandibular (inner and outer) on the jaw. Tooth plates are present on the jaws as well as on the vomer. The major function of the barbels is for prey detection.A supra-branchial or accessory respiratory organ, composed of a paired pear-shaped air-chamber containing two arborescent structures is generally present. These arborescent or cauliflower-like structures located on the fourth branchial arcs, are supported by cartilage and covered by highly vascularized tissue which can absorb oxygen directly from the atmosphere (Froese*et al.*, 2014). Since the air chamber communicates directly with the pharynx and the gill-chamber, this accessory air breathing organ allows the fish to survive out of water for many hours or for many weeks in muddy marshes (Goda et al., 2007).

### 2.1.3 Natural reproduction

*Clarias gariepinus* shows a seasonal gonadal maturation which is usually associated with the rainy season. The maturation processes of *Clarias gariepinus* are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a rise in water level due to rainfall (de Graaf*et al.*, 1995). Reproduction starts in March just after the start of the first heavy rains (as indicated by the decrease in the Gonado Somatic Index[1], G.S.I.). Natural reproduction is generally completed in July with the G.S.I. remaining low until November, thereafter the oocytes gradually maturing and becoming ripe again in March Spawning usually takes place at night in the shallow inundated areas of rivers, lakes and streams. Courtship is usually preceded by highly aggressive encounters between males. Courtship and mating takes place in shallow waters between isolated pairs of males and females. The mating posture, a form of amplexus (the male lies in a U-shape curved around the head of the female) is held for several seconds. A batch of milt and eggs is released followed by a vigorous swish of the female's tail to distribute the eggs over a wide area. The pair usually rest after mating (from a few seconds to up to several minutes) and then usually resume mating (De Graaf*et al.*, 1995).

## 2.3 Hydrated Lime

Calcium hydroxide (traditionally called slaked lime) is an inorganic compound with the chemical formula $Ca(OH)_2$. It is a colorless crystal or white powder and is obtained when calcium oxide (called *lime* or *quicklime*) is mixed, or slaked with water. It has many names including hydrated lime, caustic lime, builders' lime, slack lime, cal, or pickling lime. Calcium hydroxide is used in many applications, including food preparation. Limewater is the common name for a saturated solution of calcium hydroxide.

### 2.3.1 Properties

Calcium hydroxide is relatively insoluble in water, with a solubility product K of $5.5 \times 10^{-6}$. It is large enough that its solutions are basic according to the following reaction:

- $Ca(OH)_2$

Calcium hydroxide adopts a polymeric structure, as do all metal hydroxides. The structure is identical to that of $Mg(OH)_2$ (*brucite structure*); i.e., the cadmium iodide motif. Strong hydrogen bonds exist between the layers (Greenwood et al., 1997). Calcium hydroxide is produced commercially by treating lime with water:

- $CaO + H_2O \rightarrow Ca(OH)_2$

In the laboratory it can be prepared by mixing aqueous solutions of calcium chloride and sodium hydroxide. The mineral form, portlandite, is relatively rare but can be found in some volcanic, plutonic, and metamorphic rocks. It has also been known to arise in burning coal dumps.The positively charged ionized species $CaOH^+$ has been detected in the atmosphere of S-type stars.

Hydrated lime is produced by "slaking" quicklime. Briefly water is added to quicklime and in thisprocess $CO_2$is released from the carbonate and the quicklime picks up hydroxide from water producing hydrated lime ($Ca(OH)_2$). The product is a colorless crystal or white powder. Hydrated lime is easily converted back to calcium carbonate($CaCO_3$), in the presence of carbon dioxide ($CO_2$) in either air orin water. When hydrated lime reacts with carbon dioxide in the air the effectiveness of the product is reduced, meaning that hydrated lime has a shelf life (Ramsay et al. 2014). When hydrated lime is mixed in excess with water there is not sufficient $CO_2$to convert all the lime. This results in release of hydroxide ions (OH-), an alteration of the hydrogen ion concentration and therefore a change in pH. Saturated hydrated lime suspensions have a pH of approximately 12.7. When $Mg(OH)_2$or $Ca(OH)_2$is exposed to $CO_2$dissolved in seawater, $MgCO_3$or $CaCO_3$is also formed. The solubility of these carbonates is much lower than hydrated lime at 0.00066 g/100 gwater for $CaCO_3$ (CRC, 2005), and therefore has the potential to

precipitate out of suspension and settle to the bottom in particulate form as the reaction occurs. This is easily observed during treatment of mussel sleeves (Ramsay et al. 2014). Calcium carbonate is a common substance in rocks in all parts of the world and is the main component in shells of marine organisms, and so is considered innocuous (Locke et al. 2009). Calcium carbonate in marine sediments can also be converted to bicarbonate $2(HCO_{3-})$ and ionic calcium $(Ca_{2+})$ in the presence of dissolved $CO_2$(University of Puerto Rico, 2015).

