EFFECTS OF WASTEWATER EFFLUENT ON BONE GAMMA-CARBOXYLGLUTAMATE GENE IN *Clarias gariepinus*

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APRIL 2024

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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 AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

APRIL 2024

CERTIFICATION

DECLARATION

I, **BENEFIT PHILIP** declare that **"EFFECTS OF WASTEWATER EFFLUENT ON BONE GAMMA-CARBOXYLGLUTAMATE GENE IN CLARIAS GARIEPINUS"** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that work has not been submitted before for any other degree at any other University.

BENEFIT PHILIP

…………….

Date

DEDICATION

This work is dedicated to Almighty God for His many miracles and not failing whenever I needed help the most.

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ABSTRACT

This study was carried out to determine the genotoxic effects of varying treatments of non-alcoholic beverage industrial effluent on the expression of juvenile *Clarias gariepinus* genes. Tissue samples were extracted from juvenile *C. gariepinus* gills followed by RNA isolation, quantification and normalization of the isolated RNA, synthesis of complementary DNA, and agarose gel electrophoresis.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Despite uncontrollable factors like improper solid and liquid waste management by humans that hinders development, especially in developing countries, increasing industrial enterprise is thought to be crucial to a nation's socioeconomic growth. These factors have led to the occurrence of surface and ground water pollution by complex interacting chemicals and substances (Clement et al., 2006; Coors and Frische, 2011; Landrum et al., 2012). As a result, there has been an interruption to the aquatic biota, habitat loss, and depletion (MEA, 2005).

Anthropogenic impacts on the environment are the major cause of changes in the genetic makeup of species living that environment. Because man's survival depends so much on his surroundings, human activities has greatly exploited those resources and changed the natural status of those resources. The environment is being lost as a result of this change. For the purposes of this research, the aquatic or water body will be the main emphasis.

Industrial wastewater contamination on natural water body has become a significant issue in emerging, highly populated nations like Nigeria. The activities of the nearby communities and industrial institutions frequently damage estuaries and inland water bodies, which are the main sources of drinking water in Nigeria (Sangodoyin, 1995).

Chemical leachates, runoff, and industrial effluents from both point and non-point sources make surface and ground water extremely vulnerable to contamination. Groundwater and surface waters are essential sources of drinking water. The agricultural irrigation, brewing, food and beverage, mining, and other industrial uses of surface water sustain the economy. For their water needs, people living in rural areas rely on surface and groundwater. During the production and processing phases, the majority of industrial operations produce wastewater, which poses a threat to the environment because it is typically disposed of in nearby receiving water bodies and surrounding areas. Surface waterways are significant industrial and community sewage systems.

Washing and rinsing water used accounts for about half of the wastewater produced by the beverage and food processing sectors. In addition to other contaminants, the water used for these

washing and rinsing activities may later be comprised of caustics, detergents, soft drink and syrup residue, water from cleaning bottles and cans, and lubricants from their machinery (Chen et al., 2006).

Industrial wastewater parameters vary by industry and even within industries. The quality varies depending on the processes; for example, the quality of wastewater from a cooling tower will differ significantly from effluent from any chemical activity. However, sewage quality varies very little depending on the season, sewage system, and population lifestyle (Shivsharan et al., 2013). Water bodies that collect wastewater are polluted and tainted with unpleasant and potentially dangerous substances (Osho et al., 2010).

Bone gamma carboxyglutamate (Gla) protein (BGLAP), also known as osteocalcin, is a protein found in bones. In osteoblasts (cells that forms bone) the transcription of the BGLAP gene into messenger RNA (mRNA) is the process by which the gene is expressed. Following translation, this mRNA becomes the osteocalcin protein, which is essential for the regulation of bone production and mineralization of bone in *Clarias gariepinus*.

1.2 Statement of the problem

One major negative effect of wastewater effluents on living organisms either aquatic or terrestrial and even humans is damage to genetic materials (DNA and RNA) leading to great changes in their genetic makeup.

1.3 Aims and objectives.

The aim of this research project was to determine the effect of wastewater effluents on the bone gamma-carboxyglutamate gene expression in *Clarias gariepinus*.

The objectives of this study were to:

1. To expose *Clarias gariepinus* to varying concentrations of industrial wastewater effluents from point of discharge.
2. To extract total RNA from the *Clarias gariepinus* exposed to the industrial wastewater effluent.

3. To denature the RNA from the *Clarias gariepinus* exposed to the wastewater effluents.
4. To amplify the genes using corresponding primers.
5. To observe RNA bands.

1.4 Justification

There are many publications on the impacts of toxic substances in wastewater on aquatic organisms. However, the genotoxic effects on fishes are not very understood. The goal of this project research is to ascertain the effect of wastewater effluents on the bone gamma carboxyl glutamate gene expression in Clarias GARIENPINUS. The complementary deoxyribonucleic acid (cDNA), which was taken from the tissue of the test organism, was amplified using the polymeric Chain Reaction (PCR) technology.

CHAPTER TWO

LITERATURE REVIEW

GENETIC MATERIALS

GENE

A gene is a nucleotide-based DNA locus (or area) that serves as the molecular basis for heredity (Slack et al., 2014). Phenotypic inheritance is the result of genes being passed down from one organism to its progeny. The majority of biological traits are governed by interactions between genes and polygenes, or several distinct genes.

Different gene variants, or alleles, can arise from mutations in the gene sequence of the population. These alleles code for the same protein, yet they result in slightly different phenotypes. Every additional instance that is found updates the concept of a gene (Garickle et al., 2006). For example, a gene's coding regions can be split up into multiple exons, and the regulatory and coding sections can be kept apart. Non-coding RNAs make up some gene products, and RNA rather than DNA is used to encode the genomes of certain viruses. Therefore, according to Pearson H et al. (2006), a gene can be defined as any distinct segment of heritable genomic sequence that modifies an organism's properties through either influencing gene expression or being expressed as a functional product.

Deoxyribonucleic Acid (DNA)

Currently, the most common genetic material in the living world is DNA. But this lengthy polymer's prevalence is a result of its properties. In contrast, the structure of double-stranded RNA is comparable. Watson and Crick published their article revealing the structure of DNA. Notable characteristics of the molecule included the complementarity of the base sequences on the two strands and the double-helical form of the polymer.

It has been said that DNA offers considerable advantages over protein analysis as the most appropriate molecule for fish species identification and detection in processed foods. DNA may be found in a wide variety of tissues, is more durable at higher temperatures, and the diversity of its genetic code enables the differentiation of closely related species (Lewis, 2005).

The two strands of DNA are entangled in a right-handed double helix, which is the characteristic that currently distinguishes the molecule.

A "double helix" is found in DNA. The polymer is endowed with important physical and chemical properties by this double helical nature, even if the complementarity per sea does not require it. A basic, straight ladder structure would serve these purposes just as well. The biological function of DNA is largely determined by these characteristics. Therefore, two characteristics work together to provide the genetic activities of DNA:

- A polymer that exists as a double-helical string that facilitates the packing, accessibility, and replication of the information store.
- A tape that contains the information store and encodes the sequences of proteins and RNA molecules.

DNA DAMAGE

As per the United States National Library of Medicine's [Unified Medical Language System (UMLS)], Damage from drugs and radiation that causes DNA to deviate from its typical double-helical structure. These modifications include point mutations that break base pairs and alter the DNA sequence, which can have detrimental impacts on future generations, as well as structural deformities that obstruct transcription and replication.

