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96h Acute Sediment Toxicity Testing of Bonny Light Crude Oil on Clarias Gariepinus

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Abstract
The acute sediment toxicity test was conducted with juveniles of the freshwater – Clarias gariepinus of length (3.50 ± 0.5 cm) and body weight (0.55 ± 0.05 g) exposed to sediment spiked with Bonny light crude oil coded JL-01-BLCO, using the semi-static renewal assay technique. The sediment used was collected from a relatively pristine area of the New Calabar River, Choba, Rivers State. The results obtained from the bioassay showed the following, no toxic effect was observed in the control with over 90% survival rate obtained as required for test acceptability. During the test period, the range of temperature, pH and DO were 16.5 – 22.2OC, 6.88 – 7.77 and 7.22 – 11.2mg/l for the freshwater media. These values were within ranges conducive for survival of the test species. The median lethal concentrations (LC_{50}) obtained after 48h, 72h and 96h of exposure of the test organism, Clarias gariepinus to JL/01/BLCO was 331,267mg/kg, 194,102mg/kg and 12,359.6mg/kg respectively. Sediment avoidance, erratic swimming and mortality increased as the test concentrations increases. Sediment containing the test substance, JL/01/BLCO exhibited some level of toxicity on Clarias gariepinus. The toxic effect of the test substance increased over time and as the test concentration got higher. The implication of these findings and results is that BLCO-contaminated sediment will continually exhibit a toxic effect on sediment-dwelling species via two (2) mechanisms namely bodily contact with the sediment and exposure to water containing leached toxic components of BLCO.

Keywords: *Clarias gariepinus, Mortality, Sediment, Juveniles*

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1. Introduction

Modern societies are faced with serious concerns about some global environmental challenges; developing countries in particular, currently experience growing and complex pollution problems. The global environmental pollution, water pollution and waste management in particular, have attracted international public health attention (Nwude, *et al*., 2020). Several researchers have investigated chemicals or hazardous substances - in river water samples, sediments and some species of fish in Nigeria (Unyimadu *et al*., 2019; 2018a,b,c; 2017, Babatunde *et al*., 2013). Oil spillage as a result of petroleum industry activities is a regular occurrence in oil-producing Niger Delta region of Nigeria. The spillage is often caused as a result of pipe-line vandalization by saboteurs, who are mostly youths of the oil-producing regions clamoring for remediation of their polluted aquatic environments among other things (Ndimele 2011). There have been over 3,854 reported cases of oil spillage in Nigeria from 1986 to 2000 (Adeyemi 2004). Acute toxicity bioassay is widely used to assess the effects of pollutants on one or more organisms usually based on the determination of acute lethal toxicity and sub-lethal toxicity test using sensitive species or organisms based on their economic and ecological importance, availability and ease of handling (Fuller et al 2004). Although, the tests are mostly laboratory based, simple, are of single variable and do not necessarily simulate the field situations, they nonetheless provide useful information on the potential of the pollutant to harm the biota (Akbari et al 2004). As an indicator of general environmental pollution and as a route of human exposure, fish is often used as a biomarker for quality of water bodies since the quality of the fish depends on the quality of the water (Fakankun *et al*., 2012; Olusola and Festus, 2015; Obot *et al*., 2016; Bawuro *et al*., 2018).

It is important to monitor the aquatic environment, especially sediments, which are sinks for many pollutants (Lyytikainem et al., 2001).

Sediments can accumulate large quantities of chemicals, particularly poorly soluble organic compounds, that may be rapidly taken up by benthic fish, both through direct contact with the sediment and interstitial water, and from ingested food (Vigano et al., 2001). Thus, pollution monitoring or impact assessment in aquatic ecosystems should not be limited to the water phase, but should also include the sediment (Julianna *et al.,* 2005). Historically, the assessment of sediment quality has often been restricted to chemical analysis. However, quantifying contaminant concentrations alone cannot provide enough information to evaluate adequately the potential adverse effects, or time-dependent availability of these materials to aquatic organisms (Ingersoll, 1995). Hence, the employment of Acute sediment toxicity tests seems to be a more effective means of predicting and detecting the diverse impacts of contaminated sediments (Julianna *et al.,* 2005). In the last decade fish have been increasingly used in toxicity tests with sediment (Di Giulio et al., 1993; Vigano` et al., 1998, 2001).