### 2.3.4 Laboratory Based Toxicity Studies of Hydrated Lime

There is a considerable body of literature regarding effects of lowering pH; in addition to causing direct effects due to increasedacidity, it may make other toxicants more available, particularly in freshwater. The consequences of increased pH particularly in the marine environment, is not as well studied. Long term exposure to increased pH can result in damage to scales and mucous layers with fish but acute lethality, when observed in response to elevated pH as with liming, is likely caused by damage to gills and disruption of cellular membranes. There may also be severe osmotic stress in "unprotected" species such as the tunicate. Locke et al. (2009) conducted laboratory bioassays of hydrated lime with the bacterium Vibrio fischeri, sand shrimp (*Crangonseptemspinosa*) and threespine stickleback (*Gasterosteusaculeatus*). These authors also reported No Observable Effect Concentrations (NOEC) for the stickleback and shrimp. NOEC refers to the concentration of the compound that results in <= 10% mortality over the duration of the experiment. These results are shown in Table 2. Only in 14 day experiments with shrimp is the NOEC, expressed aspH (8.17), close to reported normal pH as measured in Malpeque Bay. Lobster larvae are known to be intolerant of quicklime (Loosanoff and Engle 1942) and the susceptibility to hydrated lime was reported in Burridge et al. 2010 (in reference to unpublished data by Doe et al. 2009). Stage III larval lobsters were exposed to a range of

concentrations and exposure scenarios, including realistic short-term pulse exposures fo
by transfers to clean water. Clearly high concentrations of lime and a consequent elevated pH a
required to kill Stage III lobsters. In tests with more realistic exposure scenarios, short term or
pulsed, higher concentrations and pH are required. In a lab-based experiment Reebs et al. (2011)
investigated responses of the sand shrimp, Crangonseptemspinosa, when given a choice of
settling on sediment that was carbonate- layered as a result of treatment with hydrated lime or
untreated sand bottoms. The shrimp showed a preference for the untreated sand. In aquaria where
lime treatment had taken place up to 75% of the shrimp died (Reebs et al. 2011). Carbonate was
injected using saturated suspension of hydrated lime to mimic "real world" addition. A
consequence of the exposure technique was a rapid and significant elevation of pH. The authors
attribute mortality to the elevated pH and speculate that, while this work clearly shows a hazard
to this marine invertebrate, there are a number of reasons to believe the risk may not be
significant (Reebs et al. 2011). The observed precipitate is likely to remain un-dissolved in
systems where there is no turnover or renewal of water as is the case in static lab-based assays.
During operationaltreatments there is constant exposure to seawater as the lime suspension
dissipates. A 48-h exposure to 9.0 pH units was foundto be lethal to *Crassostreavirginica*(eastern
oyster) and *Mercenariamercenaria*(hard clams) larvae under laboratory conditions. Similarly, a
48-h exposure to 8.5 pH units lowered the percentage of *Mulinialateralis*(coot clam) embryos
that developed normally, and a 6-8 day exposure to 9.0 pH units decreased *M. lateralis*survival at
the larval stage.Doe et al. (2009) (unpublished work reported in Burridge et al. 2010) also
reported that lobsterbehavior, as indicated by increased frequency of tail flickswhichisa known
stress response in lobsters(for example Chiasson et al. 2015), was affected by plumes of hydrated
lime. Tail flicks decrease as particles settle to the bottom of the jars. Ramsay et al. (2014) report

on unpublished work wherein exposure to CaCO3alone did not result in an increase in tails flicks but exposure to particles generated by addition of hydrated lime did. The authors suggest it is the "caustic" nature of the particles that the lobster are reacting to, i.e. the tail flicks are probably in response to encountering small particles of un-dissolved lime. Interestingly, Reebs et al. (2011) reported that when they added hydrated lime to water, 80-85% of the resulting particles were magnesium carbonate. It is therefore possible that the Stage IV lobsters were being exposed to two different types of carbonate. As stated earlier, some hydrated lime products contain important proportions magnesium.

