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HISTOPATHOLOGICAL EFFECT OF USED ENGINE OIL ON THE GILLS OF AFRICAN CATFISH (*Clariasgariepinus)* JUVENILES AND THE PHYSICO-CHEMICAL PARAMETERS OF BIOASSAY WATER.

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ABSTRACT

Engine oil, a major lubricant in automobile engines is often discharged in its spent form into the environment during engine servicing and repairs, from where it may be leached into adjoining surface water bodies during precipitation, with adverse effects on fish and other aquatic biota. The effect of sub-lethal concentrations of spent engine oil on the gills of juveniles ofClariasgariepinus was assessed in this study which was carried out in the Water Quality Laboratory of the Department of Fisheries and Aquaculture Prince Abubakar Audu University Anyigba. A range finding test was first carried out to determine the concentrations for the acute toxicity test, from which concentrations of 550, 650, 750 and 850 mg/L. 0.0 ml/l was used. Fifteen plastic 40 litres was used as the bioassay units with five treatments and three replicates and ten juveniles of Clarias gariepinus with mean weight of 18±2.0g mean length of 6-8cm stockedin each unit. The experiment was laid as Completely Randomized Design with five treatments and three replicates. The data collected was subjected to ANOVA of the log/concentration and probit analysisfor the acute toxicity bioassay and histopathology of the gill in different concentration were subjected to photo micrographic analyses. Based on the results, acute toxicity bio-assay conducted to determine the 96hr LC_{50} value was 599.95 mg/l.The impact was seen in the overall deterioration of the quality of water in the experimental tanks aside the control tanks. Gills of fishes in the control group showed little or no histological abnormalities. However there was histological abnormalities in the gills in the other units with different concentrations of spent engine oil which ranged from moderate to severe thickening and sloughing off the epithelium and necrosis, lamellar disorganization in plate2,distortion of gill filaments, epithelium rupture with haemorrhage, blanketing and blood congestionin plate3 gills epithelium rupture with haemorrhage and intense cellular necrosis in plate 4 and epithelial rupture with haemorrhage, lamellar erosion. epithelia and diffusion secondary lamellae in plate 5. The indiscriminate discharge of spent engine oil into water bodies poses a threat to aquatic organisms such as fish resulting in adverse behavioral responses, histological abnormalities in the gills and eventually death. Proper treatment and disposal of the spent oil is therefore necessary to ensure adequate protection of aquatic resources

Key word:Histopathology, Used engine oil, African Cat fish, Juveniles.

