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Assessment of Biodegradation and Toxicity of Drill-Muds Used in an Onshore Active Field Located in Edo State, Nigeria

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Abstract

The biodegradation and toxicity of two drill-muds used in an onshore oil field located in Edo State were examined. Biodegradation of drill-muds by two bacterial and fungal isolates; *Citrobacter* sp., *Staphylococcus* sp., *Aspergillus* sp. and *Penicillium* sp. were carried in a shake flask experiment using mineral salts medium at 120 rpm for 28 days. The total viable counts were monitored and ultimate biodegradability was derived from the ratio of Chemical Oxygen Demand and Biological Oxygen Demand (BOD_5), after every four days. The water based mud was more degradable than non-aqueous based mud. This was shown by the highest total viable counts recorded in consortium amended with water based mud (126 ×103 cfu/ml), and also recording the lowest chemical oxygen demand and biological oxygen demand (45 mg/l and 0.38 mg/l, respectively). There were no significant differences ($P > 0.05$) in the degradation of the muds by the isolates. The 96 hrs and 24 hrs acute toxicity bioassay were carried out using juvenile fishes *(Tilapia guineensis)* and microorganisms (*Staphylococcus* sp.and *Aspergillus* sp.), respectively. The different concentrations were prepared for fishes (in aquaria) and microorganisms (conical flask). Mortality was recorded after 8, 24, 48, 72 and 96 hrs and 0, 2, 4, 8, 12 and 24 hr at 30 0C to assess toxicity. The 96 hr lethal concentration, 50 % (LC_{50}) of water based mud and Non-Aqueous Based Mud (NABM) were greater than10, 125 mg/l and 6000 mg/l for *Tilapia guineensis*, respectively. The 24 hr lethal concentration, 50 % (LC_{50}) of water based mud was 370 mg/l for *Staphylococcus* sp. and *Aspergillus* sp. Therefore, these selected isolates have the potential applications in the bioremediation of sites polluted by these drill-muds. Also, in the interest of the environment, oil exploration and production companies operating in Edo State and other parts of Nigeria should strictly adhere to the use of non-toxic and biodegradable drilling muds during exploration activities.

Keywords: Toxicity, biodegradation, drilling muds, bacteria, fungi.

1. Introduction

In oil and gas operations, drilling fluids, also referred to as drilling muds, are used to lubricate and cool the drilling apparatus, transport drill cuttings to the surface and seal off porous geologic formations (Odokuma and Ikpe 2003; Okoro, 2011; Imarhiagbe and Atuanya, 2013; Linjun, 2013). Drilling fluids typically consist of bentonite and a range of additives mixed with fresh water or hydrocarbons. The two primary types of drilling muds are water based muds and non-aqueous based muds (Mairs et al., 1999). Water based muds consist of water mixed with bentonite clay and additives, such as barium sulfate (barite); they are used for most types of drilling. The non-aqueous drilling muds (NABM) comprise all non-water and non-dispersible based muds and they include Oil Based Muds (OBM), Low Toxicity Mineral Based Muds (LTMBM), Enhanced Mineral Oil Based Muds (EMOBM) and Synthetic Based Muds (SBM) (Mairs et al., 1999; Anwuli, 2011; Ogeleka and Tudararo-Aherobo, 2013) and are mostly used in offshore wells or other water sensitive formations.

According to Odokuma and Akpanah (2008), in Nigeria, drilling muds and cuttings are sometimes discharged into fills and from where they over flow into nearby farms and rivers. Small amounts are re-injected into special Cutting Re-Injection (CRI) wells while lesser amounts are treated in Thermal Desorption Units (TDU). The three basic types of drilling muds are water based mud, oil based mud and synthetic based mud (Okoro, 2011). Researchers have abundantly shown that drilling muds additives may contain toxic substances such as heavy metals, hydrocarbons, biocides, chromate, organic polymers and trace elements that have the tendency to bioaccumulate and interfere with normal biological activities of organisms (Odokuma and Ikpe, 2003; Odokuma and Akponah, 2008; Vincent-Akpu et al.,2010). Studies, according to Engelhard et al. (1989) and Vincent-

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Akpu et al. (2010) have been conducted with various drilling fluids in the North Sea using mortality as the criterion for determining their effects on the biota. Odokuma and Ikpe (2003) observed that water based muds were more biodegradable than oil based muds. They ascribed this observation to the greater toxicity of oil based muds.