### 2.3.5 Uses of Hydrated lime

One significant application of calcium hydroxide is as a flocculant, in water and sewage treatment. It forms a fluffy charged solid that aids in the removal of smaller particles from water, resulting in a clearer product. This application is enabled by the low cost and low toxicity of calcium hydroxide. It is also used in fresh water treatment for raising the pH of the water so that pipes will not corrode where the base water is acidic, because it is self-regulating and does not raise the pH too much.It is also used in the preparation of ammonia gas, using the following reaction:

- $Ca(OH)_2 + 2NH_4Cl \rightarrow 2NH_3 + CaCl_2 + 2H_2O$

Another large application is in the paper industry, where it is an intermediate in the reaction in the production of sodium hydroxide. This conversion is part of thecausticizingstep in the Kraft process for making pulp (Greenwood et al., 1997). In the causticizing operation burned lime is added to *green liquor* which is a solution primarily of sodium carbonate and sodium

sulfate produced by dissolving *smelt*, which is the molten form of these chemicals from the recovery furnace.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Collection of Hydrated Lime and fish

Hydrated lime was purchased from a reputable farm in .

A total of 300 apparently healthy *Clarias gariepinus* juvenile of average body weight **16.62** ± 4.36gand mean total length of 14.97± 8.94 cm was bought and transported from GBOGO **Fish** Farm. The fish was acclimatized in de-chlorinated water (tap water exposed to air for greater than 24 hours) in 10L capacity plastic bowl for 2 weeks.

### 3.2 Range Finding Test

A total of 75 fish were used for the range finding test; the fish were weighed with a sensitive weighing balance to measure the average weight and a meter rule to measure the mean total length. During the range-finding test, there were five treatments with 3 replicates each, and the treatments contained five concentrations of the lime as (0, 2, 4, 6, 10 g/l of water).Five juveniles of *Clarias gariepinus* were introduced into each of the 15 containers, and the effect of the lime on the fish was observed for 96h. At 96h, those fish that did not show any tactile response was considered dead. Thus, the concentrations that killed 50% of the fish formed a base for selecting the concentrations that were used in the definitive test.

### 3.3 Definitive Test

The definitive test was carried out after the range finding test was conducted. The control and the treatments were carried out concurrently. The test was also carried out in 15 containers, 3 containers for each treatment, with a total of 5 treatments. Five different concentrations of the hydrated lime were used as:.Out of the 300 fish samples that were acclimatized, 10 active fish was selected and put into each of the 15 containers.The effect of the lime on the fish was observed for 96h. Mortality was observed at regular intervals, and fish were considered dead when they showed no movement of the body upon gentle prodding and inability of the gills to move. Dead fishes were removed to avoid further contamination of the water. Borehole water was used throughout the experiment, and before then, the physiochemical properties of the water were determined, so that changes in the water quality during toxicity test were attributed to the effect of the lime.

### 3.4 Determination of lethal concentration $LC_{50}$

$LC_{50}$ value is defined as the concentration at which 50% of a test organism is killed by the introduction of a toxicant. Data collected on mortality (%) was subjected to Probit values while the concentrations of the lime was subjected to Logit transformation method (Finney, 1982) and the LC50 value was calculated from the graph of Mortality against the concentrations of the lime.

### 3.5 Fish behavior

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

### 3.6 Determination of physiochemical parameters in the experimental water

The physiochemical parameters of the water in the aquaria were examined at every 24hours for the 96hour toxicity test using standard methods.

a) Dissolved Oxygen (DO) Concentration:

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

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

c) Temperature:The temperature was measured and recorded using a digital thermometer.

### 3.7 Haematological analyses

The fish that survived after the 96h toxicity study was cleared of the water from the body surface, then the caudal peduncle was severed and the blood was collected using a disposable syringe from the caudal artery and transferred into heparinized tubes. Approximately 2 ml blood was drawn from the experimental fish as well as the control fish. RBC count was done with a Neubauer crystalline counting chamber as described by Sohn and Henry (1969). WBC count was determined following the procedure described by Hunter and Bomford (1963). The haemoglobin content was estimated by acid haematin method (Sahli, 1982). The haematocrit (Ht) value was calculated by the micro haematocrit method as described by Blaxhall and Daisley (1973). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC). Oxygen Carrying Capacity were determined and tabulated.

### 3.9 Histological analyses

For histological examinations the gills and liver of the dead were used. They were isolated from control and experimental fish through trans-spinal dissection, gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and adhering debris and immerse in fixative composed of glacial acetic acid , formaldehyde and ethanol (1:3:7). The fixed tissues was passed through the alcohol series 50 to 100% for dehydration and finally cleared in chloroform. Tissues were then embedded in paraffin wax (58C). After embedding, the blocks were subjected to sectioning at 5µin thickness in a rotary microtome (Leica Rotary type). The sections were stained with Haematoxylin and Eosin stains (Roberts, 1978). The stained slides was treated with xylene and mounted in DPX. The stained sections was observed under a research microscope attached with digital camera (Olympus E420 camera) and photographed.

