COMPARATIVE STUDY OF ACUTE TOXICITY OF THREE NIGERIAN CRUDE OILS USING OIL IN WATER DISPERSION (OWD) METHOD ON *CLARIAS GARIEPINUS* (AFRICAN CATFISH)

[3]Omogoriola H.O, [1]Ayoola S.O and [2]Otitoloju A.A power tussle
[1]Department of Marine Science, University of Lagos, Lagos State, Nigeria.
[2]Department of Zoology, University of Lagos, Lagos State, Nigeria.
[3]Nigerian Institute for Oceanography and Marine Research, No 3, Wilmot Point Road, Victoria Island, PMB 12927, Marina Lagos, Nigeria

ABSTRACT
Oil industry activities such as exploration, transportation, storage, use and disposal, as well as oil spills are sources of major contamination problems in Niger Delta, which have significant deleterious effects on aquatic organisms. The study was to investigate LC_{50} values obtained from acute toxicity tests on C.gariepinus using Oil in Water Dispersion (OWD) of Ebok, Meji and Erha. The acute toxicity concentrations of Ebok (0, 4, 6, 8 and 10ml/l), Meji (0, 15, 20, 25, and 30 ml/l) and Erha (37.5, 50, 62.5 and 75ml/l) were used to determine the 96h Lethal Concentration (LC_{50}) respectively. The analysis of variance (ANOVA) showed that there was a significant difference ($p<0.05$) in the quantal response of C.gariepinus to different concentrations of Ebok, Meji and Erha crude oils at 24, 48, 72 and 96hours of exposure. These results showed that $96LC_{50}$ values for Ebok, Meji and Erha crude oils on C.gariepinus were 6.35 ml/l, 18.35 ml/l and 32.04ml respectively. These showed that Ebok was three times more toxic than Meji and five times more toxic than Erha while Meji crude oil was two times more toxic than Erha on C.gariepinus. Based on the acute toxicity tests, Ebok with lower API gravity is more toxic than other crude oils in C.gariepinus. All crude oils are toxic to aquatic organisms especially the fish; their discharge into the water bodies during crude oil exploration, transportation and storage should be discouraged for a safety environment.

Keywords: *Acute Toxicity, Crude oil, Oil in water Dispersion, C. gariepinus, API gravity, Nigeria*

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 and the likes. (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). Crudev oii 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. Cyclohexacnes 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).

Previous researches on acute toxicity of Nigerian crude oils were based on Bonny light, Forcados, Qua Iboe and several other crude oils. However, information of Ebok (Heavy), Meji (Light) and Erha (Medium) crude oils in relationship with their American Petroleum Institute (API) gravity is not available. Despite the economic importance of these three variants of crude oil to Nigerian government revenues, little is known about their acute toxicity levels on fish, especially on *Clarias gariepinus*. The aim was to carry-out acute toxicity bioassay ($96LC_{50}$) using Oil in Water dispersion (OWD) prepared from the three Nigerian crude oils (Ebok, Meji and Erha) on African Catfish, *Clarias garepinus.*

MATERIALS AND METHODS

SELECTED CRUDE OILS

Crude oils selected for the purpose of this study are Ebok, Meji and Erha crude oils. 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 $< 22.3^{o}$ (density 920 to 1000 kg/m^3), therefore it float in water. The medium crude oil has API that is between 22.3^{0} and 34^{0}while light has API >340 (Veil and Quinn, 2008). The light crude oil is sweet; this comes from the low sulfur while heavy crude oil is sour with high sulfur content (NOAA, 2012).Ebok crude oil is heavy, Meji crude oil is light while Erha is medium crude oil.

SOURCES 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 Porthacourt. All the crude oils were gotten through the assistance from Department of Petroleum Resources (DPR) located in Lagos, Nigeria.

EXPERIMENTAL DESIGN

African Catfish, *Clarias gariepinus* was supplied by Aquculture unit, Department of Marine Science, University of Lagos. Fingerlings of *C.garipenus* (Total length 5.55 ± 0.59 cm and Weight 6.10 ± 0.87 g) were used for raw crude oils acute toxicity tests. 150 fingerlings were stocked in different tanks but with the same dimensions. They were held in glass tanks (60 x 45 x 30cm^2) at 22.0±1.0°C for 14

days prior to the start of the experiment. They were fed with commercial feed at 3% of their body weight twice daily, 6:00am – 8.00am and 6:00pm - 9.00pm. The habitat water in the tanks was replaced every two days. The photo-period was 12 hour light and 12 hours darkness (OECD, 2000).