1.0 INTRODUCTION.

Aquatic toxicology constitutes a comprehensive investigation into the repercussions of manufactured chemicals, alongside other anthropogenic and natural substances and activities on aquatic organisms spanning various tiers of organization. This encompasses examinations at the subcellular level, progressing through individual organisms, and extending to communities and ecosystems (Adeyemo, *et al*., 2018).Human activities in agriculture, industry, power generation, urbanization and transportation result in the emission of several wastes products in solid, liquid or gaseous forms into the aquatic environment (Ayoola, 2018). In many developing countries such as Nigeria, the activities of informal mechanical workshops, usually results in uncontrolled discharge of spent engine oil into drainage channels and canals and this eventually end up in the aquatic bodies. The indiscriminate discharge of spent engine oil into the environment and its negative effects on the environment demands the development of various control strategies (Solomon 2019). Mechanical workshops now use dispersants, emulsifiers, degreasers or detergents in washing off spent engine oil thus ensuring easy dispersal (Smith *et al*. 2018). According to Nnawuike *et, al.*, (2024), spent engine oil which is chemically referred to as used mineral-based crankcase oil is simply described as the brown to black, oily liquid removed from the engine of a motor vehicle when the oil is changed. It consists of branched alkanes; cycloalkanes; Poly-aromatic Hydrocarbons (PAHS); linear alkanes; decomposition products; additives and contaminants of worn engine parts which include heavy metals such as chromium, iron, lead, nickel, silicon, tin, aluminum, copper, magnesium (Johnson and Williams, 2019). Spent engine is a common and toxic environmental contaminant that is not naturally found in the environment. However, disposal of spent engine oil into gutters, water drains, open vacant plots and farms is commonly practice in Nigeria, especially by motor mechanics that change oil from motor vehicles and generators (Fleeger *et al*., 2013) The improper disposal of used engine oil has become a significant environmental concern due to its potential to contaminate aquatic ecosystems. Aquatic organisms, such as fish, are particularly vulnerable to the toxic effects of used engine oil due to their direct exposure to contaminated water. Among these organisms, the African catfish, *Clariasgariepinus*, has been widely used as a model species to study the acute toxicity of various pollutants, including used engine oil. Previous studies have highlighted the toxicological impacts of used engine oil on aquatic organisms. Research conducted by Smith *et al*. (2018) demonstrated that exposure to used engine oil led to adverse effects on fish behavior, growth, and reproduction. Similarly, Johnson and Williams (2019) reported a significant decrease in survival rates and increased levels of oxidative stress markers in fish exposed to used engine oil. The lack of information on the effects of used engine oil on *Clariasgariepinus* has made it difficult to develop effective strategies for reducing the impact of petroleum pollution on this species and the environment. In Anyigba where the study is conducted, there are so many mechanic workshops who discharge the spent or used engine oil on the ground, in the drainage and water ways which eventually end up in rivers such as Ofu river and oganeajiresevoir which inhabit many fish species. Much work has been done on crude oil toxicity of many fish species for example crabs, periwinkles and crayfish but there is paucity of information on the toxicity of used engine oil on the*Clariasgariepinus*juveniles in Anyigba, Kogi State.

2.0 MATERIALS AND METHODS.

2.1 Study Location

This study was conducted at the Department of Fisheries and Aquaculture Laboratory, Faculty of Agriculture, Prince AbubakarAudu University, Anyigba, Kogi State, Nigeria, located within Latitude $7^0 48^{.}5'80''$ N, and Longitude $7^{0.}.18.7'40''$ E.Anyigba is located in the Eastern part of Kogi State,Dekina local government area. Anyigba lies between latitude 7015-7029 N and Longitude 7011-7032 N and with average altitude of 420 meters above sea level. The study area falls within the tropical wet and dry climate region and the vegetation guinea savanna, with mean annual temperature of 25^0C and rainfall 1600 mm (Ifatimehin et al., 2012).

2.2 Test Organisms

Two hundred and fifty (250) healthy *Clariasgariepinus* juveniles, with mean weight of 18±2.0g mean lenght from 6-8cm, were purchased from the Department of Fisheries and Aquaculture Prince AbubakarAudu University Fish Farm and they were acclimated for 24 hours in the laboratory to condition them before the bioassay.

2.3 Spent Engine Oil

A representative composite sample of used engine oil was collected from motor mechanic workshop or local automotive service centers along Dekina road opposite Anyigba Market, Anyigba, Dekina LGA, Kogi State. The sample was stored in airtight containers to prevent contamination and degradation prior to use in the experiment

2.4 Experimental Unit

Fifteen (15) Plastic containers having capacity of 40 liters each purchased from Anyigba marketwere used as experimental units. Water quality such as temperature, pH, dissolved oxygen, and ammonia levels were monitored using instruments thermometer

Temperature and pH Probe: To measure water temperature and pH levels.

Dissolved Oxygen Meter: To determine dissolved oxygen concentration.

Ammonia Test Kit: To assess the level of ammonia in the water.

Spectrophotometer, Gas Chromatograph, was used for analyzing oil composition and potential contaminants.

