TOXICITY OF AFRICAN LOCUST BEAN PROCESSING INDUSTRY EFFLUENT TO FINGERLINGS OF NILE TILAPIA

(Oreochromis niloticus)

EKPO-JIDE, OLAJUMOKE FRANCES

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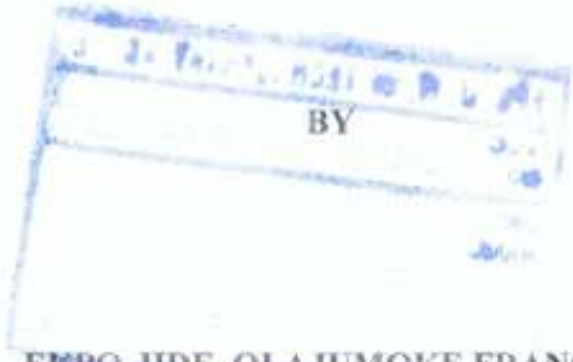

BY

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ABSTRACT

Experiments were conducted to determine the toxicity of African locust bean effluent *(Parkia bioglobosa)* to Nile Tilapia *(Oreochromis niloticus)* (Trewavas 1983) fingerlings and the effect of various concentrations on water quality. Static bioassay tests revealed that the 96 hr LC_{50} of the effluent as 3.1ml/L. Degenerative changes were observed on the parenchymatous organs of test fishes. The liver and the gills were enlarged and were perforated while the gill lamellae were extensively fused. Haematological analyses also showed a drop in the haematocrit values, as the heamatocrit values of the control blood samples were significantly higher ($P \leq 0.05$ from those of the treated blood samples and there were deformations in the blood cells. Water quality parameters monitored during the experiment showed no deviations from the control experiment. During the 96 hour sub-lethal test; fingerlings showed no mortality at concentration below 3ml/L of water and the behavioural responses exhibited by the fish showed unbalanced swimming patterns, loss of reflex and rapid opercula movements.

CERTIFICATION

We certify that this study was carried out by Mrs. Olajumoke Ekpo-Jide of the Department of Fisheries and Wildlife, School of Agriculture and Agricultural Technology, Federal, University of Technology, Akure.

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28-02-05
Date

DEDICATION

To my all in all, the Lord God Almighty who created me and saw me through this programme and to my husband Mr. Ekpobomini Jide; for your love, understanding, wisdom, goodness, generosity, you are to me, an epiphany of God's faithful love.

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CHAPTER ONE

1.0 INTRODUCTION

In Nigeria, the importance of protein is becoming greater as meat production declines (Oladimeji, 1993). Although only a small fraction of the fish consumed by the nation is from local sources at present, efforts are being made towards increasing local fisheries industry. Among the many factors that may negate this effort is pollution.

Presently, the country is faced with rapid population growth, increased rural to urban migration/drift, and the subsequent industrial expansion, all contribute to the increase in environmental pollution. Fish toxicology is the study of the effect of toxicants on fish and other aquatic organisms. Toxicity test is used to detect and evaluate the potential effect of chemicals, reagents, pollutants or other anthropogenic materials or xenobiotics organisms. Toxicity tests may be short-term (acute toxicity tests), depending on the information required. According to Oram *et al.* (1976), aquatic ecosystems are affected by poisons such as mercury and lead wastes produced by some industries. Many industrial by-products are also released in large volumes into streams and rivers deliberately or accidentally. Hazardous substances may also affect quality of fish and fish populations and such substances include among others, metal salts, pesticides, chemical fertilizer, petroleum, hydrocarbons, sewage and industrial pollutants.

Sikoki and Kolo (1993) reported that aquatic pollution is the greatest pollution problem that Nigeria has ever experienced and it is currently experiencing. Water pollution affects both man and aquatic ecosystems

directly and indirectly as industrial wastes, are uncontrollably dumped into the aquatic environment. The industrial effluent discharges exert great impact on the aquatic system even at low concentrations.

Various workers (Krous *et al.*, 1982; Ofojekwu *et al.*, 1990) have used species of test organisms, different periods of exposure to pollutants, waters of different quality and different ways of reporting the results. The acute toxicity of Rogor® was determined for the juveniles of Nile tilapia, *Oreochromis niloticus* by Annune and Ajike (1999) and it was observed that Osmoregulatory and respiratory incapacitation through mucus accumulation and epithelial detachment were the major causes of fish mortality following exposure to Rogor®.

Grizzle (1977) reported that neutrophilia was the most drastic haematological change observed when the channel catfish *Ictalurus punctatus* was exposed to 0.1mg-1.1mg of malachite green. Exposure of the Nile tilapia, *Oreochromis niloticus* to sublethal concentrations of malachite green was reported by Omoregie *et. al.* (1992) to lead to severe physiological impairment of fish after a 10 week exposure period.

Toxicants enter aquatic ecosystems from two main sources:

(1) non-point sources such as agricultural run-off from land, contaminated ground water and bottom sediment disposal, and atmospheric fall out, and

(2) point sources such as discharges (effluents) from manufacturing plants, hazardous waste disposal sites and municipal waste water treatment.

Industrial wastes consist of toxic substances which adversely affect the water quality and consequently affect the biota in water bodies. The uncontrollable discharge of the industrial wastes into the aquatic environment poses serious problems and ecological disasters of varying types and magnitude. Since discharges (effluents) affect both man and aquatic ecosystems directly or indirectly, there is a great need to combat discharges in order to conserve the aquatic ecosystem.

African locust bean *(Parkia bioglobosa)* is a source of protein in diets of Nigerians, and in the fermented form. It is one of the most important food condiments in the entire Savanna region of West and Central Africa. Apart from its flavouring attribute, it contributes significantly to the intake of protein, essential fatty acids and B vitamins particularly riboflavin. It is also known locally as "Dawadawa" (Hausa) "Ogiri Igala" (Ibo) and Iru (Yoruba). It belongs to the Family *Parkia Clappertoniana* and has species that are grown in the Sub-Saharan African (National Academy of Science, 1979).

It is known that *Parkia bioglobosa* seed contains 30-40% protein, 31-40% lipid, 11.7-15.4% carbohydrate, 8.82-9.4% crude fibre, 4.4-5.38% ash, NFE of 37% and 93.7% content of dry matter (Campbell 1980) and that the seeds are rich in calcium, sodium, potassium and phosphorus, while the pulp is exceptionally high in energy value, containing 29% crude protein, 60% sucrose and also rich in vitamin C.

The average length, width and thickness of locust bean seed are 10.80, 8.42 and 4.64mm. Carbon gum extracted from the locust bean can be added to foods and cosmetics as thickners and emulsifier or made to sizes for

textiles and leather works (Yadkin, 1985). The bean could also be processed into food flavours and additives. The seed is also suitable for the manufacturing of soap (Owoyale *et al,* 1986).

The processing of the locust bean is mainly done by women in Africa. Locust beans are boiled for about an hour to soften the seeds for removal, the hulls are removed by mashing the boiled seeds with bare feet near the river side or in a mortar and pestle (Oyeyiola, 1988). If the mortar is used, clean sand is added to ease removal of the hull. The locust beans are later boiled for additional 10-12 to soften the cotyledons. The cotyledons are allowed to undergo mild fermentation for about 2 to 3 days during which the characteristic colour (brown) and odour (ammeruated) are developed. African locust bean is prepared in the solid non-mucilaginous state ("pete") for sale or consumption in South Western part of Nigeria.

The list of toxicants contained in African locust bean *(Parkia biglobosa)* pods as reported by Odeyemi *et al* (1999) are saponin, tannins and alkaloids.

Water pollution research has been directed towards collecting data on the following.

(i) Studying the behaviour, degradation and modification of the toxicants once they get in touch with water.

(ii) Measuring their effects through toxicity tests of varying degrees of refinement and equally studying the physiological, developmental and behavioural responses or organisms that are exposed to the various concentrations.

Usually, when the pollutant (African locust bean processing industry effluent) gets into the water, it follows any of the following pathways:

(a) it may be dissolved in water;

(b) it may be absorbed on the surface of silt in the water;

(c) it may be suspended in water or occur in sediments at the bottom; and

(d) it may be taken up by living aquatic organisms.

1.1 JUSTIFICATION

The effluents generated during African locust bean production may have harmful effect on water bodies as well as organisms that inhabit such water bodies. The effluent may be introduced deliberately or accidentally into the aquatic ecosystem during washing besides rivers impairing the quality of the water and making it unfavourable for aquatic life. The environment of the fish is often altered by toxic substances and this results in death (either total or partial fish kills) especially when the concentration is higher than what the homeostasis of the fish can control. Most of the fishes migrate from the polluting source especially when the water is continuous. Information on the effects of sub-lethal and lethal exposure of most of Nigerian freshwater species to African locust bean and its effluent is scarce hence the choice of this study. It is therefore imperative to assess the quality of African locust bean effluent, the concentration that will cause harmful effects to fingerlings of the Nile tilapia, *Oreochromis niloticus*. This study therefore highlights the risk inherent in permitting indiscriminate dumping of African locust bean effluent into our water systems.

