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Toxicological Effect of Oil and Water Based Drilling Muds on Soil Nitrosomonas and *Nitrobacter* spp.

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Abstract

The toxic effect of used drill muds on soil Nitrosomonas and Nitrobacter spp *was studied. The 24 hr. acute toxicity test and the 28 day chronic toxicity tests were adopted. Findings revealed that the drilling muds were relatively acidic with a pH of 4.94 and 6.03 for oil based mud and water based mud respectively. Both types of mud contain a wide range of heavy metals, aliphatic and polycyclic aromatic hydrocarbons, petroleum hydrocarbons and volatile organic compounds. There was a progressive toxic effect on the organisms with increase in drill mud concentration. The 24 hr. acute toxicity effect of the muds to Nitrosomonas sp. recorded EC_{50} of 503,500.0mg/kg and 398,100.0mg/kg for water based and oil based muds respectively. The 24 hr. acute toxicity effect of the mud to Nitrobacter sp. recorded EC_{50} of 384,500.0mg/kg and 234,400.0mg/kg for water based and oil based muds respectively. The muds showed marked toxicological effect on the nitrogen transformation activity in the soil. The percentage inhibition of nitrite production in the soil by the drill muds ranges between 10.51 – 40.34% and 20.17- 50.28% for the water based mud and the oil based mud respectively. The percentage inhibition of nitrate production in the soil by the drill muds ranges between 11.94 – 45.63% and 20.68 – 56.08% percent for water based mud and oil based mud respectively. Elimination of Nitrosomonas and Nitrobacter organisms in drilling mud contaminated soil is a threat to food production.*

1. Introduction

Drilling mud, also known as drilling fluid, is a vital component of any drilling operation. The primary functions of drilling mud are to cool and lubricate the drill bit and drill string, assist in removal of drill cuttings from the well bore, control subsurface pressure to prevent any blowouts from the well, maintain borehole stability by protecting produced formations by minimizing formation/fluid interactions and sealing the wall of the bore hole with an impermeable cake, control corrosion of the metal components of the drilling tools, casing and rig facilities that are exposed to the corrosive marine environment and maximize drilling penetration rates [1].

There are basically three main types of drilling fluids; Water base muds, Oil base muds and the Synthetic base muds [2]. The water based mud has fresh salt or sea-water as the continuous phase. They are mainly composed of aqueous solutions of polymers and clays in water or brines, with different types of additives incorporated to the aqueous solution. The oil based muds are invert emulsions of brine into an oil major continuous phase stabilized by surfactants. Other additives are often added to the organic phase, such as organophilic modifiers of the clay surface. These drilling fluids have been developed for situations where water based mud were found inadequate [2]. Although oil based mud often give better performances, they have major drawbacks such as to be generally more expensive and less ecologically friendly than water based mud. In the mid-1990s the offshore drilling industry began phasing out the use of oil based mud and replacing them with light synthetic-based mud. Synthetic-based drilling muds are water in oil emulsion intended to replace oil based mud as a low toxicity readily biodegradable alternative to mineral oil-based muds [1]. These muds are a synthetic material as the carrier fluid is more readily biodegraded, unlike conventional oil-based mud [3]. Drilling mud often contains a variety of chemicals which are formulated as required from a generally limited list of additives [4]. The type and amount of chemical additives included in the mud formulation varies according to the required characteristics of the mud depending on the well to be drilled.

In the Nigerian oil industry both water-based and oil- based mud systems are commonly employed in oil and gas drilling operations [5]. During drilling activities, wastes which include muds and cuttings wastes are generated in considerable amounts and their discharge to terrestrial and water bodies is regulated in many countries, mainly due to concerns on the impacts of solids and toxicity of the chemical waste in the water column and benthic ecosystems [6]. Disposal of spent drilling muds is a challenge, especially for oil-based muds (OBMs) with diesel-range organics. The widespread use of diesel-based drilling muds has raised concern regarding their impacts on human and environmental health. Typically diesel-based drilling muds and cuttings are characterized by extreme ecotoxicity which can persist following bioremediation. According to [7], drilling muds and cuttings are sometimes discharged into landfills and thereafter overflow into nearby farms and rivers. During drilling, plumes (muddy) of turbid water are commonly seen trailing downstream from the drilling platform [8]. Drilling mud (drilling wastes) are sometimes unintentionally or intentionally released into water bodies and can damage the gills of prawn, shrimp and other bottom dwellers at post larvae stages.

