Acute Toxicity and Histopathological Effects of Engine Oil on *Sarotherodon melanotheron* (Black Jaw Tilapia)

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Abstract: The lethal effects of engine oil effluent on *Sarotherodon melanotheron* was investigated using pathologic lesions in the gill, kidney and muscle. Five concentrations of 0.035, 0.07, 0.14, 0.21 and 0.28 ml/l for 96hrs were used for the experiment. The randomized ANOVA for toxicity of the extract against *S. melanotheron* showed that there were significant differences in the test. The lethal concentration LC_{50} that caused 50% mortality was approximately 1.12mg/l of the engine oil. Lesion observed in the gill epithelium exposed to at the different concentrations of the engine oil were dose dependent with the highest effluent concentration inducing the highest damages which include mild congestion, severe congestion and calcification of the gill. The Kidney tissues of *S. melanotheron* exposed to the engine oil effluents showed severe congestion, inflammation, cytoplasmic vacuolations which may have been due to glycolysis leading to microsomal and mitochondrial dysfunction. While the muscle tissues were normal even after the exposure. The entire test organism in the control showed inappreciable or no histologic degradation while their staining patterns and cellular arrangement remain unaffected. The result obtained showed that engine oil effluents pose a serious damage to *S. melanotheron* and was observed that acute concentration of engine oil effluents have histopathological effects on aquatic organisms. It can be deduced that indiscriminate discharge of industrial effluents into water bodies can induce damage to the tissue and organ, which might make the fish vulnerable to diseases and eventually lead to death of prominent edible species of the aquatic environment, Therefore there is need for the adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluent and check the recurrence of oil spillage. Indiscriminate exposure of aquatic organisms to engine oil effluent should be discouraged.

Key words: Bio-indicator % Acute Toxicity % Histopathological % Engine Oil % Black Jaw Tilapia

INTRODUCTION

Most oil spill on waters rapidly spread within the water body into a slick, the most damaging effect being observed when the oil strand on shorelines or enters restricted shallow waters such as estuaries; resulting in long term negative consequences [1]. Most human activities (domestic and industrial) result in the introduction of wastes directly or indirectly into the aquatic environment. The ever increasing need to oil our cars and to service our engines in order to regulate and maintain our automobiles and aid our movement from one place to another without excessive stress thereby causing water pollution.

Motor oil, or engine oil, is used for lubrication of various internal combustion engines. While the main function is to lubricate moving parts, motor oil also cleans, inhibits corrosion, improves sealing and cools the engine by carrying heat away from moving parts [2].

Motor oil lubricates all forms of engines, these include motor or road vehicles such as cars and motorcycles, heavier vehicles such as buses and commercial vehicles, non-road vehicles such as go-karts, snowmobiles, boats (fixed engine installations and outboards), lawn mowers, large agricultural and construction equipment, locomotives and aircraft and static engines such as electrical generators. In engines, there are parts which move against each other causing friction but do not wear and tear easily because of the cooling effects of engine oil; Contact between moving surfaces also wears away those parts, which could lead to lower efficiency and degradation of the engine. In the bid to avoid the breakdown of the automobile the oil is

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evacuated and replaced with a new one periodically, the spent oil is usually emptied into a water body like streams, lakes, oceans etc.

Toxicity testing is a measure of how poisonous a substance or how large a dose is required to kill or cause damage to an organism.

The significant increase in the pollution of water bodies has lead to serious deleterious effects in the aquatic organisms [3]. Many of such chemical can induce, besides death of exposed organism, other effects like genetic disorders and phylogenetic alterations.

According to [4], some substances when present in low concentrations may not cause acute detectable effects in organisms, but may in the long run reduce their life span due to bioaccumulation. Many Nigerians especially those that live in coastal areas, rely significantly on fish consumption as their major source of animal protein. Fishes are known to serve as source of food in which some of this fishes accumulate toxic pollutants in their organs; they can cause peripheral sense organs to malfunction [5].

The test organism is *Sarotherodon melanotheron*, also known as the black jaw tilapia, is one of the most popular brackish water fish species in West Africa. They are Members of the genus *tilapia* (family *cichlidae*) having been an important source of food for man in tropical and sub-tropical Africa and have entrenched as one of the world's most important fish by the end of the twentieth century [6].

