ESTIMATION OF INDUCED DNA DAMAGE IN THE AFRICAN CATFISH (*Clarias gariepinus)* OBTAINED FROM THE UPPER STREAM OF IKPOBA RIVER, BENIN CITY, EDO STATE, NIGERIA

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UNIVERSITY OF BENIN

BENIN CITY

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AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc,) IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

FEBRUARY, 2025

CERTIFICATION

This is to certify that this research titled **"Estimation of Induced DNA Damage in the African Catfish (*Clarias gariepinus)* Obtained from the Upper Stream of Ikpoba River, Benin City, Edo State, Nigeria"** was carried out by **RUKE PRECIOUS ESIGIE** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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PROF. D.I. OLORUNFEMI **DATE**

(Project Supervisor)

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PROF. A.A. ENUNEKU **DATE**

(Head of Department)

DECLARATION

I, **RUKE PRECIOUS ESIGIE,** declare that "**Estimation of Induced DNA Damage in the African Catfish (*Clarias gariepinus*) Obtained from the Upper Stream of Ikpoba River, Benin City, Edo State, Nigeria ''** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

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RUKE PRECIOUS ESIGIE **DATE**

DEDICATION

This work is dedicated to God Almighty for His protection and preservation all through my days in school.

ACKNOWLEDGEMENTS

I thank God for his grace and love. I specially want to acknowledge the effort of my project supervisor, Professor Daniel Olorunfemi, for his guidance and encouragement and love and support throughout the period of my project.

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I want to appreciate my lovely parents Mr. and Mrs. James Esigie for their support and patience they had toward me

I wish to express my profound and deepest gratitude to the family of Pastor Victor Lawrence Agenmonmen and Pastor Bright Osemweige for your love and kindness you were not only a pastor to me but a father and a support system I lack words to say I am grateful for standing in gap as a father figure to me here in school.

I can't stop thanking and appreciating Dr. Dele I. who stood by me during this process, your support, your assistance both financially and morally and educationally during my research work am forever grateful.

Where I come from we say 'MIGWOH'.

TABLE OF CONTENTS

CERTIFICATION **iii**

DECLARATION **iv**

DEDICATION v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

ABSTRACT x

CHAPTER ONE **1**

INTRODUCTION **1**

1.1 BACKGROUND OF STUDY 1

1.2 AIM OF THE STUDY 2

1.3 OBJECTIVE OF STUDY. 3

CHAPTER TWO **5**

2.1 LITERATURE REVIEW **5**

2.2 Genotoxicity Indicator 6

2.3 Clarias gariepinus Overview 6

2.4 Potential Pollutants in Ikpoba River 7

2.5 Techniques for Evaluating Fish DNA Damage 8

2.6 Test of Micronucleus 8

2.7 Pollutants' Impact on Fish Populations 10

2.8 EFFECT ON FISH POPULATION 11

2.9 Importance of *Clarias gariepinus* as a Bioindicator Species 12

2.10 Ecological and Economic Importance 13

CHAPTER THREE **15**

MATERIALS AND METHOGS **15**

METHODOLOGY 15

3.1 Study Area 15

3.2 Sample Collection 16

3.3 Sample Preparation 17

3.4 CHALLENGES: 18

3.5 APPARATUS AND REAGENTS 20

3.6 DIGESTION PROCEDURE 20

3.6.1 Acid Digestion for Heavy Metal Analysis 20

3.6.1.1 Reagents: HNO_3, or concentrated nitric acid. 20

3.6.1.2 Method: Fill a digestion flask with around 1 g of tissue sample. 20

3.6.1.3 ANALYSIS AND CALCULATION 21

3.7 QUALITY ASSURANCE 22

CHAPTER FOUR **23**

RESULTS **23**

CHAPTER FIVE **32**

5.1 DISCUSSION **32**

5.1 DNA Damage in *Clarias gariepinus* 32

CONCLUSION AND RECOMMENDATION 35

REFERENCES **37**

LIST OF FIGURE

Study area map 16

The percentage frequency of the different aberrations induced in *Clarias gariepinus* obtained from Upper Stream of Ikpoba River 30

LIST OF TABLE

Micronucleus and other nuclear abnormalities found in *Clarias gariepinus* from Upper Stream of Ikoba River in Benin City, Edo State 23

LIST OF PLATE

Blebbed Cytoplasm	25
Heptane Nucleated	26
A-Nucleated	27
Bi-Nucleated Cell	28
Micronucleus Cell	29

ABSTRACT

This study examines how environmental pollutants affect aquatic animals' genetic integrity, with a focus on the African catfish (*Clarias gariepinus*). This study was undertaken is to evaluate DNA damage in *C. gariepinus* samples obtained from the upstream of Ikpoba River in Benin City, a freshwater body impacted by human activities notably, industrial effluent discharge from a brewery into the river. The piscine micronucleus assay was employed for identifying and measuring breaks in DNA strands in ten (10) samples of *C. gariepinus* obtained from several points along the river because of its ecological and economic importance as well as its high sensitivity to chemical stressors, serving as an ecotoxicological indicator species that offers important information about the level of pollution in aquatic habitats. Results showed that fish from the study site had significantly more DNA damage than control groups from unpolluted habitats, indicating that the Ikpoba River's contaminants cause genotoxic stress. It was revealed that heavy metals were among the possible sources of pollution that the study found to be important contributors to the observed genetic damage. Furthermore, the degree of exposure and pollutant concentration were associated with differences in DNA damage levels among the fish studied. The findings highlight how urgently the Ikpoba River and other comparable water bodies need to be regularly monitored and cleaned up. Such genetic harm has consequences for population health and ecological sustainability in addition to individual creatures, underscoring the wider environmental and socioeconomic effects of water pollution. By laying the groundwork for upcoming ecotoxicological research and conservation tactics, this study is an essential step in comprehending and reducing genotoxic dangers in aquatic systems.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The African sharptooth catfish, *Clarias gariepinus*, is one of the continent's most extensively dispersed and commercially significant freshwater fish species. In sub-Saharan Africa, the Middle East, and certain regions of Asia, it lives in rivers, lakes, ponds, and swamps. Because of its exceptional physiological and behavioral adaptations, the species can survive in a variety of environmental settings, including both clean and dirty rivers (Bruton, 1979). The African catfish is incredibly resilient to harsh environmental circumstances. It thrives in low-oxygen settings, which are frequently found in murky or stagnant bodies of water. This flexibility is made possible by its auxiliary air-breathing organ, which enables it to draw oxygen from the surrounding air when the water's dissolved oxygen content is inadequate (Viveen 1985).

DNA damage is defined as structural changes to DNA molecules that can cause genetic instability, reduced cellular function, and an increased susceptibility to illness. It is induced by both endogenous and external causes, such as reactive oxygen species (ROS), ultraviolet (UV) radiation, chemical contaminants, and physical stresses (Lindahl and Barnes, 2000). Understanding DNA damage is crucial for understanding environmental implications, particularly in aquatic creatures exposed to pollution.

The genetic instructions needed to synthesize proteins and other macromolecules necessary for cell life and function are encoded in DNA. These functions can be hampered by DNA damage, such as mutations or strand breaks, which can result in malfunctioning proteins and disturbed cellular pathways (Lindahl and Barnes, 2000). Keeping DNA intact is essential for aquatic species, whose longevity is greatly impacted by changing environmental factors, to adjust to stressors such as variations in salinity, temperature, and oxygen availability (Shugart, 2000).

Fish that are exposed to pollutants such as PAHs and heavy metals suffer from DNA damage, which compromises the integrity of their cells. Fish populations are at risk due to genotoxicity, which can cause mutations, cancer, and decreased reproductive capacity (Frenzilli 2009).

When industrial effluents are released into water bodies, heavy metals including lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) are frequently found. These metals can interfere with DNA integrity and enzyme activity, and they build up in fish tissues (Frenzilli 2009). According to Obiakor (2012), prolonged exposure to heavy metals frequently causes bioaccumulation, rendering the fish unfit for human consumption.

