



## ACUTE TOXICITY OF SOME NIGERIAN CRUDE OILS ON BLACK JAW TILAPIA (*Sarotherodon melanotheron*) JUVENILES

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### ABSTRACT

Oil industry activities are sources of major contamination problems in Niger Delta. Pollutants from these activities have significant deleterious effects on aquatic organisms. The study investigated the  $LC_{50}$  values obtained from acute toxicity tests on *Sarotherodon melanotheron* using Oil- in-Water Dispersion (OWD) of the three selected Nigerian crude oils –Ebok, Meji and Erha. The acute toxicity concentrations of 0, 2, 4, 6 and 8ml/L, 0, 10, 15, 20, and 25ml/L and 0, 25, 37.5, 50 and 62.5ml/L were used to determine the 96h Lethal Concentration ( $LC_{50}$ ) of Ebok (heavy), Meji (light) and Erha (medium) crude oils respectively. The analysis of variance (ANOVA) showed that there were significant differences ( $p < 0.05$ ) in the quantal response of *S. melanotheron* to different concentrations of three crude oils at different hours of exposure. These results showed that 96 $LC_{50}$  values for OWD-Ebok, OWD-Meji and OWD-Erha crude oils on *S. melanotheron* were 0.56ml/L, 6.78ml/L and 24.82ml/L respectively. Based on the acute toxicity tests, Ebok with lower API ( $>22.3^{\circ}C$ ) gravity was more toxic than other crudes on *S. melanotheron*. All crude oils were toxic to the fish; their discharge into the water bodies during crudes exploration and exploitation should be discouraged for a safety environment.

**Keywords:** Acute Toxicity, Crude oils, OWD, *S. melanotheron*, API gravity.

### INTRODUCTION

Spill of crude oil and its refined products occur on a frequent basis during routine operations of extraction, transportation, storage, refining and distribution (Zhu *et al.*, 2001). In Nigeria, studies have shown that the quantity of oil spilled over 50 years was a least 9-13 million barrels, which is equivalent to 50 Exxon Valdez spills (FME, *et al.*, 2006). However, the oil spills occurring in the Niger Delta have received less attention in global media, despite significantly higher impacts on human health and the local ecology (UNEP 2011). Oil exploration and exploitation is very lucrative, and a major revenue earner in Nigeria. But, like most industrial activities, it produces environmental hazards that are “slow poisons,” in that they often take months and years to cause disease and death (WHO, 2003). This is unlike the contamination of water, food, and the environment with micro-organisms, which immediately results in ill health (WHO, 2003). The covert and slow action of the hazards created by oil exploration and exploitation make it difficult to fully appreciate their contribution to the disease burden in Nigeria, especially in the oil-bearing communities, even with the emergence of non-communicable diseases as major causes of ill health in Nigeria (WHO, 2005).

Oil spills mainly impact vegetation and wildlife, such as seabirds. Most of the impacts are due to the physical characteristics of the oil. The

adhesive properties lead to reduced mobility and dissolution of natural fats and waxes on body surfaces, feathers etc. (ITOPF 2011a). Certain aromatic petroleum hydrocarbons may also cause direct toxic impacts due to ingestion or penetration through body surfaces such as gills Middleditch (1984); Jenssen (1996); Heubeck *et al.* (2003). Many of the toxic as well as non-toxic hydrocarbons evaporate and are degraded by microorganisms quite rapidly (ITOPF 2011b). However, there may be adverse long-term effects under particular conditions (Peterson *et al.* 2003). An estimated 2 million tons of oil is released into the environment annually from human and natural processes (NRC 2003). About half of this comes from natural seepage of oil into the sea and coastal environments from oil deposits on the continental shelf (NRC 2003).

