Chapter One

Introduction

1.1. Background to the Study.

Solid waste management has remained a significant problem in most parts of the world, but the problem is more pronounced in developing and underdeveloped countries[1]. A large number of major cities across the world lack effective waste management system despite the fact that they produce a large amount of solid wastes on a daily basis[2]. In most of these developing countries, household and industrial wastes are often discarded directly into open landfills where they eventually get incinerated or dispersed into gutter drains, rivers and swamps[3].

Despite the extensive use of landfills in several regions of the world, landfills continue to pose a significant threat to the environment, especially ground and surface water resources[4]. Landfill sites often contaminate surrounding water resources through direct contact or soil infiltration[5]. When solid wastes deposited in landfills come in contact with water from rainfall, a series of chemical reactions takes place. These chemical reactions break down the solid waste and leads to the formation of a liquid known as leachate[6]. The resulting leachate solution often contain toxic organic and inorganic compounds that gets deposited at the bottom of the landfill – from where it can percolate into the soil, and contaminate the groundwater resources below[7].

In order to reduce the chances of groundwater contamination from landfill leachates, landfills are now been developed into high quality facilities that are provided with bottom liners and leachate management setups have to reduce the movement of leachate into the environment[8]. Unfortunately, this improved form of landfills is only found occurring in developed regions such

as Europe, North America and parts of Asia[9]. Developing economies such as Africa and some parts of Asia have been unable to keep up with the pace of this evolution[10].

As populations and rate of urbanization continues to increase in these developing regions, the amount of solid wastes produced by the inhabitants of these regions also continues to rise[11]. Hence more solid waste finds its way into these poorly structured landfills. Due to the increasing volume and variety of solid waste finding its way into these landfills[12], there is an increasing possibility that water resources surrounding the landfills would continue to suffer from increasing level of pollution from leachate[13]. Sub-Saharan Africa is of special worry because of the large extent at which population and urbanization rate are growing. In addition, this region is characterized with improper waste management infrastructure and unsatisfactory land use planning[14].

The increase in global waste production has not been matched by a similar improvement in waste management[15], hence a large fraction of solid wastes finds their way into the environment where they threaten human and environmental health[16]. The accumulation of wastes thrown in landfills, gutters, and streets often lead to generation of an unpleasant liquid waste product known as leachate[17].

Leachates often contain various toxic chemical constituents as a result of the dissolution of solid wastes, as well as other chemical and biochemical reactions that takes place within the landfill[18]. The chemical constituents of leachates differ, as it depends on the nature of the landfill and the solid wastes contained therein[19]. Leachates can thus be categorized as a derivative of solid wastes which is enabled by rapid development, improper waste management, industrial revolution, and

increasing urbanization[20]. It is very common in developing regions of the world especially in the Africa, few regions of Asia, and Middle East nations[21].

When leachates find their way into aquatic environments, it can lead to mild and severe impacts on aquatic organisms[22], thus causing damage to biodiversity by threatening the survival of aquatic species[23]. In terrestrial environments, leachate gets into agricultural and farmlands, where it contaminates the soil with toxic heavy metals[24]. These heavy metals can get taken up into the edible parts of plants such as shoots and fruits[25]. Accumulation of toxic heavy metals in the edible parts of plants increases human exposure to these chemicals via dietary routes[26]. Leachates can also percolate through soil media down to surface and contaminate groundwater, hence increasing human exposure to toxic chemicals via water consumption[27].

Leachate production rate largely depends on factors such as the amount of precipitation, the in-situ moisture content of waste, run-off, evapotranspiration and the level of water table[28]. Water is highly fundamental in the formation of leachates because it facilitate the dissolution and degradation of solid wastes[29]. When water seeps through solid wastes, it stimulates the action of bacteria and fungi to decompose these solid wastes[30]. This decomposition process leads to the formation of leachates by exhausting any available oxygen and creating an anoxic environment[31].

Dumpsite leachate can be characterized as water based solution of four categories of contaminants namely; dissolved organic matter (alcohol, acid, aldehydes, short chain sugar etc.), inorganic macro component (common cation and anions including sulfate, chloride, iron, aluminum, zinc and ammonia), heavy metals (Pb, Ni, Cu, Hg), and xenobiotic organic compound such as halogenated organics (PCBs, dioxins, etc.) [32].

As argued by Hassan, the environment can be contaminated by leachates from landfills. This contamination occurs after the decomposition of solid wastes and the resulting residue mixed with precipitates of surface water[33,34]. Correspondingly, surface water collection system (rivers, creeks, and lakes), subsurface collection system (groundwater reservoirs) and solid system (different soil layers) become susceptible to pollution from the landfill/dumpsite[35]. Quite a number of leachate contaminations have been documented in the literature. Leachates have been shown to pollute surrounding soil, groundwater, aquifer, and surface waters[36]. There are adequate evidences from the literature that the dumpsite leachates have a potential of causing significant environmental problems through the deposition of heavy metals in the environment[37]. Several research studies carried out on different dumpsites in different parts of the world, has shown that the quality of human and environmental health is being influenced by leachate seeping into underground water bodies[38].

In order to provide a solution to this noticeable menace of leachate, it is important to characterize solid wastes and identify the nature of leachate produced by each category of solid waste[39]. This will help us understand the source and content of the wastes vis-a-vis the component of the leachate[40]. Adequate knowledge of the composition and proportion of the leachate is necessary when characterizing leachate because the composition of leachates are often influenced factors such as nature of wastes, climatic conditions, and mode of handling as well as the phase of the leachate[41].

In Nigeria, leachate continues to pose a significant threat to groundwater resources in the country[42]. This is fueled by improper waste management and poor attitude of Nigerians[43]. Mega cities in Nigeria such as Lagos, Kano and Ibadan are often characterized with waste littered street

and open dumpsites[44]. In addition to this, high poverty rate and low level of education continues to hinder behavioural changes that can help improve Nigerians attitude towards disposal and management of solid wastes[45]. Despite the obvious menace of poor solid waste disposal in Nigeria, there is a scarcity of reliable empirical evidences addressing the impact of leachate on the environment[46]. Nevertheless, few studies have provided empirical evidences to address the damaging impact of solid leachates on aquatic and terrestrial resources in Nigeria[47,48,49]

To avert the imminent danger associated with leachate contamination, frequent monitoring of generation wastes and improved management techniques are recommended to maintain low risk and unpolluted environment which will eventually further the attainment of an eco-friendly environment for sustainable development[50].

1.2. Statement of the Problem

Every day, an estimated 0.51kg of solid waste is generated by Nigerians[51]. This figure is expected to be doubled by 2050[52]. Despite the high amount of solid waste generated daily by Nigerians, the country lacks the efficient waste management system to absorb half of these wastes[53]. Hence, most of these wastes end up in open landfills, oceans, surface waters and other parts of the environment where they negatively impact environmental and human health[54].

For a very long time, landfills have been known for their potential to contaminate surface and groundwater systems through the production of leachates[55]. Several studies have documented the increased heavy metal concentrations and presence of toxic organic compounds in water resources around poorly managed landfills[56,57].

The Awotan landfill is one of the oldest and largest landfill in Nigeria[58], located in one of the most populous cities in the country. For years, Awotan landfill has served as the main deposit of solid waste for the inhabitants of Ibadan city, Oyo State[59]. The Awotan landfill is open and poorly managed, hence it has a high potential of contaminating the many surface and groundwater resources in the area. Although a few studies have provided scientific evidence to show that leachates from the dumpsite affects the surrounding water resources[60], very few studies have assessed the specific effects of these leachates on the aquatic community in the area. In a bid to fill this gap, this study will evaluate the effect of leachates from the Awotan landfill on the p53 gene of selected fish species.

1.3. Aims and Objectives of the Study

The main aim of this study is to investigate the toxicity impact of the Awotan landfill leachates on juveniles of *Clarias gariepinus*. However, the objectives of the study include:

- To evaluate the physico-chemical parameters and concentration of heavy metals in leachates from Awotan dumpsite.
- To evaluate the effect of leachates on the haematology of *Clarias gariepinus*
- To evaluate effect of leachates on the behaviour and mortality of *Clarias gariepinus*
- To evaluate the effect of leachates on the p53 tumor suppressor gene in *Clarias gariepinus*

1.4. Significance of the Study

This study will create public health awareness on the effects of the Awotan landfill leachate on the groundwater and surface water resources in Ibadan and its environs. This study shed light on the heavy metal composition of leachates from the Awotan landfill and promote out theoretical

understanding of the specific effect of leachates on the physiology, haematology, behaviour, mortality and histopathology of *the Clarias gariepinus*. Finally, the result of the study is expected to practically demonstrate the dangers on poorly engineered landfills through the production of toxic leachates.

1.5 Research Questions

The research questions in relating to the effect of leachates on *Clarias garipienus* were:

1. To what extent does leachates affect the histology of *Clarias garipienus* ?
2. To what extent does leachates affect the haematology of *Clarias garipienus* ?
3. To what extent does leachates affect the target p53 gene of *Clarias garipienus* ?
4. Whats is the effect of landfills on aquatic environment in Awotan community?

1.6 Scope of the Study

This study analyzed profound impact of leachates on juveniles of *Clarias garipienus* in Awotan, Ibadan. The sampling method employed is a simple random method sampling. Where we have 5 treatments in triplicates, the sample size is a total of 259 juveniles of *Clarias garipienus.* This study encompasses positive and negative control, since we are making use of 5 treatments in triplicates; this will gives a total of 15 samples to work with. The positive control will take one sample (0%) and the remaining will be of negative control ranging from 5%, 10%, 25%, 50% concentration of the leachates.

1.7 Operational Definition of Terms

Apoptopsis : Apoptosis eliminates the pre-cancerous and virus-infected cells. It thus maintains a balance of cells in the human body and enhances the immune system.

Reactive Oxygen Species (ROS) :consist of radical and non-radical oxygen species formed by the partial reduction of oxygen, ROS may cause DNA strand breakage due to free radical attacks on the DNA sugar-phosphate backbone.

Hyperproliferative Signals: it's a form of instruction**,** It does so by acting as a transcription factor that induces the expression of genes that induce cell cycle arrest, DNA repair

Hypoxia: Hypoxia is a condition in which the body or a region of the body is deprived of adequate oxygen supply at the tissue level.

Post-Translational Level :post-translational regulation refers to the control of the levels of active protein. There are several forms. It is performed either by means of reversible events (posttranslational modifications, such as phosphorylation or sequestration) or by means of irreversible events (proteolysis).

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Chapter Two

Literature Review

2.1. Solid Waste Management.

Improper solid waste management has become a chronic environmental issue in developing countries such as Nigeria[1]. It is estimated that the yearly production of municipal solid wastes (MSW) in Nigeria reaches 29.78 x 109 kg/year and this may further increase because of the rapid rate of urbanization and population growth in the country[2].

In Nigeria, solid wastes are commonly disposed in unrestrained and poorly engineered landfills[3]. Also, open dumps are common end sites for solid wastes in Nigeria[4]. Unrestricted incineration of dumps in dumpsites as well as burning of refuse from homes and offices is also a common waste management procedure in Nigeria[5]. As a result of the poorly engineered landfills and dumpsites in Nigeria, these sites have a high potential of releasing huge amount of toxic chemicals into the surrounding water, soil and atmospheric resources via leachate, landfill gases and ashes from incineration[6].

Landfill leachates have been studied broadly for chemical and physical composition by researchers' globally[7] and significant progress has been made in understanding the physical and chemical composition of leachates[8]. Leachates have been discovered to contain an extensive range of chemical compounds involving heavy metals, dissolved organic compounds and inorganic macro compounds[9]. All of these chemicals are capable of leaching into the ground where they can contaminate soil and water resources[10]. Aside from the toxic chemical

components, leachates may also harbor microbes such as harmful protozoa, bacteria, and viruses. These microbes are capable of producing toxins that can lead to severe public health problems[11].

These chemicals and biological component of leachates poses a significant threat not only to humans but also to other animals which may consume these leachate polluted waters[12]. It also affects general environmental well-being by polluting surface and groundwater through its various toxic components[13]. The organic carbon component of leachates affects the natural odour and taste of groundwater[14]. The nitrogen and phosphorus components facilitate eutrophication in surface waters, while the heavy metals interfere with the morphological and physiological activities of aquatic biota[15].

In a bid to increase awareness about the negative potentials of solid wastes, landfills, and leachates, several research efforts have been directed towards assessing the environmental impact of leachates[16]. Some of these studies have focused on the general environment[17], while some have narrowed their study to leachate effect of selected aquatic community such as algae[18], invertebrate[19] and fishes[20]. Amongst these studies, information on the possible toxic effects of leachate on fishes are limited, and very few of these studies have made use of tropical fishes as experimental subject. Also, very little reports are currently available about the toxicity of MSW landfill leachates in Nigeria, and such a database would be important in assessing treatment options and reuse possibilities.

Due to the crucial position occupied by fishes in the food chain[21], accumulation of pollutants in their tissues automatically increases human exposure to such pollutants[22]. As a result of this, fishes should be a major experimental model in ecotoxicological studies[23]. Adequate assessment

of how leachates impact various fish species is fundamental to designing strategies of reducing human exposure to the toxic substances contained in leachates[24].

Of all tropical fish species used in experimental models, *Clarias gariepinus* appears to be the most suitable[25]. *Clarias gariepinus* is often considered by several researchers because of factors such as high resistance to diseases, high fecundity and ease of larval production[26].

2.2. Leachates

Leachate refers to wastewater that is formed as a result of the chemical reactions that take place between solid wastes and water within a landfill or dumpsite[27]. Leachates are formed as a result of the combined action of physical, biological and chemical reaction occurring between solid wastes unloaded in a dumpsite[28]. The amount of liquid which passes through the dumpsite is a factor that determines the amount and composition of leachate that is being generated[29]. Decomposition of carbonaceous material produces a comprehensive list of substances including methane, carbon dioxide and a complex mixture of organic acids, aldehydes, alcohols and simple sugars produces volume of leachate[30]. A major contributor to production of leachate is precipitation in form of rainfall or run-offs[31].

2.2.1. Formation of Leachates.

When solid wastes get buried in a landfill, it undergoes a series of biological and chemical reaction which eventually leads to decomposition[32]. It is widely accepted that solid wastes in dumpsites goes through four different phases of decomposition which include, an initial aerobic phase, an anaerobic acid phase, initial and stable methanogenic phase[33]. The consecutive stages involved in landfill stabilization are as follows;

Phase I: Initial Adjustment - In no more than a short time after the waste is unloaded into the dumpsite, microorganism seems to be attracted to the dumpsites which could build up to a population sufficient to alter the waste[34].

Phase II: Transition – This stage shows transition from previous aerobic state to an anaerobic environment[35]. A movement toward reducing conditions, such that elements or molecules gain electrons[36]. At the later stage of this phase, quantifiable concentrations of chemical oxygen demand (COD) and volatile acids (VOAs) appear in the leachate[37].

Phase III: Acid Formation – At this stage, a fair amount of the wastes is hydrolysed, they react with water to give rise to soluble organic and inorganic products[38]. Here, anaerobic, acid-forming bacteria breakdown biodegradable organic matter in the waste, producing volatile acids[39]. The products formed of VOAs increase loads of dissolved metals and reduce the pH of the leachate. Fermentation of organic matter also takes place at this stage[40]

Phase IV: Methane Production –The presence of a group of dominant microorganisms' known as methane producing bacteria makes this phase highly distinctive[41]. These bacteria transform the organic acids produced in Phase III to methane and carbon dioxide[42]. An extremely reducing chemical environment develops and leads to the reduction of sulfates (SO_4^{2-}) to sulfide (S^{2-})[43]. The methanogenic bacteria continue to flourish and it is being supported by the pH which is maintained in the neutral range by bicarbonates (HCO^{3-})[44]. The precipitation of metals is also favored by presence of sulfides and hydroxides (OH^-)[45]. This phase is often made reference to as the methanogenic phase.

Phase V: Maturation – In this phase, biological activity reduces due to the depletion of readily-degradable organic matter and other nutrients[46]. Gas production also declines and concentrations of pollutants in leachate are lower than in previous phases[47].

2.2.2. Composition of leachates

The nature of a dumpsite, as well the solid wastes contained therein are the biggest factors influencing the composition of leachates[48]. Hence, the composition of leachates strongly differs from one landfill to another. Some other factors affecting the composition of leachates might include; age of the dumpsite, the type of waste, as well as the degree of decomposition and physical modification of waste[49].

Landfill leachate may be distinguished as a water-based solution of four categories of contaminants which include; dissolved organic matter (alcohols, acids, aldehydes, short chain sugars etc.), inorganic macro components (common cations and anions including sulfate, chloride, iron, aluminum, zinc and ammonia), heavy metals (Pb, Ni, Cu, Hg) and xenobiotic organic compounds such as halogenated organics[50]. Different hydrocarbons such as esters, alcohols, and ketones, as well as aromatic and heterocyclic compounds are range of considerable hydrocarbons compounds that are found in a landfill leachate[51]. All of these components are often found in leachate solution – especially leachates from landfills which receive solid wastes from a mixture of communal, commercial and industrial sources. Dumpsite leachate may also consist of microbes such as protozoa, bacteria and virus[52].

The presence of microbes in leachates is often influenced by the type of substrates available to support the physiological requirements of these microbes[53]. Fortunately, dumpsites and landfills

are characterized by diverse substrates hence making a good environment for microbes to thrive[54]. As a result of this, pathogens are common components of leachates obtained from any type of landfill or dumpsite.

The composition and complexity of microbial community found in leachate samples is often determined by factors such as the moisture content, nature of solid wastes, age of landfill, and nature of landfill[55]. Various species of microbes have been reported in leachate samples from different landfills across the world[56]. Some of the most common categories of microbes found in leachate samples include aerobic, psychrophilic and mesophilic bacteria, coliform and fecal coliforms, spore-forming-bacteria, and with numerous fungi[57]. Some of the species that have been reported include; *Arthorobacter*, *Bacillus,E. coli, Klebsiella, Micrococus, Proteus, Serratia marcescene, Klebsiella aerogenes, Staphylococcus aureus, Alcaligens sp, Proteus mirabilis and Salmonella, Rhizopus* and *yeast species*[58].

2.3. Environmental and Public Health Impact of Leachates.

The environment consists of all the biotic and abiotic factors which surrounds and influences human survival and well-being[59]. The abiotic factor could include water, air and soil, while the biotic factors could include human relationships with all the living component of the ecosystem[60]. The influence of dumpsite leachate on the environment is mostly as a result of the poor management of dumpsites and landfills. The absence of fitting engineered dumpsites for disposal of waste (especially in developing countries) has led to rapid formation and leachates which eventually end up in the environment via leaching or run-offs. This leachate contains toxic chemicals which can negatively affect environmental and public health[61].

Abiotic components of the environment such as surface water, groundwater, soil and air has been reported to be contaminated with dumpsite leachates on several occasions[62]. Landfill leachates have also been reported to contaminate biotic components of the environment such as fishes, plants, and livestock[63]. Contamination of both biotic and abiotic components of the environment increases human exposure to leachate toxicity.[64]

2.3.1. Leachate Toxicity in Surface Water.

Due to the toxic nature of the various components of leachates, it has been shown to negatively impact the morphological and physiological process of both aquatic and terrestrial biota[65]. In the aquatic ecosystem, the toxicity of leachates has been found to be quite severe on the various communities present in the ecosystem[66]. Leachates have been found to facilitate the growth of algae in aquatic ecosystem[67]. Leachates high in phosphorus and nitrogen have the potential of facilitating the growth of algae when introduced into the aquatic ecosystem[68]. This leads to a situation known as Alga bloom or eutrophication[69]. During eutrophication, the rapid growth of phytoplankton leads to a significant drop in the level of dissolved oxygen because the planktons make use of the dissolved oxygen for respiration and decomposition[70]. In addition, this plankton covers the surface of the water where they produce offensive odours and inhibit light penetration in the aquatic ecosystem, hence inhibiting oxygen production via photosynthesis[71]. Extreme cases of eutrophication could lead to a state of anoxia where the water body gets completely deprived of oxygen[72]. In these cases, the physiological process of all the biota becomes affected.

