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GROWTH AND MORTALITY RATES HISTOPATHOLOGY EFFECTS OF WATER SOLUBLE FRACTION OF BONNY LIGHT CRUDE OIL ON THE AFRICAN CATFISH, *Clarias gariepinus*

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Abstract
Chronic toxicity test of Water Soluble Fraction (WSF) of crude oil (Bonny Light Crude) was carried out to determine its effect on the African catfish, *Clarias gariepinus*. Effects such as growth rate, mortality rate histopathology of the fish were investigated. The fish was subjected to graded levels of concentration of WSF of the Bonny Light Crude oil in six different tanks namely 5.0ppm; 10.0ppm; 15.0ppm; 20.0ppm; 25.0ppm and 30.0ppm of toxicant respectively. Tank 7 is the control having 0.0ppm of the toxicant. The results indicated a retarded growth rate when compared to the specific growth rate (SGR) of the control which stands at 38.2% and 48.7% for Total length and Standard length respectively. Where those exposed to 5ppm had (31.2:40.2), 10ppm (28.3:35.1), 15ppm (23.1:29.5), 20ppm (23.8:23.8), 25ppm (20.1:19.4),30ppm (10.8:13.9) respectively. The specific growth rate showed similar trend as that for length. The result shows that those exposed to 5ppm of toxicant (67.8%) weight gain; 10ppm (48.6%) ,15ppm (44.2%) ,20ppm (30.9%), 25ppm (19.7%) ,30ppm (18.8%) respectively. This when compared to the control of (104.2%) gain is a great decrease in growth performance. The summary of the results indicates detrimental effect of the toxicant on the *Clarias gariepinus* both at low concentrations and at high concentrations of WSF of Bonny Light Crude Oil.

Keywords: Chronic toxicity, Water soluble fraction, growth response, histopathology

Introduction

African Catfish Clarias gariepinus is a specie of the catfish family of the Claridae, the air breathing catfishes. They are found throughout African and the Middle East and live in the freshwater lakes, rivers, and swamps, as well as human made habitats such as oxidations ponds or even urban sewage systems. The African catfish Clarias gariepinus is the most popular and widely cultivated fish in the Niger Delta of Nigeria (Olukunle, 2013). The African Catfish Clarias gariepinus is very susceptible to the effect of WSF due to its predominance in the Niger Delta where heavy oil exploration is ongoing. The tendency for this encounter between the African catfish and the WSF is very high and detrimental to the existence of the fish.

Because of the high nutritional content of the African catfish Clarias gariepinus, it is consumed in large quantities in Nigeria. A number of researchers have investigated these effects, and some of their work is included below. According to study conducted by Henry et al. (2016), being exposed to crude oil significantly increases the risk of death for invertebrates, amphibians, and a number of different kinds of fish. Alterations in the species composition, low abundance, the extinction of species, and tainting are some other impacts. When aquatic life is exposed to crude oil and its derivatives, such as refined oils, water-soluble fractions, and accommodated fractions, some other effects can occur. One of these effects is an impact on fish physiology, which can result in the fish mutating and becoming deformed, which in turn leads to a high mortality rate. (Barron et al., 2003; Couillard et al., 2005). According to the findings of investigations carried out by other experts, oil spills have a considerable impact (both deadly and sublethal) on a wide diversity of aquatic species. This is found in rabbits of a younger age that were exposed to crude oil; their growth was slowed down. Changes in the performance of liver enzymes in African catfish—Clarias gariepinus—after exposure to crude oil were also observed in pink Salmon-Oncorhyncu gorbuscha (Wang et al., 1994).

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The term "crude oil" refers to a complex mixture of hydrocarbons that is the starting point for the production of many other types of petroleum products, including gasoline, kerosene, fuel oil, lubricating oil, wax, and asphalt. It has been established that crude oil contains a number of hazardous components, some of which include polycyclic aromatic hydrocarbons and water-soluble portions (Daniel & Odioko, 2017).). It is common knowledge that refined petroleum products derived from crude oil contain lengthy organic chains of hydrocarbons. However, refined petroleum products also contain inorganic components such as nitrogen, sulfur, oxygen, and various metals like nickel, vanadium, iron, and chromium. Chronic toxicity is associated with the persistence fractions of crude oil as well as the responses of the various individual species to the specific compounds after an oil spill. This is true both before and after an oil spill. The chemical make-up of crude oil has a significant impact on these responses.

Aim and Objectives

The primary objective of this study is to determine the effect of the water-soluble fraction (WSF) of Bonny Light oil on some biological parameters of the African catfish, Clarias gariepinus. More specifically, this study was to:

1. investigated the effect of the WSF on the specific growth rate for both weight and length of the fish (standard and total length), as well as the histopathology of the fish.
2. assess the effect of the toxicant on the flesh of the fish.
3. determine the effect of the toxicant on the gills of the fish.
4. compare the general activity of the Clarias gariepinus in polluted and unpolluted aquatic systems.
5. determine the effect of Bonny Light crude oil on some physical properties of the tested water (pH, temperature, and dissolved oxygen).