Research have abundantly shown that drilling muds additives may contain toxic substances such as heavy metals, hydrocarbons, biocides, chromates, organic polymers and trace elements that have the tendency to bioaccumulate and interfere with normal biological activities of organisms [7, 9, 10]. These toxic effects may be acute or chronic and some may stimulate growth [11, 12, and 13]. The degree of effect depends on the type, dosage, and exposure duration [14].

Eco-toxicity testing provides indicative information on possible effects of toxic chemicals on biota and thus provides a basis to assess its environmental acceptability. Acute eco-toxicity testing is commonly used to predict the toxicity of drilling fluids in the marine environment. Toxicity tests are used to expose test organisms (fish, shrimps, microorganisms, earthworm etc.) to a medium-water, sediment or soil and evaluate the adverse effect of contaminant on the survival, growth, reproduction, behavior and other attributes of these organisms [2].

This study was designed to investigate the toxicological effect of spent drilling mud on species of *Nitrobacter* and *Nitrosomonas.*

2. Methodology

2.1 Drilling mud collection: The drilling mud used in this study was collected from a drilling site located in Kolo Creek, Bayelsa State Nigeria, and coded as oil based mud (OBM) and water based

mud (WBM). Both the water based drilling mud and the oil based drilling muds were collected in labeled 2 liter plastic containers and kept at 4^{o}C prior to commencement of study.

2.2 *Nitrobacter and Nitrosomonas* spp.: The *Nitrobacter* and *Nitrosomonas* sp. were isolated using the Winogradsky medium [7]. *Nitrosomonas* was isolated using Winogradsky medium for nitrification phase 1g $(NH_4)_2SO_4$, 2.0 g;K_2HPO_4, 1.0 g;$MgSO_4.7H_2O$, O.5 g; NaCl,2.0 g; $FeSO_4.7H_2O$, 0.4 g; $CaCO_3$ 0.01, agar 15.0 g; distilled water 1000 ml). *Nitrobacter* was isolated using Winogradsky medium phase 2 (KNO_2 0.1 g; $Na^{2-}Co^3$,1.0 g; NaCl, 0.5 g; $FeSO_4.7H_2O$, 0.4 g agar 15.0 g; distilled water 1000 ml). Isolates that were greyish, mucoid, flat, Gram negative, pear shaped and aerobic were selected according to the scheme of [15]. Sub cultures were made into slants of Winogradsky – *Nitrosomonas/Nitrobacter* agar and stored at 4^{o}C prior to commencement of test.

2.3 Acute toxicity of drilling muds on *Nitrosomonas and Nitrobacter* spp: The methods of [16, 17, and 9] were adopted with some modifications. A fresh dilution and culture were made from the *Nitrobacter* slant. A loop full of the bacteria was collected from the slant and dislodged in 20 ml peptone water and allowed to stand for a few hours at 30^{o}C. The stock culture was prepared by inoculating 180ml of sterilized peptone water with 20ml of the activated culture. The test concentrations were prepared using the dilution water from the bacteria's habitat; sterilized at 121^{o}C for 15 minutes.

Concentrations of 312.5, 625, 1250, 2500, and 5000mg/l of the drilling muds were prepared respectively in 250ml conical flasks. 100ml of each test concentration was put into 250ml conical flask and sterilized at 121^{o}C for 15 minutes. On cooling, 10ml of the cell suspension was added to each flask containing the different drilling mud concentrations and control. The control used was sterile dilution water. This was done in triplicate for each of the mud type. The flasks were shaken thoroughly to mix and were incubated at 30^{o}C to determine the number of viable cell at 0 (start), 8, 16, and 24 hr. 0.1ml of each test concentration and control was collected from the test solution and dispensed onto the surface of an already prepared Winogradsky *nitrobacter* agar plate. The plates were then incubated at 30^{o}C for 24h. The viable cells were counted and recorded. The EC_{50} was determined using the probit method of analysis [18].