ETIOLOGY OF DNA DAMAGE

1. **Physical and chemical factors**

   Physical or chemical elements can be used to broadly classify genotoxic substances. Certain chemicals can damage DNA due to their physical and chemical properties. For example, ionizing radiation and ultraviolet (UV) radiation are examples of short wavelength electromagnetic energy that can be harmful to DNA due to their physical properties. Low-energy electromagnetic waves, such as microwaves, radio waves, and infrared radiation (heat), can also harm DNA under specific circumstances (Deshmukh et al., 2013; Harper et al., 2010). Alkylating agents, oxidizing agents, compounds that form DNADNA or DNA protein crosslinks, and others are common chemically manufactured DNA damaging agents (Chakarov et al., 2014).

2. **Mutagens and teratogens**

   "Mutagen" describes a substance that modifies DNA, either directly or indirectly, to the point where the altered sequence or structure of the DNA finally becomes potentially heritable. Because the damage they inflict may lead to neoplastic transformation, mutagens usually have intrinsic carcinogenic properties. According to Chacarov et al. (2014), teratogens are substances that either cause or raise the risk of abnormal foetal development.

3. **Factors from Endogenous and Exogenous origin**

   Potentially harmful substances produced by normal cell metabolism as well as external sources such environmental variables (factors of endogenous origin) might damage DNA. The effects of endogenous factors on cell DNA damage may be more extensive and/or severe than those of most exogenous agents that damage DNA. DNA-damaging events caused by endogenous causes generally occur significantly more frequently than those caused by external sources. For example, thousands of nitrogenous bases are lost from DNA every day in eukaryotic cells due to spontaneous base hydrolysis alone. DNA damage is caused by the same mechanism by both endogenous and external DNA-damaging substances. According to Chakarov et al. (2014), reactive oxygen species are produced by ionizing radiation and normal oxidative phosphorylation, two environmental factors that can damage DNA in cells.

4. **Cytotoxic and genotoxic agents**

   Any agent that damages living cells has the potential to selectively damage the DNA of the cells (genotoxic agents) or to damage the cell as a whole (cytotoxic substances). Predominantly cytotoxic or predominantly genotoxic effects of the same contaminant on cells might occur depending on the type of agent, the type of cells involved, and the duration of the damaging agent's action. When exposed to large doses of ionizing radiation, for example, cells and cell populations may directly perish from the severe oxidative stress caused by the dramatic increase in the number of free radical species within the cell. Even at reduced dosages, ionizing radiation can cause significant DNA damage, albeit not all damaged cells will necessarily perish right away or as a result

delayed effect. This is referred to as genotoxicity. Lastly, cytotoxic effects can be induced by genotoxic action. For example, most chemotherapeutic anticancer drugs work by seriously damaging DNA in the hopes that this could divert cancer cells to the pathway leading to programmed cell death (Chacarov et al., 2014).

5. **Factors causing alteration in information content and structural content of DNA.**
   Any damage to DNA always modifies its informative content. A mutation is only permanently altered DNA sequence that results from specific detrimental events that aren't corrected. A DNA mismatch-causing events are just one type of them. A specialized mismatch repair system can spot and correct mismatched bases fast, but if the DNA template containing the mismatches is replicated in spite of damage, the resulting daughter strands' DNA will have a unique "meaning" (Chakarov et al., 2014). Because cells are unable to discriminate between daughter molecules that differ and those that are identical to the "maternal" molecule, the modified cell will faithfully duplicate the modified sequence during subsequent cell divisions. Generally, "nominal" transcription factors involved to replication would stop working as soon as they encountered template damage. Activating the repair machinery at the blockage area would therefore cause the damage to be repaired without producing any long-term modifications to the DNA sequence. Occasionally, methods used to treat other types of DNA damage—like some of the mechanisms leading to nucleotide reconfigurations—allow for the nucleotide or nucleotides that have been reconfigured. Stated differently, the altered nucleotide or nucleotides are not completely ignored during replication; rather, the fragment containing them is reproduced in a way that preserves the original sequence despite the template's damage. Chakarov et al. (2014) have suggested that DNA damage may also affect higher order structures, apart from nucleotide sequences.

DETECTION STRATEGIES FOR DNA DAMAGE

Techniques for detecting DNA damage as described by Kumari et al. (2008):

1. **Halo assay:** Vinograd et al. (1965) first outlined this approach, then Roti Roti and Wright (1987) refined it. During this experiment, a fluorescent dye called propidium iodide (PI) intercalates into the DNA helix, altering the supercoiling state of the DNA. Consequently, DNA appears as a fluorescent halo, the size of which varies with the amount of PI present. Lower PI concentrations (0–7.5 g/ml) relaxed the supercoils, while higher PI concentrations (7.550 g/ml) rewound the supercoils in the sense of reverse winding. This test can assess single cells and does not require the tagging of radioactive DNA (Roti Roti and Wright, 1987). But it is limited in its sensitivity. Through cell lysing, this technique makes individual nucleoids visible as "halos," which may be assessed by an image analysis system to ascertain the degree of chromatin fragility (Woudstra et al.,1998). (Malyapa et al.,1995). It was used to detect changes to DNA organization in certain cells. According to Roti Roti and Wright (1987), this assay can detect changes in DNA organization at radiation doses of up to 2 Gy, but only if the damage is not healed. When the injury heals below 10 Gy, the assay becomes less sensitive. (Wright and Roti, 1987; Jaberaboansari et al., 1988).
2. **Fluorescence in situ hybridization (FISH):** According to Murthy and Demetrick (2006), it is a non-isotopic labelling and detection technique that determines the amount or closeness of cellular DNA material that has been disrupted in nuclei or chromosomes. Because rare chromosomal abnormalities in normal urothelium prevent 100% specificity from ever being achieved, FISH is more sensitive than flow cytometry at identifying aberrant urine cells and requires less material (Sauter et al., 1997). DNA damage can be observed and estimated cell by cell using this method (Hopman et al., 1991; Kallioniemi et al., 1992). According to Sauter et al. (1997), this method is effective in locating chromosomes with numerical anomalies.
3. **Comet assay:** In 1990, Olive et al. used the term "Comet assay" for the first time. (gel electrophoresis on a single cell). As the name suggests, it is the identification of DNA damage in individual cells and the approximation of its distribution within the cell population. This method is mostly used to identify single- and double-strand breaks, oxidative DNA damage, and single-strand breaks linked to incomplete

excision repair sites brought on, among other things, by electromagnetic frequency, UV, and ultrasonic radiation.