Materials and Methods

A local fish farm in Elelenwo, Rivers State, provided the Clarias gariepinus (Juvenile) used in this study. To prevent heat fatigue, they were brought in airbags with pond water from the farm in the early morning hours to the biology lab of the Ignatius Ajuru University of Education in Rumuolumeni, Port Harcourt. The fish were weighed and measured before being immediately moved into plastic aquariums. They underwent 14 days of acclimatization. The holding aquariums were cleaned, and fresh water was added on a regular basis. Throughout the acclimation period, commercial feed (Vital) was supplied to the fish twice daily. The fish were introduced to their plastic aquariums or tanks with clean water after acclimation, and no studies were conducted until they showed no signs of stress, such as color changes or irregular movements. All throughout these studies, these fish were fed.The test substance, Bonny Light crude oil (BLCO), was acquired from the SPDC Production Lab in Port-Harcourt, Rivers State, and delivered in corked bottles to the lab. Prior to usage, it was kept in a cold, dry location.

WSF (Water Soluble Fraction): One thousand milliliters of BLCO and two thousand milliliters of portable water were combined to create the WSF. The solution was then allowed to stand for twenty-four hours after being agitated for four hours at a rate of one thousand revolutions per minute with a Lenze overhead stirrer. The WSF, which was the heavier fluid, was collected from the bottom of the funnel that was used to separate the fluids.

Making Test Solutions for Chronic Toxicity: Seven plastic aquariums need five and a half liters of portable water to fill them. Each of the five-liter plastic aquariums was filled with 0.0 milliliters, 25.0 milliliters, 50.0 milliliters, 75.0 milliliters, 100.0 milliliters, 125.0 milliliters, and 150.0 milliliters of WSF that had been prepared. This resulted in the following harmful concentrations: 0.0 ppm, 5.0 ppm, 10.0 ppm, 15.0 ppm, 20.0 ppm, and 30.0 ppm. To make sure it was uniform throughout, this was blended. Prior to preparation, this was completed in triplicate. (Ogbeibu, 2005).

Histopathological Evaluation: Fishes from each aquaria and the control were killed by a blow to the head and a part of the skin (flesh) and the gills were cut and placed in a tissue cassette and placed in 10% formalin Nalgene bottle. After processing, the samples were stretched with an albumin and distilled water solution, and this was mounted on microscope slides and air dried. The dried section was stained with Haemotoxylin and Eosin staining techniques. The stained sections were mounted with cover slides using Entellan. Every of the slide was microscopically analyzed and photomicrographs taken to demonstrate the tissues pathology and result recorded. The results were analyzed at the university of Port-Harcourt teaching hospital (UPTH Histopathology Lab).

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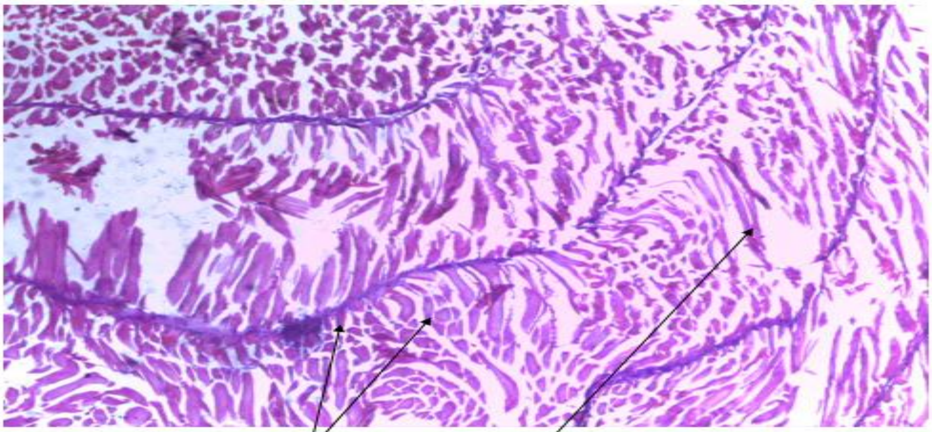
Results

Histology

The flesh (skin) and gills of the Clarias gariepinus were the subjects of the histological examinations that were performed. As a result of the experiment, we were able to determine that the flesh (skin) and gills of the Clarias gariepinus were subjected to a large amount of change.

