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Measurement of Sub-Lethal Toxicity and Effect of Kerosene Pollutant on Hematological Profile of African Catfish (*Clarias gariepinus)*

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Authors' contributions

This work was carried out in collaboration among all authors. Author EAI initiated the idea, coin the topic and drafted the manuscript. FOSJ, NEE, COA, GMU all carried out the analysis of the data generated from the study. COA and FOSJ search the literature. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: The study measured the impact of crude oil fractions and its derivatives like kerosene on the early life and sub-adults growth of African catfish, *Clarias gariepinus* in terms of mild, acute and chronic toxicity effect. It also examines the effect of indiscriminate spillage of crude oil derivatives on aquatic organisms.

Materials and Methods: Blood profiles such as Red Blood Cell count (RBC), White Blood Cell count (WBC), Blood Differentials, Hemoglobin, (HB), and Packed Cell Volume (PCV) of the treated fishes and control were estimated after exposure of the fish to the kerosene pollutant. Behavioral changes in fish exposed to8.0ml/l, 16.0ml/l, 25.0ml/l and 50.0ml/l of kerosene pollutant varied from erratic swimming, moribund movement, jumping and lack of balance.

___ **Results:** At concentration 8.0ml/L, mean total mortality was observed within 72hours of exposure. The lethal concentration (LC50) was 8.0ml/L and highest mean mortality observed with 80%

kerosene pollutant. PCV of the exposed fish was 26% when compared to the control 27%, HB was 8.9 gdl as against 9.0 gdl in the control. Total WBC was 640 x 109/L as against 1280 x 109/L in control. RBC reading was 2.1 compared to 5.3 mm³ of the control. Lymphocyte was 80%, Neutrophil was 4% and monocyte 16% compared to 62%, 8% and 30% in control respectively. There was decrease in white blood cells counts for 8.0, 16.0, 25.0 and 50.0ml/L groups (p<0.05) compared to the control.

Conclusion: The study concludes that it is necessary to ensure the safety of aquatic life forms especially fishes, by minimizing aquatic pollution with kerosene to sustain fish food quality, availability and security.

Keywords: Blood quality, kerosene fractions, fish behavior, lethal concentrations, Clariasgarienpinus.

ABBREVIATIONS

- *HB : Hemoglobin*
- *WBC : White blood cells*
- *RBC : Red blood cells*
- *PCV : Packcell volume*
- *LC : Lethal concentration*
- *EDTA : Ethylene di-amine tetraamino acid*
- *LABS :Linear Alkyl Benzene Sulphonate (LABS),*
- *STPP : Sodium tripolyphosphate (STPP)*

1. INTRODUCTION

The incessant crude oil and its derivative spillage and pollution in Nigeria and the resultant spoilage of valuable fish foods inform the need for this study. The Pollution occurs either on land, air or water. Man depends heavily on water for domestic, industrial and agricultural uses. Poorer water quality means water pollution [1]. No nation is completely free from the global menace of water pollution [2]. This era of globalization and industrialization has sky rocketed the rates of aquatic pollution due to the increasing volume of industrial and domestic effluents that find their way into fresh water and marine habitats, thus altering the balance of these ecosystems [3]. Most chemicals exhibit deleterious effects on the aquatic environment if not properly handled or controlled. Industrial products beneficial to man can also pose serious threats to man and the entire environment [4]. Most of the commonly used industrial products in Calabar, Nigeria include kerosene (energy source for the commons), detergent, fertilizers and a host of others [5]. Pollution is a global menace that affects all ecological habitats. It is the introduction of foreign toxic substances capable of causing harm to man and the entire environment [6]. The heightened activities of exploration and exploitation of crude oil in the Niger Delta region of Nigeria has elicited the spillage of crude oil and its derivatives like kerosene into surrounding water bodies thus

rendering the water and other life forms therein unfit for utilization of any sort or kind [7].

kerosene was the major refinery product for several decades until the advent of the electric lamp reduced its value for lighting [8]. Production further declined as the rise of the automobile established gasoline as an important petroleum product. Nevertheless, in many parts of the world, kerosene is still a common heating and cooking fuel as well as a fuel for lamps especially for the low and middle income earners who dominate the study area. Standard commercial jet fuel is essentially high-quality straight-run kerosene, and many military jet fuels are blends based on kerosene [9]. Despite reported cases of rapid uptake of crude oil derivatives like kerosene from water by fish and the concomitant bioaccumulation that do occur [10], little is been known about range findings behavior, sub-lethal toxicity, hematological impact, metabolism and acute toxicity level in African catfish, *Clarias gariepinus.*in Calabar, Cross River State, Nigeria. This is as a result of high level of aquatic pollution from crude oil spillage in the area.

