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Acute Toxicity of Water Accommodated Fractions (WAFs) of three Nigerian Crude Oils to *Clarias gariepinus* (Burchell, 1822)

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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 objective of this study was to report LC_{50} values obtained from acute toxicity tests on the African Catfish, *C. gariepinus* exposed to Water Accommodated Fraction (WAF) –heavy (*Ebok*), light (*Meji*) and medium (*Erha*) crude oils. Acute toxicity concentrations of 0%, 2%, 4%, 6% and 8%, 0%, 15%, 20%, 25% and 30% and 0%, 40%, 60%, 80% and 100% were used to determine the 96h Lethal Concentration (LC_{50}) of heavy, light and medium crude oils respectively. The analysis of variance (ANOVA) showed that there was a significant ($p<0.05$) difference in the quantal response of *C. gariepinus* to different concentrations of the various crude oil types at 24, 48, 72 and 96 hours exposure. These results showed that $96LC_{50}$ values for heavy, light and medium crude oils on *C. gariepinus* were 0.028 mg TPH/l, 0.177 mg TPH/l and 0.742 mg TPH/l respectively. The $96LC_{50}$ of WAF showed that the heavy crude oil was six times more toxic than light and twenty six times more toxic than medium and on toxicity categorization, the heavy, light and medium crude oils were very highly toxic, highly toxic and highly toxic on *C. gariepinus*. Based on the acute toxicity tests, heavy with lower API ($<22.3^0C$) gravity was more toxic than other crude oils on *C. gariepinus*. All crude oils are toxic to aquatic organisms especially the fish; their discharge into the water bodies during crude oil exploration, transportation, storage and even sabotage should be discouraged to protect the environment.

Keywords: Acute Toxicity, Crude oils, Water Accommodated Fraction, *C. gariepinus*, 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 at 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 chronic in nature, 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, 2011). 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 two 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 African catfish *Clarias gariepinus (*Burchell, 1822) is an economically important siluriforme species that contributes significantly to annual fish productions in many countries of the world, but most especially in Africa and many parts of Asia. It is cultured both intensively and extensively in Africa and Asia (Huisman & Richter, 1987), but most intensively in some countries in Europe and in many continents across the world (Galbusera *et al.*, 2000). This species belongs to the Clariidae family teleost catfish that is found mostly as freshwater fish, except for two families, Ariidae and Plotosidae that have marine species (Teugels, 1996). They are bottom dwellers but obligate air-breathers. They inhabit a variety of freshwater environments, including quiet waters such as lakes, ponds, and pools; are very prominent in flowing rivers, rapids, and around dams; and very adaptive to extreme environmental conditions.

C. gariepinus can live in very poorly oxygenated and eco-degraded waters (Pienaar, 1968). They can live at a pH range of 6.5–8.0; and at temperatures of 8–35^{o}C with their optimal growth temperature being 28–30^{o}C (Teugels, 1986). *C. gariepinus* has become increasingly important globally, being a good source of protein as well as being easy to culture, plus having a high growth rate and resistance to diseases. Moreover, it can tolerate high stocking densities, a wide range of temperatures, low dissolved oxygen as well as high salinity levels, and most importantly it has low production input requirements and high returns (Teugels, 1986; Omitogun and Aluko, 2002; Mahmoud *et al.*, 2009). This study carried out acute toxicity bioassay (96LC_{50}) using Water Accommodated Fraction (WAF) prepared from the three Nigerian crude oils heavy (*Ebok*), light (*Meji*) and medium (*Erha*) on *C. gariepinus*.

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 (API) gravity 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 < 22.3^0 (density 920 to 1000 kg/m^3), therefore it floats in water. The medium crude oil has API that is between 22.3^0 and 31.1^0 while light has API >31.1^0C (Veil and Quinn, 2008, NOAA, 2010) (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 a 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 Port Hacourt. All the crude oils are obtained through the assistance of the Department of Petroleum Resources (DPR) located in Lagos, Nigeria.