The *Sarotherodon melanotheron* are valuable for investigating the effects of toxic substances in the aquatic ecosystem. It was reported that the chronic carcinogenetic bioassays with small fish species are feasible and scientifically valid [7].

Physiological assays are especially useful for monitoring fluctuating or complex exposures, or acting as early warning systems for acute events [8]. The popularity of this fish as standard test is that it is easy maintain and handle in the laboratory and also as a result of information on their responsiveness to a range of classes of toxicants and carcinogens.

Besides the direct health effects, the subtle danger of pollutants like motor oil, detergents etc, lies in the fact that they may be toxic and lead to several human afflictions, like cancer, cardiovascular diseases and premature ageing [8].

Studies have been carried out on the toxicity of oil, oil spill chemicals and industrial wastewater (effluent) on different aquatic organisms including those of [9, 8] in Nigeria. Other toxicity studies carried out include those of

[10, 11]. None has however dealt with acute toxicity of the motor engine oil effluent on tilapia species, which is a common estuarine and pond-reared fish in Africa.

Histopathology is now recognized as an important tool to evaluate the effects of contaminants in vital processes such as growth, reproduction, detecting early effects in cells, tissues and organs [3].

According to [12], mucus accumulation result from increase in the activity of mucus cells subsequent to exposure to pollutants, the accumulation of the mucus on the gill filaments can cause impairment in the bronchial ion regulatory mechanism and gaseous exchange which would result in the suffocation of the fish [10].

This study is aimed at investigating and generating data that will be useful and vital in protecting the environment as well as useful to the environmental regulatory agencies in setting discharge limits for waste waters. Therefore there is need to investigate the indiscriminate discharge of engine oil into the environment and its negative effects on the environment demands and the development of various control strategies.

The aim of this work is therefore to investigate the lethal (acute) toxicity of motor engine oil effluent on the common tilapia specie, *Sarotherodon melanotheron*. The information would give a clue to the approximate concentrations that could be referred to as safe concentration and would enable prediction of the effluent effect in the field. This intend to investigate the acute toxicity effect of engine oil on *Sarotherodon melanotheron* fresh water system and examine the histopathological alterations of kidney, gill and muscle on exposure to engine oil.

MATERIALS AND METHODS

Three hundred species of *S. melanotheron* with the mean weight of 5.0±0.3g and mean standard length of 5.0±0.1cm were used for the experiment. This is due to the more sensitive nature of juveniles than adult for toxicity test. They were purchased from the Department of Marine sciences Aquaculture unit, University of Lagos, Akoka Lagos State, Nigeria.

In the laboratory the fishes were acclimatized for two weeks during which they were fed with commercial floating pellets at 10% of their body weight.

Unconsumed feed and feaces were removed and water replenished regularly as recommended by [13]. Dechlorinated tap water of temperature = 26.0 ±0.8°C, pH = 7.0 and dissolved oxygen 6.3±0.1 mg/L were used.

Feeding was stopped 24hours before they were introduced into the test concentrations. The pH and temperature of the water samples were determined using a Huribar U-10 by immersing the probe into the water sample. Buffer solution of known pH was then used in calibrating the pH meter until it gave a stable measurement.

The temperature of the water sample was determined by immersing the glass thermometer in it and the temperature was recorded.

Dissolved oxygen (DO) was determined using Huribar U-10. The equipment was calibrated first by dropping the probe in the solution until it gave a stable measurement.

Test Chemical: African petroleum engine oil is effective oil used for lubrication of various internal combustion engines. The main function is to lubricate moving parts; it also cleans, inhibits corrosion, improves sealing and cools the engine by carrying heat away from moving parts. Motor oils are derived from petroleum-based and non-petroleum-synthesized chemical compounds. Motor oils today are mainly blended by using base oils composed of hydrocarbons, polyalphaolefins (PAO) and polyinternal olefins (PIO), thus organic compounds consisting entirely of carbon and hydrogen. The base oils of some high-performance motor oils contain up to 20% of esters.

A predetermined volume of this toxicant was measured out and mixed out and mixed with dechlorinated water to obtain required solution (7 liters) for the acute tests. The mixture of water and test compounds was done in bioassay tanks of (20x18x38) cm.