I. Control Sample (0.0 ppm of WSF)



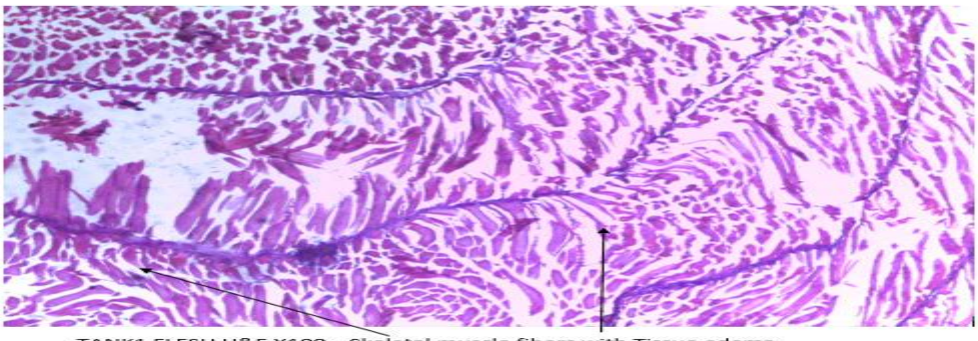
CONTROL FLESH H&E X100 skeletal muscle fibers with tissue edema

HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING NO TISSUE EDEMA.

PLATE 1 **FLESH**: Control Sample (0.0 ppm)

Tank 1 (5.0 ppm of WSF)

Tank 1 (5.0 ppm of WSF)



TANK1 FLESH H&E X100 Skeletal muscle fibers with Tissue edema

HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING MILD TISSUE EDEMA.

PLATE 2 **FLESH**: Tank 1 (5.0 ppm)

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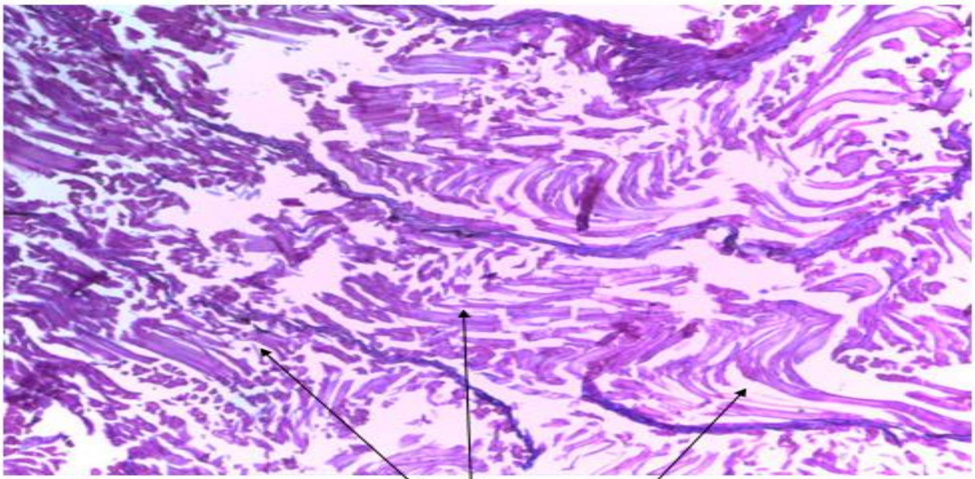
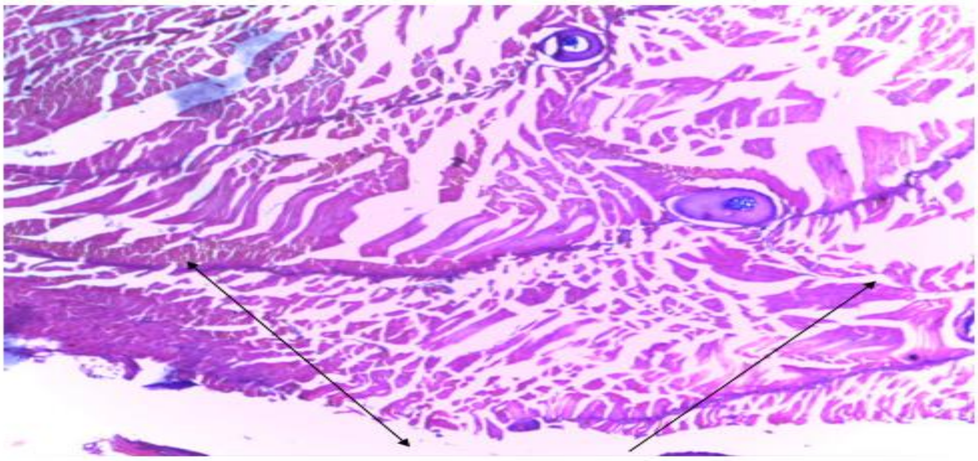
II. Tank 2 (10.0 ppm of WSF)



TANK2 FLESH H&E X100 Skeletal muscle fibers with Tissue edema
HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING MILD TISSUE EDEMA.

PLATE 3 **FLESH**: Tank 2 (10.0 ppm)

III. Tank 3 (15.0 ppm of WSF)



TANK3 FLESH H&E X100 Skeletal muscle fibers with Tissue edema
HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING MILD TISSUE EDEMA.