### 3.11 Statistical Analysis

The results were subjected to Two-way analysis of variance using the Statistical Package for Social Science Version 2.0. Parameters where significant difference occurred were separated using the Duncan new multiple range test at (P= 0.05). The $LC_{50}$ of the fish was determined using the Biotic Ligand model version 2.0.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1Water quality parameters

The water quality parameters tested were temperature, pH and dissolved oxygen concentration. The results are presented in Table .The table shows that as the time of the experiment increased, dissolved oxygen concentration reduced. The longer the toxicant the smaller the amount of dissolved 0xygen in the water. Also from the result with hours as the variation, it could be observed that the pH value of the water was near alkaline.

Furthermore, from the result, it could be observed that temperature of the water was quiet stable around $24^0$C

**Table 1: Physiochemical parameters of test medium with treatment and hours as variation**

| Variation | Parameters | Dissolved oxygen | PH | Temperature(°C) |
|---|---|---|---|---|
| Hours | 24 hours | 6.38±0.63$^{c}$ | 7.89±0.62$^{a}$ | 24.38±0.12a |
| | 48 hours | 5.93±0.05$^{b}$ | 7.58±0.02$^{a}$ | 24.16±0.02$^{a}$ |
| | 72 hours | 5.20±0.11$^{b}$ | 7.76±0.02$^{a}$ | 24.48±0.02$^{a}$ |
| | 96 hours | 4.97±0.12$^{a}$ | 7.49±0.06$^{a}$ | 24.43±0.02$^{a}$ |
| Treatments | Control | 6.25±0.06$^{b}$ | 7.90±0.17$^{ab}$ | 24.93±0.40$^{ab}$ |
| | T1 | 5.21±0.42$^{a}$ | 7.58±0.11$^{a}$ | 24.25±0.96$^{a}$ |
| | T2 | 5.33±0.38$^{a}$ | 7.66±0.09$^{a}$ | 24.35±0.10$^{a}$ |
| | T3 | 5.64±0.24$^{a}$ | 7.56±0.10$^{a}$ | 24.33±0.08$^{a}$ |
| | T4 | 5.86±0.46$^{ab}$ | 7.79±0.13$^{a}$ | 24.35±0.10$^{a}$ |
| | T5 | 5.88±0.29$^{ab}$ | 7.77±0.11$^{a}$ | 24.30±0.07$^{a}$ |

Mean ± S.E with different superscript within column is significantly different at $p< 0.05$

## 4.2 Fish Behavior and Mortality

During exposure, the test fish exhibited the following behavioral patterns before death occurred; restlessness, respiratory distress, loss of balance, gasping for air, vertical movement, excessive accumulation of mucus on the skin and death. The reaction to the toxicant was more pronounced in the container containing the lowest amount of water of 4.8L, that is the highest concentration. The observation made with control group were however different, they had stable swimming and no respiratory stress.

## 4.3 Estimation of Lethal Concentration (LC50)

The result obtained from this research work showed that the 96 hour LC50 was determined to be 1g/5.0ltr(Fig.1).

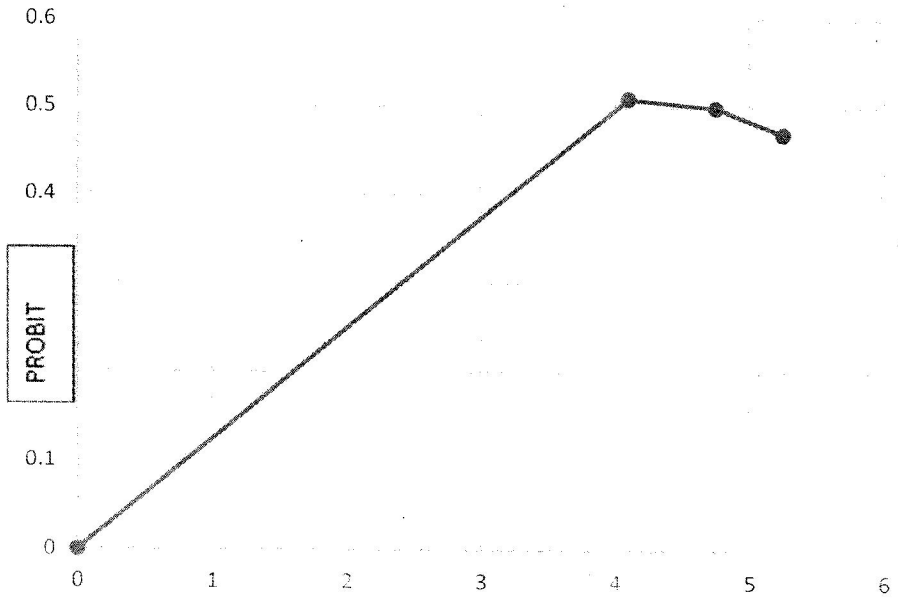


**Figure 1:** Mortality (96h – $LC_{50}$) of *Clarias gariepinus* exposed to different concentrations of hydrated lime (LC 50 = 1g/5.0 ltr)