According to Ogeleka and Tudararo-Aherobo (2013), an average of 7000 to 13000 bbl of waste per well, composing approximately 1400 to 2800 bbl of drill cuttings depending on the depth and diameter of the well when water based muds were use during drilling, with an average volume estimation of 2000 to 8000 bbl waste per well when oil based mud was used. In most oil producing countries, drill-muds and drill-cuttings are discharged on site (Ogeleka, and Tudararo-Aherobo, 2013) including Nigeria (Anwuli, 2011; Odokuma and Akponah, 2008); the improper disposal of drilling muds and cuttings have been found to pose a significant stress on the ecosystem of the receiving environment (Odokuma and Ikpe, 2003). Edo State is one of the states in the Niger-Delta region of Nigeria, playing host to several oil and gas exploration companies. The present work, therefore, assess the biodegradation and toxicity of the two main classes of drilling muds (water based mud and non-aqueous based mud) now commonly employed in drilling operations in this part of Niger-Delta region in Nigeria..

2. Material and Methods

2.1. Source of Test Isolates

The test isolates, employed in the preset study, were earlier isolated from drill cuttings obtained from a land rig situated in an oil-producing community in Edo State (Imarhiagbe, 2012).

2.2. Collection of Drilling-Muds

The drilling-muds used in the present study were collected from the drilling site located in Edo State oil-producing community (the Geographic Position System (GPS) coordinate of the well was E: 350020.000 m, N: 229477.600 m) and was coded as non-aqueous based mud and water based mud. Samples were transported to the laboratory aseptically for evaluation, in labeled plastic containers.

2.3. Monitoring the Biodegradation

Biodegradation of drill muds by microorganisms were carried in a shake flask experiment using mineral salts medium. The mineral salt medium composed of the following: MgSO4.7H2O, 0.42 g/l, KCl, 0.30 g/l, KH2PO4, 0.8 g/l, K2HPO2, 1.3 g/l, NaNO3, 0.42 g/l and agar 15 g/l (Okpokwasili and Okorie, 1988). Two predominant drill cuttings isolated bacteria (*Citrobacter* sp., *Staphylococcus* sp.) and fungi (*Aspergillus* sp. and *Penicillium* sp.) were selected for this test. One hundred and fifty milliliters (150 ml) of the mineral salt medium was dispensed into five (5) different 250 ml conical flasks in duplicate and 10 ml of each drilling mud was added. Bacterial and fungal inoculants for this experiment was prepared by suspending a loopful of each isolate in 2 ml of mineral salt medium. Each organism was introduced into separate conical flask, while a consortium of the bacterial and fungal isolates was transferred into separate conical flasks. The control conical flask remained uninoculated. All flasks were incubated at room temperature on a rotary shaker operating at 120 rpm for 28 days. The total viable counts were monitored and ultimate biodegradability was derived from the ratio of Chemical Oxygen Demand and Biological Oxygen Demand (BOD_5), after every four days.

2.4. Toxicity Assay

The 96 hr acute toxicity bioassay was carried out using juvenile fishes (*Tilapia guineensis)* according to Organization for Economic Co-operation and Development [OECD] (1995). Non-aqueous base mud and water base mud were separately prepared into six different aquaria while the seventh was used as control, without the test chemical. The different concentrations, 1000 mg/l, 4000 mg/l, 5000mg/l, 6000 mg/l, 8000 mg/l and 10000 mg/l of the test chemicals were prepared. The fishes were distributed randomly in batches of ten per concentration into the seven aquaria. The organisms were not touched with bare hands during selections so as to avoid stress due to handling. The fishes were exposed to an initial period of acclimatization. The experiment was observed hourly for any death. Mortality was recorded after 8, 24, 48, 72 and 96 hours. Method used for 24 hrs lethal toxicity assay was adapted from Odokuma and Ikpe (2003) using bacteria (*Staphylococcus* sp.) and fungi (*Aspergillus* sp.). A loopful of the bacterial and fungal cells were collected from their individual slants and dislodged in 10 ml of normal saline, and then allowed to stand for few hours. An approximate cell dilution was chosen (*Staphylococcus* sp was 4.0x103 cfu/ml and *Aspergillus* sp was 3.3 x103 cfu/ml). Thereafter, 10 mg/l, 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l and 250 mg/l concentration of the drilling muds were prepared, respectively, in 250 ml conical flasks. The control was distilled water. The mixture was vigorously shaken for even mixing. One milliliter of each set-up including control was plated out at 0, 2,4, 8,12 and 24 hr at 30 ^{0}C to determine the viable cells and to assess toxicity. While the bacterial isolate was plated on nutrient agar, the fungal isolate was plated on potato dextrose agar incorporated with chloramphenicol. At the end of incubation, viable cells were counted and recorded. The lethal concentration (LC 50/24) values were extrapolated from the graph of mortality against concentration.