1.2 OBJECTIVES OF THE STUDY

The objective of this study is to evaluate the acute tests/definitive tests and the effects of sublethal concentration of African locust bean processing industry effluent on the behaviour, survival, some haematologial parameters, and histology of the gills and liver of Nile tilapia, *O. niloticus*, fingerlings. The specific objectives of this study were to determine:

(a) the LC_{50} values of Nile tilapia fingerlings exposed to different concentrations of African locust bean effluent.

(b) the effects of sublethal concentration of African locust bean effluent on the histology of gills and liver cells of Nile tilapia fingerlings.

(c) the effects of sublethal concentration of African locust bean effluent on some haematological characteristics of Nile tilapia fingerlings.

CHAPTER TWO

2.0 LITERATURE REVIEW

Oreochromis niloticus commonly known as the Nile tilapia, is a conspicuous member of the freshwater fauna in the tropics, where water temperatures are suitable for their growth and reproduction. Stickney (1979) reported that several species of tilapia including *O.niloticus* are able to survive well at dissolved oxygen concentrations as low as 1mg/litre and continue to grow rapidly if the exposure period is not prolonged. The fish is well suitable for pond culture in the tropics where it attains maturity in less than six months.

O. niloticus is a member of the family Cichlidae and the order is perciformes. It has a relatively high growth rate and reproduces at a relatively larger size than the other tilapia species. The genus was changed from *Sarotherodon* (Trewavas) to Oreochromis, however the use of common names such as giant *Tilapia* or Nile Tilapia or Nilotical helped to promote the popularity in rearing the species. They are widely distributed in Africa and Asia, and is one of the most widely farmed freshwater fish in the tropics. *O. niloticus* is laterally compressed and deep-bodied with dorsal fins. They may reach a size of 60.0cm and 450 grams and are more tolerant than most commonly farmed freshwater fish exposed to water temperature, low dissolved oxygen, high salinity and high ammonia concentrations. They are highly resistant to viral, bacterial and parasitic diseases especially at

optimum temperature for growth. They are good fish for warm water aquaculture because they are easily spawned, use a wide variety of natural foods as well as artificial feeds, tolerate poor water quality and grow rapidly at warm temperatures.

2.1 ENVIRONMENTAL PROBLEMS IN NIGERIA

According to Purdon (1983), man has exploited and modified the environment to his advantage in many ways, because of quest for a comfortable and a convenient life, thus inducing large enough perturbations in the ecosystem which can permanently upset the balanced state of the natural cycles. Furthermore, man's activities on earth due to industrialization have resulted in the production or discharge of materials (wastes) or energy such as organic materials and toxic chemicals into land, water and air. These have resulted in the distortion of the natural environment.

Fagade *et al,* (1993) emphasised that the aquatic environment, such as the brackish water in Nigeria, is no longer as productive as it should be because it is not able to tolerate human abuse on it. In the oil producing areas, oil exploration, extraction, production activities have induced perturbations and occasionally damaged the environment. Witter (1993) further stated that, the world is presently facing a terrible situation on the global scale with forest losses, global warming rising sea levels, pollution and contamination. In Nigeria, as in many other countries these problems are mirrored on a local and regional scale and much will be lost by the destruction of a wide variety of ecological zones and habitats, and rich biodiversity.

Presently, man is the major cause of pollution in the flowing waters, since they have turned the waters to the medium for getting rid of wastes, thus introducing poisonous substances into them, which consequently pollute the water bodies. Sikoki and Kolo (1993) noted that high concentration of these wastes, noted adversely affect the physicohemical properties of the water and consequently affect the biota in such water bodies.

2.2 THE IMPACT OF POLLUTION ON THE AQUATIC ECOSYSTEM.

Salvato (1982) noted that land disposal of water effluent has led to public health hazards which include: possible inhalation of pathogenic microorganisms, particularly bacteria and viruses from spray irrigation, activated sludge and tricking filter treatment systems.

Examples of Impact of pollution on the Aquatic ecosystem.

(a) Heavy metals or other toxic materials taken up by crops during growth

(b) Contamination of groundwater through infiltration and percolation of wastewater microorganisms and chemicals into a groundwater aquifer which serves as a source of drinking water.

(c) Runoff from land areas receiving wastewater effluent, to surface waters used as sources of drinking, shellfish, bathing water, or other recreational purposes.

(d) possible cross-connection between potable and non-potable water systems.

(e) Build-up of detrimental chemicals in the soil.

The various hazardous substances which can affect water quality and fish populations include pesticides, chemical fertilizers, sewage and industrial pollutants. According to Morgan (1972), Moss *et al.* (1980) and Godfrey (1982), agricultural pesticide application to rice paddy water contaminated or killed fish raised for human food. Similarly, the expanding use of chemical fertilizers can stimulate water weed and algal growth in reservoirs and primary canals, and led to eutrophication of fresh waters.

As a result of the eutrophication of streams and estuaries from fertilizer run-off or municipal wastewater, Jorgensen and Johnsen (1981) reported that organisms and the ecosystem undergo over stimulation and the ecological balance is also disturbed. The changes brought about by sewage include depletion of Oxygen, by action of biological oxidations and floating scums. Domestic sewage contains a lot of organic matter and other toxic substances which bring about changes in the chemistry of the water and the physical nature of the water and the physical nature of the substratum.

According to Jorgensen and Johnsen (1981),wastewaters and the different inherent effluents fall into six groups namely;

i. organic residues such as domestic sewage, effluent from food processing industries, ensilage, manure heaps and cattle yards, laundries and paper mills. These effluent vary a great deal, but they have much in common. They all contain complex organic compounds in solution and/or suspension, some times with toxic substances and various salts. Their basic property, however are unstable compounds

are readily oxidized and deplete the dissolved oxygen in the water. Some of these compounds are said to be more readily decomposed than others, for example, slaughter house wastes oxides rapidly, whitewood pulp is comparatively stable.

ii. Poisons in solution which occur in the waste-waters from many industries. They include acids, alkalis, oil, heavy metals and toxic organic compounds mainly from chemical industries, gas works and use of insecticides.

iii. Inert suspensions of freely divided matter which result from many types of mining and quarrying and from washing processes, such as those of coal and root crops.

iv. Other inorganic agents, such as salts or reducing agents. For example, Sulphides, Suphites and ferrous salts which occur as constitutents of the effluent of several types of industry. Minor discharges of salts are generally harmless to the environment but reducing compound use up the oxygen in the receiving body of water and have the same effects as organic residues.

v. Hot water which is produced by many industries that use water for cooling purposes. They often use river water, which is pumped through the cooling system and sometimes raised to very high temperature during the process.

vi. Bacteriological contamination of waters which originate mainly from domestic sewage, food processing industries, manure heaps and cattle ranch are also sources of this type of pollution.

However, waste waters emanate from four primary sources which are the municipal sewage, industrial waste waters, agricultural run off and storm water/urban run off. The problem of the first group is related mainly to organic residues and nutrients, while the second group encompasses the entire spectrum of pollution problems, although discharge of heavy metals and organic compounds are the most serious.

Clegg (1982), reported that physiological toxic substances such as persistent pesticides eventually get leached into rivers, lakes and oceans, where they become serious water pollutants. The Chlorinated hydrocarbons, Dichloro-diphenyltri chloroethane (DDT), Benezene Hexa Chloride (BHC), Dieldrin and Aldrin and the organ phosphorus compounds, parathion and malathion, have entered aquatic ecosystems and caused wildlife catastrophes, especially seabirds.

Furthermore pollutants that reduce dissolved oxygen consist mainly organic matter such as domestic sewage, animal excreta from intensive farming units and organic effluents from paper mills, food processing factories, dairies, breweries and silage.

This is broken down by aerobic saprophytic bacteria, which reduce the oxygen content of the water as they do so. The reduction of the oxygen content of the water according to Clegg (1982) can lead to the death of aquatic animals and a disruption of the aquatic ecosystem, this problem is compounded by thermal (heat) pollution which is invariably associated with such organic effluents as an increase in temperature decreases the solubility

of oxygen in water, as well as increasing the metabolic rate of organisms so that the animal population in the water requires more oxygen.

The physico-chemical changes which sewage causes in the quality of water in some rivers were described by Oladimeji and Wade (1984) who noted that these changes in turn caused an ecological imbalance especially in the distribution and abundance of organisms.

2.3. SPECIES SENSITIVITY

Jones (1977) suggested that important sensitive species must be identified from freshwater, estuarine (if possible) and marine environments and their biology must be fully studied so that effects of pollutants can be properly assessed. It is difficult to make generalizations about species sensitivity because of the differences in reporting values in the literature and in test methodologies.

It is also difficult to generalize about the toxicity of compounds because of lack of data for group of organisms (e.g. mollusc) when variables have not been controlled or at least specified, they may be the source of differences incorrectly regarded as due to species sensitivity.