ACUTE TOXICITY STUDY (96h LC_{50})

RANGE FINDING 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 48 hours. These were determined based on 0% - 100% mortality of tested organism in 48 hours (Solbe, 1995, Rahman *et al*, 2002). The following concentrations were used for OWD-Ebok: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml, for OWD-Meji: 5,10,15,20, 25, 30, 40, 50 and 60ml, for OWD-Ebok: 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 30, 40, 50, 60, and 70ml.

PREPARATION OF OIL WATER DISPERSION (OWD)

The samples of crude oils were directly dropped in water to disperse. This was used after preliminary range finding had been established on *Clarias garipenius* for acute toxicity test.

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: 0ppm, 4ppm, 6ppm, 8ppm and 10ppm, for Erha crude oil: 0ppm, 37.5ppm, 50ppm, 62.5ppm and 75ppm and Meji crude oil:0ppm, 15ppm, 20ppm, 25ppm and 30ppm, these were exposed to *Clarias gariepinus*. 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 *C.gariepinus* 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 (OECD, 2000 and USEPA, 2002 and). Different concentrations were used because these concentrations were drawn from established range test values. Mortalities were assessed every 24h over a 96-hours experimental period. The experimental

animals were taken as dead when there were no opercula and other forms of body movements even on probing with a glass rod.

MONITORING OF WATER QUALITY PARAMETERS

Water quality parameters were monitored before the start of experiment, and also specify (daily) according to standard method (OECD, 2000) for 96 hours. Parameters that were monitored include; Dissolved Oxygen (DO), hydrogen ion concentration (pH) and temperature (^{O}C)

PROBIT CALCULATIONS

The indices of toxicity measurement derived from this analysis were median lethal concentration that caused 50% mortality (response) of exposed organism (LC_{50}), lethal concentration that causes 90% mortality (response) of exposed organism (LC_{90}) and sub lethal concentration that causes 10% mortality (response) of exposed organism (LC_{10}) and their 95 confidence limit (CL). The toxicity factor 1 (TF1) for different crude oils was determined using the formula described by Odiete (1999) while the toxicity factor 2 (TF2) was gotten using Zinc Sulphate as reference toxicant on the same species (Ololade and Oginni, 2009).

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

$$\text{TF1 = Toxicity Factor 2 } = \frac{LC_{50} \text{ of Test Compound at 96 Hours}}{LC_{50} \text{ of Reference Compound (Zinc Sulphate) at 96 hours}}$$

STATISTICAL ANALYSIS

All values were expressed as mean $\pm$ SD and analyzed by SPSS for Win 20.0 software computer program and micro-soft Excel 2010. Acute toxicity test, the concentrations were converted into a logarithm and the corresponding percentage (%) mortality was transformed into probit values (Sprague, 1964 and Finney, 1971). 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

Relative and Comparative Toxicity of Oil in Water Dispersion (OWD) of Ebok, Meji and Erha Exposed to *C. gariepinus*

The relative toxicity values (LC_{50}, LC_5 and LC_{95}, probit equations and toxicity factors) for oil in Water Dispersion (OWD) of Ebok, Meji and Erha in African Catfish (*C.gariepinus*) are reported in Table 1 while the mortality percentage in relation to exposure period are reported in Table 2.

The acute toxicity level based on the $96LC_{50}$ at 95% confidence intervals of Ebok, Meji and Erha were found to be 6.45ml/l (5.41 – 7.53 ml/l), 18.35ml/l (15.81-20.32) and 32.04ml/l (11.41 – 40.06 ml/l) (11.41 – 40.06 ml/l) respectively.

Zero mortality was recorded at 24hours for Ebok on *C. gariepinus.* However, these results showed that OWD-Ebok was more toxic followed by Meji while Erha was least toxic. The TF1 in all the toxicant increases as the exposure period increased and TF2 were 0.05, 0.07 and 0.09 for Ebok, Meji and Erha respectively (Table 1).