Like many urban and semi-urban water bodies, the Ikpoba River is vulnerable to contamination from a variety of sources. Fish populations and aquatic ecosystems are seriously threatened by these contaminants, which come from home, agricultural, and industrial sources.

1.2 AIM OF THE STUDY

The purpose of this study is to evaluate the degree of DNA damage caused to populations of *Clarias gariepinus* that live in the upper Ikpoba River stream. Using cutting-edge genotoxicity test, the study aims to:

i. Assess environmental contaminants that may be found in the upper Ikpoba River.

ii. Assess the degree of DNA damage in *Clarias gariepinus* using sensitive and trustworthy biomarkers, such as the micronucleus test, which may be used as a gauge of environmental genotoxicity.

iii. Make connections between the reported DNA damage in *Clarias gariepinus* and the exposure to pollutants. This entails investigating the ways in which particular pollutants, either by themselves or in combination, contribute to genotoxic consequences.

iv. Examine the effects on the environment and human health hazards to people that arise from the bioaccumulation of contaminants in fish that local populations feed on.

v. Participate in environmental management by providing baseline data so that environmental regulators and policy makers are aware of the condition of the Ikpoba River's aquatic ecosystem. Strategies to lower pollution and protect biodiversity may be developed with the use of this knowledge.

1.3 OBJECTIVE OF STUDY.

The study's goals are to methodically address the ecological ramifications of genotoxic effects and DNA damage on fish populations, particularly on *Clarias gariepinus* in the upper stream section of the Ikpoba River in Benin City, Edo State. These goals are as follows:

(i) To examine and quantify the levels of possible pollutants in water and sediment samples from the upper Ikpoba River stream, including heavy metals, pesticides, hydrocarbons, and other organic and inorganic contaminants. Linking environmental pollutants to biological impacts requires an understanding of pollutant dispersion (Livingstone, 2001).

(ii) To determine DNA damage in the *Clarias gariepinus* obtained from the river

(iii) To ascertain the connection between DNA damage and pollutants

(iv) To examine the effects of DNA damage on *Clarias gariepinus* populations' survival, reproductive success, and general health, as well as the wider ramifications for ecological stability.

(v) To create baseline information on the genotoxicity of *Clarias gariepinus* in the Ikpoba River. Future attempts to reduce pollution and conserve biodiversity can be

guided by this knowledge, which is essential for environmental risk assessments (Obiakor 2012).

CHAPTER TWO

2.1 LITERATURE REVIEW

This is the biomarker of environmental contamination and ecosystem health, DNA damage in aquatic organisms especially fish has attracted a lot of interest. An economically significant and extensively dispersed fish species, *Clarias gariepinus*, is frequently used in ecological studies to evaluate the effects of contaminants. An enormous amount of anthropogenic activity, such as home trash, agricultural runoff, and industrial discharges, have affected the Ikpoba River, a crucial water resource in Southern Nigeria. These activities expose aquatic creatures to a variety of contaminants that may be genotoxic.

The versatility and capacity to thrive in diverse aquatic environment makes the African catfish (*Clarias gariepinus*), is a perfect bioindicator for tracking environmental stress. The health of the aquatic environment is reflected in the build-up of contaminants in its tissues and the resulting harm to its DNA. Cellular survival, reproduction, and function depend on DNA integrity, and its degradation can have detrimental effects on the environment and evolution (Frenzilli *et al*., 2009). The objective of this paper is to present a thorough analysis of the causes and consequences of DNA damage in *Clarias gariepinus*, as well as how the contaminants present in the upper Ikpoba River stream affect these factors.

The toxicity, bioaccumulation potential, and persistence of heavy metals including lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) make them especially dangerous. These metals have the ability to either directly bind to DNA and form complexes that disrupt transcription and replication processes, or they can indirectly harm aquatic ecosystems by causing oxidative stress (Frenzilli 2009). Heavy metals have complicated and multidimensional impacts on aquatic species due to their bioavailability and toxicity, which are regulated by biological and water chemistry variables.

2.2 Genotoxicity Indicator

Aquatic creatures' DNA damage has emerged as a key indicator of environmental stress brought on by contaminants. Direct contact with genotoxic chemicals or indirect oxidative stress brought on by reactive oxygen species (ROS) can both cause this damage. In ecotoxicology, biomarkers including chromosomal abnormalities, micronuclei production, and DNA strand breaks are widely utilized to evaluate the genotoxic potential of environmental pollutants (Shugart, 2000).

DNA Damage Mechanisms:

(vi) **Direct Genotoxicity**: Adducts formed by heavy metals such as lead and cadmium can damage DNA's structure and functionality (Phillips 2013).

(vii) **Oxidative Stress**: Base alterations and DNA strand breaks are caused by ROS, which are produced by metals like chromium and mercury (Livingstone, 2001).

Relevance to Fish: Because they are constantly exposed to aquatic contaminants, fish are especially susceptible to DNA damage. Since DNA integrity is essential for cellular survival, reproduction, and function, it is a vital metric for evaluating the health of the environment (Frenzilli 2009).

2.3 Clarias gariepinus Overview

African catfish, or Clarias gariepinus, is a hardy and commercially important fish species in aquaculture. Because of its versatility in many habitats and capacity to bioaccumulate contaminants, it is frequently employed in ecological and toxicological research (Obiakor 2012).

Characteristics:

- Habitat: Found in freshwater habitats such as ponds, rivers, and streams.
- Physiology: It is a good model for research in polluted regions because it has adaptable respiratory systems that allow it to survive in low oxygen situations (Clay, 1979)
- Relevance to Study: *Clarias gariepinus* is a dependable sentinel species for monitoring water pollution due to its high metabolic activity and ability to collect contaminants (Adedeji 2014).

2.4 Potential Pollutants in Ikpoba River

Given its strategic location in a heavily populated region, the Ikpoba River is exposed to a variety of contaminants brought in by human activity. The following categories of pollutants have been identified by studies:

Heavy Metals

Lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg) are among the heavy metals that are frequently found in household and industrial wastewater that is dumped into rivers. These metals cause structural and functional disturbances because of their strong affinity for nucleophilic sites in DNA. They provide a continuous hazard to aquatic life due to their bioaccumulative nature (Frenzilli 2009).

Pesticides

Fish are known to experience oxidative stress and DNA strand breakage due to the introduction of organochlorines and organophosphates from agricultural runoff (Livingstone, 2001).

Aromatic hydrocarbons that are polycyclic (PAHs)

According to Phillips (2013), PAHs are lipophilic substances that enter cellular membranes and interact directly with DNA to produce genotoxic effects. They are found in oil spills and industrial effluent.

Eutrophication

Nutrient overloading from untreated home and agricultural waste leads to eutrophication, lowering oxygen levels and generating stress in fish populations. Stress increases the generation of ROS, which worsens DNA damage (Shugart, 2000).

Microplastics and Plastic

According to Browne et al. (2007), microplastics serve as carriers of harmful compounds such as pesticides and heavy metals, intensifying their genotoxic effects on aquatic life.

2.5 Techniques for Evaluating Fish DNA Damage

Fish DNA damage is measured using a variety of tests, which offer both quantitative and qualitative information on the genotoxicity of environmental contaminants.

Comet Assay (Single-Cell Gel Electrophoresis)

This technique measures the migratory pattern of fragmented DNA under an electric field to identify breaks in individual DNA strands. Ecotoxicology uses it extensively and it is quite sensitive (Collins, 2004).

2.6 Test of Micronucleus

The micronucleus test finds tiny, extranuclear entities in cells to reveal chromosomal damage. This is particularly beneficial for analyzing cytogenetic effects of genotoxicants (Fenech, 2000). IT Because adult fish erythrocytes are nucleated, big, and abundant, the Fish MN test

has the benefit of making micronucleus scoring technically considerably simpler and faster. As long as the nuclei are visible during the interphase stage, the test may be utilized in any population of proliferating cells, independent of the species' karyotype.

Analysis of DNA Adducts

In order to provide information on pollutant exposure and its impacts, this entails identifying certain DNA adducts created by direct interaction with genotoxic chemicals (Phillips, 2013).

