Effects of Diesel on the Behavior and Histopathology of the Gills of Fresh Water Fish (Clarias gariepinus)

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Abstract— **Effects of Diesel on Fresh water fish (Clarias gariepinus) was carried out in the Fish Pathology Laboratory of the Department of Fisheries and Aquaculture, University of Calabar, Nigeria. About 200 fish were purchased from the University Fish farm, acclimated for 48h in the laboratory. The diesel used in this experiment was obtained from the University of Calabar power generating unit. Experiment was conducted in plastic containers of 10l capacity, each containing 10 fish and 5l of diesel in different concentrations (0.25, 0.37, 0.50 and 0.75g/l). The control tanks had similar number of fish and same volume of water without diesel. The tests and control tanks were replicated three times under same laboratory conditions. The duration of the experiment was 96h with hourly observations for abnormal fish behavior and mortality. Erratic swimming behavior, fast opercula movement and hanging on the water column and weakness were observed in test tanks only whereas fish in the control were calm and normal. Mortality of fish (3, 5, 7 and10) occurred in the test concentrations respectively within the duration of the experiment. Histopathology of gills of affected fish showed damaged gill lamellae, similar changes were not observed in the histological sections of the control fish. Mortality data obtained were analyzed graphically by plotting % fish mortality against log concentrations of diesel. The concentration of diesel in which 50% fish mortality (LC50) occurred was 0.37g/l. The results of the experiment led to the conclusion that diesel at different concentrations has deleterious effects on fresh water fish (C. gariepinus) and could cause mortality on repeated exposure to the organisms at higher concentrations. Fresh or used diesel should not be discarded into the environment to safeguard freshwater fish and other organisms. Further study on the effects of diesel on the food chain is recommended.**

Index Terms— **Diesel, C. gariepinus, Histopathology, Gill**

I. INTRODUCTION

Diesel is a refined petroleum product from crude oil with a chemical formula $C_{12}H_{24}$.. Its Power efficiency and dependability makes it the fuel of choice for farms and constructive equipment, container ships, marine workboats and freight railway locomotives etc [1].

According to [2], diesel fuel and its constituents represent one of the toxins most commonly encountered by people and animals living in both urban and, rural areas of the world. It is responsible for long term, sub-lethal and chronic effects on fish [3].

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A good number of studies have been carried out on the toxicity of diesel on fishes and other aquatic organisms.

[4]reported that oil pollution causes complex changes in the structure and functions of natural ecosystems as well as violation of metabolic processes, production and destruction of organic matter, therefore reduces the diversity of species composition, structure and monotonous to the loss of stability of ecosystems.

Clarias gariepinus is one of the most consumed fresh water fish in Nigeria. It is also a popular aquaculture candidate because of its excellent flesh quality and ability to tolerate harsh environmental conditions in culture systems [5]. The present study examines the effects of diesel contaminants on the behavior and histopathology of the gills of *C. gariepinus*.

II. MATERIALS AND METHOD

The research work was carried out in the fish pathology laboratory of the Department of Fisheries and Aquaculture, Faculty of Oceanography, University of Calabar, Nigeria. The *C. gariepinus* post fingerlings used in this study was purchased from University of Calabar fish farm. Two hundred post fingerlings were collected in plastic container and transported to the laboratory. The fish were acclimated to laboratory conditions for 48h before the experiment.

The experiment was conducted in plastic containers of 10L capacity. Each container contained 10 fish in triplicates in 5ml of water. The toxicant (diesel) was measured into different concentrations (0.1, 0.5, 1, 1.5 and 2mg/l). Preliminary test was conducted to guide in the selection of test concentrations. The toxicant was dissolved in 10ml dimethysulphoxide (DMSO) to ensure solubility in water. The experiment last for 96h with hourly observations of fish behavior and mortality. Dead fish was taken out of the test medium immediately it was noted.

Mortality data were plotted into a probit graph (mortality against concentration). The concentration in which 50% mortality (LC_{50}) occurred was extrapolated from the probit graph.