The analysis of variance (ANOVA) showed that there were significant differences ($p<0.05$) in the quantal response at 24h, 48h, 72h and 96h of exposure for the three toxicants. 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 toxicants except for Ebok and Erha at 24h and 48h respectively (Table 2).

The probit analysis showing the Log concentration plotted against the probit percentage mortality of the *C.gariepinus* against OWD-Ebok, Meji and Erha were presented in Figures 1a-c. The coefficient of determination (R^2) in all crude oil were strong and positive (R^2 = 0.95, 0.91 and 0.92) for Ebok, Meji and Erha respectively that showed greater than 90% of the association was dependent on the variable (log concentration and probit mortality). In general, trends indicate that mortality percentage of *C.gariepinus* increased as the concentration of the toxicants 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

Table 1: RELATIVE TOXICITY FACTOR OF OWD EBOK, MEJI AND ERHA CRUDE OILS AGAINST *CLARIAS GARIEPINUS*

Exposure Time (Hrs)	LC_{50} (95%CL) (ml/L)	LC_5 (95%CL) (ml/L)	LC_{95} (95%CL) (ml/L)	Slope ± S.E	Probit Line Equation	TF1	TF2
OWD-EBOK							
24	----------	----------	----------	----------	----------	------	-------
48	12.08 (10.42-197.57)	19.25 (13.29-108.29)	7.57 (1.68-8.66)	8.12±3.69	Y = -8.78+8.12x	1	
72	8.89 (7.52-12.61)	22.43 (14.67- 93.06)	3.53 (1.39-4.71)	4.09±1.16	Y = -3.89+4.09x	1.36	
96	6.45 (5.41 – 7.53)	74.71 (11.19-29.01)	2.83 (1.35-3.80)	4.59±1.06	Y = -3.72+4.59x	1.87	0.05
OWD-MEJI							
24	44.35 (0.00-0.00)	384.37 (0.00-0.00)	5.76 (0.00-0.00)	1.75±1.31	Y = -2.88+1.75x	1	
48	21.08 (17.86-24.41)	45.37 (34.39 – 104.39)	9.79 (4.01 – 13.13)	4.94±1.32	Y =-6.54+4.94	2.10	
72	19.19 (16.38-21.47)	31.12 (26.65-44.50)	10.33 (5.64-13.11)	6.10±1.41	Y = -7.83+6.10x	2.31	
96	18.35 (15.81-20.32)	31.59 (27.02 – 44.69)	10.66 (6.48 – 13.17)	6.97±1.50	Y = -8.81+6.97x	2.42	0.07
OWD-ERHA							
24	102.184 (79.96– $6.77X10^{12}$)	180.74 (106.33-0.00)	57.77 (0.00 – 70.51)	6.64±3.38	Y= -13.35+6.64x	1	
48	71.81 (64.52 – 89.89)	122.12 (95.03-263.13)	42.23 (27.28-49.31)	7.13±1.90	Y = -16.65+7.13x	1.42	
72	43.38 (28.10 – 51.10)	110.46 (80.75– 408.39)	17.03 (2.32 -26.95)	4.05±1.31	Y = -6.63+4.05x	2.35	
96	32.04 (11.41 – 40.06)	74.07 (59.73 – 195.93)	13.86 (0.75 – 23.72)	4.52±1.60	Y = -6.80+4.51x	3.19	0.09

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

Table 2: Percentage Mortality of *Clarias Gariepinus* Exposed to Different Concentrations of OWD-Ebok, Meji and Erha Crude Oils

Concentration(mg TPH/l)	N	24H	48H	72H	96H
OWD-Ebok					
0	21	--------	0.00 [a]	0.00 [a]	0.00 [a]
4	21	---------	0.00 [a]	8.25 [a]	14.29[ab]
6	21	---------	0.00 [a]	24.73 [bc]	47.62 [bc]
8	21	---------	9.53 [a]	16.49 [bc]	71.33 [c]
10	21	--------	33.33 [b]	43.64 [c]	76.19[c]
OWD-Meji					
0	21	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
15	21	23.81 [ab]	26.51a	33.33 [a]	33.33 [a]
20	21	19.05 [ab]	4.76 [ab]	38.09 [ab]	47.62 [ab]
25	21	38.09 [b]	8.25[bc]	80.95 [b]	85.71 [b]
30	21	42.85 [b]	12.59 [c]	80.95 [b]	95.24 [b]
OWD-Erha					
0	21	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
37.5	21	0.00 [a]	0.00 [a]	35.95[ab]	66.67 [b]
50	21	0.00 [a]	14.29 [a]	21.82 [b]	76.19 [b]
62.5	21	14.29 [b]	42.86 [a]	21.82 [b]	85.71 [b]
75	21	14.29 [b]	21.82 [a]	16.49 [b]	100 [b]