Leachates have also been shown to severely affect the community of macroinvertebrates in the aquatic ecosystem[73]. Several studies have emphasized the negative effect of different categories of leachate on the macroinvertebrate community[74]. For instance, a previous study showed that

cigarette butt leachates increase the rate of mortality of invertebrates such as *Planorbis planorbis*, *Polycelis nigra* and *Dreissena polymorpha* by 60%[75]. Also, Hicham and Lofti[76] showed that leachate disrupts the community structure of macroinvertebrates by allowing pollution tolerant species such as Chironomidae to thrive and causing a significant decline in the population of sensitive groups such as *Ephemeroptera*, *Plecoptera*, and *Trichoptera.*

Leachates have also been shown to have adverse effects on fish communities in the aquatic ecosystem. Another study showed that leachates from textile waste induces mortality in the embryo of Zebrafishes, reduces locomotion response in fish larval, increases EROD activities in the liver of Brown trout, and induces toxicity of fish cell lines[77]. Similarly, another study showed that the heavy metal contents in leachate adversely affect the genetic composition of the Common Roach (*Rutilus rutilus*)[78].

2.3.2. Leachate Toxicity in Groundwater.

Groundwater resources are a very important part of the ecosystem because they supply the bulk of the water used for drinking and irrigation[79]. These groundwater resources have become highly vulnerable to leachate contamination because of the ease at which leachates can seep into the ground[80]. When leachates come in contact with groundwater resources, it compromises the quality of such water and makes it unfit for drinking or irrigation. The degree and nature of contamination often depends on the composition and concentration of the leachate. In most cases, leachates alter the pH of groundwater, increase its heavy metal contents and introduce harmful pathogens into the water[81].

Several studies have reported the contamination of groundwater resources by leachate from surrounding solid waste dumps[82]. Several studies have reported leachate toxicity to groundwater as a result of different kinds of pollutants such as heavy metals, nitrogen species, chlorinated hydrocarbons phenols, cyanides, and bacteria among others[83]. These studies are more pronounced in developing regions where the formation of leachate is very common.

For instance, Akinbile and Yussof[84], reported high concentrations of heavy metals such as zinc, lead and chromium in the groundwater resource near a poorly managed dumpsite in Akure Nigeria. The study also showed an increased count of bacteria strains such as *Escherichia coli* in the groundwater resource.

Longe and Balogun[85] reported a low level of contamination in the groundwater resources around a municipal landfill in Lagos, Nigeria. The minimal impact of landfill leachate on the groundwater resource was due to the soil stratigraphy of the area. Landfill sites with clay or silty clay soil fosters natural attenuation of leachate in groundwater resources. Hence, areas with such soil stratigraphy often witness a minimal contamination of groundwater from surrounding landfills.

Ugwoha and Emete[86] reported a high level of contamination in the groundwater resources surrounding an open dumpsite in Port-Harcourt. The study revealed a Water Quality Index (WQI) score which indicates that the water is unfit for drinking. The high level of contamination in this water was attributed to leachates from the open dumpsite. The results also showed that the impact of leachates of groundwater resources decreases significantly with distance.

A study evaluated the physico-chemical and heavy metal parameters of groundwater resources around the Emirin dumpsite in Ekiti State, Nigeria. The results of the study showed that the groundwater resource in the area is contaminated with four heavy metals including nickel, iron, lead and chromium. The presence of these heavy metals was traced to the leachates produced within the Emirin dumpsite[87].

Leachate contamination of groundwater is a global challenge and has also been reported in other parts of the world[88]. For instance, a report from the agricultural district of Chachoengsao, in the east of Bangkok, showed that the local villagers had lost their main water source as a result of leachates generated from piles of electronic wastes. A Chinese-run factory in the region started bringing in foreign e-waste items such as crushed computers, circuit boards and cables for recycling to mine the electronics for valuable metal components like copper, silver and gold. These items contain lead, cadmium and mercury, which are highly toxic. The wastes from these activities were improperly disposed in landfills. During rainfall, the water toxic components from these wastes and found its way into the village water system. Afterwards, members of the community started to perceive foul odour from their water system and some of them started to develop skin diseases[89]. A physico-chemical analysis of the water showed that water is contaminated with toxic heavy metals such as iron, manganese, lead, nickel and in some cases arsenic and cadmium.

These empirical evidences have shown that the ecological balance of the aquatic environment may suffer a destructive outcome as a result of leachate contamination of the surface and groundwater[90]. The diversity of aquatic biota may reduce based on the magnitude of contamination[91]. Aquatic biota may also accumulate heavy metals in their tissues and pass it on

to humans and carnivore who occupy the top of the food chain[92]. The magnitude of leachate pollution in the aquatic ecosystem often depend on; the volume and sensitivity of the receiving water bodies, basic quality, concentration and variation of the leachates[93]. Evaluating groundwater quality and developing a plan of actions to protect aquifers from leachate pollution are vital for appropriate planning and designing water resources.[94]

2.3.3. Leachate Contamination in Soil.

Leachates are known to cause pollution within and around dumpsite soil[95]. Leachate may contain various toxic components like chemicals, heavy metals, organic compounds, microbes[96] etc. The migration of leachate into the soil makes the soil a potential sink for leachates produced in surrounding landfills[97].

Heavy metals such as copper, zinc, iron, lead, manganese, cadmium and chromium may cause significant environmental problems when they find their way into soil via leachates[98]. This is because these heavy metals are non-biodegradable[99]. When these heavy metals accumulate in the soil, they alter soil physiology[100]. For instance, heavy metals alter enzymatic activities in the soil by disrupting the balance of the microbial community. Heavy metals often alter the overall activity of soil microbes, their diversity, and population[101]. A study by Lin *et al,*[102] showed that leachate from landfill has the ability to inhibit soil functionality and constitute prolonged health risks.

Agricultural lands are at more risk of soil contamination with leachates because it could result in low crop quality and a decrease in overall productivity[103]. Various pollutants contained in leachates could affect soil functionality in different ways with a resultant effect of poor crop

yield. For instance, high concentration of Pb in agricultural lands may inhibit important plant processes such as; photosynthesis, mitosis and water absorption with toxic symptoms of dark green leaves[104]. Cadmium may lead to the overproduction of oxidative markers, while nickel could impair plant metabolism and inhibit photosynthesis[105]. Other effect of leachate soil contamination may include; modification of soil composition, landscape change and visual discomfort, and abnormal plant growth and development[106].

2.4. Landfills and Leachates as an Environmental and Human Health Issue.

Leachate from dumpsite contains a large number of pathogenic and opportunistic microorganisms, according to different studies that have been reported.[107] Growth of microbial pathogens such as *Arthorobacter, Bacillus, E. coli, Klebsiella, Micrococus, Proteus, Serratia, Aspergillus, Fusarium, Mucor, Penicillum, Rhizopus* and *Saccharomyces* are facilitated by the disposal of raw human faeces and other human waste in dumpsites and landfills[108]. The uncontrolled growth of these pathogens can lead to an outbreak of infectious diseases among landfill workers and the community at large[109].

Scavengers and small mammals which move from one dumpsite to another are capable of carrying these harmful pathogens across the environment, hence exposing human and wildlife to different types of diseases[110]. Several illnesses such as abdominal pain, cough, skin irritation, malaria, respiratory diseases, asthma, recurring flu, eye irritation, body weakness, cholera, tuberculosis, diarrhea etc. have been reported among people that live in proximity to landfills[111].

According to a study landfills and dumpsites should not be situated in regions close to residential areas or other delicate regions like rivers, market place, and streams[112]. This is because closeness to dumpsite and landfills increases environmental and human exposure to leachate contamination.

Farmlands close to dumpsites and landfills should be considered unsafe and a public health threat. This is because edibles such as vegetables, fruits and other food items being produced on such farmlands are exposed to contamination from the dumpsite[113]. Consumption of food products from such farmlands leaves the consumer at a high risk of contamination. This is even more pronounced among vulnerable group such as children, aged, and immune-deficient individuals[114]. A few of the pathogens that have been associated with dumpsite leachate in the literature include*; E.coli, Enterobacter, Bacillus, Salmonella, Aspergillus niger, Aspergillus flavus, Rhizopus, yeast species, Serratia marcescene, Klebsiella aerogenes, Staphylococcus aureus, Alcaligens species* and *Proteus species*[115]. Disease outbreaks such as typhoid, cholera, diarrhea, skin rash etc., may evolve from these pathogens through contamination in water, plant or food sources[116].

The unethical disposal of dangerous waste such as electronic waste, batteries, and construction and demolition wastes has increased the rate at which heavy metals are found occurring in leachate solutions[117]. Most studies on leachates have revealed the high heavy metal content found present in leachate samples. Leachate may contain different metals depending on the type of wastes it is produced from and the age of dumpsite[118].

Approximately 30 heavy metals and metalloids are potentially toxic to humans when compared to the other ninety-two naturally occurring elements. Some of these toxic heavy metals include: Be, B, Li, Al, Ti, V, Cr, Mn, Co, Ni, Cu, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, W, Pt, Au, Hg, Pb, and Bi[119]. Lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are known to

have no positive effect on human and even at low concentration of consumption[120]. Heavy metals may get into the human body throug the ingestion of heavy metal contaminated plants and water[121]. For example, Cadmium spread in the environment may remain in the soils and sediments for decades – from where plants gradually take up the accumulated metal and store it in their edible tissues such as stems, shoots and fruits. This makes such plants an exposure route for humans that feed on them[122].

Based on previous studies, legumes and vegetables are some of the plants with the highest rate of heavy metal accumulation from the soil[123]. Hence, it is important to discourage the planting of vegetables and legumes around dumpsite should to prevent the transfer of toxic metals into human system[124]. The uptake of metal by plant from soil at high concentration has great health risk for mankind if consumed[125]. Other effects from leachates may also occur through the ingestion of other organisms such as fish and aquatic plants whose habitat is contaminated by leachates[126]. The presence of toxic heavy metal in leachate contaminated soil indicates that there is appreciable contamination of the soil and if actions are not taken, pollutants will eventually migrate through soil strata and may contaminate ground water after a certain period of time which can create serious problem because these metals cannot be degraded[127]. Heavy metals being proven as toxic to human health have been regarded as a major threat, associated with several health risks and illness such as reduced growth and development, nervous system damage, organ damage, and increase cancer rates when it is being consumed[128].

2.5. Fishes as Indicator of Leachate Toxicity.

Fishes are extensively regarded as a good bio-indicator of stress and pollution in the aquatic ecosystem[129]. Hence, they are often used in studies involving pollution and toxicity[130]. Fishes are

mostly found at the end of the food chain in the aquatic ecosystem; thus, they are a good indicator of heavy metal pollution in the aquatic ecosystem[131].

When toxic substances are willingly or accidentally discharged into the aquatic ecosystem, it compromises the integrity of the ecosystem and subjects the biotic community to a new level of stress[132]. Aquatic organisms adapt to this stress by altering their physiological activities[133]. However, when the concentration of the pollutant is too high and the stress becomes unbearable, the aquatic organisms start to die as a result of their inability to adjust to the new level of stress and toxicity[134].

This characteristic of aquatic organisms adapting to changes in their environment until they no longer can, makes them a suitable model for toxicity studies[135] and a preferable indicator over plankton and invertebrate for toxicity studies[136]. Fishes are also preferred to macroinvertebrates and algae because fishes are relatively large, easy to sample and they respond to toxicity more than plankton and macroinvertebrates[137].

Some of the common species of fish used in bioassay experiments in tropical regions such as Nigeria include *Clarias gariepinus*, *Anabas testudineus, Pangasius sutchi, Cyprinus carpio, Orechromis niloticus* and *Orechromis mossambicus*[138]. Most of the previous studies test for chemical contents of the leachate and the concentration of heavy metals such as Pb, Cr, Zn, Fe and Mn. Other countries such as Brazil, fish species such as *Leporine obtusidens, Danio rerio* and *Geophagus brasilienis* have mostly used in bioassay experiments[139]. Studies from Egypt ofen use *Orechromis niloticus* as their testing organism[140]. In Japan, Japanese *Medaka* or *Oryzias latipes* is one of the popular fish used in fish bioassay[141]. *Gambusia affinis* was used to test the toxicity produced by textile wastewater in India [142]. *Fundulus heteroclitus* was used in Canada to

test the toxicity of Benzo[a]pyrene (BaP) in fish[143]. In Spain, *Sparusaurata* and *Solea senegalensis* were used to test for As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, PCBs, and PAHs toxicity in the sediment[204]. Fishes are often selected for bioassay test based on their presence in the region of the study and based on their reputation as a model for toxicity tests[145].

2.6. Mechanism of Pollutants Absorption in Fishes

The ability of fishes to accumulate toxic pollutants such as heavy metals in their tissues has been a topic of public health concern for a very long time[146]. Due to the importance of the topic, several research efforts have been channelled towards understanding the mechanism of heavy metal uptake in fishes[147].

The absorption of metals by aquatic organism involves the movement of metals into the circulatory system across the epithelial barrier of gills, digestive systems or integument[148]. This movement via the epithelial cells consists of three processes. The first one is the ingestion by the apical membrane – which is the interface with the external environment. The second process is the movement through the cell and interaction with intracellular ligands and the third process is the discharge via the basolateral membrane, the interface with the circulatory system[149]. Organs that serve as the region for ingestion (gills, intestine and digestive gland) are also likely to become concentrated with metals and therefore, reveal relatively high potentials for bio-accumulation[150].

As the concentration of the heavy metal increases in the environment, the more it may be taken up and accumulated in the fish tissues[151]. Water hardness (mostly related to calcium

concentration) affects the ingestion of metals via the gill epithelium[152]. The excessive accumulation of nutritive metals such as Fe, Cu and Zn can be harmful to the fish[153].

Fish exposed to organo chlorine pollutants will be directly ingested across gill membranes, and the gastrointestinal membrane via the food uptake[154]. All organo chlorine pollutants have the capacity to bio concentrate and bio-accumulate. Previous studies have shown that the concentration of pollutants in fish tissues is not directly related to the corresponding concentrations in water[155]. This suggests that some of the pollutants taken up by fishes do not accumulate in the tissues. Often times some of these pollutants are returned to the environment during excretion[156].

Metals accumulate in different tissues of fishes, but the most common tissues that favour metal accumulation include gills, muscles, stomach and liver[157]. All heavy metals have varying affinities for these tissues. For instance, studies have shown that the concentration of copper is often highest in the liver, while mercury is often found in the liver and muscle[158]. There are three openings through which fishes can take in heavy metals, and they include: through the gills, skin and mouth[159]. Gill is the first focus of waterborne metals since it is a respiratory organ exposed to the exchange of ions in the aquatic environment[160]. Different studies have claimed that the main route of bio ingestion for metals that are concentrated in fish is via the gill epithelium[161]. The accumulated rate revolves around the balance between the ingestion rate, metabolism of the chemical and excretion rate[162]. In most studies, the gills have often shown the highest rate of heavy metal accumulation[163]. This is so because of the gills constant contact with water. Studies have also shown that the liver often accumulate a large concentration of heavy metals such as lead and cadmium[164]. This can be attributed to the fact that the liver plays a major role in fish

metabolism. Muscles have been reported to have the lowest heavy metal accumulation rate because of its passive role in feeding and metabolism[165].

2.7. *Clarias gariepinus* as a Model for Toxicity Tests.

Clarias gariepinus (African catfish) is a benthopelagic freshwater fish. *Clarias gariepinus* is a tropical fish widely distributed across African waters[166]. The ecological distribution and feeding habits of *Clarias gariepinus* makes it highly vulnerable to xenobiotics[167]. *Clarias gariepinus* has been used in many research studies to evaluate the cytogenotoxic effects of communal landfill leachate[168], e-waste leachates, and textile leachates among others[169].

Clarias gariepinus is often a preferred fish for toxicity studies because it is easy to culture. *Clarias gariepinus* is easy to culture because of its rapid turnover rate and its quick adaptation to new environments such as laboratory conditions. In addition, *Clarias gariepinus* has a high growth rate and a high tolerance for stress and pollution. All these factors make *Clarias gariepinus* a good candidate for toxicity study[170].

Due to the ease of cultivation and its appealing taste and texture, *Clarias gariepinus* is one of the most commercially farmed fished in Nigeria and Africa as a whole[171]. The ability of *Clarias gariepinus* to endure adverse environmental condition makes it a leading choice among fish farmers in Africa[172]. *Clarias gariepinus* is also capable of using atmospheric oxygen and it effectively converts feed to flesh. These traits also make *Clarias gariepinus* a very popular choice for toxicity studies.[173]

2.8. Genotoxicity of *Clarias gariepinus*

Genotoxicity encompasses the interaction of DNA dangerous agents such as heavy metals as environmental toxicants with the cell's genetic material taking into consideration consequent results on health of the organisms[174]. DNA integrity can get lost as a result of environmental toxicants as regard to cases of mutations, chromosomal aberrations and cancer in vertebrates and genotoxic disorders in invertebrates[175]. Therefore, it is highly important to elucidate the results of genotoxins discharge in freshwater system and the number of DNA strand breakage in order to show the level genotoxicity and an essential biomarker in ecological monitoring[176].

Comet assay is mostly applied to assess the effects of genotoxic contaminants on fish with the analysis of DNA damage to weigh up the genetic risk associated with xenobiotic exposures[177]. A dependable, efficient and rapid technique is the comet assay for the detection of DNA strand breakage and alkali-labile sites, which are induced in organism's cells by agents[178]. Fishes are exposed to numerous stressors in the water, and heavy metals are the extensively reported ones[179]. At a higher concentration, they are generally toxic and the continuous accumulation may cause biochemical and genetic alterations[180]. Biomarkers, which depict the type, degree, and level of alterations, need to be investigated thoroughly to understand the magnitude of the damage, which is an integral aspect of Ecotoxicological research[181].

Metallothionein (MT) and glutathione peroxidases (GPX) genes elicit their response in fishes on the intake of heavy metals from the surrounding[182]. Metallothionein proteins (MT) are the products of such gene elicitation[183]. Metallothionein is found to be present in all vertebrates and some invertebrates, to detoxify and scavenge reactive oxygen species (ROS). Glutathione peroxidase (GPX) aids to continue maintaining the homeostasis between the imbalanced anti-

oxidants and ROS[184]. Thus, sensing the previous amount of heavy metal in fish tissues, removal strategies should be designed.

Aquatic environment is deleteriously affected by man-made activities and pollutants (heavy metals, PAH, pesticides etc.) and consequently, the ecosystem quality is reduced and its balance is lessened as well[185]. A considerable quantity of unprocessed or inadequately treated wastewater from domestic, industry, and agriculture released into rivers, which deteriorate the quality of water and produce conspicuous negative influence on freshwater organisms[186]. These human-related activities amplify heavy metals in the environment that cause serious danger to human health as a result of their toxicity, persistency, abiotic degradation and bioaccumulation[187]. However, heavy metals accumulation to toxic levels in water, sediments and aquatic organisms lead to ecological damage which endanger human health throughout the food chain[188]. Therefore, the pollutants in aquatic environments with an extensive scale of contaminants have been an essential matter of concern over the last few decades.

