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Determination of Lethal Concentration (LC_{50}) Values of Spent Automobile Engine Oil on *Clarias gariepinus* (African Catfish)

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Abstract— In this study, the mortality and lethal concentration (LC50) values of spent automobile engine oil on *Clarias gariepinus* (African catfish) were investigated. The experimental design followed Completely Randomized Design comprising 5 levels of treatment in three (3) replicates. The setup constituted a total of 150 fish samples with 30 fish in each group. The treatment, spent engine oil was introduced into the aquaria at 5 ml, 10 ml, 15 ml, 20 ml concentrations and 0 ml as the control. The setup was allowed to stand for 96 hours to determine the mortality rate of African catfish to the selected concentrations of spent engine oil. Percentage mortality of fish was calculated in these concentrations. Mortality was observed across treatments exception of the control. The result obtained indicates that spent engine oil triggers environmental stress for the test organism with respect to concentrations. While the 48 hr LC_{50} value (with 95% confidence limits) of the safe dose of spent engine oil was estimated at LC_{50}=24.1 ml, 72 h LC_{50} value=15.75 ml, and 96 h LC_{50} value= 9.89 ml. However, fish mortality was significantly attributed to DO depletion and asphyxiation due to change in fish environment.

Index Terms— acute toxicity, *Clarias gariepinus*, LC_{50}, lethal bioassay, mortality, spent engine oil,

1 Introduction

African sharp-tooth catfish is one of the most abundant, widely distributed and commercially cultured fish in Nigeria. It is of the Clariidae family named by Burchell in 1822 as *Clarias gariepinus*. This category of fish is typically scaleless, and has bony elongated body, a helmet like head and air-breathing respiratory organ. These enable it to stay for some time outside water, making use of atmospheric oxygen [1] Hence, they can tolerate very low oxygen concentrations and survive for reasonable period out of water, via the use of a specialized suprabranchial organ. This organ is a large paired chamber with branches above the gill arches specifically adapted for air breathing [2].

They are adoptive to extreme environmental conditions and can live in pH range of 6.5 - 8.0. They are able to live in very turbid water and can tolerate temperature of 8-35°C [3] They are of great economic importance as food fish and vital in the sustainability of aquaculture [4]. Many species of fish are produced all over the world, but Catfish is taking the lead because of its uniqueness [5], and It serves as a source of income, reduces the rate of unemployment in the economy and increases GDP [5].

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Spent engine oil is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain [6].

It is a common environmental contaminant which is toxic and not naturally occurring in the environment [7], it is also known as used mineral based crankcase oil and is a mixture of various chemicals [8], including high and low molecular weight (C15-C20) aliphatic hydrocarbons, aromatic hydrocarbons, chlorodibenzofurans, polychlorinated biphenyls, decomposition products, lubricative additives and also heavy metal contaminants such as chromium, tin, aluminum, manganese, lead, nickel, and silicon which come from engine parts as they wear down [6].

The indiscriminate disposal of spent engine oil in Nigeria has been persistently problematic; yet the local consumption of engine oil in Nigeria is increasing at a very high rate due to the drastic increase in vehicles and other machines that make use of this lubricant [9]. Since it is liquid, spent engine oil will migrate into the environment and eventually find its way to contaminate either water bodies or agricultural field [10].
This study demonstrated the percentage of death of African catfish (*Clarias gariepinus)* exposed to spent automobile engine oil polluted water with the aim of determining the LC_{50} of exposure time.

MATERIALS AND METHODS

Experimental animals

150 samples of *C. gariepinus* collected from the fresh water Fish Farm of Michael Okpara University of Agriculture, Umudike at fingerlings growth size with initial recorded mean body weight of (4.0±0.52 g) and mean body length of (4.56±0.57 cm) was transported to the laboratory, distributed into 15 plastic aquarium (of 25 L capacity) and acclimated for 30 days. The acclimated fish was carefully observed till juvenile size with a recorded mean weight of 12.50±0.5 g and mean length of

10.21±7.25 cm after a daily two ration of feeding frequency with 2 mm pelletized fish feed (vital ©) obtained from a local market.