2.6 Causes of DNA Damage

Endogenous Sources: One of the main causes of DNA damage is ROS produced during regular cellular metabolism. Oxidative stress can result from ROS-induced SSBs, DSBs, and base alterations (Cooke 2003).

External Sources: One of the main causes of DNA damage in aquatic species is exposure to environmental contaminants, including hydrocarbons, pesticides, and heavy metals. Through the production of ROS, these contaminants either directly or indirectly interact with cellular DNA (Livingstone, 2001).

Genotoxicity in the Environment

One accurate indicator for determining genotoxicity in organisms exposed to contaminated environments is DNA damage. Because contaminants have a tendency to collect in water bodies, aquatic species like Clarias gariepinus are especially susceptible to genotoxic chemicals. Heavy metals such as lead and cadmium, for example, can cause oxidative stress and interfere with DNA repair processes, which can lead to instability due to genetics (Frenzilli 2009).

2.7 Pollutants' Impact on Fish Populations

Fish DNA damage brought on by pollutants has a significant impact on ecological stability, population dynamics, and human health.

Reproductive Impairment

Fish reproductive systems are impacted by genotoxicants, which can result in decreased fertility, aberrant development, and population losses (Livingstone, 2001). By imitating or inhibiting natural hormones, pollutants, particularly heavy metals like lead (Pb) and cadmium (Cd), can interfere with the endocrine system. The hypothalamic-pituitary-gonadal (HPG) axis is disrupted by these endocrine-disrupting chemicals (EDCs), which results in changes to the levels of reproductive hormones such as testosterone and estrogen. Fish ovulation, spawning behavior, and gamete production can all be negatively impacted by such disturbances (Jobling 2006).

Modifications in Behavior

Notable deviation from an organism's typical patterns of behavior is referred to as a behavioral alteration. This occurs frequently as a result of exposure to external stressors, contaminants, or internal physiological problems. Such changes are employed as markers of stress and toxicity in species, particularly in aquatic habitats, in the context of environmental neurological processes, which impacts fish behavior, including eating, breeding, and avoiding predators (Frenzilli 2009).

Deaths and Population Reductions

Fish population declines and pollution-induced mortality are important consequences of environmental contamination. These impacts, which affect both individual individuals and entire fish populations, are frequently connected to the intensity, kind, and persistence of pollutants in aquatic environments.

According to Obiakor (2012), prolonged exposure to genotoxic contaminants raises death rates, endangering fish populations and upsetting aquatic ecosystems.

Magnification and Bioaccumulation

Fish tissues allow pollutants to bioaccumulate, which causes biomagnification throughout the food chain. People that eat tainted fish run serious health risks as a result (Browne 2007

2.8 EFFECT ON FISH POPULATION

Genotoxicity

Fish that are exposed to pollutants such as PAHs and heavy metals suffer from DNA damage, which compromises the integrity of their cells. Fish populations are at risk due to genotoxicity, which can cause mutations, cancer, and decreased reproductive capacity (Frenzilli 2009).

Deficits in Reproduction

Fish hormone control is disrupted by endocrine-disrupting chemicals (EDCs), which include industrial substances and certain pesticides. Offspring grow abnormally, reproductive cycles are changed, and fertility is decreased as a result (Livingstone, 2001).

Biomagnification and Bioaccumulation

Fish tissues gradually acquire contaminants such as heavy metals and persistent organic pollutants (POPs). As they move up the food chain, these toxins endanger the health of both humans and other predators (Shugart, 2000).

Behavioral and Physiological Stress

Fish behavior, including as eating, swimming, and avoiding predators, can be changed by pollution-induced stress. Survival rates are decreased by physiological impacts such as diminished immune responses, damaged gills, and poor oxygen delivery (Phillips, 2013).

Population Reductions

Because of increased mortality, infertility, and habitat degradation brought on by prolonged exposure to pollution, fish populations decline. The biological equilibrium of the river environment is upset by declines in important species (Obiakor *et al*., 2012).

2.9 Importance of *Clarias gariepinus* as a Bioindicator Species

The African catfish, or Clarias gariepinus, is a species that has been extensively researched in toxicological and ecological studies. It is a useful bioindicator for evaluating the condition of aquatic ecosystems and the effects of environmental contaminants due to its biological, ecological, and physiological traits. Clarias gariepinus is a crucial bioindicator species for the reasons listed below:

Ecological Adaptability and Broad Distribution

Throughout Africa and certain regions of Asia, Clarias gariepinus is indigenous to a variety of freshwater habitats.

It grows well in a variety of aquatic situations, such as lakes, ponds, rivers, and contaminated areas.

It is a perfect choice for tracking the impacts of pollution in both pure and damaged ecosystems because of its capacity to endure low oxygen levels, high turbidity, and destroyed habitats.

Potential for High Bioaccumulation

Pollutants such pesticides, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) build up in the tissues of this species.

Aquatic environment contamination levels may be directly inferred from the concentrations of these contaminants in *Clarias gariepinus*.

Exposure to Pollutants

In response to environmental stresses, including as oxidative stress and contaminant-induced DNA damage, *Clarias gariepinus* displays physiological and biochemical reactions. It's a dependable organism for identifying genotoxic effects since these reactions may be statistically evaluated utilizing biomarkers like the comet assay and micronucleus test.

2.10 Ecological and Economic Importance

Clarias gariepinus is economically significant in aquaculture and fisheries and is a significant source of protein in many areas.

The total safety of the aquatic food chain and its hazards to human consumers are reflected in its health.

Size and longevity are rather large.

Clarias gariepinus's comparatively big size makes tissue collecting and analysis simple.Because of its durability, it may be used to investigate long-term, chronic pollution exposure.

Clarias gariepinus is an essential bioindicator species for determining the magnitude and effects of environmental contaminants on aquatic environments. It is a dependable model for

tracking ecological health, directing pollution mitigation methods, and preserving biodiversity and human health because of its plasticity, bioaccumulation capacity, and sensitivity to toxins.

CHAPTER THREE

MATERIALS AND METHOGS

METHODOLOGY

Estimation of induced DNA Damage in *Clarias gariepinus* Obtained from the Upper Stream of Ikpoba River,Benin City, Edo State, Nigeria.

3.1 Study Area

One of the most notable rivers in Benin City, which is situated in Edo State, Southern Nigeria, is the Ikpoba River. The river rises in the middle of the state and empties into the Niger River after flowing southeastward and ultimately joining other tributaries. about in latitude 6°20′N and longitude 5°37′E.

The river flows through both urban and rural areas for more than 50 kilometers.

The study was conducted at this site because of its closeness to industrial regions, which raises the possibility of heavy metal pollution, which causes DNA demage in the fish in the river (Ezemonye *et al*., 2016). Intense metals including nickel (Ni), cadmium (Cd), and mercury (Hg) may build up in the water due to the intense traffic and industrial activity in these locations, which may subsequently have an impact on the fish in the river.

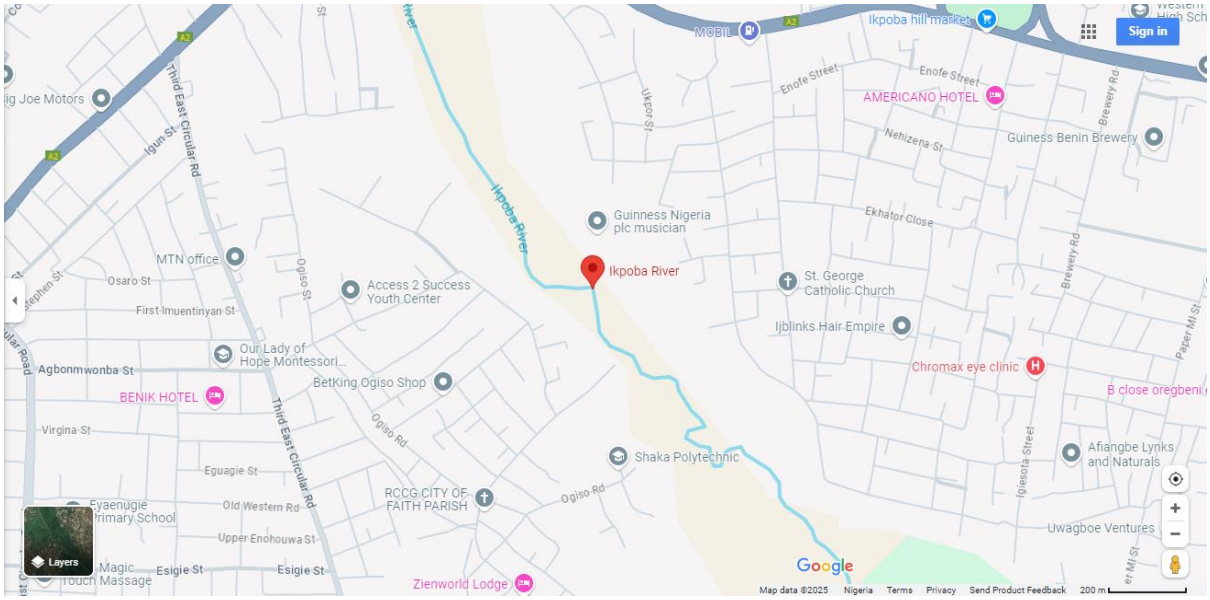

Figure 3.1 Study area map.