A. *Histopathology of the gills*

Gills of test fish were removed with the help of a pair of scissors and forceps, fixed in 10% phosphate buffered formalin (PBF) for 48h. They were manually processed through graded series of alcohol, cleared in xylene, impregnated in paraffin wax at 60^{o}c, embedded in wax, sectioned with a rotary microtome, mounted on a glass slide with Canada balsm. The tissues were stained with haematoxylin and eosin solution, dehydrated in alcohol, cleared in xylene for microscopic examination.

III. RESULTS

A. *Behavioral Changes*

Observed behavioral changes on test fish included fast opercula movement, weakness, hanging on the water column, restlessness and fish mortalities. Above changes in fish behavior were more pronounced in higher concentrations of diesel than in low concentration as shown on table 1.

Fish mortalities increased in a concentration-related manner (Table 1). Similar changes were not observed in the control.

TABLE I. Effects of Diesel on the behavior of *C. gariepinus* at 24, 48, 72 and 96 h of exposure.

	MEAN FISH MORTALITY (%)				OBSERVATIONS			
CONC. (G/L) OF DIESEL	24	48	72	96	Mortality	SD	Mean % Mortality	Behavioral Changes
CONTROL (0)	0	0	0	0	0	0	0	Normal
0.25	2	1	0	0	3	0.96	30	Hanging on the water column
0.37	2	3	0	0	5	1.5	50	Increase opercula movement
0.5	3	2	1	1	7	0.96	70	Moribund swimming
0.75	5	3	2	-	10	2.08	100	Extreme weakness and death

B *Histopathological Changes*

Microscopic changes observed on affected fish gills were fused gills lamellae, loss of secondary lamellae and gill erosion (Plate 1). These changes were not observed in the control.