Animal Acclimation

Fries of African Catfish, *Clarias gariepinus* were supplied by Aquaculture Unit, Department of Marine Sciences, University of Lagos. A total of seven hundred post fry (mean weight 0.72 ± 0.10 g; mean total length 4.56 ± 0.20 cm) of *C. gariepinus* 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 used for both preliminary ranging and semi acute toxicity tests. They are fed three times a day at the rate of 3% of their body weight. The water in the tanks was changed every two days. The photo-period was 12 hours 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 ten *C. gariepinus* in three replicates were exposed to several concentrations for 96 hours. These were determined based on 0% - 100% mortality of tested organism in 96 hours (Solbe, 1995, Rahman *et al*, 2002).

Preparation of Water Accommodated Fraction (WAF)

To carry out toxicity bioassays on the crude oils, the water accommodated fraction (WAF) was prepared. The water accommodated fraction is a solution free of particles of bulk material (i.e., droplets > 1 µm diameter) derived from mixing (no vortex) test material and water (Aurand and Coelho, 1996). The principle of this experiment is to introduce oil at the water surface of a closed flask. Two litres of borosilicate conical flask was used and closed off with cotton bud and foil paper. It was filled with 1 L of dilution water (borehole water from Marine Science Department, University of Lagos) and 25grams of each of the crude oil samples was added leaving a 20% headspace above the liquid. A stir bar was used to stir the mix on a magnetic stir plate for 22 hours in darkness. The solution was allowed to settle for an hour and the prepared solution was separated via a port at the bottom of the conical flask. The mixture was used immediately after preparation (USEPA, 2010; Singer *et al.,* 2000). The prepared WAFs were used for *Clarias garipenus* acute toxicity tests.

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

API Type	API Gravity	Density	Sulfur Content
Light (Meji)	>31.1^0 and above	<870kg/m^3	<0.42 (sweet)
Medium (Erha)	Between 22.3^0 and 31.1^0	8m70 to 920 kg/m^3	<0.42 (sweet)
Heavy (Ebok)	<22.3^0 and below	920 to 1000kg/m^3	>0.50 (sour)

Source: Vein and Quinn, 2008, NOAA, 2010

Semi-Static Acute Toxicity with WAFs Crude Oils

Water Accomodated Fractions acute toxicity bioassay was performed for 96 hours (USEPA, 2002) on *C.gariepinus* with 10 organisms in three replicates at different concentrations. On the three oils, the percentages of WAF used for Ebok were: 0%, 2%, 4%, 6%, and 8%, for Erha: 0%, 40%, 60%, 80% and 100%, and Meji: 0%, 15%, 20%, 25% and 30%. 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 use. *Clarias gariepinus* were gently caught using a hand net in order to avoid stress. Borosilicate glass beakers of 250 ml were used as exposure chambers with 200 ml of respective test solutions. Three replicates of each concentration with 10 organisms each were run concurrently (OECD, 2000 and USEPA, 2002). 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, 1964 and Finney, 1971). The indices of toxicity measurement and their 95 confidence limit (CL) derived from this analysis were:

LC_{50} = Lethal concentration that causes 50% mortality (response) of exposed organism.

LC_{95} = Lethal concentration that causes 95% mortality (response) of exposed organism.

LC_{5} = 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 Odiete (1999) while the toxicity factor 2 (TF2) was obtained using Zinc Sulphate as reference toxicant on *C. gariepinus* (Ololade and Oginini, 2009).

$$\text{Tf1} = \text{Toxicity Factor 1} = \frac{LC_{50} \text{ of Test Compound at 24 hours}}{LC_{50} \text{ of Test Compound at others hours (48, 72, 96 hrs)}}$$

$$\text{TF2} = \text{Toxicity Factor 2} = \frac{LC_{50} \text{ of Test Compound at 96 hrs}}{LC_{50} \text{ of Ref. Compound (Zinc Sulphate) at 96 hours}}$$

Monitoring of Water Quality Parameters

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

Statistical Analysis

All values were expressed as mean $\pm$ SD and analysed by SPSS for Win 20.0 software computer programme 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. There were significant ($p<0.05$) differences in the concentrations of dissolved oxygen, pH and temperature for WAF-Ebok, Meji and Erha crude oils exception of temperature for WAF-Ebok and Meji at different treatment.