### 4.4 Haematological examination

Table 2 shows the result of the hematological examination of the fish after exposure to the toxicant. From the result it was observed that the WBC, PCV and the Lymphocyte decreased across the treatments, while there was no effect on the Eosinophil.

**Table 2 Haematological Parameter of African Catfish exposed to Toxicant (hydrated lime)**

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **control** | **6.0L** | **5.5L** | **5.0L** | **4.8L** | **4.5L** |
| **RBC** | 15.17± 1.33$^{ab}$ | 44.50±2.84 $^{d}$ | 29.00± 2.08$^{c}$ | 18.33± 4.49$^{ab}$ | 12.50± 2.60$^{b}$ | 8.00± 2.26$^{a}$ |
| **WBC** | 2.00± 0.58$^{a}$ | 9.27± 0.54$^{d}$ | 7.00± 1.26$^{c}$ | 5.00± 0.51$^{bc}$ | 4.33± 1.30$^{b}$ | 3.50± 1.00$^{ab}$ |
| **PCV** | 32.33±3.38$^{a}$ | 80.00± 1.16$^{d}$ | 60.00± 6.43$^{c}$ | 40.17± 4.76$^{b}$ | 42.17± 0.67$^{b}$ | 38.00± 2.78$^{ab}$ |
| **Lymphocytes** | 20.33± 6.74$^{a}$ | 80.00± 2.89 | 65.00± 2.89$^{e}$ | 55.00± 5.77$^{d}$ | 40.33± 1.86$^{c}$ | 35.33± 3.53$^{b}$ |
| **Eosinophil** | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| **Neutrophil** | 79.67± 6.74$^{e}$ | 20.00±2.89 $^{a}$ | 35.00± 2.89$^{b}$ | 45.00± 5.77$^{bc}$ | 59.67± 1.86$^{c}$ | 64.67± 3.53$^{d}$ |

Mean ± S.E with different superscripts across rows are significantly different at $p< 0.05$

## 4.5 HISTOPATHOLOGY OF LIVER AND GILLS OF THE TEST

### Liver

The histology of control fish liver revealed normal (Plate 1) typical paranchymatous appearance. The liver was made up of hepatocytes, which were polygonal cells with a central spherical nucleus. There were degeneration of hepatocytes (plate 2 and 3) and mild vacuolation of hepatocytes and multi-focal areas of hepatocytes degeneration, respectively. Plates 4 and 5 showed a centrilobular degeneration of hepatocytes. Mild vacuolation of hepatocytes and severe necrosis were also observed. The liver cells were degenerated with necrosis which appeared as focal areas with lymphocytic infiltration (Plate 6).