Acute Toxicity Bioassays
Toxicity Tests: A Semi-static bioassay procedure was adopted for all the toxicity tests, in which the test media was renewed every 24hrs at the same concentration for 96 hours. Preliminary screening was carried out to determine the appropriate concentration range for testing chemical. Seven *S. melanotheron* juvenile per concentration of toxicant were used in triplicates each for 96h. Based on this, five concentrations (0.0, 5, 10, 20, 30 and 40ml) of the lubricant were prepared and tested on the *S. melanotheron* juveniles for the preliminary (range finding) test.

The bioassays were carried out in glass tanks measuring (20x18x38) cm for the length, breadth and depth respectively.

Dechlorinated water from the tap was used as the medium for the entire bioassay test conducted. Predetermined volumes of engine oil were measured using measuring cylinder and introduced into the appropriate volumes of dechlorinated tap water. There was also a controlled medium but no toxicant was introduced.

Test organism were exposed to various concentrations of test compound African Petroleum Motor oil which were determined from range tests carried out and these concentrations were used including control 0.0, 5.0, 10.0, 20.0, 30.0 and 40.0ml. This was done in triplicates.

Mortality was assessed every 3 hours for the 96hour period. Dead fishes were removed to prevent contamination of the water.

Histopathology: Organs, such as kidney, gill and muscle were collected and preserved in 10% neutral buffered formalin from Fishes that died after 96 hours of the bioassay test to avoid autolysis and was processed for histopathology studies.

Statistical Analysis: Toxicological data involving quantal response (mortality) were analyzed by probit analysis after [14]. One way analysis of variance, ANOVA and Student Newman-Keul's, (SNK) test were used to test for significant difference (5% level) for 96hrs, the indices of toxicity measurement derived from the analysis were:

LC_{50} = The concentration that kills 50% of the test population.

LC_{95} = The concentration that kills 95% of the test population.

TF = Toxicity factor for relative potency measurements.

RESULTS

Behavioural Responses/ Observation: The average behavioural changes noticed was that the fishes had slower, non- directional movements and some just stayed afloat and mortality of the fishes occurred. It was observed that the higher the concentration, the higher the mortality rate.

The parameters that were measured were Dissolved Oxygen, Hydrogen ion (pH) concentration and Temperature which was presented in Table 1 below.

Table 1: Mean physico- chemical parameters of experiment

Effluent concentration ml/L	Dissolved O$_2$	pH	Temperature °C
Control	6.2±0.04^a	7.5±0.03^a	27.2±0.02^a
0.035 (5.0ml)	6.1±0.02^a	5.8±0.03^b	26.5±0.02^b
0.07 (10.0ml)	5.8±0.02^{bc}	5.3±0.03^c	26.9±0.02^{bc}
0.014 (20.0ml)	5.4±0.02^{cd}	4.9±0.03^{cd}	27.5±0.02^{cd}
0.21 (30.0ml)	5.1±0.02^{cd}	4.2±0.03^{de}	27.8±0.02^{cd}
0.28 (40.0ml)	4.6±0.02^{cd}	3.7±0.03^{cd}	27.9±0.02^{de}

*Means followed by the same superscript letter in a column are not significantly different in the SNK test (P>0.05)

Toxicity (LC$_{50}$) Value: Based on these, five concentrations (0.0, 5.0, 10.0, 20.0, 30.0 and 40.0ml) of the lubricant, the LC$_{50}$ value was 1.12mg/l (i.e. concentration that will affect 50% mortality in the experiment) and was presented in Fig. 1.

Analysis of variance (ANOVA) computed for the acute test showed that there was significant difference. (P < 0.05) in the quantal response of test organism to diffe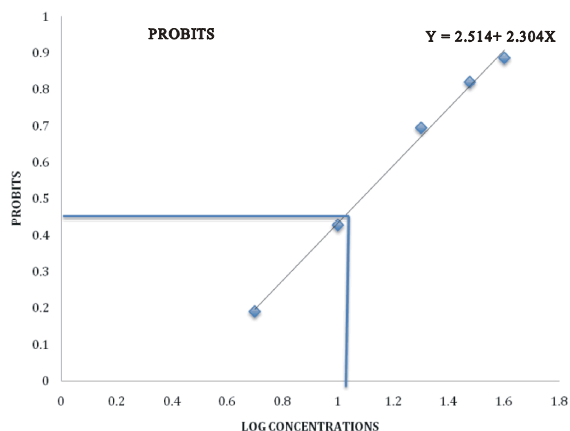rent concentration of African petroleum oil at 24, 48, 72 and 96 hours of exposure. The histopathological examinations observed were presented in plates 1a to 3f.