The Africa catfish of the genus *Clarias* are a highly esteemed group of fishes in tropical Africa and they command high market value. They are of the genus *Clarias,* a highly esteemed group of fishes in tropical Africa and they command high market value (Ugwu *et al*., 2011). Their hardy nature and possession of accessory air-breathing organs enable them tolerate adverse aquatic conditions (Reed *et al* 1967). Nonetheless, *Clarias gariepinus* fingerlings are very delicate and sensitive to aquatic pollutants including crude oil and other petroleum products (Ugwu *et al*., 2011). The objective of this study is to investigate levels at which Sediment containing the test substance, Bonny light crude oil coded JL/01/BLCO exhibited acute toxicity on *Clarias gariepinus* and the levels at which it will continually exhibit a toxic effect on sediment-dwelling species via two (2) mechanisms namely; bodily contact with the sediment and exposure to water containing leached toxic components of JL/01/BLCO, with a view of ascertaining their level of tolerance and suitability as a bio-indicator in freshwater environment.

2. Materials and methods

2.1 Collection and storage of test organism

Juveniles of *Clarias gariepinus* (3-6 months, 250-400 mg) that have not shown signs of sexual differentiation was used in the test. The test organisms were obtained from Aqua-Green culture centre in Onne, Rivers State. Selected samples were subjected to a TPH and PAH test to determine the baseline before using it for the test, and the baseline analysis showed no contaminants in the fish, during acclimation, with less than 0.2% mortality. The Juveniles were about 3.50 $\pm$ 0.5cm in length and 0.55 $\pm$ 0.05g in weight, with lipid content of 1.04$\pm$0.02%. This specie was selected for the following reasons:

a. They are known sediment-dwellers.
b. They are easy to maintain in the laboratory.
c. Considered as one of the most important tropical catfish species for aquaculture
d. They are of "least concern (LC)" according to the IUCN red list and as such, can be used for research purposes to ascertain pollutant effects on aquatic species.

On receipt at the laboratory, the test organisms were transferred to the flow-through aquaculture system and acclimated for 10-12 days with constant aeration. They were fed on 3% bulk weight of fish food throughout the acclimation period.

2.2 Overlying water

Overlying water (freshwater) used for the test was prepared in the laboratory using analytical grade salts as described by Okoroafor, 2011. The water was synthesized in the laboratory, then its baseline physico-chemistry (pH: 7.02, Hardness: 55.8mg/l, Alkalinity: 40mg/l, Ammonia: 3.86mg/l, and PAHs: <0.001mg/l) was established before using it for the definitive test.

2.3 Collection of pristine sediment

Since this research is on sediment associated contaminants, pristine sediment is needed and which was collected from the field. Freshwater sediment used for sediment toxicity and bioaccumulation tests was collected from a relatively pristine area of the new Calabar River, Rivers State. On arrival at the laboratory a sample was taken immediately for Physico-Chemical characterization of their particle size distribution, percent moisture, percent total organic and the rest stored at 22$\pm$2℃. The physico-chemical characteristics of the freshwater sediment is as shown on Table 1. The Moisture Content of the sediment is 58.2%. This means that 100g of wet sediment contains 58.2g of water and 42.8g of dry sediment. Therefore, to achieve 100g dry weight of sediment, 233.64g of wet sediment is needed.

Table 1: Weights of sediment and volume of JL-01-BLCO used in preparing the test concentration

Nominal Concentration (mg/kg)	Dry weight of Sediment for Test (g)	Wet weight of Sediment Used (g)	Volume of Test Substance Used (µl)	Volume of Overlying Water Used (ml)
Control	100	233.6	0.00	700
123.6	100	233.6	12.40	700

2.4 Test substance (Bonny light crude oil)

The Test substance is Bonny light crude oil coded JL-01-BLCO. The test sample was duly assigned a lab number, logged into the sample register, and stored at 22+2℃ until the acute sediment toxicity and bioaccumulation studies. The test substance has a density of 0.91g/ml as determined in the laboratory using a hydrometer.

2.5 96h acute aquatic toxicity testing/ sediment toxicity test

Test species was acclimated and then exposed to varying concentrations of the Test substance-BLCO (in sediment) and a reference toxicant for 96 hours. A preliminary range-finding test was performed to establish a working range for the definitive test by obtaining the least concentration that gives no effect and the highest concentration that gives 100% death. Mortality was recorded after every 24 hours. The aim of the toxicity test was to: (1) Determine the median lethal concentration (LC50) of Bonny light crude oil (BLCO), and (2) Screen for survival/determine the "No observable effects concentration (NOEC)." The NOEC brought about the selection of the two (2) concentrations for the bioaccumulation test. This is to ensure that the test organisms do not die within the period for the bioaccumulation test.