The African catfish (*Clariasgariepinus***)** African catfish (*C. gariepinus*) are sharp tooth catfish, eel-like in nature, usuallydark gray or black with coloration on the back, fading to a white belly. In Africa, thecatfish has been reported as being second in size only to the Vundu of the Zambesianwaters [11]. Because of its wide mouth, it is able to swallow relatively large prey whole. It is able to crawl on dry groundto escape drying pools and it is also able to survive in shallow mud for long periods of time in between raining seasons [12]. It spawns around inundated areas of river, lakes and streams mostly at nights [13]. One of the reasons for the choice of this genus of fish for this research work is because it has been found tobe a biomarker in the aquatic environment [14]. Other reasons for this choice of this fish includes its' hardiness and ability to tolerate adverse water quality conditions, its

ability to grows fast and feed on large variety of agriculture by products and its ability to tolerates difficult conditions in captivity [15].

The high demand for this giant African catfish, owing to its rapid growth and flavor, necessitated an investigation into the effect of this kerosene which is the common household energy sources, on juveniles and sub-adults life forms of the fishes stocked on water polluted with kerosene and other crude oil derivatives. [16] posited that about five million red blood cells (RBC) are contained in one cubic milliliter $(1m^3)$ of blood sample of vertebrates [17]. reported that the exposure of fish to crude oil fractions such as kerosene compounds resulted in the destruction of the RBC and WBC components of the blood as well as the alteration of the immune response and liver metabolism among other damages especially to the juveniles and sub-adults.

Hence, a detail haematological analysis is therefore required to determine the effect of crude oil fractions like kerosene infiltration into the juvenile and sub-adults catfish blood systems and the subsequent toxicity it impacts on the fishes. It has been reported that the uptake of crude oil fractions by fishes in water bodies and the subsequent translocation of the fractionated compounds in fish is through the gills, gut or the intestinal walls [18], where the compounds solubilizes the cell membranes and are carried via the erythrocytes to the general circulation of the blood.

Thus the response of sub-adults of African catfish (*Clariasgariepinus*) to kerosene related water pollution is determine by changes through expressions of several key enzymes and the biotransformation systems which include mild, acute and chronic toxicity of the pollutants to the marine life forms and the attendant bioaccumulation and biomagnifications [19] in the food chain. Hence, the study sought to measure the impact of indiscriminate spillage of crude oil fractions and its derivatives like kerosene on the blood parameters in early life and sub-adults growth of African catfish, *Clarias gariepinus* in aquatic ecosystem in terms of mild, acute and chronic toxicity.

2. MATERIALS AND METHODS

2.1 Study Location

The University of Calabar is located in between the Calabar municipality and Calabar south local government areas of Cross River State, Nigeria. The study was carried out in the laboratory of the Department of Zoology and Environmental biology, University of Calabar, from March, 2016 to June, 2019. It is bounded to the east by the great quo river,Calabar is the capital of Cross River State, Nigeria. It is located geographically at 40 57" North 80 191 0' East. Cross River State is one of the States in the Niger Delta, South South Region of Nigeria. The State shares a maritime boundary with Cameroon in the east, AkwaIbom State in the South, Abia and Ebonyi States in the West and Benue State in the North. It is geographically located at 50451N, 80301E /50751N, 8.50E, [20]. The State according to National Population Commission (NPC) [21] has a population of 3.2 million people with a land mass of 20, 156 km2 (7,782sqm).

Activities in the rural areas are mainly agriculture and petty-trading, the urban areas are characterized by heavy commercial activities, industrialization and tertiary education. Fish farming is also one of the major human activities in both rural and urban areas of the study location [21].

2.2 The Fish Farm

The University of Calabar Fish Farm is located about 1 kilometer away from the research laboratory (Fig.1). The fish farm harbours various sizes and species of fishes ranging from fry, fingerlings sub adults and adults. This research was carried out in the Fish Pathology Laboratory of the Faculty of Oceanography, University of Calabar. Collection of samples and gathering of materials was preceded by a thorough reconnaissance survey.