2.4 COMMON PROBLEMS IN FISH TOXICOLOGY

2.4.1 Problems associated with therapeutic drugs

Chemicals usually used in the treatment of water and fish diseases must be applied at accurate doses to avoid fatal effects on the fish and other aquatic organisms. These chemicals include common salts (for the treatment of leeches), formalin (external protozoa and flukes) and malachite green (external protozoa and fungi). The use of some of these chemicals is still

dependent on colour index i.e. persistent pink colouration (potassium permanganate, $KMnO_4$) and attainment of light green colouration (malachite green). Even where the actual dosage is known these dosage is often dependent on physicochemical parameters such as temperature, hydrogen concentration (p^H) and alkalinity.

It is also important to note that species differ in their tolerance to treatment dosage and that the state of the fish before the chemical is applied on body, administered orally or mixed with feed. The problem associated with treatment according to Salami *et al.* (1991) may be either the use of wrong chemicals often due to inaccurate diagnosis, misleading text and the application of a wrong dosage, the causes of which had been mentioned above.

Tucker (1987) reported that chemicals used in the treatment of fish have to be effective to conquer pathogens and must neither be toxic to fish nor pollute the water media. Massive fish kills have been observed in fish hatcheries and farms where overdose of $KMnO_4$ have been applied. Salami *et al.* (1990) reported the 72 h-LC_{50} value of 3.4-5.2 mg/litre and 9.98 mg/litre for *Heterobranchus bidorsalis* and *Clarias gariepinus*, respectively, for $KMnO_4$ in pond water. The 96 h LC_{50} values of 0.8 mg/litre and 60 mg/litre for $KMnO_4$ in both pond water and spring water respectively for *C. gariepinus* and a 96 h LC_{50} value of 6.0 mg/litre and 3.0mg/litre of $KMnO_4$ for *Oreochromis niloticus* (Salami *et al.*, 1991). They observed that *C. gariepinus* and *H. bidorsalis* fingerling exhibited erratic swimming, loss of reflex, discolouration, peeling and eventual death. Examination of the gills

showed some alterations as the gills were swollen and the lamellae were fused. In addition, the cells of the liver, were deformed while the kidney lost their colouration.

2.4.2 Changes in the body composition

Toxic pollutants alter the composition of the fish flesh when taken up through the gills or the muscle of the fish. Stoner and Livingston (1978) reported that the exposure of finfish to 1% bleached kraft mill effluent resulted in reduced total lipid content and increased protein content. Rathore *et al.* (1979) found that the total protein content of the midge *(Chironomus tentans)* larvae was reduced when exposed to cadmium. It has also been observed that the appearance of fishes cultured in water receiving sewage were dull ie the fishes lost their irradiscient colour and that such colours were replaced only when the fishes are allowed to stay in clean water for sometime before being sold to fish mongers Salami et al. (1990).

2.4.3 Inhibition of growth

Sub-lethal doses of pollutants are reported to enhance growth, reduce growth or to have no effect on the growth of fishes in the receiving water. Webb and Breth (1972) recorded reduced growth is sockeye salmon (*Onchorhynchus nerka*), blue gills (*Mepomis macrochirus*) and fathead minnows (*Pimephales promelas*) exhibited retarded growth in zinc toxicity. Pickering, (1968) and the pesticides, endrin and malathion reduced growth in the flag fish, *Jordanella floridas*. Stoner and Livingston (1978) recorded no effect of bleached kraft mill effluent on growth, measured as length, in the pinfish, *Lagodon rhombiodea*.

2.4.4 Behavioural problems

Sub-lethal concentrations of toxic pollutant may effect the behaviour of organisms and the disruption of behaviour may reduce the fitness of a natural population (Mason, 1983). The swimming performance of fish may be reduced, caused by a reduction in oxygen uptake as has been described for sub-lethal concentrations of pulpwood fibre and Sub-acute concentrations of DDT caused changes in the orientation, movements, feeding, aggression and comfort behaviour of the fish, *Therapon jarbus* (Lingaraja *et al.*, 1979).

Chlorine and chloramines reduced the feeding rate of the rotifier, *Branchionus plicatilis* (Capuzzo, 1979) while detergents damaged the taste buds of the American channel catfish (*Ictalurus natalis*) such that they were unable to find food. Davis (1973) found that the precopultory behaviour of the amphipod, *Anisogammenus pugethensis*, was impaired in the presence of bleached kraft pulp mill effluent with the resultant effect that the paired animals separated.

Ajao (1985) reported that textile mill waste water and a detergent wash caused the hermit crab, *Clibinatium africanus*, to crawl in all directions with high concentration causing the crabs to climb on each other in a way suggestive of an avoidance reaction. Salami *et al.* (1991) reported the moulting, erratic swimming pattern and loss of barbels in two African catfishes namely C. *gariepinus* and *H. bidorsalis*.

2.4.5 Respiratory problems

Zinc reduced the oxygen level of blood leaving the gills, but when zinc was injected into the blood system, it had no effect. Zinc therefore,

reduces the efficiency of oxygen transport across the gill membrane so that the fish die of hypoxia (Skidmore, 1970). Sellers *et al* (1975) reported that zinc reduced the pH of the blood, probably because metabolic products such as lactic acid were increased. Large quantities of detergent lead to fish kills and in some cases lead to damage of cell membrane therefore leaving other toxicants to act and kill the animal (Salami, 1991).

Duffus (1980) noted that oil coating on living organisms may cause death by asphyxiation. He observed that oil films on the surface of natural waters reduce light transmission and hence, photosynthetic primary production. Such films also retard oxygen uptake by water and cause a lower dissolved oxygen concentration and the death of many organisms. Salami (1991) reported that low boiling point saturated hydrocarbons, at least up to octane, can produce anesthesia and necrosis in many lower animals. Benezenes characteristically inhibit blood cell formation in bone marrow and the low boiling point aromatic hydrocarbons cause local irritation of the respiratory system.

2.4.6 Effects on target organs

The water balance of fishes is affected by toxicants. The urine flow in the rainbow trout increased with an increase in the external ammonia concentration until the highest concentration of 20 mg/litre of nitrogen at which time all the fishes died (Lloyd and Swift, 1976). Mason (1983) noted that the mechanism by which ammonia increased the water uptake of fish is unknown, though it could be related to increased activity, which may increase permeability.

Smart (1978) suggested that ammonia disturbs cellular metabolism, resulting in an increasing oxygen requirement; the hyper-metabolic state thus accelerated the utilization of tissue energy reserves. Thus, high energy compounds may become depleted in the brain with the characteristics symptoms which include hyperexcitability and hyperventilation which has been reported in fishes.

Adeney (1977) reported increase in ventilatory and coughing frequency, and response to anoxic of treatment exposed to copper sulphate solution of 6 mg/litre showed increased in their activity of up to 4-6 times that of the controls during the first eight hours of exposure, while the activity pattern returned to normal after three days. Bilinnki and Jonas (1973) also noticed extensive degeneration of gill structure, detachment of the epithelia hypertrophy and hyperplasia of the interlamella epithelium and a significant depression of lactate oxidation in exercise gill filaments from surviving rainbow trout exposed to cadmium. Smith (1977) reported impaired eggs viability following gametogenesis and embryogenesis in acidified water.

Cadmium caused increases in plasma glucose levels, whereas methyl mercury resulted in increases in haemoglobin and plasma sodium and chloride. Lead inhibits enzyme activity and acts at a large number of biochemical sites. Mason (1983) reported that at low levels of pollution the organism is maintained in health by normal homeostatic mechanisms. As level rise compensation occurs such that normal functioning is maintained without significant changes but a higher levels still the organism becomes stressed and physiological breakdown occurs, the organism is unable to

repair damage and become disabled. Much higher loadings of pollutants result in physiological failure and death.

Oxygen is the first limiting component of the aquatic environment and the minimum tolerated O_2 level varies with species. Tilapias, for example, are relatively hardy and will tolerate DO as low as $3mg.l^{-1}$ whereas salmonids repond badly below $5mg.l^{-1}$ or higher (Ross & Ross 2002).

2.4.7 Municipal Effluent

It causes serious Biological Oxygen Demand (BOD) problems and the case worsens when the effluent is untreated. The excessive growth of plants (algae and water hyacinth) according to Dore (1993) aggravate the problem by their own oxygen demand. In enclosed water bodies (lakes, lagoons, mangrove swamps), the loss of oxygen reduces the biological diversity. In extreme cases, organic matter together with toxic materials can cause anoxic condition. Shellfish contamination particularly filter feeders clams, oysters, mussels) of micro-orgnaisms and organochlorines from sewage pose serious health hazards when consumed by man. Also, high concentration of halogenated hydrocarbons, which have a tendency to accumulate along food chains are reported to have lethal effects on fish, plankton, crustaceans and mollusc (Dore, 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION AND CONDITIONING OF EXPERIMENTAL FISHES

A total of 240 live fingerlings of *Oreochromis niloticus* used for this study were purchased from Agricultural Development Project (ADP) Fish Farm at Alagbaka, Akure between January 2000 and August 2000. They were transported in cylindrical plastic buckets to the Federal University of Technology, Akure, laboratory. The fingerlings were weighed and the mean weight was 2.13 $^{\pm}$S.E0.00 at the start of the exposure period. The fingerlings were acclimated to laboratory conditions for seven days, during which they were fed with a commercial fish diet twice daily (9-9.30h) and (6-6.30h) at 3% of their body weight before the commencement of the toxicity tests.