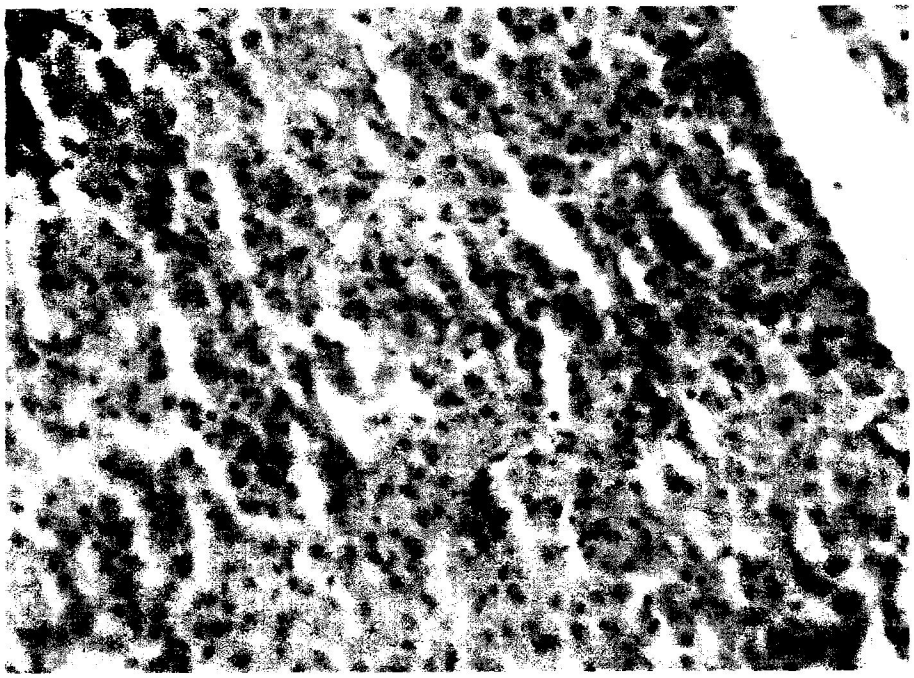

**Plate 1: Photomicrograph of liver section from control juvenile *C. gariepinus* showing the central vein (V) and normal place of hepatocytes. (mag x100)**

**Plate 2: Photomicrograph of liver of *C. gariepinus* in 6.OL of water showing mild vacuolation of hepatocytes. (mag x100)**

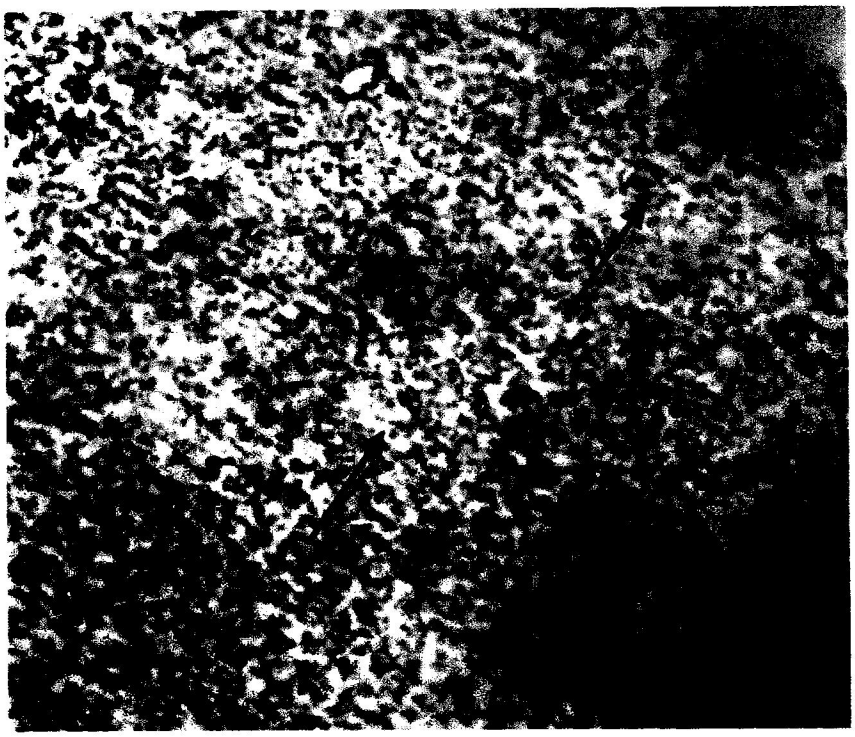

**Plate 3: Photomicrograph of liver of *Clarias gariepinus* in 5.5L of water showing multifocal areas of hepatocyte degeneration. (mag x100)**