2.3 Sources of the Pollutants

The kerosene was purchased from Ekoson filling Station, Calabar in Cross River State, Nigeria.

2.4 Field Investigation

A trip was taken to the university of Calabar fish farm to ascertain the availability of African catfish (*Clariasgariepinus*) sub adults. After confirming the availability, the desired quantity was booked for. Similarly, the chosen toxicants were sort for in the various shops in Calabar.

Fig. 1. Map of the University of Calabar showing the fish farm *(Source: https://www.unical.edu.ng./handbook)*

2.4.1 Collection and transportation of the study fish

A total of 600 *C. gariepinus*sub adults with a mean weight of 8.5 ± 0.2 g were purchased from the University of Calabar fish farm. Samples were carefully collected and transferred into a

plastic container and transported to the Faculty of Oceanography, University of Calabar, Cross River State, Nigeria. The study fishes were transported to the laboratory in transparent plastic containers by car [22] to the laboratory which is about five min. drive from the fish farm.

2.4.2 Laboratory studies

A whole day was used in arranging the laboratory. Unwanted and obstructive materials were removed and the needed aquaria thoroughly washed and dried. The laboratory was thoroughly disinfected.

2.5 Acclimatization of Study Specimens

The sub adult fish were transferred into a laboratory aquarium (80 x 30 x 30 cm3) and allowed to acclimatize in this holding tank in the laboratory condition for one weeks at a temperature 30.02 ± 0.09 OC and a pH of 8, the sub adults were fed once daily with commercial feed (Copen's) at 5% of their body weight. The unconsumed feeds and faeces were removed from the holding tank and the water in the tank was changed every 24 hours as recommended by [23].

2.6 Pollutants Used for Experiments

The pollutants selected for the experiment is the crude oil derived kerosene which chemical formula is shown in Fig. 2 below.

Fig. 2. Chemical structure of kerosene

2.7 Stocking of the Test fish

Ten (10) sub adults were carefully were introduced to each of the plastic aquaria (Fig. 3A) containing the measured toxicant and another ten (10) introduced to the control aquaria (Fig. 3B) using a hand sieve [24]. This procedure was repeated for all the experiments.

2.8 Range Finding Tests

Series of range finding tests were conducted using the different concentrations of the toxicant on the sub-adult fish to determine the

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concentration boundaries before the actual experiment.

2.9 Toxicity Experiments with Kerosene

The experiment was carried out in triplicates of four treatments i.e. 0.00 ml/L (control), 8.0 ml/L, 16.0 ml/L and 25.0 ml/L and 50.0 ml/L (Fig. 3A). Ten (10) sub adults of *C. gariepinus* were stocked in each of the four glass aquaria (25 x 15.5 x 15.5 cm3) in triplicates and used for the experiment. The experiment was monitored periodically; observation and responses were taken at intervals of 24, 48, 72 and 96hour respectively.

2.10 Haematological Studies (Blood Profile)

Collection of blood samples from the treated and control fishes were obtained by inserting a needle directly from the anterior part of the anal fin about 2-5mm behind the genital papilla to draw the desired amount of blood. Blood profiles such as Red Blood Cell count (RBC), White Blood Cell count (WBC), Blood Differentials, Hemoglobin, (HB), and Packed Cell Volume (PCV) of the treated fishes and control were estimated after exposure of the fish to the kerosene toxicity . The Haematological indices were carried out at the Hematology Department of the University of Calabar Teaching Hospital, Calabar, Cross River, Nigeria. Blood samples were collected from the fish into anticoagulant (EDTA) bottles and rocked gently to ensure proper mixing while avoiding hemolysis.

2.11 Statistical Analysis

Data generated from the study were analyzed using analysis of variances (ANOVA) for significant difference (p<0.05) at 95% confidence limit. Significant treatment means were separated using the Fischers' least significant difference (LSD) test.

3. RESULTS

3.1 Fig. 3 shows the results of a lethal concentraton on a probit curve of concentrations 8.0, 16.0, 25.0 and 50.0 ml/l generated against mean mortalities. It showed the mean lethal concentration (LC50) at which the kerosene toxicant killed 50% of the test fish, *C.gariepinus*at 8m/l as shown in Fig. 3. This was accessed from all the replicates.