4. **Polymerase Chain Reaction (PCR):** It is an in vitro genetic approach that enables the exponential enzymatic synthesis of a specified area of DNA in huge quantities. DNA polymerase—enzymes that cells utilize to copy their DNA—helps create DNA in the same way that it is seen in vivo, or in cells (Mullis, 1990).
5. **Immunological assay:** Although it is one of the most used methods for identifying oxidative DNA damage, it has limitations since antibodies may react with DNA bases that are not damaged. This method was used to discover thymine glycols (Leadon and Hanowalt, 1983). Secondary antibodies that are fluorescent, radiochemical, or enzyme conjugated (CPDs) can be used to quantify UV photoproducts. This experiment allows for the very efficient detection and measurement of DNA damage using an immunoblot-blot system with chemiluminescent detection, secondary antibodies conjugated to alkaline phosphatase enzymes, and secondary antibodies conjugated to radioactive iodine (Kriste et al., 1996; Wani et al., 1987; Plaza et al., 1991).
6. **Radio immunoassay (RIA):** Initially, DNA antibodies that had been exposed to carcinogens and anti-carcinogen adducts were used in RIA. Equal volumes of radiolabelled (inhibitor) and non-radiolabelled (tracer) antigen are combined with increasing inhibitor concentrations in a set volume to construct standard curves (Poirier et al., 1977; Umbenhauer et al., 1985). (1999; Santaella). It is capable of identifying 6-4 photoproducts and cyclobutene dimers in DNA (Mitchell et al., 1985). According to Jeffrey et al. (1996), Miller et al. (1999), and Mitchell (1996), radioimmunoassay was found to be very effective at detecting extremely low amounts of cyclobutane pyrimidine dimer (CPD), which is formed by UV-B radiation and found in marine viruses and bacterioplankton.
7. **Enzyme-linked immunosorbent assay (ELISA):** It is possible to stop the plate from binding to antigens (modified DNA) by soaking the wells in a diluted protein solution. Following a similar mixing procedure with the antibody, unknown samples are subsequently put to the plate. After incubation and the removal of non-bound material, enzyme-conjugated secondary antibodies are employed to assess bound primary

antibodies (Santella, 1999). Specifically designed RIAs were used to track anti-body binding sites associated with 6-4 photoproducts and cyclobutene dimers. This technique makes it possible to anticipate the biological function of sixty-four photoproducts (Mitchell and Rosenstein, 1987).

8. **Gas chromatography-mass spectrometry (GC-MS):** When γ-rays contact with DNA, they hydrolyse water by producing reactive oxygen species that can react with DNA to cause a range of DNA damage, including strand breaks, changed bases, a basic site, and DNA-protein crosslinks. Ward (1988). (Von Sonntag, 1987; Dizdaroglu, 1985). The diagnosis of oxidative DNA damage is commonly performed using GCMS because of its ability to distinguish between a wide range of DNA base products. This method's derivatization process converts polar bases into mass-spectra bearing derivatives that are thermally stable. However, this method may overstate the amount of oxidative damage because of the derivatization of hydrolysed DNA at higher temperatures in the presence of air, which leads to a greater amount of 8OH guanine, 8-OH adenine, and 5-OH cytosine (Jenner et al.,1998).
9. **Immunohistochemical assay:** This technique is used to remove proteins and RNA that can have a harmful interaction with DNA from fixed cells (lymphocytes, bladder cells, etc.) after they have been treated with RNase and protease. Propidium iodide counterstaining is used in immunofluorescence to reveal the nuclei of adduct-negative cells. It works with very small amounts of materials and can identify adsorbed molecules in specific tissue cell types (Santella, 1999). Without the need for DNA extraction and hydrolysis, this approach can be used to localize epitopes and select specific regions. It can be used in vitro and in vivo for research on cancer, neurodegenerative diseases, oxidative stress-related pathologic disorders like aging, ischemia reperfusion injury, and other issues. (Toyokuni and associates, 1997). HER 2/neu (c-erbB-2) mutations in breast cancer patients have recently been found using this technique in conjunction with FISH (Sidoni et al., 2006).
10. **Terminal deoxyribonucleotidyltransferasemediated deoxy uridine triphosphate nick end Labelling (TUNEL assay):** Since the TUNEL assay detects DNA fragmentation by fluoresceinating the free ends of the DNA, a fluorescence microscope can be utilized to detect apoptosis (Bruggeman et al., 1997). It can also

detect fractures in both single and double strands (McGahon et al., 1994; Migheli et al., 1995). When DNA strands break and fragment, the 3'-OH termini are released. Digoxigenin and a modified nucleotide called dUTP are then added as enzyme labels to the freed termini, which are then identified by an anti-digoxigenin antibody (Pulkkanen et al., 2000). However, its specificity and sensitivity are limited. Because of DNA condensation and the protein milieu surrounding DNA, this experiment might not go as planned in the apoptotic cell (Gold et al., 1994). It has a high propensity for fixations (Lucassen et al., 1995). Chen et al. (1997) employed the TUNEL assay to locate DNA strand breaks in the rat brain after a brief period of focal ischemia and reperfusion. Pietruszewska et al. (2005) used the TUNEL assay to find a significant correlation between the apoptotic index, the degree of polymorphism in neoplastic cells, and tumour progression. The TUNEL assay was used to analyse apoptosis in the brainstems (hypoxic ischemic injury), and the variability in the apoptotic index between the nuclei supports the idea that the brainstem nuclei are vulnerable in different ways (Stecco et al., 2005).

Ribonucleic Acid (RNA)

In cells, RNA is found in three primary forms: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). These different kinds of RNA are found in both prokaryotic and eukaryotic cells. Ribosomal RNA molecules comprise 75% of the total cellular RNA. The 28S (or 25S), 18S, and 5S in eukaryotic ribosomes and the 23S, 16S, and 5S in bacterial ribosomes are the three unique molecules that they are present in ribosomes as, based on their sizes. Transfer RNA is the smaller molecule that supplies amino acids to ribosomes so that they can make proteins. Its approximate molecular weight is 25,000. Merely 1 to 5% of all cellular RNA is composed of messenger RNA, an RNA that encodes proteins. In various ways, the correct translation of genetic information (in the form of DNA) into protein depends on all three forms of RNA. In eukaryotic cells, heterogeneous nuclear (or pre-messenger) RNA and small nuclear RNA are two additional RNA types that are involved in this mechanism. Isolating intact RNA and purifying the mRNA fraction are necessary for the conversion of mRNA to complementary DNA (cDNA) (Krieg, 1996).

MOLECULAR BIOLOGY TECHNIQUES RELEVANT TO THIS STUDY

Polymerase Chain Reaction

It was made by Kary Mullis in 1983 (Saiki et al., 1985). The polymerase chain reaction (PCR), a molecular biology technique, amplifies a single copy or a small number of copies of a piece of DNA to make hundreds to millions of copies of a specific DNA sequence. This simple and reasonably priced technique amplifies a specific DNA segment, which is essential for several applications such as investigating the function of the targeted segment, detecting, and tracking genetic abnormalities, and identifying criminals (in forensics) (Bartlett et al., 2003). One of the first DNA sequences to be amplified by PCR was the human β-globin gene. (Mullis et al., 1986; Mullis and Faloona 1987; Saiki et al., 1985). The identification of genetic fingerprints—which are used in forensic sciences and DNA paternity testing—the detection and diagnosis of infectious diseases, DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes are just a few of the many uses for which PCR is now a commonly used and frequently indispensable technique in medical and biological research labs. In 1993, Mullis and Michael Smith were granted the Nobel Prize in Chemistry for their research on PCR. In order to induce enzymatic DNA replication and DNA melting, a process called thermal cycling entails periodically heating and cooling the reaction.

Types of Polymeric chain reaction techniques

In order to achieve the amplification of other research-relevant molecules, such as RNA, and to improve performance and specificity, the basic PCR process has undergone revisions or versions recently. Among these variations are the following:

- Semiquantitative PCR determines the relative number of nucleic acids present in a sample (Ehtisham, 2016).
- Nested PCR improves the specificity of the amplified product for a second PCR with new primers that hybridize within the first PCR’s amplified fragment (Ehtisham, 2016).
- Real-time PCR quantifies nucleic acid copies obtained by PCR in absolute or relative terms (Ehtisham, 2016).
- RT-PCR produces RNA amplification by synthesis of cDNA (DNA complementary to RNA), which is then amplified by PCR (Ehtisham, 2016).

