



(RESEARCH ARTICLE)



Toxicity of bitumen on *Tilapia guineensis* from Nigerian Institute for Oceanography and Marine Research (NIOMR), Buguma, Rivers State

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Abstract

This study evaluated the toxicity of bitumen on apparently health *T. guineensis* (condition factor of 1.976) over a period (24 hrs, 48 hrs, 72 hrs and 96 hrs) using standard analytical procedures. The density, API and flash point of the bitumen used in this study at 15°C was 0.9898 g/cm³, API at 60°F value of 11.46 and >200°C respectively. The total TPH and PAH constituent of the bitumen was 20,549.77 mg/l and 924.09 mg/l respectively. Analysis of the effect of different concentrations (10mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) of bitumen on *T. guineensis* revealed that the mortality of the organism increased as the concentration of the toxicant and time of exposure increased. The concentration with the longest exposure time where lowest mortality was observed was 40mg/l for 24 hours. The probit analysis on the lethal concentration (LC₁₀, LC₂₀ and LC₅₀) of the toxicant on *T. guineensis* when exposed for 96 hours revealed a varying mortality status. The LC₁₀, LC₂₀ and LC₅₀ of the bitumen on the *T. guineensis* was 6.746 mg/L, 10.773 mg/L and 26.38 mg/L respectively. Continuous discharge of untreated wastes especially petroleum-based products should be discouraged as it poses serious threat to aquatic habitat and produces.

Keywords: Bitumen; Toxicity; Tilapia; Probit; Mortality

1. Introduction

Petroleum is still the principal energy source for industries and industrial uses, even for some domestic uses. Despite its importance in society, petroleum is a major source of pollution in the environment. Certain petroleum hydrocarbons are carcinogenic and mutagenic [1,2] and pose a serious threat to human, plants, and animals' health. Pollution of the ecosystem is a matter of global concern as it leads to contamination of the food chain. Contamination of water bodies by oil-related operations, which is the most obvious industrial activity in the Niger Delta, is a chronic problem that has drawn considerable attention in the past few years. The enormous growth of the oil and gas industry and its steady advancement into the deeper waters have raised concern about the impact of these activities on the environment, fisheries and other legitimate uses of the water [3].

Fishes are known to be sensitive to changes in their environment including the presence of hydrocarbons. Environmental pollution in aquatic ecosystems is usually at a low level but chronic in nature. Studies have shown that chronic toxicity testing is more relevant to environmental management than acute toxicity results as aquatic organisms will be exposed in the longer term to pollution [4]

Bitumen is a sticky black highly viscous liquid or semi-solid form of petroleum. The highly viscous bitumen is usually facilitated by dilution with lighter petroleum hydrocarbons to ease transportation. They may enter the environment via leaks [5] and pipeline rupture [6]. The effect of petroleum products has been studied for years and several reviews have been published on the toxicity of major constituents of bitumen, specifically naphthenic acids [7], PAHs [8], metals and

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their mixtures [5, 9]. However, despite several spills and the large volume of bitumen transported in Niger Delta region of Nigeria, there is little information on the effects of bitumen spills on brackish water *Tilapia guineensis*. This study was designed to investigate the toxicity of bitumen on brackish water *Tilapia guineensis* (finfish) which were collected from Nigerian Institute for Oceanography and Marine Research (NIOMR), Buguma, Rivers State, Nigeria.

2. Materials and Methods

2.1. Sample Collection

Bitumen used for this study was collected from an artisanal refinery located in Bodo town, Gokana Local Government Area of Ogoni, Rivers State, Nigeria. Bodo city is situated on a geographic grid reference of longitude 4°30N and latitude 7°15E. The samples were collected in sterile four (4) liter plastic containers and transported to the laboratory. The brackish water *Tilapia guineensis* (finfish) were collected from Nigerian Institute for Oceanography and Marine Research (NIOMR), Buguma, Rivers State. The habitat water used for acclimatization and toxicity test was also gotten from Nigerian Institute for Oceanography and Marine Research (NIOMR), Buguma, Rivers State. The test organisms were collected in an oxygen plastic bash bag. The test organisms were aerated in the bash bags as soon as they were collected. The organisms were immediately transported to the laboratory for acclimatization. Some important criteria were put into consideration during the selection of the test organisms. The test organisms were collected and transported in an oxygen plastic bash bag to the laboratory for acclimatization.