2.4 Chronic toxicity effect of drilling muds on nitrogen transformation activity in the soil: The method according to [19] was adopted in this study. The effect of the two types of drilling muds on the nitrifying bacteria *Nitrosomonas* and *Nitrobacter* was determined. This test was used to detect the long term (chronic) adverse effects of drilling muds to the process of nitrogen transformation activity in the soil. Pristine soil was taken from a depth of 0 to 20cm from a garden in Warri Delta State, Nigeria and was transported in an ice-chest at 4^{o}C to guarantee the initial soil properties were not significantly altered.

In the laboratory soil samples were kept in the refrigerator at 4± 2^{o}C when they could not be used immediately. The soil was dried, sieved and treated with five concentrations (3125 mg/kg, 6250 mg/kg, 12500 mg/kg, 25000 mg/kg and 50000 mg/kg) of drilling mud or left untreated (control). After day 0, 7, 14 and 28 treated and control samples were extracted and analyzed for ammonia, nitrite, nitrate and *Nitrosomonas* and *Nitrobacter* spp. counts. The rate of nitrite and nitirate formation in treated soil was compared with the rate in the controls and the percent deviation of the treated from control was calculated. Enumeration of nitrogen transformation bacteria was also done to correlate the microbial growth with the transformed nitrogen. Results from the test of multiple were analyzed using a regression model (ANOVA) and the EC_{50} was calculated. All analysis was done by ASTM method. The control contained only the soil. A geometric series of five concentrations were used. Three replicates for both treatments and control were also used.

The test was carried out in the dark at 25^{o}C ±2^{o}C where 90 ml of sterile distilled water was added to each tank to achieve moisture content of between 40 – 60%. The content of the tank was mixed thoroughly and covered with perforated polythene to prevent excessive evaporation of water and

volatile fractions. Moisture content of between 40 – 60% of the maximum water holding capacity was maintained during the test by watering at intervals with distilled water. The duration of the test was 28days. Composite soil sampling was done on days 7 and 28 and the soil samples were analyzed for some physic-chemical parameters such as pH, total organic carbon, nitrite/nitrate and ammonia. Bacteriological parameters included enumeration of total *Nitrobacter/Nitrosomonas* sp. count. Enumeration of *Nitrobacter/Nitrosomonas* spp was done with Winogradsky medium. The quantity of ammonia and nitrite/nitrate formed and *Nitrobacter*/*Nitrosomonas* spp. counts obtained in each replication test soil were recorded. Mean values of all replicates were determined and a dose response curve was prepared for the estimation of the effective concentration causing 50% reduction (EC_{50} value). The rate of nitrite/nitrate formation in treated samples was compared with the rate in the control and percent deviation/inhibition of the treated from the control was calculated after 28days using the Equation 1 [20]:

$$\text{Inhibition (\%)} = \frac{C_{ref} - C_{sample}}{C_{ref}} \times 100 \quad (1)$$

Where C_{ref} is concentration of nitrite/nitrate formed in control, $C_{control}$

2.5 Determination of Nitrite: The spectrophotometric method as described by [21] was adapted in the estimation of the nitrite profile of the respective soils. Five (5) g of the soil sample was weighed and oven-dried at 55 °C in an oven for 12-16 hours. The dried soil was dissolved in water, shaken thoroughly and filtered. The respective filtrates were then centrifuged for about 10 min. The supernatant liquid was taken and 1 mL of 5% EDTA solution was added to the liquid. An aliquot of 1-2 mL of the supernatant liquid was transferred onto a 25 mL calibrated tube. About l mL of potassium iodide solution followed by 1 mL of 6 M hydrochloric acid was added. The mixture was gently shaken till the appearance of yellow colour was developed, which indicated the liberation of iodine. Then, 1 mL of leucocrystal violet (LCV) was added and the pH of the solution was adjusted to 4.0 with drop wise addition of phosphate buffer solution. The content was then kept on a water bath for 2-3 min at 40-45 °C. The mixture was allowed to stand for 20-25 minutes which was the time duration required for complete colour development of the final solution. For the final extraction, the colored solution was transferred in to a 250 mL separating funnel and 5 mL of extracting solution of isoamyl alcohol was added and the funnel was gently shaken. The violet extract was separated and absorbance was measured at 590 nm against a reagent blank ascertain the nitrite concentration present in the soil filtrate.

