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Range finding, acute and sublethal test of petrol and engine oil mixture on African catfish, *Clarias gariepinus* (Burchell, 1822)

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Abstract
Range finding, Acute and sub-lethal toxicity test of a 3:2 mixture of petrol and engine oil on the African catfish, *Clarias gariepinus* fingerlings (0.83±1.23g) using 96-hour bioassay was carried out. Range finding test using ten (10) concentrations of 0.50, 0.67, 0.83, 1.00, 1.67, 1.83, 2.00, 217 and 3.33 ppt; was carried out to determine the definitive concentration. The acute test determined graphically revealed lethal median concentration (LC_{50}) of 2.06 ppt and the safe concentration of 2.0 ppt. Mortality was higher within the first 24hrs and increased with increase in concentration of the toxicant. Significant differences ($p<0.05$) in mortality occurred between the various concentrates (2.00, 2.07, 2.14, 2.21, 2.28 ppt) used in the acute test. *Clarias gariepinus* fingerlings exposed to sub-lethal test with concentrations 0.83, 0.90, 0.97, 1.04 and 1.11 ppt had 0, 40, 50 and 100% mortality within 14 days of experiment. Fish also showed abnormal behaviour, examples are; restlessness, sudden quick movement, erratic swimming pattern, loss of reflex and discolouration. Histopathological changes in gills and skin of the catfish showed alteration of the gill and separation of the epithelial layer from the supportive tissue respectively. Water quality was poor in treatments with higher concentrations as 96-hour bioassay days progresses, specifically, dissolved oxygen greatly reduced in value within the 96 hours bioassay.

Keywords: Acute, range finding, sub-lethal, toxicity, petrol, engine oil

1. Introduction
According to WorldFish [17], Nigeria has become the largest aquaculture producer in the Sub-Sahara Africa and *Clarias gariepinus* is the leading aquaculture species in Nigeria which accounts for 64% aquaculture food production in 2015. This is because of its hardness, ability to grow better on an artificial food supply, reproduce with greater success in cultured facilities, withstand poor water quality and greater resistance to diseases commonly encountered in the fish culture [15].

However, fish seed is still at the minimal production level in Sub- Sahara Africa, as a result of water pollution causing considerable loss to fish farmers [12]. Many factories in Nigeria are located on River Banks and use the rivers as open sewers for their waste products. The major industries responsible for this include petroleum, mining, textiles, plastics, paint and food, of all these, the petroleum industry presents the greatest threat to water quality [9]. When petroleum is refined, [14] the general types of associated materials are petroleum, fuels, kerosene, lubricants, acids, alkalis, gasoline, naphthalene paints, paraffin, pesticides, petroleum hydrocarbons, solvents, tar, derivatives and waxes. Many of these water-soluble components of crude oil and refined products have a toxic effect on aquatic organisms, their eggs and young stages being highly vulnerable.

It has been reported that conferences and symposia have been held globally to access the impact of oil and refined oil products on aquatic ecosystems [11].

The consensus has always been that oil could be dangerous to live organisms, both in and around the waters.

In a report, the effect of oil spillage on fishing sites in Ibem and Oyakama town, Cross River State in Nigeria showed that large or small amount of oil can be harmful to both aquatic plants and animals [1].

The incessant power outage has led to an increased level of purchase of generator sets and also there are more vehicles on our roads now more than ever before.

These engines are serviced and the mixture of worn-out engine oil and petrol are drained into the drainage which finds its way into nearby water bodies after rainfall.

There is an immediate need for empirical studies on the joint effect of mixtures of toxicants especially on the contribution of small fractions of the toxic units of individual components and the relationship between long and short term lethal and non-lethal joint effects [2].

The objectives of this study are to; determine the toxicity of different concentrations of the binary mixture of petrol and engine oil on *Clarias gariepinus* fingerlings, determine the range finding, lethal and sub-lethal doses of the mixture on *Clarias gariepinus* and study the effect of exposing the fish to sub-lethal dose on the histopathology of the gill and skin of the catfish. Little work has been done on these and it is therefore imperative to carry out this study.

Materials and Method

The experimental fish, *Clarias gariepinus* fingerlings (0.83±1.23g) of the same genetic stock were obtained from a reputable fish farm and acclimatized in rectangular glass tanks (75 x 40 x 40 cm, 60L capacity) filled to half capacity in the laboratory for seven days prior to commencement of the acute toxicity test. Fish were fed 35% crude protein commercial diet and feeding discontinued two days before the experiment to minimize the amount of waste in the test media and depletion in oxygen level.