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

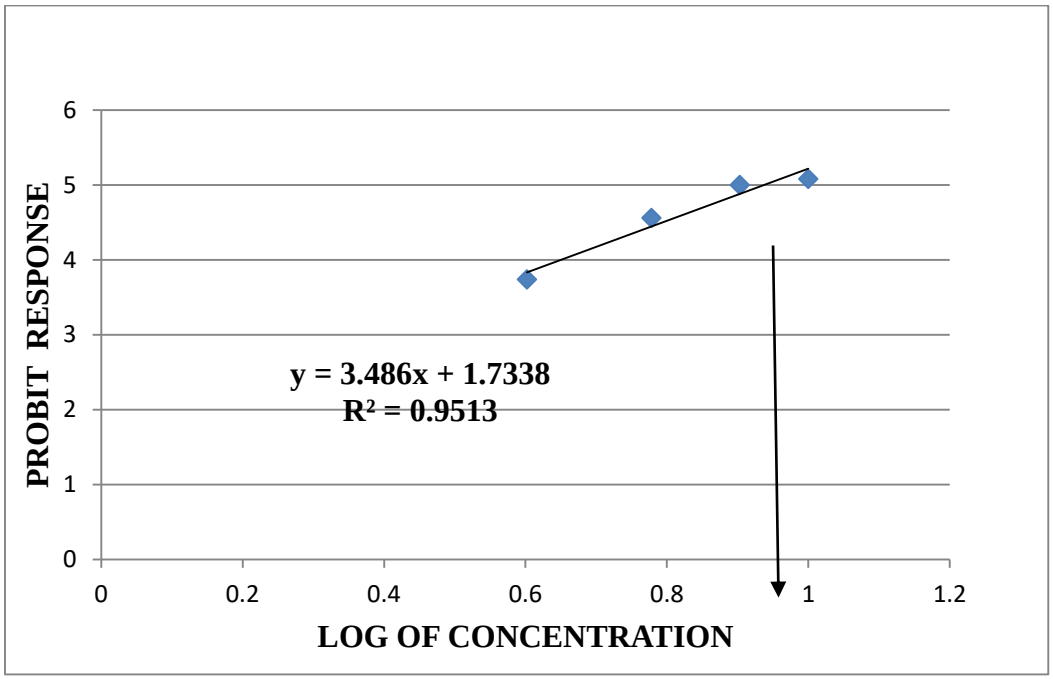

Figure 1a: Probit response against log concentration of OWD-Ebok crude oil to *Clarias garipenius*