3.2 Sample Collection

The focus was on adult *Clarias gariepinus* specimens because of their increased propensity to bioaccumulate contaminants with time.

1. Sampling tools such as bucket, gloves, Gill Nets was provided before sampling commenced.

2. The sampling method adopted was

- The focus was on adult *Clarias gariepinus* specimens because of their increased propensity to bioaccumulate contaminants with time.
- To guarantee consistency and minimize variation in outcomes, fish were chosen according to their size (length: 25–30 cm; weight: 500–800 g).
- To prevent damage, captured fish were carefully taken out of the nets. To preserve their physiological condition while being transported to the lab, live specimens were kept in aerated plastic containers with river water.

- To guarantee that only live specimens were examined, dead or damaged fish were thrown away.
- For tracking purposes, a unique identification number was attached to each specimen's label.
- Before being analyzed in a lab, samples were carried in a bucket filled with water to maintain tissue integrity.

3.3 Sample Preparation

- Acclimate to laboratory investigations in an aerated tank for a minimum of two weeks.
- Five fish per group should be exposed to the test solution and the negative control group; the test solution should be changed every 24 hours depending on the characteristics of the test substance.
- Using a syringe, draw 0.05–0.1 mL of blood from the fish's heart.
- Using a clean slide (spreader) positioned at angle 450, create a tiny smear of peripheral blood on grease-free, spotless slides.
- Let the slides air dry at ambient temperature for the whole night in a dust- and moisture-free environment.
- After 20 minutes of fixing the slides in 100% methanol, let them air dry for at least an hour. After 10 minutes of staining the slide with 10% May Grunwald's solution, rinse it with distilled water and let it air dry.
- After 30 minutes of counter-staining with 5% Giemsa solution, thoroughly rinse the slides in distilled water. Overnight, let the slides air dry.

- To make the slide permanent, add two to three drops of DPX or Canada balsam, dip in xylene, and then cover with a cover slip.
- For micronucleated erythrocytes, score at least 3000 cells per animal at 1000x (oil immersion).
- Get ready Three of the four slides or fish might be chosen for observation.
- The cytoplasm is distinct and the nucleus and micronuclei tint blue. The comparison of MN frequencies between the treatment and negative control groups is the test's endpoint. Cells that overlap shouldn't be scored.
- Micronuclei are defined as non-refractive micronuclei that are between 1/3 and 1/20 of the size of the major nucleus and do not come into contact with it *(Al-Sabti & Metcalfe, 1995).*

In addition to MN for cytotoxicity, other nuclear abnormalities as categorized by (*Carrasco* 1990) might be scored. The nuclear membrane, which contains chromatin, evaginates very little in blebbed nuclei;

3.4 CHALLENGES:

1. Difficulties with Logistics

One major problem is getting living specimens, such *Clarias gariepinus*, to the lab without causing them undue stress or death *(Akinsorotan 2018).* Along the Ikpoba River, limited access to isolated or challenging terrain frequently necessitated improvised storage and transit arrangements. Furthermore, it was especially difficult to keep fish samples viable in aerated containers during long-distance transporation since insufficient aeration might result in hypoxia (Ezeonyejiaku, 2021).

2. Environmental Difficulties

The ease of sample collection was influenced by environmental conditions, such as changing water levels throughout the dry season. Finding the best sample locations became challenging due to the river's increasing turbidity caused by organic waste, algae, and sediments (Ogbeibu & Anagboso, 2020).

3. Contamination of the Sample

One of the most important aspects of sampling preparation is avoiding contamination during collection and transit. Inappropriate handling methods or the use of non-sterile equipment may contaminate samples, which would reduce the precision of further tests (Adesuyi 2015). Furthermore, it was difficult to identify the precise contaminants causing DNA damage due to pollution from agricultural runoff and effluent discharges.

4. Concerns about Ethics and Regulation

Following legal and ethical requirements was necessary in order to catch *Clarias gariepinus.* These rules' restrictions occasionally limited the amount of samples that could be gathered, which might have decreased the study's statistical power (Ikenweiwe 2019).

5. Conservation and Storage

Another major concern was preserving the integrity of tissue and water samples while they were being stored. Biased findings might arise from improper preservation techniques, such as failing to acidify water samples or maintain a constant temperature, which could change the concentration of contaminants and other characteristics (Nwani 2013). Similarly, if not sufficiently protected during transit, *Clarias gariepinus* tissue samples were prone to deterioration.

3.5 APPARATUS AND REAGENTS

- Aerated tank
- Methanol
- Syringe
- 10% May Grunwald's solution
- Distilled water
- 5% Giemsa solution
- xylene
- 2- 3 drops of Canada balsam
- Slides

3.6 DIGESTION PROCEDURE

- Gathering Samples of Tissue Under sterile circumstances,
- Tissue samples from the obtained Clarias gariepinus specimens, including skin and are dissected To get rid of outside impurities
- Samples are washed with distilled water right away Before being digested, each type of tissue is kept apart in sterile, labeled containers at -20°C.

3.6.1 Acid Digestion for Heavy Metal Analysis

This process releases bound metals by decomposing organic matter:

3.6.1.1 Reagents: HNO_3, or concentrated nitric acid.

As an oxidizing agent, use hydrogen peroxide ($H2O_2$) or perchloric acid ($HClO_4$)

3.6.1.2 Method: Fill a digestion flask with around 1 g of tissue sample.

Ten milliliters of strong nitric acid should be added.

Let the mixture sit at room temperature for the entire night to pre-digest.

On a hot plate, heat the mixture to 120–150°C until the solution turns clear or pale yellow, signifying full digestion.

To improve digestion, add two to three milliliters of hydrogen peroxide or perchloric acid if needed.

After letting the solution cool, dilute it with 50 milliliters of purified water.

Pour the solution into a sterile volumetric flask after filtering it with Whatman No. 42 filter paper.

Storage: To prepare the digested samples for analysis by inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectrophotometry (AAS), store them in acid-washed polyethylene bottles.

3.6.1.3 ANALYSIS AND CALCULATION

Three single elemental standards were created by diluting stock solutions of each element (Pb, As, Hg, Ni, Cd, and Cr) by 1000 mg/l.

4 .Every day, at least five standard functioning solutions were created using the stock solutions. The range of the solution was 0.1 mg/l to 10 mg/l.

5 De-ionized water and a set of calibration standards for every element were utilized for external calibration. After that, a calibration curve was created for every metal.
6. The absorbance readings were then obtained by running the digested samples and blank on the AAS.

7 .The calibration curve's equation was used to determine the metal concentrations in the sample.

3.7 QUALITY ASSURANCE

All sampling and analysis procedures will adhere to ethical guidelines. Informed consent will be obtained from participants, and confidentiality of data will be maintained throughout the study.