2.6 Preliminary range finding test

A preliminary range-finding test was performed to establish a working range for the definitive test by obtaining the least concentration that gives no effect and the highest concentration that gives 100% death. The freshwater sediment (100g dry weight) was spiked with the test substance, JL/01/BLCO to obtain a ten-fold series of concentrations ranging from 10 – 1,000,000mg/kg (dry weight). Afterwards, overlying water (laboratory formulated freshwater) was introduced gently to achieve a 1:3 sediment to water ratio. Six (6) test organisms were then placed in each test trough and observed for mortality over a 48hr period alongside a control. Based on observations made from the range finding test, five (5) test concentration were selected for the definitive sediment toxicity test.

2.7 Definitive test

Following the preliminary range finding test and using a dilution factor of 0.6, test concentrations of 64,800mg/kg, 108,000mg/kg,180,000mg/kg,300,000mg/kg and 500,000mg/kg (dry weight) were selected and prepared for the definitive test.

2.7.1 Preparation of test medium

The various concentrations of the test sediment were prepared by spiking the freshwater sediment with calculated amounts of JL-01-BLCO as shown in Table 2. The spiked sediments were well homogenized in a porcelain dish before being transferred to the test troughs. Overlying water was carefully dispensed through a narrow tube to avoid sediment disturbance and then aerated for 20mins before introduction of the test organisms (20 individuals per test trough). All test concentrations were set up in triplicate**s.**

Table 2: Preparation of Test concentrations of JL-01-BLCO for Definitive test

Nominal Concentration (mg/kg)	Dry weight of Sediment for Test (g)	Wet weight of Sediment Used (g)	Volume of Test Substance Used (ml)	Volume of Overlying Water Used (ml)
Control	100	233.6	0.00	700
64,800	100	233.6	7.12	700
108,000	100	233.6	11.87	700
180,000	100	233.6	19.78	700
300,000	100	233.6	32.97	700
500,000	100	233.6	54.95	700

2.7.2 Determination of endpoints

Acute toxicity data were obtained from analysis of mortality data obtained for the five (5) test concentrations over 48, 72 and 96 hours. Data analysis to determine the median lethal concentrations (LC_{50}) and associated 95%

confidence intervals was done by probit method using SPSS 24 software. The median lethal times (LT_{50}) for the observed effects (i.e., mortality) were also computed for each test concentration using the same software and method.

3. Results

3.1 Probit analysis for toxicity testing

The results of statistical probit analysis indicating the most logical approach of fitting a regression of the response versus the concentration is presented in Tables 3 – 5. The probit concentrations 4.812, 5.033, 5.255, 5.477 and 5.699 of JL-01-BLCO on *Clarias gariepinus* were 3, 4, 7, 7 and 14 respectively after 48hrs exposure, this indicates that the LC_{50} occurred between 5.477 and 5.699 probit concentrations. For 72hrs exposure, 4.812, 5.033, 5.255, 5.477 and 5.699 probit concentrations yielded 4, 4, 8, 12 & 19 mortality response respectively, signifying LC_{50} between 5.255 and 5.477 probit concentrations. While 96hrs exposure resulted in 5, 8, 12, 18 & 20 mortality responses respectively for the same probit concentrations, with LC_{50} occurring between 5.033 and 5.255. Tables 6 - 10 represent the probit analysis for median lethal times (LT_{50}). Consequently, the mortality response for 0.602, 0.903, 1.380, 1.681, 1.857 and 1.982 median lethal time (LT_{50}) of JL-01-BLCO on *clarias gariepinus* were 0, 0, 2, 3, 4 and 5 respectively for a dose of 64,800mg/kg. Correspondingly, dose of 108,000mg/kg of 0.602, 0.903, 1.380, 1.681, 1.857 and 1.982 time, 0, 0, 1, 4, 4 and 8 mortality response were observed. 0, 0, 2, 7, 8 and 12 was also observed for 180,000mg/kg dose. 300,000mg/kg elicited a mortality response of 0, 0, 3, 7, 12 and 18. And a mortality response of 0, 0, 4, 14, 19, 20 was observed for 500,000mg/kg dose.