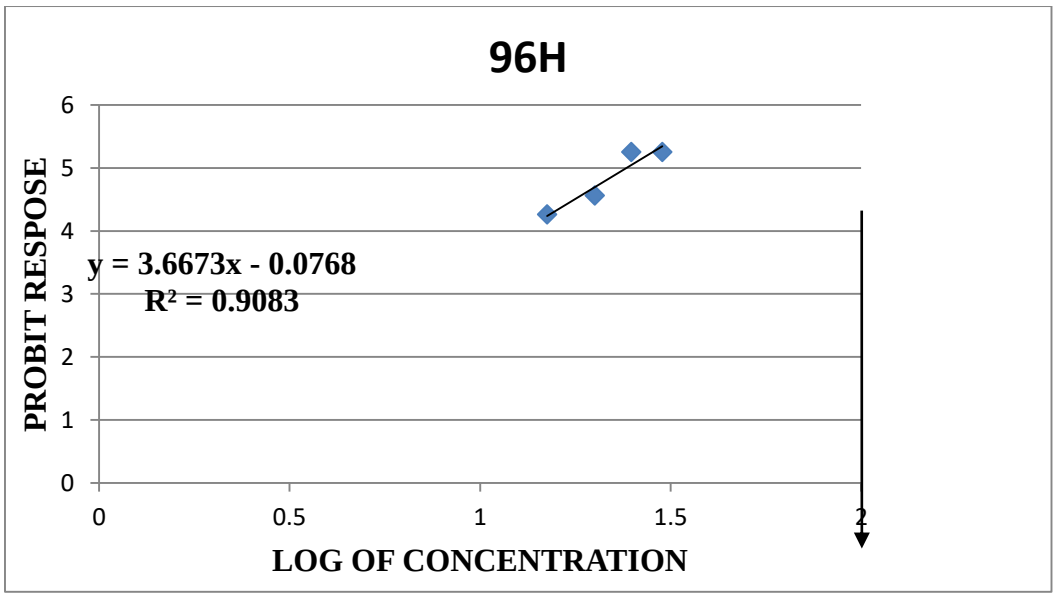

Figure 1b: Probit response against log concentration of OWD-Meji crude oil on *Clarias garipenius*

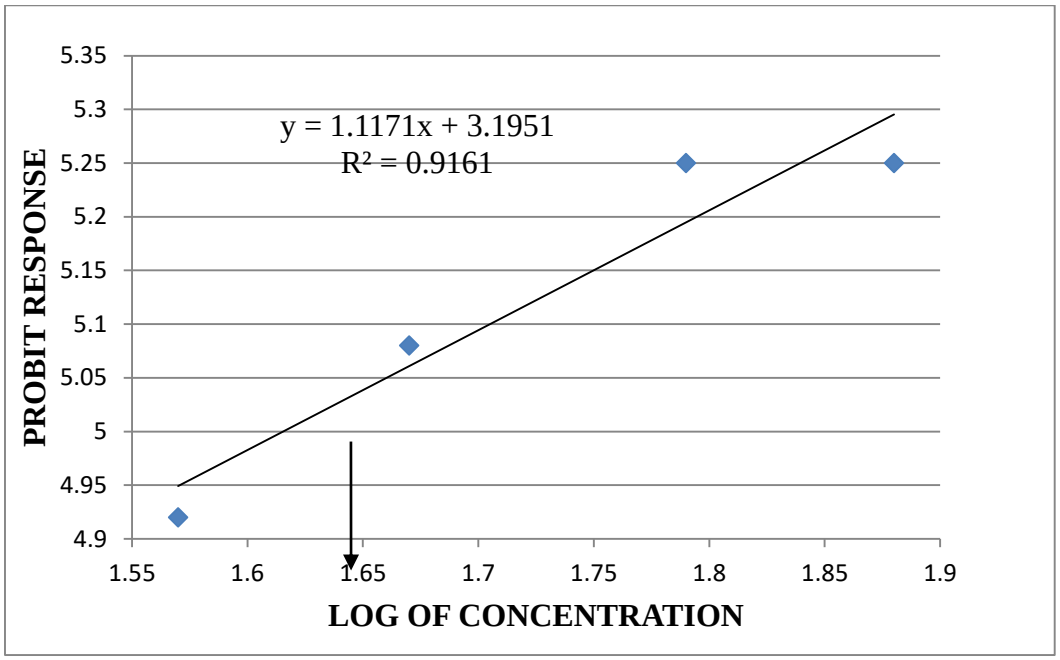

Figure 1c: Probit response against log concentration of OWD-Erha crude oil on *Clarias gariepnius*

DISCUSSION

In the current study, the LC_{50} for OWD-Ebok, Meji and Erha were 6.45, 18.35 and 32.04ml/l respectively. No mortality was observed in the highest concentration within the first 24 hours of exposure for OWD-Ebok crude oil while highest mortality was observed within the 24 hours of exposure to OWD-Meji (Table 1 and 2). These results showed that Ebok crude oil was five times toxic than Erha crude oil and three times more toxic than Meji crude oil while Meji crude oil was two times toxic than Erha crude oil using oil in water dispersion (OWD) method of preparation on *C.garipenius*. 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 there was no mortality in the highest concentrations of OWD-Ebok within 24 hours, this suggested that the heavy crude oil toxicity effect was gradual and more toxic as the exposure days were increased. The results from OWD-Ebok, Meji and Erha showed that mortality increased as the crude oil concentration increased and the exposure days increased and these differences were significant ($p<0.05$) (Table 1 and 2). This result agrees with the study of Olaifa (2005) which studied