PLATE 4 **FLESH**: Tank 3 (15.0 ppm)

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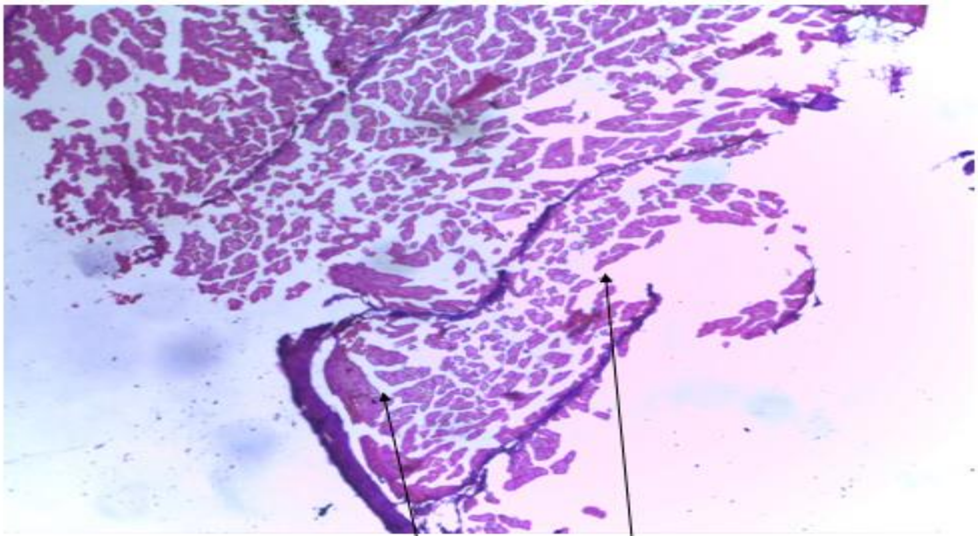
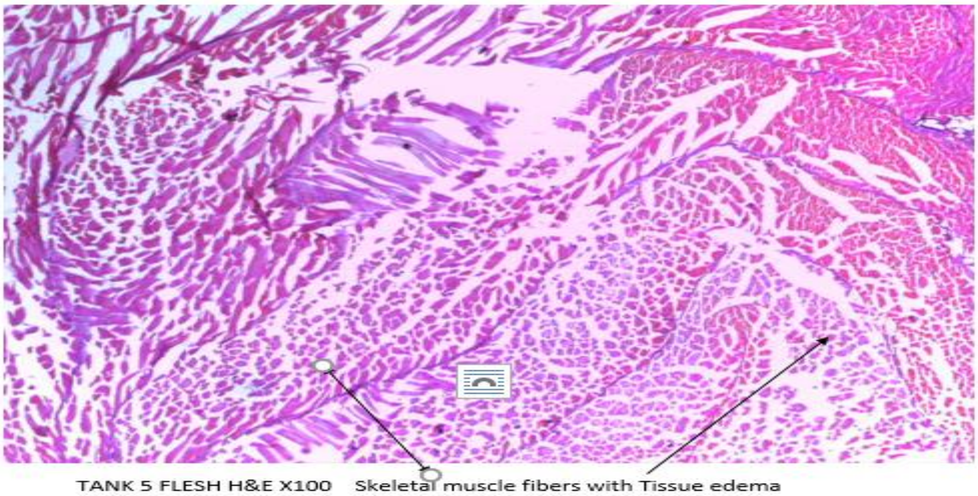
IV. Tank 4 (20.0 ppm of WSF)



TANK4 FLESH H&E X100 Skeletal muscle fibers with Tissue edema
HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING MILD TISSUE EDEMA.

PLATE 5 **FLESH**: Tank 4 (20.0 ppm)

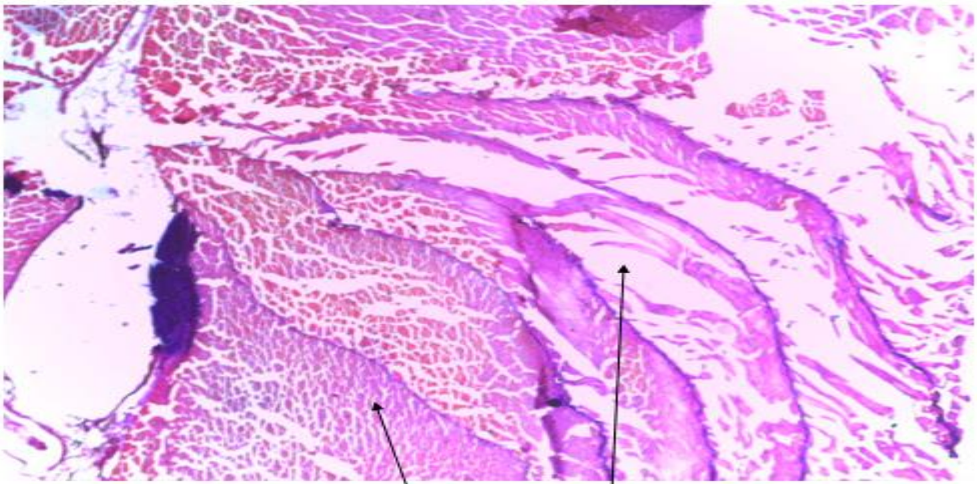
V. Tank 5 (25.0 ppm of WSF)