Table 3: 48hr probit analysis for JL/01/BLCO; *Clarias gariepinus*

	Cell Counts and Residuals					
	Number	**a_conc**	**Number of Subjects**	**Observed Responses (Mortality)**	**Expected Responses**	**Residual**
Probit	1	4.812	20	3	2.461	.539
	2	5.033	20	4	4.256	-.256
	3	5.255	20	7	6.646	.354
	4	5.477	20	7	9.438	-2.438
	5	5.699	20	14	12.302	1.698

Table 4: 72hr probit analysis for JL/01/BLCO; Clarias gariepinus

	Cell Counts and Residuals					
	Number	**a_conc**	**Number of Subjects**	**Observed Responses (Mortality)**	**Expected Responses**	**Residual**
Probit	1	4.812	20	4	2.285	1.715
	2	5.033	20	4	5.199	-1.199
	3	5.255	20	8	9.340	-1.340
	4	5.477	20	12	13.673	-1.673
	5	5.699	20	19	17.010	1.990

Table 5: 96hr probit analysis for JL/01/BLCO; Clarias gariepinus

	Cell Counts and Residuals					
	Number	**a_conc**	**Number of Subjects**	**Observed Responses (Mortality)**	**Expected Responses**	**Residual**
Probit	1	4.812	20	5	3.815	1.185
	2	5.033	20	8	8.550	-.550
	3	5.255	20	12	13.897	-1.897
	4	5.477	20	18	17.706	.294
	5	5.699	20	20	19.418	.582

Table 6: 64,800mg/kg probit analysis ($\boldsymbol{LT_{50}}$) for JL/01/BLCO; *Clarias gariepinus*

	Cell Counts and Residuals					
	Number	**a_time**	**Number of Subjects**	**Observed Responses (Mortality)**	**Expected Responses**	**Residual**
Probit	1	.602	20	0	.068	-.068
	2	.903	20	0	.244	-.244
	3	1.380	20	2	1.264	.736
	4	1.681	20	3	2.837	.163
	5	1.857	20	4	4.207	-.207
	6	1.982	20	5	5.380	-.380

Table 7: 108,000mg/kg probit analysis ($\boldsymbol{LT_{50}}$) for JL/01/BLCO; *Clarias gariepinus*

	Cell Counts and Residuals					
	Number	**a_time**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**
Probit	1	.602	20	0	.006	-.006
	2	.903	20	0	.062	-.062
	3	1.380	20	1	.941	.059
	4	1.681	20	4	3.163	.837
	5	1.857	20	4	5.424	-1.424
	6	1.982	20	8	7.411	.589

Table 8: 180,000mg/kg Probit Analysis ($\boldsymbol{LT_{50}}$) for JL/01/BLCO; *Clarias gariepinus*

	Cell Counts and Residuals					
	Number	**a_time**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**
Probit	1	.602	20	0	.010	-.010
	2	.903	20	0	.117	-.117
	3	1.380	20	2	1.912	.088
	4	1.681	20	7	5.884	1.116
	5	1.857	20	8	9.258	-1.258
	6	1.982	20	12	11.779	.221

Table 9: 300,000mg/kg Probit Analysis ($\boldsymbol{LT_{50}}$) for JL/01/BLCO; *Clarias gariepinus*

	Cell Counts and Residuals					
	Number	**a_time**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**
Probit	1	.602	20	0	.000	.000
	2	.903	20	0	.028	-.028
	3	1.380	20	3	2.037	.963
	4	1.681	20	7	8.512	-1.512
	5	1.857	20	12	13.446	-1.446
	6	1.982	20	18	16.297	1.703

Table 10: 500,000mg/kg Probit Analysis ($\boldsymbol{LT_{50}}$) for JL/01/BLCO; *Clarias gariepinus*

	Cell Counts and Residuals					
	Number	**a_time**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**
Probit	1	.602	20	0	.000	.000
	2	.903	20	0	.006	-.006
	3	1.380	20	4	3.550	.450
	4	1.681	20	14	14.957	-.957
	5	1.857	20	19	18.903	.097
	6	1.982	20	20	19.762	.238

3.2 Establishing the $\boldsymbol{LC_{50}}$ and $\boldsymbol{LT_{50}}$ through acute toxicity testing

The results obtained in the definitive sediment toxicity testing ranges between 64,800 and 500,000mg/kg using a control and nominal concentrations selected on the basis of a preliminary range finding test. The median lethal concentrations LC_{50} that is, the time at which 50% mortality of the exposed population occurred and their 95% confidence intervals for 48hrs, 72hrs and 96hrs obtained from the bioassay of Bonny light crude oil coded JL/01/BLCO are presented in Table 11. The LC_{50} for 48hrs exposure period was 331,267mg/l with upper limit of 226,705mg/l and lower limit of 731,507mg/l. Correspondingly, 72hrs exposure yielded an LC_{50} of 194,102mg/l with an approximate lower and upper limit of 149,788mg/l and 257,707mg/l respectively while LC_{50} for 96hrs exposure period was 123,596mg/l with a lower and upper limit of 95,096mg/l and 153,794mg/l respectively. The median lethal times, LT_{50} recorded in this study is summarized in Table 12. The result precisely indicates that for 64,800mg/kg of the test chemical, LT_{50} occurred at 244.673hrs, as 108,000mg/kg had LT_{50} at 134.989hrs while LT_{50} for 180,000mg/kg, 300,000mg/kg and 500,000mg/kg occurred at 78.329hrs, 54.121hrs at 35.895hrs respectively.