Collection of spent engine oil

Samples of spent oil were collected from the engine drain points of four different mechanic workshops in Ohiya Mechanic Village (the Central Automobile Repairs/Servicing Workshop) in Umuahia South L.G.A. Abia State. The collected spent oil was properly mixed by hand inversion in a clean 4-liter container rinsed with distilled water; this was done to represent the various sources of spent engine oil that contaminates the aquatic environment through runoff. However, prior to the study, a toxicity range-finding test was conducted using five widely spread concentrations of the spent oil to determine the concentration of the toxicant to be used.

Experimental design and layout

The experiment was laid out in a Completely Randomized Design (CRD) with five (5) levels of treatment replicated three (3) times (giving 10 fish per replicate and 30 fish per level). The concentrations of spent engine oil used were; 0 mls (**T1 as** control), 5 mls (**T2**), 10 mls (**T3**), 15 mls (**T4**) and 20 mls (**T5**). The experiment was conducted under room temperature (27°C±2.0) using 15 perflourocarbon plastic container (of 25 L capacity), having the dimension of 45 cm x 28 cm x 28 cm dimension. The fish were not fed during the non-renewal bioassay, however careful observations were made within 12 hours, and 12 hourly intervals for 96 hours.

The characteristics of the test media were determined for changes in the water quality parameter after the introduction of the determined concentrations of spent oil; water quality indices parameters such as temperature, pH, dissolved oxygen and conductivity were determined across treatment tanks.

The behaviour and general condition of fish were observed at the time of toxicant introduction till the termination of the 96hours end point. Mortality was used as a measure of toxicity, and the numbers of dead and active fish were recorded. Fish were considered dead when no gill (opecular) movement was observed and when they lie suspended in test water ventral side upwards and without response to gentle prodding with a glass rod. Hence Mortality was mathematically determined.

Mortality (M) was calculated as:

$$M = \frac{(No - Nt) \times 100\ \%}{No}$$

Where:

No = Number at the start of the experiment

Nt = Number at the end of the experiment

The susceptibility of fish to the toxicant was measured in terms of 96hours LC_{50} (concentration of spent engine oil in water that kill 50% of the fish population in 96 hours) this was expressed in percentage (%) according to Finney (1971)).

Statistical Analysis

The experiment was performed in triplicate. Data were analyzed with SPSS statistical analysis software (Version 20.0) using Probit Analysis Statistical Method. The LC_{50} values (with 95% confidence limits) were calculated. Differences among the results were considered to be statistically significant when *P* value was < 0.05. Also, the MS Excel 2007 was used to find regression equation (Y=mortality; X=concentrations), the LC_{50} was derived from the best-fit line obtained.

RESULTS AND DISCUSSION

Characterization of the Test Media

The characteristics of the test media revealed obvious changes in the water quality indices after the introduction of the determined concentrations of spent oil. The result showed that water quality parameters changed with relative to toxicant concentrations variation (Table 1). In details, the mean pH values obtained from the test chambers: T_1, T_2, T_3, T_4 and T_5 were 7.54, 6.24, 6.01, 5.83 and 5.65 respectively. Aside the control (**T_1**) which had a mildly alkaline pH, the test media (**T_2**) with the lowest concentration (5 ml) had the highest pH value which was slightly acidic while the test media (**T_5**) bearing the highest concentration (20 ml) gave a low pH value which appeared medium acidic. This implies that the toxicant acidified the test media. Sequentially, the values obtained for DO ranged from 3.93 mg/l – 6.30 mg/l throughout the test period showing significant variances between the test chambers and the control with the mean of 9.00 mg/l. This shows a severe reduction in DO due to the spent engine.