- Multiplex PCR amplified several DNA sequences at the same time (usually exonic sequences) (Ehtisham 2016)

Stages in PCR Amplification

- **Denaturation:** The Taq polymerase can only tolerate a maximum temperature of 94–95°C for 30 cycles or longer before denaturation takes place in PCRs. During the first cycle, denaturation is carried out for five minutes in order to guarantee that the lengthy template DNA molecules are completely denaturated. Such extended denaturation, nevertheless, occasionally has drawbacks. Denaturation at 9495°C for 45 seconds is advised for routine amplification of linear DNA templates with G+C contents of 55% or less. Raising the temperature may be necessary for denaturation of templates with higher G+C content. Additionally, it takes longer for denaturation to occur the longer the DNA templates. If the denaturation temperature is too low or the duration is too short, only the A-T rich sections of the template DNA will be denatured. This type of DNA will re-anneal later in the PCR cycle when the denaturation temperature is lowered. (Ukwubile, 2013).
- **Annealing:** A suitable temperature that is dependent on the estimated melting temperature (Tm) of the primers is utilized (5°C below the Tm of the primer) since complementary sequences have a chance to hybridize during annealing. The length and sequence of the primer determine the annealing temperature, which can range from 55 to 65 degrees Celsius. The oligonucleotide primers anneal poorly, and the amplified DNA is too low if the annealing temperature is set too high. Unwanted DNA segments may be amplified if the annealing temperature is too low because primers may not anneal precisely. To find the ideal annealing temperature, do a series of trial PCRs at room temperatures between 2 and 100°C below the melting points of oligonucleotide primers. In a standard PCR, a range of annealing temperatures can also be employed (Ukwubile., 2013).
- **Extension/Elongation:** At 70 to 72°C, DNA polymerase activity is at its peak, and primer extension can happen at up to 100 bases per second. The extension of oligonucleotide primers is catalysed by DNA polymerase, which creates a new strand with complementary sequences to the template strand. The ideal temperature for DNA

synthesis can change slightly depending on the DNA polymerase that is being utilized. The optimal temperature range for Taq polymerase-mediated DNA synthesis is 72–78 degrees Celsius. Taq polymerase can insert roughly 2000 nucleotides per minute at this temperature (Ukwubile, 2013).

Selective DNA isolation

Through the process of selective amplification of a specific DNA segment, PCR facilitates the separation of genomic DNA from fragments of DNA. A number of methods, such as DNA cloning and the development of hybridization probes for Northern or Southern hybridization, which require greater quantities of DNA representing specific DNA regions, are improved by this use of PCR. Since PCR yields vast quantities of pure DNA, these methods can analyse DNA samples even from extremely small amounts of starting material. A DNA sequence can also be isolated to expedite recombinant DNA technologies, which entail inserting a DNA sequence into a plasmid, phage, cosmic, or other size-dependent organism's genetic code. These applications of PCR include DNA sequencing to identify unidentified PCR-amplified sequences in which one of the amplification primers may be used in Sanger sequencing. PCR is a fast method for checking bacterial colonies (such E. Coli) for accurate DNA vector constructions (Pavlov AR et al., 2006). PCR is also employed in genetic fingerprinting, a forensic method that compares experimental DNA samples using a variety of PCR-based techniques to identify a person or an entity. Certain polymerase chain reaction (PCR) "fingerprints" methods, utilized in paternity testing, provide significant discriminatory ability, and can be applied to ascertain genetic connections between individuals, including parent-child or sibling relationships. This approach can also be used to ascertain the evolutionary relationships of other animals (e.g., the microbes' recA and 16S rRNA genes) when particular molecular clocks are employed (Pavlov AR et al.,2006).

Electrophoresis

Most PCR methods involve thermal cycling, which involves gradually raising and lowering the PCR sample's temperature within a preset range. High temperatures are involved in the first stage, known as DNA melting, which physically splits the DNA double helix into two strands. The target DNA only is amplified in the second phase, which involves lowering the temperature and using the two DNA strands as templates for DNA polymerase. Using primers corresponding to the area of DNA that will be amplified under particular heat cycling conditions allows PCR to

be highly selective. Proteins, RNA, and DNA can all be separated using gel electrophoresis. DNA fragments are sorted according to size. Proteins can be divided into groups based on size and charge (different proteins have different charges). Gel electrophoresis has a wide range of applications, such as (Ukwubile, 2013):

- To see if a PCR reaction is working.
- For forensic reasons, to get a DNA fingerprint.
- To look for genes linked to a certain illness.
- To get a DNA fingerprint to investigate the evolutionary connection between organisms.
- To get a DNA fingerprint to do paternity testing.

Electrophoresis has been successfully applied in numerous investigations to detect genetic variation in Clarias gariepinus (Majolagbe, 2012; Popoola et al., 2014).

DNA AMPLIFICATION AND QUANTITATION

Because PCR amplifies the targeted regions of DNA, it can evaluate minuscule amounts of sample. This is usually important for forensic analysis when there is only a small amount of DNA available as evidence. Tens of thousands of years old DNA can also be examined using PCR. The applications of these PCR-based techniques range from Egyptian mummies to the identification of a Russian tsar and the body of English ruler Richard III. They have also been employed on animals, including a forty-thousand-year-old mammoth, as well as human DNA. Quantitative PCR is a widely used technique to measure the levels of gene expression by estimating the amount of a certain sequence contained in a sample. A tried-and-true technique for determining the quantity of DNA product generated is quantitative PCR (Ukwubile, 2013).

THE AFRICAN CATFISH

Many countries in Europe, Asia, and Africa are home to a fish known as Clarias gariepinus (Saad et al., 2009). *Clarias gariepinus* is Africa's second-most significant freshwater fish, after tilapia. With the exception of Nigeria, where the productivity of Clarias gariepinus—which accounts for 70–80% of freshwater fish production—outperforms that of tilapia. These regions have a particular fondness for the fish due to its succulent flesh, unparalleled resilience, swift development, and premium cost. The Clarias species is a native fish to Nigeria and can be found in freshwater environments all around the country. The fish species thought to have dispersed the

greatest over Africa is Clarias gariepinus (Skelton, 2001). North African catfish can be found in a variety of wet environments, including lakes, ponds, and pools. They are common in fast-moving rivers, rapids, and dams. They can endure in a pH range of 6.5 to 8.0 and are highly tolerant of hard conditions. They can withstand temperatures ranging from 8 to 35 degrees Celsius as well as very muddy circumstances. Temperatures between 28 and 30 degrees Celsius are ideal for their growth. (Teugels, 1986).

Their primary habitat is at the base of the food chain, which makes them bottom feeders. They need to breathe; therefore, they have to stay on the surface for a while. According to Pienaar (1968), this species is among the few that can live in oceans with low oxygen levels. In order to stay wet in a drying body of water, they might make mucus and excavate burrows in the muddy ground (Skelton, 1994). Tropical, freshwater, and other pelagic, benthic, lakes and ponds, rivers and streams, temporary pools, and brackish water are some of their habitats. Other aquatic biomes include benthic, lakes and ponds, rivers and streams, and temporary pools.

Taxonomic tree of African Catfish

Domain: Eukaryota

- Kingdom: Animalia
- Phylum: Vertebrate
- Subphylum: Vertebrate
- Infraphylum: Gnathostomata
- Class: Teleostei
- Order: Siluriformes
- Family: Clariidae
- Genus: Clarias
- Species: Clarias gariepinus, Burchell 1822

(Eschemeyer et al., 1998)

Physical description of African Catfish.