2.6 Determination of Nitrate: Nitrate analysis of the soil samples was conducted by adapting the spectrophotometric procedure as described by [22]. One gram (1g) of the respective soils was weighed and transferred into a 25 mL beaker and a soil extract was prepared three mL (3 ml) portions of 0.5% sodium carbonate solution. The soil extract was then filtered through Whatman no. 41 filter paper and the filtrate was collected and made up to 25 mL with distilled water. An aliquot of 1-2 mL of the diluted filtrate was transferred in to a 10 mL calibrated flask. About, 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L-1 HCl solution, shaken thoroughly for 5 minutes was added to the flask followed by the addition of 1 ml of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solutions respectively. The mixture was further diluted with the addition of 10 mL of distilled water. The absorbance of the red colored solution was then measured at 493 nm against the corresponding reagent blank to ascertain the nitrate concentration present in the soil filtrate.

3. Results and Discussion

Table 1A: Physicochemical Properties of Drilling Mud Samples

Parameters	Oil Based Mud	Water Based Mud
pH	4.94	6.03
EC (µS/cm)	1,400	3,196
SO_4^{2-} (ppm)	350.23	799.01
TOC (%)	7.605	7.488
CL^- (ppm)	276.76	664.23
Total N (%)	0.355	0.376
NO_3 - N (ppm)	1.571	1.664
NO_2 - N (ppm)	1.167	1.236
NH_4 - N (ppm)	0.457	0.485
Total P (%)	0.0207	0.0028
Exchangeable Acidity (Cmol/kg)	0.86	0.61
Organics		
TPH (mg/kg)	1800.34	1026.79
Oil & Grease (mg/kg)	3613.56	1008.31
Aliphatic (mg/kg)	378.60	320.93
PAHs (mg/kg)	354.46	297.59
VOCs (BTEX) (mg/kg)	89.48	14.05
Cations		
Ca (ppm)	713.60	106.10
K (ppm)	37.57	5.51
Mg (ppm)	10.21	3.23
Na (ppm)	137.94	35.59

Overall mean value

Table 1B: Heavy Metals in Drilling Mud Samples

Parameters (ppm)	Oil Based Mud	Water Based Mud
Al	147.48	93.71
Ba	0.0453	0.0324
Cd	0.2948	0.3033
Co	0.4347	0.5332
Cr	44.4227	12.5279
Cu	1.1678	0.4122
Fe	507.89	143.00
Mn	14.1287	4.0648
Ni	0.2349	0.1996
Pb	1.1855	0.1100
V	3.1620	2.5236
Zn	3.7617	1.0580
As	0.0088	0.0316
Hg	0.0152	1.0821