3.2 COLLECTION OF TEST POLLUTANT

African locust bean processing industry effluent was obtained from an African locust bean processing factory at New Oke-Aro in Akure, Ondo State. The effluent was collected from the portion where the waste was flowing out through a gutter by placing a plastic container that was covered with its tightly sealed cover immediately after collection and was transported from the producer to the laboratory in the morning time.

3.3 BIOASSAY PROCEDURE

The fingerlings were acclimated and undamaged before the commencement of the toxicity tests. The fingerlings were placed in 100 ml of water inside conico-cylindrical plastic buckets for two days, to enable

them adjust to test laboratory conditions such as light intensity and temperature. Feeding was discontinued during these two days in order to avoid pollution of the water with feed wastes.

3.4 WEIGHTING AND SORTING OF FINGERLINGS

A top loading mettler balance was used to weigh the experimental fish individually. The fingerlings were randomly distributed into each bucket containing 100 ml of water at ten fingerlings per container. The mean weight of the fingerlings was 2.13± S.E 0.00

3.5 RANGE-FINDING TEST

The range finding test was carried out by using ten conico-cylindrical plastic buckets, each filled with 100 ml of water prior to the introduction of the effluent. Each of the five varying concentrations (5ml, 10ml, 15ml, 20ml and 25ml) of African locust bean effluent was introduced with a pipette into the containers at 09.h and the mixture was later stirred. Two replicates of the control were also prepared by placing ten fingerlings in each of the two buckets already filled with 100 ml of water without the effluent. A total of 120 *O. niloticus* fingerlings (mean weight 2.13 ± S.E 0.00) were used for this test.

3.6 DEFINITIVE TEST

The definitive test was carried out by using ten conico-cylindrical plastic buckets each filled with 100ml of water. Five varying concentrations of African locust bean effluent (1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0ml) were duplicated and each was placed in each of the plastic containers. Two replicates of the control were also prepared by placing ten fingerlings in each

of the plastics containers already filed with 100 ml of water without the effluent. A total of 120 *O niloticus* fingerlings (mean weight 2.15± S.E 0.00) were used for this test.

3.7 DETERMINATION OF THE LETHAL CONCENTRATION

The 96h-LC_{50} value is defined as the concentration of a toxic material which kills 50% of test organisms within 96h. This was determined using logarithmic transformation (Hewlett and Placket 1979).

3.8 BIOLOGICAL DATA COLLECTION

The number of dead and living fingerlings in each plastic containers was recorded every 24h. General observations on the fingerlings were also made at six hour intervals, starting from 08h, during which the behaviour of the fingerlings were thoroughly monitored. Observations made include mortality rate, lack of movement and lack of reaction to gently prodding as described by Ward and Parrish (1982). The percentage mortality in each concentration was determined.

3.9 WATER QUALITY MONITORING

During the exposure period which lasted for four days, the temperature, p^H and dissolved oxygen (DO) concentration were determined at 24 hour intervals (09h) everyday, using the standard methods described by APHA (1989).

3.9.1 Temperature

This was determined using mercury-in-glass thermometer calibrated in degrees centigrade (°C). It was inserted into the different plastic containers

containing the different treatments for two minutes then the readings were taken.

3.9.2 pH

This was determined by using p^H meter (model, METTLER TOLEDO 320). The probe was inserted into the sample bottles containing the different samples (e.g. African locust bean processing industry effluent.

3.9.3 Dissolved oxygen (DO) concentration

This was determined with a digital dissolved oxygen meter (DO) model 90,71). The probe was inserted into the sample bottles containing the different treatments. The unit of measurement is mg/litre.

3.10 HISTOLOGICAL EXAMINATION OF ORGANS OF *O. niloticus*

Immediately after death, gills and liver of the fingerlings were excised upon dissecting the fish. They were fixed in 10% formalin for three days to preserve the organs. The fixed organs were dehydrated in graded levels of alcohol (50%, 70%, 90%, 100%) after which they were cleared in 50/50 mixture of alcohol and xylene for three hours followed clearing in 100% xylene for three hours, after which they were embedded in Petri dishes with wax. The specimens were later mounted on wooden blocks and sectioned with the aid of a microtome to 7um sections before staining in haematoxylin and eosin. The stained specimens were observed under a light microscope.

3.11 HAEMATOLOGICAL CHARACTERISTICS

Two fingerlings from each of the experimental and control media were placed on a table immediately after death and blood samples were collected from them by caudal puncture into 2.5 ml syringe already treated with

ethylene amino tetra acetic acid (EDTA) to prevent coagulation. Packed cell volume (PCV), haemoglobin concentration, red blood cells and the count were estimated using various appropriate laboratory methods as described (Unified method 1991).

3.11.1 Packed cell volume (PCV)

The blood samples obtained from the test tubes were collected using 100mm capillary tubes which were already treated with (EDTA). The blood was drawn by cappilary tubes until four-fifths (4/5) full. The dry end of the capillary tubes was then sealed immediately, with plasticine. The capillary tubes were placed in microhaematocrit centrifuge and were then spun at 10,000 revolutions per minutes (rpm) for 5 minutes. The PCV was then established by placing the spun tubes into the haematocrit already calibrated in percentage.

3.11.2 Erythrocyte Count

The blood samples was diluted with the Hayem's fluid, comprising of 1g Nacl, 5g Na_2 SO_4, 0.5g $HgCl_2$ and 200 ml dilute water. A haemacytometer placed on a compound microscope was used to estimate the erythrocyte number. The number of cells counted was multiplied by a diluting factor of 200 and volume factor (VF of 50) as according to Svobodova *et al* (1991).

3.11.3 Haemoglobin Concentration

This was determined by the indirect acid haematin (salilic) method. It was carried out by using special haemoglobin meter and a pipette. The reagents and the process were as according to Kelly (1979).

The haemoglobin is converted to acid haematin by using Salilinometer N/10Hcl and).02 ml pipette. The salilinometer was filled to the 20ml mark with N/10HCl and 0.02ml blood added and mixed perfectly. This was kept for five minutes, with the distilled water being added drop by drop until the colour matches that of the standard sample.

The standard sample is the colour produced by a known haemoglobin concentration and the blood was obtained by measuring the amount of oxygen or iron in the haemoglobin. The amount of solution in the graduated tube gives the haemoglobin concentration as a percentage, where the value obtained is multiplied by 17.2g per 100ml and later divided by 100.

3.11.4 Blood smear preparation

A drop of blood was drawn across a microscope slide, and then another microscope slide was used to create smears. Smears were air-dried at ambient temperature 5^{0}C before fixing in methanol for five minutes staining followed with Giensa for 30 minutes. The stain solution was drained and the smear washed with a p^{H} buffered phosphate solution then dried again. The blood smears were later examined under-oil immersion.

3.12 STATISTICAL ANALYSIS

Data collected were subjected to the Two-way analysis of variance (ANOVA). Differences among the means were separated by Duncan multiple range test at P=0.05. Duncan 1955. The main effects considered were time and concentration.

CHAPTER FOUR

RESULTS

4.1 WATER QUALITY

The water parameters monitored are temperature, dissolved oxygen and p^H. The parameters were measured at 09h each day and at 24 hours interval. The water quality values did not change throughout the test (Table 1).

4.2 OBSERVATIONS MADE ON *O. niloticus* FINGERLINGS

The mortality of *O. niloticus* was observed in all concentrations (1ml,2ml,3ml,4ml,5ml) of the test toxicant as shown in Table (2). It was observed that no fish died after 24 hours of exposure. No mortality was observed on exposure of tilapia fingerlings to 1ml or 2ml of effluent throughout the experiment however, mortality was found between 24 and 96 hours of exposure to 3ml-5ml. On introduction into the toxicant concentrations, abnormal behaviour was observed among the fishes Table 3 and this increased with increase in concentration of the toxicant. Moulting was observed on all the fishes treated with the toxicant, but at different degrees, as they showed variation in tolerance to the toxicant at different levels of concentrations. Also, mortality increased with increase in concentration. The LC_{50} obtained graphically for the fish exposed to locust bean effluent within the 96 hours duration was 3.1ml (Fig 1) and the stastical analysis (ANOVA Table) revealed that there is a significant difference between the control treatment and the test media. $P<0.05$. Table 5.

Table 1.

MEAN VALUES OF THE PHYSICO-CHEMICAL ANALYSIS OF WATER CONTAINING THE AFRICAN LOCUST BEAN PROCESSING INDUSTRY EFFLUENT DURING THE 96 HOURS PERIOD.