Crude oil is a complex combination of hydrocarbons consisting predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen, and sulfur compounds and trace amounts of metals (iron, nickel, vanadium, and arsenic). This category encompasses light, medium, and heavy petroleum. Most of them are hydrocarbons that consist of three major types: alkanes, cycloalkanes, and aromatics (Mason, 2002). Alkanes are a class of aliphatic hydrocarbons characterized by open chains of carbon atoms with only single bonds between adjacent

carbon atoms. Simple alkanes include methane, ethane, propane, and hexane. Cyclohexanes are ringed alkanes. They are rather unreactive, non-polar, not readily biodegradable and moderately toxic to aquatic organisms (Irwin, 1997). Aromatic hydrocarbons are composed of hydrogen and carbon, arranged in benzene rings, with low water solubility, and high lipophilicity (Maliszewska-Kordybach, 1999). The aim of this study was to carry-out acute toxicity bioassay (96LC<sub>50</sub>) using Oil in Water dispersion (OWD) prepared from the three Nigerian crude oils (Ebok, Meji and Erha) on Black Jaw Tilapia, *S. melanotheron*.

## MATERIALS AND METHODS

### Selected Crude Oils

Crude oils selected for the purpose of this study were Ebok crude oil, Meji crude oil and Erha crude oil. The selection was based on the American Petroleum Institute gravity (API) and the sulfur content of the crude oil. The API is an inverse measure of petroleum and water. Heavy crude oil has API gravity of < 22.3<sup>0</sup> (density 920 to 1000 kg/m<sup>3</sup>), therefore it floats in water. The medium crude oil has API that is between 22.3<sup>0</sup> and 31.1<sup>0</sup> while light has API >31.1<sup>0</sup>C (Veil and Quinn, 2008) (Table 1). The light crude oil is sweet; this comes from the low sulfur while heavy crude oil is sour with high sulfur content (USEPA, 2011). Ebok crude oil is heavy; Meji crude oil is light while Erha is medium crude oil.

### Source of Crude Oils

Meji, Erha and Ebok crude oils were provided by Chevron, ExxonMobil and Oriental respectively. They all supplied through their loading stations in Portacourt. All the crude oils were gotten through the assistance from Department of Petroleum Resources (DPR) located in Lagos, Nigeria.

### Experimental Design

*S. melanotheron* were supplied by Aquaculture unit, Department of Marine Sciences, University of Lagos. Juveniles of *S. melanotheron* (8.7 ± 0.3) cm were exposed to raw crude oils for acute toxicity tests. *S. melanotheron* was held in glass tanks (60 x 45 x 30cm<sup>2</sup>) at 22.0±1.0°C for 14 days prior to the start of the experiment. They were fed three times a day (3%) of their body weight. The water in the tanks was changed every two days. The photo-period was 12 hour light and 12 hours darkness (OECD, 2000).

### Preliminary Ranging Test

A preliminary toxicity range-finding test was done for the three crude oils (Ebok, Erha and Meji). Range finding is a process where the maximum concentration of toxin is determined in which the organism can survive and the minimum concentration which the organism cannot survive. Groups of three organisms (3) were exposed to several concentrations for 96 hours to carry out preliminary ranging test. The concentration used for Ebok, Meji and Erha crude oils ranged from 1ml/l to 12ml/L, 20ml/L to 40ml/L and 15ml/L to 75ml/L respectively. These were determined based on 0% - 100% mortality of tested organism in 96 hours (Solbe, 1995, Rahman *et al*, 2002)

### Preparation of Oil-in-Water Dispersion (OWD)

The samples of crude oils were measured and directly dropped in water to disperse. Different concentrations were used after preliminary ranged finding had been established on *S. melanotheron* for each crude oil.