The genotoxic and carcinogenic ability of heavy metals can induce oxidative stress, activating the production of reactive oxygen species (ROS), which lead to damage in DNA and death of cell[189]. The production of ROS can be proliferated, specifically as a result of the exposure to transition metals [Cr, Mn, Fe, Co, Ni, Cu, and Zn] reason that in nature they are redox active chemicals[190]. These free radicals lead to changes in DNA structures. Consequently, the elimination of ROS and/or other free radicals are vital for maintaining physiological balance. Antioxidant defense system is present for neutralization of free radicals in the animal that encompasses both enzymatic and nonenzymatic systems. The responsibility of superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST) are commonly known while

reduced glutathione (GSH) is one of the most essential non-enzymatic antioxidants. When accessible antioxidants are insufficient to suppress all free radicals then DNA damage with concomitant tissue destruction get higher[191]. Malondialdehyde (MDA), one of the breakdown products of lipid peroxidation as thiobarbituric acid reactive substances, is regarded as a practical indicator of oxidative damage as a result of the vulnerability of membranes to be targeted by ROS[192]. Different research work about the effect of metal pollution in aquatic system on oxidative stress markers such as thiobarbituric acid reactive substances, SOD, CAT, GSH, GR, GPx, GST, G6PDH etc[193].

2.9. Fish as a Bio-Indicator For p53 Gene

The tumour suppressor p53 gene is a vital cellular stress sensor that induces apoptosis, arrest of cell-cycle, and a sequence life biology processes by responding to environmental stresses such as DNA damage, hyperproliferative signals, and hypoxia[194]. The correlating cellular responses being controlled by p53 rely on its transcriptional factor role trigger particular target genes[195].

The task of p53 require tight limitations to the steadiness of cell and the protein level of p53 is stunted in normal cells[196]. Prior scientific work has revealed that p53 takes part in the defense against viral infection relying on its ability to induce cell-cycle arrest or apoptosis through the transcription of target genes[197]. p53-dependent apoptosis has been recognized as a potent control to keep under control virus infection, such as by inhibiting the infections of vesicular stomatitis virus (VSV), influenza A virus (IAV), herpes simplex virus (HSV), and poliovirus[198].

The word apoptosis was initially introduced by Kerr and colleagues in 1972 to illustrate the form of cellular death coexisted by shrinkage of cytoplasm, condensation of nuclear chromatin,

nuclear fragmentation of the plasma membrane[199]. This term is derived from ancient Greek words 'apo' and 'ptosis', which in conjunction depicts petals falling off from flowers. Apoptosis is a programmed contingency that takes part in an important role in sustaining bodily homeostasis and occurs in the entire process of normal ontogenesis. Apoptosis can also play a role of a protective mechanism that gets rid of impaired cells upon physiological and pathological stimuli. Tumour cells are caused by the action of repression of this programmed cell suicide which leads to the accumulation of virtually immortal cells[200]. Apoptosis can be activated by extra- and intracellular signals. The extrinsic or death receptor routes involve the ligation of the surface tumour necrosis factor (TNF) receptors and death-inducible signaling complex (DISC) emergence. Immediately DISC is formed, it induces caspase-8 and commences the apoptosis execution phase. The intrinsic or mitochondrial pathway is mediated by the adversarial association between the pro- and antiapoptotic proteins of the Bcl-2 family. Results of pro-apoptotic stress, such as ER stress and DNA damage, the effector proteins of the pro-apoptotic Bcl-2 family, BAK and BAX homo-oligomerize inside the outer membrane of mitochondria and activate mitochondrial outer membrane permeabilization (MOMP) with the release of apoptotic activators such as cytochrome c and endonuclease G[201]. Lately, a third pathway has been uncovered which entails the regulation of granzyme A and B[202]

However, Cellular tumour antigen p53 has been identified about thirty years ago, but remains concern most of the consecration in the fields of cancer research[203]. The Cellular tumour antigen p53 or p53 is a protein encoded by the TP53 gene which is most vital a tumour suppressor gene. Extensive research work has been carried on this gene in humans and mammals except in some fishes. It is also called tumour suppressor p53 or phosphor-protein p53 or antigen NY-CO-13 or p53 or Transformation-related protein 53 (TRP53) which play a vital role in apoptosis i.e.

programmed cell death in development of tumour and genomic stability[204]. The tumour suppressor p53 protein plays a role of transcription factor to regulate expression of several genes in its association network, which comprises upstream regulators and downstream target genes. Hence, if p53 is mutated, cell growth ensues leading to emergence of tumour. The activity and expression of p53 are keep tab on by different layers of control, mostly by ubiquitin ligases such as Mdm2 and Mdm4 at the post-translational level[205]. In relation to mammals, the strength and purpose of p53 is control by a number of post-translational modifications whereas in Zebrafish, modulation at both the mRNA and protein a level in response to different types of stress has been identified[206].

Tumour is caused by mutation of p53 gene and thus, tends to subdue the tumour suppression mechanism and the factors. The single amino substitution will also affect the expression of p53. The loss of purpose of p53 as a result of mutations has been well examined in mouse models. Diverse work has been carried out on p53 in humans and mammals, but in case of fish, very few works has been reported. Recently, in the age of bioinformatics, different tools and algorithms have been developed for understanding biological molecules down to atomic level and envisaging fundamental mechanisms. For instance, a study[207] reported that advance understanding of tumour suppressor p53 regulation in fishes using cellular mechanisms along with protein modifications will enable us to understand in vivo underlying mechanisms that regulate the tissue specific response of p53.

Recently, p63 and p73 of other organisms has recently been shown to be similar to the structure of the p53 gene. A study recently reported that the human p53 gene produces nine forms of isoforms, as examined by cap dependent PCR amplification of RNA species, due to alternative

splicing, alternative promoter usage and alternate initiation in translation. Additionally, it has been revealed that the structure of the p53 gene, most especially the production of RNA potentially encoding N terminally truncated isoforms, is retain in *Drosophila melanogaster*, *Danio rerio* (zebrafish) and *Homo sapiens*, suggesting a conservation of the function of p53 and its isoforms through evolution. Recently, studies discovered that of the two p53-like genes in the Deer Tick genome one is truncated and in fact an extraordinary close homologue of the human and zebrafish Δ113p53 proteins[208]. The Δ113p53 isoform of ZFp53 in zebrafish and its orthologous equivalent Δ133p53 in human are controlled by an internal promoter in intron 4 of the p53 gene and this promoter relies on p53 for transcription[209].8,13 The human Δ133p53 isoform is deficient of the transactivation domain and part of the DNA binding domain but conserves the tetramerization domain of p53. It has been reported that Δ133p53 has a particular dominant negative activity on p53 after transfection into H1299 cells, is overexpressed in breast cancers[210], oral lichen planus[211] and after chemotherapeutic treatment in AML patients[212]. It is able to eventfully stimulate the number of division cycles that primary cells can pass through before replicative senescence and suppress the degree of the mir34aA microRNA[213].

Additionally, zebrafish have also been reported to express Δ113p53 which has been discovered at the mRNA level. Using a reporter system Δ113p53 expression was found to regulate apoptosis through the regulation of B-cell lymphoma-extra-large BclxL[214]. A study reported that Bony fish comprises of all three genes, p53, p63, and p73, and the role of these three transcription factors diversify in the higher animals[215].

Mammalian p53 homologs have been examined in several fish species. These include rainbow trout[216], zebra fish[217], channel catfish, flounder[218], medaka[219], orange-spotted grouper[220]; tilapia[221],

and whitefish[222]. Generally, the studies showed a high level of functional homology with mammalian p53[223]. Out of the few studies that have been carried out with fish, the regulation of p53 levels in teleosts is revealed to differ from the regulation in mammals. This has been seen most clearly with in vitro studies on cell lines. Numerous chemotherapeutic agents that are identify to cause damages to DNA increase p53 levels in mammalian but not piscine cell lines[224].

Little studies have emphasized on p53-mediated signal transduction pathways and the processes that they control in fish. Many studies have been carried out with zebra fish, with p53 being discovered to be involved in different classic activities. These include up-regulating gene expression, inducing apoptosis after UV irradiation, and suppressing tumour formation[225]. The Knock down of Mdm2, the principal negative regulator of p53, in zebrafish embryos was known to cause extreme apoptosis and early growth arrest. On the other hand, upregulation of Mdm2 in zebrafish liver does not cause hyperplastic livers or liver cancer but lead to liver atrophy[226]. Whether p53 will work similarly in other teleosts is undecided. Interestingly UV failed to activate p53 in the medaka, indicating that p53 behaves distinctively in this species. Even amidst the mammals the probabilities that p53 regulated tumour suppression acts distinctively between mouse and humans have been upraise[227]. Thus, further p53 research work to other fish species is needed. One method to achieve this is to use cell lines, which are accessible from many species[228], and to make use of p53 inhibitors to strive to understand the functions in fish cells.

2.9.1. p53 Activators and Inhibitors

Activators and inhibitors of p53 have been recognized and explored as latest pharmaceuticals for a diverse number of human diseases[229]. Diseases such as atherosclerosis, diabetes, Alzheimer's, Parkinson's, and Huntington's have been associated with action of p53[230]. Nevertheless, the

entire concentration has been focused on cancer treatments because p53 is the most regularly mutated gene in human cancers. Frequently, the p53 mutations in human tumours are missense mutations and lead to the expression of entire mutant p53 protein[231]. Normally p53 moderate tumour repression through arrest of cell cycle, senescence and apoptosis[232] (Eischen & Lozano, 2014). Often these tumour suppressive actions are not visible in the mutant p53. A term described as mutant p53 gain-of-function is as a result of few mutant p53 proteins which acquire oncogenic functions, such as tumour cell proliferation promotion and apoptosis blockage[233].

Two approaches are being employed to target mutant p53s. Inhibitors might suppress the gain - of-functions that aid tumour development. However, the main favorable strategy appears to be to recognize compounds that reactivate or reinstitute the wild type p53 functions[234]. For example, compound NSC-319725 reinstates the structure and function of the R175H mutant p53 and leads to apoptosis in tumour of mice. PRIMA-1 is an additional molecule in the process of investigation, which converts mutant p53 conformation to wild type and sensitizes tumour cells to chemotherapy[235].

Furthermore, drugs that target wild type p53 are being invented to enhance treatments of cancer. Oftentimes irradiation or chemotherapy gets rid of tumour cells by inducing p53 and activating apoptosis. Therefore, wild type activators of p53, such as nutlin, have been invented to improve the process. Also, amidst these therapies the normal cells surrounding the tumour need to be guarded. Therefore, inhibitors of wild type p53 are being designed for use with treatment of cancer to protect healthy cells surrounding tumours. One group of inhibitors is referred to as pifithrin for p53 three inhibitor. The two major types are pifithrin-α2-(2-imino-4,5,6,7-

tetrahydrobenzothiazole-3-yl)-1-p-tolyethanonehydrobromide), with an abbreviation of PFT-α, and pifithrin-μ (2-phenylacetylenesulfonamide), occasionally abbreviated as PFT-μ or PES.

2.9.2. p53 Inhibitor Pifithrin-α (PFT-α)

PFT-α was described originally as a compound that inhibit p53-mediated apoptosis[236]. Subsequently PFT-α was shown to repress the transcriptional activity of p53 by holding back the p53 binding to its DNA sites[237]. PFT-α lessen the activation of p53-regulated genes, including cyclin G, p21WAF1, 14-3-3-σ and MDM2 without converting the amount of p53 protein itself[238]. Several data with adult rats suggested that PFT-α did not have effect on the synthesis of p53 but works by suppressing p53 nuclear translocation and inhibiting its binding to its specific DNA sites[239]. In several research work PFT-α works as designed and protected mammalian cells from apoptosis[240]. For instance, PFT-α has been described to prevent apoptosis in HCT116 cells after gamma irradiation[241] and in neurons after treating it with amyloid β-peptide[242]. Therefore, PFT-α is sold and generally used as an inhibitor of p53 that inhibits p53-dependent transcriptional activation and apoptosis.

Although, few paradoxical or off-target actions of PFT-α have been studied and documented. A cytotoxic effect of PFT-α was seen with the mouse epidermal JBC C1 41 cell line[243], two wild type p53 human tumour cell lines[244] and murine myoblast cell line, C2C12[245].

2.9.3. p53 Inhibitor PES

PES was described in a screen for compounds that would hinder the localization of p53 to the mitochondria and inhibit apoptosis[246]. p53 deactivates anti-apoptotic protein Bcl-xL and Bcl-2 on the mitochondrial outer membrane by producing complexes with them, which will cause MOMP

and release of apoptotic activator proteins naturally[247]. With the help of PES, it blocks apoptosis by forcefully preventing the translocation of p53 to mitochondria and lessening the connection of p53 to anti-apoptotic protein Bcl-xL and Bcl-2. PES did not have effect on the transcriptional activity of p53 but solely p53 mediated apoptosis acting through mitochondria[248].

Prior to its original discovery, PES was described to have effects that potentially not be mediated through p53. B-chronic lymphocytic leukemia (CLL) cells from human patients in a p53 independent way were cytotoxic with PES when they bind[249]. In addition, PES was described as an inhibitor of HSP70. PES was able to bind HSP70 at its C-terminus and interfere with the interaction between HSP70 and its co-chaperones within the human osteosarcoma and melanoma cells. The interruption of HSP70 caused protein accumulation and lysosome membrane disruption which eventually causes cell death with weakened autophagy[250].

2.9.4. p53 Inhibitors and Fish

Inhibitors of p53 seem not to have been used in fish systems. Moreover, the utilization of inhibitors potentially produces insights into the actions of p53 in fish. The understanding of how fish cells acknowledge how p53 inhibitors have an additional function. Drugs that target p53 are noticeably to be more widely utilized in the future and pharmaceuticals that have broad usage have the potential to be released into the aquatic environment. More so, knowledge of how p53 drugs might act on fish cells might help assess the risk of such a release.

2.9.5. Cellular Processes Regulated by p53

The activities of p53 control a web of interconnected cellular processes. The tumour repressive function of p53 is established through p53 cell cycle arrest, senescence and mediating

apoptosis[251]. The response is contingent on the degree of the stress. With minimal stress, activation of p53 triggers cell cycle arrest; with extreme stress, p53 activation results in apoptosis. The stresses include ionizing radiation leading to damage to DNA, chemotherapeutic drugs, and aberrant growth signals[252]. Further extensive cellular processes that triggers p53 regulates are autophagy[253] and endoreplication. Asides stressful conditions, p53 works to control the normal or constitutive activities of the cell required for cellular homeostasis[254]. These include energy metabolism[255] and antioxidant defenses[256].

2.9.6. Transcription-Dependent and Independent p53 Mechanisms

Occasionally p53 control cellular processes by acting as a transcription factor[257], but latest discovery has shown transcription-independent functions for p53 as well[258]. This is evident in cell death mechanisms and in energy metabolism.

Frequently p53 seems to regulate apoptosis by a transcription-dependent mechanism, but p53 can also trigger apoptosis in a transcription-independent manner. With extreme stress, such as DNA damage, p53 activation can also cause apoptosis[259]. Activated p53 controls the transcription of genes vital to apoptosis, such as PUMA, Bax and Noxa. These proapoptotic factors result in the permeabilization of the mitochondrial outer membrane (MOMP). Moreover, p53 can lead to MOMP and apoptosis in a transcription-independent way. During stress, p53 aggregates in the cytoplasm and mitochondria. The p53 executes directly in the outer mitochondrial membrane to dislocate pro-apoptotic proteins from their negative regulators and results in oligomerization and activation of Bak and cytochrome c release.

A transcription-independent action of p53 potentially occurs with another mode of cell death, necrosis[260]. Oxidative stress caused p53 to aggregate in the mitochondrial matrix in mouse embryo fibroblasts. The p53 then interacted with cyclophilin D and activate mitochondrial permeability transition pore (PTP) opening and necrosis.

The participation of p53 in energy metabolism has been shown to be through transcription-dependent and transcription-independent actions. Illustrations are seen in glutamine metabolism and the pentose phosphate pathway (PPP). Mitochondrial glutaminase converts glutamine to glutamate, resulting in the formation of α-ketoglutarate, an intermediate in the tricarboxylic acid cycle (TCA). Expression of the glutaminase gene is triggred by p53. Cytoplasmic p53 can transiently interact with the rate-limiting enzyme of PPP, glucose-6phosphate dehydrogenase (G6PDH), and block its activity.

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Chapter Three

Methodology

3.1. Study Area.

This research was conducted in the Awotan Landfill, located in the city of Ibadan, Oyo-state, Nigeria. The city of Ibadan is the largest settlement (approximately 3123km^2) in tropical Africa, south of theSahara. With an estimated population of 3.4 million people,Ibadan ranks as the third most populatedcity in Nigerian[1]. Ibadan has a tropical climate, which is characterized by two marked seasons: the wet season (April to October) and the dry season (November to March). Ibadan city has amean annual rainfall of about 1150 mm and a mean annual temperature of 25.9^{o}C.

Ibadan consists of eleven Local Government Areas (LGA) – and only four of these LGAs have approved dumpsite/landfills (Figure 3.1). All these approved dumpsites are located in the suburbs, where they receive commingled waste from the entire metropolis: The landfills include; Aba-Eku landfill, Ajakanga landfill, Awotan landfill and Lapite landfill.

Awotan landfill is located onlatitude 7.463^{o}N and longitude 3.849^{o}E (Figure 3.2), along the Apete-Awotan-Akufo Road in Awotan, Ido Local Government Area of Oyo State. Awotan community encompasses quite a number of institutions, commercial firms and residential settlements close to the landfill area. Thisfacility, which is owned by the Oyo State Government, is managed by the Oyo State Waste Management Authority (OYWMA). The Awotan landfill is the oldest landfill in Ibadan and it receives and estimated 78,000 tonnes of waste annually[2]. The landfill was constructed in 1998.The Awotan landfill is the second largest landfill(20.26 hectares) in Ibadan city and it is listed among the50 biggest dumpsites worldwide[3]

Plate 3.1. Picture showing different dumplings at Awotan.

Source: Authors Field Work, 2022.

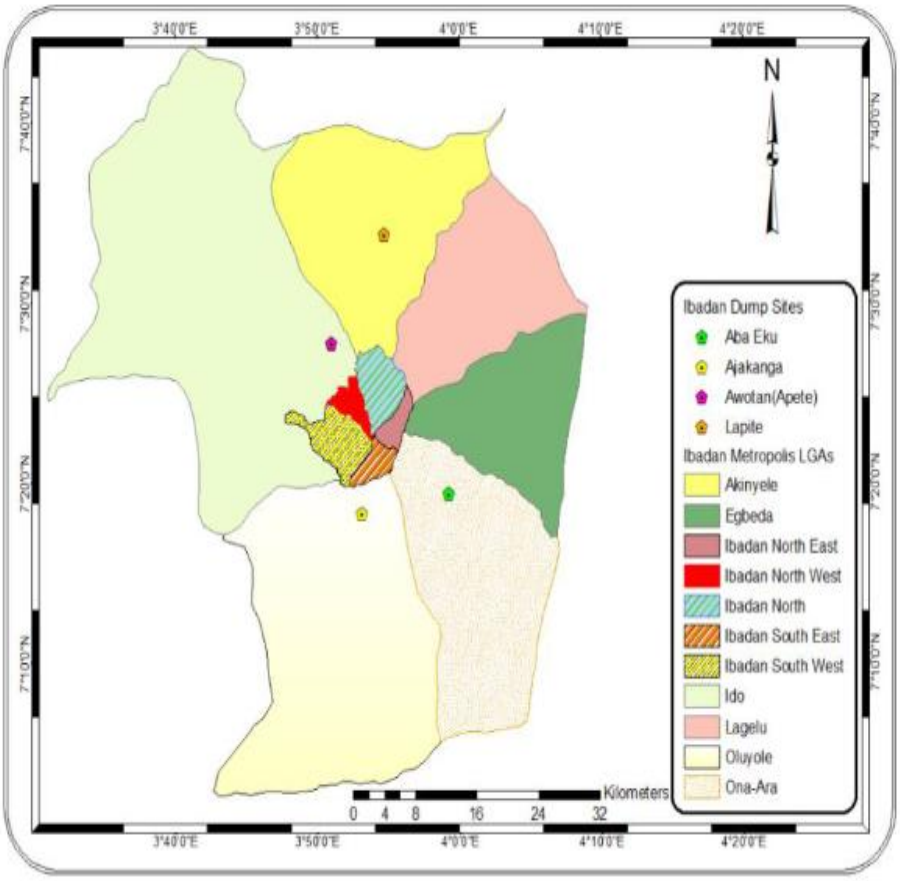


Figure 3.1. Map of Ibadan showing approved landfills

Source: Authors Field Work, 2022.