The cylindrical body flattened bony head, small eyes, prolonged spineless dorsal fin, four pairs of barbels around a large mouth, and scaleless skin all serve to distinguish *Clarias gariepinus*. In juvenile fish, the top of the skull is smooth, but in adult fish, it is coarsely granulated (Van Oijen, 1995). There are distinct structures for the anal, caudal, and dorsal fins. A prominent sexual papilla situated directly outside the anal hole helps identify males from females. Female fish are devoid of this sexual papilla. According to Van Oijen (1995), the underside of the body is creamy-white, and the body is greyish black. The ventral portion of the head has a noticeable black longitudinal stripe on either side. Young fish less than 9 cm (about the length of a credit card) lack this stripe. Fish bigger than 9 cm have a speckled grey-khaki coloration. It is established that the amount of light and substrate in culture systems affect the skin's colour.

Ecosystem roles of African Catfish

African catfish are necessary in some habitats. As farm fish, they are now available in Thailand. The walking catfish, a natural species found in the marshes and swamps where they are bred, is in risk of becoming extinct. The massive population expansion of North African catfish is the cause of this. It is also because of the hybrid that results from the walking catfish's backcrossing. As a result, the genetic diversity of the native walking catfish is likewise decreasing (NaNakorn et al2004). Regarding rice fields, this plant is crucial for recycling nutrients. According to d'Oultremont and Gutierrez (2002), rice yields are increased by the movement of nutrients from the pond to the rice via fish excrement.

TOXICITY TESTING

The term "toxicity" describes a material's characteristic or characteristics that are harmful to a living organism. A chemical is called a toxicant if it has this biological effect. Assessing a chemical's toxicity entails determining if it has the capacity to function poisonously, determining the conditions under which this potential will manifest, and characterizing the behaviour of the substance in issue.

The goal of a toxicity test Is to determine the quantities of a test material that, in a population exposed to controlled conditions, might cause a particular response, which is typically not favourable. Even though eliminating risks to humans is the primary goal of most toxicity research, most toxicity testing is done on experimental animals. The current quantitative

structure-activity relationships (QSAR) data do not allow for precise extrapolation to novel medications, which makes this toxicity assessment vital. Understanding side effects such irritation, nausea, allergies, scent perception, and other higher nervous system functions is vital, even though collecting experimental human data is difficult due to ethical concerns (Hodgson et al., 2010).

There are two categories of materials that are added to the surroundings. Sewer releases, streams of industrial waste, locations for disposing of hazardous waste, and unintended spills are all examples of point discharges. It is quite simple to characterize point discharges in terms of the components released, the rates of release, and the total volumes. Agricultural runoff, air deposition, contaminated soils and aquatic sediments, and urban runoff are examples of sources of nonpoint discharges, on the other hand. Classification of a nonpoint discharge is much harder. Non-point source releases usually comprise intricate blends with challenging-to-characterize quantities of toxicants, and their frequency and timing are as erratic as the weather.

Test Organisms

Two of the most crucial aspects of a toxicity test are the appropriateness and health of the test organisms, or, in the case of multispecies toxicity studies, the imported community. It's critical to understand the goals of the toxicity test. If maintaining a certain economic resource—like a salmon fishery—is crucial, it may be necessary to use a salmonid and its food sources as test species. To thoroughly comprehend the extent of a chemical's harm to various species, toxicity tests are employed. This is the reason why species used in labs are only thought to be representative of particular classes or, frequently, entire phyla. The following factors should be taken into account when choosing a test species for toxicity testing:

1. The organism must be readily available and able to flourish in a lab environment. Due to inadequate knowledge of their food needs, overcrowding, and stress brought on by the mere presence of laboratory personnel, many species do not thrive in the laboratory.
2. It should be simple to get the test organism by field collection, hatchery or other facility procurement, or laboratory culture. Field gathering is frequently necessary since marine organisms are frequently challenging to adequately nurture in a lab.
3. It is important to understand the genetics, genetic makeup, and history of the culture.

4. The test species' relative susceptibility to different kinds of toxicants should be known in relation to the endpoints that will be examined.
5. The sensitivity of the test species must match that of the phylum or class to which it belongs (Landis et al., 2003).

Acute toxicity

The adverse effects that manifest soon after receiving a single dosage of a test chemical are assessed by acute toxicity testing protocols. Acute toxicity data can be utilized to establish dose levels in repeated dose studies, as well as to help determine the mode of harmful action of a chemical. It can also provide information on dosages associated with target organ toxicity and mortality. The diagnosis and treatment of unfavourable human reactions may benefit from a wider application of this knowledge. The selection of new study subjects can be aided by the use of data from acute toxicity tests to evaluate the toxicity and dosage response of members of various chemical classes. Numerous regulatory applications can be implemented using the outcomes of acute toxicity studies. Among these are the requirements for childproof packaging, the necessity of re-entry intervals after pesticide application, worker training in chemical use, the foundation for that training, the necessity of protective gear and clothing, and the question of whether pesticides should be registered or limited to certified applicators (Hodgson et al., 2010). According to Reish and Oshida (1987), the most popular test is the 96-hour test. During these periods, the effects of the test material often manifest quickly and are plainly discernible. Certain test materials will not reach their "threshold" (the point at which there is no discernible increase in mortality or impact) in 96 hours.

Classification of toxicity assessment

The vast array of organisms and ecosystems that environmental toxicology has studied has led to a significant number of toxicity trials. But the tests can be categorized based on the intricacy of the biological community and the duration of the trials in respect to the organism's life cycle. For a brief period of time throughout an organism's existence, acute toxicity testing is conducted. We've given time spans of 24 to 48 hours (about 2 days) to rats, birds, fish, daphnia, and other species. The quick-living Daphnia magna can undergo its initial molt in as little as 48 hours. Animals with extended lifespans receive even less time for these toxicity trials. Contrary to popular belief, acute toxicity testing does not often involve experiments conducted over similar

time periods using bacteria, protists, or algae. Just ideal conditions, a multitude of germs may proliferate in just an hour. Throughout a day or two, binary fission can happen in most protists and algae. One generation of an algal cell can be compared to a 24-hour period. Growth toxicity testing or chronic toxicity testing are more appropriate for tests involving unicellular organisms. According to Landis et al. (2003), studies of chronic and sublethal toxicity often span a sizable amount of an organism's lifecycle.

Exposure Scenarios

In aquatic test systems, for instance, exposure to a diluted stock effluent solution usually entails exposure to the entire body of the test organism. Through the skin, cell walls, respiratory system (gills, stomata), and ingestion, the toxicant (effluent) can enter the body. Toxicants are occasionally injected into aquatic species as part of toxicity studies to see what effects they have, though this does not happen very often. Interacting with animals on land results in fewer whole-body exposures. Normally, a xenobiotic dose is given by intramuscular, intraperitoneal, or vein injection based on the weight of the toxicant per unit weight of the animal (intravenous). In other toxicity experiments, known as gavages, a fixed amount of material is injected into the stomach through a tube, allowing the precise control of the amount of chemical that enters the organism. To ensure adequate distribution of the toxin, feeding tests are conducted using a specific toxin concentration combined with either food or water. The test organisms quit feeding right away because so many of the substances make them uncomfortable. Exposure to air pollutants can also occur through inhalation. After the first air exposure, dermal exposure may occur often. An alternative method to guarantee inhalation exposure is to use an airtight or watertight seal that restricts exposure to the breathing apparatus. Rats' paws and coats can be kept clean by using nose-only exposures. Pollutants from polluted soils or air deposition are more easily absorbed when exposed to the skin. Toxicological testing may take use of additional exposure pathways for species that live in plants, soil, and sediment. Usually, soil or atmospheric deposition occurs to plants. Soil invertebrates are often placed in standardized soil that has been infused with a particular concentration of the test medication.