Fig. 1: Linear relationship between probit response and log concentration of AP Engine Oil on *Sarotherodon melanotheron*
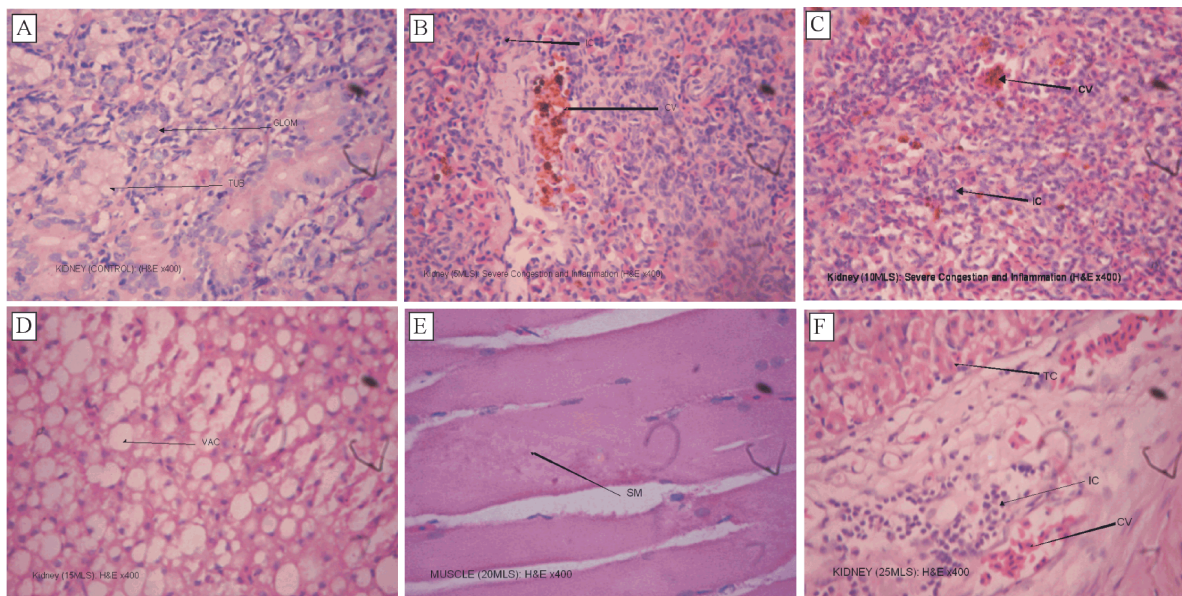

Plate 1A: Control Kindney of *S. melanotheron* x 400. The 96h exposure to engine oil showing glomeruli (GLOM) and tubules (TUB)

Plate 1B: (Kindney of *S. melanotheron* x 400) The 96h exposure at 5mls pg engine oil showing IC-Inflammatory cytoplasm, CV- Congested Vessels Moresevere cong stion is seen within the renal parenchyma, as well as inflammatory cell infiltrates

Plate 1C: The Kidney of *S. melanotheron* x400 to engine oil at 10mls Showing IC -Inflammatory cells, CV-Congested vessels. More severe congestion is seen within the renal parenchyma as well as inflammatory cell infiltrates

Plate 1D: Kidney of *S. melanotheron* x400. The 96h exposure at 20mls of engine oil showing- Vacuoles. The renal parenchymal cells have a vacuolated appearance due to the presence of cytoplasmic vacuoles

Plate 1E: Kidney of *S. melanotheron* x400. The 96h exposure at 30ml of engine oil showing VAC- Vacuoles, The renal parenchyma cells have vacuolated appearance due to the presence of cytoplasmic vacoulation