Table 11: Median lethal concentration ($\boldsymbol{LC_{50}}$) of JL/01/BLCO for the freshwater species, *Clarias gariepinus*.

Exposure Period	**Median Lethal Concentration, (LC_{50}) (mg/l)**	**95% Confidence Limits**	
		Lower Limit	**Upper Limit**
48 Hours	331,267	226,705	731,507
72 Hours	194,102	149,788	257,707
96 Hours	123,596	95,096	153,749

Table 12: Median lethal times ($\boldsymbol{LT_{50}}$) for JL/01/BLCO

Test Species	Median Lethal Times, $\boldsymbol{LT_{50}}$ ($\boldsymbol{hrs}$)				
	64,800mg/kg	**108,000mg/kg**	**180,000mg/kg**	**300,000mg/kg**	**500,000mg/kg**
Clarias gariepinus	244.673	134.989	78.329	54.121	35.895

4. Discussion

4.1 Physicochemical characteristics of sediment and overlying water

Three basic physico-chemical parameters were taken during the uptake and depuration phase of the exposure of test specie to JL-01-BLCO contaminated sediment. The measured mean values for the respective physicochemical parameters during the uptake phase are reported as; pH (7.18), Temperature (20.4) ℃ and DO (6.28) mg/l/. Correspondingly, the mean of the recorded parameters during the depuration phase were; pH (8.15), Temperature (19.2) ℃ and DO (12.7) mg/l for the fresh water medium. Water temperature recorded for all troughs fell within the lower limit of the recommended range of (20-35℃) proposed by Akinyemi (1988) as a suitable temperature range for catfish culture. Water pH for the analytical setup were within the optimal recommended range of 6.5 to 9.0 for catfish culture according to recommendations by Boyd and Tucker (1998), while the DO recorded were above the minimum recommended threshold of 5 mg/l (Water Resources Commission, 2003). These discussions on the measured parameters (temperature, pH and DO) are in agreement with the studies of (Ezike and Echor, 2018 and Amponsah *et al.,* 2021), as conditions conducive for survival of the test species.

4.2 Dose effect of the toxicant on *Clarias Gariepinus* using probit analysis

In evaluating the toxicity or effectiveness of a toxicants on a specific population, statistical software to calculate and compare the lethal dose is required (Lei and Sun, 2018). This can be achieved by testing the response of an organism under various concentrations of a chemical and then comparing the concentrations at which a response (Mortality) is encountered in this case, the probit regression software is used. Mortality increased with concentrations of JL/01/BLCO (which means toxicity is directly proportional to mortality rate), and this is in agreement with the study of Davies *et al*. (2019), whereby in the study probit was used in analysing the number of mortalities recorded and their percentage mortality in other to determine its 96hr LC_{50} and LT_{50}. It appeared that there was statistical significance (P>0.05) in the number of mortalities observed in the six concentrations from 24 hours to 96 hours of exposure and high percentage mortalities were recorded as the concentration of the toxicant increased and no mortalities were recorded in the control. As an established fact by this investigative assessment, that percentage mortalities are concentration – dependent is also supported by similar reports presented by Ogundiran *et al*., (2010) when investigating toxicological impacts of detergent effluent in fingerlings of *Clarias gariepinus*. Several other studies also agree with the finding that percentage mortalities are concentration – dependent in their various toxicological evaluations such as Calta *et al*. (2004) when studying acute toxicity of the synthetic pryrethroid deltamethrin to young minnow cap (*cyprinus carpio)*; Ayotunde *et al*. (2011) when investigating the toxicity of *Carica papaya* on adult *C. gariepinus*; Ayuba and Ofojekwu (2002) when investigating on acute toxicity of diazinion to African catfish *C. gariepinus*. Therefore, it is affirmed that severity of response is established by dose-effects [the relationship between dose and the magnitude of defined biological effects either in individual or in a population sample as per crump *et al.* (1976)].