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Fig. 3A. African Catfish (*Clariasgarienpinus*) in control water

Fig. 3B. African catfish (*Clariasgarienpinus*) in Kerosene submerge water

Fig. 4. Graph of concentration against percent mortality showing LC₅₀=8ml/L of kerosene

SD = standard deviation

Result from range findings experiment with kerosene toxicant revealed normal behaviour of fishes in the control aquaria. At the concentration 8.0ml/l, the fish exhibited erratic movement with 50% mean mortality recorded. The fish were weak with increased opercula movement at 16.0ml/l concentration recording 60% mean mortality. At 25.0 ml/l, the fish exhibited moribund behaviour with 90% means mortality. Total mortality was recorded within 96 hours of exposure at concentration of 50.0 ml/l. These mortalities were recorded at intervals of 24, 48, 72 and 96 hours as shown in Table 1.

These mortalities were recorded at intervals of 24, 48, 72 and 96 hours against the concentrations used and presented in bar chart as shown in Fig. 4. The multiple bar chart revealed an increasing mean mortality with increasing concentrations of kerosene. The higher the concentrations of kerosene used or applied, the higher the mean mortality recorded from *C. gariepinus*.

Results of the impact of kerosene on the blood parameters of *C. gariepinus* are presented in Figs. 5-10. Fig. 5 revealed that there a significant decrease in pack cell volume counts for 8.0,

16.0, 25.0 and 50 ml/L groups (p<0.05) compared to the control. Fig. 6 shows that there was an insignificant decrease inhaemoglobin counts for 80ml/L(p>0.050} and significant decrease for 16, 25.0 and 50ml/L groups (p<0.05) compared to the control Fig. 7 shows that there was a significant decrease in white blood cells counts for 8.0, 16.0, 25.0 and 50.0ml/L groups (p<0.05) compared to the control. Fig. 8 revealed an insignificant increase in lymphocyte counts for 8.0ml/L group (p>0.05) and significant increase for 16.0, 25.0, and 50.0ml/L group (p<0.05) compared to the control. What this implies that, as the concentration increases, lymphocytes counts increases correspondingly. Fig. 9 also revealed there was insignificant decrease in neutrophil counts for 8.0 and 16.0ml/L groups (p>0.05) and significant decrease for 25.0 and 50.0ml/L groups (p<0.05) compared to the control. This means as the kerosene concentration increased, the neutrophil count decreased, while Fig. 10 shows a significant decrease in monocyte counts for 8.0, 16.0, 25.0 and 50.0ml/L groups (p<0.05) compared to the control. This implies that even at the lowest concentration, kerosene toxicant had impact on the monocytes of the exposed fish as shown in Fig. 10.

Fig. 5. Significant decrease of pack cell volume counts for 8.0, 16.0, 25.0 and 50.0ml/L group (p<0.05) compared to the control

Concentration of kerosene

Fig. 6. Insignificant decrease of haemoglobin counts for 80ml/L(p>0.050 and significant decrease for 16, 25.0 and 50ml/L groups (p<0.05) compared to the control

Fig. 7. Significant decrease of white blood cells counts for 8.0, 16.0, 25.0 and 50.0ml/L groups (p<0.05) compared to the control

Fig. 8. Insignificant increase of lymphocyte counts for 8.0ml/L group (p>0.05) and significant increase for 16.0, 25.0, and 50.0ml/L group (p<0.05) compared to the control

Fig. 9. Insignificant decrease of neutrophil counts for 8.0 and 16.0ml/L groups (p>0.05) and significant decrease for 25.0 and 50.0ml/L groups (p<0.05) compared to the control

Concentration (ml/L) of kerosene

Fig. 10. Significant decrease of monocyte counts for 8.0, 16.0, 25.0 and 50.0ml/L groups (p<0.05) compared to the control