Plate 1F: Kidney of *S. melanotheron* x400. The 96h exposure at 40mls of engine oil showing TC -tabular Cells, IC-Inflammatory Cells, CV-Congested Vessels
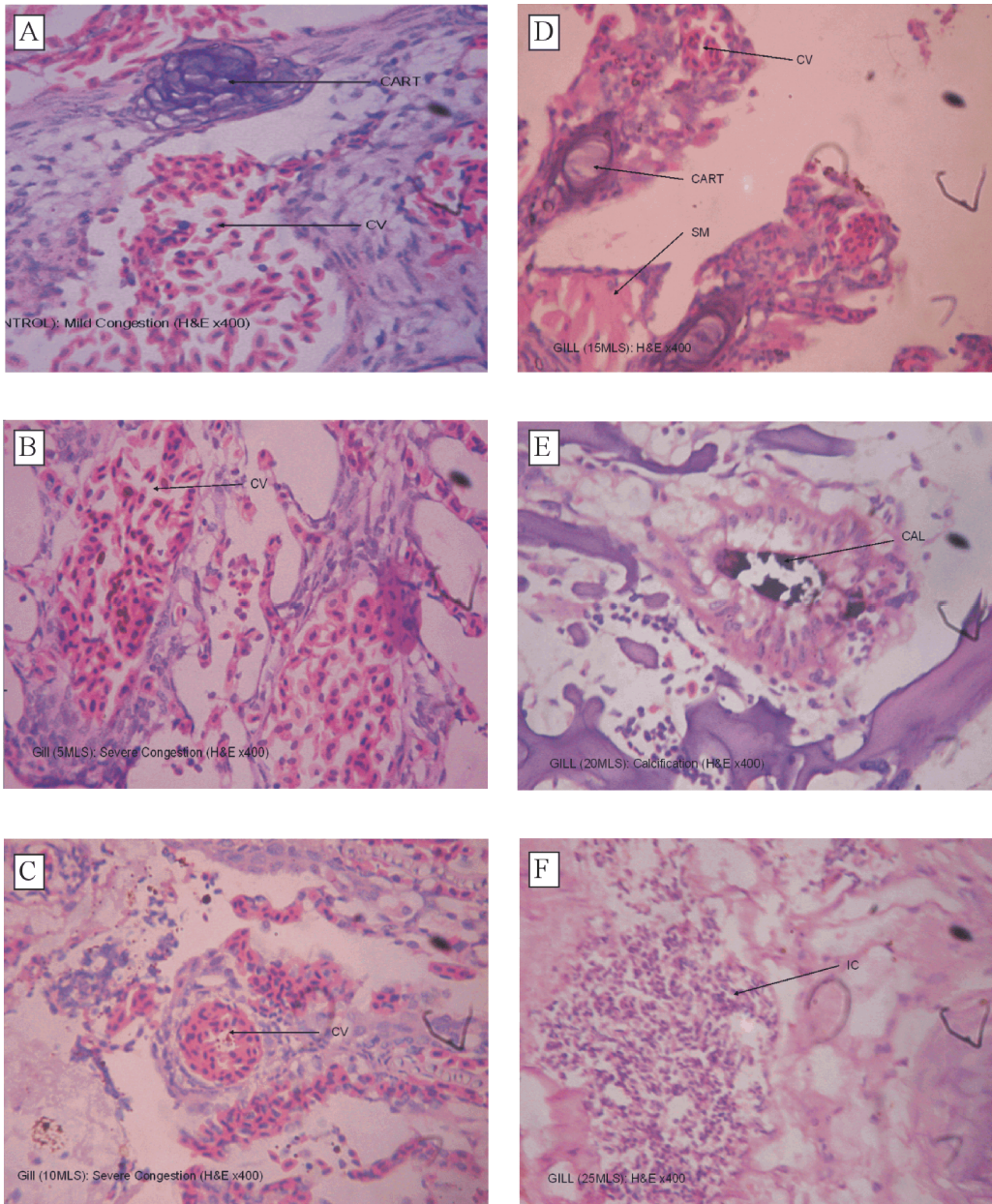
Plate 2A: Control gil of *S. melanotheron* x400. The 96h exposure to engine oil showing CART-Cartiladge CV- Congested vessels. Thereisno congestion of vessels, no inflammatory cells are seen

Plate 2B: Gill of *S. melanotheron* x400. The 96h exposure at 5mls showing CV- Congested Vwsswls more Congestion is seen within the gills

Plate 2C: Gills of *S. melanotheron* x400. The 96h exposure at 10mls showing CV- Congested vessel, There is more severe congestion of vessels. No inflammatory cells