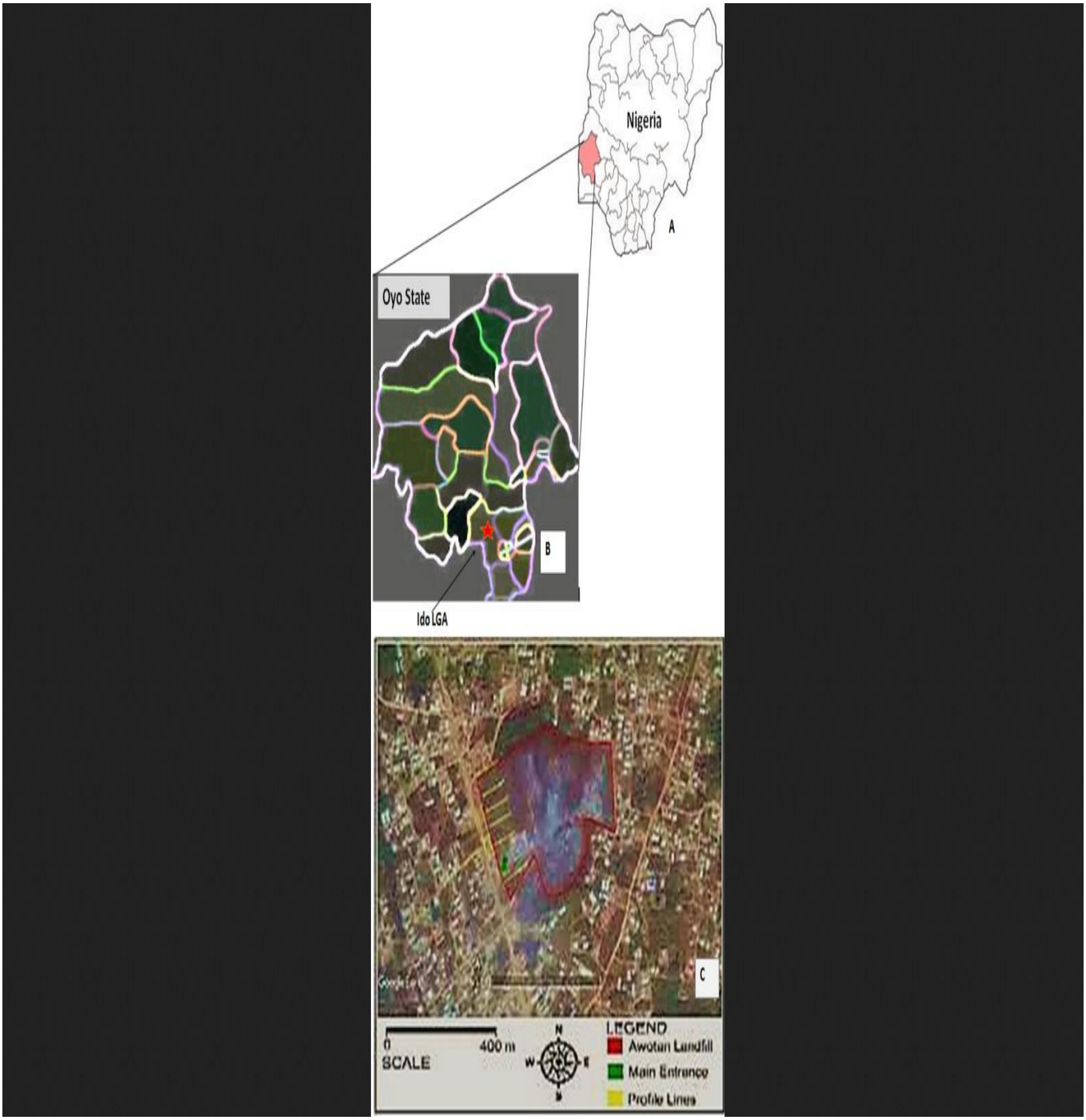


Figure 3.2. Map of Ibadan showing Awotan landfill

Source: Authors Field Work, 2022.

3.2. Leachate Sampling.

Raw leachate samples were collected from the Awotan leachate wells shown in Plate 3.2. The leachate samples were collected in prewashed 50L plastic containers and dark amber colored bottles. All leachates samples were then transported to the laboratory.

In the laboratory, the leachate samples in the prewashed 50L plastics were filtered to remove debris and solid particles. The filtrate was collected in prewashed containers and stored at a 4^{o}C temperature for 48hrs. The samples collected in the amber colored bottles were kept in a dark cupboard for five days for the measurement of Biological Oxygen Demand (BOD). After 48hrs, the leachate samples were labeled as stock solutions and diluted samples were prepared from the stock solutions following the standard procedure for short term static bioassay as described by Bailey[4].

The physical and chemical properties of the leachate samples were measured using the standard procedures described by APHA[5]. Chemical parameters such as pH, Dissolved Oxygen (DO), Electrical Conductivity (EC), and Total Dissolved Solids (TDS) were measured using a well calibrated multi 3630 digital meter. Other chemical parameters such as Chloride (Cl), Nitrate (NO_3), Nitrite (NO_2), Sulphate (SO_4), Phosphate (PO_3), Alkalinity (as $CaCO_3$), Acidity (as $CaCO_3$), and water Hardness (as $CaCO_3$) were measured using the standard titrimetric methods. Biological Oxygen Demand (BOD) was calculated by measuring the DO of the leachate samples collected in the amber colored bottle on the fifth day and subtracting it from the DO measured on the day of collection. Temperature was also measured using the multi 3630 digital meter, while turbidity was measured using a Secchi Disc. The concentration of six heavy metals including Cu, Cd, Mn, Fe, Pb, and Zn was measured using the Atomic Absorption Spectrophotometry (AAS) method as described by Welz & Sperling[6].

Plate 3.2. Picture showing leachate section of Awotan landfill.

Source: Authors Field Work, 2022.

3.3. Collection of Fish Samples

Fingerlings of *Clarias gariepinus* were obtained from the University of Ibadan Aquaculture Department. A total of 259 fingerlings of *Clarias garipienus* were obtained and transported to the animal house in Lead City University, Ibadan. The fingerlings were transported to the laboratory in an exposed 50L keg filled halfway with chlorine-free borehole water. In the laboratory, the fishes were acclimatized in 40L plastic containers at 26^0c for 14 days, during which they were fed twice a day with 5g of commercial fish feeds (Plate 3.3) sourced from the Departmentof Aquaculture and Fisheries Management at the University of Ibadan During this period, the water housing the fishes were changed every other day to ensure that the amount of oxygen within the water does not deplete to dangerous levels as a result of accumulation of metabolic wastes. At the end of the acclimation period, the fishes have grown in size, with their mean weight reaching 3.01 ± 0.33g on the 14^{th} day of acclimation. Throughout the acclimation period no fish mortality was recorded.

3.4. Toxicity Assessment Setup

After the acclimation phase, the experimental phase was initiated by setting up five treatment plastics, four of which werefilled with different concentrations of leachates from the Awotan landfill, and one filled with dechlorinated tap water. The leachate concentrations used include 5%, 10%, 25% and 50%. Three replicates were created for each of the treatment setup, hence a total of fifteen treatment plastics were set up. In each treatment plastic, fifteen samples of *Clarias gariepinus* were introduced and were frequently observed for changes in behavioural patterns such as abnormal behavior, rolling movement, air gulping, skin discoloration, loss of reflex, erratic swimming and finally mortality for a period of 21 days.

Plate 3.3. Picture showing weighing of fish meal

Source: Authors Field Work, 2022.

During the experiment phase, the fishes were thoroughly monitored for behavioural and physiological changes, as well as mortality every hour. A fish was considered dead if it shows no noticeable opercula movement when prodded using a glass probe.

3.5. Histopathology Analysis

For the histopathology analysis, four juveniles from each treatment were randomly selected and sacrificed for the procedure. All selected fishes were dissected by carefully opening their abdominal cavities and removing the liver. The removed livers were fixed in 10% buffered formalin before being subjected to histopathology analysis.

3.6. Haematological Analysis

For the haematological analysis, four fishes from each group were also subjected to a similar procedure as in the histopathology analysis. Blood samples of the test organism were taken from the tail region using caudal puncture and transferred into an EDTA bottle and immediately covered to prevent clotting of blood. The blood samples were then taken for analysis at the Veterinary Laboratory in the University of Ibadan.

3.7. Molecular Analysis

For the molecular analysis, four fishes were also selected from each test group and their blood samples were collected in clean and well labeled containers. The blood samples were taken from the tail region using caudal puncture and transferred into an EDTA bottle and immediately covered to prevent clotting of blood. The blood samples were then transported to the laboratory for genomic DNA extraction, polymerase chain reaction (PCR) analysis and sequencing.

3.8. Statistical Analysis

Descriptive statistics such as mean and standard deviation were used to analyse and present the physico-chemical and heavy parameters of the leachate samples. The One-Way ANOVA and Dunn Post-hoc inferential statistics were used to establish the toxicity of different leachate concentrations on the haematological features of *Clarias gariepinus*.

Endnotes

1. A.A. Ayorinde, A. Adefioye, O. Oladipo, & O.O. Fabiyi, *Flood Risk Management Projects*. Handbook of Flood Risk Management in Developing Countries. 2023.

2. C. Ogbonna, F. Nwafor, & E.A. Ugbogu, *Physiochemical Properties and Anticipated Performance of Selected Plant Species in Lokpaukwu Quarry Site in Abia State, Nigeria*. **Journal of Environmental Pollution Human Health**, 7(1), 2019, 7-14.

3. A. Maalouf, & A. Mavropoulos, Re-Assessing *Global Municipal Solid Waste Generation*. **Waste Management & Research**, 41(4), 2023, 936-947.

4. D.P. French-McCay, T.F. Parkerton, & B. De Jourdan, *Bridging the Lab to Field Divide: Advancing Oil Spill Biological Effects Models Requires Revisiting Aquatic Toxicity Testing*. **Aquatic Toxicology**, 2023, 106389.

5. M. Tumolo, V. Ancona, D. De Paola, D. Losacco, C. Campanale, C. Massarelli, & V.F. Uricchio, *Chromium Pollution in European Water, Sources, Health Risk, and Remediation Strategies: An Overview*. **International Journal of Environmental Research and Public Health**, 17(15), 2020, 5438.

6. J.C. Gosse, & F.M. Phillips, *Terrestrial in Situ Cosmogenic Nuclides: Theory and Application*. **Quaternary Science Reviews**, 20(14), 2001, 1475-1560.

Chapter Four

Results and Discussion of Findings

4.1. Physico-chemical parameters of Leachates

The values for the measured physico-chemical parameters are shown in Table 4.1. The odor and taste of the leachate samples were objectionable as opposed to the SON and USEPA recommended standards. The values recorded for pH, chloride, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were all within the SON and USEPA recommended standards. The values reported for turbidity, nitrate, nitrite, sulphate, water hardness, electrical conductivity, and total dissolved solids all exceeded the SON and USEPA recommended standards.

4.2. Heavy Metal Content of Leachates

The concentration of selected heavy metals measured in the leachate samples from the Awotan Landfill in comparison with the SON and USEPA recommended limits is shown in Table 4.2. Heavy metals such as chromium, lead and cadmium were undetected in leachate samples. Heavy metals such as copper, manganese and iron were detected in very high concentrations exceeding the SON and USEPA recommended standards.

4.3. Effects of Leachates on the Haematology of *Clarias gariepinus*

Table 4.3 shows the effect of the various concentrations (5%, 10% 25%, 50%, and 0%) of leachate on selected haematological features of *Clarias gariepinus*. The Packed Cell Volume (PCV), Hemoglobin (Hb) and Red Blood Cell (RBC) all had their highest value in the control set up and their lowest value in 50% leachate.

Table 4.1. Physico-Chemical Parameters of Leachates from Awotan Landfill

Parameters	Mean ± SD	SON	USEPA
Odour	objectionable	Unobjectionable	Unobjectionable
Taste	objectionable	Unobjectionable	Unobjectionable
pH	7.75 ± 0.12	6.5 – 8.50	6-9
Turbidity	7.0 ± 1.2	0-5	0-5
Chloride (Cl) mg/L	200 ± 2.2	250	250
Nitrate (NO_3) mg/L	87.00 ± 5.7	0-50	10
Nitrite (NO_2) mg/L	0.90 ± 3.1	0-0.2	0-0.2
Sulphate (SO_4) mg/L	940.00 ± 10.3	100	250
Phosphate (mg/L)	3.134 ± 0.11	-	0.1
Alkalinity (as $CaCO_3$) mg/L	4680.00 ± 15.33	-	-
Acidity (as $CaCO_3$) mg/L	80.00 ± 7.4	-	-
Hardness (as $CaCO_3$) mg/L	740.00 ± 12.1	150	180
Total Dissolved Solids (mg/L)	2450 ± 14.4	500	500
Total Suspended Solid (mg/L)	130.00 ± 7.7	-	-
Total Solid (mg/L)	2580.00 ±13.2	-	-
Conductivity (µS/cm)	4200 ± 13.0	1000	1000
BOD (mg/L)	1.30 ± 0.03	6	6
COD (mg/L)	115.60 ± 6.4	30	30

SON (Standard Organization of Nigeria)

USEPA (United States Environmental Protection Agency)

Table 4.2. Heavy Metal Content of Leachates from Awotan Landfill

Parameters	Mean	SON	USEPA
Copper (mg/L)	9.28 ± 3.2 *	1.00	1.00
Chromium (mg/L)	0.0 ± 0.0	0.05	0.05
Lead (mg/L)	0.0 ± 0.0	0.01	0.015
Manganese (mg/L)	45.64 ± 6.3 *	-	0.05
Cadmium (mg/L)	0.0 ± 0.0	0.003	0.005
Iron (mg/L)	604.48 ± 10.62 *	0.30	0.30

SON (Standard Organization of Nigeria)

USEPA (United States Environmental Protection Agency)

*** Lower or higher than recommended limit**

Table 4.3. Haematological Parameters of *Clarias gariepinus*

Parameters	5%	10%	25%	50%	Control	F	p
PCV	23.25 ± 0.26 ^{a,e}	28.08 ± 1.06 ^{a,b,c}	25.08 ± 0.58 ^{b,d,f}	22.05 ± 0.58 ^{c,d,g}	29.00 ± 0.34 ^{e,f,g}	34.83 ***	1.986×10^{-6}
Hb	7.3 ± 0.37 ^{a,b}	8.7 ± 0.08 ^{a,c}	8.4 ± 0.49	7.2 ± 0.11 $^{c, d}$	9 ± 0.04 $^{b, d}$	38.54 ***	1.00×10^{-7}
RBC(x10^{6}uL)	1.73 ± 0.07 ^{a}	2.14 ± 0.03 $^{a, b, c}$	1.80 ± 0.10 ^{b}	1.66 ± 0.02 $^{c, d}$	2.24 ± 0.01 ^{d}	76.94 ***	8.23×10^{-7}
WBC(x10^{3}uL)	10.6 ± 0.61 $^{a, b}$	13.8 ± 0.34 $^{a, c}$	12.15 ± 0.04	11.45 ± 0.08 $^{c, d}$	13.65 ± 0.03 $^{b, d}$	70.26 ***	1.568×10^{-9}
Platelet (uL)	102.67 ± 1.53 $^{a, b}$	119.01 ± 0.09 ^{a}	112.02 ± 0.03 ^{c}	122.98 ± 0.71 $^{b, c, d}$	112.03 ± 0.06 ^{d}	230.7 ***	2.83×10^{-13}
LYM (%)	62.01 ± 0.35 $^{a. b}$	62.10 ± 0.17 $^{c, d}$	67.09 ± 0.09 $^{a, c}$	63.11 ± 0.08	64.00 ± 0.20 $^{b. d}$	424.9 ***	3.09×10^{-15}
HET (%)	33.02 ± 0.08 $^{a, b, c}$	30.98 ± 0.10 ^{d}	25.01 ± 0.07 $^{a, d}$	28.01 ± 0.06 ^{b}	28.02 ± 0.07 ^{c}	6196 ***	6.18×10^{-24}
MON (%)	0.00 ± 0.00 $^{a, b}$	2.02 ± 0.07 ^{c}	5.00 ± 0.08 $^{a, c, d}$	4.00 ± 0.01 ^{b}	2.03 ± 0.05 ^{d}	17.37 ***	0.001525
EOS (%)	4.05 ± 0.06 ^{a}	5.00 ± 0.05 $^{b, c}$	3.09 ± 0.03 $^{a, b, d}$	4.05 ± 0.06 ^{d}	3.12 ± 0.04 ^{c}	1076 ***	3.05×10^{-18}
BA (%)	1.00 ± 0.01 $^{a, b. c}$	0.00 ± 0.00 $^{a, d}$	0.00 ± 0.00 $^{b, e}$	1.00 ± 0.01 $^{d, e, f}$	0.00 ± 0.00 $^{c, f}$	13.73 ***	0.00146

***** Highly significant variation**

Means with similar superscripts a, b, c, d, e, and f shows that their values significantly differ from one another.

All three parameters also showed a significant variation across all concentrations as indicated by p values < 0.05 obtained through the one-way ANOVA test. For PCV, the Dunn-post hoc test further revealed that values recorded at each concentration significantly differ from other concentrations. For the Hb test, the Dunn-post hoc test showed that the Hb values recorded in 5% and 50% concentration significantly differs from those recorded in 10% and 0%. For RBC, the Dunn Post-Hoc test showed that values recorded in 10% significantly differs from the values recorded in 5%, 25% and 50%, while the value recorded in 50% significantly differ from the values recorded in 0%.

The number of White Blood Cells (WBC) was highest in 10% leachate and lowest in 5% leachate. The one way ANOVA test showed a significant difference across the various concentrations. The Dunn Post-Hoc test further revealed that the values recorded at 5% and 50% significantly differs from the values recorded at 10% and 0%. Platelets had the highest value in 50% leachate and the lowest value in 5% leachate. The one way ANOVA test showed a significant difference across the various leachate concentrations. The Dunn-Post Hoc test further revealed that the values recorded in 50% leachate differs from the values recorded in 10%, 25% and 0% leachate. Also, the value reported in 5% leachate significantly differs from the values recorded in 10% leachate.

Lymphocytes (LYM) and Monocytes (MON) both had their highest counts in 25% leachate concentration and their lowest count in 5% leachate concentration. Both parameters showed a significant variation across the various leachate concentrations as shown by the one-way ANOVA test. For LYM, the Dunn Post-Hoc test showed that the values recorded in 5% and 10% leachate concentrations significantly differ from the values reported in 25% and 0%.