2.5 Experimental Procedures

2.5.1 Preparation of Used Engine Oil Solutions.

Various concentrations of used engine oil were prepared by diluting the stock oil with clean borehole water. Using syringe, 5ml of the spent engine oil was diluted with 95ml of borehole water which gives 100 ml of solution. From the solution, 55, 65, 75, and 85 ml were taken to represent 5.5, 6.5, 7.5, and 8.5-ml stock concentrations respectively (Nwamba *et al.,* 2001) The concentrations were then converted to ml/L. which gave 0.55, 0.65, 0.75, and 0.85 ml/L due to the negative outcome of the log of concentration of the value in ml/L which affected the graph to the negative side, the concentrations were further converted to mg/L which finally gave 550, 650, 750 and 850 mg/L. 0.0 ml/l was used as control (Smith, *et al.*, 2018). The experiment was laid as Completely Randomized Design (CRD) with five treatments and three replicates.

2.5.2 Acute Toxicity Tests

Each experimental unit was filled with borehole water and appropriate serial concentration of spent engine dropped in them and stirred vigorously with glass rod for ten minutes. The controlled units did not received the spent engine oil.

Clariasgariepinus juveniles (ten each) were randomly stocked into the experimental units, with the initial body weight taken prior to stocking.

The fish were exposed to the various concentration of spent oil solutions for 96 hours in bioassay water in which mortality and LC50 of the fish monitored and water quality parameters measured (Ayoola and Alajabo 2012).

2.5.3 Histopathological analysis

At the end of the exposure period (96hrs), a fish from each experimental unit was randomly taken, sacrificed under chloroform anesthesia by placing the fish in a closed glass chamber containing soaked chloroform in a cotton wool. They were later dissected and the gills needed for histopathology were removed and fixed in Bouin's fluid.

The fixed target organ (gills) in Bouin's fluid were removed, dehydrated and treated with acetone. The organ was then cleared in xylene to enhance tissue transparency, followed by impregnating and embedding in paraffin wax. The tissue was then sectioned at and cleared (dewaxed) for staining with hematoxylin and eosin (Madu*et. al*., 2013).

2.6 Data Analysis

Data collected on the observable fish behavior were recorded at different intervals water quality of different concentration was subjected to ANOVA and the gill in different concentration were subjected to photo micrographic analyses.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 The physico-chemical parameters of water exposed to spent engine oil

The mean values of physico-chemical parameters of bioassay water is shown in table 1. The pH values obtained from the test chambers: T_1, T_2, T_3, T_4 and T_5 were 5.59, 5.63, 5.72, 5.75 and 5.80 respectively. Aside the control (T_1) which had a mildly alkaline pH, the test media (T_2) with the lowest concentration (550 ml) had the highest pH value which was slightly acidic while the test media (T_5) bearing the highest concentration (850 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 5.18 mg/l – 5.42 mg/l throughout the test period showing significant variances between the test chambers and the control with the mean of 5.3mg/l. This shows a severe reduction in DO due to the spent engine.

The variation in temperature was obvious between treatments with test chamber T_5 (850 ml) having the highest temperature similar to the control, while the test chamber T_3 (750 ml) having the lowest temperature with a mean of 27.25^0C, this was attributed to the kinetic energy of the surviving active fish in T_1 and T_2 however dissimilar to T_3 where few fish were active due to mortality. Correspondingly, variations were observed in conductivity, the highest mean value for conductivity was 143.80 from test media T_5 (850 ml) and the lowest was 110.13 obtained in test media T_2 (550 ml) compared to the control which gave a value of 97.12.

Table 1: Water quality parameters of experimental units during acute toxicity test of *Clariasgariepinus* exposed to spent engine oil.

S/N	Treatment Mg/L	pH	TEMP (^{0}C)	TDS (ppm)	EC (µS/cm)	DO (mg/L)
1	T1 (control)	5.59±0.01^a	27.47±0.03^{ab}	46.60±0.05^a	97.12±0.10^a	5.37±0.03^e
2	T2 (550)	5.63±0.03^a	27.40±0.06^a	54.82±0.17^b	110.13±0.35^b	5.40±0.01^d
3	T3 (650)	5.72±0.02^b	27.25±0.03^{ab}	59.22±0.05^c	123.05±0.10^c	5.42±0.01^c
4	T4 (750)	5.75±0.03^b	27.42±0.03^b	65.25±0.15^d	129.10±0.31d	5.41±0.00^c
5	T5 (850)	5.80±0.02^c	27.37±0.03^{ab}	71.34±0.06^f	143.80±0.12^f	5.18±0.00^a
	P-Value	0.100	0.001	0.002	0.001	0.002

Mean in the same column with different superscripts differ significantly ($p<0.05$).