the toxicity of Nigerian Qua Iboe Light crude oil on *Clarias gariepinus*. It also agreed with the findings of Sogbanmu and Otitoloju (2014) which also studied the toxicity of Forcados Light Crude Oil on the same species of fish. It also supported the findings Ayoola and Alajabo (2012) of acute toxicity of engine oil on Black jaw Tilipia *S.melanotheron*. Murakami (2008) and NRC (2003) stated that increased toxicity of light crude oils is primarily caused by two factors: (1) light crude oils often have concentrations of aromatic hydrocarbons, and (2) light crude oils are usually less viscous than heavy one thus requiring less mixing energy for toxic concentrations to be mixed into the water. Neff *et al*. (2000) had shown that the toxicity of heavy oils to be of physical or mechanical nature and chemical toxicity due to light oils. The light oils are rich in aromatic hydrocarbons. These are known to be readily soluble and toxic (Neff et al; 2000).

According to the 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.

Neff *et al.*(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. 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 Aguigwo (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 (Tables 1 and 2). This supported 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 C. gariepinus for the three 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.

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 also to determine water quality criteria.

CONCLUSIONS

Ebok crude oil was more toxic than other two Nigerian crude oils on *C. gariepinus*. Due to the properties of the crude oils, Ebok crude oil was considered to be heavy and less toxic compared to light crude oils, therefore, a lot of factors have to be 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 gave baseline information on the three Nigerian crude oils based on their different American Petroleum Institute (API) gravity which showed that heavy crude oil could be more toxic than light crude oil because of the high viscosity.

REFERENCES

Aguigwo J.N. (2002). The toxic effect of cymbush pesticide on growth and survival of African catfish, *Clarias gariepinus* (BURCHELL1822). *J. aquat. sci.* 17 (2): 81-84.

Ayoola S.O. (2008). Histopathologyical effects of glyphosate on juvenile African catfish (Claria gariepinus). *Am. Env. J. Agric. Environ. Sci.* 4:362-367.

Ayoola S.O, Alajabo O.T. (2012). Acute Toxicity and Histopathological Effects of Engine Oil on *Sarotherodon melanotheron* (Black Jaw Tilapia). *American-Eurasian Journal of Toxicological Sciences*.;4(1): 48-55.

De Zwart, D. and Slooff, W. (1983). The Microtox as an alternative assay in the acute toxicity assessment of water pollutants. *Aquatic Toxicology*, **4**: 129-138.

Federal Ministry of Environment (FME, 2006), Abuja, Nigerian Conservation Foundation Lagos, WWF UK and CEESP-IUCN Commission on Environmental, Economic, and Social Policy, May 31,(2006). Niger Delta Resource Damage Assessment and Restoration Project.

Finney, D.J. (1971). Probit Analysis, Third Editions. Cambridge University Press, London, UK, 318pp.

Fryer J.D. Weed control handbook Vol.1 Edited by Make Peace. 1977;384-389.

Heubeck, M., Camphuysen, K.C.J., Bao, R., Humple, D., Sandoval Rey, A., Cadiou, B. and Bräger, S. (2003). Assessing the impact of major oil spills on seabird populations. *Marine Pollution Bulletin*, **46**: 900–902.

Imevbore, A.M.A., Adeyemi, S.A. and Afolabi, O.A. (1987). The toxicity of Nigerian crude oils to aquatic organisms. The petroleum Industry and the Nigerian Environment: Proceeding of 1987 by The Nigerian National Petroleum Corporation, pp 171 - 176.

Irwin, R.J. (1997). Environmental contaminants encyclopedia.National Park Service, Water Resources Divisions. Fort Collings, Colorado.

ITOPF (2011a). Effects of oil pollution on the marine environment. International Tankers Owners Pollution Federation (ITOPF).

ITOPF (2011b). Fate of marine oil spills. International Tankers Owners Pollution Federation (ITOPF).

Jenssen, B.M. (1996). An overview of exposure to, and effects of, petroleum oil and organochlorine pollution in grey seals (*Halichoerus grypus*). *Science of the Total Environment*, **186**: 109–118.

Maliszewska-Kordybach, B. (1999). Sources, concentrations, fate and effects of polycyclic aromatic hydrocarbons (PAHs) in the environment. Part A: PAHs in air. *Polish Journal of Environmental Studies*, **8**: 131-136.