Heterophils (HET) were recorded in 5% leachate concentration and the lowest value in 25% leachate concentration. The one-way ANOVA test showed that the values recorded across the different concentrations varied significantly. The Dunn Post-Hoc test further revealed that the values at 5% significantly differ from the values recorded at 25%, 50% and 0%, while the values recorded at 10% significantly differ from those recorded at 25%. Eosinophils (EOS) recorded the highest value at 10% and the lowest value at 25%. The one-way ANOVA test showed that there was a significant difference in the values recorded across various concentrations. The Dunn Post-Hoc test further revealed that the values recorded at 25% significantly vary with the values recorded at 5%, 10% and 50%. Also, the values recorded at 10% significantly vary with those reported at 0%. Basinophilis (BA) was only detected in 5% and 50% where it had similar counts. The one-way ANOVA and the Dunn Post-Hoc test showed that the values recorded at 5% and 50% vary significantly with the values recorded across other concentrations.

4.4. Behavioural Response of *Clarias gariepinus* to Varying Concentrations of Leachate

The behavioural response of *Clarias gariepinus* to various concentrations of leachates at different time periods is shown in Table 4.4 Five behavioural patterns including rolling movement, air gulping, skin coloration, loss of reflex, and erratic swimming was observed. At 6 hours, mild erratic swimming was noticed in all concentrations except the control.

4.4.1. Rolling Movement

At 6hours, rolling movement was mildly observed in LC_{25} and LC_{50} only. This continued until 108hours where rolling movement was also mildly observed in LC_{10}. The mild rolling movement continued in LC_{10}, LC_{25} and LC_{50} till 168 hours. Rolling movement was not observed at all in control and LC_5 for the whole 144 hours. .

Table 4.4. Behavioural Response of *Clarias gariepinus*

Concentration %	Rolling movement	Air gulping	Skin discoloration	Loss of reflex	Erratic swimming
6hr					
Control	-	-	-	-	-
5	-	-	-	-	+
10	-	-	-	-	+
25	+	+	-	-	+
50	+	+	-	+	+
12hr					
Control	-	-	-	-	-
5	-	-	-	-	-
10	-	-	-	+	-
25	+	+	-	+	+
50	+	+	-	+	+
24hr					
Control	-	-	-	-	-
5	-	-	-	-	-
10	-	-	-	+	-
25	+	+	-	+	+
50	+	+	-	+	++
36hr					
Control	-	-	-	-	-
5	-	-	-	-	-
10	-	-	-	+	-
25	+	+	-	+	-
50	+	+	-	+	++
48 hrs					
Control	-	-	-	-	-
5	-	-	-	-	-
10	-	++	-	+	-
25	+	++	-	+	+
50	+	++	-	+	++
60hr					
Control	-	-	-	-	-
5	-	-	-	-	-
10	-	-	-	+	+
25	+	+	-	+	+
50	+	++	+	+	++

72hr					
Control	-	-	-	-	-
5	-	-	-	-	-
10	-	-	-	+	+
25	+	+	-	+	+
50	+	++	+	+	++
84hr					
Control	-	-	-	-	-
5	-	-	-	+	+
10	-	-	-	+	+
25	+	+	+	+	+
50	+	+	+	+	+
96hr					
Control	-	-	-	-	-
5	-	-	-	+	+
10	-	-	-	+	+
25	+	+	+	+	+
50	+	+	+	+	+
108hr					
Control	-	-	-	-	-
5	-	-	-	+	+
10	+	-	-	+	+
25	+	+	+	+	+
50	+	+	+	+	+
120hr					
Control	-	-	-	-	-
5	-	-	-	+	+
10	+	+	-	+	+
25	+	+	+	+	+
50	+	+	+	+	+
132hr					
Control	-	-	-	-	-
5	-	-	-	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
144hr					
Control	-	-	-	-	-
5	-	+	-	+	+

10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
156hr					
Control	-	-	-	-	-
5	-	+	-	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
168hr					
Control	-	-	-	-	-
5	-	+	-	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+

- No observation, + Mild observation, ++ Strong observation

4.4.2. Air gulping

Mild observations of air gulping were made in LC_{25} and LC_{50} from 6 hours till 36 hours. At 48 hours, strong observations of air gulping were made in LC_{10}, LC_{25} and LC_{60}. Strong observation of air gulping continued in LC_{50} only till 72 hours, where mild observation of air gulping was made in LC_{10}, LC_{25} and LC_{60} till 132hr. At 144hr, mild observation of air gulping was made in LC_5 for the first time and persisted until 168 hours. Air gulping was not observed in the control throughout the whole period.

4.4.3. Skin discoloration

The first observation of skin discoloration was made after 60 hours in LC_{50}. The observation was mild and persisted till 168 hours. Mild observations of skin discoloration was also made in LC_{25} at 84 hours and also persisted till 168 hours.

4.4.4. Loss of Reflex

Mild observations of reflex loss were first made in LC_{50} at 6 hours. At 12 hours mild observations were made in LC_{10}, LC_{25} and LC_{50} and this persisted till 168 hours. A mild observation of loss of reflex was first made in LC_5 at 84hours and persisted till 168 hours.

4.4.5. Erratic swimming

Erratic swimming was mildly observed in all concentrations except control at 6hrs, and persisted in LC_{25} and LC_{50}. At 24hrs strong observations of erratic swimming were made in LC_{50} and it continued until 72hrs. At 84 hours, mild observation of erratic swimming in all concentrations except control was made, and it persisted till 168 hours.

4.5. Effect of Leachate on *Clarias gariepinus* Mortality

Table 4.5 and Figure 3 show the rate of fish mortality based on their exposure to different concentrations of leachates for a given number of hours. At six and 12 hours of exposure, no mortality was detected for all leachate concentration. One death was recorded in the L_C leachate solution at 24hrs of exposure. At 36 hours of exposure, no mortality was recorded. At 48 hours of exposure, only one death was recorded in the LC_{50}. At 60 hours of exposure one death was recorded in L_{25} and another one in LC_{50}. Zero mortality was recorded at 72, 84 and 96 hours of exposure. At 108 hours of exposure, two deaths were recorded in L_{50}. At 120 of exposure one death was recorded in L_{50}. At 132 hours of exposure, one death was recorded in LC_{50}. At 144hours of exposure, one death was recorded in LC_{25} and another recorded in LC_{50}. At 156 hours of exposure, one death was recorded in LC_{25} and another one recorded in LC_{50}. At 168 hours of exposure, one death was recorded in LC_{25} and another two were recorded in LC_{50}.

4.6. Effect of Leachates on the Histology of *Clarias gariepinus*

The effect of the various leachates on the histology of *Clarias gariepinus* is shown in plates4.1-4.6. Plate 1 shows the tissues of the liver were unaffected at LC_0. Plate 2 showed mild signs of necrosis at LC_5. Plates 3 and 4 also show mild signs of necrosis at LC_{10}. Plate 5 shows intense cytoplasmic vacuolation at LC_{25}, while Plate 6 shows severe steatosis in the liver tissues at LC_{50}.

Table 4.5. Effect of Leachate on *Clarias gariepinus* Mortality

Mortality time (hrs)	L_C	L_5	L_{10}	L_{25}	L_{50}	Total
6	0	0	0	0	0	**0**
12	0	0	0	0	0	**0**
24	0	0	0	0	0	**0**
36	0	0	0	0	0	**0**
48	0	0	0	0	1	**1**
60	0	0	0	1	1	**2**
72	0	0	0	0	0	**0**
84	0	0	0	0	0	**0**
96	0	0	0	0	0	**0**
108	0	0	0	0	2	**2**
120	0	0	0	0	1	**1**
132	0	0	0	0	1	**1**
144	0	0	0	1	1	**2**
156	0	0	0	1	1	**2**
168	0	0	0	1	2	**3**
Total	**0**	**0**	**0**	**4**	**10**	**14**

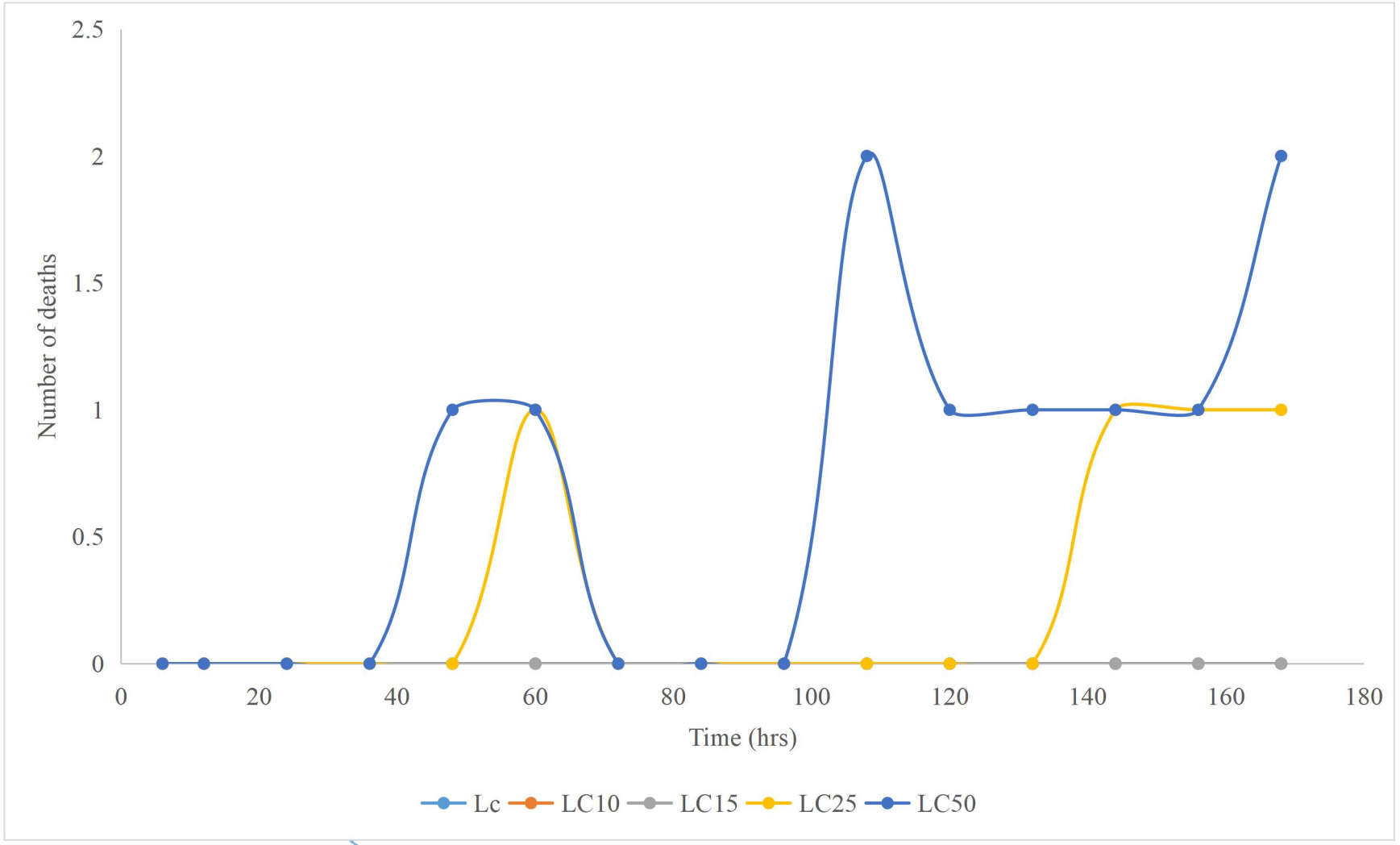
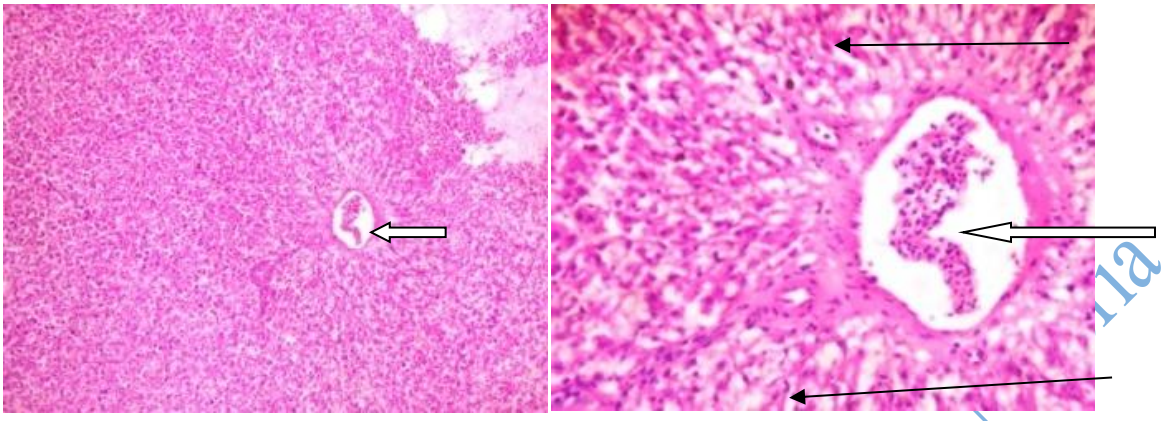
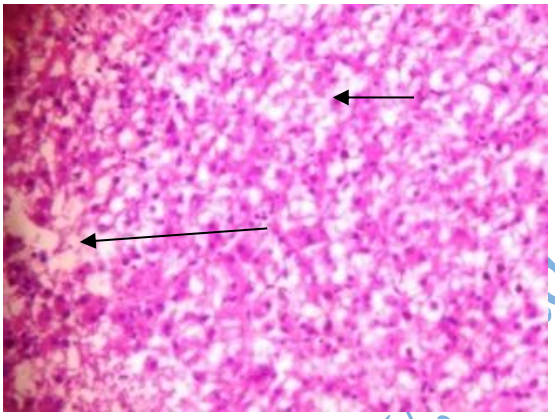


Figure 4.3. Mortality of *Clarias gariepinus* based on time of exposure to varying leachate concentrations

0%



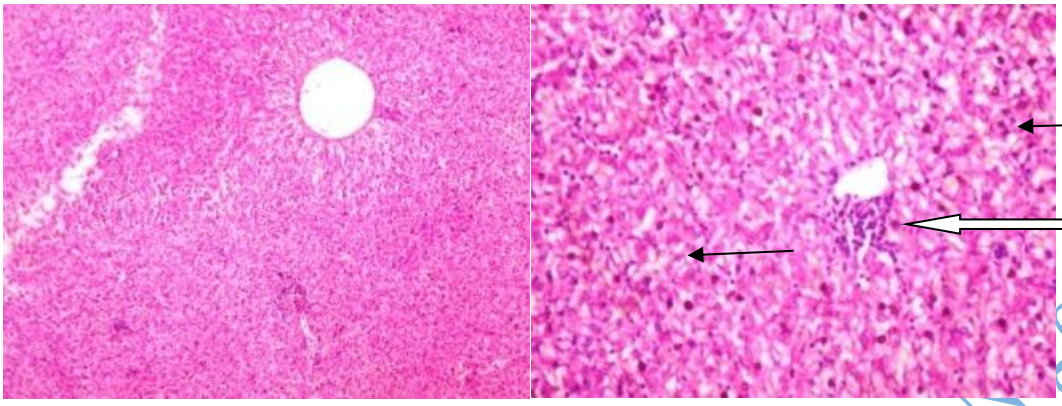
X100 x400



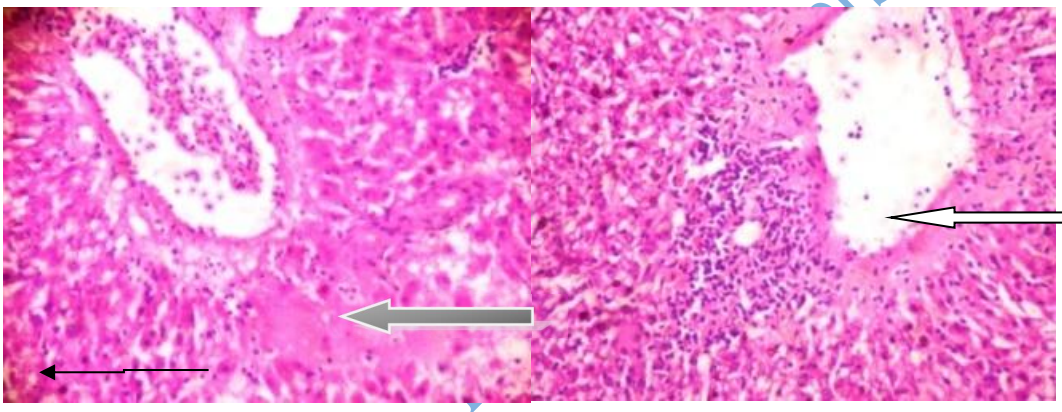
X400

Plate 4.1. Photomicrograph of a fish liver section stained by H&E, showing normal uncongested venule (white arrow), however, the liver hepatocytes show cytoplasm with glycogen infiltration (slender arrow)

5%



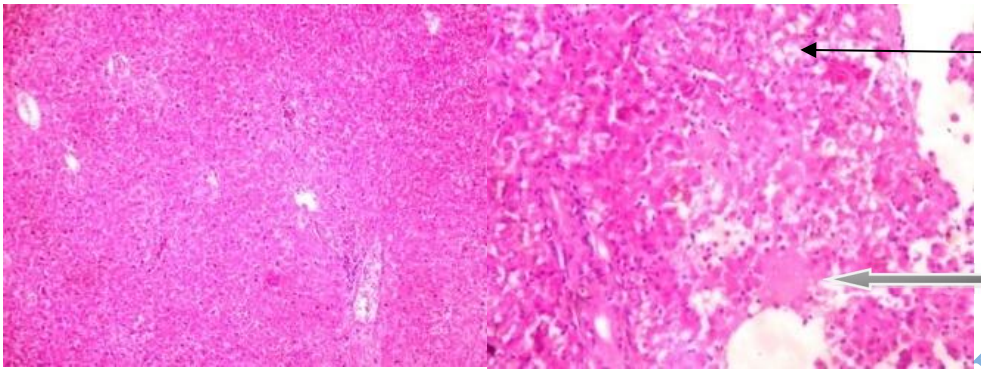
X100 x400



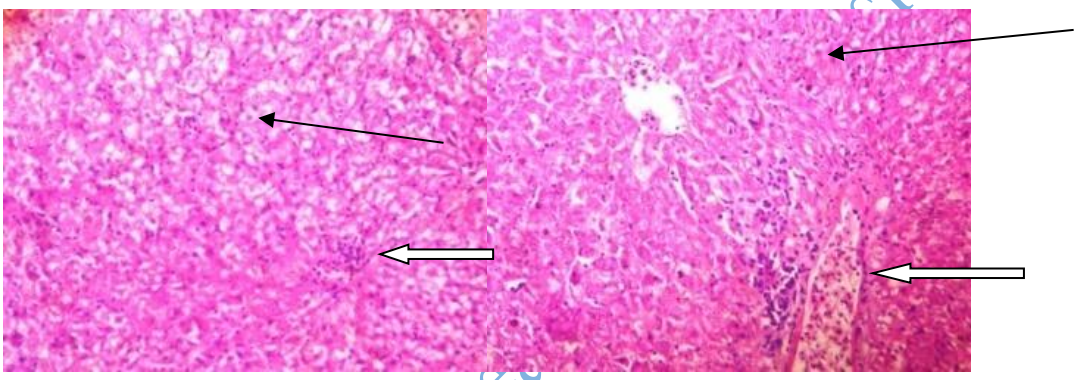
X400 x400

Plate 4.2. Photomicrograph of a fish liver section stained by H&E, showing venule with moderate perivascular infiltration of inflammatory cells (white arrow), there is focal area of necrosis seen (green liver) hepatocytes show cytoplasm with glycogen infiltration (slender arrow)