3. Results

3.1. Monitoring the Biodegradation

Tables 1(A-B) and figures 1(A-D) show the ability of single cultures of *Citrobacter* sp, *Staphylococcus* sp., *Aspergillus* sp., *Penicillium* sp., and their mixed cultures to degrade water based mud and non-aqueous based mud as depicted in their Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5) graph trends. The biodegradation potentials of the above isolates were monitored over a period of twenty-eight (28) days by assessing their total viable counts, chemical oxygen demand and biological oxygen demand. From the results, cultures containing consortiums (mixed isolates) showed remarkable biodegradation potential when compared to single isolates; and the water based mud showed a higher degree of biodegradation when compared with non-

aqueous based mud. The results showed that the highest total viable counts were observed in culture medium amended with water based mud (126x 10^3 cfu/ml and 10.5x 10^3 cfu/ml) for bacterial and fungal counts, respectively. Whereas, single cultures had their maximum growth peaks at day 16, mixed cultures had their peaks at day 24, 28(bacterial counts) and day 20 (fungal counts).

Table 1a. Total Viable Counts of Bacterial Isolates in Culture Medium Amended With Drilling Mud (10^3 Cfu/Ml)

	initial		Day 4		Day 8		Day 12		Day 16		Day 20		Day 24		Day 28	
	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM
Citrobacter sp.	3.5	3.5	4.7	4.4	5.3	4.4	45.1	10.0	48.0	15.2	47.2	32.0	60.0	15.3	19.0	11.0
Staphylococcus sp.	4.1	3.7	5.1	4.9	5.3	5.3	47.0	25.0	56.0	52.0	45.0	38.0	41.0	43.0	36.0	25.0
Citrobacter + *Staphylococcus* sp.	4.5	4.0	4.5	4.6	5.9	5.7	82.0	62.0	90.0	64.0	99.0	67.0	112.0	83.0	126.0	75.0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Over all mean values. Control contained no isolate.

Table 1b. Total Viable Counts Of Fungal Isolates In Culture Medium Amended With Drilling Mud (10^3 Cfu/Ml).

	initial		Day 4		Day 8		Day 12		Day 16		Day 20		Day 24		Day 28	
	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM
Aspergillus sp.	3.0	3.0	3.4	3.1	4.1	3.4	5.2	3.8	5.8	4.4	6.5	5.1	6.6	5.1	6.6	4.9
Penicillium sp.	2.9	3.0	4.6	4.9	4.8	5.1	5.2	5.5	7.0	5.7	7.0	6.0	7.1	6.0	7.2	5.7
Aspergillus sp. + *Penicillium* sp.	3.0	3.0	6.3	6.3	7.5	7.5	8.9	8.9	9.1	9.1	9.1	9.1	9.1	9.1	7.0	7.0
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Over all mean values. Control contained no isolate.

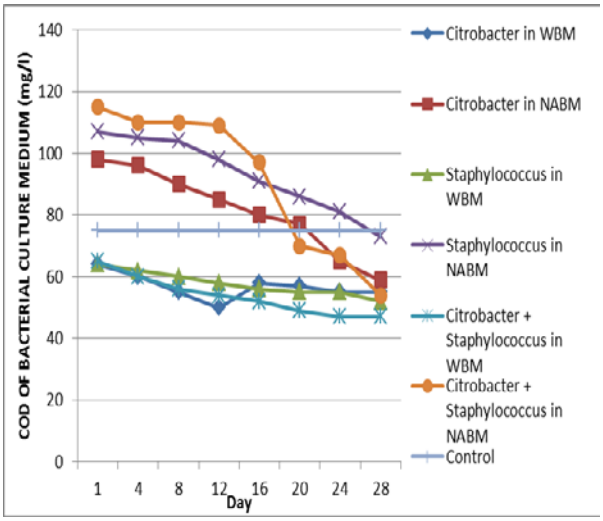

Figure 1A. Chemical oxygen demand of bacterial culture medium amended with drilling mud

