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Histopathological Effects Of Acutely Toxic Levels Of Palm Oil Mill Effluent On Gill And Liver Of Nile Tilapia Fingerlings

AKINSOROTAN, A. M

Abstract --- The toxicity of Palm Oil Mill Effluent (POME) was investigated with emphasis on histopathological effects of Nile Tilapia (*Oreochromis niloticus*) juvenile. Static bioassay was conducted to determine the LC_{50} of POME to Nile tilapia fingerlings. The fishes were exposed to 0, 5, 25, 50 mg/l of POME. Histopathological examinations were performed on the gills and liver of Nile tilapia fingerlings exposed to POME under standard laboratory condition. 120 live and apparently healthy *O. niloticus* fingerlings measuring 9.3-10.6cm standard length and weighed between 5.8g and 6.5g were randomly distributed into twelve (40cm x 29cm x 28cm) glass tanks of 60 litres capacity each were filled with 20litres aerated unchlorinated well water at ten fish/tank for the experiment. The toxicant was introduced at different concentrations in duplicate per treatment. The lethal concentration (LC_{50}) value of POME was 9.19mg/l for 96h of exposure. The total mortality occurred in the concentration of 50mg/litre within 24hours of exposure period. Toxic reactions exhibited by the fish include erratic movement, air gulping, loss of reflex, molting, barbell deformation, hemorrhage and excessive mucus secretion in fish exposed to higher concentration of POME. Histopathology of the organs after 96 hr exposure revealed cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting. In the liver, there was vacuolation of hepatocytes and necrosis. The changes in these tissues occur predominantly in the 96 hr exposure. Respiratory stress, erratic swimming and instant death of fish were observed in exposed fish, which varied with the concentration of the toxicant. Histopathological examination of the gills and liver of Nile tilapia fingerlings showed varied degrees of degenerative changes including vacuolation and necrosis which worsened with increasing concentration of the effluent. Observations on the bioassay test indicated hyper exetability and the eagerness of the test fish to jump out of the pollutant. This is a confirmation that fish in river Oluwa where Palm Oil Mill Effluent had been discharged into over decades must have either migrated out of the zones or died due to POME toxicity. POME is highly toxic to *Oreochromis niloticus*, therefore it's discharged directly into water bodies, near fish farms or in areas close to aquatic bodies should not be encouraged.

Index Terms— necrosis Palm oil mill effluent, River Oluwa, toxicity, histopathological

1 INTRODUCTION

The constant flow of agricultural effluents into fresh water often leads to a variety of pollutant accumulation, which becomes apparent when considering toxic pollution [22]. Palm oil is obtained from the fleshy mesocarp of oil palm fruits (*Elaeis guineensis*) which contain 45-55% oil [30]. In 1978, Okitipupa Oil Palm Mill was established, and since then the effluent, is being discharged into River Oluwa [2]; [4]. The effluent from the discharge is a mixture of sterilizer condensate from the hydrocyclone unit, sludge from bottom sludge tanks and others from factory drains. Presence of the effluent in the river constitutes environmental harzard as it causes oxygen sag apart from unpleasant odour and other bioactivities with detrimental effect on aquatic life [4], & [11]. Pollutants in water significantly affect the ability of fish to detect and respond to chemical stimulus. Feeding, Growth, and reproductive performances could also be seriously affected by such polluted habitat. Pollution of aquatic habitat may result in mass fish mortality or their failure to breed in the polluted environment. These chemical affect not only the physiology and survival of aquatic organisms including fish but also can interact with their genetic material which may lead to the mutations and/or carcinogenesis [15].

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety. Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals [24]. Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metal [23]. Also the liver is a very important organ which breaks down chemicals and as a result, liver cells are often among those that are damaged by toxic chemicals.

Effects of glyphosate herbicide on Tilapia was investigated by [7], filament cell proliferation, lamellar

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fusion, lamellar cell hyperplasia and epithelial lifting were observed. The major effects observed on the gills were Oedema, epithelial lifting, and thickening of the primary lamellar epithelium and fusion of secondary lamellae. In view of the need for knowledge of the aquatic side-effects of POME, the objective of this study is to determine the lethal concentration and the acute toxic effect of POME with emphasis on the histopathology on *Oreochromis niloticus.*

RESEARCH METHODOLOGY

A 96 –hour short-term static bioassay was conducted using the fingerlings of *Oreochromics niloticus* as test organisms. This was done in order to study the toxicity of POME on fish, and determine allowable levels or concentrations of POME for very short exposures.