Sediment testing often uses contaminated sediments or substances added to a normal sediment (Landis et al., 2003).

Toxicity testing with aquatic organisms.

Test subjects for acute testing are exposed to test solutions containing the compounds at varying concentrations; alternatively, aquatic species can be utilized to evaluate the toxicity of substances by injecting or feeding the substances to the test subjects. To assess the experiment's acceptability, one or more contemporaneous controls are carried out, in which the organisms are kept under similar circumstances but are not exposed to the toxicant. The trial settings, handling protocols, and dilution water appropriateness are all demonstrated by the control trials. The 96-hour test is commonly used as a threshold for a change, while it can also be extended to examine other physiological, biochemical, or behavioural changes. The term "lethal concentration," or "LC50," refers to the concentration at which a 50% live death response occurs.

CHAPTER THREE

MATERIALS AND METHODS

Study Area Description

The 7up bottling company (PLC) at Iguosa, Utekun village, Benin, Edo state, Nigeria, was the site of the research. Situated near Oluku Junction in Benin City, Edo State, on the Benin-Lagos highway, the company is situated in the South-South area of Nigeria. The facility is located quite a distance from the Ise-uvbi river, which is the location of the bottling industry's discharge. Situated within the community that was previously mentioned. The river is surrounded by lush grasses and towering trees. It is also characterized by a marshy landscape.

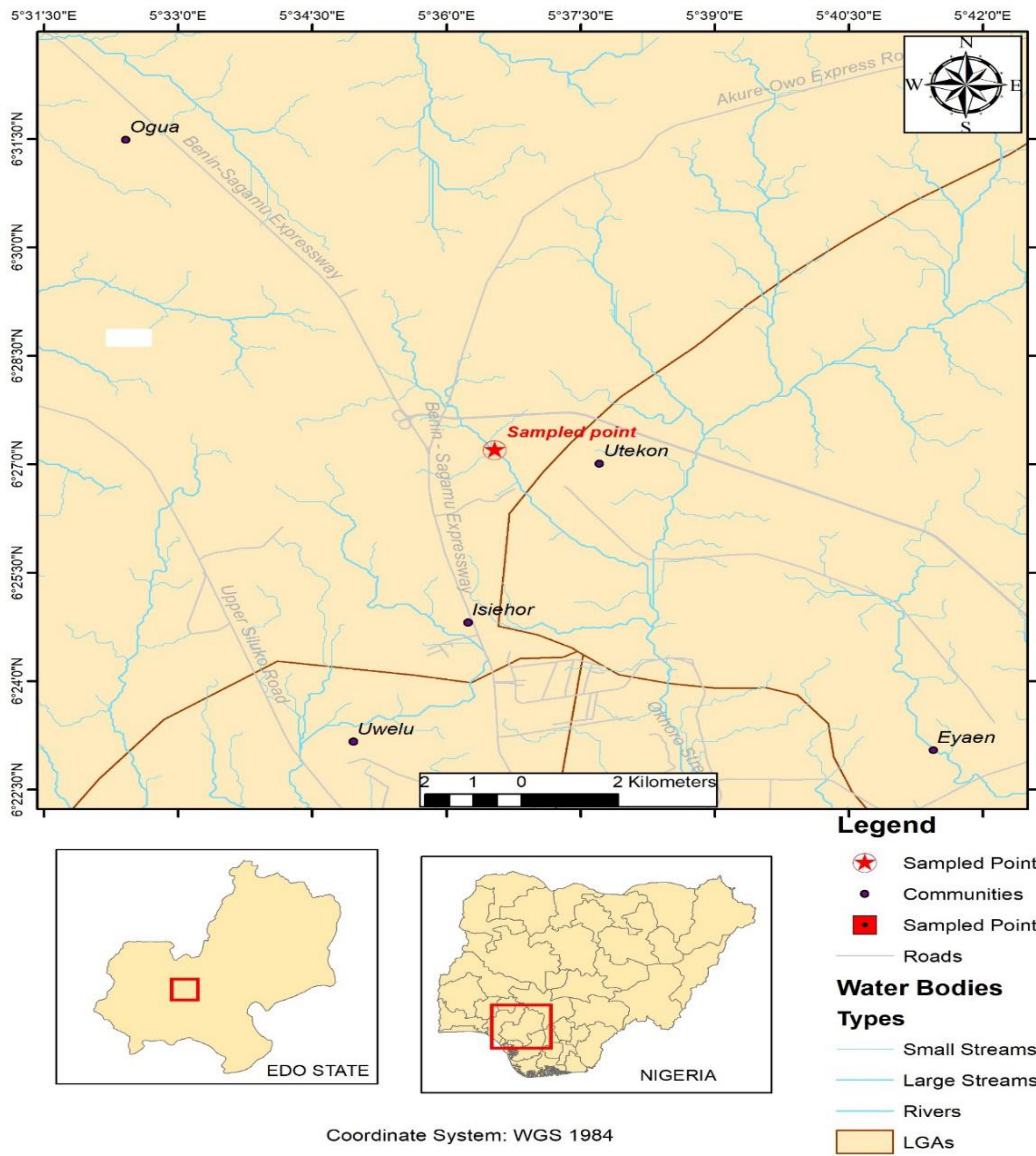


Fig 3.1: Map of study Area

Effluent Collection

Twenty-five (25) litre rigid plastic jerry cans were used to collect the effluent straight from the 7up Bottling Company effluent discharge site in Oluku, Benin City, Edo State. The samples were labelled suitably and stored at room temperature.

Experimental procedure duration

The experiment is in two phases. The first phase was an Acute toxicity study that lasted for 96 hours, and the second phase was DNA extraction.

Collection of Experimental Organism

African catfish (Clarias gariepinus) juveniles with a mean weight of 8–9g were purchased from a commercial fish farm in Benin City, Nigeria. The fish samples were moved to a storage area that was left open. The fish were housed in an aquarium using water from a borehole. During the acclimation period, all fish were fed commercially available fish meals. Following the period of acclimatization, the fish were placed at random into Preparation of the Various Concentrations of 7up effluent.

Preparation of the Various Concentrations of 7up effluent

There were five (5) concentrations, of which four (4) had sub-lethal concentrations of 0.5%,1.5%, 3.5%, and 5.0%, with the control concentration of 0% being the fifth concentration. The 0% was made up of clean borehole water and ten (8) Clarias gariepinus juveniles alongside the sub-lethal concentrations and their replicates of which the sub-lethal concentrations were marked as "A" of the concentration and its replicate marked as "B" of the concentrations making a total of 72 Clarias gariepinus juveniles across the various concentrations and its duplicates. These were put in their various containers which was made up of the percentage of the concentration of the effluent in millilitre to 1000 ml of water and this was done four times to meet the 3000ml mark. This experiment lasted for a period of 96hours (4 days).

Concentrations

- Control: This is clean distilled water used to measure the activity of the juveniles under normal conditions, it contains 0% of the effluent.