Relative Toxicity Factors

The relative toxicity values (LC_{50}, LC_{5} and LC_{95}, probit equations and toxicity factors) for Water Accommodated Fraction (WAF)-Ebok, Meji and Erha in Catfish (*C.gariepinus*) are reported in Table 3. The acute toxicity level based on the 96LC_{50} values of WAF-Ebok, WAF-Meji and

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

WAF Conc.	Dissolve oxygen	(mg/L)	pH		Temperature ^{O}C	
	Mean and SE	**Range**	**Mean and SE**	**Range**	**Mean and SE**	**Range**
			WAF- Ebok (heavy)			
0%	6.47 ± 0.17^{c}	6.20 – 6.80	7.40 ± 0.21^{c}	7.10 – 7.80	27.50 ± 0.28^{a}	27.00 – 28.00
2%	6.00 ± 0.12^{abc}	5.80 – 6.20	6.93 ± 0.08^{bc}	6.80 – 7.10	26.73 ± 0.28^{a}	26.40 – 27.30
4%	5.80 ± 0.12^{ab}	5.60 – 6.00	6.70 ± 0.45^{bc}	5.80 – 7.20	27.00 ± 0.26^{a}	26.50 – 27.40
6%	6.20 ± 0.31^{bc}	5.80 – 6.80	5.73 ± 0.18^{ab}	5.80 – 6.40	27.50 ± 0.45^{a}	26.10 – 27.60
8%	5.50 ± 0.21^{a}	5.20 – 5.90	6.57 ± 0.26^{a}	5.30 – 6.20	27.50 ± 0.57^{a}	27.40 – 27.60
Mean	5.99 ± 0.11	5.20 – 6.80	± 0.19	5.30 – 7.80	27.14 ±0.13	26.10 – 28.00
WAF-Meji (Light)						
0%	6.17 ± 0.12^{c}	6.00 – 6.40	7.13 ± 0.67^{d}	7.00 – 7.20	26.60 ± 0.20^{a}	26.20 - 26.80
15%	6.00 ± 0.12^{bc}	5.80 – 6.20	6.93 ± 0.67^{cd}	6.80 – 7.00	26.83 ± 0.03^{a}	26.80 – 26.90
20%	5.63 ± 0.21^{ab}	5.20 – 5.90	6.80 ± 0.00^{bc}	6.80 – 6.80	27.07 ± 0.07^{a}	27.00 – 27.20
25%	5.70 ± 0.06^{ab}	5.60 – 5.80	6.53 ± 0.13^{b}	6.40 – 6.80	27.10 ± 0.55^{a}	26.00 – 27.70
30%	5.40 ± 0.12^{a}	5.20 – 5.60	6.13 ± 0.13^{a}	6.00 – 6.40	27.00 ± 0.00^{a}	27.00 – 27.00
Mean	5.78 ± 0.09	5.20 – 6.40	6.71 ± 0.38	6.00 – 7.20	26.92 ± 0.43	26.00 – 27.70
WAF-Erha (Medium)						
0%	6.33 ± 0.07^{b}	6.20 – 6.40	7.30 ± 0.15^{b}	7.00 – 7.50	26.50 ± 0.25^{a}	26.00 – 26.76
40%	6.07 ± 0.07^{ab}	6.00 – 6.20	7.20 ± 0.11^{b}	7.00 – 7.40	26.92 ± 0.13^{ab}	26.70 – 27.14
60%	5.80 ± 0.12^{ab}	5.60 – 6.20	6.47 ± 0.26^{a}	6.00 – 6.90	27.28 ± 0.15^{b}	27.12 - 27.58
80%	5.53 ± 0.35^{a}	5.00 – 6.20	6.63 ± 0.88^{a}	6.50 – 6.80	27.54 ± 0.20^{b}	27.14 – 27.78
100%	5.60 ± 0.12^{a}	5.40 – 5.80	6.63 ± 0.67^{a}	6.20 – 6.40	27.55 ± 0.21^{b}	27.14 – 27.80
Mean	5.87 ± 0.10	5.00 – 6.40	6.78 ± 0.12	6.00 – 7.50	27.15 ± 0.13	26.00 – 27.80

Means with the same superscript letter(s) in a column are not significantly different in the DMRT (p=0.05).
WAF = Water Accommodated Fraction