Table 1. Mean and standard deviation of the characteristics of the test media for 96 hours acute toxicity

Treatment	pH	DO (mg/l)	Temp (°C)	Conductivity (mg/l)
T_1 Control (0 ml)	7.54	9.0	26.63	160.33
T_2 (5 ml)	6.24	6.30	26.23	207.33
T_3 (10 ml)	6.01	5.37	26.07	254.00
T_4 (15 ml)	5.83	4.83	25.98	291.67
T_5 (20 ml)	5.65	3.93	25.78	361.33

The variation in temperature was obvious between treatments with test chamber **T_2** (5 ml) having the highest temperature similar to the control, while the test chamber **T_5** (20 ml) having the lowest temperature with a mean of 25.78°C, this was attributed to the kinetic energy of the surviving active fish in T_1 and T_3 however dissimilar to T_5 where few fish were active due to mortality. Correspondingly, variations were observed in conductivity, the highest mean value for conductivity was 361.33 from test media **T_5** (20 ml) and the lowest was 207.33 obtained in test media **T_2** (5 ml) compared to the control which gave a value of 160.33

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Mortality

The impact of the different concentrations of spent engine oil on the test organisms (juveniles of *Clarias gariepinus)* showed marked variation during the course of the experiment. No mortality was observed in the control experimental tank; however, mortality rate increased with the increase in concentration in other experimental tanks (Figure 1).

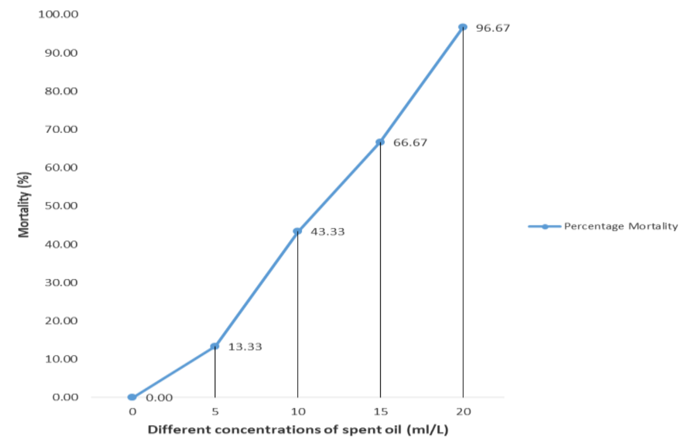
MACROSCOPICAL OBSERVATION AND MORTALITY DETERMINATION OF THE TEST ORGANISM (LC_{50}) TO THE SPENT ENGINE OIL

Table 2: Macroscopic observation of fish across treatment tanks

T_1 (0 ml CTL)	There were no observed changes in the Control tanks. Fish exhibited normal life style as well as feeding and swimming characteristics.
T_2 (5 ml)	Gradual retardation in swimming was observed as fish adjusted due to presence of environmental stressor **(plate 3).** Mortality was observed in T_2 tanks after 72 hours accounting to 13.33% of total mortality
T_3 (10 ml)	General aggressive movement was observed in this treatment level (T_3). Fish showed signs of restlessness followed by sudden retardation and breathlessness. Mortality commenced after about 48 hours accounting for 43.33% of total mortality.
T_4 (15 ml)	Extended oil film formed was observed over the test media. Rapid movement was also observed in the aquaria with fish settling at the depth/base of aquarium and maintained calmness for about 30 minutes. Operculum motilities of fish were seen often after 5 minutes. Breathless was observed in fish leading to mortality. This level accounted for about 66.66% of total mortality **(plate 4).**
T_5 (20 ml)	Dense oil film spread over T_5 test media at the introduction of 10 ml of spent engine oil. There was sudden increase in the fish swimming pace. Rapid movement, breathless and lethargy was also observed after about 30 minutes. Operculum motilities of fish became irregular within 2 hours and finally total mortality was observed at approximately 8 hrs. after toxicant introduction. **(plate 5)**

There were 13.33% mortality in T_2 which was the least concentration containing 5 ml of the spent engine oil, 43.33% mortality in T_3 containing 10 ml of the spent engine oil, 66.67% mortality in T_4 containing 15 ml of the toxicant and 96.67% mortality in T_5 containing 20 ml of the toxicant. This finding show that spent engine oil has varying effect on *Clarias gariepinus* with respect to increasing concentration.