Plate 2D: Gills of *S. melanotheron* x400. The 96h exposure at 20mls showing Skeletal muscle, NUC- Nucleus, CYT- Cytoplasm. No Abnormality was seen

Plate 2E: Gills of *S. melanotheron* x400. The 96h exposure at 30mls of engine oil showing CAL- Calcification. Foci of calcificationnis seen within the gill stroma

Plate 2F: Gills *S. melanotheron* x400. The 96h exposure at 40mls of engine oil showing IC- Inflammatory Cells are seen within the gill stroma
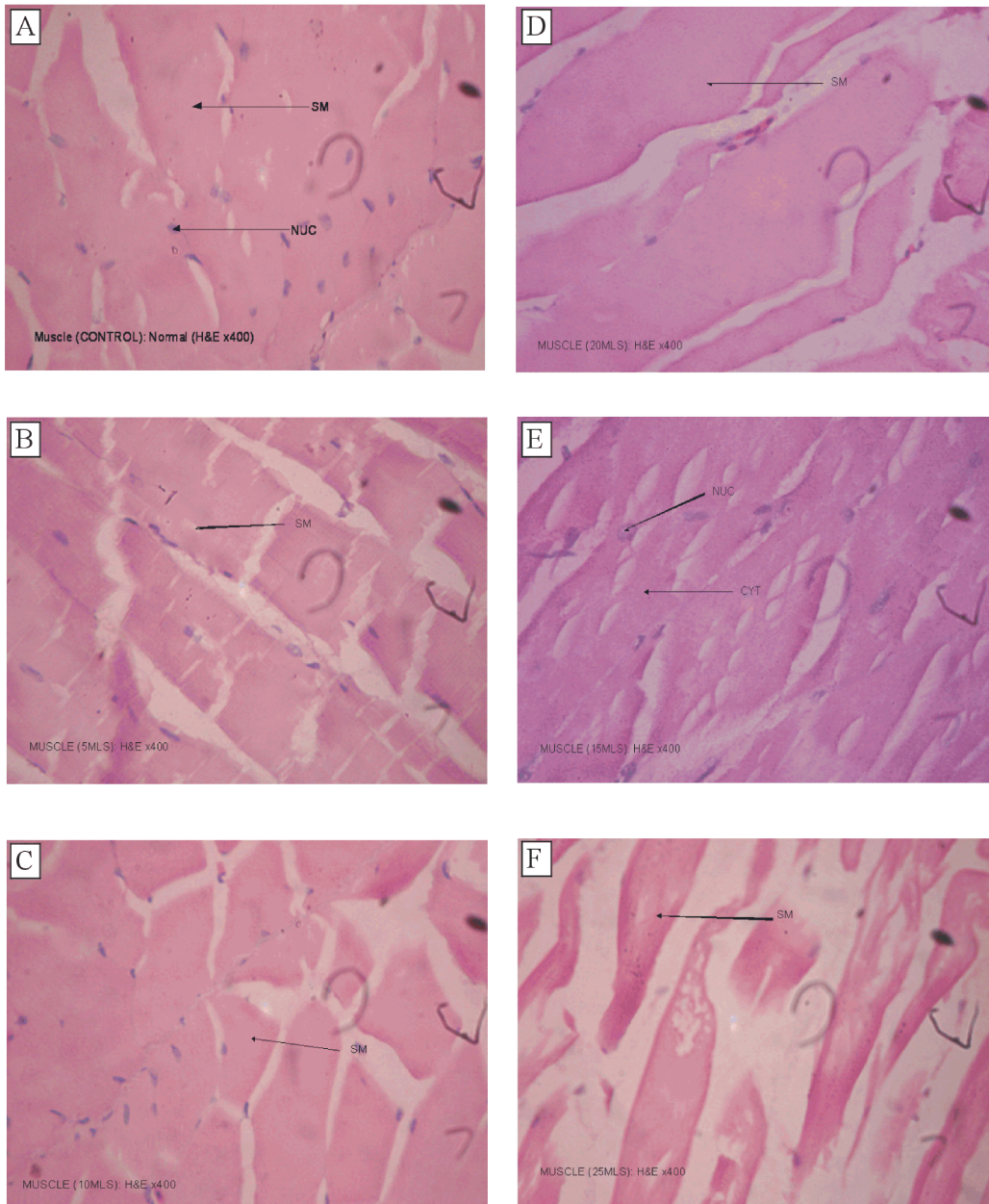
Plate 3A: Control muscle of *S. melanotheron* x400. The 96h exposure to engine oil showing SM- Skeletal muscle cytoplasm, NUC- Nucleus. No abnormality is seen within the skeletal muscles