The results of impact of kerosene on the blood cells hemolysis of *C. gariepinus* are presented in Fig. 11-15. Fig. 11 is lymphocytes and monocytes in control stained with haematoxylin. Figs. 12,13,14 and 15 had various degrees of alteration and haemolysis when compared to control as indicated with arrows and shown in Fig. 11-15. Fig. 11 presents the lymphocytes and monocyte counts in the control experiment with kerosene treatment. Lymphocyte counts was 62% while monocyte count was 30%. The value of monocytes decreased from 30 to 17 as the concentration of kerosene in the blood increased from 0.00 to 50m/l as depicted in Fig. 12. The rate hemoglobin in the red blood cells decreased from 5.3 in the control plate to 2.1 when the concentration of kerosene in the blood increased to 50m/l as shown in Fig. 13. Fig. 14 shows the effect of increased concentration of kerosene in neutrophils count of test fishes. Neutrophils counts decreased from 8.00 to 6.00% with increase in concentration of kerosene from 0.00 to 50m/l while Fig. 15 revealed the increase in lymphocyte cells from 62 to 77% with increase in concentration of kerosene in the blood from 0.00 to 50m/l.

Fig. 11. Lymphocytes and Monocytes in Control

The results of effect of kerosene spills of *C. gariepinus*haematological parameters are presented in Table 2. Analyzed samples of blood of *C. gariepinus*exposed to kerosene were highly haemolyzed. The RBC value dropped from 5.3 (control) to 2.1 mm3 due to haemolysis. PCV dropped from 27% to 16%. WBC value dropped from 1280 x109/l to 602 x 109/l, haemoglobin value dropped from 9.0 Gdl (control) to 5.3 gdl. In the differential count, Lymphocyte value increased from 62% to 77%, Neutrophil control

value dropped from 8 to 6% while monocyte dropped from 30 percent to 17%. All these alteration in values may not be unconnected with the effects of this toxicant (kerosene) on the blood profile of the test fish as shown in Table 2.

4. DISCUSSION

According to [25], Kerosene is a well-known fraction of hydrocarbon with a history of toxicity on land and aquatic life. Behavioural changes of fish exposed to different concentration of kerosene were erratic movement, increase opercula movement, weakness, moribund behavior and death. These observations were not seen in the control. Mortality rate was directly proportional to increase in concentration. Total mortality was observed at 50m/L concentration within 96hrs exposure as shown on the LC50 probit curve in Fig. 13. Increase in opercula movement may not be unconnected with suffocation and gasping for breath as a result of toxicant impact on the fish [26].

Fig. 12. Monocytes in 50ml/L of kerosene (x10)

 Fig. 13. Haemolyzed RBC (Arrow) in 50ml/L of kerosene (x10)

Fig. 14. Neotrophils (arrow) in 50ml/L of kerosene (x10)

 Fig. 15. Lymphocytes count (arrow) in 50ml/L of kerosene (x10)

L = Lymphocyte cell counts; N = Nutrophils cell counts; M = Monocytes cell counts; PCV = Pack cell volume; HB=Hemoglobin; WBC=White Blood cells; RBC = Red blood cells

Statistical analysis and graphical presentation showed the concentration at which 50% of the experimental fish are killed by the toxicant (kerosene) at 8m/L. Graph and Bar chart of concentration against cumulative fish mortality exposed to kerosene at timely intervals (24h, 48h, 72h and 96h) showed that mortality increased with increase in concentration as shown in Fig. 14. There was total mortality at concentration 50m/L of kerosene (which is the highest concentration used). Haematological analysis indicated severe haemolysis of the blood samples. Another stunning discovery was that the experimental fishes became severely anemic during blood samples collection. Haemolysis and Anaemia in fishes can be attributed to environmental factors, toxicants and pesticides according to [27,28,29]. Fluctuations in the RBC, WBC and differential counts readings as compared to the control could be caused by haemolysis and anaemia as a result of environmental factors, toxicants and
pesticides [30]. The more pronounced pesticides [30]. The more pronounced observations during the experiment were severe laceration of the fish skin within 48h of exposure.

Comparison of PCV, HB, WBC and RBC with the control showed decreased values and readings.This could have been caused by anaemia and haemolysis which agrees with [31,32,33]. A comparison of differential count (%) between control and kerosene toxicant revealed elevated level of lymphocytes (lymphocytosis) and a decrease in monocyte count. This finding agrees with [34,35,36,37]. A comparison of total RBC (mm3) of the exposed fish with the control showed a drastic decrease in the RBC count.