Range finding test

A range finding test was done using nine treatments (0.5, 0.6, 0.8, 1.0, 1.7, 1.8, 2.0, 2.2 and 3.3 ppt). Each treatment had three replicates with ten (10) fishes introduced into them. The experiment was carried out under standard bioassay procedures [3] and the experiment was static. The test materials, petrol and engine oil were obtained at a reputable gas station in Akure, Ondo State. The mixture of the materials was prepared in a beaker using ratio 3:2 of petrol and engine oil. *Clarias gariepinus* fingerlings were weighed using Top loading metler balance and introduced at the same time into rectangular glass tank water (30 litres) already containing the mixture of the test materials at different concentrations. The experiment was monitored at 3 hours interval and lasted for 72 hours [16]. *Clarias gariepinus* mortality was recorded and failure to respond to external stimuli was used as criteria for death.

The definitive test (acute toxicity test)

This was conducted under standard bioassay procedure with five treatments (2.0, 2.07, 2.14, 2.21, and 2.28 ppt). The same experimental design used in range finding test was repeated in this test. Mortality was monitored for 96 hours and Temperature, pH and dissolved oxygen level were monitored using standard methods.

Sub-lethal test

The experimental design used in range finding test was repeated in this test. The varying concentrations used were 0.83, 0.90, 0.97, 1.04 and 1.11 ppt. Mortality was also monitored and abnormal behaviour such as restlessness, sudden quick movement, erratic swimming pattern, loss of reflex and discolouration were reported. At the end of the experiment two fishes were taken from each tank, weighed on a Metler top-loading balance (Model P13 8001), killed by decapitation and vital organs such as the gills and skin removed. These were fixed for 24 hrs in formalin saline solution made of equal volumes of 10% formalin and 0.9% NaCl solution. Histology of 5µm thickness was prepared following standard procedure [7]. Photomicrographs were taken with a Leitz (Ortholux) microscope and camera. Temperature, pH and dissolved oxygen level were also monitored using standard methods.

Results

In acute toxicity test, fish showed stressed condition immediately the toxicant was introduced into the water. Mortality was higher within the first 24 hours and increased with a higher concentration of the toxicant. The 96 hours LC_{50} was recorded graphically at 2.06 ppt as shown in figure 1.

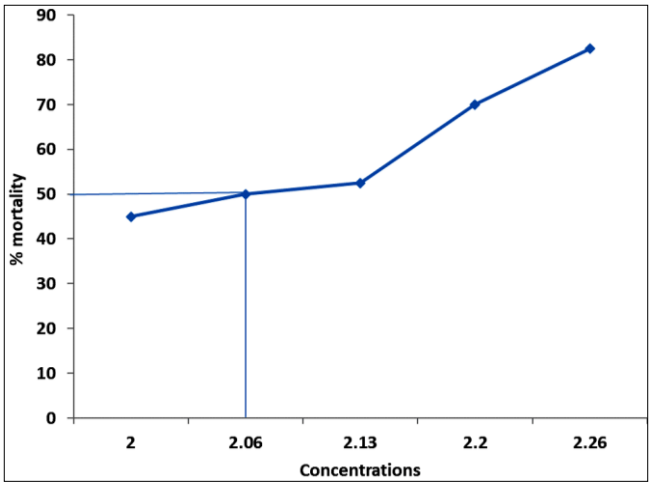


Fig 1: LC_{50} (median lethal concentration) for 96h bioassay (2.06 ppt)

There were significant losses of fish with an increase in petrol and engine oil mixture (p<0.05). As shown in table 1, at concentrations less than 2.00 ppt, the mortality rate was less than 50% but increased greatly to 70-100% at concentrations higher than 2.00 ppt.

Table 1: Range finding test: Percentage mortality of *C. gariepinus* exposed to different levels of petrol and engine oil mixtures

	Concentration (ppt)									
Time elapsed hr	**0**	**0.50**	**0.67**	**0.83**	**1.00**	**1.67**	**1.83**	**2.00**	**2.17**	**3.33**
24	0	0	0	0	0	20	30	70	70	100
48	0	0	0	0	0	30	40	70	70	100
72	0	0	0	0	0	30	40	70	70	100
96	0	0	0	0	0	30	40	70	70	100

In acute toxicity test as shown in table 2, the addition of toxicant resulted in 50% mortality within the first 24 hours in all the treatments except in 2.00 ppt concentration which was 40%. At 96-hour, mortality increases as concentration increases and concentration 2.28 ppt had the highest mortality.