Acidified deionized water is first aspirated as blanks in duplicate. Duplicates and laboratory control samples are run as QC samples

CHAPTER FOUR

RESULTS

Results of the investigation of nuclear anomalies in *Clarias gariepinus* from Upper Stream of Ikoba River in Benin City, Edo State is presented in Table 4.1

Table 4.1: Micronucleus and other nuclear abnormalities found in *Clarias gariepinus* from Upper Stream of Ikoba River in Benin City, Edo State

Sample	MN	BL	NC	AN	BN	TOTAL NAs	TOTAL CELLS	%FREQUENCY OF NAs
1	58	60	12	68	41	239	4000	5.98
2	37	69	50	56	50	262	4000	6.55
3	37	40	17	63	77	234	4000	5.85
4	35	75	24	32	96	262	4000	6.55
5	26	57	61	36	38	218	4000	5.45
6	30	80	60	25	58	253	4000	6.33
7	22	19	54	75	55	225	4000	5.63
8	32	47	45	25	41	190	4000	4.75
9	38	36	55	41	87	257	4000	6.43
10	44	85	36	32	99	296	4000	7.40
Mean								243.6±0.55***
Control 1	3	3	5	14		25	4000	0.63
Control 2	1	-	-	7		8	4000	0.02
Mean								0.55±0.28

MN=micronucleus, BL=blebbed cells, NC=notched cells, AN=anucleated, BN=binucleated, NA=nuclear aberrations. *** statistically significant at $p \leq 0.001$.

The table compares two control groups and an experimental group to assess nuclear abnormalities (NAs) in *Clarias gariepinus* samples. Micronuclei (MN), blebbed cells (BL), notched cells (NC), anucleated cells (AN), and binucleated cells (BN) are among the abnormalities evaluated. When compared to the control groups, the experimental group exhibits substantially greater frequency of all nuclear abnormalities.

The experimental group's total NAs vary from 190 to 296 with an average of 243.6 ± 0.55, whereas the controls' values are significantly lower (25 and 8, mean 0.55 ± 0.28). Compared to the controls (mean 0.55%), the experimental group's percentage frequency of NAs (mean 6.09%) is much greater. The experimental group's increased nuclear abnormalities point to environmental stress or pollution and may indicate exposure to genotoxic chemicals. The modest abnormality levels in the control groups verify that baseline differences are not the cause of the harm.