### Static Acute Toxicity with OWD Crude Oils

Once the approximate range to be used was determined, acute toxicity bioassays were performed for 96 hours (USEPA, 2002). The concentrations used for Ebok crude oil were: 0ml/L, 2 ml/L, 4 ml/L, 6 ml/L and 8 ml/L, for Erha crude oil: 0 ml/L, 25 ml/L, 37.5 ml/L, 50 ml/L, and 62.5 ml/L and Meji crude oil: 0 ml/L, 15 ml/L, 20 ml/L, 25 ml/L and 30 ml/L, these were exposed to *S. melanotheron*. These concentrations were used after preliminary ranged finding has been established on *S. melanotheron* for different crude oils. New glassware was washed with 10% hydrochloric acid and rinsed with deionized, and dilution water. All containers and equipment were flushed with dilution water before using. The *S. melanotheron* juveniles were gently caught using a hand net in order to avoid stress, into glass tanks measuring 25 X 10 X 15cm from an acclimatized tank. The glass tank was filled with 5 liters of dilution water while treatment tanks were varies depending on the amount of raw crude oils introduced. Three replicates of each concentration with 7 organisms each were run concurrently (USEPA, 2002 and OECD, 2000). Mortalities were access every 24h. The experimental animals were taken as dead when there were no opercula and other forms of body movements even on prodding with a glass rod.

For Acute toxicity test, the concentrations were converted into a logarithm and the corresponding percentage (%) mortality was transformed into probit

values (Sprague, J.B., 1964 and Finney, D.J. 1971). The indices of toxicity measurement and their 95 confidence limit (CL) derived from this analysis were:

LC<sub>50</sub> = Lethal concentration that causes 50% mortality (response) of exposed organism.

LC<sub>95</sub> = Lethal concentration that causes 95% mortality (response) of exposed organism.

LC<sub>5</sub> = Lethal concentration that causes 5% mortality (response) of exposed organism.

The toxicity factor 1 (TF1) for different crude oils was determined using the formula described by Odieta (1999) while the toxicity factor 2 (TF2) was gotten using Zinc Sulphate as reference toxicant on *C. gariepinus* (Ololade and Oginini, 2009).

### Monitoring of Water Quality Parameters

Water quality parameters were monitored before start of experiment, and also specify (daily) according to standard method (OECD, 2000). Parameters that were monitored include; Dissolved Oxygen (DO), hydrogen ion concentration (pH) and temperature (°C). The hydrogen ion concentration was measured using Philip meter (pH-009 111) with glass electrode. The dissolved oxygen (DO) level of the water was taken with (DO) meter (model Eutex DO 600). Water temperature was determined by simple mercury thermometer, calibrated in centigrade (°C).

### Statistical Analysis

All values were expressed as mean + SD and analyzed by SPSS for Win 20.0 software computer program and micro-soft Excel 2010. Analysis of Variance (ANOVA) was used to test for significant differences in the number of survivors in the concentrations of the test toxicants (Ebok, Meji, and Erha crude oils) followed by post hoc test with Duncan Multiple Range Test (DMRT).

## RESULTS

### Water Quality Parameters

The water quality parameters were measured in all the experimental tanks. The results are presented in Table 2 below. The temperature ranged between 24.0 and 30.0°C, pH ranged between 6.6 and 7.5 while dissolved oxygen ranged from 6.3 to 7.8mg/L over the 96h acute toxicity evaluations.

### Relative Toxicity Values and Factors

The relative toxicity values (LC<sub>50</sub>, LC<sub>5</sub> and LC<sub>95</sub>, probit equations and toxicity factors) for oil in Water Dispersion (OWD)-Ebok, Meji and Erha in black jaw Tilapia, *S. melanotheron* are reported in Table 3. The acute toxicity level based on the 96LC<sub>50</sub> values of OWD-Ebok, OWD-Meji and OWD-Erha were found to be 0.56ml/L with 95% confidence intervals of (0.00 – 0.00 ml/L), 6.78ml/L with 95% confidence intervals (0.132 – 10.32 ml/L) and 24.82ml/L with 95% confidence intervals of (13.86 – 16.19 ml/L) respectively when tested against *S. melanotheron*. These results showed that OWD-Ebok was more toxic followed by OWD-Meji while OWD-Erha was least toxic. The TF1 in all toxicants increased as the exposure period increased exception of Erha crude oil. The TF2 ranged between 0.1(Ebok) and 2.3(Erha) (Table 3).