Plate 3B: Muscle *S. melanotheron* x400. The 96h exposure at 5mls showing SM- Skeletal Muscle cell. Abnormality is rarely noticeable in the Skeletal muscle

Plate 3C: Muscle *S. melanotheron* x400. The 96h exposure at 10mls of engine oil showing SM- Skeletal muscle cell Abnormality is reaely noticeable in the Skeletal muscle

Plate 3D: Muscle of *S. melanotheron* x400. The 96h exposure at 210mls of engine oil showing SM- Skeletal muscle cell Abnormality is reaely noticeable in the Skeletal muscle

Plate 3E: Muscle of *S. melanotheron* x400. The 96h exposure at 30mls of engine oil showing SM- Skeletal muscle cell, CYT- cytoplasm. Abnormality is reaely noticeable in the Skeletal muscle

Plate 3F: Muscle of *S. melanotheron* x400. The 96h exposure at 40mls of engine oil showing SM- Skeletal muscle cell Abnormality is reaely noticeable in the Skeletal muscle

DISCUSSION

In this study the concentration that affected 50% mortality of *Sarotherodon melanotheron* was investigated to be 1.12mg/l. The result of this study showed that engine oil have considerable effects on the gill, muscle and kidney of the juvenile *S. melanotheron* and is in concordance with [15], who worked on the impact of refined petroleum spills on water quality macro- invertebrates and microbial communities of a tropical aquatic environment.

This study shows that no recognizable changes were observed on the muscle, kidney and gills of the control fishes. The muscles exposed to acute concentration of engine oil showed no recognizable changes. This is in agreement with [16] who worked on the histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane.

In the morphology of the gill, mild congestion was observed on the gill tissues of the lower concentration, while severe congestion was observed on the gill tissues of the higher concentration with severe inflammation and calcification of the gill was observed. The observed respiratory distress must have been due to decreased dissolved oxygen by the effluent which resulted in continuous oxidative biodegradation of the constituents of the engine oil which may cause oxygen tension in such solution by diverting the much needed dissolved oxygen for this biodegradation process. Similar result was shown by [17] in the gill morphology of *Clarias gariepinus* juveniles exposed to extract of *Parkia biglobosa* and *Raphia vinifera* in which the gill showed severe inflammation and degeneration.

Severe congestion and Inflammation were observed on the kidney tissues of the lowest concentration, Cytoplasmic vacuolations of the Kidney tissues of the higher concentration was also observed. Vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. The Kidney is another key important organ in the excretory pathway which showing the effect of chemical on the excretory system. This in turn provide toxicologist with a definitive site for investigation of the nephrotoxic potential of a pollutant. Once absorbed, toxicant is transported by blood observed. The inflammation observed is an indication of a secondary defense mechanism of the body against infections and this in conformity with the submission of [4, 8, 3] observed the same when circulation to liver for transformation and/or storage and if transformed in the kidney it may be excreted through the bile or pass back into blood for possible excretion by kidney or gill [18],

he worked on the histopatholgical effect of paper mill effluent on the kidney lesions which observation is similar with this study were dose dependent with the highest effluent concentration inducing the highest damages which are degeneration of the some veins and inflammation, were visible. This is in conformity with [18].

It has been discovered that toxic environmental conditions can result in structural changes in tissues of test organisms. Majorly are from direct toxic effect of the pollutant leading to degeneration and necrosis of vital organs usually at the cellular level.

In conclusion, this study showed that engine oil effluents pose severe damage to *Sarotherodon melanotheron* and it was observed that the acute concentration of engine oil have histopathological effects on aquatic organisms. It can be deduced that indiscriminate discharge of industrial effluents into water bodies can induce damage to the tissue and organ, which might make the fish vulnerable to diseases and eventually lead to death of prominent edible species of the aquatic environment. Therefore there is need for the adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluent and check the recurrence of oil spillage.

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