Table 2: Acute toxicity test: Percentage Mortality of *C. gariepinus* exposed to different levels of petrol and engine oil mixtures

	Concentration (ppt)					
Time elapsed hr	**0**	**2.0**	**2.07**	**2.14**	**2.21**	**2.28**
24	0	40	50	50	70	80
48	0	40	50	50	70	80
72	0	50	50	50	70	80
96	0	50	50	60	70	90

Table 3 showed low mortality rate in the sub-lethal test. Concentration 0.83 and 0.90 ppt had no mortality while concentration 0.97 and 1.04 ppm had 10% mortality and concentration 1.11 ppt had the highest mortality of 20%.

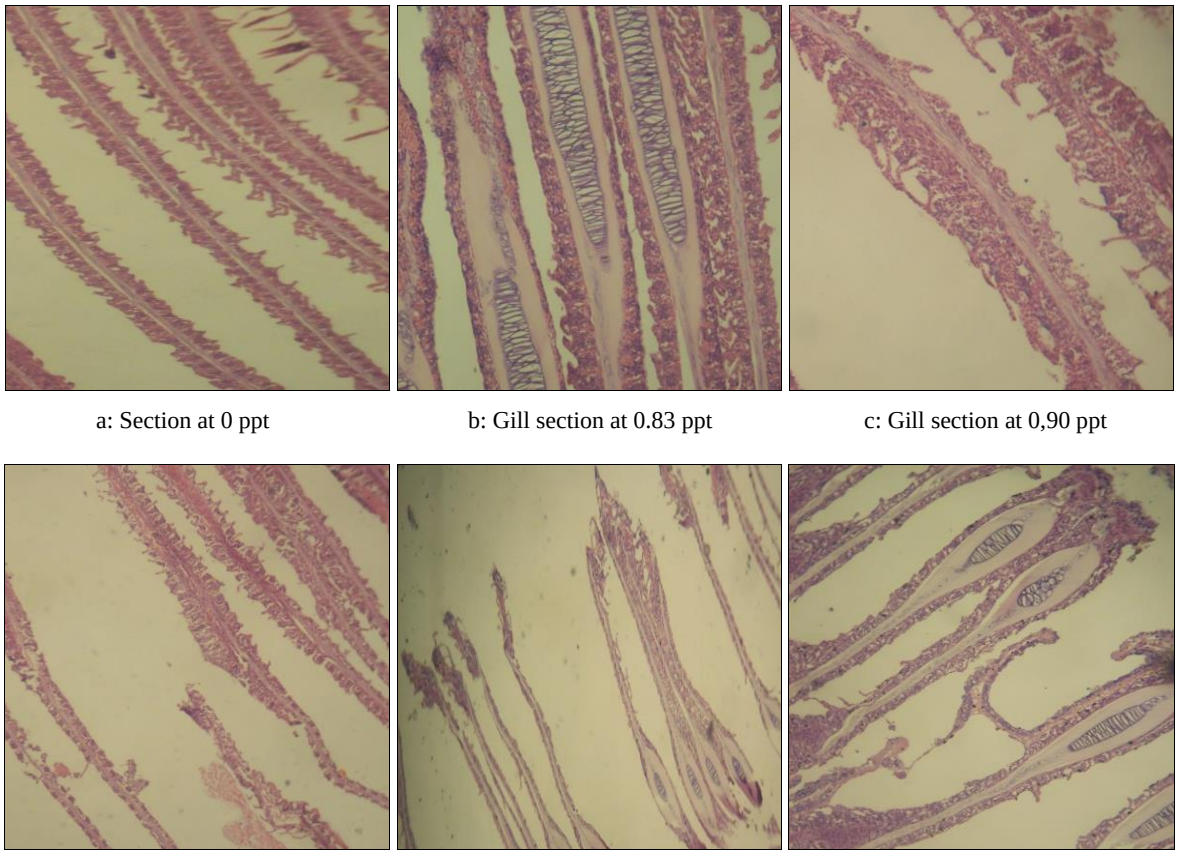
Table 3: Sub-lethal test; percentage mortality of *C. gariepinus* exposed to different levels of petrol and engine oil mixtures