Some studies have identified only a few individual aromatic compoundsin the juvenile fish livers [38] or bile [39] of fish that have been exposed to crude oil and a distillate fraction of petroleum that contains only a portion of the aromatic compounds found in the crude oil and absorbed in the fish causing different degrees of toxicity ranging from mild, acute to chronic toxicity of the petroleum fractions in the fish. The results of the present study is in line with this reports.

Toxicity of kerosene on the haematological parameters of the African catfish (*C. gariepinus*) sub adult was investigated. The experiment was conducted in triplicates of four treatments. Behavioral changes in fish exposed to different concentration of kerosene ranged from erratic swimming, moribund movement, jumping and lack of balance. Similar changes were not observed in the control throughout the experiment. This observation is similar to that of [40,41,42]. kerosene is composed of linear Alkyl Benzene Sulphonate (LABS), sodium tripolyphosphate (STPP), sodium carbonate, sodium sulphate, sodium perborate and sodium silicate (perfume) as active ingredients. The ability of these chemicals to cause behavioral changes in *C. gariepinus* has been reported by [43,44] and [45,46]. In this present study, the fish were exposed to concentrations 8.0ml/L, 16.0ml/L, 25.0ml/L and 50.0ml/L. At 16ml/L concentration, the mean mortality was 80% with an initial erratic swimming [47,48] It was further observed that mean mortality increased drastically with increase in concentration of the crude oil pollutant [49,50]. At concentration 25.0ml/L, total mortality was observed within 72hours of exposure preceding moribund swimming while total mortality was observed in 50.0ml/L. The concentration at which 50% of the experimental fish were killed (LC50) was 8.ml/L. Results from statistical analysis indicated that mortality varied significantly with concentrations as higher values recorded higher mortalities. However, mean values showed highest mortality (74%) with Kerosene [50].

A bar chart of concentration against cumulative fish mortality exposed to kerosene at timely intervals of 24h, 48h, 72h and 96hr revealed that mortality increased as concentration increased. Total mortality of exposed fish was recorded mainly at 48hr and 96hr exposure. This finding corroborates with that of [50] and [49,50].

Haematological parameter of the blood cells of the fish exposed to kerosene revealed severe haemolysis of the blood cells when viewed with a motic electron microscope at x10 magnification. Images from the control showed normal distribution of blood cells. Images from the histopathology of the blood cells revealed severe damages to the lymphocytes, monocutes, neutrophils and RBC cell counts respectively at 50ml/l concentration of kerosene whereas the control showed a normal distribution of lymphocytes and monocytes cell counts [49,50]. In all, a two way Analysis of variance on effect of kerosene toxicant with different concentrations on haematological parameters of the catfishes at the end of 96hr bioassay showed significantly different at P<0.05 among the various concentrations studied.

5. CONCLUSION

The degree of exposure of marine organisms to crude oil and its derivatives is becoming worrisome and a threat to food security and sustainability in most coastal regions and especially in the Niger Delta region of Nigeria where crude oil spillage has left the water bodies in the region unusable. This menace is usually and often accessed by measuring the body burden of crude oil and its derivatives like kerosene compounds, which are potentially harmful to aquatic life forms that utilize them in their livers and predominantly excrete them into bile. The pollution of water by petroleum effluents like kerosene and xenobiotics may play a major role in the decline of aquatic animals and fishes. Increasing awareness on the adverse effects of anthropogenic activities and crude oil fraction pollutants on aquatic environment should focused interest on health of fish populations and the possibilities to utilize these health parameters for assessing the quality of coastal aquatic environment. With the current threat to food availability and security post by the global COVID 19 pandemic, it thereforebecomes pertinent for all hands to be on deck to ensure the safety of aquatic life forms especially fishes, by minimizing aquatic pollution with crude oil derivatives like kerosene, to sustain fish food quality, availability and security.

6. SIGNIFICANCE STATEMENT

This study discovered and unveiled the sublethal toxicity and range testing of kerosene pollutant on aquatic life forms including fishes and other sea foods. The study revealed the toxic effects of water pollution by kerosene and xenobiotics and shows the major role it plays in the decline of aquatic animals and fishes interms of the damage to their hematological indices. Thus the study will increase awareness on the adverse effects of kerosene pollutants on aquatic environment and advocate for a focused interest on health of fish populations and the possibilities to utilize these health parameters for assessing the quality of coastal aquatic environment. This study will help researcher to uncover the critical impact of kerosene pollutant that many researchers were not able to explore.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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