### Mortality Percentages

The analysis of variance (ANOVA) showed that there was a significant differences (p<0.05) in the quantal response at 24h, 48h, 72h and 96h of exposure for the three toxicants with the exception of Meji and Erha at 24h. Furthermore, the analysis using DMRT showed that there was significant difference (p<0.05) in quantal response at the 24h, 48h, 72h and 96h of exposure period and at different concentrations for the three toxicant with the exception of Erha at 48hours (Table 4).

### Probit Analysis

The probit analysis showing the Log concentration plotted against the probit percentage mortality of the *S. melanotheron* in OWD-Ebok, Meji and Erha were presented in Figures 1-3. The coefficient of determination (R<sup>2</sup>) in all crude oil were strong and positive (R<sup>2</sup> = 0.58, 0.90 and 0.99) for Ebok, Meji and Erha respectively which also showed that greater than 90% of the association is dependent on the variable (log concentration and probit mortality) exception of Ebok in *S. melanotheron*. In general, trends indicate that mortality percentage of *S. melanotheron* increased as the concentration of the toxicant and exposure period increased.

No adverse behaviour changes or any mortality were recorded in the control fish throughout the period of the bioassay. The symptoms of toxicosis observed in the fish behaviour were sudden quick movement, erratic swimming, restlessness and rolling movement and swimming on their back. These made the exposed fish very weak, settle at bottom and died

$$\text{TF1} = \text{Toxicity Factor 1} = \frac{\text{LC}_{50} \text{ of Test Compound at 24 hours}}{\text{LC}_{50} \text{ of Test Compound at others hours (48, 72, 96 hours)}}$$

$$\text{TF2} = \text{Toxicity Factor 2} = \frac{\text{LC}_{50} \text{ of Test Compound at 96 Hours}}{\text{LC}_{50} \text{ of Reference Compound (Zinc Sulphate) at 96 hours}}$$

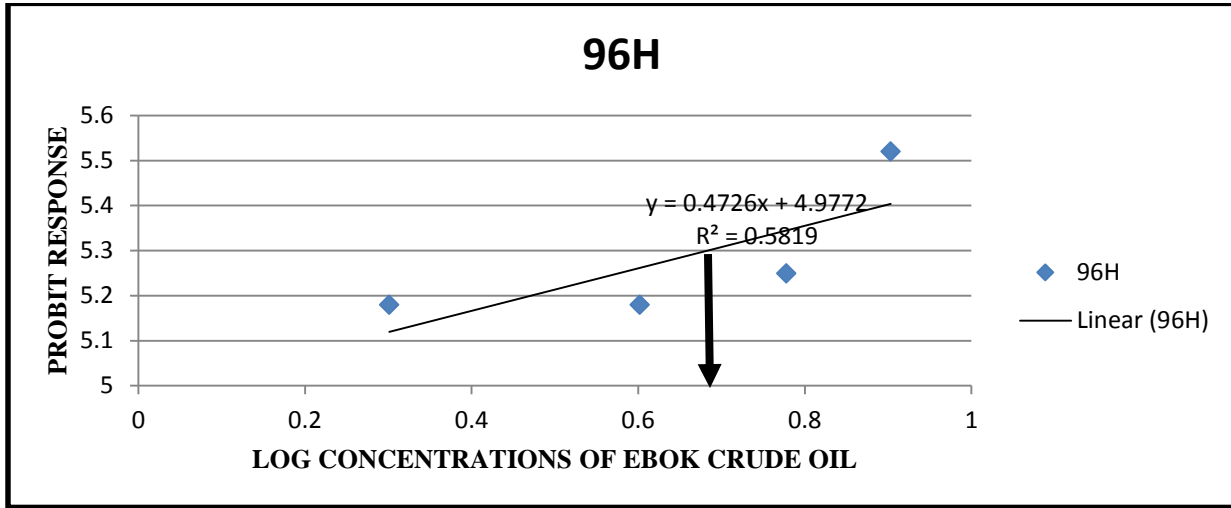


Figure 1: Probit response against log concentration of OWD-Ebok crude oil to *S. melanotheron*