TANK 5 FLESH H&E X100 Skeletal muscle fibers with Tissue edema
HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING MILD TISSUE EDEMA.

PLATE 6 **FLESH**: Tank 5 (25.0 ppm)

VI. Tank 6 (30.0 ppm of WSF)



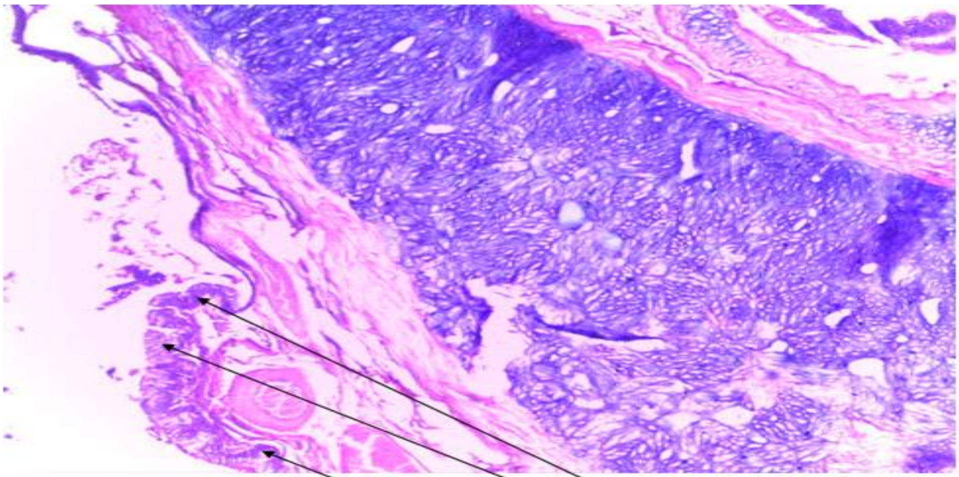
TANK6 FLESH H&E X100 Skeletal muscle fibers with Tissue edema

HISTOLOGIC SLIDE FROM A FISH FLESH SHOWING MILD TISSUE EDEMA.

PLATE 7 **FLESH**: Tank 6 (30.0 ppm)

Gills

I. Control (0.00 ppm of WSF)

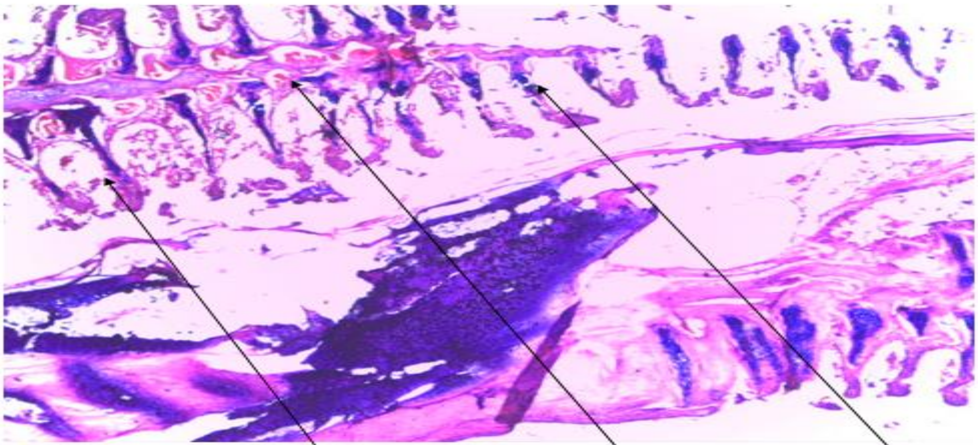


CONTROLGILL H&E X 200 gill filaments gill rakers gill arch

HISTOLOGIC SLIDE FROM A FISH GILL SHOWING NO HISTOLOGIC CHANGE,

PLATE 8 **GILLS**: Control (0.0 ppm)