Sources and collection

The choice of *Oreochromics niloticus* was informed by its ability to withstand stress and its high commercial value in Nigeria. Nile Tilapia juveniles, *Oreochromics niloticus* averaging 3.8 ± 0.13g and length range of 4-6cm obtained from Agricultural Development Project office, Alagbaka-Akure in Ondo State were used for the experiment.

Acclimatization of fish

The fish were held in 36.5cm by 25cm by 26cm, aquarium containing non-chlorinated water. The fish were allowed to acclimatize for more than one week under laboratory conditions to allow them adapt to experimental conditions (27 ± 2 °C). The period of acclimatization was extended beyond one week to ascertain the condition of the fish. The fish were inspected for disease conditions and general fitness. The fish were fed during the period of acclimatization and the water was changed every two days in order to remove faecal and unconsumed feeds. Feeding was discontinued during the 96-hour test period.

The determination of the physico–chemical parameters of the water

The physico–chemical parameters of the water used were examined. These parameters included temperature, dissolved oxygen (D.O.) and the hydrogen ion concentration (pH). The temperature was measured with a clinical thermometer and the dissolved oxygen of the water was measured with a digital meter (Jenway9071), while the pH was measured using the HANNA HI 9813 GRO CHEK meter.

GENERAL BIOASSAY TECHNIQUES

The bioassay was carried out in a rectangular glass tank. The top was covered with mesh net aided by elastic rubber band to prevent the fish from escaping. Each tank size of 36.5 cm by 25 cm by 26 cm contained ten fish. After a range – finding test, the concentrations prepared for the experiment were 0 mg/L, 5 mg/L, 25 mg/L and 50 mg/L, with two replicates. The amount of POME which contained the require mg of POME was determined by dissolving a known weight in a litre of distill water.

The behavioural pattern of the fish and other external changes in the body of fish were observed accordingly. Dead fish were identified by an absolute lack of movement. They were removed as soon as this was noticed, and disposed. The LC_{50} value of the *Oreochromics niloticus* for 96 hrs was calculated using the probit analysis.

Histopathology studies

At the end of the experiments, one fish per treatment were sampled after 96hour of exposure to POME for histological analysis. The fish was sacrificed with a blow on the head, using a mallet and was dissected to remove the liver and the gill. The organs were fixed in 10 % formalin for 3 days after which the tissue was dehydrated in periodic acid Schiff"s reagent (PAS) following the method of Hughes and Perry [18] in graded levels of 50%,70%,90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The organs were then embedded in molten wax. Tissue were sectioned into a thin sections (5-7µm) by means of a rotator microtome and were dehydrated and stained with Harris haematoxyllin-Eosin (H&E) stain. Bancroft and using a microtone and each section was cleared by placing in warm water (38^0C) where it was picked with clean slide and oven dried at 58^0C for 30 minutes to melt the wax. Slides containing sectioned materials/tissue was cleared using xylene and graded levels of (50%, 70%, 90% and 100%) of alcohol for 2 minutes each.

The section was stained in haematoxyline Eosin for ten minutes. The stained slides were observed under a light microscope. At varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus PM C35 AD4) a camera (OlympusC40 AB -4)

Statistical analysis: The dose response of mortality were analysed by probit analysis [14] based on a computer programme by Ge Le PaHoure, Imperial College, London. This was used to derive the LC_{50}.

LC_{50} = Median lethal concentration that causes 50% mortality of exposed animals.

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RESULTS

The physico-chemical characteristics of the raw POME

The physico-chemical properties of POME are presented in Table 1, which showed that the pH was acidic and critical to survival of aquatic organisms. The COD, BOD and T°C values were far above the values for other rivers in Nigeria. While conductivity was far less than values reported for other rivers. This indicates danger to aquatic lives where POME was discharged. Also the high levels of minerals concentration in POME is an indication of possible minerals toxicity to the biodiversity in the discharged areas.

The physico-chemical characteristics of the water

The physico-chemical properties are presented in Table 2, which showed that the pH of water in POME exposed experiments (5.43±0.2) was acidic and critical to survival of aquatic organisms. The water in the control experiment had almost neutral pH (7.20±0.01).