4.3 Median lethal concentrations $\boldsymbol{LC_{50}}$ and median lethal time $\boldsymbol{LT_{50}}$

Median lethal concentrations (LC_{50}) is the dose required to kill half the members of a test population after a specific test duration. Similarly, median lethal time (LT_{50}) is the time taken for half the members of a tested population to be killed after exposure to a toxic substance or stressful conditions. The LC_{50} of JL/01/BLCO was assessed for 48h, 72h and 96h exposure of the test organism, *Clarias gariepinus* to PAHs of the test chemical using 96hr acute toxicity testing protocol as described in EPA/600/R-99/064 (USEPA, 2000). Correspondingly, the five concentrations (64,800mg/kg, 108,000mg/kg, 180,000mg/kg,300,000mg/kgand500,000mg/kg) of the test chemical was used to determine the LT_{50}. Thus, the LC_{50} values reported for 48hr, 72hr and 96hr were 331,267mg/kg, 194,102mg/kg and 123,596mg/kg respectively and the median lethal

times (LT_{50}) for the highest concentration tested (i.e., 500,000 mg/kg) was 35.9hrs as presented in Table 15 and 16 respectively. Crude oil has been reported to produce 100% mortality of fish in 96hrs by other researchers such as George *et al*. (2014) who recorded the percentage mortality of *C. gariepinus* in the water-soluble fraction of Qua Iboe Light crude oil in two batches A and B, ranging from 0-100% in both batches at the end of the 96hrs test. In this study of the mortality response of the test specie to varying concentrations of JL/01/BLCO, it was observed that at the highest concentration of JL/01/BLCO (500,000mg/kg) for 96hr all fishes died (100% mortality) and JL/01/BLCO displayed characteristics of being toxic by increasing mortality rate at short period of fish exposure - 48h. Similar trend of highest concentration of bonny light crude oil causing 100% mortality rate was reported in the work of Seiyaboh *et al* (2013), thereby affirming Bonny Light Crude oil is toxic to *Clarias gariepinus*. Among petroleum hydrocarbons, PAHs as contained in Bonny Light Crude Oil are also considered to be the most acutely toxic components (Cherr *et al*., 2017).

Clarias gariepinus exhibited high tolerance to the effect of JL/01/BLCO polluted sediment at periods before 48hrs which dwindled progressively from thence as the cumulative mortality rose from 20.0 – 88.3% within the 48-96hr period. This means, the LC_{50} for the respective concentrations exhibited an inverse relationship with time from 48-96 hr as shown in Figure 9. Also, as the sequence of toxicity level, LC_{50} values along the period of exposure became more toxic from 48hr LC_{50} (331,267mg/kg), 72hr LC_{50} (194,102mg/kg) and 96hr LC_{50} (123,596mg/kg) and this agrees with the reports of Ndimele & Jenyo-Oni (2010) when studying BLCO on *Tilapiaguineensis* for which the LC_{50} at 48-hr, 72-hr and 96-hr were 316.23, 281.84 and 125.89 mg/l respectively. Similarly, Ndimele (2011) in his work, BLCO on *Desmocaris trispinosa* averred that LC_{50} at 72hr and 96hr were 281.84 mg/l and 120.23 mg/l respectively. Udeme (2010) when studying Qua Iboe Light Crude Oil against a fresh water fish, *Oreochromis niloticus*, 96hrs LC_{50} value was shown to be 1.069ml/l while 72hrs LC_{50} value was 1.432ml/l. These results lend credence to the popularly held notion that different types of crude oil vary in their toxicities to different species of animals (Udeme, 2010).

For the median lethal time, LT_{50} was observed to decrease with increased concentrations of test chemical as reported in Table 16. Ndimele & Jenyo-Oni (2010) study shows median lethal time (LT_{50}) at crude oil concentrations of 160 mg/l, 240 mg/l and 320 mg/l being 92hrs, 82hrs and 49hrs respectively, and Ndimele (2011) also indicated LT_{50} at crude oil concentrations of 160 mg/L, 240 mg/L and 320 mg/L to be 89.5hrs, 80.7hrs and 53.3hrs respectively. The observed difference in median lethal concentrations obtained in these studies might be due to the method of bioassay employed and the type of crude oil administered (Fuller *et al*., 2004) and the nature of the compositions of the various crude oil which according to USEPA (2002) varied greatly in composition of hydrocarbon content. However, in the control set-up, no toxic effect was noticed with over 90% survival rate obtained as required for test acceptability of the United State Environmental Protection Agency (USEPA). The implications of this findings are that BLCO-contaminated sediment will continually exhibit a toxic effect on sediment-dwelling species via two (2) mechanisms namely bodily contact with the sediment and exposure to water containing leached toxic components of BLCO.