Overall mean value

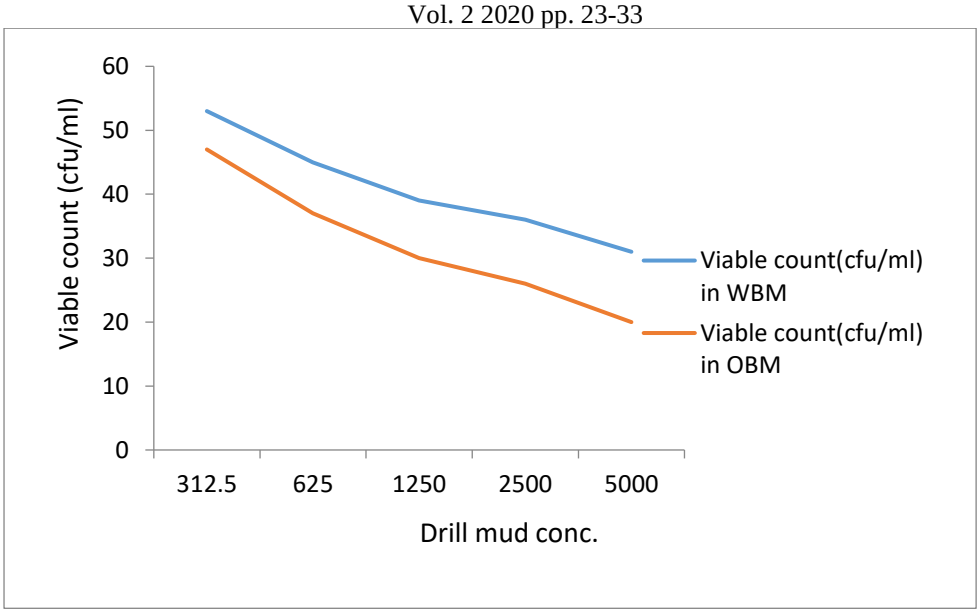

Figure 1: 24 hr. toxicity effect of water and oil based drilling muds to *Nitrosomonas* sp as represented by the total viable counts

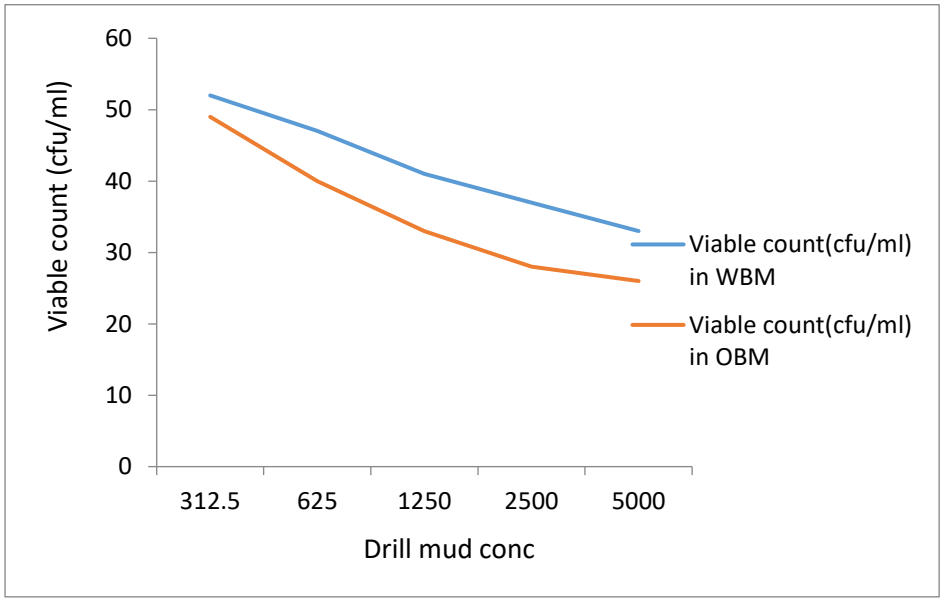

Figure 2: 24 hr. toxicity effect of water and oil based drilling muds to *Nitrobacter* sp as represented by the total viable count

Table 2: Effective concentrations of used drilled muds on the bacterial isolates

Bacterial isolates	EC_{50} 24 hrs		EC_{50} 28 days	
	OBM (mg/l)	WBM (mg/l)	OBM (mg/l)	WBM (mg/l)
Nitrosomonas	398.1	503.5	25,882.1	33,884.7
Nitrobacter	234.4	384.5	50,466.1	169,817.7