II. Tank 1 (5.0 ppm of WSF)

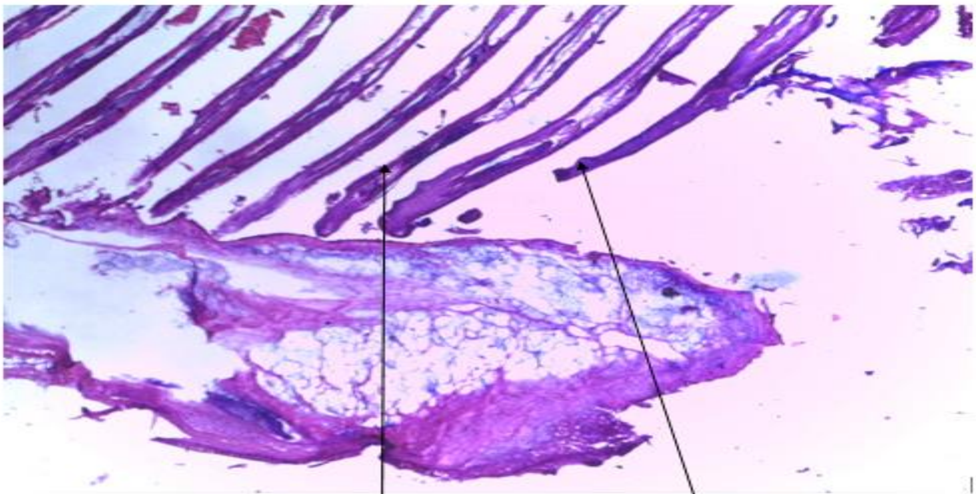


TANK 1 H&E X 2000 Mild destruction of gill filaments gill rakers Mild damage gill arch

HISTOLOGIC SLIDE SHOWING DESTRUCTION OF GILL FILAMENTS AND RAKERS

PLATE 9 **GILLS**: Tank 1 (5.0 ppm)

III. Tank 2 (10.0 ppm of WSF)



TANK2 GILL H&E X200 Mild destruction of gill filaments gill rakers Mild damage

HISTOLOGIC SLIDE SHOWING DESTRUCTION OF GILL FILAMENTS AND RAKERS

PLATE 10 **GILLS**: Tank 2 (10.0 ppm)

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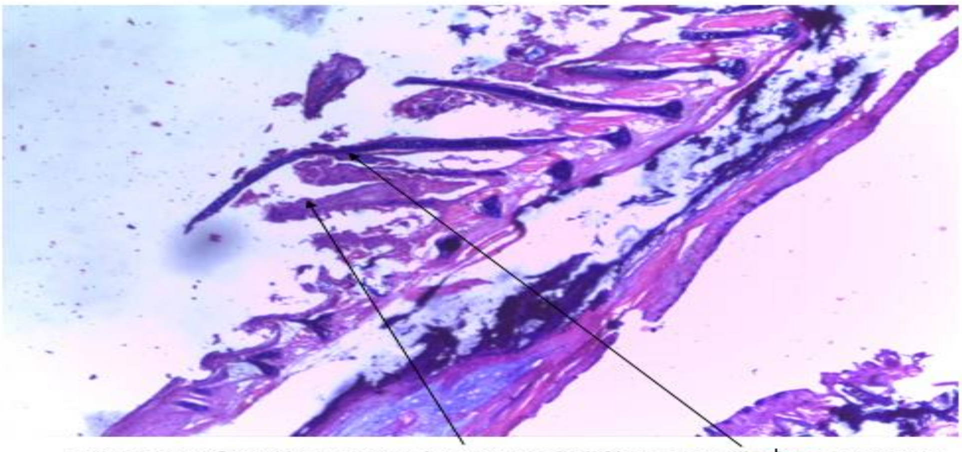
IV. Tank 3 (15.0 ppm of WSF)

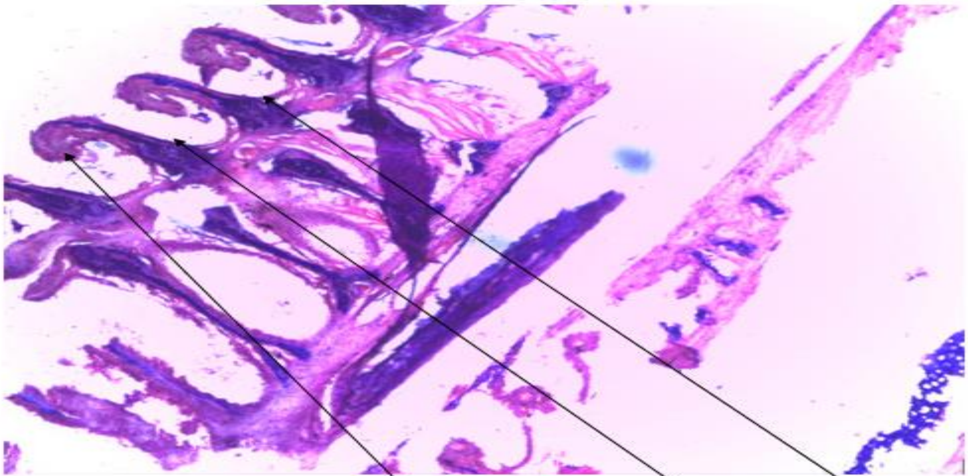


TANK3 GILL H&E X 100 Severe destruction of gill filaments Gill rakers destruction