WAF-Erha were found to be 0.028mg TPH/l with zero 95% confidence intervals, 0.177mg TPH/l with 95% confidence intervals (0.103 – 0.212 mg/l) and 0.742 mg TPH/l with 95% confidence intervals of (0.632 – 0.914 mg/l) respectively when tested against *C. gariepinus*. These results showed that OWD-Ebok was more toxic followed by OWD-Meji while OWD-Erha was least toxic. Ebok was six times more toxic than Meji and twenty six times more toxic than Erha. The TF1 ranged between 48h (1.00) and 96h (24.68), 24h (1.00) and 96h (78.93) and 24h (1.00) and 96h (3.61) for WAF-Ebok, Meji and Erha respectively. This means toxicant effects increases as the exposure period increased. The TF2 were 0.05, 0.07 and 0.09 for WAF-Ebok, WAF-Meji and WAF-Erha respectively (Table 3). Different crude oils tested on fish species are compared with the three Nigeria crude oils in the present study. Previous studies ranged from the Bonny light (> 0.01 mg TPH/l) to the Nigeria crude oil (5.06 mg TPH/l) (Table 4).

Percentage Mortality

The mortality percentages in relation to exposure period are reported in Table 5. The analysis of variance (ANOVA) showed that there was a significant difference ($p<0.05$) 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 expected at 24h, 48h and 72hours of exposure for WAF-*Ebok* (Table 5).

Toxicity Categorization

US EPA's 96LC_{50} aquatic toxicity scale for laboratory-generated aquatic toxicity data was used to determine the toxicity category of the three crude oils. Toxicity levels of WAF-*Ebok, Meji* and *Erha* on *C.gariepinus* were very highly toxic, highly toxic and highly toxic respectively (Tables 6 and 7).

Probit Analysis and Equations

The probit analysis showing the Log concentration plotted against the probit percentage mortality of the *C. gariepinus* on WAF-Ebok, *Meji* and *Erha* were presented in Figures 1-3. The coefficient of determination (R^2) in all crude oil were strong and

Table 3: Relative Toxicity Factor of WAF-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**
				WAF-Ebok			
24	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 ± 0.00	0	------------	
48	0.691 (0.00 – 0.00)	0.001 (0.00 – 0.00)	24.805 (0.00 – 0.00)	0.82 ± 0.611	y = 0.821+0.824x	1.00	
72	0.206 (0.00 – 0.00)	0. 00 (0.000 - 0.004)	14.963 (0.00 – 0.00)	0.68 ± 0.534	y = 0.473+0.69x	3.35	
96	**0.028 (0.00 – 0.00)**	0.00 (0.00 – 0.00)	1.328 (0.00 – 0.00)	0.77 ± 0.768	y = 1.188+0.768x	24.68	0.67
				WAF-Meji			
24	13.97 (0.00 - 0.00)	0.006 (0.00 - 0.00)	1033.4 (0.00 – 0.00)	0.69±1.39	y = -0.785+0.686x	1.00	
48	0.364 (0.284 -1.565)	0.644 (0.001 - 0.112)	0.952 (0.507 - 70.470)	3.08±1.18	y = 1.348+3.075x	38.38	
72	0.267 (0 . 2 4 3 - 0.593)	0.046 (0.01- 0.088)	0.706 (0.431 - 9.945)	3.03±1.07	y = 1.741+3.034x	52.32	
96	**0.177 (0.103- 0.212)**	0.029 (0.000 - 0.067)	0.478 0(.334 - 3.172)	2.97±1.06	y = 2.233+2.971x	78.93	2.15
				WAF-Erha			
24	2.679 (0.00 – 0.00)	0.701 (0.00 – 0.00)	5.58 (0.00 – 0.00)	0.84±1.25	y = 1.430+0.840x	1.00	
48	2.311 ($1.211-8.7X10^9$)	0.23 (0.000 - 0.423)	8.24 ($2.569 - 4.2X10^{21}$)	1.73±0.95	y= -0.655+1.728x	1.16	
72	1.17 (0.901 - 3.165)	0.165 (0.11 - 0.299)	3.448 (1.810 - 66.832)	2.72±0.84	y = 0.118+2.724x	2.29	
96	**0.742 (0.632 - 0.914)**	0.16 (0.042 - 0.260)	1.728 (1.241 - 4.124)	3.49±0.85	y = 0.453+3.490x	3.61	0.09

LC = Lethal Concentration, CL = Confident Limit, WAF = Water Accommodated Fraction, S.E = Standard Error

positive (R^2 = 0.99, 0.84 and 0.97) 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) in *C. gariepinus*. 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, settling at the bottom and death occurs eventually.