Plate 1: Showing Effects of Diesel on the Gills Lamellae

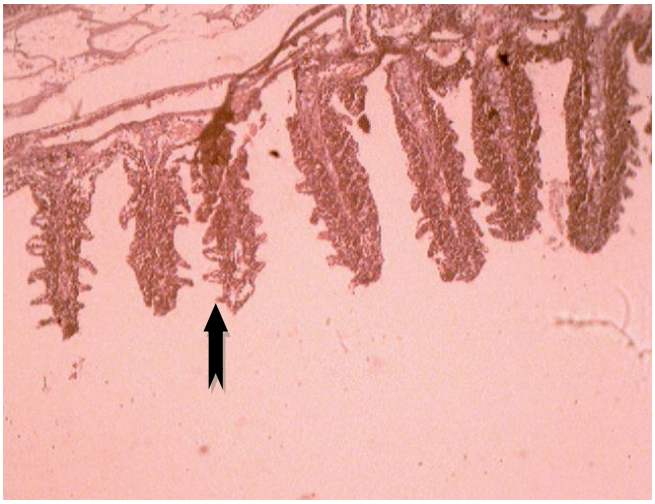

Plate 2: Showing Normal Gill Lamellae in the Control

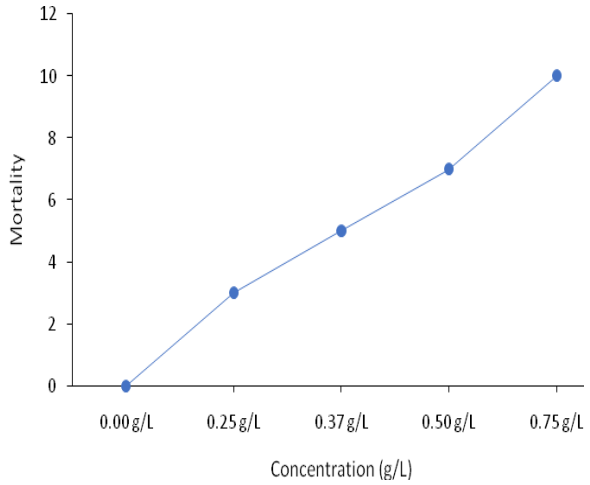

Plate 3: Showing the graph of Concentration against mean fish mortality

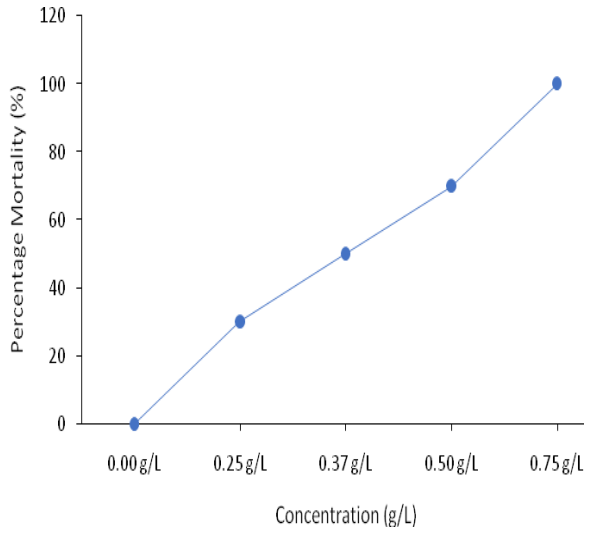

Plate 4: Showing the graph of Concentration against mean (%) fish mortality

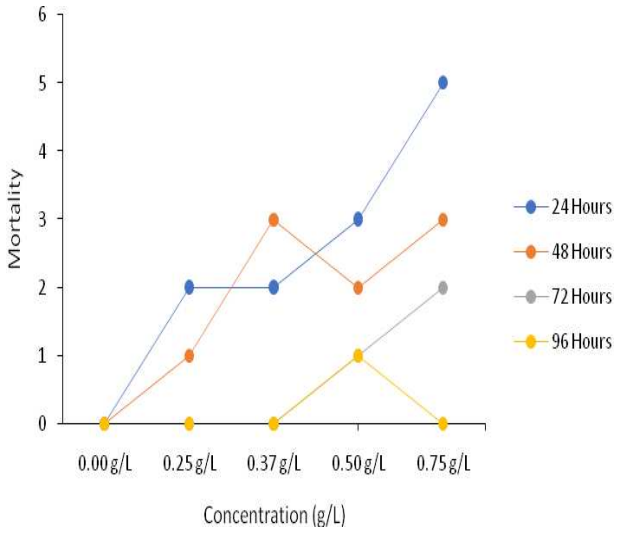

Plate 5: Showing the graph of fish mortality against exposure time

IV. DISCUSSION

A. *Behavioral Changes*

Test fish behavioral changes observed in this study are attributable to the presence of diesel, because similar changes were not observed in the control containing no diesel. Increase opercula movement is an indication of respiratory difficulty. The gills are respiratory surfaces for gaseous exchange but when they are blocked by an environmental toxicant, the surfaces available for gaseous exchange are reduced, resulting in respiratory difficulty.

According to [6] Fish gills are one of the vital organs that are affected by diesel oil . Fish gill is an organ with a large surface area, highly sensitive to the effect of toxicant. Epidemiologists have attributed respiratory morbidity in human and some animals to diesel exhaust [7].

It was also observed that affected fish were hanging at the water column, which is an indication of oxygen deficiency in the water. According to [8] diesel is a complex mixture of toxic compounds with wide variability of deleterious effects in human and animal studies.

B. *Fish Mortality*

There was increase fish mortality with increase in the concentration of diesel. This is expected due to toxic effects on the vital organs of affected fish. The polyaromatic hydrocarbons (PAHs), such as naphthalene, fluorine and phenanthrene [9] are among the chemical components of diesel oil that pose the greatest environmental hazard [10].

C. *Histopathology of the gills*

Loss of secondary gill lamellae and lamellae erosion of the primary organs of gaseous exchange were observed in the gills of affected fish. The affected gills as shown in the result could not function for gaseous exchange. The gill surfaces have a direct contact with aquatic toxicants and are easily affected. Moreover, the presence of polycyclic aromatic hydrocarbons (PAH) in diesel causes long term, sub-lethal and chronic effects on fish respiration, growth, reproduction and survival [11].

Diesel causes damage to important vital organs and reduces the survival, growth and the reproduction performance of aquatic organisms when concentration exceeds tolerant level [12].

V. SUMMARY AND CONCLUSSION

All the behavioral and histopathological changes observed in this study could be attributed to diesel contamination because similar changes were not observed on fish in the control tanks which contained no diesel. These changes can result in economic losses to fish culturists due to fish mortality and retard growth and reproduction. Introduction of diesel into the aquatic environment should be controlled to reduce its effects on aquatic life.

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