The physico–chemical parameters of the water values are: Dissolved oxygen (D.O.) 1.25± 0.05- 9.28 ±0.08 mg/L, Temperature 26.75 ±0.1 – 28.88±0.13°C and the pH–5.43 ±0.20-7.20± 0.01

Table 1: Physico-chemical properties of raw Palm Oil Mill Effluent.

Parameters	
Appearance	Yellowship and emulsified
pH	4.83± 0.04
COD (mg kg^{-1})	1547.4±21.1
BOD (mg kg^{-1})	1048.9 ±16.5
Conductivity (µs/m)	9.85± 0.00
Temperature (°C)	38.5± 0.00

Table 2. Physico-chemical properties of water in different treatment

Parameters	A	B	C	D
pH	5.43 ±0.20[a]	6.38± 0.07[b]	6.97± 0.03[c]	7.20± 0.01[d]
Dissolved oxygen (DO) mg kg^{-1}	1.25± 0.05[a]	2.80± 0.15[b]	5.88± 0.15[c]	9.28 ±0.08[d]
Biochemical oxygen demand (BOD) (mg kg^{-1})	91.1 ± 0.64[a]	39.0 0.34[b]	10.2±0 .04[c]	6.21±0.04 [d]
Chemical oxygen demand (COD) (mg kg^{-1})	261.6± 2.18[a]	127.0± 0.62[b]	57.6± 0.45[c]	9.54± 0.18[d]
Conductivity (µs/m)	1.73 ±0.03[a]	0.57± 0.12[b]	0.53± 0.01[b]	0.06± 0.00[c]
Temperature (°C)	28.9 ± 0.13[a]	27.0 ± 0.00[b]	27.0 ± 0.00[b]	26.8 ± 0.25[b]

Acute toxicity

The results of the acute toxicity test are presented in Table 1. The LC_{50} value based on probit analysis was found to be 9.19 mg/L for 96 hrs of exposure to the POME (Fig.3). The results obtained showed that there was no mortality of fish in the control experiment throughout the 96 hrs. There was 40% mortality of the fish exposed to 5 mg/L while at 50 mg/L, 100% mortality was observed. During this study the behaviour of the control fish was normal, while the fish introduced into the different concentrates of the herbicides showed different abnormal behaviour. Abnormal behaviour such as erratic swimming, sudden quick movements and restlessness were observed in fish exposed to the chemical. At high concentration of 50mg/L, the fish became very weak and settled at the bottom. Normal colour and behavioural response was observed in the control experiment.

HISTOPATHOLOGICAL EFFECT

Liver: Transverse section through the liver showed normal cellular pattern, normal central vein,

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billary epithelium, hepatic plate and hepatocytes. No lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen in the control (Fig.2). There were areas of slight lesion, necrosis, malignancy, pigment, inclusion bodies and inflammation in the livers exposed to the POME (Fig 3 and 4). Vacuolation and disarrangement of tissue was seen in concentration of 50mg/l of POME treated fish. Shrinkage of cell and hyperplasia of cell was observed. Complete degenerated tissue was observed in this highest concentration of 24.0mg/l within 96 hours (fig. 5)

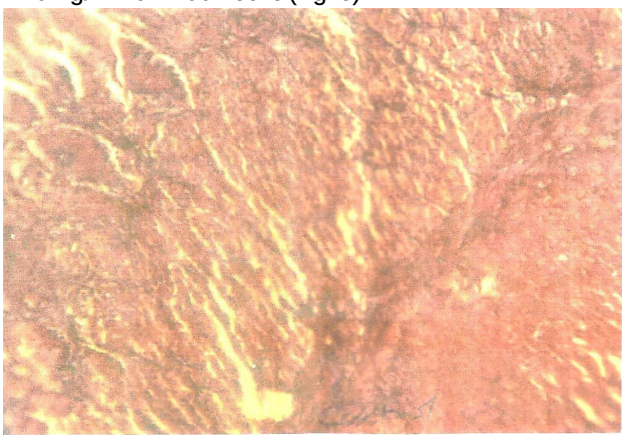

Fig 2: Ultra structure of liver of Oreochromics niloticus fingerlings exposed to 0mg/l of POME