2.2. Determination of Total Petroleum and Polycyclic Aromatic Hydrocarbon (PAH) of Toxicants

To determine the total petroleum content in the samples, one hundred (100) ml of the sample was measured into a separating funnel and 10 ml of Dichloromethane: Hexane (1:1) was added into it. The mixture was shaken gently and vented for 5 minutes. The aqueous layer was allowed to separate and was decanted. The extracts were concentrated by rotary evaporator into 1 ml. Precisely 1.0 µL of the extracts was injected into a pre-programmed Hewlett-Packard HP 5890 GC-FID. The concentration of TPH was calculated from the peak area of the calibration standards. The GC operational conditions employed for determining the TPH is as follows: Initial oven temp-50 °C, Initial Hold time - 2.0 mins, Ramp-10 °C/min to 300 °C, Final Oven Temp - 320 °C, Detector Temp - 340 °C, Injector Temp - 250 °C, Carrier gas - Helium, Ignition gas - Hydrogen and air [10].

To determine the PAH concentration, precisely, 100 ml of the sample was also measured into a separating funnel and 20 ml of dichloromethane was added and shaken for 5 minutes. The extract was concentrated to 2 ml in a rotary evaporator. 20 ml 0.5 M KOH in 100 ml of methanol was added and the mixture was refluxed for 30 minutes in a water bath at 60 °C. 10 ml Deionized water was added and extracted with hexane (10 ml). The extract was dried over anhydrous sodium sulphate and concentrated at 60 °C in a rotary evaporator to 2 ml. The extract was then passed through a silica gel column which had been pre-conditioned with hexane and eluted with 10 ml of hexane for aliphatic fractions. To same column, 10 ml of dichloromethane was added for the elution of PAHs and the eluent was concentrated to 1 ml and solvent exchanged with 1ml of acetonitrile. 1 µL of the extract was injected into a pre-programmed HP 5890 GC-FID. The concentration of the PAHs was calculated from the peak area of the calibration standards. The operational condition for PAH analysis were as follows: Initial oven temp - 100 °C, Initial Hold time - 0.5 minutes, Ramp - 15 °C/min to 200 °C, then 20 °C/min to 300 °C, Final Oven Temp - 300 °C, Detector Temp - 340 °C, Injector Temp - 250 °C, Carrier gas - Helium, Ignition gas - Hydrogen and air. The PAHs was determined in selective ion-monitoring mode with ionization energy of 70 eV. The m/z peaks corresponding to the molecular masses of individual PAH were used for identification and quantification [10].

2.3. Physiochemical Parameter of Bitumen, API Gravity of Bitumen (Hydrometer Method)

Using ASTM 2012 and ASTM 2026 standard tables, gravities are calculated at 60°F (15.56°C) or converted to values at 60°F. It made use of the thermohydrometer. The samples' temperatures were changed in line with the information in the following table. The temperature of the test samples and the hydrometer cylinder was similar. In order to avoid the development of air bubbles and to minimize the evaporation of the lower boiling components of the more volatile samples, the samples were put into the clean hydrometer cylinder without splashing. Siphoning was used to move the more volatile samples to the hydrometer cylinder. Before inserting the hydrometer, the samples' surface bubbles were cleared by contacting them with a fresh piece of filter paper. The samples were placed in a cylinder that was vertically positioned and out of the way of air currents. In order to avoid the samples' temperatures altering much while the test was being conducted, precautions were taken. During this time, the surrounding medium's temperature was carefully controlled to keep changes from going above 5°F (2°C). Once it had settled, the hydrometer was carefully lowered into the samples, sunk about two scale divisions into the liquid, and then released. The remainder of the stem was maintained dry because extra moisture on the stem influences the instrument's effective weight, which in turn affects the reading