Table 4: Median Lethal Concentrations (LC50) and Median Lethal Loadings (LL50) for Different Crude Oil Toxicity Tests on Different Fish Species

Fish species name	Crude oil Type	96- h LC_{50} (mgTPH/l)	96- h LL_{50} (mg/l)	Reference
Clarias gariepinus	Qua Ibeo light	1.58	NA	Olaifa, 2005
Clarias gariepinus	Nigeria crude	2.19	NA	Awoyinka *et al*, 2011
Claria anguillarias	Nigeria crude	1.22	NA	Awoyinka *et al*, 2011
Clarias gariepinus	Forcados Light crude	5.06	NA	Sogbamu and Otitoloju,2014
Sarotherodon melanotheron	Bonny light	0.01	NA	Seiyaboh *et al*, 2013
Menidia beryllina	Alaska North Slope	0.35	3520	Rhoton *et al.* (2001)
Menidia beryllina	Prudhoe Bay	> 0.5	> 8152	Rhoton *et al.* (2001)
Piaractus brachypomus	Louisiana sweet	2.05	17700	Reátegui-Zirena *et al*, 2013
Piaractus brachypomus	Peruvian	> 4.00	> 50000	Reátegui-Zirena *et al*, 2013
Pimephales promelas	Peruvian	1.83	22920	Reátegui-Zirena *et al*, 2013
Clarias gariepinus	Ebok heavy crude	0.028	NA	Present study
Clarias gariepinus	Meji light crude	0.177	NA	Present study
Clarias gariepinus	Erha medium crude	0.742	NA	Present study

Note: TPH = Total petroleum hydrocarbons, NA = not available.

Table 5: Percentage Mortality of *Clarias Garipenus* Exposed to Different Concentrations of WAF- Ebok , Meji and Erha Crude Oils

Concentration (%)	N	24H	48H	72H	96H
WAF-Ebok					
0	30	3.33^{a}	3.33^{a}	3.33^{a}	3.33^{a}
2	30	0^{a}	16.67^{a}	26.67^{a}	46.67^{b}
4	30	0^{a}	6.67^{a}	30.00^{a}	50.00^{b}
6	30	0^{a}	6.67^{a}	26.67^{a}	56.67^{b}
8	30	13.33^{a}	36.67^{a}	46.67^{a}	66.67^{b}
WAF-Meji					
0	30	3.33^{a}	3.33^{a}	3.33^{a}	0.00^{a}
15	30	6.67^{a}	13.33^{a}	26.67^{ab}	43.33^{b}
20	30	13.33^{a}	20.00^{ab}	33.33^{ab}	53.33^{b}
25	30	13.33^{a}	26.67^{ab}	33.33^{ab}	66.67^{b}
30	30	10.00^{a}	43.33^{b}	63.33^{b}	76.67^{b}
WAF-Erha					
0	30	0^{a}	0^{a}	0^{a}	0^{a}
40	30	6.67^{a}	13.33^{a}	13.33^{a}	20^{a}
60	30	0^{a}	6.67^{a}	20^{ab}	30^{ab}
80	30	6.67^{a}	20^{a}	40^{bc}	60^{b}
100	30	10^{a}	30^{a}	53.33c	63.33^{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, WAF = Water Accommodated Fraction