- 0.5% Concentration: This is the first sub-lethal concentration; it is made up of 0.5% of the effluent in the concentration measured at a ratio of 5 millilitres of the effluent concentration in every1000 millilitres of distilled water to make up the 3,000 millilitres concentration mark. The same procedure was done for the replicate being “0.5% B”.
- 1.5% Concentration: This is the second sub-lethal concentration; it is made up of 1.5% of the effluent in the concentration measured at a ratio of 15 millilitres of the effluent concentration in every1000 millilitres of distilled water to make up the 3,000 millilitres concentration mark. The same procedure was done for the replicate being “1.5% B”.
- 3.5% Concentration: This is the third sub-lethal concentration; it is made up of 3.5% of the effluent in the concentration measured at a ratio of 35 millilitres of the effluent concentration in every1000 millilitres of distilled water to make up the 3,000 millilitres concentration mark. The same procedure was done for the replicate being “3.5% B”.
- 5% Concentration: This is the fourth sub-lethal concentration; it is made up of 5% of the effluent in the concentration measured at a ratio of 50 millilitres of the effluent concentration in every1000 millilitres of distilled water to make up the 3,000 millilitres concentration mark. The same procedure was done for the replicate being “5% B”.

Plate 3.1: Juvenile Catfish placed into concentration(a)

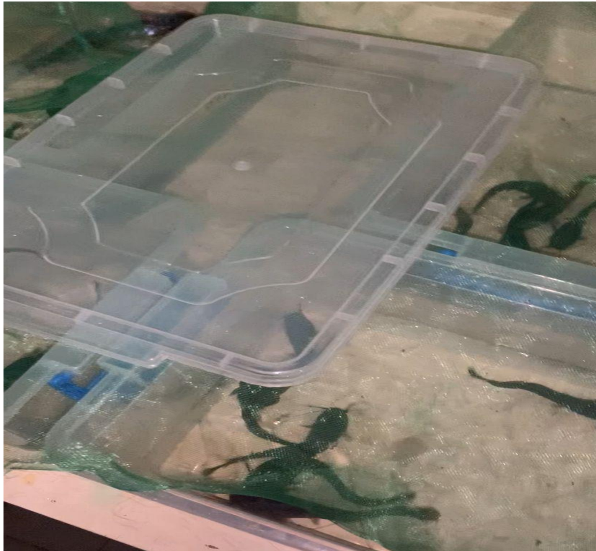


Plate 3.2: Juvenile Catfish in concentration (b).

TISSUE SAMPLE FROM JUVENILE AFRICAN CATFISH

Fish were dissected using sterile instruments to extract the gill organs as fresh as possible. The thoracic region was opened in order to remove the organ, which was then placed in EDTA tubes for storage. In order to prevent denaturing, the gill organ was kept at freezing temperatures. Reverse transcriptase enzyme conversion to cDNA and PCR technology's use to measure gene expression to assess gene integrity. The gills are extremely susceptible to alterations in the quality of the water, such as the presence of harmful materials. Furthermore, the gills are a perfect organ for evaluating the immediate impacts of chemicals on aquatic species because of their enormous surface area and direct interaction with the surrounding environment. As such, it is selected in accordance with the OECD's list of organs for toxicant exposure and is thus the most appropriate organ for this study.

Plate 3.3: Gill organ being extracted.

REAGENTS AND SOLUTIONS

- 95µl Solid tissue buffer
- 10µl Proteinase K
- DNA Elution buffer
- DNA prewash buffer
- DNA wash buffer
- Primers (forward and reverse)
- Taq polymerase (DNA polymerase) thermo-stable polymerase
- 95µl Nuclease free water

Plate 3.4: Agarose powder.

Plate 3.5: primers

EQUIPMENTS

- Eppendorf tubes
- Micropipettes
- centrifuge machine
- UV spectrophotometer
- PCR machine
- Electrophoresis gel
- EDTA tubes
- Water bath
- Blue light transilluminator

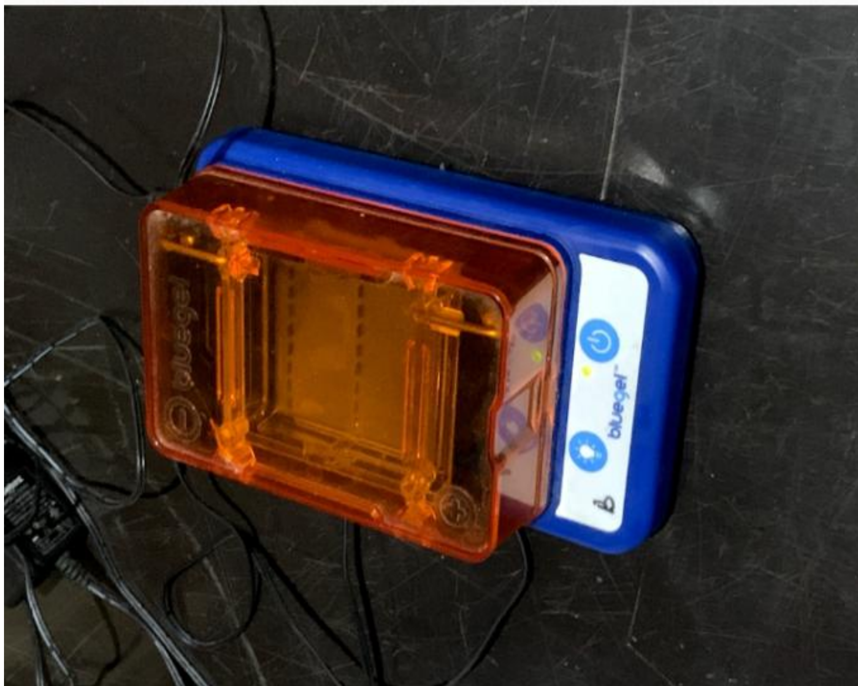


Plate 3.6: A gel electrophoresis

Plate 3.7: Water bath

Plate 3.8: A PCR machine

Plate 3.9: Micropipette

Plate 3.10: A centrifuge machine

Plate 3.11: A Vortex

DNA EXTRACTION

Following the gill organ extraction from the sample, it was transferred into an Eppendorf tube. To reveal the cell nucleus, a mixture of 10µl proteinase K, 95µl solid tissue buffer, and 95µl water was pipetted into the Eppendorf tube holding the organ. Using a centrifuge machine to homogenize the tissue broke down the tissue's nuclear membrane and plasma. The tissue was then allowed to solubilize for one to three hours at 55°C in the Eppendorf tube. Following incubation, the tubes underwent another one-minute vortex. Three stages were identified in the cell material following centrifugation: RNA, DNA, and protein. Certain contaminants and the supernatant are still contained. After carefully pipetting the sample into a fresh Eppendorf tube, two volumes of genomic binding buffer were added to the supernatant. This mixture was then completely mixed or vortexed for ten to fifteen seconds. In a collection tube, the mixture was sent to a zymo-spin column. Centrifuge for one minute at ≥ 12,000 x g. The flow through was discarded.400µl DNA pre-washed buffer was added to the spin column in a new collection. Centrifuge again at ≥ 12,000 x g for 1 minute.