that will be taken. When testing samples with low viscosity, the instrument was given a short spin before being let go, allowing the sample to float easily away from the hydrometer cylinder's walls. The hydrometer was given enough time to totally stop moving and for all air bubbles to rise to the surface. The hydrometer was read to the closest scale division once it had settled, floating freely, and the sample's temperature had stabilized to 0.2°F (0.1°C). The hydrometer scale's cutoff point, where the liquid's surface meets the scale, yields the proper reading. This point was identified by placing the eye just below the liquid's surface and gradually lifting it until the surface, which was initially visible as a warped elliptical, appeared to transform into a straight line that cut the hydrometer scale. To get a reading, the hydrometer's eye was placed just above the liquid's plane surface, and the point on the scale was watched to note when the samples rose above it. The reading needed to be corrected. By watching how high the samples rose on the hydrometer scale relative to the liquid's main surface, the correction was found for the particular hydrometer in use. This was accomplished by submerging the hydrometer in a clear liquid with a surface tension similar to bitumen. Prior to and following the observation of the gravity, the temperature of the samples was measured to the nearest 0.25°F (0.1°C), the liquid in the cylinder being thoroughly but carefully agitated with the thermometer, and the entire mercury thread being immersed. Before and after the final hydrometer measurement, the mean of the thermometer reading was recorded to the nearest 1°F, and this temperature served as the test temperature.

2.3.1. Calculations

The approach was used to deduct the adjustment from the hydrometer measurement when gravities were noticed in opaque liquids. Using Tables 2, all hydrometer data were adjusted to 60°F (15.56°C). The corrected hydrometer reading was written down as API Gravity or degrees API (°API). The samples matched the requisite limiting conditions and sample type as given in the table below.

Table 1 ASTM 2012

S/N	Sample Type	Gravity Limits	Initial Boiling Point Limits	Other Limits	Test Temperature
1	Highly volatile	lighter than 70° API			Cool to 35°F (2°C) or lower in original closed container.
2	Moderately volatile	heavier than 70° API	below 250°F (120°C)		Cool to 65°F (18°C) or lower in original closed container.
3	Moderately volatile and viscous	heavier than 70° API	below 250°F (120°C)	Viscosity too high at 65°F (18°C)	Heat to minimum temperature for sufficient fluidity.
4	Nonvolatile	heavier than 70° API	above 250°F (120°C)		Any temperature between 0 and 195°F (-18 and 90°C) as convenient. 60 ± 0.25°F (15.56 ± 0.1°C)
5	Mixtures of nonpetroleum products or				

2.4. Measurement of Test Organisms

The length and weight of the test organisms was checked using a metre rule and a weighing balance and recorded.



Figure 1 The length of test *Tilapia guineensis* (fin fish)

2.5. Acclimatization of *Tilapia guineensis*

Acclimatization was done for (10) days at $28 \pm 2^\circ\text{C}$ for *T. guineensis*. This was conducted using dark glass tanks (440 mm × 255 × 330 mm), oxygen (air) was supplied via agitation with the aid of an aerator connected to the glass tanks [11]. Organisms were fed on 3% of bulk weight daily using fish feed (pellets) bought from a local feed shop. The fishes (post juvenile) were fed 3 times daily on a 9.5 - 10cm fish feed weight. The fish feed weighed 1kg. Water in acclimatization tanks was renewed with water from brackish water habitat daily, to increase nutrient availability and remove unwanted pollutants. The outer wall of each acclimatization tank glass was lined with polythene sheets for light control. Twelve (12) hours of light and 12 hours of darkness was maintained. Prior to commencement of the test, each tank was cleaned with detergent free water, and allowed to dry to avoid contamination. This is in accordance with the recommendation of DPR [12].

2.6. Range Finding Test for *Tilapia guineensis* (aqueous toxicity)

This test was done to evaluate approximately the concentration to be applied in the definitive toxicity test. Four (4) bioassay tanks were prepared. Bioassay tanks were previously washed, double rinsed and dried. The range of concentrations were prepared in geometric ratios such as 0.01, 1.00, 10.00 and 100.00 gram of toxicant per litre of habitat (dilution water) that will kill all organisms and others that will kill very few or no organisms. Bioassay tanks were labelled with concentrations and test organisms alongside date and time. Ten (10) test organisms were added to each labelled bioassay tank. The results were then used to determine the range of the definitive toxicity test. Mortality rate was recorded after 24 hour of test organisms against concentration of toxicant using this format below. This is in accordance with the recommendation of DPR [12].

Table 2 Format for toxicity report

Parameters	10 (0.01g/L)	mg/L	100 (0.1g/L)	mg/L	1000 (1.0g/L)	mg/L	10000 (10g/L)	mg/L
No. of Test Organisms	10		10		10		10	
Mortality	2		3		6		10	
Percentage mortality (%)	20		30		60		100	
Percentage Survival (%)	80		70		40		0	

(EGASPIN, 2018)

For definitive test, geometrically spaced series of concentrations between the highest (killed no, or only a few, test organisms) and the lowest concentration (killed most or all test organisms) were selected. The results are then used to determine the range of the definitive toxicity test.