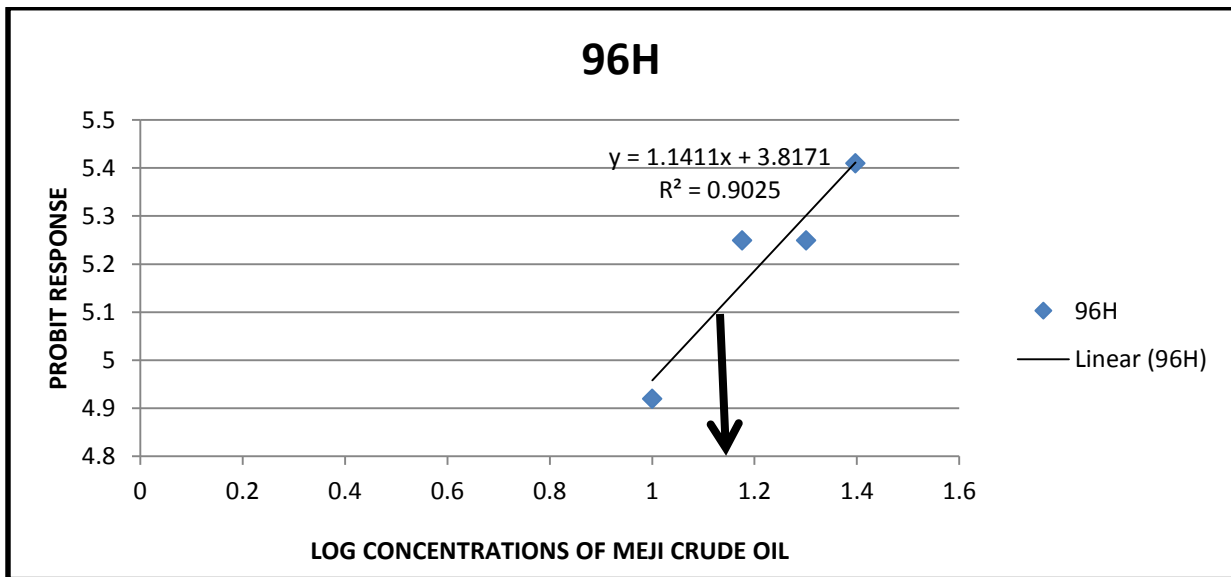


Figure 2: Probit response against log concentration of OWD-Meji crude oil to *S. melanotheron*