10%



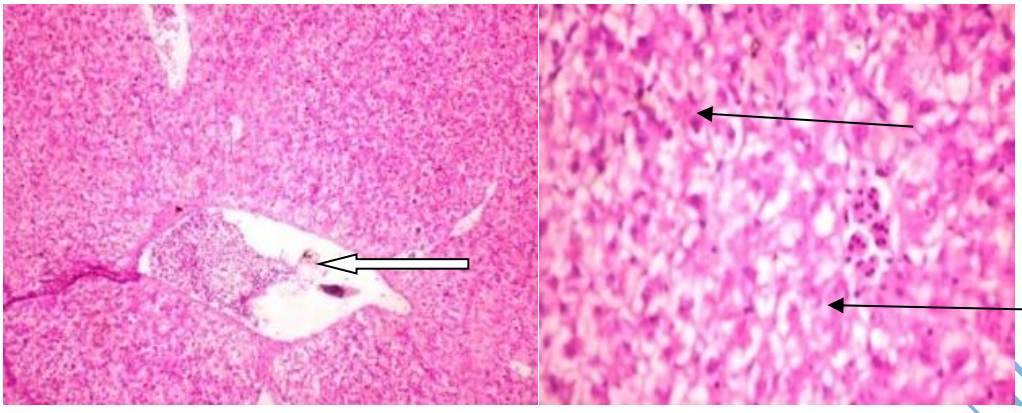
X100 x400



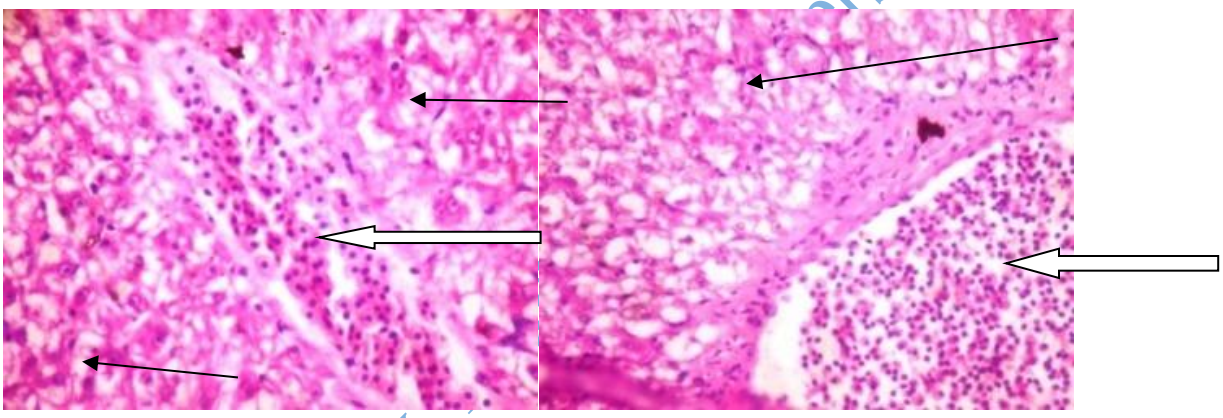
X400 x400

Plate 4.3. Photomicrograph of a fish liver section stained by H&E, showing venule with moderate perivascular infiltration of inflammatory cells (white arrow), there is focal area of mild necrosis seen (green liver) hepatocytes show cytoplasm with glycogen infiltration (slender arrow)

10%



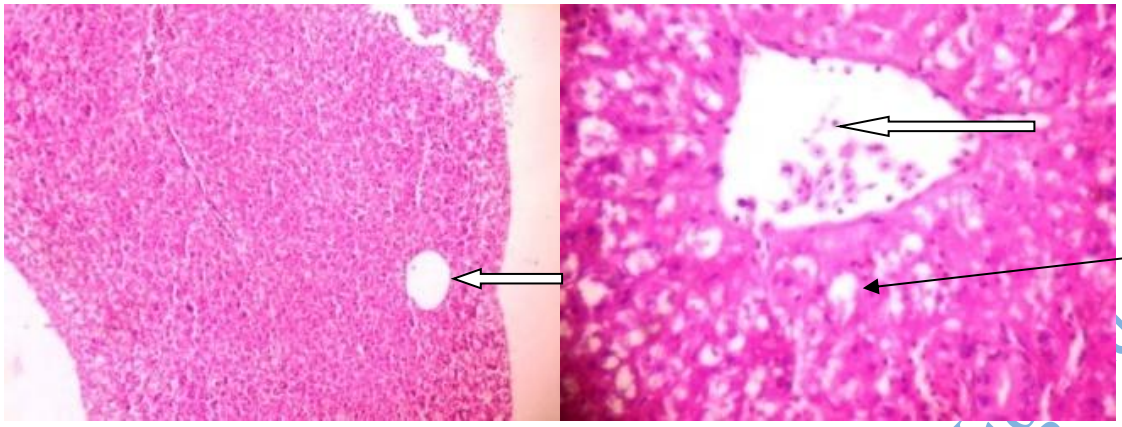
X100 x400



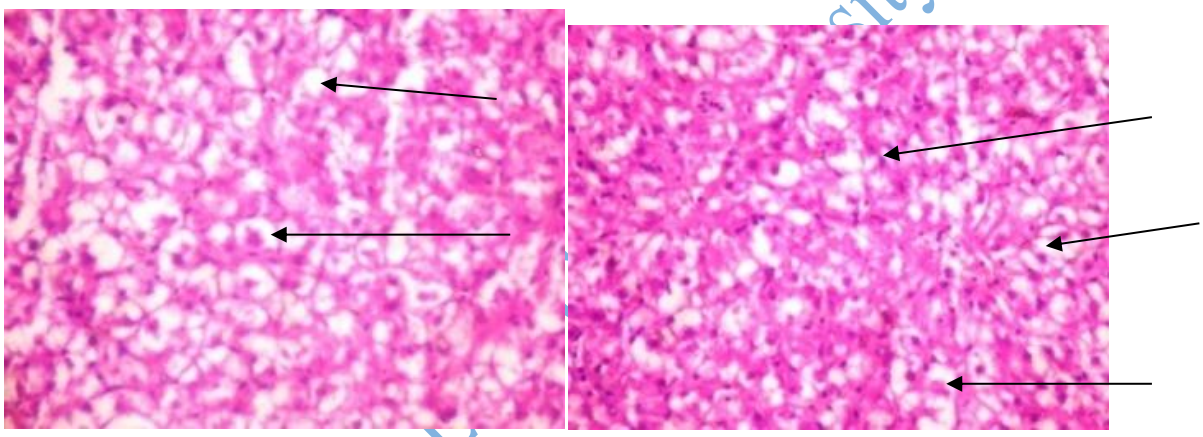
X400 x400

Plate 4.4. Photomicrograph of a fish liver section stained by H&E, showing venules with mild congestion (white arrow), the hepatocytes morphology show cytoplasmic vacuolation with abundant glycogen infiltration (slender arrow)

25%



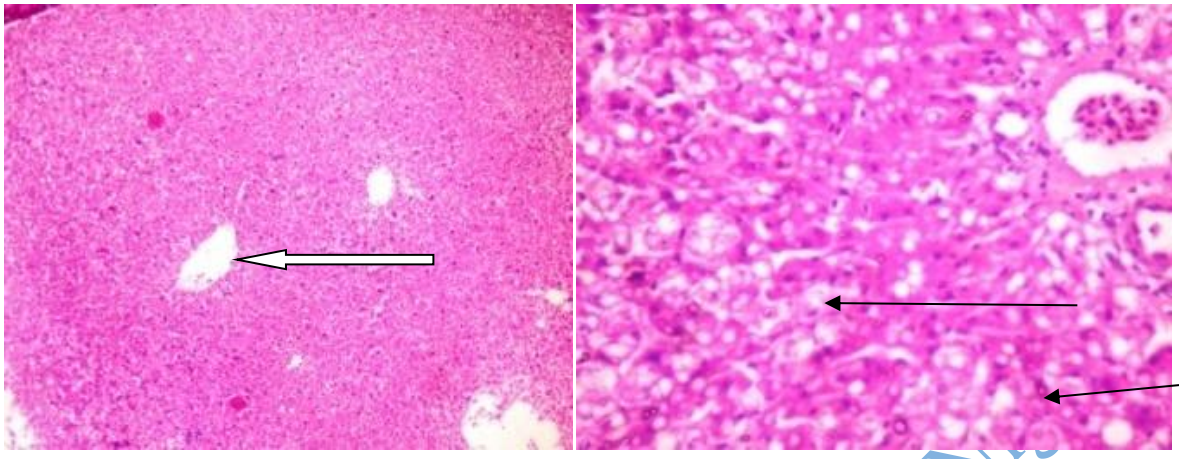
X100 x400



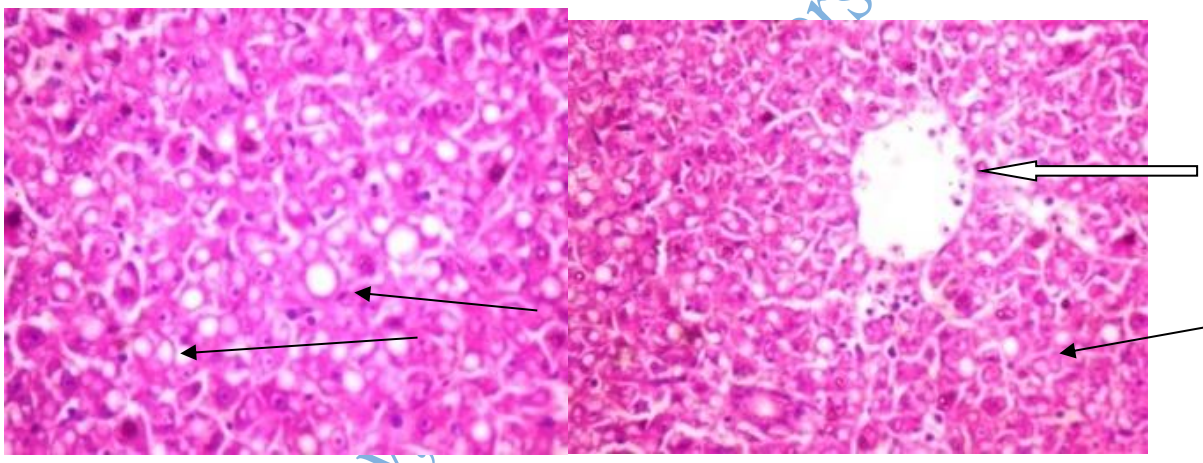
X400 x400

Plate 4.5. Photomicrograph of a fish liver section stained by H&E, showing normal venules without congestion (white arrow), the hepatocytes morphology shows cytoplasmic vacuolation with severe fat and glycogen infiltrating the cytoplasm (slender arrow).

50%



X100 x400



X400 X400

Plate 4.6 Photomicrograph of a fish liver section stained by H&E, showing normal venules without congestion (white arrow), and the hepatocytes morphology show severe steatosis; there is severe infiltration of liver cell cytoplasm by fat (slender arrow)

4.7. Polymerase Chain Reaction (PCR) Analysis on the P53 Gene in *Clarias gariepinus*

Figure 4.4 diagram reveals the gel electrophoresis of Polymerase chain reaction (PCR) on the tumor protein p53-inducible nuclear protein 2 (tp53inp2) genes. Detection of p53 gene by PCR using primer 5'GGATGGAGAGACAGAGAGAGAT3'and 3'AGAGGTGAGGGACAGAAAGA5' (forward and riverse primer). PCR analysis showed that 1,2,3,4,5, gave a specific amplification band of 700bp which is identical to Marker M. However, DNA band was found to be less thicken in well 1 and 2 representing the control experiment and 5% leachate treatment, which is suggesting smaller size of the DNA. This could be due to various factors, such as the presence of different alleles, mutations, or variations within the target region being analyzed. The well (3, 4, and 5) with a leachate concentration of 10%, 25% and 50% has a thick band at 700 base pairs meaning they are of similar sizes.

4.8. Effect of Leachates on p53 genes in *Clarias gariepinus*

Table 6 shows the evolutionary distance between the p53 genes in *Clarias gariepinus* samples and those of other selected species. In the control and all the test groups (5%, 10%, 25% and 50%), the distance recorded for the p53 gene in *Clarias gariepinus* is 0.000 which is significant, i.e the genes are present and identical. The distance however diverges in comparison to other species, the paire waise distance is pointing at evolutionary distance between the genes of *Clarias garipienus* and that of the selected species. Also, figure 4.5 showed that the phylogenetic tree of *Clarias garipienus* and other selected species of fishes and human. This results shows that the phylogenetic relationship of *Clarias garipienus* is far away from human (*Homo sapiens*). Importantly, it's seen that only the p53 of *Notobrnachius khuntae* have close evolutionary relationship with Clarias garipienus. This can indicate that the species are subjected to same environmental pressure or mutate at some region.

However, figure 4.6 helped us to understand the multiple alignment sequence of p53 genes in *Clarias gariepinus* and some selected species.

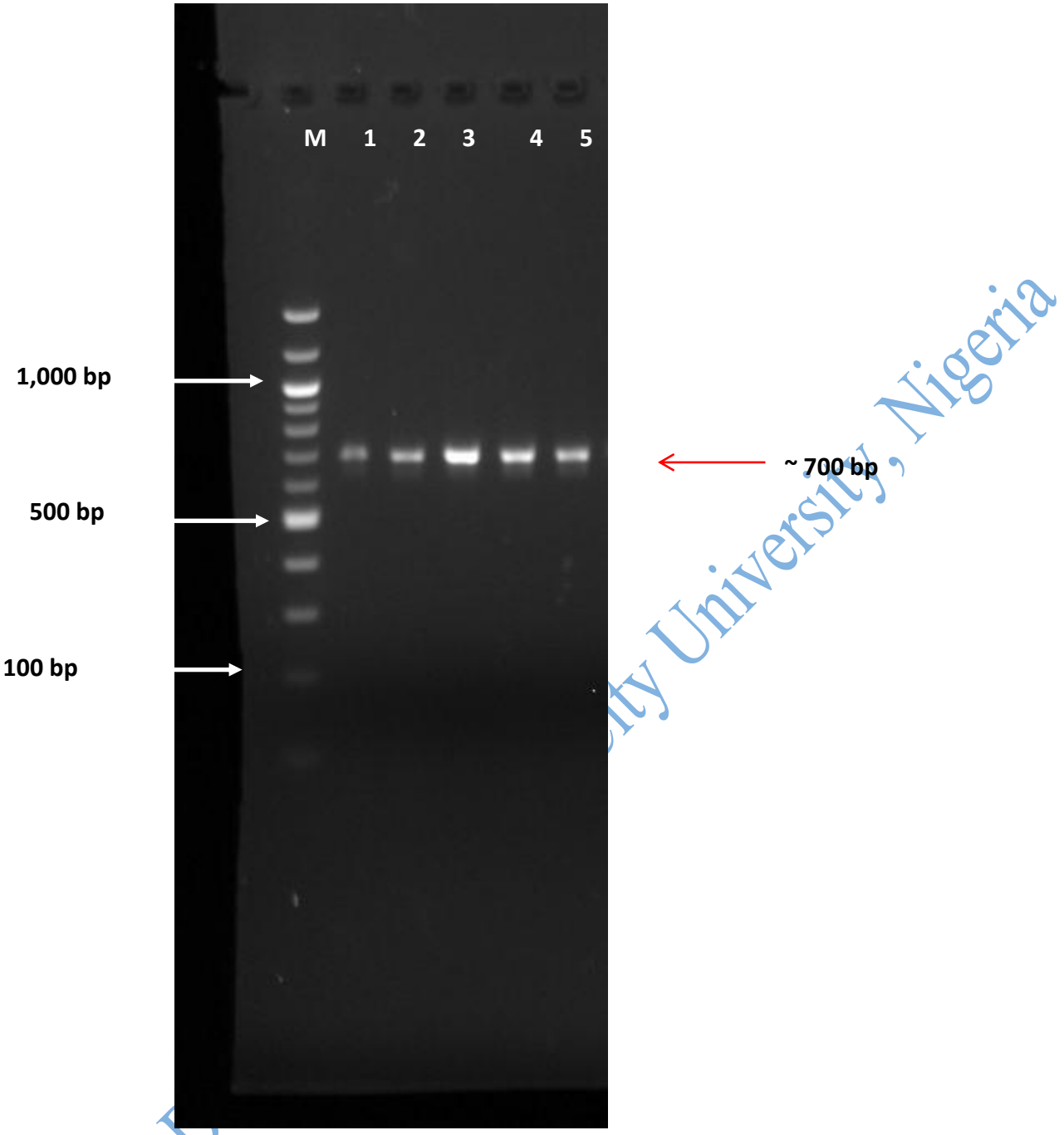


Figure 4.4: Gel electrophoresis of Polymerase chain reaction (PCR) on the tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene from *Clarias gariepinus* fed with Awotan landfill leachates.