2.7. Definitive Toxicity Test

After the expiration of the range finding test, the concentrations for the definitive test were determined from 20-50 % mortality result from the range finding. A definitive test on *Tilapia guineensis* was carried out using habitat water of the test organisms as diluent and control. The control, which was set up, was 100 % brackish water and no toxicant was

added. The habitat water used as diluent was used after filtration on stainless steel mesh sieve (60 µm). The pH, conductivity and temperature of the habitat water were ascertained before being added to the test tanks.

Five (5) range of concentrations were prepared in arithmetic ratios such as 0.02, 0.04, 0.06, 0.08 and 0.10 gram of toxicant per litre of habitat (dilution water) [13]. Bioassay tanks were labelled with concentrations and test organisms alongside date and time. A sterile hand net was used to transfer ten (10) healthy test organisms of exact same size carefully from the acclimatization tanks into each of the test bioassay tanks. A hand net was used to avoid stressing the test organisms from handling with bare hands. After the addition of the test toxicants to the respective bioassay tanks, the concentrations (solutions) were stirred for 5mins and subsequently at 8 hourly intervals for even distribution of toxicants. Food was withheld from the test organisms throughout this test period. At the end of each exposure period all dead organisms were removed. Removal of dead organisms was carried out to avoid contamination of live test organisms through decaying of dead test organisms by bacteria. There was adequate light control, 12 hours of light and 12 hours of darkness were observed. Mortality was recorded every 24 – 96 hours (i.e., 24 hr, 48 hr, 72 hr and 96 hr). Dead organisms were removed immediately on detection using this format. The treatments were in duplicates [12,13,14].

2.8. The Percentage Mortality of *Tilapia guineensis*

The probable toxic effect of bitumen on *T. guineensis* was accessed as a baseline for other aquatic organisms. The formula for the percentage mortality was adopted from APHA [11]. The percentage mortality was derived by dividing the number of organisms that died at each exposure hour by the total test organisms and multiplying by 100.

The formula for %Mortality will be;

$$\text{Number of dead organism} \times 100 \div \text{Total number of organisms}$$

3. Results and Discussion

Bitumen is a mixture of organic liquids that are highly viscous, black, sticky and entirely soluble in carbon disulphide. Although no two bitumen are chemically identical and chemical analysis cannot be used to define the exact chemical composition of bitumen, elemental analysis indicates that most bitumen contains 79-88 % carbon; 7-13 % hydrogen, traces to 3% nitrogen; 8% sulphur; 8% oxygen by weight [15]. The density of the bitumen used in this study at 15°C was 0.9898 g/cm³ with an API at 60°F value of 11.46 (Table 3). The Flash Point of the bitumen sample was >200°C. Based on flash point of the bitumen sample, it can be rated as a good bitumen. According to Mumah and Muktar [16], the flash point of a good grade bitumen sample lies in the range of 245-352°C.

Table 3 Physiochemical Proprieties of Bitumen

Parameter	Method	Bitumen Result
Density @15°C (g/cm ³)	ASTM-D4052	0.9898
API @ 60°F (°API)	ASTM -D287	11.46
Flash Point (°C)	ASTM D92	>200
Basic Sediment and Water (BS&W) (%)	ASTM-D1796	<0.1

Analysis of the total petroleum hydrocarbon constituent (TPH) and Polycyclic Aromatic Hydrocarbon (PAH) of the toxicants (Bitumen) revealed that the total TPH and PAH constituent of the bitumen was 20,549.77 mg/l and 924.09 mg/l respectively. A summary of the TPH and PAH constituent and their concentration is as presented on Figure 1 and 2 respectively.