TREATMENT

Water parameter	Control	1.0mls	2.0mls	3.0mls	4.0mls	5.0mls
Mean Temperature (^{O}C)	26±0.00	26±0.00	26±0.00	26±0.00	27±0.00	27±0.00
Mean P^{H}	6.70±0.00	6.68±0.00	6.67±0.00	6.63±0.00	6.59±0.00	6.51±0.00
Mean DO Concentration	6.80±0.00	6.80±0.00	6.68±0.00	6.60±0.00	6.50±0.00	6.41±0.00

Means ± Standard Error (SE)

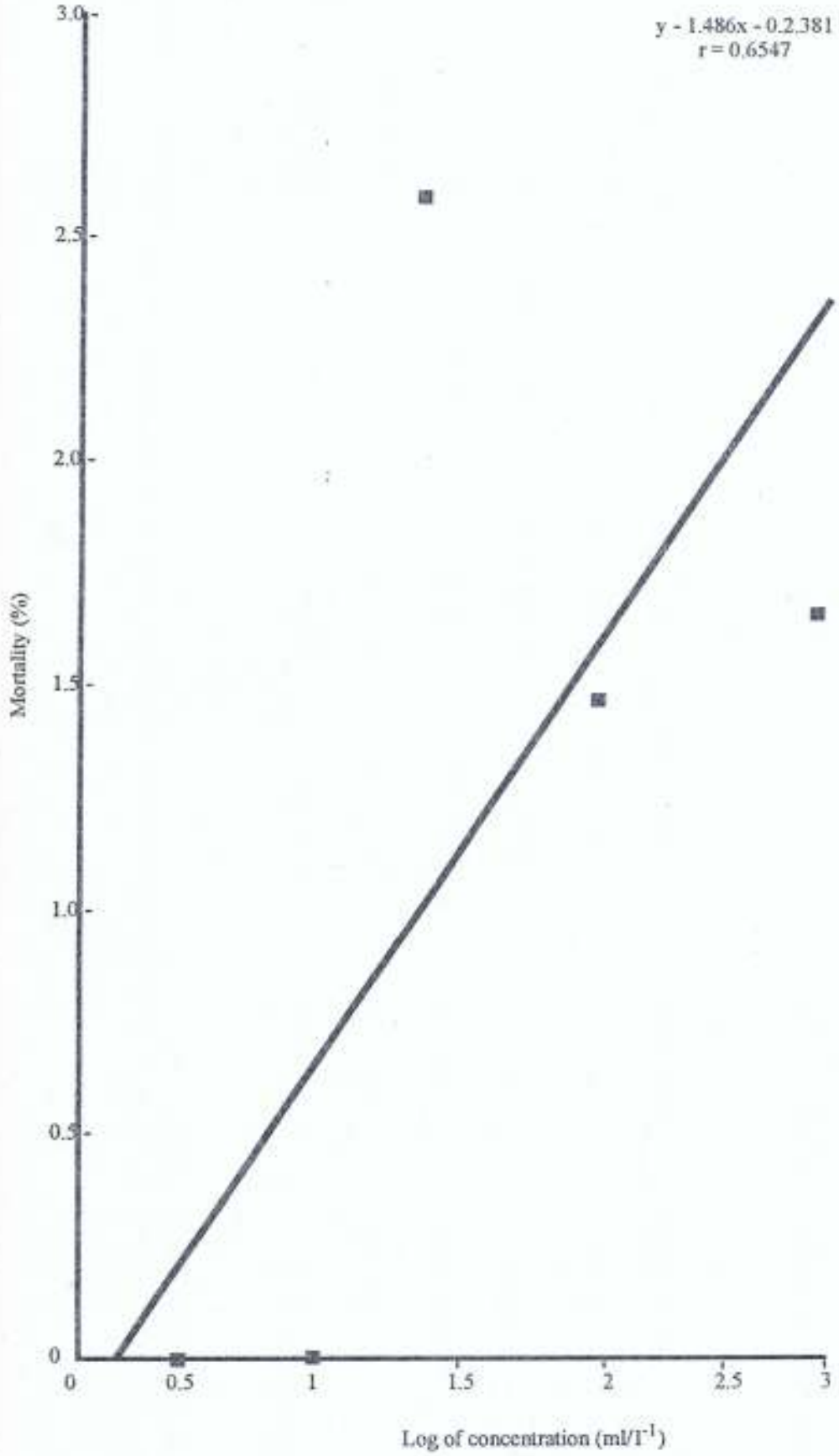


Figure 1:- Mortality (96h - LC_{50}) of *O. niloticus* fingerlings in different concentrations of *Parkia bioglobosa* effluent

TABLE 2 – Descriptive statistics of *O. niloticus* in tap water polluted with African locust bean processing industry effluent at different concentrations in definite test.

Concentration	6 hours	12 hours	24 hours	48 hours	72 hours	96 hours
1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	0.00±0.00	0.00±0.00	10.00± 0.00	10.00± 0.00	10.00± 0.00
4	0.00±0.00	0.00±0.00	10.00± 0.00	10.00± 0.00	10.00± 0.00	10.00± 0.00
5	0.00±0.00	0.00±0.00	10.00± 0.00	10.00± 0.00	10.00± 0.00	30.00±0.00

Means of two replicates and ± Standard Error

4.3 RANGE FINDING TEST.

The exposure of tilapia to 5ml and 10ml of African locust bean effluent resulted in 20% and 70% mortality respectively within 96 hours while exposure to 15ml, 20ml and 25ml resulted in 70%, 80% 90% and 100% mortality, respectively, within the exposure period. (Table 4).

4.4 HAEMATOLOGICAL EXAMINATION

The concentrations of the African locust bean processing industry effluent used for the haematological test were 1m/L, 2m/L, 3m/L, 4m/L and 5m/L for a period of 4days (96 hours), Appendix IV. Result of the haemoglobin values, the red blood cells (RBC) and packed cell volume of tilapia fingerlings exposed to 1ml, 2ml, 3ml, 4ml and 5ml of the effluent decreased significantly compared to those of the control fish while the values of erythrocyte of the fish increased at the end of the exposure period compared to the value recorded in the control which indicate that the higher the concentrations, the less the values of the blood indices (Haemoglobin, packed cell volume and the red blood cell. During the test, fishes showed stress condition immediately they were introduced into the treatment, they exhibited erratic swimming pattern, loss of reflex, change of behaviour by remaining upright within the treatment and hyperventilation. Table 3.

4.5 HISTOLOGICAL CHANGES

The gills and liver of the fish were examined to assess the toxicological effect of the African locust bean effluent on them. The various organs were exposed to different level of concentrations of the toxicants, that is 1ml, 2ml, 3ml, 4ml and 5ml.

TABLE 3: Effect of African locust bean processing industry effluent on body features of *O.niloticus* fingerlings

M1/1	24 HOURS							48 HOURS						72 HOURS						96 HOURS					
Concentration	1	2	3		4	5	Control	1	2	3	4	5	Control	1	2	3	4	5	Control	1	2	3	4	5	Control
Erratic Swimming	-	-	+		+	+	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-
Loss of Reflex	-	-	+		+	+	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-
Changes in Behaviour	-	-	+		+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-
Hyper Ventilation	-	-	+		+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-
Moulting	-	-	+		+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-
Death	-	-	+		+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-

Keys

\+ = present

\- = Not present

TABLE 4 – Descriptive statistics of *O. niloticus* in tap water polluted with African locust bean processing industry effluent at different concentrations in range finding test.

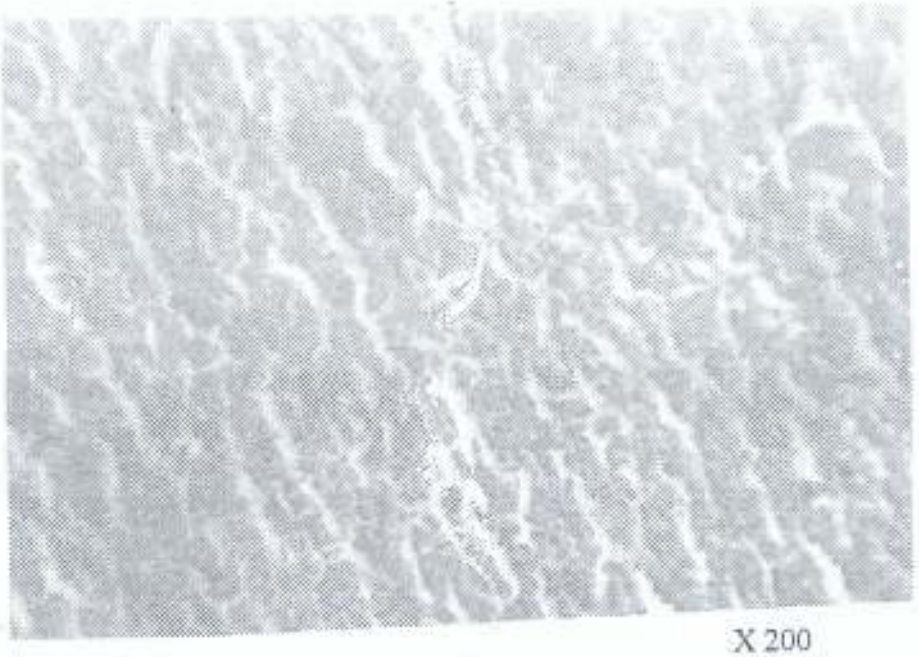
Concentration M/L	6 hours	12 hours	24 hours	48 hours	72 hours	96 hours
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5	0.00±0.00	0.00±0.00	10.00±0.00	10.00±0.00	20.00±0.00	30.00±0.00
10	30.00±0.00	30.00±0.00	40.00±0.00	50.00± 0.00	50.00± 0.00	60.00± 0.00
15	30.00±0.00	50.00±0.00	50.00± 0.00	60.00± 0.00	65.00± 0.00	70.00± 0.00
20	40.00±0.00	60.00±0.00	65.00± 0.00	70.00± 0.00	75.00± 0.00	80.00±0.00
25	65.00±0.00	70.00±0.00	75.00±0.00	80.00±0.00	100.00±0.00	100.00±0.00

Means of two replicates and ± Standard Error.