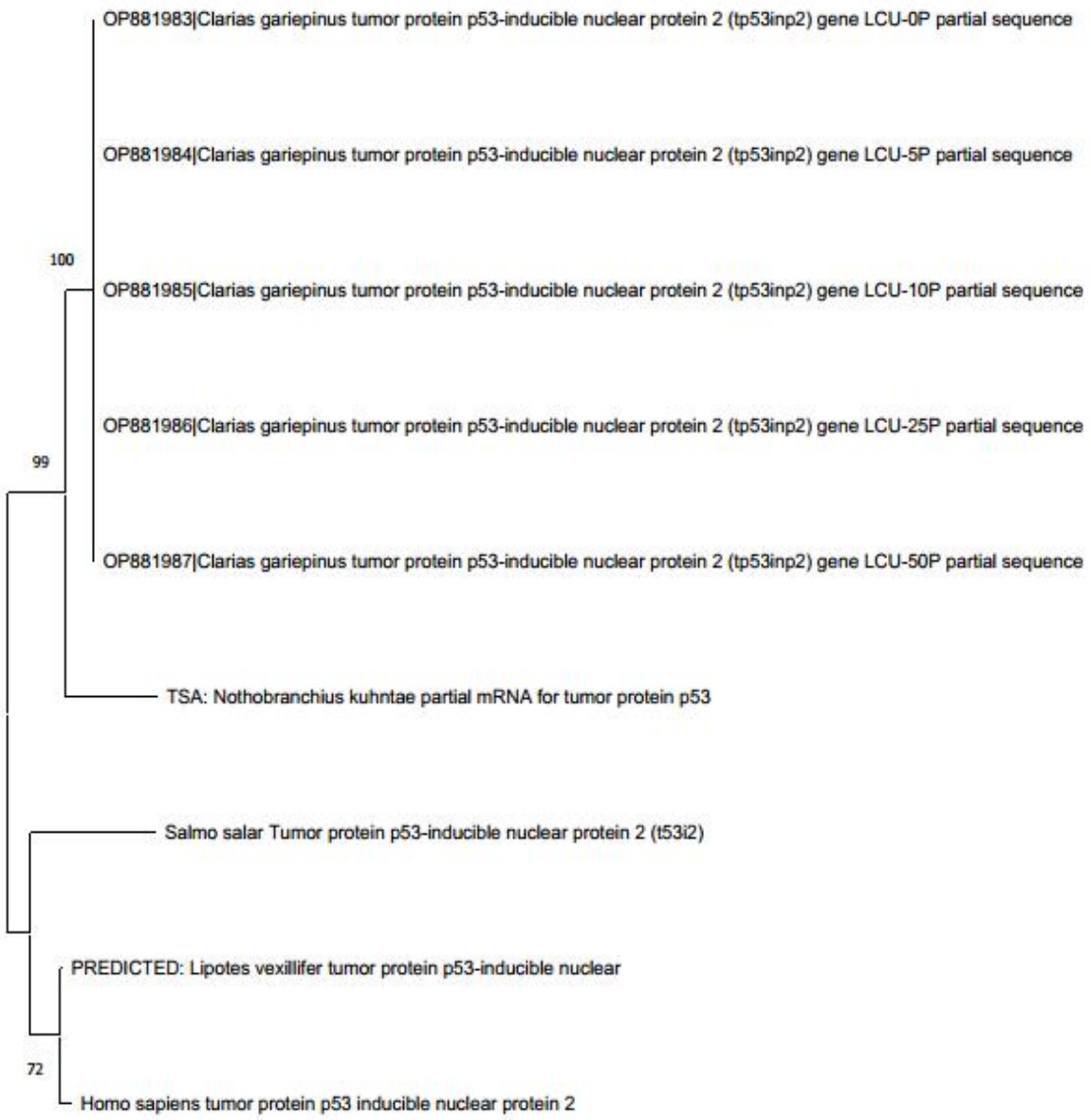


Figure 4.5 : Phylogenetic tree showing comparison between *Clarias gariepinus* p53 gene and p53 gene of Selected Species

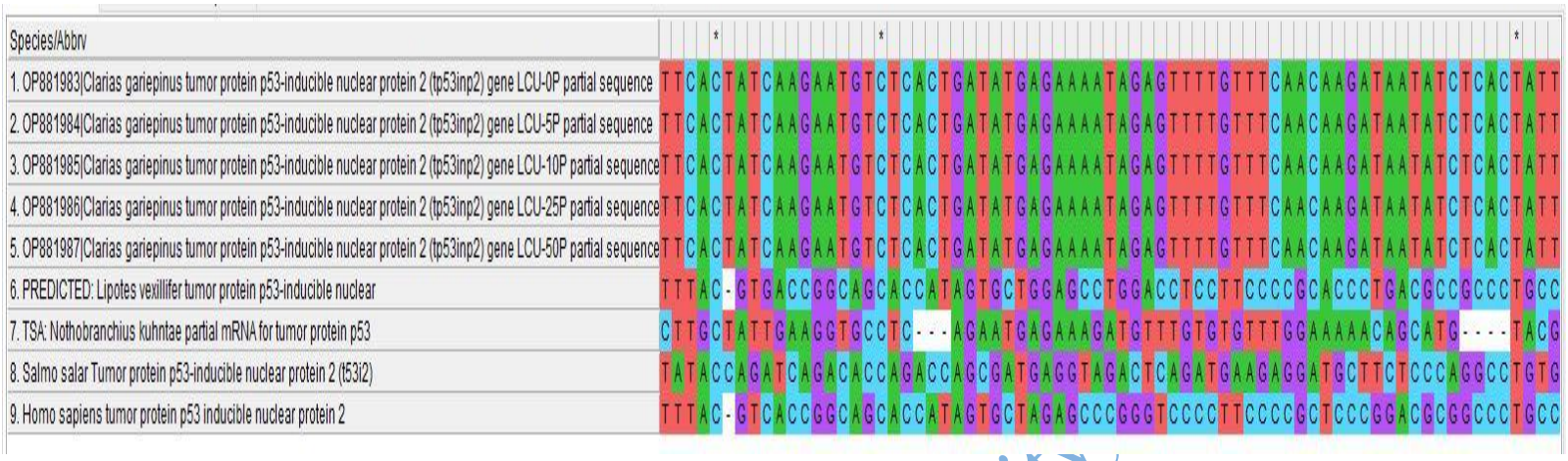


Figure 4.6: Multiple Sequence Alignment of *Clarias gariepinus* p53 gene and p53 gene of Selected species.

Table 4.6: Paire Waise Distance Showing Evolutionary Distance between *Clarias Gariepinus* P53 Gene and P53 Gene of Selected Species

	2	3	4	5
Clarias gariepinus tumour protein p53 gene LCU-0P partial sequence				
Clarias gariepinus tumour protein p53 gene LCU-5P partial sequence				
Clarias gariepinus tumour protein p53 gene LCU-10P partial sequence	0.000			
Clarias gariepinus tumour protein p53 gene LCU-25P partial sequence	0.000	0.000		
Clarias gariepinus tumour protein p53 gene LCU-50P partial sequence	0.000	0.000	0.000	
Predicted: *Lipotes vexillifer* tumour protein p53 inducible-nuclear	1.6358	1.6358	1.6358	1.6358
TSA: *Nothobranchius Kuhntae* partial mRNA for tumour protein p53	1.3048	1.3048	1.3048	1.3048
Salmo salar tumour protein p53-inducible nuclear protein 2 (t53i2)	3.3592	3.3592	3.3592	3.3592
Homo sapiens tumour protein p53 inducible nuclear protein 2	1.8082	1.8082	1.8082	1.8082

4.9. Discussion of Findings

For a very long time, leachates from dumpsites all across the world have been reported to pollute surface and freshwater systems to varying degrees[1],[2]. In order to determine the exact effect of these leachates on the physiology of *Clarias gariepinus*, this study collected samples of leachates from the Awotan dumpsite and introduced them at different concentrations into cultures of *Clarias gariepinus* under laboratory conditions.

Physico-chemical analysis is a traditional and highly effective method of determining the integrity of leachate samples[3]. Several studies over the years have made use of physico-chemical analysis to evaluate the level of contamination in leachate samples. The physico-chemical analysis of the leachate samples collected from the Awotan dumpsite showed that the leachate samples are highly polluted. This was indicated by the values of physico-chemical parameters such as total dissolved solids (TDS), Electrical Conductivity (EC), turbidity, NO_3^-, and SO_4^{2-}, all exceeding the national and international recommended standards.

Total dissolved solids, which refers to the amount of organic and inorganic substances dissolved in water, are a great indicator of contamination[4]. High levels of TDS indicate that leachate contains a lot of dissolved organic and inorganic substances which could increase its toxicity in the aquatic ecosystem[5]. The leachate samples from Awotan dumpsites have a very high TDS of 2450mg/l which is approximately five times the recommended national and international standard. This indicates a very high level of leachate contamination at the Awotan dumpsite. Although similar cases of high leachate TDS have been reported in Nigerian leachate toxicity studies, no study has reported values as high as 2450mg/l. Nevertheless, several studies have also reported TDS values exceeding national and international recommended standards, For instance, a TDS value of 630mg/L was reported in leachate samples from Warri municipal dumpsite[6], and

a value of 2176mg/L was reported in leachate samples from the Delta municipal dumpsite[7]. Similar to TDS, high levels of Electrical Conductivity (EC) also suggest low leachate quality and high level of contamination because EC is a measure of ionic substances present in a leachate solution. The higher the TDS of a leachate solution, the higher its EC[8]. The EC value of 4200µS/cm reported in this study is one of the highest yet among other documented studies in Nigeria.

High concentration of NO_3^-, and PO_4^{2-} in leachates solutions are also widely known for their potential to contaminate groundwater and freshwater resources[9]. When leachates high in NO_3^-, and PO_4^{2-} gets into freshwater system, they can increase the level of freshwater NO_3^-, and PO_4^{2-}, hence causing eutrophication and disrupting the normal physiological process of aquatic organisms[10]. The value of NO_3^-, recorded in leachate samples from the Awotan dumpsite far exceeds the national and international recommend standards, indicating a high level of leachate contamination[11]. Similarly, the concentration of PO_4^{2-} recorded in these samples were far higher than recommended standards. This shows that leachate from the Awotan dumpsite has a very high potential of contaminating freshwater ecosystems and groundwater resources in the surrounding areas.

Reports of high concentrations of NO_3^- and PO_4^{2-} in leachate solutions are not very uncommon in Nigeria and other parts of the world. This can be attributed to the high level of bacteriological activities that take place within the dumpsite. These activities are often aerobic, hence facilitating the conversion of NH_3 to NO_3^-[12]. The high concentration of PO_4^{2-} can be attributed to the large amount of phosphate based wastes such as detergents, sewage sludge, animal dung and fertilizers often disposed of at the Awotan Dumpsite. Previous studies have also reported very high

concentrations of NO_3^- and PO_4^{2-} in leachate samples from various dumpsites in Nigeria such as Warri dumpsite and Igando dumpsite[13,14].

To further assess the quality of leachates from the Awotan dumpsite, heavy metal analysis was conducted on all the collected leachate samples. Determination of heavy metals is an effective method of determining the toxicity of leachate samples[15]. This approach is very popular among researchers because it is easier and provides accurate information concerning the toxicity of leachates[16]. Leachate samples with high concentrations of heavy metals pose a serious threat to aquatic ecosystems and the biota contained therein[17].

In this study, the concentration of six heavy metals including Copper (Cu), Chromium (Cr), Lead (Pb), Manganese (Mn), Cadmium (Cd) and Iron (Fe) were evaluated. Chromium, Cd and Pb were undetected in all the leachate samples collected from the Awotan Dumpsite. This was surprising because of the widespread report of Cr, Cd and Pb in leachate samples from other dumpsites in Nigeria[18,19]. However, this could be attributed to the fact that waste materials present in Awotan dumpsites are mainly residential wastes which are low in Cr, Cd and Pb. Similar to this study, previous studies have also reported negligible concentrations of Cr, Cd and Pb especially in dumpsites receiving low amounts of industrial wastes and effluents such as the Soluos dumpsite[20]. Unlike this study, other studies have reported moderate to high concentrations of Cr, Cd and Pb in various dumpsites, especially those that receive industrial effluents such as the Olusosun dumpsite and Emirin dumpsite.[21,22]

Unlike Cd, Cr and Pb, the concentrations of Cu, Fe and Mn in the leachate samples from the Awotan dumpsite were very high and exceeded the national and international recommended standards. The high concentration of these metals can be attributed to their extensive presence in household wastes. For instance, Cu and Fe are commonly used in the production of wires and

several household electronics, scissors, gardening tools, and screws among others[23], while Mn is also used in the production of textiles, glass and bricks[24]. These materials are very common constituents of municipal waste often dumped within the Awotan dumpsite. When these materials decompose as a result of constant reaction with heat and water, heavy metals such as Cu, Fe and Mn can easily be produced, hence increasing their concentration in leachate samples from these dumpsites. The high presence of heavy metals such as Cu, Fe and Mn in leachate samples is not uncommon and has been reported extensively in leachate toxicity literature[25].

After identifying the toxicity potential of leachate samples from the Awotan dumpsite, the study went further to examine the effect of these leachates on the haematology of *Clarias gariepinus*. The results showed that leachate samples from the Awotan Dumpsite had a significant effect on some haematological features of *Clarias gariepinus*. For instance, the amount of Red Blood Cells (RBC) in *Clarias gariepinus* showed a significant reduction when exposed to 50% leachate concentration. A similar observation was made with other red blood cell indices such as Packed Cell Volume (PCV), and Hemoglobin (Hb). Although all these features showed a reduction in cell count in all the concentrations (5%, 10%, 25% and 50%), the reduction was most significant in 50% concentration, hence showing that increasing concentration of the leachate has a detrimental effect on the red blood cell components of *Clarias gariepinus*. This is further proven by the fact that the number of cells increases as the concentration of the leachate decreases. The detrimental effect of Awotan leachates on the RBC components of *Clarias gariepinus* can be attributed to the presence of toxic heavy metals and organic substances present in the leachates as indicated by a high level of EC and TDS. Previous studies have shown that leachate samples with high amount of toxic metals and organic substances could cause bone marrow failure or damage the red blood cells mechanically, hence leading to low production of RBC[26,27,28].

Considering the fact that the leachate also suppressed the production of haemoglobin, it could be possible that certain components of the leachate are interfering with oxygen uptake in the *Clarias gariepinus* samples. This has also been reported in other studies, using species such as *Tilapia zilli* and *Oreochromis niloticus*[29,30].

Similar to the RBCs, the White Blood Cells (WBC) of the *Clarias gariepinus* samples also showed a sharp decrease in cell count when introduced to 50% leachate concentration and the number of cells increases as the concentration decreases, except in 5% leachate concentration where the number of WBC is the lowest. The decrease shown by the WBC can also be attributed to toxic components contained in the leachates.

Other WBC components such as eosinophils, monocytes and heterophils showed increased cell count when introduced into the various leachate concentrations with the highest increment seen in 10% and 25%. The increment in the number of these cells can be attributed to the specific immune response of *Clarias gariepinus* to the toxic components of the leachate samples. This anomalous observation has also been reported in earlier studies conducted on *Clarias gariepinus*[31].

After establishing the effects of leachates from the Awotan Dumpsite on the haematological features of *Clarias gariepinus*, the study further examined the effect of these leachate samples on the behaviour and mortality of *Clarias gariepinus*. Throughout this test, all *Clarias gariepinus* samples in the control group showed no behavioural responses. Also, behavioural changes were hardly noticeable in the 5% and 10% leachate concentration groups. However, strong observations of behavioural responses such as air gulping and erratic swimming were seen especially in the 25% and 50% groups after 48 hours of exposure. This shows that the leachate is highly toxic to *Clarias gariepinus* especially at very high concentrations and during long

exposures. The increased air gulping witnessed at high concentrations could be due to a shortage of oxygen intake as a result of toxic organic components such as benzene, toluene and ethylbenzene. This also provides an explanation for the significant reduction of haemoglobin (Hb) when *Clarias gariepinus* samples were exposed to 50% leachates in this study. Previous studies have also shown that *Clarias gariepinus* can experience air gulping when exposed to high concentrations of leachate samples containing toxic organic substances such as benzene[32] or inorganic metals such as Cu[33].

During the mortality test, there was a 100% survival rate in the control, 5% concentration and 10% concentration groups irrespective of the hours of exposure. A 40% mortality was recorded in the 25% leachate concentration group, while a 100% mortality was recorded in the 25% leachate group. This shows the acute toxicity of the leachate samples in concentrations greater than 25%. This could be attributed to the fact that the amount of toxic metals and harmful organic components of the leachate solution increases as the concentration increases, hence increasing its ability to interfere with physiological processes such as oxygen uptake and cell formation. Several studies have also reported acute toxicity of landfill leachates at high concentrations, using species such as *Sarotherodon mossambicus* and Zebrafish amongst others[34,35].

The study also examined the effect of leachates on the histopathology of *Clarias gariepinus* by exposing the fish samples to varying concentrations of the leachates and examining samples of the fish liver after 100 hours of exposure. The results showed that the leachate has a moderate effect on the liver cells at 5% concentration, with moderate cases of glycogen infiltration and perivascular infiltration of inflammatory cells. The impact of the leachate on the liver cell gradually worsened with increasing leachate concentration. At 10% leachate concentration,

glycogen infiltration became abundant and traces of cytoplasmic vacuolation were noticed. At 25% severe glycogen infiltration and cytoplasmic vacuolation of the hepatocytes. At 50%, severe steatosis of the liver was observed. The gradual increase of cytoplasmic vacuolation of the hepatocyte can be attributed to the presence of bacteria, viral pathogens or low-molecular weight compounds in the leachate solutions[36], which increases as the concentration of the leachate increases. Cytoplasmic vacuolation of hepatocytes is a common reaction to toxicity in *Clarias gariepinus* and this has been reported in literature using various types of substances such as landfill leachates[37], atrazine[38] and Zinc sulphate[39].

Although the cause of steatosis in the hepatocytes of fishes is not clearly understood among researchers, the reports of steatosis in fishes upon exposure to toxic substances is very common in the literature[40]. Due to the paucity of information concerning the causes of liver steatosis in the literature, the severe steatosis observed in this study will be attributed to the presence of the toxic substances in the leachate solutions from the Awotan dumpsite.

The study also examined molecular analysis of a specific gene, p53, the effect of leachates on the p53 of *Clarias gariepinus*. The polymerase chain reaction (PCR) showed a band length of 700 base pairs meaning that the region of the p53 gene being amplified is approximately 700 base pairs long.

While in well 1 and well 2 in polymerase chain reaction, the band are less visible band at this region suggest that the targeted gene p53 have smaller size in this treatment concentration which are the 0% and 5% leachate concentration. The presence of smaller and larger bands suggests that the DNA sample contains a mixture of DNA fragments with different sizes. This could be due to various factors, such as the presence of different alleles, mutations, or variations within

the target region being analyzed[41]. It's also possible that the sample contains multiple PCR products as it was amplified using primers that target different regions of the DNA[42]. The multiple alignment sequences above are for the tumor protein p53-inducible nuclear protein 2 (TP53INP2) gene in a variety of species. P53 is a protein that plays a role in cell growth, apoptosis, and autophagy. Mutations in the p53 gene have been linked to a number of diseases, including cancer and neurodegenerative disorders[43]. The sequences provided show that the p53 gene is highly conserved across species. This means that the gene has a similar structure and function in all of the species that you have listed. The only significant differences between the sequences are a few single nucleotide polymorphisms (SNPs). The SNPs in the *Clarias gariepinus, Nothobranchius kuhntae, and Salmo salar* sequences are in the untranslated region of the gene, which means that they are not likely to affect the function of the protein. The SNP in the *Lipotes vexillifer* sequence is in the coding region of the gene, but it is a silent mutation, which means that it does not change the amino acid sequence of the protein. The SNP in the Homo sapiens sequence is also in the coding region, and it changes the amino acid sequence of the protein. However, it is not known whether this SNP is harmful or harmless.SNPs are changes in a single base pair in the DNA sequence. SNPs can be harmless, or they can be harmful and lead to disease. This mean that no definite mutation has occurred within various regions of the genes in *Clarias garipienus* of the control experiments and the leachate experiment since there is no perfect alignment among them.

However, figure 4.5 show the phylogenetic tree of p53 gene of *Clarias garipienus* and other selected species of fishes and human (*Homo sapiens*). The result shows that the phylogenetic relationship of *Clarias garipienus* is far away from human (*Homo sapiens*). Importantly, it's seen that only the p53 of *Notobranchius khuntae* have close evolutionary relationship with

Clarias garipienus. This can indicate that the species are subjected to same environmental pressure or mutate at same region. For the pairwise distance, a pairwise distance of 0 between genes indicates that the sequences being compared are identical[44]. In this context, if two genes have a pairwise distance of 0, it suggests that they have the same sequence or are highly similar.

On the other hand, if the pairwise distance between genes from different species is 0.4555, it indicates that there are differences in the sequences. A pairwise distance of 0.4555 suggests that the genes have diverged or evolved over time, resulting in sequence variations between the species. The implications of these differences in gene sequences could include functional variations or adaptations specific to each species. These sequence differences may result in variations in gene expression, protein structure, or biological functions[45]. *Nothobranchius kuhntae* has a pairwise distance of 1.6358 with *Clarias gariepinus* 0: This suggests that there are significant sequence differences between the p53 gene in these two species.

Overall, the pairwise distances reflect the degree of sequence divergence and evolutionary changes between the p53 gene in *Clarias gariepinus* and other species. These variations may have implications for the functional roles and adaptations of the p53 protein in each species, reflecting their distinct evolutionary histories. The implication include; functional adaptations, species-specific trait.