Keys
PH= Potential hydrogen
TEMP= Temperature
TDS= Total dissolved solids
EC= Electrical conductivity
DO= Dissolved oxygen

3.1.2 Histopathological analysis of the gills of *Clariasgariepinus*exposed to used engine oil.

Gills of *Clariasgariepinus* in the control tank, plate 1, shows normal gill architecture with gill filaments (black arrow), the lamellae(L), a pillar cell(white arrow) , primary lamellae(PL) and secondary lamella(SL). In plate 2, the gills exposed to 550mg/l of spent engine oil shows lamellar disorganization(black arrow) hyperplasia of lamellar epithelium cell(white arrow), deformed secondary lamellae(D), erosion of epithelium lining of the secondary lamellae(E). In plate 3, the gills exposed to 650mg/l of spent oil shows distortion of gill filaments as some have blanketed and eroded(black arrow) , epithelium rupture with haemorrhage, blanketing and blood congestion(white arrow) and haemorrhages(H). In plate 4, the gills exposed to 750mg/l of spent oil shows distortion of gill filaments as some have blanketed and eroded (black arrow) and epithelium rupture with haemorrhage(F) , intense cellular necrosis(N) and blanketing and blood congestion(white arrow). In plate 5, the gills exposed to 850mg/l of spent oil shows epithelial rupture with haemorrhage(black arrow) lamellar erosion(white arrow) and epithelial and diffusion secondary lamellae(D).

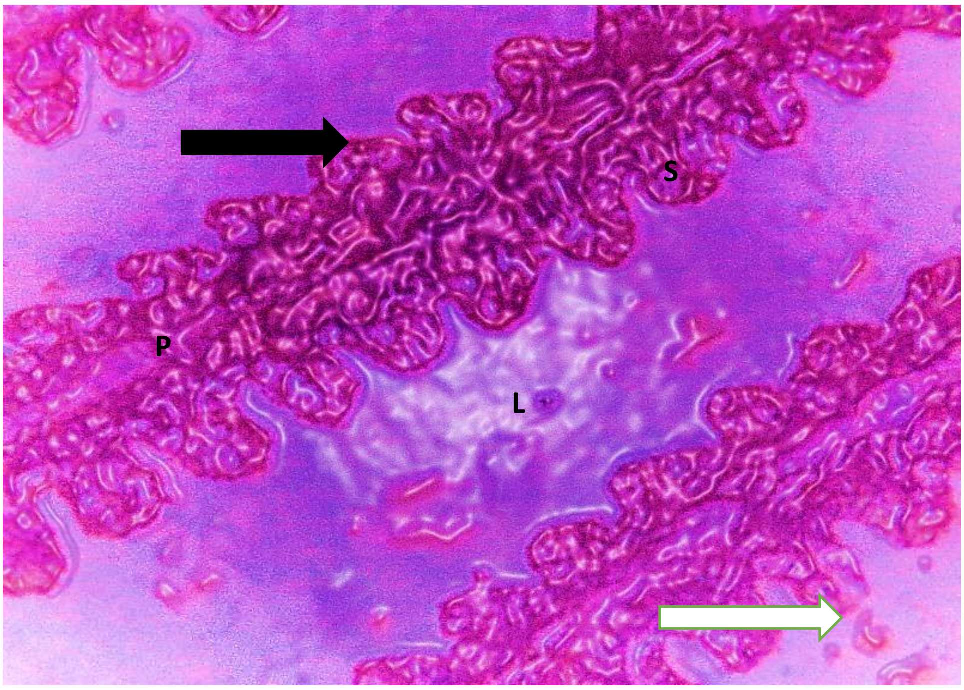

Plate 1: Photomicrograph of the gills of *Clariasgariepinus*in the control tank showing normal gill architecture with gill filaments (x400).