Mason, C. (2002). Biology of Freshwater Pollution. Fourth Edition. Prentice Hall.

Middleditch, B.S. (1984). Ecological effects of produced water effluents from offshore oil and gas production platforms. *Ocean Management*, **9**: 191–316.

Murakami, Y., Kitamura, S.I., Nakayama, K., Matsuoka, L. and Sakaguchi, H. (2008). Effects of heavy oil in the developing spotted halibut, Verasper variegates. *Marine Pollut. Bull.*, **57**: 524-528.

National Research Council (NRC) (2003). Oil in the sea III. Input, fates and effects. Washington. DC: National Academic Press.

Ndimele P.E, Jenyo-Oni A, Jibuike C.C. (2010). Comparative Toxicity of Crude oil, Dispersant and Crude Oil-Plus-Dispersant to *Tilapia guineensis*. *Research Journal of Environ.Toxicol*., 4:13-22.

Neff, J.M., Ostazeski, S., Gardiner, W. and Stejskal, I. (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. *Environ Toxicol. Chem.,* **19**: 1809-1821.

Odiete, W.O. (1999). Environmental Physiology of Animals and Pollution Diversified Resources Ltd Lagos. 261p. Olaifa, F. E. (2005). Assessment of toxicological impact of light crude oil on *Clarias gariepinus* (Burchell, 1822) fingerlings. *Afr. J. of Livest. Ext.,* **4**: 42-46.

OECD. 2000. Guidance document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 23. Paris, September 2000.

Olaifa, F. E. (2005). Assessment of toxicological impact of light crude oil on *Clarias gariepinus* (Burchell, 1822) fingerlings. *Afr. J. of Livest. Ext.,* **4**: 42-46.

Ololade, I.A. and Oginni, O. (2009). Behavioural and hematological effects of zinc on African catfish, *Clarias gariepinus*. *International Journal of Fisheries and Aquaculture*, **1**: 22-27.

Peterson, C.H., Rice, S.D., Short, J.W., Esler, D., Bodkin, J.L., Ballachey, B.E. and Irons, D.B. (2003). Long-term ecosystem response to the Exxon Valdez oil spill. *Science*, **302**: 2082–2086.

Rahman MZ, Hossain Z, Mullah MFR, Ahmed GU (2002). Effect of Diazinon 60EC on *Anabus testudineus*, *Channa punctatus* and *Barbades gomonotus*. NAGA. The ICLARM Quarterly, 25: 8-11.

Sogbanmu, T.O. and Otitoloju, A.A. (2014). Joint Action Toxicity and Biochemical effects of Binary Mixtures of Forcados Light Crude Oil and Three Dispersants against *Clarias gariepinus. Int. J. Environ. Res.*, **8**(2): 395-340.

Solbe JF (1995). Fresh water in: Handbook of Ecotoxicology (Edited by Peter Collins) Black Well Science Ltd. Osneymeed OX 20EL. 683pp.

Sprague, J.B., 1964. Lethal concentrations of copper and zinc for young Atlantic salmon. Journal of the Fisheries Research Board of Canada 21, 17-26

United Nations Environmental Programme (UNEP. (2011). Environmental Assessment of Ogoniland. Published by United Nations Environmental Programme, Nairobi, Kenya. 7-49pp.

USEPA (2011). 2011 Edition of the Drinking Water Standards and Health Advisories. EPA 820-R-11-002. U.S. Environmental Protection Agency, Washington, D.C.

USEPA, (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th ed. EPA-821-R-02-012. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USEPA, 1996. Ecological effects test guidelines. EPA 712-C-96-118. U.S. Environmental Protection Agency, Washington, D.C.

Veil, J.A. and Quinn, J.J. (2008). Water issues associated with heavy oil production. Argonne National Laboratory ANL/EVS/R-08/4.

WHO, (2005): Facing the facts: The impact of chronic disease in Nigeria. Geneva. [Last accessed on 2011 Mar 12]. Available from: http://www.who.int/chap/chronicdisease report/en/

WHO, (2003). The World Health Report: 2002: Reducing risks, promoting healthy life. Geneva: World Health Organization. 1–71pp.

Zhu, X., Suidan, M.T. and Lee, K. (2001). Guidelines for the Bioremediation of Marine Shorelines and Fresh Water Wetlands.US Environmental Protection Agency, Cincinnati, OH.