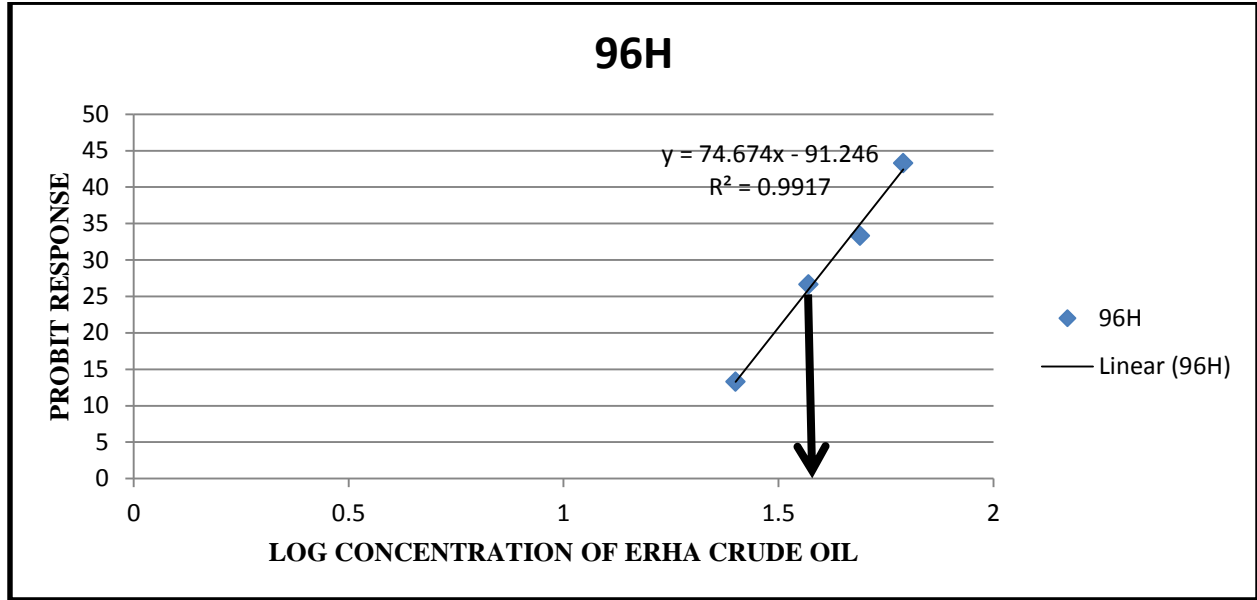


Figure 3: Probit response against log concentration of OWD-Erha crude oil to *S.melanotheron*.

Table 1: API Gravity and Density of Classes of Crude oils

API Type	API	Density	Sulfur content
Light (Meji)	>31.1 <sup>0</sup> and above	<870kg/m <sup>3</sup>	<0.42 (sweet)
Medium (Erha)	Between 22.3 <sup>0</sup> and 31.1 <sup>0</sup>	870 to 920 kg/m <sup>3</sup>	<0.42 (sweet)
Heavy (Ebok)	<22.3 <sup>0</sup> and below	920 to 1000kg/m <sup>3</sup>	>0.50 (sour)

API: American Petroleum Institute

Table 2: Water Quality Parameters of the Experimental Tanks for WAF-Ebok, Meji and Erha on *C. gariepinus*

Parameters	OWD-Ebok		OWD-Meji		OWD-Erha	
	Range	Mean and Standard Deviation	Range	Mean and Standard Deviation	Range	Mean and Standard Deviation
Ph	6.6 – 7.3	6.7±0.62	6.8 – 8.0	7.2±0.78	6.8 – 7.5	6.9±0.66
Dissolved Oxygen (DO)(mg/l)	6.3 – 7.2	6.9±0.28	6.5 – 7.5	7.0±0.23	6.7 – 7.8	7.0±0.40
Temperature(°C)	25.5 – 30.0	28.0± 1.49	26.5 – 28.0	27.5±0.21	24.0 – 28.0	26.0±1.56

OWD = Oil-in-Water Dispersion

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**Table 3: Relative Toxicity Factors of OWD-Ebok, Meji and Erha Crude Oils against *S.melanotheron***