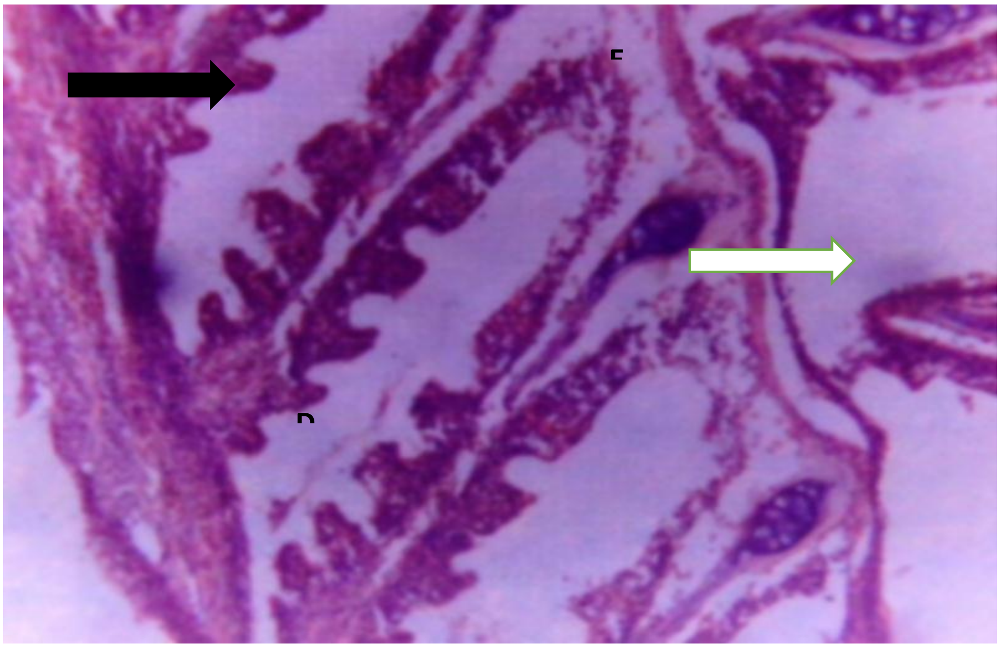

Plate 2: Photomicrograph of the gills of *Clariasgariepinus*exposed to 550 mg/l of spent engine oil showing lamellar disorganization (x 400).