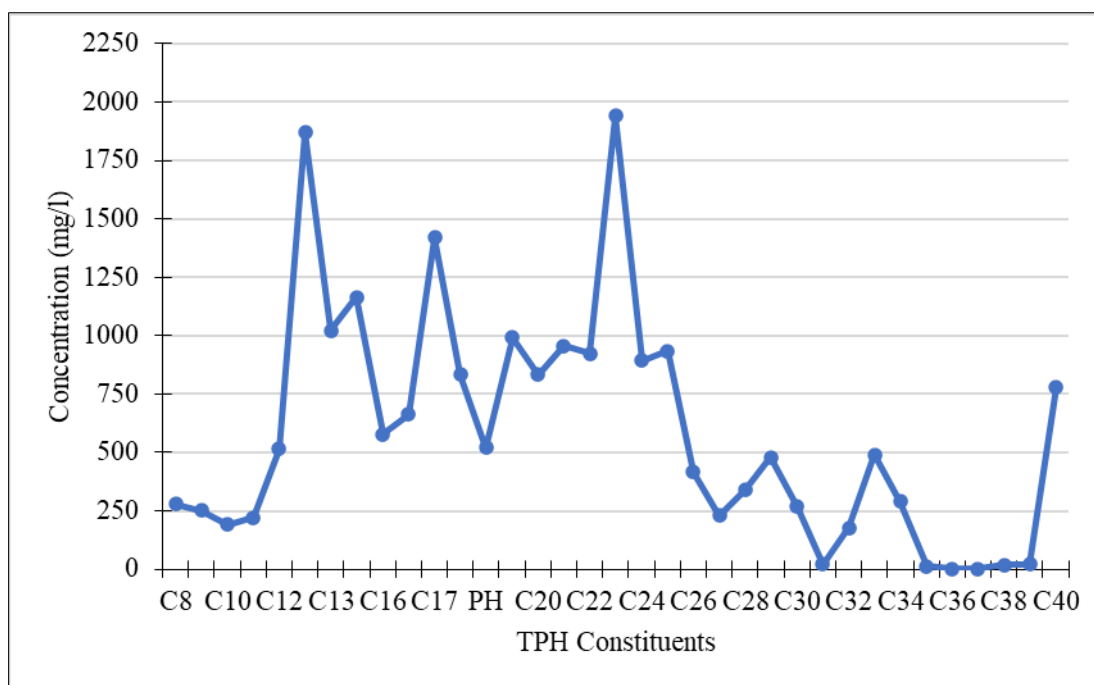


Figure 2 Total Petroleum Hydrocarbon Constituent of the Bitumen

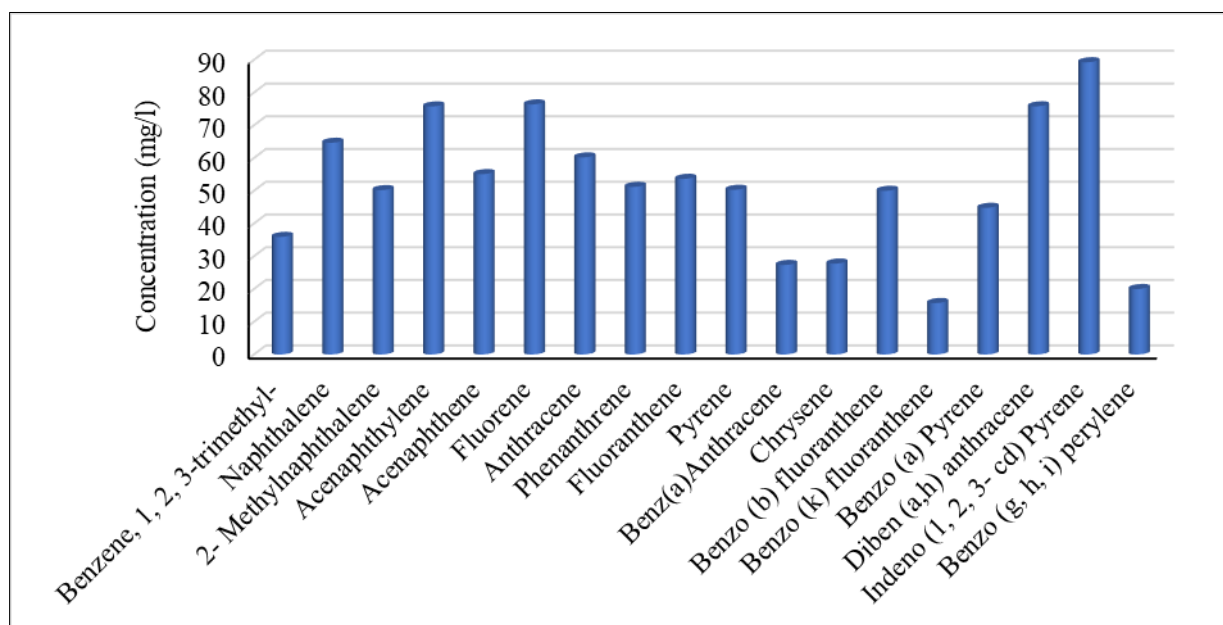


Figure 3 Polycyclic Aromatic Hydrocarbon Constituent of the Bitumen

The condition factor of the test organisms was determined using standard techniques. Analysis revealed the mean length and weight for the *T. guineensis* sample was 4.0 ± 0.5 cm and 0.86 ± 0.05 g, respectively (Table 4). The condition factor of the test organisms revealed a greater than one (1) value (1.976) indicating the samples were apparently healthy. The behavioral responses observed in the fish can be said to be in response to toxicants present in the sample at different duration of exposure and the prevailing specific environmental conditions as opined by Bobmanuel et al., [17]. Contamination of aquatic ecosystem with hydrocarbon or its products affects fishes in various ways including increased mortality [18], kill or cause sub-lethal damage to fish eggs and larvae e.g., morphological deformities, reduced feeding and growth rates, increase vulnerability to predators and starvation [19,20], habitat degradation, loss of hatching ability of eggs, fouling of gill structures, impaired reproduction, growth, development, feeding, respiration [21].

Table 4 Measurement, Health, and Condition factor of Test Organisms

Test Organism	Average Length (mean ± SD)	Average Weight (mean ± SD)	Condition Factor (k-value)
<i>Tilapia guineensis</i>	4.0 ± 0.5 cm	0.86 ± 5 g	1.976

Key: K value > 1 suggest that the fish is healthy; K value < 1 suggest that the fish is unhealthy; K = 1 or health and condition factor of the fish [22].

Analysis of the effect of different concentration (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) of bitumen on *T. guineensis* over a period (24 hrs, 48 hrs, 72 hrs and 96 hrs) revealed that the mortality of the organism increased as the concentration of the toxicant and time of exposure increased. There was no mortality observed in the negative control. The concentration with the longest exposure time where lowest mortality was observed was 40mg/l for 24 hours. A summary of this result is as presented on Figure 4.15