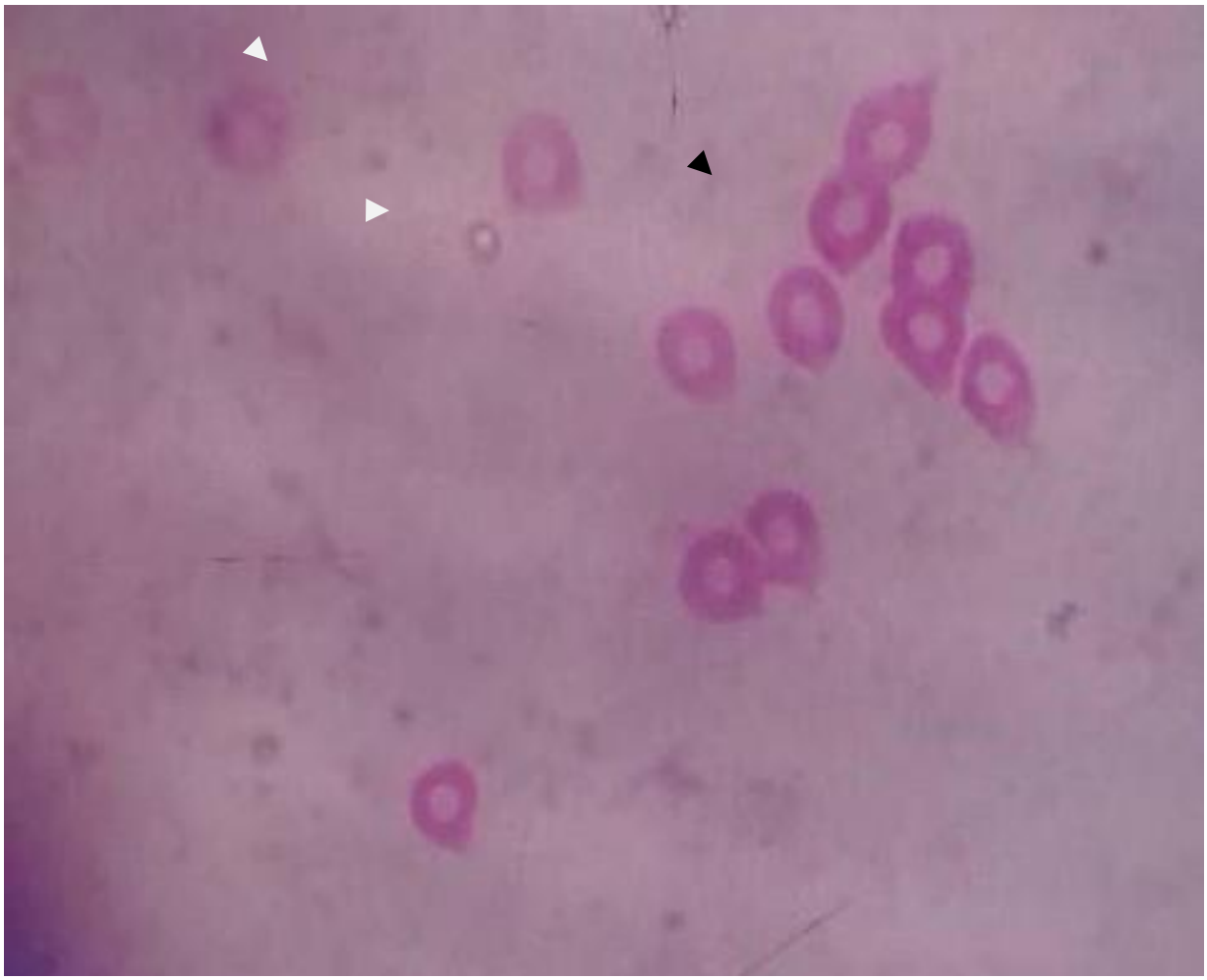

PLATE1: Blebbed Cytoplasm

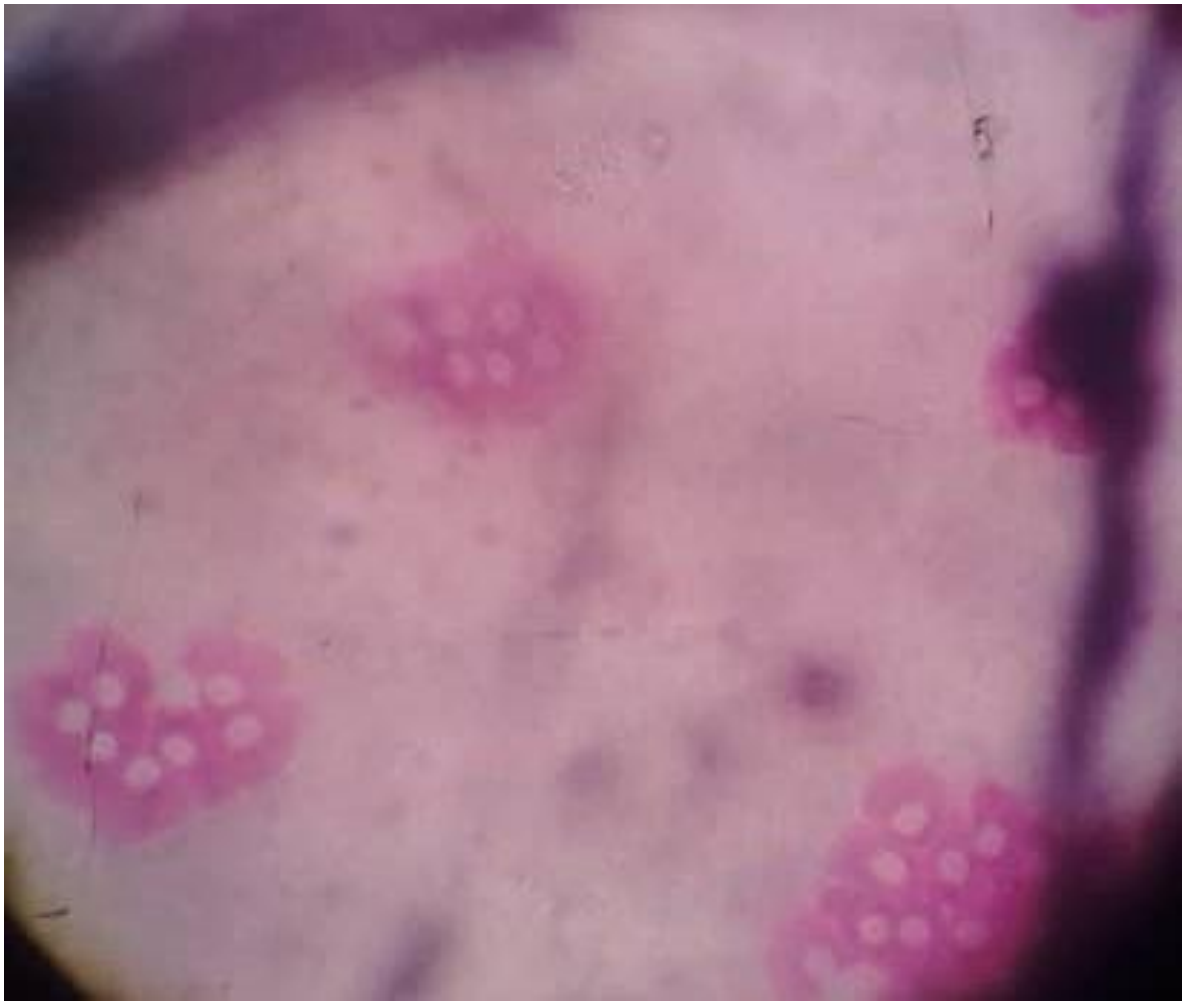

PLATE2: Heptane nucleated

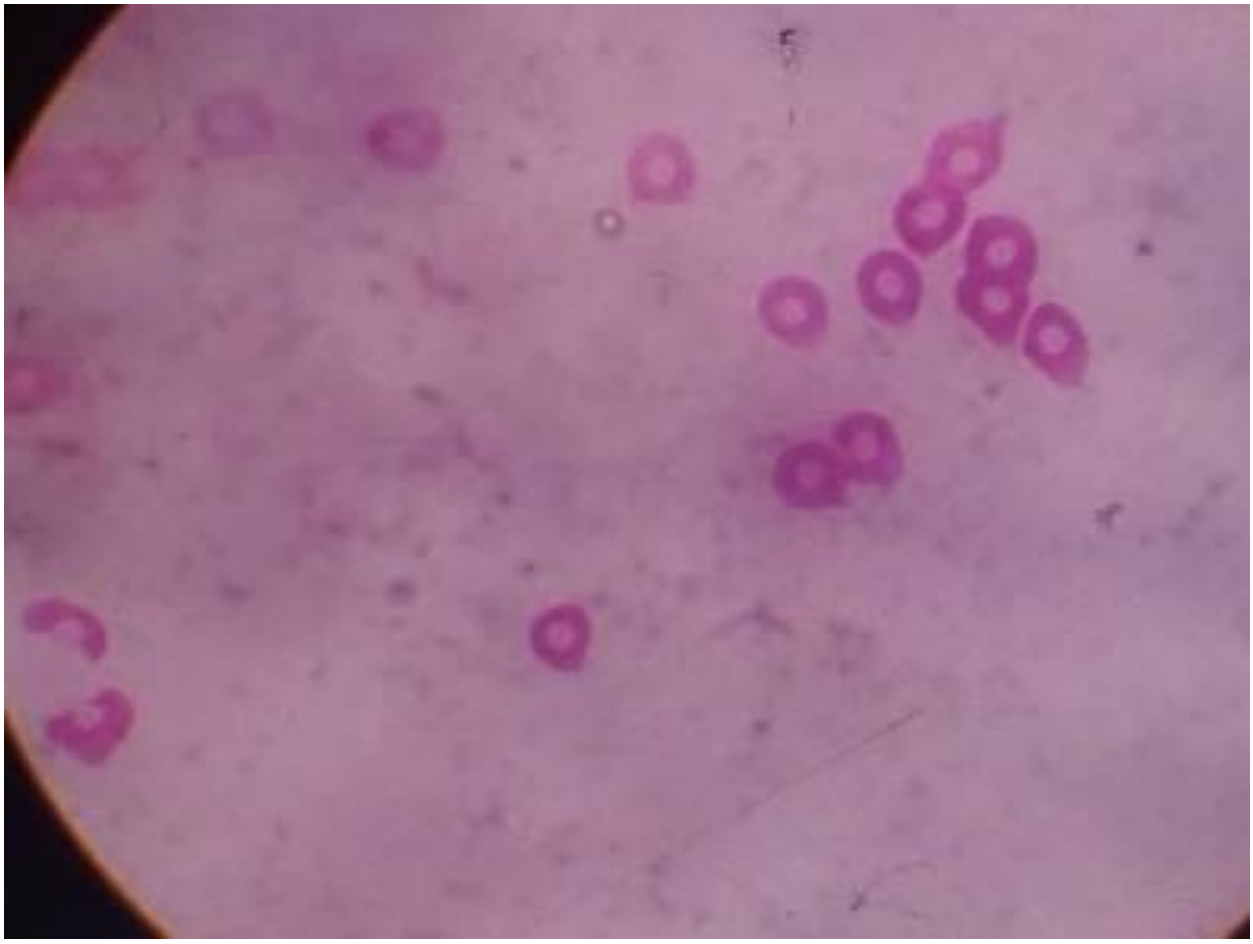

PLATE3: A-NUCLEATED

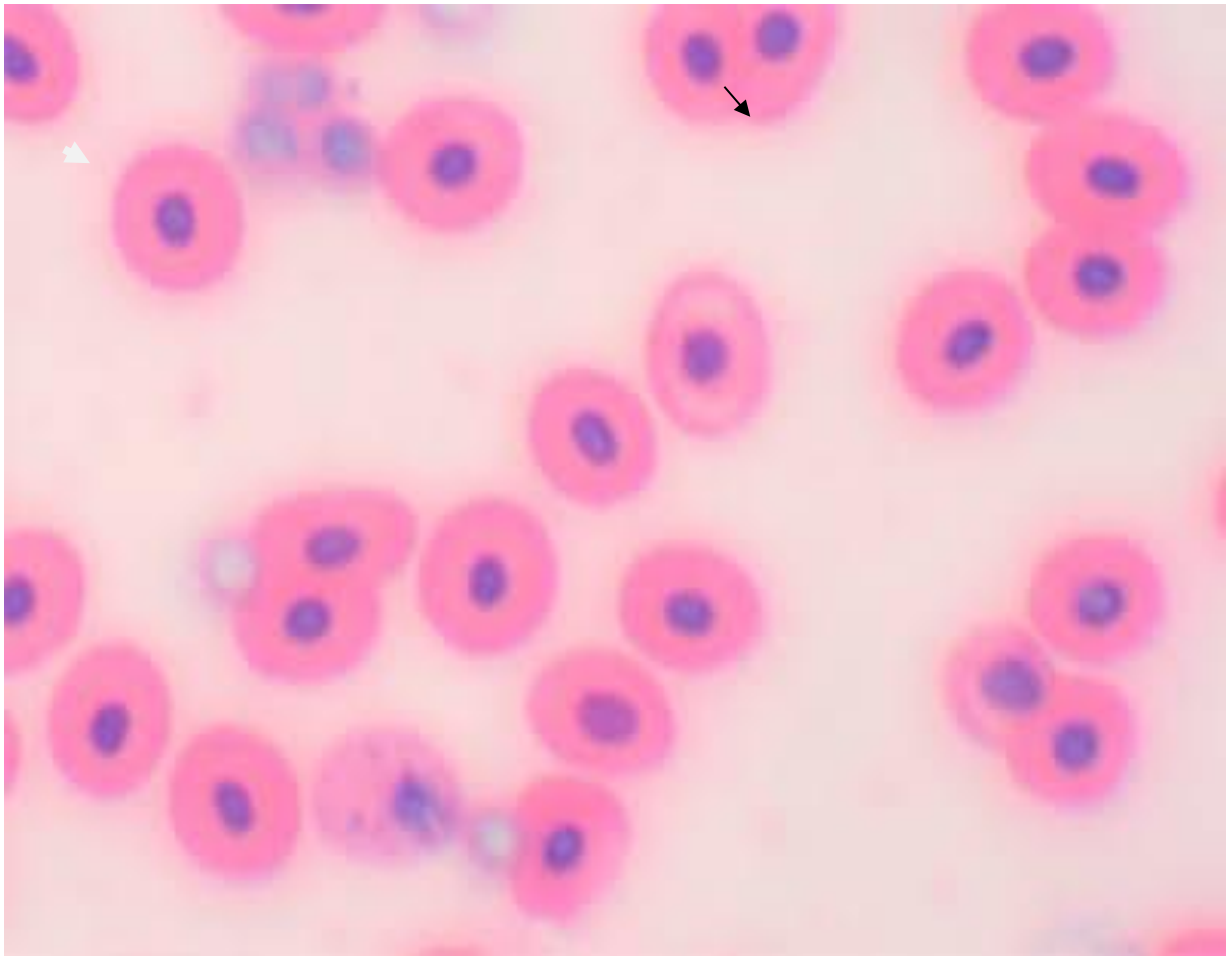

PLATE4:BI-NUCLEATED CELL