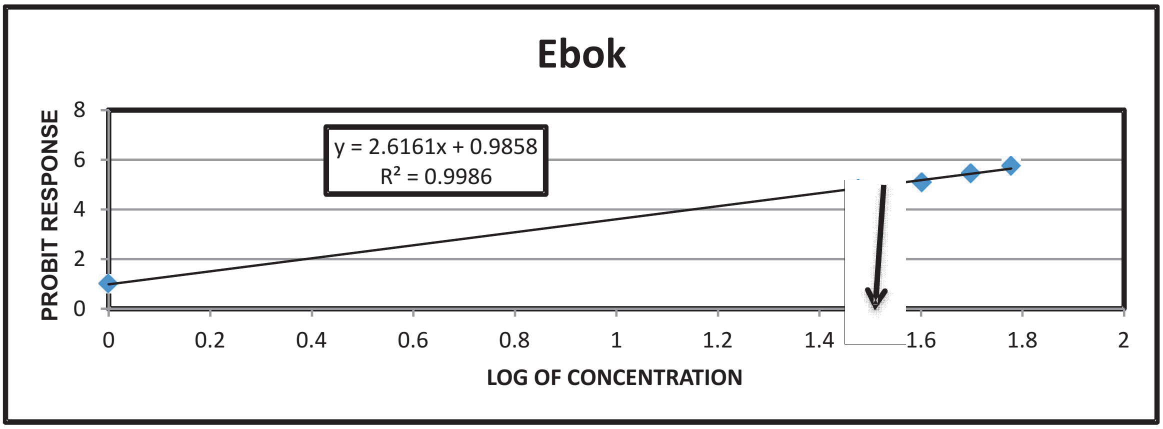

Figure 1: Probit response against log concentration of WAF-Ebok crude oil to *Clarias gariepinus*

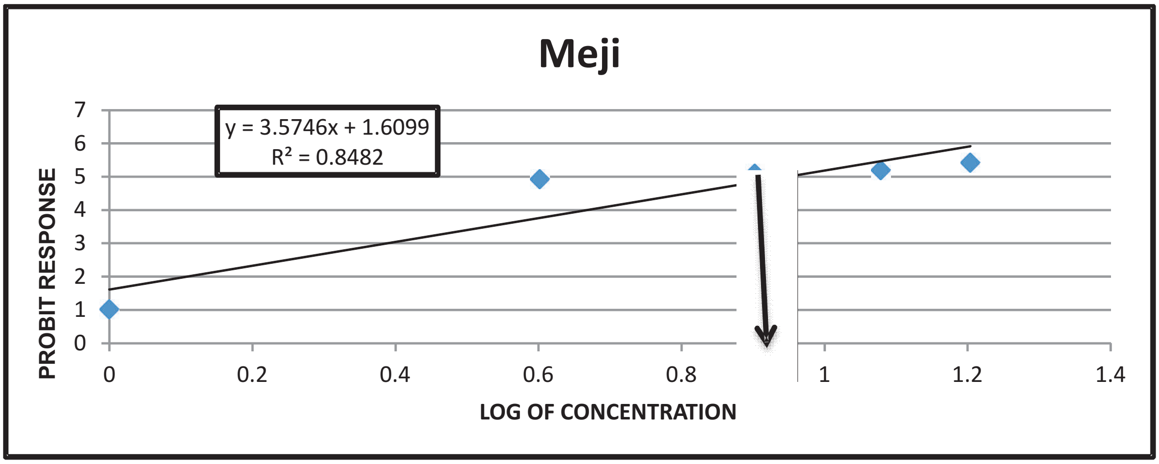

Figure 2: Probit response against log concentration of WAF-Meji crude oil to *Clarias gariepinus*

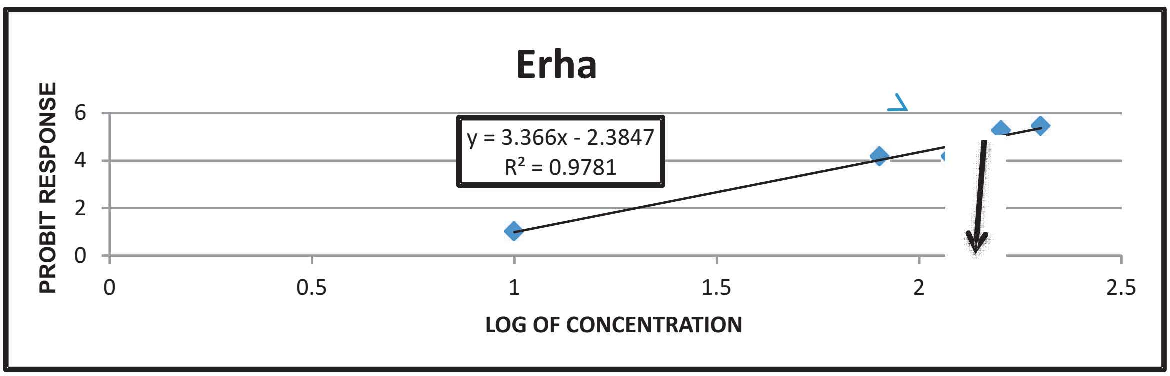

Figure 3: Probit response against log concentration of WAF-Erha crude oil to *Clarias gariepinus*