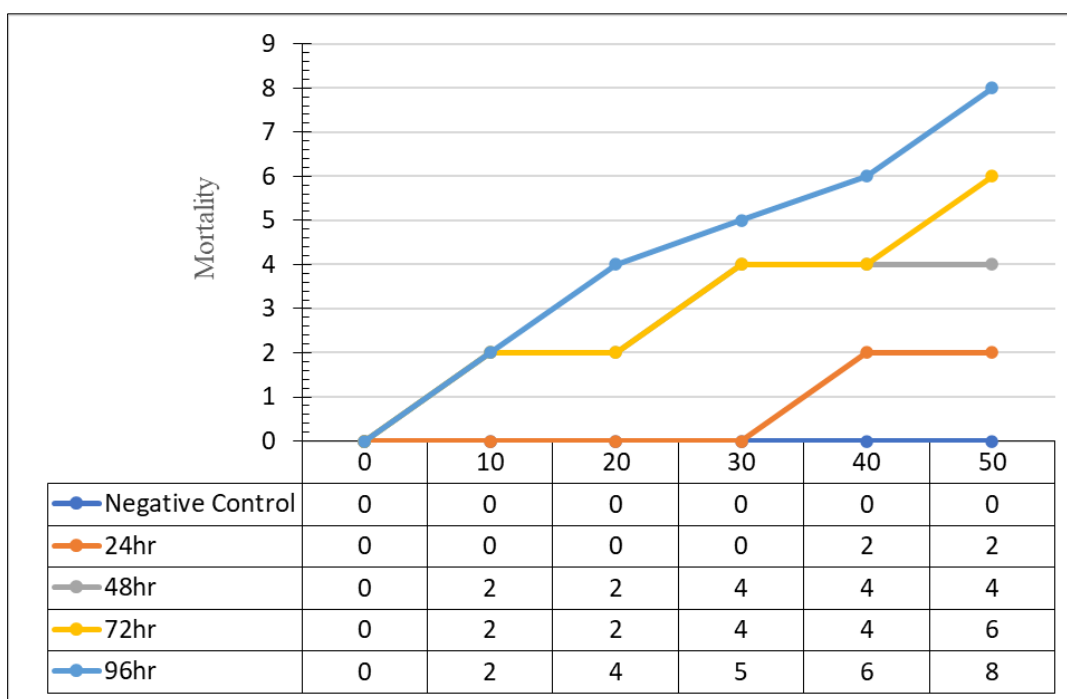


Figure 4 Mortality of *Tilapia guineensis* when Exposed to Bitumen

The probit analysis on the lethal concentration (LC₁₀, LC₂₀ and LC₅₀) of the toxicant on *T. guineensis* when exposed for 96 hours revealed a varying mortality status. The LC₁₀, LC₂₀ and LC₅₀ of the bitumen on the *T. guineensis* was 6.746 mg/L, 10.773 mg/L and 26.38 mg/L respectively. The response of *T. guineensis* to bitumen after exposure of 96 hours is as illustrated on Figure 3.

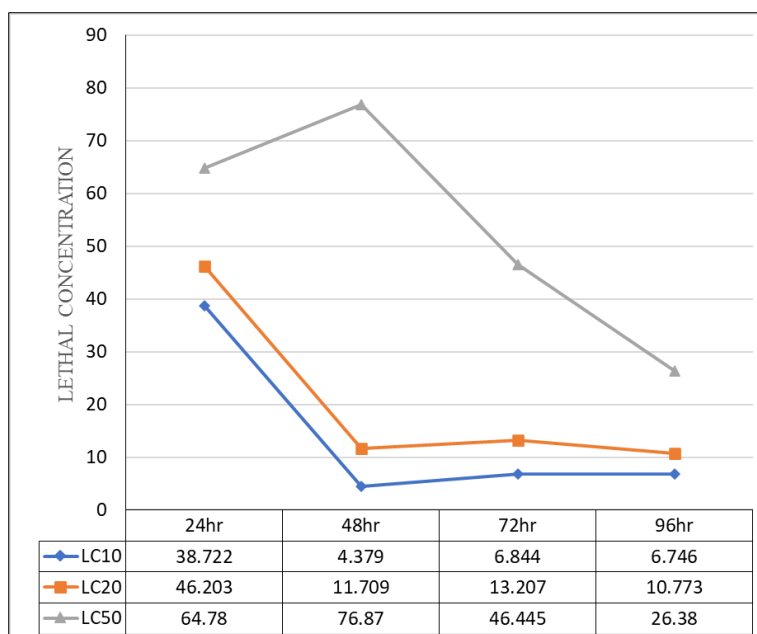


Figure 5 Response of *Tilapia guineensis* to Bitumen (96hr exposure of 10 *Tilapia* to toxicant)

Findings from the study revealed that the toxicity of the bitumen on *T. guineensis* increased with increasing concentration. This result agrees with the study of Olaifa [23] which studied the toxicity of Nigerian Qua Iboe Light crude oil on *Clarias gariepinus*. It also agreed with the findings of Sogbanmu and Otitolaju [24] which also studied the toxicity of Forcados Light Crude Oil on the same species of fish. It also supported the findings Ayoola and Alajabo [25] of acute toxicity of engine oil on Black jaw Tilapia (*Sarotherodon melanotheron*). Neff et al. (2000) reports that the toxicity of heavy oils is more of a physical or mechanical nature and chemical toxicity due to light oils. The light oils are rich in aromatic hydrocarbons. Petroleum products has the potential to kill fishes quickly by coating and interfering with gas exchange necessary for life. The findings also agree with Sloman and Wood [26] who reported that physical environmental disturbances directly affect fish at the ecological level by rupturing fish assemblages and causing fragmentation and loss of genetic variability in fish populations. Thus, crude oil exploration poses grave danger to the biodiversity of the aquatic ecosystem.

4. Conclusion

The findings of the study revealed that the toxicity of bitumen on apparently healthy brackish water fish (*T. guineensis*) increases as the concentration of the toxicant and time of exposure increased. The concentration with the longest exposure time where lowest mortality was observed was 40mg/l for 24 hours. Continuous discharge of untreated wastes especially petroleum based products should be discouraged as it poses serious threat to aquatic habitat and produces.

Compliance of ethical standard

Disclosure of conflict of interest

The authors have declared that there is no conflict of interest.

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