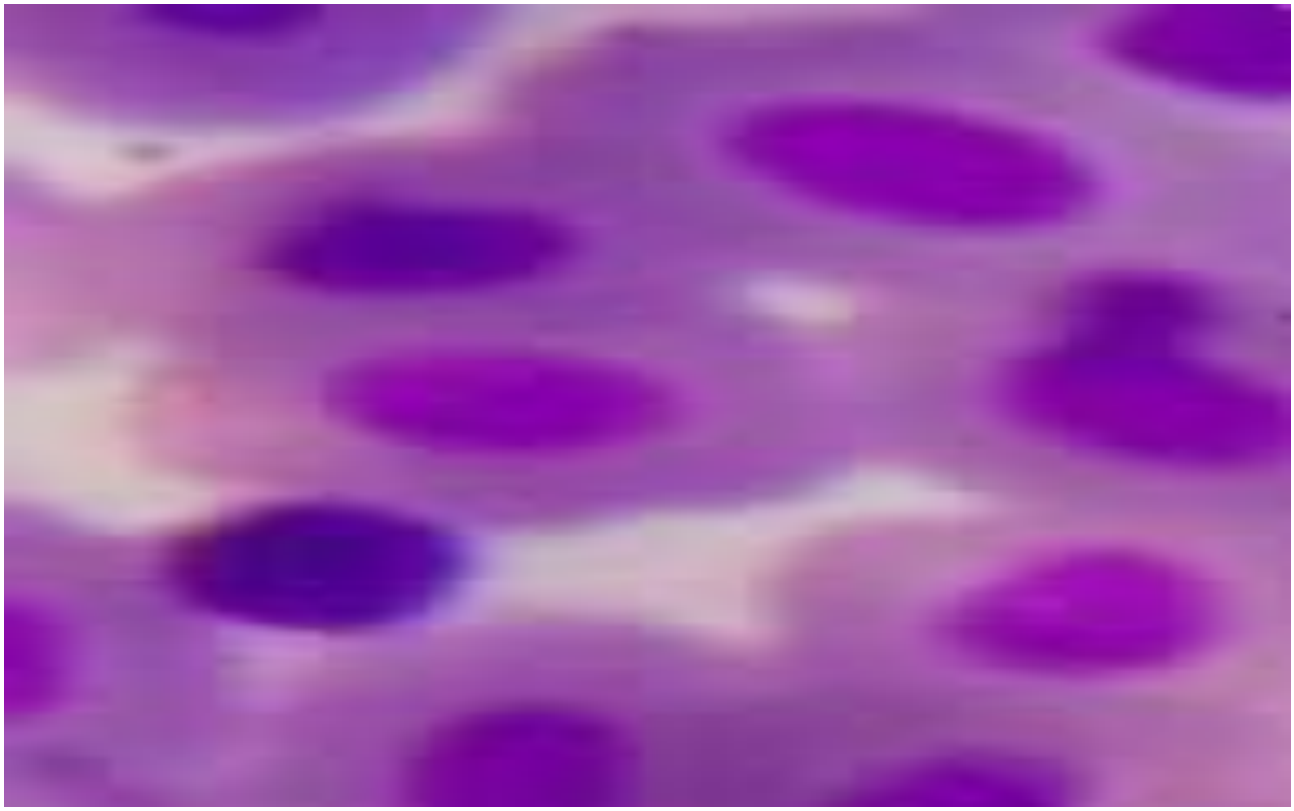

PLATE 5: Micronucleus cell

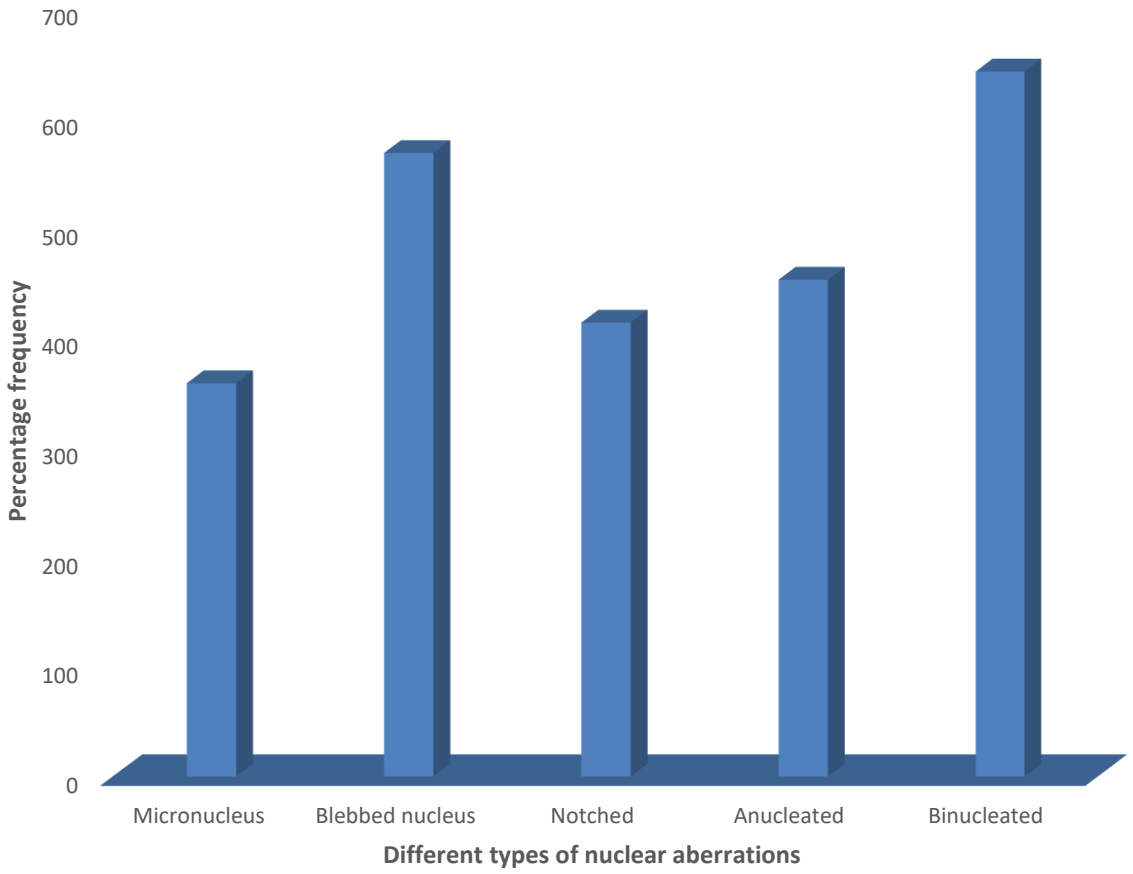

Figure 1: The percentage frequency of the different aberrations induced in *Clarias gariepinus* obtained from Upper Stream of Ikpoba River