Table 6: USEPA's 96LC_{50} Aquatic Toxicity Scale

96LC_{50}	Toxicity Category
<0.1mg/l	Very highly toxic
0.1 – 1 mg/l	Highly toxic
1 – 10 mg/l	Moderately toxic
10-100 mg/l	Slightly toxic
>100mg	Practically non-toxic

Source: USEPAS, 2002

Table 7: Toxicity Category of the three Nigerian Crudes on *C. gariepinus* based on USEPA Toxicity Scale

Type of Crude	96LC_{50}	95CL	Toxicity Category
WAF-EBOK	0.028	------------	Very highly toxic
WAF-MEJI	0.177	0.103-0.202	Highly toxic
WAF ERHA	0.742	0.632 0.914	Highly toxic

Discussion

The 96LC_{50} of WAF-Ebok is lower than the 96LC_{50} of WAF-Meji and WAF-Erha which contradict the findings of Dupuis and Ucan-Marin, (2015) which stated that, light crude oil are generally considered to be more toxic than heavy crude oils. However, Neff *et al*., (2000) proved that the toxicity of heavy oils said to be the physical or mechanical properties rather than the chemical components compared to light oils. This result suggests that heavy crude oil toxicity effects are gradual and more toxic as the exposure days are increased. Also, the light crude oil toxicity is immediate and reduces as the exposure time increases. According to NRC (2003), and Murakami (2008), the increased toxicity of light crude oils is primarily caused by two factors: (1) light crude oils often have higher 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. The light oils are rich in aromatic hydrocarbons, these are known to be readily soluble and toxic (Neff *et al.,* 2000). This result agreed with the study of Olaifa (2005) who studied the toxicity of Nigerian Qua Iboe Light crude oil to *Clarias gariepinus*. It also agreed with the findings of Sogbanmu and Otitoloju (2014), who also studied the toxicity of Forcados Light Crude Oil to the same species of fish. It also supports the findings of Ayoola and Alajabo (2012) of acute toxicity of engine oil on Black jaw Tilipia *S.melanotheron*. It is also in line with the results of Rhoton *et al*., (2001) on Alaska North Slope and Prudhoe Bay using Inland Silverside (Menidia beryllina).

On the toxicity USEPA's scale, very highly toxic, highly toxic and highly toxic recorded for the Ebok, Meji and Erha were different from the findings of Reátegui-Zirena *et al*., (2013) of moderately toxic of Water Accommodated Fractions (WAFs) of Peruvian crude on red pacu (*Piaractus brachypomus*) and Fathead minnow *Pimephales promelas*. It also contradicts the findings of Artin and Thomas (2011) on Louisiana sweet crude (LSC) who reported non-toxic effects of inland silverside fish using USEPA toxicity scales.

Different crude oils tested on fish species are compared with the three Nigeria crude oils in the present study. Previous studies ranged from the Bonny light (> 0.01 mg TPH/l) to the Nigeria crude oil (9.35 mg TPH/l) (Table 4). However, comparisons on effects of crude oil WAF are difficult since the composition of hydrocarbons in the oils vary depending on their density and origin (Neff *et al.,* 2000). Other factors influencing the widely different results is the preparation method of the WAF between studies, which include room temperature, mixing energy, settling period, and the tolerance to crude oil of the species tested (Singer e*t al.,* 2001). Furthermore, toxicity of crude oil seems to be lower in marine species compared to freshwater probably due to hydrocarbon solubility and lower bioaccumulation in fish when salinity is increased (Ramachandran *et al.,* 2006).

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 WAF-Ebok, Meji and Erha showed that mortality increased as the crude oil concentration increased and the exposure days increased. 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 *C. gariepinus* for all the three crude oils. This agreed with the findings of Ndimele *et al.* (2010) who recorded 0.98 coefficient of determination on *Tilapia guineensis* using Bonny light crude oil. According to the study of 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 determine water quality criteria.

Conclusion

Ebok crude oil is more toxic than other two Nigerian crude oils on *C. gariepinus*, this is due to the high viscosity of the heavy crude oil.

Therefore a lot of factors have to be into considered to draw out a conclusion on the toxicity of chemicals, these include; physical and chemical properties of a toxicant, exposure duration, preparation of exposure media, exposure method, species and species habitat. Bioassays are important tools 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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