Mucus accumulation was observed on body surfaces and gill filaments of dead fish. Examination of the gills of untreated (control) *O. niloticus* fingerlings (Plate 7) revealed a normal gill filament consisting of primary lamellae with its arrays of delicate secondary lamellae, primary epithelium and secondary epithelium covering the primary and secondary lamellae respectively and no vacuolation. There was little or no discernible change in gill structure of *O. niloticus* fingerlings exposed to low concentrations (1ml and 2ml) of the effluent. At higher concentrations however (3ml and 5ml) Plate 11 and 12, the gill structure showed detachment of the epithelial cells in both the primary and secondary lamellae, there was also vacuolation of the filament and influx of cells into the tissue which resulted into degeneration of the tissue.

Histological studies revealed that low concentrations (1ml and 2ml) of the locust bean effluent did not cause any histological damage to the *O. niloticus* liver. In higher concentration (3ml to 5ml), the liver exhibited histological changes as indicated by loss of nuclei in some hepatocytes, which also did not show distinct cytoplasmic boundaries as seen in the control fish liver (plate 1). There was space formation in the parenchyma tissue and the nuclear cells had thick dark look (pykuosis) as seen in plate 4-6. The cellular arrangement of the liver cells was also distorted. At some places, necrotic zones were observed where complete cell death was evident. Infiltration was also clearly evident (plate 6). Lesions were more prominent in the central zone of the liver than in the periphery.

The above results confirmed that the African locust bean effluent had a direct impact on the tissues of the *Oreochromis niloticus*.



X 200

Plate 1: Liver of *O. Niloticus* in the control experiment

X 200

Plate 2: Liver of *O. niloticus* exposed to 1 ml/l African locust bean processing factory effluent.

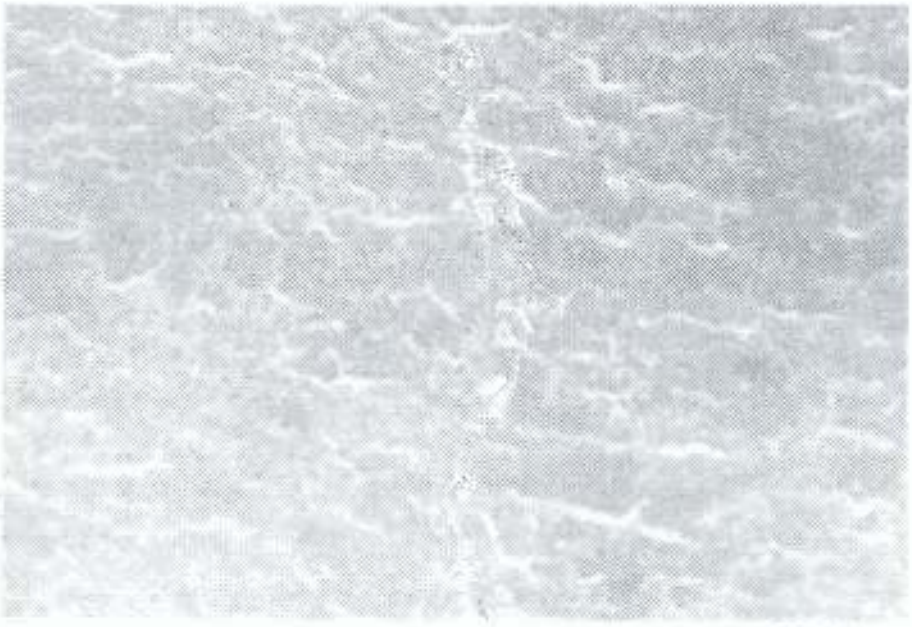


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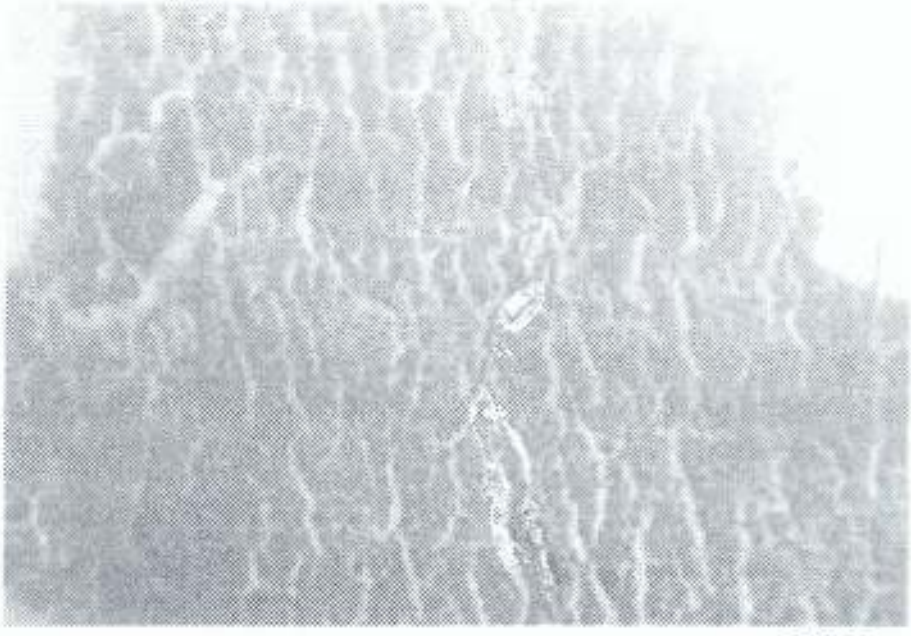
Plate 3: Liver of *O. niloticus* exposed to 2 ml/l African locust bean processing factory effluent.



X 200

Plate 4: Liver of *O. niloticus* exposed to 3 ml/l African locust bean processing factory effluent.

X 200

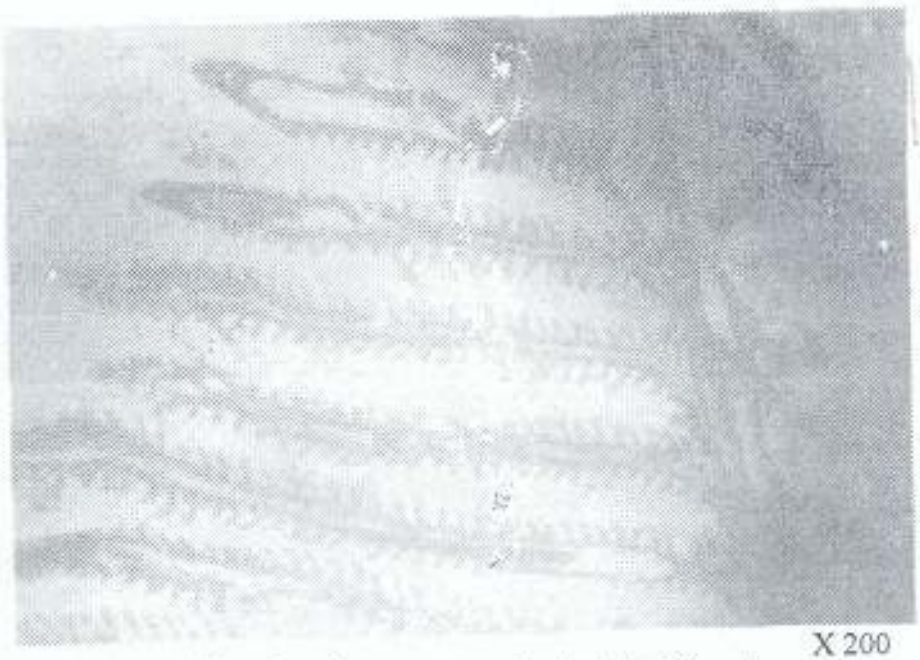
Plate 5: Liver of *O. niloticus* exposed to 4 ml/l African locust bean processing factory effluent.