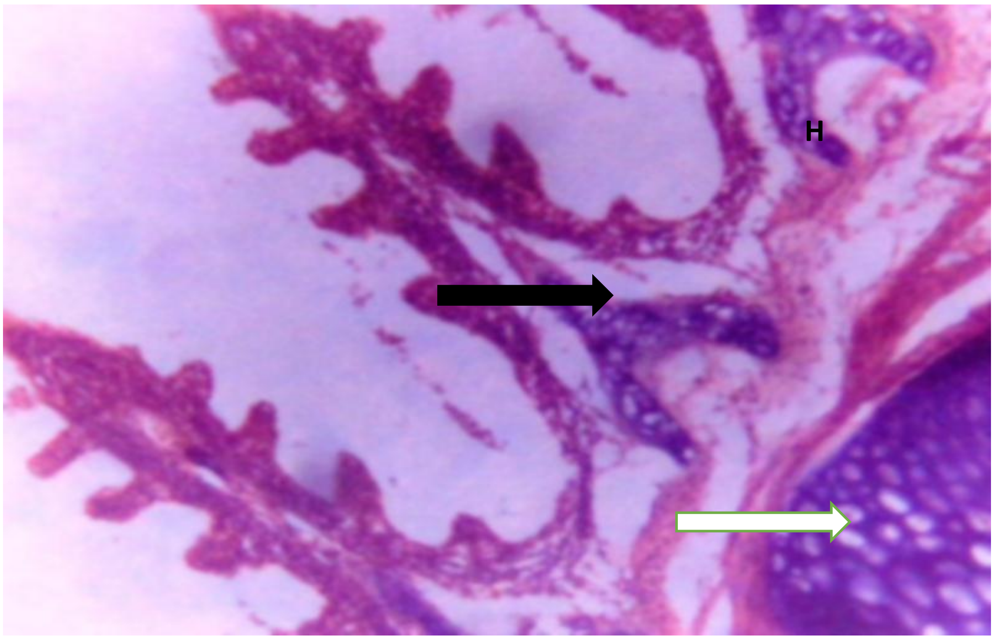

Plate 3: Photomicrograph of the gills of *Clariasgariepinus*juveniles exposed to 650 mg/l of spent engine oil showing distortion of gill filaments (x 400).

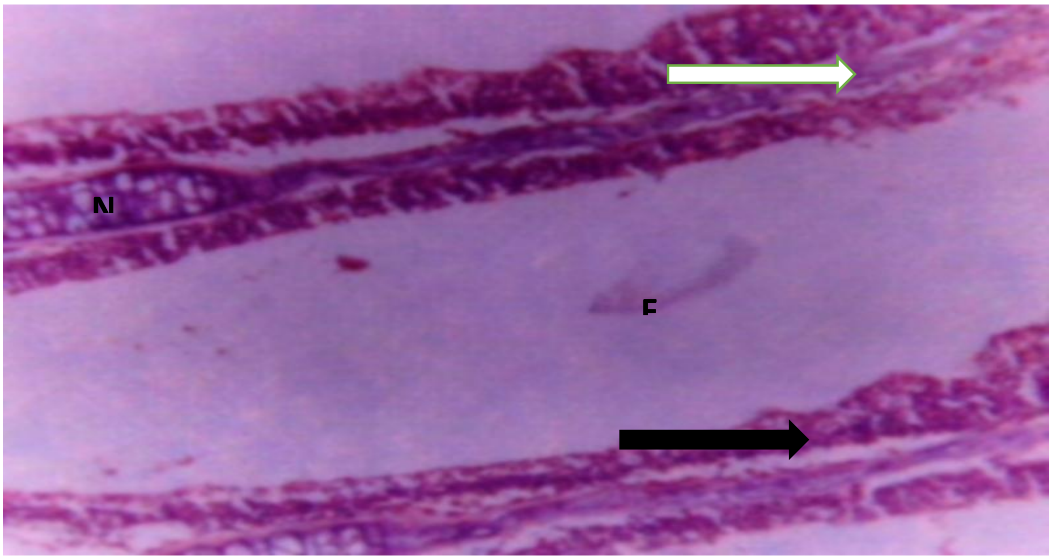

Plate 4: Photomicrograph of the gills of *Clariasgariepinus*exposed to 750 mg/l of spent engine oil showing distortion of gill filaments (x400).