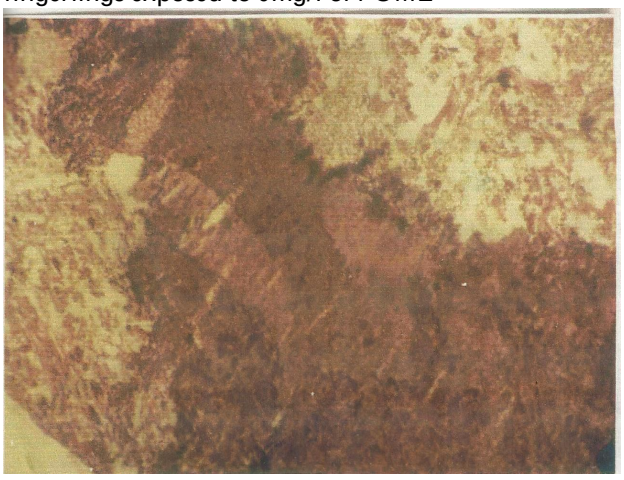

Fig 3: Ultra structure of liver of Oreochromics niloticus fingerlings exposed to 5mg/l of POME

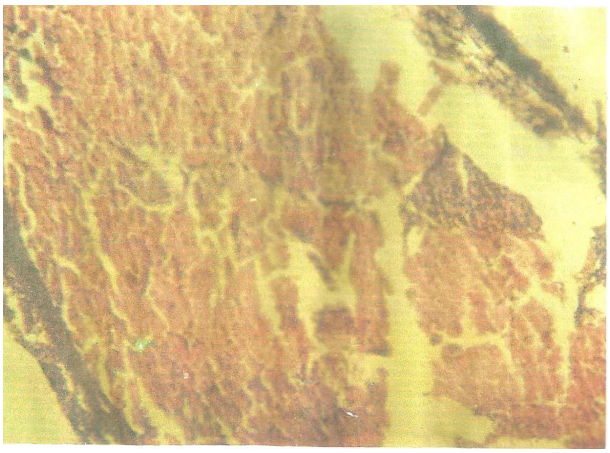

Fig 4: Ultra structure of liver of Oreochromics niloticus fingerlings exposed to 25mg/l of POME

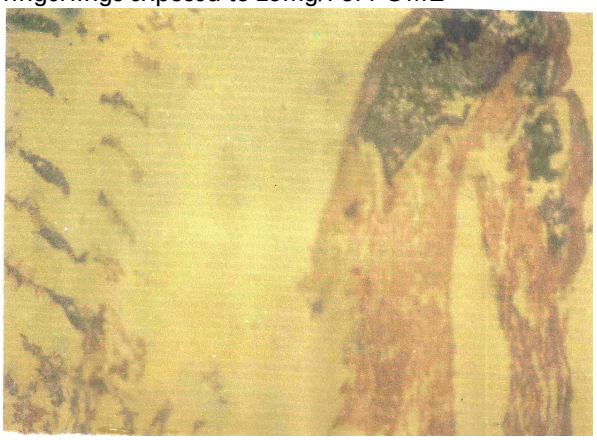

Fig 5: Ultra structure of liver of Oreochromics niloticus fingerlings exposed to 50mg/l of POME

Gill: Sections through the gill showed normal cellular pattern, ranging from gill arch, gill rakers, filament, venus, sinus, cartilaginous support, pseudo-brachial lamella, ceratobrachial bone of the arch, mucous epithelium lining on the membrane and branches of the afferent and efferent arterioles, and nucleous (Fig.6). No lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen. Moderate and severe areas of lesion, necrosis, malignancy, pigment and inclusion bodies were observd in fish exposed to POME in Fig. 7. Degeneration of lamellar and hypertrophy of cell (fig.8) occurred in concentration of 25mg/l of POME treated fish while Fig.9 also shows hypertrophy of gill arch and complete degeneration of filament in highest concentration of 50.0mg/l of POME treated fish within 96 hours period.