X 200

Plate 6: Liver of *O. niloticus* exposed to 5 ml/l African locust bean processing factory effluent.

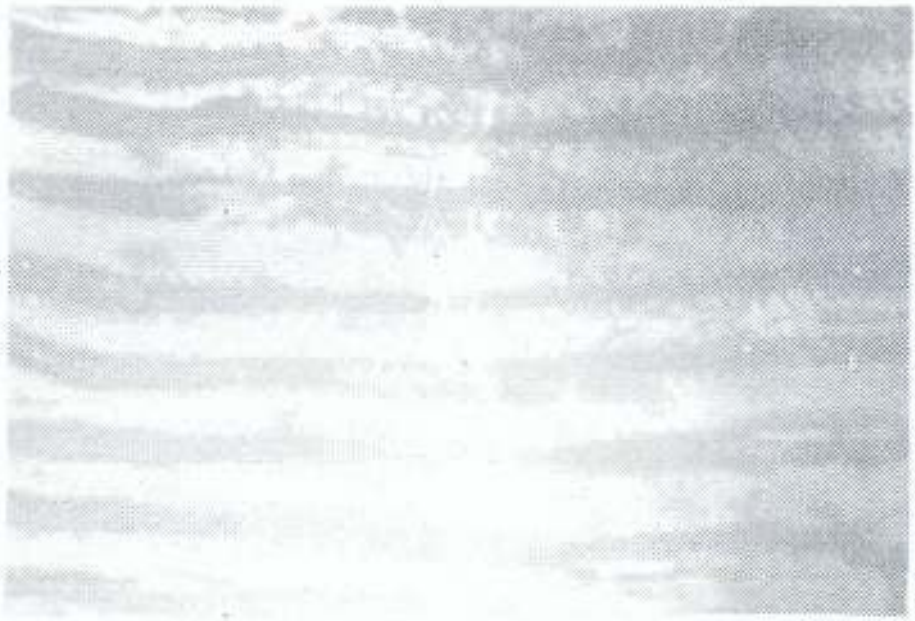
X 200

Plate 7: Gills of *O. niloticus* in the control experiment.



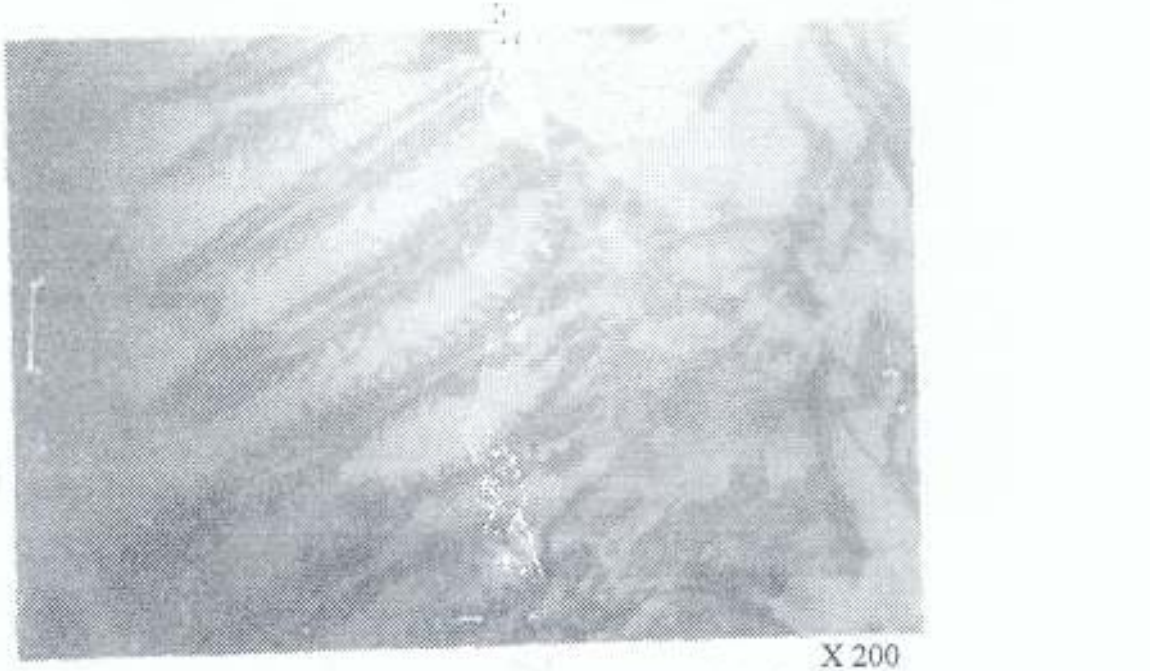
X 200

Plate 8: Gills *O. niloticus* exposed to 1 ml/l African locust bean processing factory effluent.



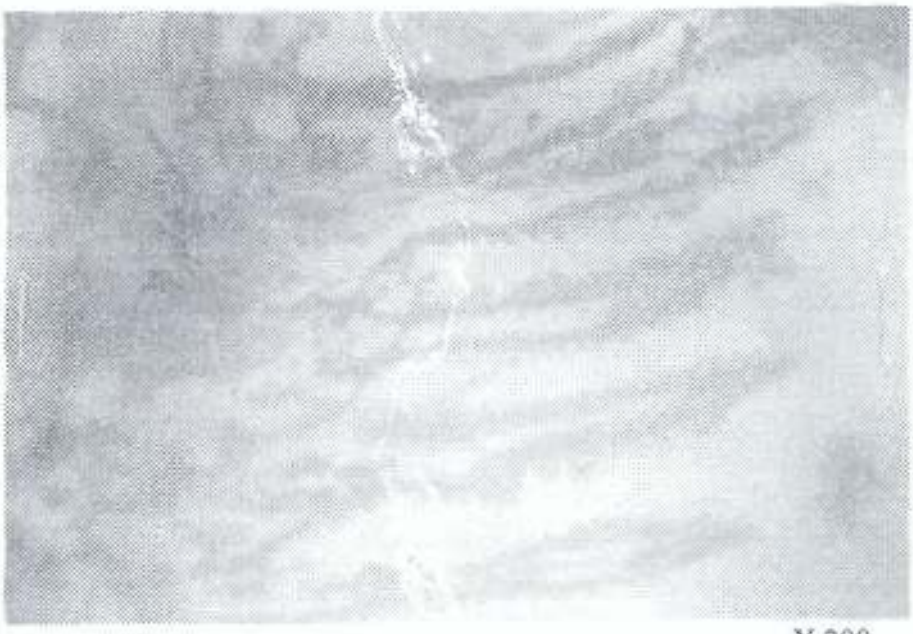
X 200

Plate 9: Gills of *O. niloticus* exposed to 2 ml/l African locust bean processing factory effluent.



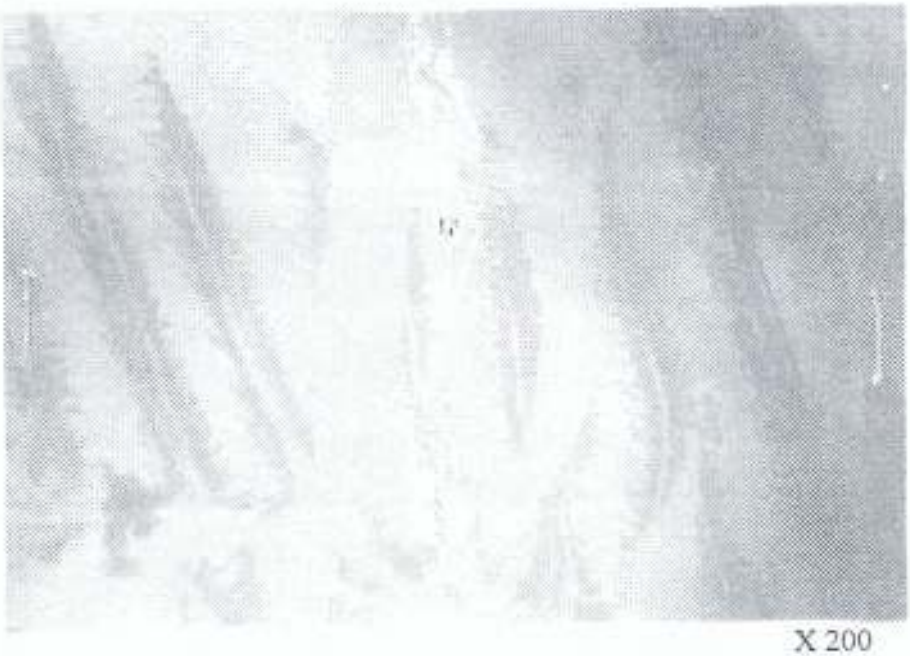
X 200

Plate 10: Gills of *O. niloticus* exposed to 3 ml/l African locust bean processing factory effluent.