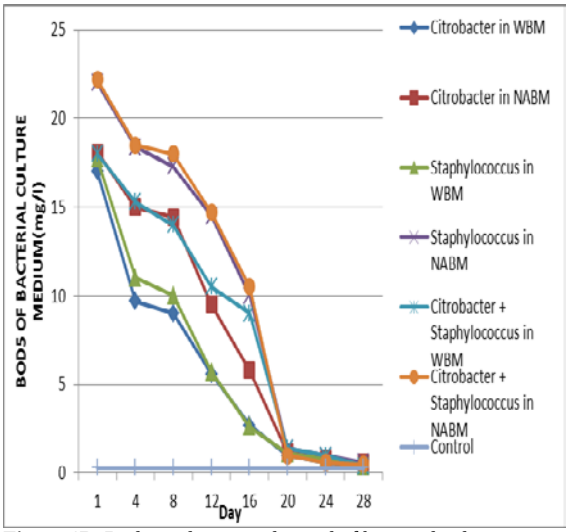

Figure 1B. Biological oxygen demand of bacterial culture medium amended with drilling mud

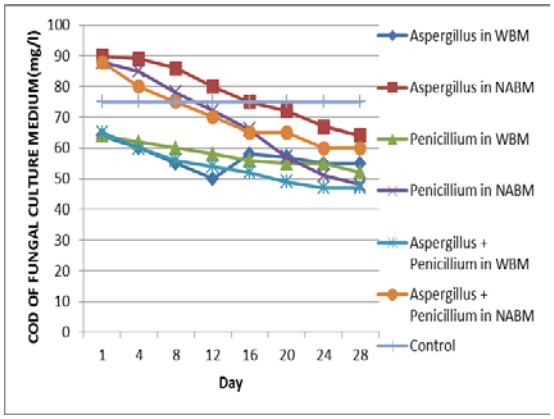

Figure 1C. Chemical oxygen demand of fungal culture medium amended with drilling mud

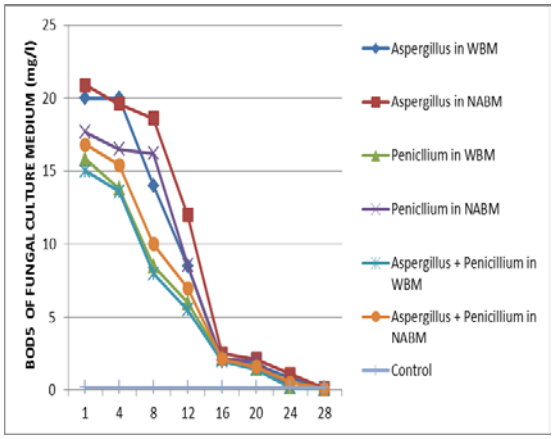

Figure 1D. Biological oxygen demand of fungal culture medium amended with drilling mud

3.2. Toxicity assay

The results of the toxic effect of the drilling muds on selected test organisms are presented in tables 2-3 (A-C).

Table 2 shows lethal concentration (LC_{50}) of the drill muds used at the location. The results showed that the non-aqueous based mud was more toxic to the test organisms than the water based mud. The 96 hours LC_{50} of WBM was greater than 10,125 mg/l while the 96 hrs LC_{50} of NABM was greater than 6000 mg/l for *Tilapia guineensis*. The 24 hrs LC_{50} of WBM was greater than 370 mg/l for *Staphylococcus* sp. and 300 mg/l for *Aspergillus* sp., and 24 hrs LC_{50} of NABM was 280mg/l and 255 mg/l for *Staphylococcus* sp. and Aspergillus sp. respectively. The effective dead time (tables 3 A-C) of the test organisms at different concentrations of the drilling muds revealed that at concentrations 250 mg/l, death of *Staphylococcus* sp. and Aspergillus sp. occurred within 12 to 24 hours of exposure to water-based mud with mortality rate of 1.38x103 cfu/ml and $1.5x10^2$ cfu/ml, $5.2x10^2$ cfu/ml, respectively; while at concentrations 100 mg/l of non-aqueous based-mud, death occurred in less than 2 hrs of exposure with mortality rate of 4.7 x 10^2 cfu/ml and $7.5x10^2$ cfu/ml, respectively. The effective time of *Tilapia guineensis* at varied concentrations of water based mud and non-aqueous based mud was observed to be 48hrs and 96 hrs of exposure, respectively.