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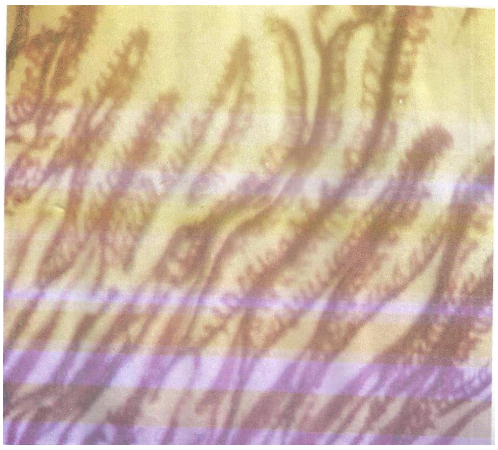

Fig 6: Ultra structure of gills of Oreochromics niloticus fingerlings exposed to 0mg/l of POME

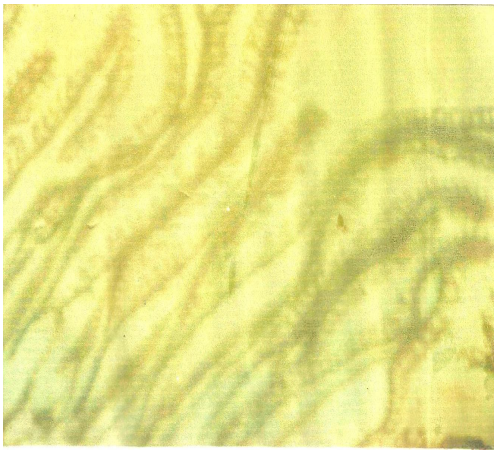

Fig 7: Ultra structure of gills of Oreochromics niloticus fingerlings exposed to 5mg/l of POME

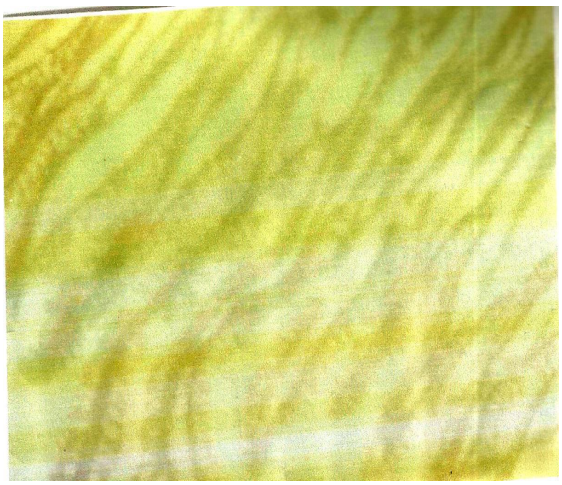

Fig 8: Ultra structure of gills of Oreochromics niloticus fingerlings exposed to 25mg/l of POME

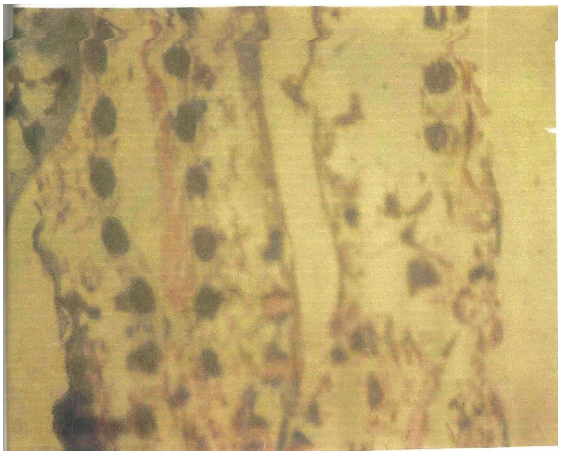

Fig 9: Ultra structure of gills of Oreochromics niloticus fingerlings exposed to 50mg/l of POME

Mortality (%) of *O. niloticus* fingerlings exposed to different concentrations of POME (bioassay test) (Table 3) showed that the fish was sensitive to concentrations from 5- 50ml L^{-1}. The table indicated that within 96h, about 40% of the fish died in concentration of 5ml L^{-1}, while 90% died in concentration of 25ml L^{-1}, suggesting that the 96h LC_{50} of POME might lie between 5 and 25ml L^{-1}. At concentrations between 25 and 50ml L^{-1}, all the fish died within 48h. The concentration values were converted to Logit, while the mortality (%) was converted to Probit values according to methods of Hewlett & Plackett [16], and the transformed values were used to determine the 96h LC_{50} graphically. Figure 1 presents the LC_{50} graph with the regression equation $Y = 4.4439x + 0.72$, where y = probit response and x = logit (log-dose). From the equation, the 96h LC_{50} was calculated as 9.19 ml L^{-1}.

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Table 3: Mortality(%) of *O. niloticus* fingerlings exposed to different concentrations of Palm Oil Mill Effluent.