Concentration (ppt)						
Time elapsed hr	**0**	**0.83**	**0.90**	**0.97**	**1.04**	**1.11**
24	0	0	0	0	0	0
48	0	0	0	0	0	0
72	0	0	0	0	0	0
96	0	0	0	0	0	0
120	0	0	0	0	0	0
144	0	0	0	0	0	0
168	0	0	0	0	0	0
192	0	0	0	0	0	10
216	0	0	0	0	0	10
240	0	0	0	0	10	20
264	0	0	0	10	10	20
288	0	0	0	10	10	20
312	0	0	0	10	10	20
336	0	0	0	10	10	20

Histopathological findings

Histopathological changes in gills and skin

Histopathological changes in gills and skin of *Clarias gariepinus* showed alteration in the structure of the organs. Normal gill architecture was observed in fish from the control treatment. Both the lamellae and the gill filaments were visible with the gill filaments well separated from each other (Plate 1a). At high concentration of the toxicant, the normal gill architecture was destroyed and the gill lamellae extensively fused together (Plate 1b).

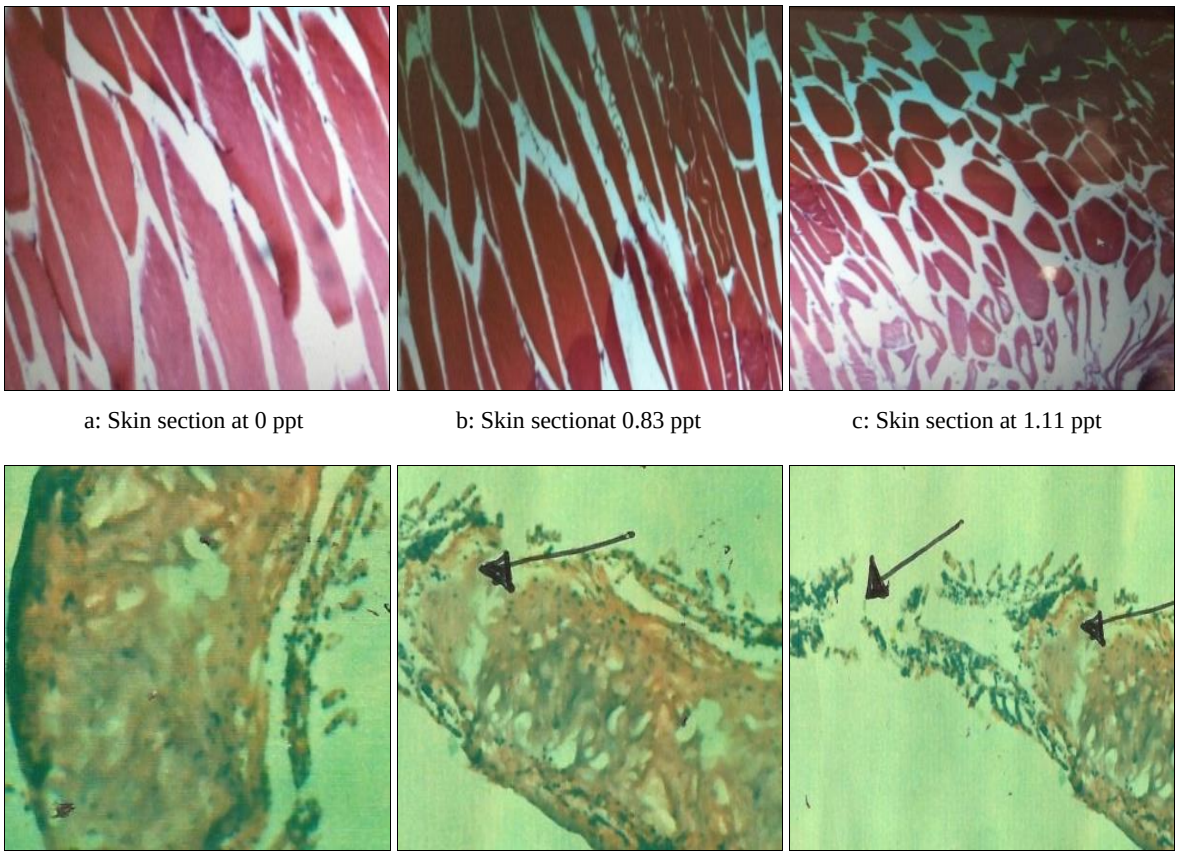


a: Section at 0 ppt b: Gill section at 0.83 ppt c: Gill section at 0,90 ppt

d: Gill section at 0.97 ppt e: Gill section at 1.04 ppt f: Gill section at 1.11 ppt

Plate 1: Histopathological changes in gills of *C. gariepinus*

The skin of the fish changed in colour and took after the colour (brown) of the tested material at (Plates d, e, and f) higher concentrations; the epidermal cells were also deformed.



a: Skin section at 0 ppt b: Skin sectionat 0.83 ppt c: Skin section at 1.11 ppt

d: Skin section at 0.97 ppt e: Skin section at 1.04 ppt f: Skin section at 1.11 ppt

Plate 2: Histopathological changes in the skin of *C. gariepinus*

Table 4 revealed that dissolved oxygen decreased as concentration of petrol and engine oil and time increases. There were little differences in both temperature and pH values throughout the period of the experiment.