Endnotes

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Chapter Five

Conclusions

5.1. Summary of Findings

This study examined the toxicity of leachate samples from the Awotan dumpsite on Clarias gariepinus, with the aim of establishing how toxic substances in leachates affect the haematology, histopathology, and molecular functions of *Clarias gariepinus*, using an experimental research design methodology. Leachate samples from the Awotan dumpsite were taken and underwent physico-chemical and heavy metal analysis tests.

Additionally, juveniles of *Clarias gariepinus* were obtained from the Department of Aquaculture and Fisheries Management at the University of Ibadan. The fish specimens were acclimated for 14 days in the Animal House, Faculty of Natural and Applied Sciences, Lead City University in 40-litre plastic aquariums (20x15x30cm) filled with chlorine-free borehole water. The juveniles were given 5 g of fish meal, or 3% of their body weight, twice daily (Plate 2). To avoid an increase in metabolic waste, the water in each tank was changed every two days. Throughout the adaptation and experimentation periods, the natural photoperiod was maintained.

Following the acclimation phase, the toxicity evaluation set-up was started by establishing five treatments, including the control treatment and treatments with 0%, 5%, 25%, and 50% of the total dose. 15 acclimated juvenile fish were added to each treatment setup in triplicates of each treatment. After twenty-one days of observation, the setups were removed, and fish samples were taken for haematology, histology, and molecular analyses.

Key pollution indicators like EC, TDS, nitrate, and phosphate all exceeded the SON and WHO-approved criteria, according to the study's physico-chemical findings, suggesting a high amount of leachate contamination. The heavy metal analysis also revealed that metals including Fe, Mn, and Cu are present in the leachate in extremely toxic concentrations that surpass the SON and WHO recommended limits. According to the behavioural and mortality tests, concentrations of leachate above 25% cause behaviours like gulping air and swimming erratically, as well as eventual death.

The haematological test showed that concentrations of leachates exceeding 25% cause a significant reduction in the production of red blood cells, white blood cells, haemoglobin and packed cell volumes, suggesting that the leachate contains a toxic substance that attacks bone marrows or suppresses oxygen uptake. However, few blood components, such as monocytes and heterophils, showed an increase in cell number, which suggests a specific immune response of *Claris gariepinus* to different types of toxic substances.

The histopathology test showed that the leachate at every concentration has a detrimental effect on the liver cells of *Clarias gariepinus*. However, the effect increases as the concentration of leachate increases, with severe cases of cell vacuolation and steatosis observed at 25% and 50% concentrations. Finally, the molecular analysis revealed that p53 wasn't suppressed in the fish despite the toxicity of the leachates.

5.2. Conclusion

The study has shown that leachates produced at the Awotan dumpsite can be classified as highly toxic to humans and aquatic organisms. The leachate samples have been shown to contain high concentrations of toxic heavy metals such as Cu, Mn, and Fe, exceeding the WHO recommended standards. Furthermore, the leachate from Awotan landfill has been shown to contain a very high amount of dissolved solids as well as high concentrations of NO_3^- and PO_4^{2-} which pose a great threat to the surface water and groundwater resources in the area. This study further confirmed that the leachate from the Awotan dumpsite is highly toxic to *Clarias gariepinus* as it contains highly toxic organic and inorganic substances that inhibit the production of red and white blood cells in the fish. Also, toxic substances in the leachate affect the physiology of *Clarias gariepinus* by inhibiting respiration in this fish species. Also, Awotan leachate also causes steatosis of the liver in *Clarias gariepinus* when exposed to very high concentrations. Finally, leachate samples from the Awotan dumpsite can result in fish mortality at very high concentrations. The degree of expression or non-expression of p53 in catfish can be used to determine the level of toxicity. However for this study, it was shown that the p53 wasn't mutated, and this could be attributed to short period of the experiment which was not able to capture the degree of mutation.

5.3. Recommendations

In light of the findings made in this study, the following recommendations were made:

1. **Proper management of landfills in Nigeria**: While landfills remain the major source of waste disposal in many parts of Nigeria, it is important that these landfills are properly engineered to reduce the chances that leachates from the landfill will find their way into

the ground and surface water in the surrounding area. A well-engineered land will also reduce the production of leachates.

2. **Utilizing other waste management methods, such as recycling,** Landfills in Nigeria are often filled with tens to thousands of recyclable materials, such as plastics, metal containers, papers, glass bottles, and cardboard, among others. Rather than disposing of these materials in landfills, people can be encouraged to discharge their recyclable wastes at fixed recycling stations all across the country. This will help reduce the amount of waste going into landfills, thereby reducing the chances of leachate formation.
3. **Conducting further studies on p53 genes of *Clarias gariepinus***: While this study has come to a unique conclusion that the p53 gene in *Clarias gariepinus* isn't suppressed, it is important that more studies are conducted to approve or refute this conclusion. Hence, the study will recommend that more research efforts are channeled towards examining the effect of toxic substances on p53 genes in *Clarias gariepinus*.

5.4. Contribution to Knowledge

According to this study, the Awotan dumpsite creates extremely hazardous leachate that can harm the community of Ibadan's freshwater and groundwater resources. By emphasizing that both humans and aquatic organisms rely on these local groundwater and freshwater resources, the interconnectedness of ecosystems and the potential impacts of leachate on multiple aspects of the environment. This insight contributes to a broader understanding of the environmental consequences of landfill practices.

These conclusions are relevant to landfill management and can assist Awotan dumpsite management in reviewing their management tactics to lower leachate generation in the landfill.

Additionally, the landfill's already-produced leachates can be biologically-treated to minimise the risk they provide to the water and soil resources in the Ibadan community and its surroundings. The knowledge generated can inform the development of mitigation strategies to reduce the impact of leachates on catfish and other aquatic organisms, potentially leading to more sustainable aquaculture practices. By studying the histopathological effects ofleachates on catfish, it has provided insights into broader ecological impacts. For example, if catfish are affected, it may suggest wider implications for the aquatic ecosystem.

Importantly, the p53 is involved in cell cycle regulation. Its role in halting the cell cycle allows damaged DNA to be repaired before the cell proceeds through the cycle, preventing the propagation of genetic errors.

5.5 Suggested Area for Further Research

1. Additional research is needed to investigate and identify p53 gene in C*larias garipeinus* to know if they exhibit homologous function as that of other selected fish species and humans.
2. Investigate the correlation between p53 gene expression levels and the severity of leachate-induced toxicity in catfish.
3. Rigorous assessments of the histopathology changes in catfish tissues, particularly those related to p53-regulated processes, upon leachate exposure.
4. Investigate the potential protective effects of antioxidant or cytoprotective agents on leachate-induced p53-mediated toxicity in catfish.
5. Assessment of the activation of p53-dependent signaling pathways in catfish exposed to leachates, such as DNA damage response, cell cycle regulation, and apoptosis.

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APPENDIX

Appendix I: Picture Showing Cleaning of Waste of Juveniles Of *Clarias garipienus*

Source: Authors Field Work, 2022.

Appendix II: Picture Showing Juveniles Of *Clarias garipienus* in Plastic Aquaria

Source: Authors Field Work, 2022.

Appendix III: Partial Sequence of *clarias garipienus* with their Accession Number

881983|*Clarias gariepinus* tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene, LCU-0P partial sequence

CCTCACCTCTATCCCTCATTTCCTTTTGCCTTGCAGTCACACATGTTTCTCATTACCAC
TTGTTATTTATACCCTCCTTCCCCCCCCTGTGTGTGTGTGTGTGTGTTTAAAGTAA
AGAATGAGTAGCTGCTACAGTACGGAGTCGTGCTGTTATCGTCTTTACCCTCGAGTA
TTGCGTGTGTCACTGGTGCTGACCCCTGTCTGCTATTTCTTGTTTGTCAGGTTGCATTT
CACTGCTGTTTTCCTGTATTCACACTGAACTCATATACTGGCCTGTACGCATTGCTGC
ATGCAAGGAGAAAAATTCCCGCCTTATTACAAGAAGACCGTCTATTCACTATCAAGA
ATGTCTCACTGATATGAGAAAATAGAGTTTTGTTTCAACAAGATAATATCTCACTAT
TATACTAAAATCGTCTCATTCTTACTGAAATCCTGCAGTGGGTTTGACTTTATATGGA
GGTATTGTACATGGGACAACACTGAAGCAGGTTATAAATATGTTCTTATTAAAATAT
ATACTTGTGATATCTGATTCAGATGATCAATATTTATTATTATTGACTGCTATGTAAA
ACATTATTGTGATAAAACTTTTGCCTGGCCTTCAGTAGAGAGAACATGGGACCACAT
TGAGGTGGACTGTATCTGGGAGAACACTGAAATGGCTTGACTG

>OP881984|*Clarias gariepinus* tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene, LCU-5P partial sequence

CACCTCTATCCCTCATTTCCTTTTGCCTTGCAGTCACACATGTTTCTCATTACCACTTG
TTATTTATACCCTCCTTCCCCCCCCTGTGTGTGTGTGTGTGTGTTTAAAGTAAAGA
ATGAGTAGCTGCTACAGTACGGAGTCGTGCTGTTATCGTCTTTACCCTCGAGTATTG
CGTGTGTCACTGGTGCTGACCCCTGTCTGCTATTTCTTGTTTGTCAGGTTGCATTTCA
CTGCTGTTTTCCTGTATTCACACTGAACTCATATACTGGCCTGTACGCATTGCTGCAT
GCAAGGAGAAAAATTCCCGCCTTATTACAAGAAGACCGTCTATTCACTATCAAGAAT
GTCTCACTGATATGAGAAAATAGAGTTTTGTTTCAACAAGATAATATCTCACTATTA
TACTAAAATCGTCTCATTCTTACTGAAATCCTGCAGTGGGTTTGACTTTATATGGAGG
TATTGTACATGGGACAACACTGAAGCAGGTTATAAATATGTTCTTATTAAAATATAT
ACTTGTGATATCTGATTCAGATGATCAATATTTATTATTATTGACTGCTATGTAAAAC
ATTATTGTGATAAAACTTTTGCCTGGCCTTCAGTAGAGAGAACATGGGACCACATTG
AGGTGGACTGTATCTGGGAGAACACTGAAATGGCTTGACTGGA

>OP881985|*Clarias gariepinus* tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene, LCU-10P partial sequence

CCTCACCTCTATCCCTCATTTCCTTTTGCCTTGCAGTCACACATGTTTCTCATTACCAC
TTGTTATTTATACCCTCCTTCCCCCCCCTGTGTGTGTGTGTGTGTGTTTAAAGTAA
AGAATGAGTAGCTGCTACAGTACGGAGTCGTGCTGTTATCGTCTTTACCCTCGAGTA
TTGCGTGTGTCACTGGTGCTGACCCCTGTCTGCTATTTCTTGTTTGTCAGGTTGCATTT

CACTGCTGTTTTCCTGTATTCACACTGAACTCATATACTGGCCTGTACGCATTGCTGC
ATGCAAGGAGAAAAATTCCCGCCTTATTACAAGAAGACCGTCTATTCACTATCAAGA
ATGTCTCACTGATATGAGAAAATAGAGTTTTGTTTCAACAAGATAATATCTCACTAT
TATACTAAAATCGTCTCATTCTTACTGAAATCCTGCAGTGGGTTTGACTTTATATGGA
GGTATTGTACATGGGACAACACTGAAGCAGGTTATAAATATGTTCTTATTAAAATAT
ATACTTGTGATATCTGATTCAGATGATCAATATTTATTATTATTGACTGCTATGTAAA
ACATTATTGTGATAAAACTTTTGCCTGGCCTTCAGTAGAGAGAACATGGGACCACAT
TGAGGTGGACTGTATCTGGGAGAACACTGAAATGGCTTGACTGG

>OP881986|*Clarias gariepinus* tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene, LCU-25P partial sequence

CTCACCTCTATCCCTCATTTCCTTTTGCCTTGCAGTCACACATGTTTCTCATTACCACT
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GAATGAGTAGCTGCTACAGTACGGAGTCGTGCTGTTATCGTCTTTACCCTCGAGTAT
TGCGTGTGTCACTGGTGCTGACCCCTGTCTGCTATTTCTTGTTTGTCAGGTTGCATTT
CACTGCTGTTTTCCTGTATTCACACTGAACTCATATACTGGCCTGTACGCATTGCTGC
ATGCAAGGAGAAAAATTCCCGCCTTATTACAAGAAGACCGTCTATTCACTATCAAGA
ATGTCTCACTGATATGAGAAAATAGAGTTTTGTTTCAACAAGATAATATCTCACTAT
TATACTAAAATCGTCTCATTCTTACTGAAATCCTGCAGTGGGTTTGACTTTATATGGA
GGTATTGTACATGGGACAACACTGAAGCAGGTTATAAATATGTTCTTATTAAAATAT
ATACTTGTGATATCTGATTCAGATGATCAATATTTATTATTATTGACTGCTATGTAAA
ACATTATTGTGATAAAACTTTTGCCTGGCCTTCAGTAGAGAGAACATGGGACCACAT
TGAGGTGGACTGTATCTGGGAGAACACTGAAATGGCTTGACTGGAG

>OP881987|*Clarias gariepinus* tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene, LCU-50P partial sequence

TCACCTCTATCCCTCATTTCCTTTTGCCTTGCAGTCACACATGTTTCTCATTACCACTT
GTTATTTATACCCTCCTTCCCCCCCCTGTGTGTGTGTGTGTGTGTGTTTAAAGTAAAG
AATGAGTAGCTGCTACAGTACGGAGTCGTGCTGTTATCGTCTTTACCCTCGAGTATT
GCGTGTGTCACTGGTGCTGACCCCTGTCTGCTATTTCTTGTTTGTCAGGTTGCATTTC
ACTGCTGTTTTCCTGTATTCACACTGAACTCATATACTGGCCTGTACGCATTGCTGCA
TGCAAGGAGAAAAATTCCCGCCTTATTACAAGAAGACCGTCTATTCACTATCAAGAA
TGTCTCACTGATATGAGAAAATAGAGTTTTGTTTCAACAAGATAATATCTCACTATT
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GTATTGTACATGGGACAACACTGAAGCAGGTTATAAATATGTTCTTATTAAAATATA
TACTTGTGATATCTGATTCAGATGATCAATATTTATTATTATTGACTGCTATGTAAAA
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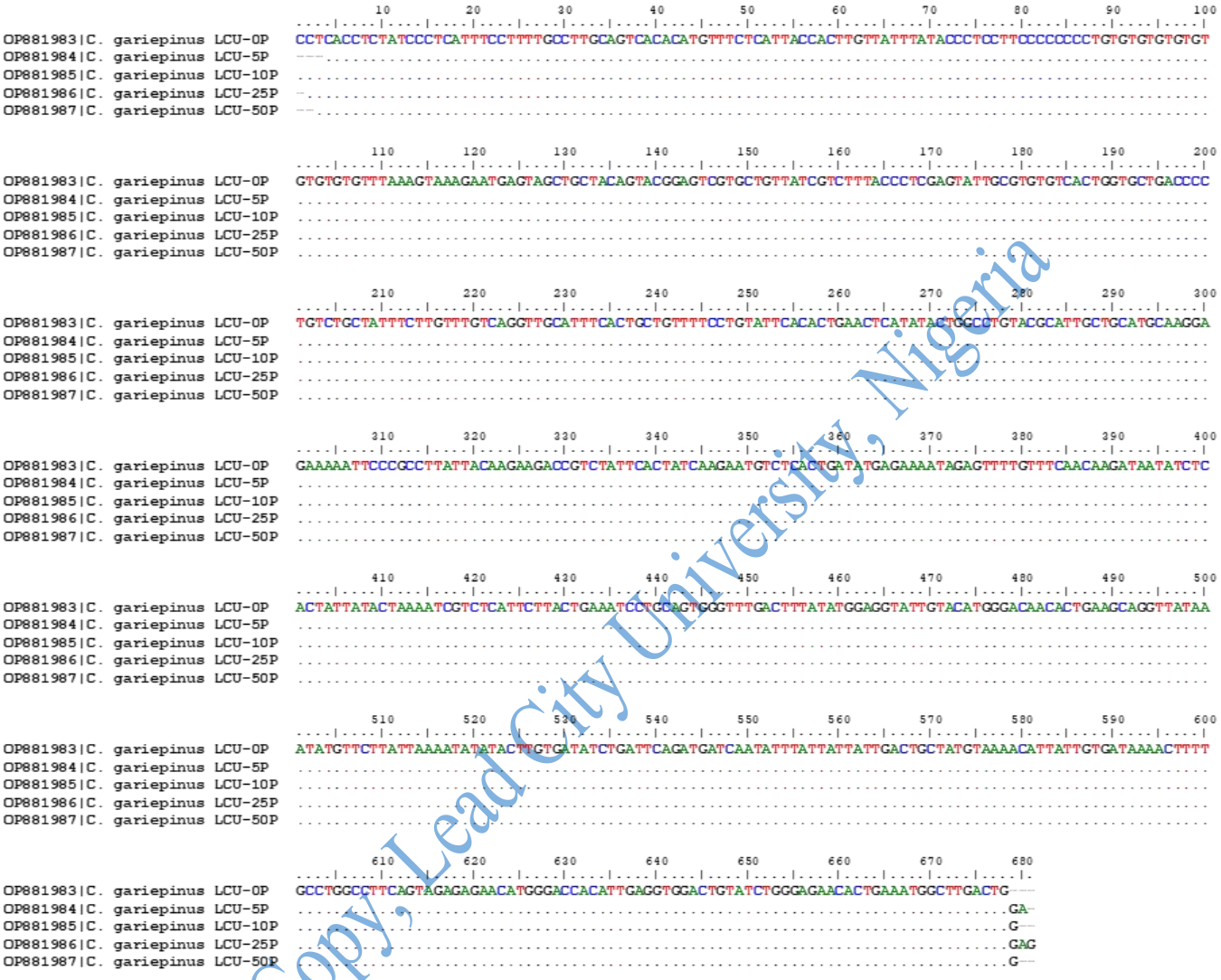

Appendix IV: Nucleotide characteristic of the tumor protein p53-inducible nuclear protein 2 (tp53inp2) gene from five *Clarias gariepinus*

Bio-data

A. PERSONAL DATA

Full Name	**John Opeoluwa OTITOOLA**
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Date of Birth	April 9th, 1997
Place of Birth	Okuku
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B. EDUCATIONAL BACKGROUND

Educational Institutions Attended with Dates and Qualifications

Obafemi Awolowo University, Ile-Ife (BSc.)	2013-2018
Folorunso Memorial College, Oyan, West African Examination Council	2006-2012

C. WORK EXPERIENCE WITH DATES

Federal Aviation Authority of Nigeria (FAAN)	2018-2019
Zenith Bank PLC	2023

D. PUBLICATIONS

Thesis/Dissertation

1. Toxicological Assessment on *Clarias garipienus* (Cat Fish) Exposed to Leachates from Awotan Landfill, Ibadan Oyo State, Nigeria

Papers Accepted for Publication:

2. O.J Otitoola , O.Bakare. "*Toxicological Assessment of Clarias gariepinus Exposed to Leachates from Awotan Landfill, Ibadan Oyo State, Nigeria."* **Pan African Journal of Life Sciences volume** *7, 2023, 1-14.*

E. Major Conferences/Workshops Attended

(1) Submitted my abstract in Lead paper presentation on "Climate change, Floodrisk and Sustainable Development" organized by the Lead City University, Post Graduate College, Ibadan, 2023.

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…….. ……

Signature **Date**

The University Compliance Certification

This is to certify that, this Thesis was written by **John Opeoluwa OTITOOLA with Matric Number LCU/PG/002583** of the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan and it is in full compliance with the approved University format and style.

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Signature **Date**