Concentration (mg l^{-1})	Time (Hours) 0	24	48	72	96
0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	10.0	30.0
25	0.0	80	20	0.0	0.0
50	0.0	100	0.0	0.0	0.0

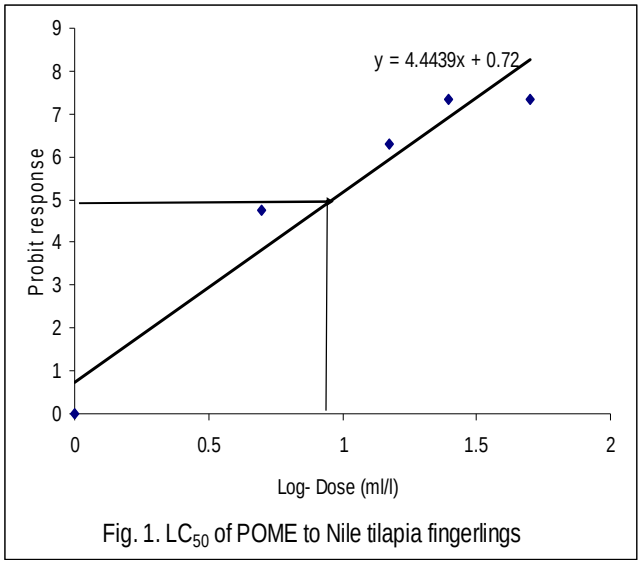

Fig. 1. LC_{50} of POME to Nile tilapia fingerlings

DISCUSSION

The POME exerted toxic effect on the fish in the present study and toxicity increased with increased concentration. The physico-chemical properties of POME clearly indicated that it is a pollutant, as its presence in water changed the physical and chemical qualities of water to critical levels that could hardly support aquatic productivity. In (1997), Aiyesanmi & Ipinmoroti studied the impact of POME on some water quality variables on river Oluwa and concluded that POME constituted environmental hazards as it changed the colour of the water and with high values of pollution characteristics. As expected, high concentration of POME in the areas resulted in increased water temperature with corresponding increase in BOD, COD, conductivity and reduction in dissolved oxygen concentrations. Conductivity is the expression of the levels of ions in the water that maintains ionic balance and offer buffer in the ecosystem. But the levels observed in the studied areas were far below values reported for good productivity in tropical rivers FAO [13] and pond waters [19]. This could be attributed to very high concentration of POME which interfered with ionic dissociations.

Abnormal behaviours such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercular acivities, surface to bottom movement, sudden quick movement and resting at the bottom observed in this study were similar to the observations of Omitoyin et. al[28] and Fafioye [12]. The fish were stressed progressively with time before eventually dying. The stressful ailment of respiratory impairment due to the toxic effect of POME on the gills was similar to the report of Omitoyin et al. [28]. The observed increasing state of inactivity with both increasing concentrations and exposure period agree with the report of Ayoola [7]. Water quality parameters had little variation, physicochemical parameter measured seemed to be within optimum range for fish culture as reported by Omitoyin et al. [28] and Olaifa et al. [26].

The extremely, high levels of BOD and COD in POME resulted in a rapid consumption of the DO in the receiving water leading to a phenomenon referred to as oxygen sag. This process has also been described by Fair [11], [17] & [2]. [25] similarly reported oxygen sag in a river in South Western Nigeria due textile mill effluent pollution. The acidic pH condition of POME led to acidic water quality. This has implication of killing the organisms or reducing aquatic productivity. [2] suggested that the acidity of the water could be ascribed to the presence of free fatty acids (FFA) in the effluent.

The 96h LC_{50} of Nile tilapia which is 9.19 ml L^{-1} is a pointer to what happens to the organisms in the river when hundreds ml L^{-1} of POME is discharged. Therefore, safe level of POME to be released in the water must be lower than 9.19 ml L^{-1} and the value could be even lower when micro-organisms are considered. The revelation from the bioassay test on how the fish were attempting to jump out of the experimental tanks when in contact with POME toxicant suggests that most of the fish in polluted areas might have migrated out of the zones.

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CONCLUSION

The results of the present study revealed that POME is toxic to fish organs and causes histopathological changes in different organs such as gills and liver; therefore, indiscriminate discharge into water bodies by farmers should be discouraged particularly in aquatic bodies.

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