Parameter	unit	SAMPLE ID	NESREA[a]	USEPA[b]
Mercury	mg/kg	0.0575	0.2	0.05
Lead	mg/kg	0.691	0.05	0.02
Iron	mg/kg	0.357	—	0.3
Cadmium	mg/kg	0.27	0.2	0.01
Chromium	mg/kg	0.1805	0.05	0.1

Table 4.2: Some physicochemical parameters of *Clarias gariepinus* samples obtained from Upper stream of Ikpoba River

CHAPTER FIVE

5.1 DISCUSSION

5.1 DNA Damage in *Clarias gariepinus*

An important source of information about the genotoxic effects of industrial pollution on aquatic life is the evaluation of DNA damage in *Clarias gariepinus*. A common method for assessing how environmental pollutants affect aquatic animals is the micronucleus test. It is a commonly used assay to identify genotoxicity and damage to DNA (Obiokor, 2018) Gentic abnormalities were considerably more common in the Ikpoba River samples indicating that the pollutants in the river are most likely harming the fish's genetic makeup. The results of this study are greatly impacted by the Ikpoba River's closeness to a brewery, which release untreated effluent into the river. Industrial waste as a genotoxicant source which are frequently linked to genotoxicity in aquatic organisms, are among the industrial effluents that end up in the Ikpoba River. In the experimental samples, the increase in micronuclei (MN) is a definite indication of DNA damage.

Micronuclei are produced during cell division when chromosomes particles are not incorporated into the nucleus. This type of DNA damage has been observed in fish exposed to a wide range of pollutants, such as heavy metals, pesticides, and chemicals used in industry, all of which are common contaminants in lakes and rivers such as the Ikpoba river (Frenzilli, 2009). According to research by Akinbile and Yusoff (2011), fish exposed to agricultural wastewater, including fertilisers and pesticides and the release of waste from factories produced more micronuclei which causes more DNA damage in species that are exposed. One of the main causes of the DNA damage seen in *Clarias gariepinus* is the untreated wastewater that neighbouring factories release into the Ikpoba River.

Other nuclear abnormalities observed in this study, such as anucleated cells (AN) notched cells (NC), and binucleated cells (BN) blebbed cells (BL) also show significant cellular and chromosomal failure. Blebbed cells are typically associated with death and damage to their outer membrane, whereas notch cells exhibit aberrations in chromosomal separation. Anucleated cells are the result of incomplete mitosis These aberrations, which point to several disruptions in the cell phase and mitotic processes, were likely caused by *Clarias gariepinus* being exposed to environmental genotoxins in the Ikpoba River (Ugbome, 2018) found that fish exposed to industrial effluents had higher amounts of micronuclei The fact that these fish have more binucleated cells than micronuclei while observing through the microscope indicates that the following is the main cause of chromosomal damage:

(i) Challenges in Cytokinesis: The last stage of cell division, known as cytokinesis, is when the cell physically divides into two daughter cells. Binucleated cells arise as a result of this mechanism being disturbed.

(ii) Spindle Disruption: During cell division, the spindle apparatus is in charge of chromosomal separation. Aneuploidy (an aberrant number of chromosomes) and binucleated cells can develop from spindle disruptions that cause abnormalities in chromosomal segregation. The control samples, which should be indicative of the initial state of healthy, unexposed fish, had very few nuclear aberrations (0.63% in Control 1 and 0.02% in Control 2). The study which reported that fish in the unexposed control group exhibited barely noticeable chromosomal abnormalities. The statistics indicate an important distinction between the experimental and control groups, with the mean frequency of chromosomal defects in the experimental samples being 243.6±0.55 and in the control samples being 0.55±0.28 According to Okoh (2021), the genotoxic effects of Ikpoba River pollution are the cause of the increase in chromosomal abnormalities. The distinction is statistically significant at

$p \leq 0.001$, suggesting that the harmful effects of Ikpoba River pollution, not coincidence, are responsible for the increase in chromosomal aberrations. The mean frequency of chromosomal aberrations in the samples used for experiments ranged from 4.75% to 7.40%; sample 10 had the highest percentage of irregularities at 7.40%. The differences may be due to the presence of different quantities of pollutants in the water at various sampling locations. Certain parts of the river may be more polluted than others, which could lead to different levels of exposure resulting in different levels of genotoxic impact to the fish (Ogbeibu, & Anagboso, 2020).

Because they are persistent and bioaccumulative, heavy metals like chromium (Cr), lead (Pb), and cadmium (Cd), which are commonly found in manufacturing effluents, are especially dangerous. According to Frenzilli *et al*. (2009), the build-up of heavy metals in fish tissues is closely linked to genotoxic consequences. Long-term exposure to industrial discharges is suggested by the amounts of heavy metals in *Clarias gariepinus* samples taken from the IkpobaRiver.

Heavy metal analysis was conducted using the skin of the Fish as a source for the analysis and it was observed that lead (Pb) was high. The amount of lead found in the fish sample is significantly greater than the allowable limits established by the USEPA and NESREA. This implies that the environment of the river is heavily contaminated with lead.

Bioaccumulation: Through their nutrition and absorption from the water, fish can build up lead in their tissues. Fish with elevated lead levels are a sign of lead contamination in the river. Environmental Contamination Sources: Industrial discharges, agricultural runoff, lead pipe corrosion, and even natural sources like lead-bearing rocks could all contribute to the river's lead contamination. Health Risks: Eating fish high in lead can have major negative effects on

one's health, especially for young children and expectant mothers. Lead can harm the kidneys, impair cognitive development, and harm the nervous system. (Browne *et al*., 2007).

The study's findings demonstrate how urgently environmental laws pertaining to industrial waste management need to be enforced more strictly. Untreated wastewater discharged into the Ikpoba River by factories is a clear breach of environmental safety regulations. Implementing wastewater treatment technology is crucial to reducing the genotoxic effects of industrial pollution on aquatic organisms, according to Akinbile and Yusoff (2011).

CONCLUSION AND RECOMMENDATION

This study emphasises how contaminants have a major genotoxic effect on *Clarias gariepinus*, especially in river systems that are subjected to industrial discharge. The micronucleus assay, which analyses DNA damage, showed that fish living in the upper stream of the Ikpoba River experience high levels of genotoxic stress. The ongoing pollution of the river by untreated wastewater from adjacent factories, which contains heavy metals and other dangerous materials, is the cause of this stress (Livingstone, 2001).

The results highlight how urgently sustainable industrial practices and efficient pollution control are needed to slow the decline of aquatic habitats. Maintaining the general integrity of the aquatic food chain and protecting the health of indicator species like *Clarias gariepinus* depend on this (Frenzilli *et al*., 2009). Additionally, because humans who depend on fish and water from these habitats may be impacted by the bioaccumulation of toxins, the study emphasises the ecological and public health hazards associated with damaged water supplies.

Application of Wastewater Treatment To reduce the amount of genotoxic materials released into the environment, factories that discharge wastewater into the Ikpoba River should install cutting-edge wastewater treatment systems. According to research by Akinbile and Yusoff

(2011), industrial pollution's genotoxic effects can be considerably decreased by treating wastewater effectively.

Regular Water Quality Monitoring

To evaluate pollution levels and their effects on aquatic life, the Ikpoba River must be regularly monitored. Consistent monitoring programs can spot early indicators of environmental degradation and direct mitigation actions, as noted by Obiakor *et al*. (2012).

To guarantee that businesses operating close to bodies of water comply, government agencies should vigorously enforce the current environmental standards. Strong legal frameworks are essential for reducing industrial pollution and safeguarding aquatic environments, according to Browne *et al*. (2007).

To educate the people on the ecological effects of pollution and the need of protecting water resources, community education programs ought to be started. Involving local communities can encourage teamwork in minimising environmental damage.

A more thorough grasp of the health of the ecosystem can be obtained by extending research on the physiological and genetic impacts of contaminants on other aquatic species in the Ikpoba River. According to Livingstone (2001), addressing complex environmental concerns requires multidisciplinary research.

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