Table 2: Lethal Concentration (Lc_{50}) Of Drill Muds Used in the Drilling Muds.

DRILLING MUD TYPE	*Staphylococcus* sp.(24hrs LC_{50}) mg/l	*Aspergillus* sp. (24hrs LC_{50}) mg/l	Tilapia (96hrs LC_{50}) mg/l
WBM	>370	>300	>10, 125
NABM	>280	>255	>6000

Values represent means of duplicates

Table 3 A. Effective Time for Concentration of Drill Muds Toxicity Test on *Staphylococcus* Sp.

	0hr		2		4		8		12		24		TOTAL MORTALITY		% MORTALITY	
	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM
Control	-	-	-	-	-	-	-		-	-	-	-	-	-	0	0
10 mg/l	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
50 mg/l	-	-	-	-	-	$1.5x10^2$	-	$1.0x10^2$	-	$1.0x10^3$	-	$1.7x10^2$	-	$1.42x10^3$	0	35.5
100 mg/l	-	$4.7x10^2$	-	$1.0x10^3$	-	$1.5x10^3$	-	-	-	-	-	-	-	$2.97x10^3$	0	74
150 mg/l	-	$7.0x10^2$	-	$2.0x10^3$	-	$6.9x10^2$	-	-	-	-	-	-	-	$3.39x10^3$	0	85
200 mg/l	-	$4.0x10^3$	-	-	-	-	-	-	-	-	-	-	-	$4.0x10^3$	0	100
250 mg/l	-	$4.0x10^3$	-	-	-	-	-	-	-	-	$1.38x10^3$	-	$1.38x10^3$	$4.0x10^3$	35	100

An inoculum of 4.0 x 10^3 cfu/ml was introduced into each solution; (-) means no mortality; WBM = water Based Mud; NABM = Non-aqueous Based Mud.

Table 3b. Effective Time for Concentration of Drill Muds Toxicity Test on *Aspergillus* Sp.

	0hr		2		4		8		12		24		TOTAL MORTALITY		% MORTALITY	
	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM
Control	-	-	-	-	-	-	-		-	-	-	-	-	-	0	0
10 mg/l	-	-	-	-	-	$2.5x10^2$	-	$1.0x10^2$	-	$1.7x10^3$	-	$1.0x10^3$	-	$3.05x10^3$	0	92.4
50 mg/l	-	-	-	$1.0x10^2$	-	$1.0x10^3$	-	$1.0x10^2$	-	$1.8x10^2$	-	-	-	$2.3x10^3$	0	69
100 mg/l	-	$7.5x10^2$	-	$1.0x10^3$	-	$2.0x10^3$	-	-	-	-	-	-	-	$2.85x10^3$	0	86
150 mg/l	-	$7.0x10^2$	-	$2.5x10^3$	-	-	-	-	-	-	$1.9x10^2$	-	$1.9x10^2$	$3.3x10^3$	5.76	100
200 mg/l	-	$2.0x10^3$	-	$1.3x10^2$	-	-	-	-	$1.0x10^2$	-	$2.0x10^2$	-	$3.0x10^2$	$3.3x10^3$	9.1	100
250 mg/l	-	$3.3x10^3$	-	-	-	-	-	-	$1.5x10^2$	-	$5.2x10^2$	-	$6.7x10^2$	$3.3x10^3$	20	100

An inoculum of 3.3×10^3 cfu/ml was introduced into each solution; (-) means no mortality; WBM = water Based Mud; NABM = Non-aqueous Based Mud.

Table3 C. Effective Time for Concentration of Drill Muds Toxicity Test on *Tilapia Guineensis* (Fingerlings).