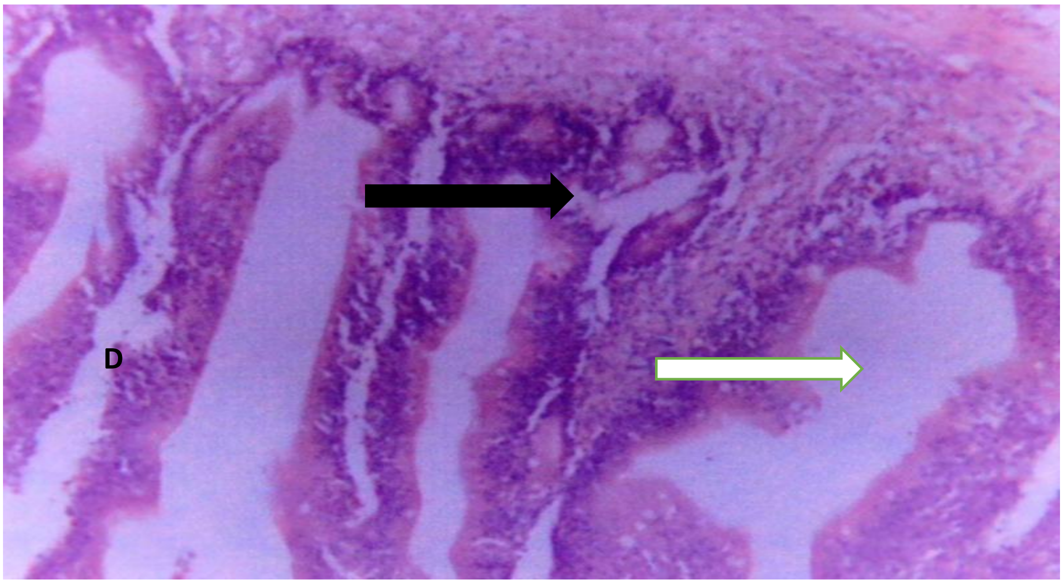

Plate 5: Photomicrograph of the gills of *Clariasgariepinus*exposed to 850 mg/l of spent engine oil showing epithelial rapture with haemorrhage(x 400).

3.2 Discussion:

The results of the study showed the adverse effect of spent automobile engine oil on African catfish (*Clariasgariepinus*).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 5.59-5.80, 5.18-5.42, 27.25-27.47, 97.12-143.80 respectively.

Whereas, T1 (the control tank) values for PH, DO, Temperature and conductivity were recorded as 5.59 (which is mildly acidic), 5.37, 27.47^{0}c and 97.12 respectively. T5 which is the highest concentration recorded the least PH(5.80) which is medium acidic, DO (5.18), temperature (27.37), the highest conductivity range (143.80) and recorded the highest mortality (93%). This therefore agrees with the reports that spent automobile engine oil pollutes water bodies and pose grievous health and environmental hazards according to Martinez and Silva, (2017). The macroscopic observation of the treatment tanks revealed the spread 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 agreed with the report ofSmith *et al.,*(2018) 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 (Smith *et al.*, 2018). 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 also the additives and contaminant contained in spent engine also makes it toxic. At the lowest dose of exposure, the gills showed mild lamellae disorganization with intermittent fusion at the secondary lamellar indicating inflammatory changes and there was also an indication of increased mucus secretion which agreed with the reported ofJohnson and Williams (2019) when *Clariasgariepinus*was exposed to crude oil. The severity of gill epithelia distortions and gill filament ruptures with hemorrhage showedpoison effect of the used engine oil on the *Clariasgariepinus* juveniles. This caused the drastic reduction in the uptake of dissolved oxygenand consequently caused the mortality. This observation is similar to the report of Okeke. *et.al*., (2020) and Adebisi*et al*(2018)*Clariasgariepinus* was exposed to chloropyfos pesticide and crude oil respectively.

The mortality recorded as 0 %, 40 %, 60 %, 80 % and 93 % for T1 (0 ml control) T2 (550ml) T3 (650 ml), T4 (750ml) and T5 (850 ml) respectively shows that as with increase in toxicant concentration, mortality increased. However, the peak of mortality (93 %) was recorded from T5 containing 850 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 caused by poison of spent engine oil.

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion:

The Indiscriminate disposal of spent automobile engine oil is a persistent problem in Nigeria. 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 and when they get to the environment they find their way into water bodies. The findings from this study showed that spent engine oil is poisonous to African catfish at considerable concentration. The toxicant altered the water quality which led to a decrease in water pH and DO and increased conductivity. The study also showed that as concentration increased mortality increased

4.2 Recommendation:

We recommend 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 that legislative body should be conscious of environmental pollution from spent automobile engine oil and legislate against the indiscriminate disposal of spent engine oil.

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