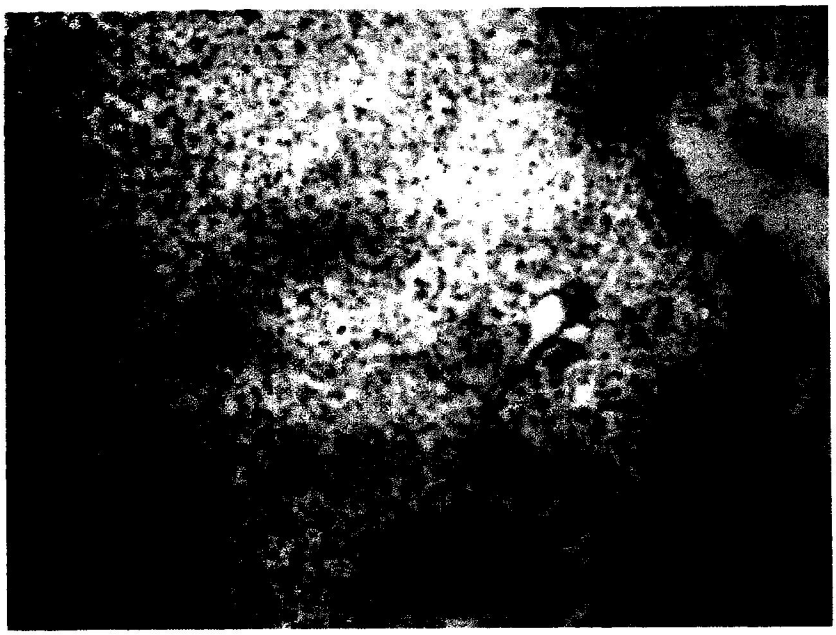

Figure 1

**Plate 4: Photomicrograph of liver of *Clarias gariepinus* in 5.0L of water showing centrilobularvacuolation of hepatocytes. (mag x100)**

**Plate 8: Photomicrograph of gills of *Clarias gariepinus* in 6.0L of water showing raised lamellar. H&E (mag×100).**

**Plate 9: Gills of juvenile *Clarias gariepinus* in 5.5L of water showing oedema (D) of the lamellar epithelia. H&E(mag×100)**

changes depict possible gill damage that has affected the respiratory system of the fish. The degrees of the pathological changes in the gills were directly concentration dependent.

**Plate 7: Gills of control of *C. gariepinus* showing normal filaments and well separated lamellar. H&E (mag ×100)**

**Plate 5: Photomicrograph of liver of *Clarias gariepinus* in 4.8L of water showing centrilobular degeneration of hepatocytes. (mag x100)**

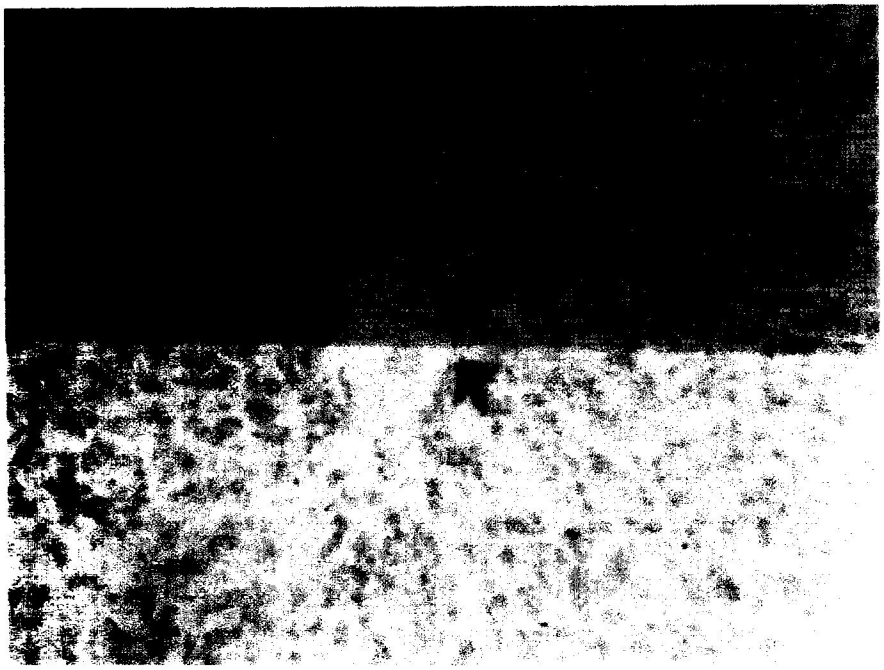

**Plate 6: Photomicrograph of liver of *Clarias gariepinus* in 4.5L of water showing severe necrosis. (mag x100)**

**Gills**

No recognizable changes were observed in the gills of the control fish. Each gill consisted of a primary filament and secondary lamella (Plate 7). At different liters of water in each tank, there were raised lamellar (Plate 8). Most of the primary and secondary lamellae were distended with oedema in the lamellar epithelia (Plate 9). However, mild oedema and rising of the filament in (Plate 10 and 11), loss of lamella epithelium was also observed (Plate 12). These

Plate 12: Gills of *C. gariepinus* in 4.5L of water showing loss of lamellar epithelium. (mag x100)

**Plate 10: Gills of juvenile *Clarias gariepinus* in 5.0L of water showing mild oedema and raising of the filaments. ( mag x100)**