	8hr		24hr		48hr		72hr		96hr		TOTAL MORTALITY		% MORTALITY	
	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM	WBM	NABM
Control	-	-	-	-	-	-	-		-		-		0	0
1000 mg/l	-	-	-	-	-	-	-		-		-		0	0
4000mg/l	-	-	-	-	-	-	-		-		-		0	0
5000mg/l	-	-	-	-	-	-	-		-	2	-	2	0	20
6000mg/l	-	-	-	-	-	1	-	2	-	3	-	6	0	60
8000mg/l	-	-	-	-	-	2	-	3	2	3	2	8	20	80
10000mg/l	-	-	-	-	-	3	-	4	4	3	4	10	40	100

Ten (10) fingerlings were introduced into each tank; (-) means no mortality; WBM = water Based Mud; NABM = Non-aqueous Based Mud

4. Discussion

In the present study, a twenty-eight day monitoring of the biodegradation potential of *Citrobacter* sp., *Staphylococcus* sp., *Aspergillus* sp. and *Penicillium* sp. revealed a consistent increase and decrease of the total viable counts (cfu/ml). The results showed that the highest total viable counts were observed in culture medium amended with water based mud ($126x\ 10^3$ cfu/ml and $10.5x\ 10^3$ cfu/ml) for bacterial and fungal counts, respectively. The presence of relatively toxic oil in the liquid phase of non-aqueous base mud may have contributed to its low microbial counts; this was contrary to that of water base (Odokuma and Ikpe 2003). The highest total viable counts (cfu/ml) were recorded for the broth batches containing consortium of isolates (*Citrobacter* sp. + *Staphylococcus* sp WBM broth; *Citrobacter* sp.+ *Staphylococcus* sp in NABM broth; *Aspergillus* sp + *Penicillium* sp in WBM broth and *Aspergillus* sp + *Penicillium* sp in NABM broth). The experimental results also showed that while axenic (single) cultures had their maximum growth peaks at day 16, mixed (consortium) cultures had their peaks at day 24, 28(bacterial counts) and day 20 (fungal counts). This is in line with previous works that had suggested that mixed microbial cultures are better degraders of organic pollutants (Okpokwasili and Okorie, 1988; Odokuma and Ikpe, 2003). Thus, flasks containing consortium showed marked biodegradation potential in comparison with flasks containing single isolates. Enhanced degradation observed by the microbial consortium in the present study may be attributed to the fact that an organism may have acted as primary utilizer, utilizing substrate molecules while the other acted as secondary utilizer, utilizing the breakdown products of substrate after initial attack by primary utilizer (Okpokwasili and Okorie, 1988). Statistical analysis revealed no significant differences ($P > 0.05$) in the degradation of the muds by the isolates. According to Okerentugba and Ezeronye (2003) the isolation of certain oil-degrading micro-organisms in a polluted environment is an indication that these micro-organisms are the active degraders of that environmental pollutant. Alan (2006) earlier showed that the authochthonous aerobic microbial populations have the capability to utilize the non-aqueous base fluid as their sole carbon and energy sources. It therefore showed that these selected isolates have potential applications in the bioremediation of sites polluted by water based mud and non-aqueous based mud.

The eco-toxicity analysis of these two types of drilling muds with reference to their composition showed that the non-aqueous based mud was more toxic to the test organisms than the water based mud (Tables 2-3) and may be ascribed to their chemical composition. The lethal concentration, 50 % (LC_{50}) of the drilling muds again buttressed the obvious fact that water based muds are relatively less or non-toxic when compared with non-aqueous base muds. From the study, lethal concentration, 50 % (LC_{50}) of water base mud was greater than 10, 125 while that of the non-aqueous base mud was greater than 6000. The increase in percentage mortality as concentration of drilling muds increased over time of exposure in this study, which is in agreement with previous findings (Ekpo and Ekanem, 2000; Vincent-Akpu, 2010). Vincent-Akpo (2010) and Ogeleka, and Tudararo-Aherobo (2013) had earlier shown a positive correlation between

toxicant concentrations (drilling muds) and fish mortality rate in treated tanks.

Several authors had shown that the toxicity of drilling muds may be linked to their chemical entities such as the base fluid types (Ekpo and Ekanem, 2000; Odokuma and Ikpe 2003), concentration, water solubility (Odokuma and Akponah, 2008) and genetic constitution of the organism (Dutton et al., 1990). Neff et al. (2000) had attributed the toxicity of drilling muds to their hydrocarbon content. The low hydrocarbon content along with other chemical compositions of drilling muds are responsible for their toxicity and also the fact that synthetic based mud does not disperse in water is an additional contributing factor to its toxicity and low biodegradation.

5. Recommendations

Oil exploration and production companies operating in Edo State, Nigeria and other parts of the world, should strictly adhere to the use of non-toxic and biodegradable drilling muds in the interest of safeguarding their immediate environment. Also, the legislative ban on the use of toxic oil-based mud should be properly monitored and enforced by their appropriate regulatory agencies.

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