HISTOLOGIC SLIDE FROM THE GILLS SHOWING DESTRUCTION OF GILL FILAMENT AND RAKERS

PLATE 11 **GILLS**: Tank 3 (15.0 ppm)

V. Tank 4 (20.0 ppm of WSF)

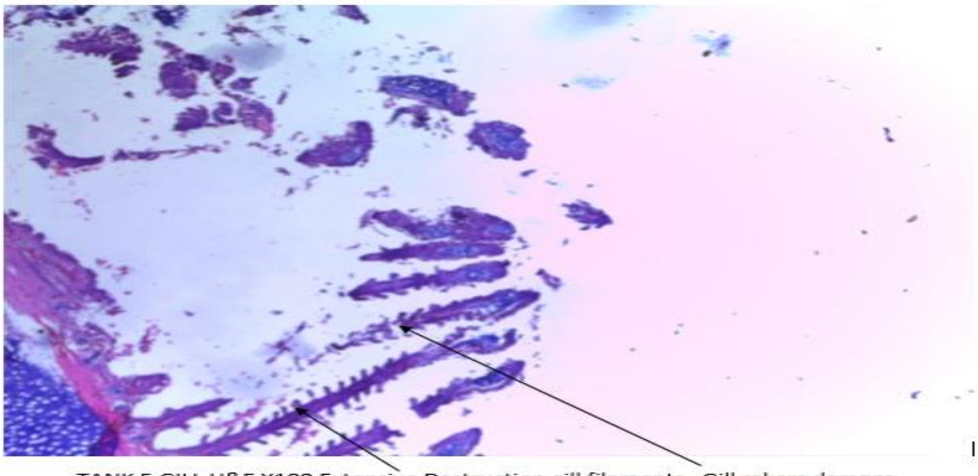


TANK 4 GILL H&E X severe destruction of gill filaments destruction of gill rakers gill arch

HISTOLOGIC SLIDE SHOWING SEVERE DESTRUCTION OF GILL FILAMENTS AND RAKERS

PLATE 12 **GILLS**: Tank 4 (20.0 ppm)

VI. Tank 5 (25.0 ppm of WSF)

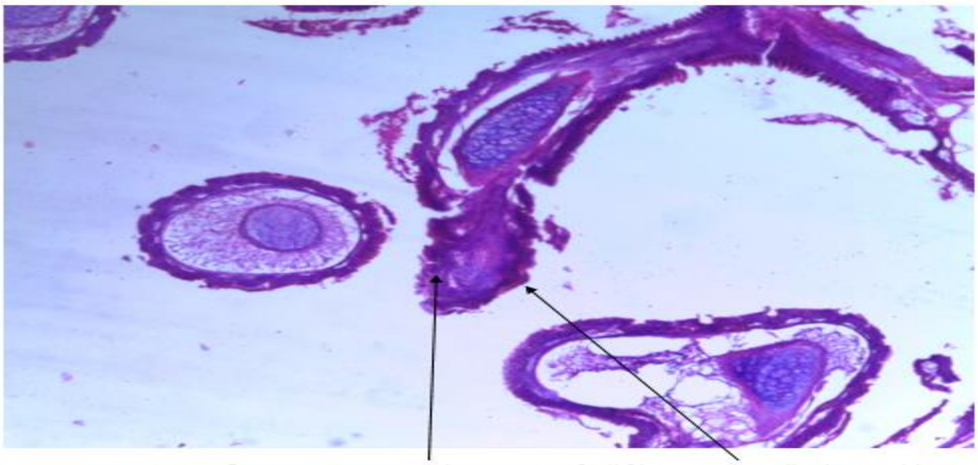


TANK 5 GILL H&E X100 Extensive Destruction gill filaments Gill rakers damage

HISTOLOGIC SLIDE SHOWS DESTRUCTION OF GILL FILAMENTS AND RAKERS

PLATE 13 **GILLS**: Tank 5 (25.0 ppm)

VII. Tank 6 (30.0 ppm of WSF)



TANK 6 GILL H&E X Extensive destruction of gill filaments Gill rakers Mild damage

HISTOLOGIC SLIDE SHOWS DAMAGE OF GILL FILAMENTS AND RAKERS

PLATE 14 **GILLS**: Tank 6 (30.0 ppm)

Discussion

In this study, the significant effect that the toxicant, Water Soluble Fraction of the Bonny Light Crude Oil, had on the Clarias gariepinus and its responses to these changes in its ecosystem for its ultimate survival resulted in some key changes in some parameters. These changes are discussed further below. It is well recognized that Clarias gariepinus can thrive in a wide variety of fresh water settings, including lakes, rivers, and muddy ponds. The fish will exhibit a spotted gray appearance while they are under pressure, and the return of their natural colouring signals that they have become accustomed to their surroundings and are under less pressure. This was seen to be the case when these fish were allowed to become accustomed to their surroundings in the test aquariums. This acclimation and return to natural coloration occurred extremely quickly and on schedule, which was one of the most noticeable things that happened, but when the fish were exposed to contaminated aquariums, they maintained the color shift for two days afterward. This demonstrates that the existence of the WSF in the ecosystem in which the fish live has a major impact on the fish's ability to adapt to new environments.