**Plate 11: Gills of *C. gariepinus* in 4.8L of water showing oedema (E) and raising of the filaments. ( mag x100)**

# CHAPTER FIVE

## 5.0 DISCUSSION

*Clarias gariepinus* juveniles were stressed progressively with time before death during this experiment. The stressful behavior of respiratory impairment due to the toxic effect of hydrated lime on the gills was similar with the reports of (Omitoyin et al., 2006) and (Omoniyi et al., 2002) that toxicant impairs respiratory organs. Death could therefore have occurred either by direct poisoning or indirectly by making the medium un-conducive or even by both, whichever is the case, the source of death was hydrated lime. Several abnormal behavior such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increased opercula activities, surface to bottom movement, sudden quick movement, resting at the bottom were similar to the observations of (Aguigwo, 2002, Rahman et al., 2002). The stressful and erratic behavior of *Clarias gariepinus* juvenile in the experiment indicates respiratory impairment; probably due to the effect of the toxicant hydrated lime on the gills. The fish became inactive at lower quantity of water with increased time of exposure to toxicant. Results obtained from this study show that percentage mortality of *C. gariepinus* fingerlings increased with increase in concentration of the toxicant. This is in consonance with a similar study by Nivedita et al. (2002) on toxicity effect of DEP on a fresh-water fish, *Cirrhinusmrigala.*

The observed decrease in the haemoglobin concentration is in agreement with some earlier studies. Van Vureen (1986) reported that metasystox caused decreased haemoglobin concentration in *Labeoumbratus* while Omoregie et al. (1990) observed decrease in haemoglobin in *O. niloticus* exposed to gammalin 20 and actellic 25EC. Other studies (Santhakumar et al.,

1999; Mgbenka et al., 2003) reported that monocrotophos and acetellic exposures decreased the haemoglobin concentration in *Anabas sp.* and *C. albopunctatus*, respectively. The decreased haemoglobin concentration observed in this study is an indication of impaired oxygen delivery to the tissues. The reduction in erythrocytes count in *C. gariepinus* is due to hydrated lime exposure is in consonance with the report of Santhakumar et al. (1999) on *Anabas sp.* exposed to monocrotophos and in *C. albopunctatus* treated with actellic 25 EC (Mgbenka et al., 2003). Similar results were reported for *Oreochromis mossambicus* exposed to copper (Nussey et al., 1995). The general reduction in haemoglobin concentration, erythrocyte count and PCV in the fish exposed to hydrated lime in varying concentration was an indication of anaemia in the fish. The observed decrease in the PCV, Hb and erythrocyte count of *C. gariepinus* juvenile after 96hours exposure to hydrated lime could be indicative of heamodilution due to erythrocyte sequesteration. Some scholars have attributed changes in such blood parameters to erythrocyte swelling (Annune and Ahuma, 1998) or haemolysis (Annune et al., 1994). The reduction in the erythrocyte counts and the haemoglobin concentrations in *C. gariepinus* have demonstrated that hydrated lime may have affected the erythropoieticcentres in the kidney thereby limiting erythropoiesis in the fish. The literature on histopathology effects of hydrated lime on fish is still rare. Muhammed et al., (2011) conducted a toxicity test on *Clarias gariepinus* juvenile exposed to hydrated lime but didn't focus on histopatholical examinations of the fish.

In the present study, damages of the gills indicated that the sublethal concentration of hydrated lime caused impairment in gaseous exchange efficiency of the gills. The major changes were raised lamellar, oedema of the lamellar epithelia, mild oedema and raising of the filament, and loss of lamellar epithelium. Histopathological changes of gill such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported

after the exposure of fish to a variety of noxious agents in the water such as herbicides, phenols and heavy metal (Nowak,1992, Zuberi et al., 2015). In vertebrate including fish, liver is the main organ that play important role in detoxification of pesticides. During metabolism, liver has the ability to break down these harmful substances, but beyond a certain limit these toxic compounds disturb the regulating mechanism of the liver and cause morphological alteration (Brusle et al., 1996, Zuberi et al., 2015). Liver is especially a useful organ in assessing the possible impact of pollutant in fish. This is because chemical tends to concentrate there. This is also a major site for biotransformation of toxic chemicals which usually makes them less toxic and more easily excreted. Necrosis of the liver tissues in the study as observed, probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis. This also agrees with Babu et al. (2007) in the exposure of fish to fenevalerate on the liver tissues of *C. mrigala*, when necrosis of tubular epithelium and pycnotic nuclei in the hematopoietic tissue occurred.

## 5.1 CONCLUSION AND RECOMMENDATIONS

The LC50 of hydrated lime to *Clarias gariepinus* juveniles was calculated as 1g/5.0Ltr. Application of excess hydrated lime to ponds could affect the liver and gills of fish. Therefore, I hereby recommend that the relevant government agencies in charge of extension services should use the findings of this research to sensitize fish farmers on the need to be careful I applying hydrated lime in ponds as excess may result in fish kills.

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