5. Conclusion

Sediment containing the test substance, Bonny light crude oil coded JL/01/BLCO exhibited some level of toxicity on *Clarias gariepinus* even at concentration of 68,000mg/kg. The toxic effect of the test substance increased as the test concentration increased and yielded an LC_{50} of 123,596mg/kg after 96 hrs exposure. The LC_{50} for the respective concentrations exhibited an inverse relationship with time from 48-96 hr. It was further observed that toxicity increased over time. This was as a result of either or a combination of the following: (1) The gradual leaching of toxic components trapped in the sediment matrix into the overlying water, and (2) Increased exposure of the organisms as they made repeated bodily contact with toxic components in the sediment. As the test concentration increased, the test organisms exhibited pathological changes and mortalities. The mortality versus concentration curves established followed a sigmoid pattern and can be deduced that mortality increases with concentrations in the JL/01/BLCO (Which means toxicity is directly proportional to mortality rate). To further assess the effect of exposure time on toxicity, the median lethal time (LT_{50}) was determined. The results obtained showed that it takes about 35.9 hours for sediment containing as much as 500,000mg/kg of BLCO to kill 50% of organisms exposed (i.e., LT_{50}). The least concentration of 68,000mg/kg

gave an LT_{50} of 244.7 hours. The implication of these results and findings is that BLCO-contaminated sediment will continually exhibit a toxic effect on sediment-dwelling species via two (2) mechanisms namely bodily contact with the sediment and exposure to water containing leached toxic components of BLCO. Other sub-lethal changes observed in the test organism were sediment avoidance, air gulping, leaping, erratic swimming and lethargic reactions even before mortality was observed in every treatment.

References

Adeyemi O. T., (2004) Oil exploration and environmental degradation: the Nigerian experience. Environ Inform Arch 2:387-393.

Akbari S., Law A. T., Shariff M., 2004 Toxicity of water-soluble fractions of crude oil to fish, Lutjanus argentimaculatus and shrimp, Penaeus monodon. Iran J Sci Tech 28:169-175.

Akinyemi O. (1988). Water Quality and Pond Management. In A. M. Ajani (Ed.), Proceedings of the Nationwide Fish Farmers Workshop. Lagos, Nigeria, pp. 101-106.

Amponsah, S. K., Agodzo, S., Agbeko, E., & Osei, E. A. (2021). Impact of tank geometry on production of African Catfish (Clarias gariepinus). African Journal of Agricultural Research, 17(1), 165-172.

Auta J and Ogueji EO (2007) Acute Toxicity and behavioural effects of chlorpyrifos-Ethyl pesticide to juvienile of Clarias gariepinus Teugels. Procceeding, 22nd Annual Comferenceof Fisheries Society of Nigeria (FISON) held at School of Nursing, Birnin Kebbi, 12th -16thNovember, 2007. 264-272.

Babatunde. BB; Dike. CS; Ibekwe. CJ (2020). Assessing Uptake Kinetics and Natural Depuration of Lead and Cadmium in African Catfish Juvenile. J. Appl. Sci. Environ. Manage. Vol. 24 (2) 207-211.

Bawuro, AA; Voegborlo, RB; Adimado, AA (2018). Bioaccumulation of Heavy Metals in Some Tissues of Fish in Lake Geriyo, Adamawa State, Nigeria. Journal of Environmental and Public Health, Article ID 1854892, 7 pages.

Boyd CE, Tucker CS (1998). Pond Aquaculture Water Quality Management (1st edition.). Springer US

Di Giulio, R.T., Habig, C., Gallagher, E.P., 1993. Effects of black rock harbour sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. Aquat. Toxicol. 26, 1 – 22.

Ezike, C.O.; Echor, F.O. Polycyclic Aromatic Hydrocarbons (PAH) in Fingerlings of Clarias gariepinus (Burchell, 1822) to Petroleum. Preprints 2018, 2018010009 (doi: 10.20944/preprints201801.0009.v1)

Fakankun, OA; Babayemi, JO; Akosile, SO (2012). Evaluation of fish gills as potential target organ for accumulation of heavy metals. Afri. J. Animal and Biomedical Sci. 7(1): 15-18.

Fuller J., Bonner J., Page C., Ernest A., McDonald T., McDonald S., 2004 Comparative toxicity of oil, dispersant, and oil-plus-dispersant to several marine species. Environ Toxicol Chem 23:2941-2949. doi: 10.1897/03-548.1

Gehring, PJ, PG Watanabe, and GE Blau. 1976. Pharmacokinetic studies in evaluation of the toxicological and environmental hazard of chemicals. New Concepts Saf Eval 1(Part 1, Chapter 8):195-270.