Exposure Time (Hrs)	LC <sub>50</sub> (95% CL) (ml/L)	LC <sub>5</sub> (95% CL) (ml/L)	LC <sub>95</sub> (95% CL) (ml/L)	Slope ± S.E	Probit Line Equation	TF1	TF2
<b>OWD-EBOK</b>							
24	75.35 (0.00-0.00)	1.95 (0.00-0.00)	2917.25 (0.00-0.00)	1.04 ± 0.89	Y = -1.94 + 1.04x	1.00	
48	6.78 (4.72 – 22.74)	0.81 (0.01 – 1.79)	56.64 (19.09 – 5.12x10 <sup>4</sup> )	1.79 ± 0.67	Y = -1.48 + 1.78x	11.11	
72	1.72 (0.00-0.00)	0.07 (0.00-0.00)	0.24 (0.00-0.00)	1.16 ± 0.63	Y = -0.28 + 1.17x	43.81	
96	0.56 (0.00-0.00)	0.03 (0.00-0.00)	10.25 (0.00-0.00)k	1.29 ± 0.78	Y = -0.33 + 1.29	135.76	0.1
<b>OWD-MEJI</b>							
24	0.00 (0.00-0.00)	1.16x10 <sup>13</sup> (0.00-0.00)	0.00 (0.00-0.00)	-0.07 ± 1.03	Y = -0.71 + -0.07x	-----	
48	13.89 (0.00-0.00)	0.58 (0.00-0.00)	329.50 (0.00-0.00)	1.19 ± 0.93	Y = -1.37+1.19x	1.00	
72	11.09 (3.46 – 14.32)	2.58 (0.01 – 5.73)	46.99 (28.49 – 1089.05)	2.61 ± 0.98	Y = -2.72+2.61x	1.26	
96	6.78 (0.13 – 10.31)	1.67 (0.00- 4.38)	27.61 (19.35 – 60 87)	2.69 ± 1.14	Y = -2.24+2.69x	2.05	1.45
<b>OWD-ERHA</b>							
24	400.84 (0.00-0.00)	8.79 (0.00-0.00)	1.8x10 <sup>4</sup> (0.00-0.00)	0.99±1.13	Y= -2.58+0.99x	1.00	
48	13.26 (1.03-21.99)	49.94 (39.66 – 82.91)	188.02 (100.43 –473.00)	2.85±0.99	Y = -4.85+2.85x	30.23	
72	32.30 (16.77 –40.93)	8.06 (0.19 – 16.01)	129.43 (70.88 – 123.92)	2.73±0.97	Y = -4.12+2.73x	12.41	
96	24.82 (13.86 –16.19)	9.61 (1.60 – 15.96)	64.08 (49.68 – 141.80)	3.99±1.16	Y = -5.57+3.99x	16.15	2.3

LC = Lethal Concentration, CL = Confident Limit, S.E = Standard Error, TF = Toxicity Factor

**Table 4: Percentage Mortality of *S. melanotheron* Exposed to Different Concentrations of OWD-Ebok, Meji and Erha Crude Oils**

Concentration(ml/L)	N	24H	48H	72H	96H
<b>OWD-EBOK</b>					
0	21	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
4	21	4.76 <sup>ab</sup>	19.05 <sup>b</sup>	52.38 <sup>b</sup>	80.95 <sup>b</sup>
6	21	9.53 <sup>ab</sup>	33.33 <sup>c</sup>	66.67 <sup>b</sup>	80.95 <sup>b</sup>
8	21	14.29 <sup>b</sup>	38.09 <sup>c</sup>	76.19 <sup>b</sup>	85.71 <sup>b</sup>
10	21	14.29 <sup>b</sup>	61.90 <sup>d</sup>	80.95 <sup>b</sup>	100.00 <sup>b</sup>
<b>OWD-MEJI</b>					
0	21	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
15	21	19.05 <sup>ab</sup>	42.86 <sup>a</sup>	47.62 <sup>a</sup>	66.66 <sup>a</sup>
20	21	19.05 <sup>ab</sup>	52.38 <sup>ab</sup>	61.91 <sup>ab</sup>	85.71 <sup>ab</sup>
25	21	23.81 <sup>b</sup>	57.14 <sup>bc</sup>	71.43 <sup>b</sup>	85.71 <sup>b</sup>
30	21	23.81 <sup>b</sup>	61.90 <sup>c</sup>	85.71 <sup>b</sup>	95.24 <sup>b</sup>
<b>OWD-ERHA</b>					
0	21	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>
37.5	21	9.53 <sup>a</sup>	19.05 <sup>a</sup>	42.86 <sup>ab</sup>	57.14 <sup>b</sup>
50	21	19.05 <sup>a</sup>	38.09 <sup>a</sup>	47.62 <sup>b</sup>	66.67 <sup>b</sup>
62.5	21	19.05 <sup>b</sup>	47.62 <sup>a</sup>	71.43 <sup>b</sup>	85.71 <sup>b</sup>
75	21	19.05 <sup>b</sup>	61.90 <sup>a</sup>	80.95 <sup>b</sup>	100.00 <sup>b</sup>

Means with the same superscript letter(s) in a column are not significantly different in the DMRT (p<0.05).N = Number of Animals, OWD= Oil-in-Water Dispersion