Table 4: Sub-lethal test water quality parameters monitored for 96 Hours

Conc. (ppt)	Day 1			Day 2			Day 3			Day 4		
	ºC		Mg/l	ºC		Mg/l	ºC		Mg/l	ºC		Mg/l
	Temp	pH	DO	Temp	pH	DO	Temp	pH	DO	Temp	pH	DO
0	25.0	10.1	4.1	25.0	10.0	4.0	25.1	10.0	3.9	25.1	10.0	3.9
0.83	25.8	10.7	-2.9	26.1	10.4	-3.1	26.4	10.3	-3.3	26.3	10.4	-3.4
0.90	25.9	10.7	-2.7	26.3	10.4	-2.8	26.6	10.4	-2.9	26.6	10.4	-3.5
0.97	25.8	10.9	-2.9	26.2	10.5	-3.2	26.5	10.5	-3.5	26.6	10.3	-3.6
1.07	26.1	11.1	-2.9	26.6	10.8	-3.2	26.8	11.1	-3.4	26.9	10.9	-3.7
1.11	26.7	11.2	-3.1	26.9	11.4	-3.7	26.9	11.2	-3.8	27.1	11.0	-4.0

After 24hrs into the acute toxicity test, the surviving fish were stationary and in most cases converged at the edges of the glass tanks. Furthermore, fish exposed to the toxicant exhibited a change in behaviour such as jumping out of the tank, chasing each other and being confrontational. They also showed an erratic swimming pattern, loss of reflex, discolouration and moulting, these reactions increased with increase in concentration (Table 5).

Table 5: Physical reaction of fish to petrol and engine oil mixtures in an acute toxicity test

	The physical reaction of fish				
Conc. (ppt)	Aggressiveness	Erratic swimming pattern	Loss of reflex	Discolouration	Moulting
0	-	-	-	-	-
2.00	+	-	-	-	-
2.07	+	+	+	-	+
2.14	+	+	+	+	+
2.21	+	+	+	+	+
2.28	+	+	+	+	+

Key: - = Absent, + = Present

Discussion

The histological section of gill and skin of *Clarias gariepinus* studied showed normal architecture in the control experiment. This is in agreement with [4] who studied acute toxicity and histopathological effects of engine oil on *Sarotherodon melanotheron.* The results of the toxicity tests revealed that

Clarias gariepinus fingerlings of the same age and size react differently to petrol and engine oil mixtures as fish mortality occurred at different periods. These reports are similar to the findings of [8, 10] who showed that hybrid of striped bass of the same age exhibited differential tolerance to the lethal ammonia concentration.

Highest percentage of mortality was recorded in acute toxicity test within the first 24 hours; this was similarly reported by [6] who recorded total mortality within 24 hours when copper concentrations of 2.61 and 5.60 mg/l were used on juvenile red drum, *Sciaenops ocellatus* and 97% mortality within 24 hours when 1.36 mg/l of copper was used.

A very significant observation was the change in colour of the catfish to light yellow which may be taken after the colour of engine oil used. Tiny oil droplets were observed on the water surface, this droplets retard oxygen uptake by water and so caused a lower dissolved oxygen concentration and mortality of the fish. In the entire toxicological test, dissolved oxygen level reduced greatly within the 96 hours bioassay, this may act as a criterion for mortality, this report corroborates [4] Dissolved oxygen reduced as the concentration of petrol and engine oil increases. A thin layer of the mixture covered the surface of the water used which could be the reason for the decrease as reported by [5] where environmental toxicology was studied.

Sub-lethal concentration affected the behavioural pattern of the fish and they displayed erratic swimming pattern, loss of reflex and death [13], reported the same three stages of behavioural reaction of *Pangasius sutchi* to the mixture of grease and engine oil.

In conclusion, the results have shown that petrol and engine oil mixture is toxic to *Clarias gariepinus* at all concentration therefore, more control on its disposal is advocated.

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