Figure 1 Variation of percentage mortality of *Clarias*



***gariepinus* (African Catfish) with concentration of spent engine oil after 96 hrs. exposure**

Meanwhile, observations made from the highest concentration; T5 (20 ml/l) showed a more adverse effect of the toxicant on the test organisms giving a rapid mortality rate, with increasing mortality at 24 hrs, 48 hrs and 72 hrs however a decline at the 96 hrs endpoint. This shows an obvious significant difference between the various concentrations of spent engine oil used.

96 hours lethal concentration (LC_{50}) determination using probit

The probit plot of mortality against concentration (Fig 2) shows a linear relationship between the toxicant concentrations and mortality values. The 48, 72 and 96 hrs LC_{50} for spent engine oil were 24.1, 15.75 and 9.89 ml/L respectively with the confidence limits (Table 3).

Table 3: Result of 48 hrs, 72 hours and 96 hours mean lethal concentrations (LC_{50}) of spent engine oil on *Clarias gariepinus*

Exposure Time (Hour)	LC_{50} (ml/l) for *Clarias gariepinus*
48	24.1
72	15.75
96	9.89

The result revealed that, as concentration increased more death occurred but as time increased the LC_{50} reduced. This however implies that mortality reduced as the toxicant concentration reduced.

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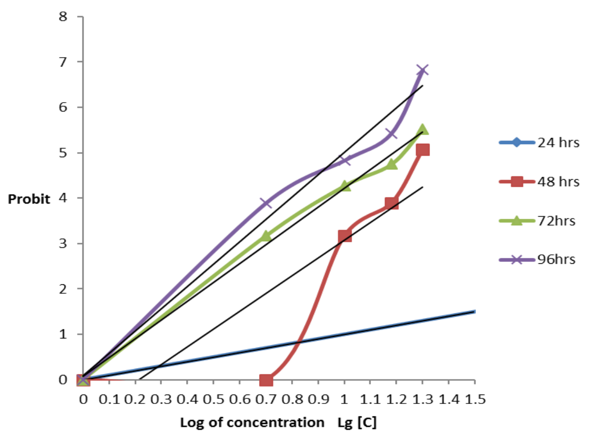

Figure 2: Probit plot of mortality against log of concentration for Juvenile of *C. gariepinus* exposed to spent engine oil.

Discussion

This present study reveals that the effect of spent automobile engine oil on African catfish (*Clarias gariepinus*) was an adverse one. the impact was seen in the overall deterioration of the quality of water in the experimental tanks aside the control tanks. From the study, pH, dissolved oxygen, temperature and conductivity in the toxicant treated media were observed at 5.65-6.24, 3.93-6.30, 25.78-26.23 and 207-361 respectively. Whereas, T_1 (the control Tank) values for pH, DO, Temperature and conductivity were recorded as 7.54 (which is mildly alkaline), 9.0, 26.63°C and 160.33 respectively. T_5 which had the highest concentration recorded the least pH (5.65) which is medium acidic, DO (3.93), temperature (25.78), the highest conductivity range (361.33) and recorded the highest mortality (100 %). This therefore agrees with the reports of [11] that spent automobile engine oil pollutes water bodies and pose grievous health and environmental hazards. The macroscopical observation of the treatment tanks revealed the spread over of oil film over the surface of the test media with increasing density with regards to increasing concentration, thereby trapping oxygen and quickening fish mortality through asphyxiation. This conforms with the postulation established by [10] that spent oil impacts on the aquatic systems begins with the formation of tiny films or sheens on the surface of the water which reduces the amount of oxygen penetration thereby depriving fish and other living organisms that comprises the aquatic food chain sufficient dissolved oxygen. Fish reaction to the chemical toxicant included; rapid movement, increased swimming pace, gradual retardation of movement and eventually mortality. This conforms to the fact that generally, reduction in oxygen penetration into the water automatically leads to suffocation and death of aquatic organisms due to asphyxiation as declared by [11] adding that the additives and contaminant contained in spent engine also makes it toxic. This kind of contamination can as well ruin the use of such water for drinking depending on the concentration. The hazards of water contaminated with waste oil include symptoms such as accumulation of toxic compounds in the liver, complete impairment of body functions and even death of aquatic organisms as noted by [12]. This also settles with the reports of [13]; [14] and [15] that exposure of aquatic organisms to crude and refined oils, water soluble and water accommodated fractions of crude oil impacts on various aspects of fish physiology and sometimes leading to large scale mortality. The morality recorded as 0 %, 13 %, 43 %, 70 % and 100 % for T_1 (0 ml control) T_2 (5ml) T_3 (10 ml), T_4 (15ml) and T_5 (20 ml) respectively shows that as with increase in toxicant concentration mortality increased. However, the peak of mortality (100 %) was recorded from T_5 containing 20 ml of spent engine oil. The Increased mortality in this test medium as well as other examined concentrations indicate severe attack on the fish gills and respiratory system due to environmental stressors in the fish environment and sequential adoptive mechanism to dissolved oxygen reduction.