A number of studies have demonstrated that the presence of petroleum hydrocarbons or fractions in an ecosystem causes an increase in the rate of death that is proportional to the degree or concentration of the contaminant that is present (Dede & Kaglo 2001; Bob-Manuel, 2012). The pattern that was observed in this result is consistent with what Bob-Manuel (2012) found in his earlier work, which showed that the death rate increased along with an increase in the concentration of the toxicant. We were able to see that as the concentration of the toxicant increased from 10.0ppm; 15.0ppm; 20.0ppm; and 25.0ppm; and 30.0ppm; the mortality rate also increased in direct correlation as the toxicant concentration. The lowest concentration of the toxicant (5.0ppm) gave a total mortality of 2, while the investigation lasted for a total of 6 weeks. Initially two deaths (5.0ppm), then three deaths (10.0ppm and 15.0ppm), then four deaths (20.0ppm and 25.0ppm), and eventually five deaths (30.0ppm). In the Control group (0.0ppm), there was not a single fatality. This may be the result of oxygen stress brought on by the WSF or a blockage of the respiratory structure of the Clarias gariepinus, both of which are possible causes (Dede & Kaglo 2001).The results acquired from the initial water properties and the final water properties reveal that considerable changes occur when WSF of petroleum (BLC) are exposed to the marine ecosystem. These results were obtained from comparing the initial water properties with the final water properties. The results demonstrated a drop in dissolved oxygen (DO), which is essential to the survival of most aquatic species and especially the Clarias gariepinus. The control sample had 7.55 mg/L of DO, whereas the system with 30.0ppm of toxicant only had 4.55 mg/L of DO. The fishes would eventually experience oxygen depletion as a result of this. Additionally, the presence of the WSF was responsible for the rise in pH that was observed across the ecosystem. Outside of this pH range, the Clarias gariepinus do not perform to their full potential. The optimal pH range for this species is between 7 and 8. (Uzoka et al., 2015). Their growth pattern is disrupted, the mortality rate is raised, and it is possible that this will also influence several histopatological characteristics.

Histopathology is the field of study that focuses on the microscopic investigation of abnormalities and injuries that occur in tissue. Monitoring the toxicity of the environment through the gills and the skin are both effective methods (Hart et al., 2007). According to the findings, we can see that neither the skin nor the gills of the control were damaged in any way. On the other hand, the skin and gills of the specimen were significantly distorted and damaged when they were exposed to different doses of the toxicant. According to the findings of the histopathology performed on the flesh, all of the specimens in the different tanks had mild tissue endema, however the specimen in the control tank has no tissue endema. While the histopathology studies on the gills showed considerable damage at all toxicant exposures, this damage was only found in one concentration. With 5ppm indicating a moderate level of destruction of Gill Filaments and Gill Rakers, 10.0ppm indicating a moderate level of destruction of Gill Filaments and Gill Rakers, 15.0ppm indicating a severe level of destruction of Gill Filaments and Gill Rakers, 20.0ppm indicating a severe level of destruction of Gill Filaments and Gill Rakers, and 25.0ppm and 30.0ppm indicating an extensive level of destruction. Using a control concentration of 0.0 ppm, we found that there was no histolic change in the gill filaments or rakers. This corroborates the findings of Ojolo et al. (2005), which stated that the degree of deformation of the gills and liver is related to the amount of time spent being exposed to the toxicant as well as its concentration. So it is dose and timing dependant. Because the length of time exposed to the toxicant was the same throughout this experiment (six weeks), the only factor that determined the degree of distortion or damage was the concentration of the toxin. This can be seen in the degree to which the gills and skin were damaged, as shown by the results.

The ability of fish to continue feeding and growing normally is an important measure of how they are reacting to the contaminated environment. An increase in a living thing's mass, length, and/or width is what we mean when we talk about growth. The Specific Growth Rate (Weight gain) of the Control in this experiment was 104.2% (as the mean weight of the fish doubled), as compared to the results obtained in the various toxicant concentrations

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of 5.0ppm (67.8%); 10.0ppm (48.6%); 15.0ppm (44.2%); 20.0ppm (30.9%); 25.0ppm (19.7%); and 30.0ppm (18.8%). In this experiment, feeding was kept constant throughout the duration of the test.

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