The collection tube was emptied.700µl DNA washed buffer was added to the spin column and centrifuged at ≥ 12,000 x g for 1 minute. The collection tube with the flow through was discarded again. The spin column was transferred to a clean microcentrifuge tube ≥ 50µl DNA elution buffer was added directly on the matrix. It was incubated for 5 minutes at room temperature, it was then centrifuged at maximum speed for 1 minute to elute the DNA. The

genes of interest were amplified using a developed and optimized set of primers, which included forward, and reverse primers and the matrix metallopeptidase 9 gene was expressed and the PCR Master Mix catalyses the amplification for 30 cycle. The DNA polymerase extends the primers, adding nucleotides onto the primers in a sequential manner, using the target DNA as the template. A 1% agarose gel electrophoresis was performed on the amplicons obtained from RT-PCR products to facilitate their migration from the anode to the cathode.

An even voltage between 20 and 40 to 100 volts was used for the electrophoresis. The molecules travel more quickly as the voltage rises. A slower migration rate may make it easier to get definitive results. The Image J program was used to quantify the intensity and density of gene bands.

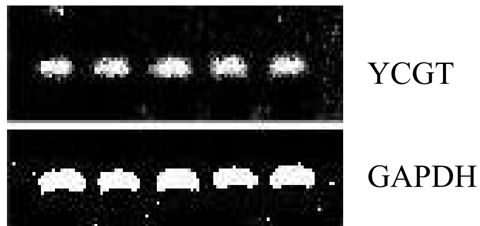
CHAPTER FOUR

Results

The effect of exposure of *Clarias gariepinus* to wastewater effluent were observed at different applied treatment levels, which were 0.5, 1.5, 3.5 and 5.0. The gene assessed during this study is the bone gamma-carboxyglutamate gene. figure 4.1 show the result for the expression level of the gene on exposure to wastewater effluent.

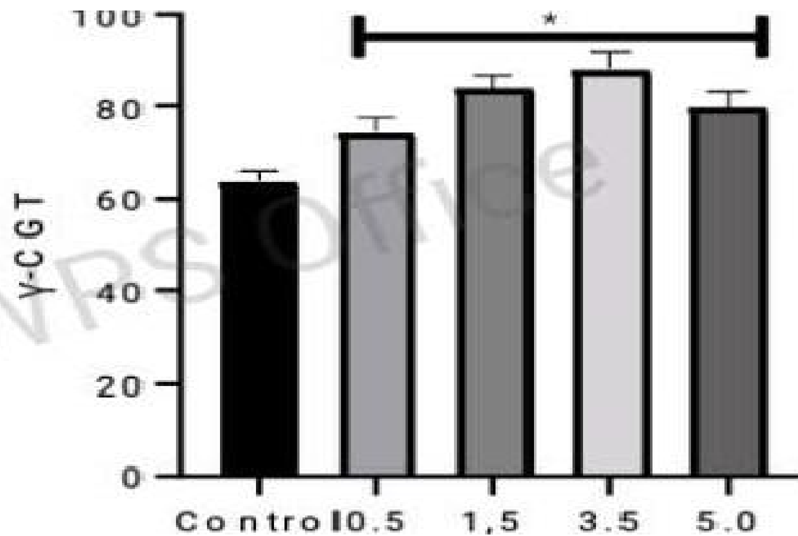
Expression of bone gamma-carboxyglutamate gene

Figure 4.1 shows the variations in the gamma-carboxyglutamate gene response to stress at different levels. When the effluent concentration level reached 3.5, the highest increase in the expression levels of bone gamma-carboxyglutamate gene was observed. The result demonstrates or shows that bone gamma-carboxyglutamate gene expression increased in proportion to the concentration of the wastewater effluent at 0.5, 1.5, 3.5 and declined at 5.0 concentration.



YCGT

GAPDH



mRNA expression of gamma CGT in *Clarias gariepinus* exposed to wastewater. •k Indicates statistical difference ($p < 0.05$) to control.

ACUTE TOXICITY TEST TABLE

TREATMENT GROUP	DUPLICATE W1					DUPLICATE W2				
	MORTALITY (HOURS)					MORTALITY (HOURS)				
CONTROL (%)	**12**	**24**	**48**	**72**	**96**	**12**	**24**	**48**	**72**	**96**
O.5%	0	3	3	0	1	3	0	3	0	0
1.5%	1	2	0	0	0	3	1	2	0	2
3.5%	2	2	4	0	0	3	2	3	0	0
5.0%	3	1	3	0	2	0	0	3	0	1

Table 4.1

CHAPTER FIVE

DISCUSSION

Discussion

Osteocalcin, sometimes referred to as bone gamma-carboxylglutamate protein (BGLAP), is produced by the "Bone gamma-carboxylglutamate (Gla) gene". Bone mineralization and structural integrity are greatly dependent on this protein. Gamma-carboxylation is necessary for this protein to become active, as it is dependent on vitamin K. This gene encodes a highly abundant bone protein secreted by osteoblasts that regulates bone remodelling and energy metabolism. The encoded protein contains a Gla (gamma carboxyglutamate) domain, which functions in binding to calcium and hydroxyapatite, the mineral component of bone. The BGLAP gene's protein product, osteocalcin, is a crucial modulator of bone remodelling and creation. It works by attaching itself to the hydroxyapatite crystals in the bone matrix, which makes it easier for them to deposit and encourages mineralization. Furthermore, osteocalcin controls osteoblast activity and differentiation, which affects bone turnover and the equilibrium between bone resorption and creation (Ducy et al., 1996). Numerous substances, including as cytokines, mechanical stimuli, hormones (like vitamin D and oestrogen), and transcription factors (like Runx2), influence the expression of BGLAP (Ducy et al., 1996; Komori et al., 1997).

Osteocalcin has become an important regulator of systemic metabolism in addition to its conventional function in bone metabolism (Ferron et al., 2010). It emphasizes the complex interactions between bone and other metabolic tissues via influencing glucose homeostasis, insulin sensitivity, and energy metabolism.

Fig 4.1 shows the changes in bone gamma-carboxylglutamate gene expression levels following exposures to various treatment levels of wastewater effluent and demonstrates how in comparison to the control sample, minimal increase in expression were observed at 0.5, 1.5 and 5.0. When the treatment level was 3.5, There was a rise in expression of the gene. These upregulations were found to be statistically significant ($p< 0.05$). This shows that a treatment of wastewater effluent with concentration of 3.5 may be harmful and deleterious to *Clarias gariepinus.* Which is why the gene expression was upregulated. Numerous bone conditions, such as osteoporosis, osteogenesis imperfecta, and bone metastases, have been linked to

dysregulation of BGLAP expression (Lee et al., 2007). Changes in osteocalcin activity or levels can upset the balance of minerals in the body, which can result in low bone mass, a higher risk of fracture, and problems with skeletal function. Although the biology of the bone gamma-carboxylglutamate gene and osteocalcin has advanced significantly, many problems still need to be answered, and research is still needed in a number of areas. Subsequent investigations ought to concentrate on clarifying the intricate processes involved in the regulation of the BGLAP gene, investigating the practical implications of osteocalcin isoforms and post-translational modifications, and interpreting the molecular routes that facilitate the metabolic impacts of osteocalcin in various tissues.

CONCLUSION

The purpose of this study was to evaluate the integrity of *Clarias gariepinus* gene expression in response to wastewater effluent. *Clarias gariepinus* genes were found to be upregulated in this study. Gene expression regulates the creation of proteins by functioning as an on/off switch and a volume control that increases or decreases the number of proteins generated. Evidently, a cell's tightly regulated mechanism of gene expression allows it to react to its ever-changing surroundings. This research establishes that wastewater effluents are environmental pollutants that negatively impact *Clarias gariepinus*. To avoid negative effects on aquatic life, it is advised that industrial wastewater effluent be appropriately and better treated before being released into the sea.

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