Significantly, the study explored the lethal concentration (LC_{50}) values of different concentrations of spent automobile engine oil on *Clarias gariepinus* (African catfish). Our results reveal that spent engine had hostile effect on the test animal giving an outrageous lethal concentration (LC_{50}) values. Based on the findings of this study, percentage mortalities and LC_{50} values of Clarias *gariepinus* exposed to spent engine oil was determined as 24.1 ml for 48 hours, 15.75 ml for 72 hours and 9.89 ml for 96 hours. However, there were no LC_{50} data obtained for 24 hours lap because specimen mortality only occurred in one treatment tank (T_5) among the setup which is the tank with the highest concentration of spent engine oil.

Conclusion

The Indiscriminate disposal of spent automobile engine oil is a persistent problem in Nigeria. It is estimated the total national used oil generating capacity in Nigeria to be over 200 million liters per annum in 2004 and over 75 % of this figure represented contribution from used crankcase engine oil while the remaining 25 % came from industry based used oil. These spent oils and solvents form part of the most hazardous wastes commonly generated in auto-repair workshops and mechanic villages around cities in Nigeria. The spent engine oil gets to the environment due to discharge and disposal by motor and generator mechanics from the exhaust system and due to engine leaks the findings from this study reveal that spent engine oil is poisonous to African catfish at considerable concentration. The toxicant altered the water quality which led to a decreased in water pH from mildly alkaline to mildly acidic. Other changes observed are, decreased water DO and increased conductivity. Though oil is a known to be poor conductor the presence of minerals in the spent oil may have influenced this character. Hence, the study shows that as concentration increased more death occurred but as the time increased the LC_{50} reduced. However, this implies that mortality reduced as the concentration reduced.

Recommendation

The researcher recommends that the indiscriminate disposal of spent automobile engine oil should be discouraged through intensified public awareness in the society and through the deployment of a systematic periodic collection of spent automobile engine oil at their various point of removal for recycling, enhancement and re-use. The researcher also recommends for legislative body to accommodate the concerns of environmental pollution from spent automobile engine oil in its regulations.

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Plate 1

Plate 2

Plate 3

Plate 4

Plate 5

Plate 1: The researcher carrying out the range finding test
Plate 2: The researcher introducing the toxicant (spent automobile engine oil into the treatment tanks
Plate 3: Treatment 2 experimental tank (5 ml) showing fish sample in retarded swim
Plate 4: Treatment 4 experimental tank (15 ml) showing rapid fish swim and bottom settling
Plate 5: Treatment 5 experimental tank (20 ml) showing dense oil film spread over with high mortality