## DISCUSSION

In the current study, the 96LC<sub>50</sub> for OWD-Ebok, Meji and Erha were 0.55 ml/L, 6.78 ml/L and 24.82ml/L respectively (Table 2). The Ebok crude oil was heavy, which USEPA (2011) describes as viscous, black, and having low toxicity. The Meji sweet crude oil was light, described as highly fluid and toxic. Also, since the 96LC<sub>50</sub> established for Ebok crude was lower compared to the 96LC<sub>50</sub> of other crude oils, this suggested that the heavy crude oil toxicity effect was gradual and more toxic as the exposure days were increased. Again, the toxicity of crude oils is not only based on the chemical properties but as well on the physical properties. The viscosity of the Ebok could then be ascribed to its toxicity. Ebok crude settled at the top and took hours before dispersed into the water column which made fish to die as a result of suffocation rather than toxic effects. This result supports the findings Ayoola and Alajabo (2012) of acute toxicity of engine oil on Black jaw Tilapia *S.melanotheron*. It agrees with the study of Olaifa (2005) who studied the toxicity of Nigerian Qua Iboe Light crude oil to *Clarias gariepinus*. It also agrees with the findings of Sogbanmu and Otitolaju (2014) who reported the 96LC<sub>50</sub> of 5.06 ml/L of Forcados Light Crude Oil to *Clarias gariepinus*. Murakami et al., (2008) and NRC (2003) stated that increase toxicity of light crude oil is primarily caused by concentrations of aromatic hydrocarbons and less viscosity than heavy one thus requiring less mixing energy for toxic concentrations to mix into the water column. Neff (2000) had shown that the toxicity of heavy oils to be of physical or mechanical nature and chemical toxicity due to light oils. Neff et al. (2000) also stated that light oils are rich in aromatic hydrocarbons and known to be readily soluble and toxic. In toxicity tests, zinc is used as a reference toxicant, that is, to demonstrate acceptable laboratory performance, and to assess the sensitivity and health of organisms (USEPA, 2002).

The toxicity factor 2 of Ebok was lower compared to other crude oils which showed that *S.melanotheron* was more sensitive to Ebok crude oil. Several abnormal behaviour such as incessant jumping and gulping of air, restlessness, surface to bottom movement, sudden quick movement, resting at the bottom were similar to the observations of Omoniyi et al. (2002), Rahman et al. (2002) and Aguiwo (2002). The significant differences ( $p < 0.05$ ) from OWD-Ebok, Meji and Erha showed that mortality increased as the crude oil concentration increased and the exposure days increased (Table 2 and 3). This supports the observation of Fryer (1977) and Ayoola (2008), who

found that in all toxicant; a threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. There were strong and positive coefficient of determinations in the log concentration and probit mortality in *S. melanotheron* for Meji and Erha crude oils. This agreed with the findings of Ndimele et al. (2010) who recorded the 0.98 coefficient of determination on *Tilapia guineensis* using Bonny light crude oil. According to work by Imevbore et al. (1987), some of the Nigerian crude oils associated with high toxicity level are Forcados Blend (FB), Bonny Light (BL) and Bonny Medium (BM), to mention a few ones. Acute toxicity bioassays are a pre-screening tool for the chemical assessment of polluted water (De Zwart and Slooff, 1983). USEPA (1996) stated that the purpose of acute toxicity tests with fish is to compare them with other species' acute testing and to help to determine water quality criteria.

## CONCLUSION

Ebok crude oil is more toxic than other two Nigerian crude oils on *S. melanotheron*, this is due to the high viscosity of the heavy crude oil. Therefore, a lot of factors have to put into consideration to draw out a conclusion on the toxicity of a chemical, these includes; physical and chemical properties of a toxicant, exposure duration, preparation of exposure media, exposure method, species and species habitat. Bioassays are an important tool used to provide background information for risk assessment of chemicals. This study gives baseline information on the three Nigerian crude oils based on their different American Petroleum Institute (API) gravity.

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