X 200

Plate 11: Gills of *O. niloticus* exposed to 4 ml/l African locust bean processing factory effluent.



X 200

Plate 12: Gills of *O. niloticus* exposed to 5 ml/l African locust bean processing factory effluent.

CHAPTER FIVE

5.0 DISCUSSION

The trend of results obtained from this study, plates 1-12 revealed that *O. niloticus* fingerlings of the same age and size exhibited different degrees of hyper-activities, stress, lesions, necrosis and mortality to different concentrations of diluted African locust bean effluent. This observation was similar to the findings of James and Chery (1993), they noted that hybrid striped bass of the same age exhibited differences in their tolerance to lethal ammonia concentrations.

Upon the addition of the locust bean effluent, especially the higher concentration, fish showed initial disturbed swimming movements rapid opecular movements and surfacing behaviour. This indicates an avoidance response to the effluent. Lower concentration of the effluent did not produce obvious behavioural change that shows serious threat to the live of the fish.

Table 1 shows the values of physico-chemical parameters of both the control and the treated fish used during the research period. The table revealed that there was no significant difference between the water quality parameters of the control experiment and the treated experiment. It was ascertained that African locust bean processing industry effluent did not have adverse effects on the water quality, so the mortality could not have resulted from the physico-chemical parameters.

As presented in plates 8-12 histological examination revealed that from the treated fish species, the gills were swollen and the lamellae were extensively fused and congested with blood at the end of the 96 hours

exposure. Similar observations were made by Onwumere (1986), who expressed that the histology of the organs (liver and gills) of *O. niloticus* fingerlings exposed to 30, 40 and 50% effluent from the NNPC Refinery at Kaduna showed that the gills were swollen and bulged the opercula. Aderiye (1998) also stated that the gill structure of the fish *O. niloticus* treated with petrol and engine oil mixture was fused together and that there was extensive hyperplasia and separation of the epithelial layer from the supportive tissues.

All fishes exposed to the different levels of the effluent showed the separation of respiratory epithellum from the underlying supportive tissue. This phenomenon is related to the gill function disorders which may affect the physiology or cause the death of the fish (Smart, 1976).

In the liver, denegeration vacuole and irregular nuclear was noted and these was observed in newborn guppies *Lebistes reticulates* (Crandall and Goodnight 1962), disintegration and necrosis in common carp, cyprinus carpio (Wong *et al.* 1977) and vacuolation, necrosis and appreance of some globular bodies in *Puntius eonchonium* (Kumar and Pant 1981) due to zinc toxicity have been reported.

Exposure of *O. niloticus* to sub-lethal concentrations of African locust bean caused a significant decrease in PCV, RBC and HB of the fish. Similar reduction in blood parameters have been reported by Sampath et al. (1993) and Omoregie et al (1994) when they exposed *Oreochromis niloticus* to polluted environment under laboratory conditions. The significant reduction in these parameters is an indication of severe anaemia caused by the effluent on the exposed fish. Anaemic response could be as a result of destruction of erythrocyte production (Wintrobe 1978, Omoregie 1995), haemodilution

(sampath et al. (1993) and it could be as a result of the destruction of intestinal cells (Gardner and Yerich 1970). Similarly, Rambhaskar and Rao (1990) noted that changes in haematological parameters due to unfavourable exogenous factors such as adverse water quality, overstocking and starvation are indices of ill health in cultivated fish. Ayotunde (1997) also reported that stress factors result in changes in haematological characteristics in Fish.

The fact that some of the fishes exposed to the locust bean were alive, did not stop the gills from being ruptured, filaments eroded and eventual swollen with blood. The rate at which the gill lamellae were affected increased as the concentration of the toxicant increased.

It is therefore concluded that some parameters and organs are affected negatively by increasing concentrations of the effluent and this will guide in policy formulations against the effect of African locust bean effluent on water pollution.

CONCLUSION AND RECOMMENDATIONS

This study revealed that discharges from African locust bean products introduced deliberately or accidentally into aquatic ecosystem during washing besides rivers have resulted to degenerative changes (such as necrosis, lesions, lamellae infiltration etc) in the gill and liver of *O. niloticus*. The effluent will definitely affect both man and aquatic ecosystems directly or indirectly. There is therefore a great need to combat the discharges by way of pollution control in order to conserve the aquatic ecosystem. The followings are recommended for immediate action.

(a) One of the effective methods of managing the living resources of Nigerian Marine and fresh water is the enforcement of fisheries regulations and bye laws. This would not only prevent pollution of the waters and over-exploitation but will provide high quality fish for consumption by the Nigerian teaming population. The enforcement of the existing laws and regulations will arrest the prevalent neglect and abuse suffered by the Nigerian water bodies. Laws for proper land use and for the protection and preservation of our water resources must be made and enforced by the relevant agencies.

(b) The Government should also mount public awareness programmes on the environment as a result of the high illiteracy rate and high percentage of rural settlement in the nation.

(c) Research programmes on the conservation of the coastal mangrove ecosystem in order to stabilize the coastal habitat should be supported and encouraged by the government.

(d) Environmental Impact Assessment (EIA) should be carried out by the Government in all new developmental programmes and activities and the on-going ones should be subjected to environmental auditing as well.

NON-GOVERNMENTAL ORGANIZATIONS (NGOS)

The services of non-governmental or private groups in creating awareness on the need to protect the environment in general and the aquatic resources in particular should be ensured. NGOs can be involved in the dissemination of environmental knowledge among the public especially through the electronic and print media and publications like pamphlets, handbills and posters. Seminars could be organized across the States of the Federation targeted at the local communities.

International pressures can be secured on governmental agencies when a particular ecosystem is under threat. This will help in the conservation of genetic biodiversity in our ecosystem.

INDUSTRIALIZATION

The involvement of the private sector in refuse collection, environmental quality standards and safe methods in the use of water for industrial processing should be introduced.

EDUCATIONAL INSTITUTIONS

Education is primarily about communication of values to the next generation. The value formation programmes should be introduced in schools to inculcate a respect for the environment and the earth planet. The need for the conservation and preservation of water resources could also be attained through talks to school children on occasions like World Environmental Conservation Day. Schools and Colleges can introduce into their curriculum well designed ecological and nature-related courses and studies for the education of the children in their formative years.

ROLE OF THE INDIVIDUAL

Each and everyone of us should have a sense of respect for all living beings. We must cooperate with other members of the Community in the campaign for environmental protection and conservation. It is in cultivating a sense of conservation ethics that our environment can be judiciously and sustainably used for the benefits of the children and those yet unborn.

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APPENDIX I

Mean Mortality of *Oreochromis niloticus* fingerlings exposed to different concentrations of African locust bean effluent.

ANOVA TABLE

Tests of Between-Subjects Effects

Dependent Variable: Mortality

Source of variation	Sum of Squares	Df	Mean Square	F	P value Sig.
HOURS	15.638	4	3.910	5.341	.001
CONC	23.269	5	4.654	6.357	.000
Error	36.603	50	.732		
Total	180.000	59			

a. R Squared = 797 (Adjusted R Squared = .756)

P<0.05 (significant)

APPENDIX II

Mortality

Duncan [a,b]

Hours	N	Subset	
		1	2
6 Hours	12	707107a	
24 Hours	12	707107a	
96 Hours	12		1.551528b
48 Hours	12		1.762633b
72 Hours	12		1.869910b
Sig.		1.000	

Means for groups in homogenous subsets are displayed

Based on Type III sum of Squares

The error term is Mean Square (Error) = 732.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha= .05

APPENDIX III

Mortality

Duncan [a,b]

Concentration	N	Subset	
		1	2
Control	10	707107a	
1 ML	10	707107a	
2 ML	10		1.720412b
3 ML	10		1.973739b
4 ML	10		2.102471b
5 Ml			
Sig.		1.000	.353

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square (Error) = .732

a. Uses Harmonic Mean Sample Size = 10.000

b. Alpha = .05.

APPENDIX IV

Effect of Concentration on Haematological Parameters

Haematological Parameters	Concentration	N	Mean	Standard Error
ESR (MM)	Control	2	3.9	0.1
	1 ml	2	5.1	0.1
	2 ml	2	5.4	0.1
	3 ml	2	5.95	0.05
	4 ml	2	6.4	0.1
	5 ml	2	7.5	0
PCV (%)	Control	2	29	1
	1 ml	2	27	1
	2 ml	2	28.5	0.5
	3 ml	2	24.5	0.5
	4 ml	2	23.5	0.5
	5 ml	2	20.5	0.5
RBC (mm^3)	Control	2	15350000	50000
	1 ml	2	1460000	10000
	2 ml	2	1490000	10000
	3 ml	2	1340000	10000
	4 ml	2	1343000	5000
	5 ml	2	1170000	10000
Hb (g/mm^3)	Control	2	25	1
	1 ml	2	24.5	0.5
	2 ml	2	25.5	0.5
	3 ml	2	24	1
	4 ml	2	23.5	0.5
	5 ml	2	21.5	0.5