George, Ubong U., Urom, Sunday. E. and Etanketuk, Nseabasi. (2014). Acute Toxic Effect of Qua Iboe Light Crude Oil on The Gills of Clarias Gariepinus Juveniles. International Journal of Environment and Pollution Research. Vol.2, No.2, pp.16-30.

Imevbore, A. M. A., Adeyemi, S. A. and Afolabi, O. A., 1986. "The Toxicity of Nigerian Crude Oils to Aquatic Organisms" In: Proceedings of 1986 International Seminar on the petroleum Industry and the Nigeria Environment. pp 171 - 176.

Ingersoll, C.G., 1995. Sediment tests. In: Rand, G.M. (Ed.), Fundamentals of Aquatic Toxicology. Effects, Environmental Fate and Risk Assessment, 2nd ed. Taylor & Francis, Washington, pp. 231 – 249.

Juliana S. Almeida, Paulo C. Meletti, Cla´ udia B.R. Martinez., (2005). Acute effects of sediments taken from an urban stream on physiological and biochemical parameters of the neotropical fish Prochilodus lineatus. Comparative Biochemistry and Physiology, Part C 140 (2005) 356 – 363

Lyytika¨inem, M., Sormunen, A., Ristola, T., Juvonen, R., Kukkonen, J.V.K., 2001. Toxicity of freshwater sediments in the vicinity of an old sawmill: application of three bioassays. Arch. Environ. Contam. Toxicol. 40, 318 – 326.

Ndimele, Prince Emeka. (2011). Acute toxicity of Nigerian crude oil (Bonny Light) to Desmocaris trispinosa (Crustacea, Palaemonidae). AACL Bioflux. 4. 606-610.

Ndimele, Prince Emeka & Jenyo-Oni, A.. (2010). Evaluation of toxicological impact of Nigerian

crude oil (Bonny Light) to Tilapia guineensis. Journal of Environmental Extension. 8. 10.4314/jext.v8i1.52422.

Nwude, DO; Babayemi, JO; AJIBODE, CP (2020). Heavy Metals Level in Clarias gariepinus (Catfish), Oreochromis niloticus (Tilapia) and Chrysichthys nigroditatus (Bagrid catfish) collected from Ogun River, Ogun State, Nigeria. J. Appl. Sci. Environ. Manage. Vol. 24 (8) 1433-1440

Obot, OI; Isangedighi, AI; David, GS (2016). Heavy metals concentration in some commercial fishes in the lower Cross River estuary, Nigeria. Nig. J. Agric. Food Environ. 12(4):218-223.

Okoroafor U. C. (2011). A handbook of Methodologies for the Evaluation of Acute Aquatic Toxicity of Chemicals and Effluents. Paragraphics, Port Harcourt. ISBN: 978-2954-70-5.

Olusola JO; Festus AA (2015). Levels of Heavy Metal in Some Selected Fish Species Inhabiting Ondo State Coastal Waters, Nigeria. J Environ Anal Toxicol 5: 303.

Reed, W. J. Burchard, Hopson, A.J., Jonathan, J. and Ibraham, Y. (1967). Fish and fisheries of Northern Nigeria. Govt. Press, London, 226pp.

Udeme U. Udofia (2010). Acute Toxicity of Qua Iboe Light Crude Oil on A Fresh Water Fish, Oreochromis niloticus. Global Journal of Pure and Applied Sciences VOL 16, NO. 3, 2010: 295-302.

Ugwu, L.L.C., Ude E.F., Nwamba, H.O. and Chima, I.N. (2011). Effect of Crude Oil and Some Petroleum Products on Clarias Gariepinus Fingerlings (Catfish: Claridae). Continental J. Fisheries and Aquatic Science 5 (1): 24 - 30

Unyimadu, JP; Osibanjo, O; Babayemi, JO (2019). Concentration and Distribution of Organochlorine Pesticides in Sediments of the Niger River, Nigeria. Journal of Health and Pollution: 9 (22): 190606.

USEPA (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA-821-R-02-012. Fifth Edition. Office of Water (4303T), Washington, DC.

Vigano, L., Arillo, A., Falugi, C., Melodia, F., 1998. Histochemical and biochemical markers in trout larvae exposed to river sediments. Chemosphere 37, 2797 – 2807.

Vigano`, L., Arillo, A., Falugi, C., Melodia, F., Polesello, S., 2001. Biomarkers of exposure and effect in flounder (Platichthys flesus) exposed to sediments of the Adriatic Sea. Mar. Pollut. Bull. 42, 887 – 894.

Water Resources Commission (2003). Ghana Raw Water Quality Criteria and Guidelines, GRWQCG: Aquaculture Water Use - Volume 5. Accra, Ghana.