Over all mean values

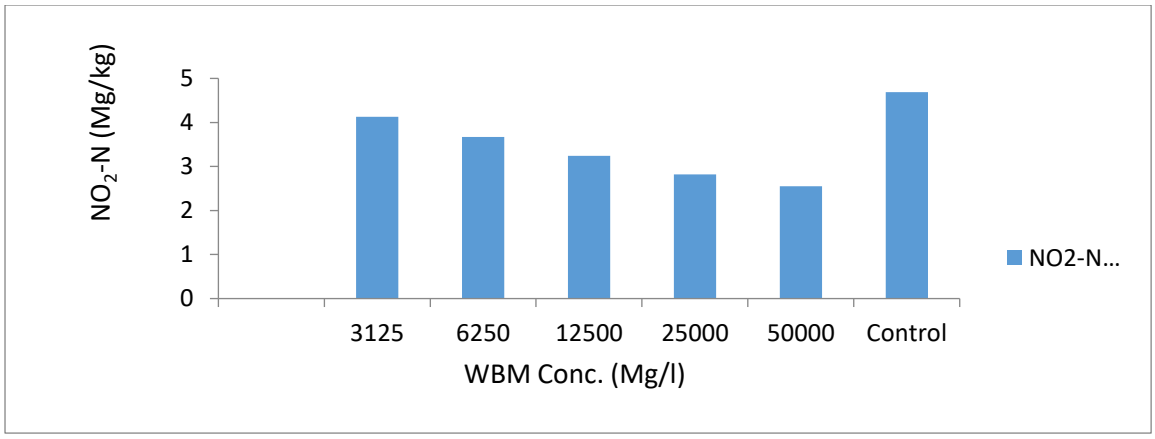

Figure 3: Effect of water based mud on NO_2-N production in soil

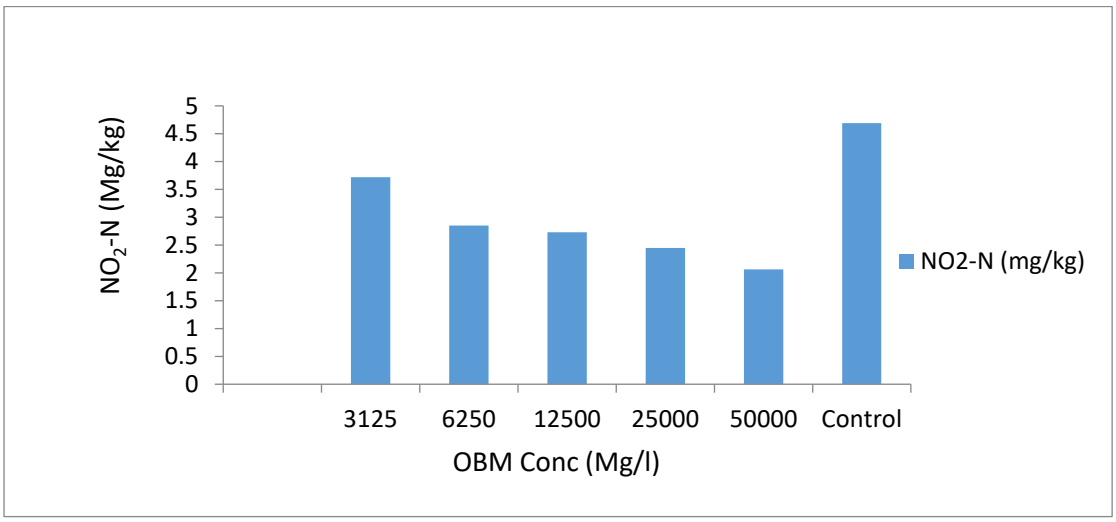

Figure 4: Effect of oil based mud on NO_2-N production in soil

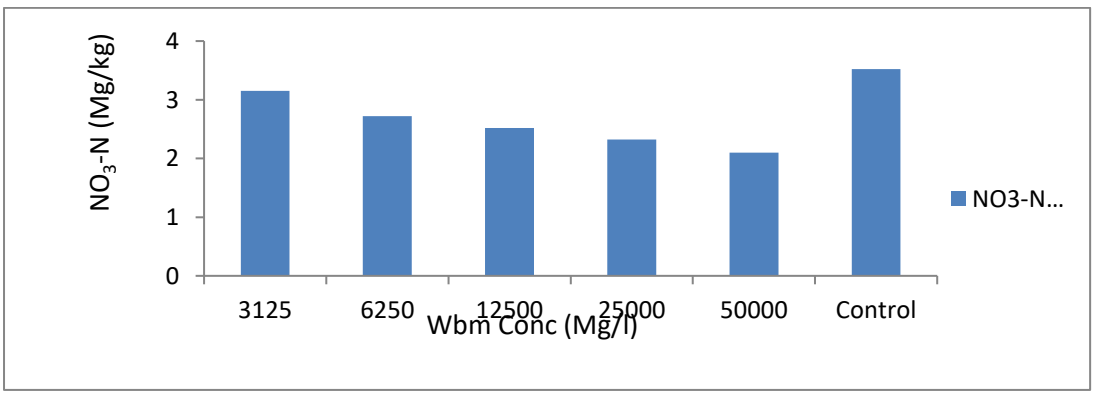

Figure 5: Effect of water based mud on NO_3-N production in soil

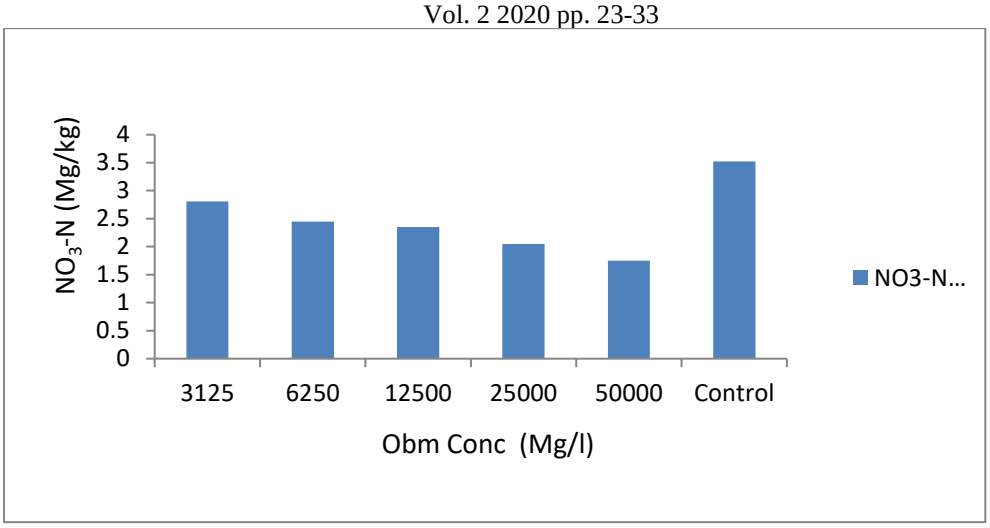

Figure 6: Effect of oil based mud on NO_3-N production in soil

Table 3A: Percentage (%) inhibition of Nitrite formation in water and oil based drilling mud contaminated soil

WBM Concentration mg/l	**% Inhibition of nitrite**	**OBM Concentration mg/l**	**% Inhibition of nitrite**
3125	11.94	3125	20.68
6250	21.75	6250	39.23
12500	30.92	12500	41.79
25000	39.87	25000	47.76
50000	45.63	50000	56.08

Overall mean value

Table 3B: Percentage (%) inhibition of Nitrate formation in water and oil based drilling mud contaminated soil

WBM Concentration mg/l	**% Inhibition of nitrate**	**OBM Concentration mg/l**	**% Inhibition of nitrate**
3125	10.51	3125	20.17
6250	22.73	6250	30.40
12500	28.41	12500	33.24
25000	34.09	25000	41.76
50000	40.34	50000	50.28

Overall mean value

The results of the physicochemical properties of the drilling muds are shown in Tables 1A to 1B. The drilling muds were observed to be relatively acidic with pH values of 4.94 (oil based mud) and 6.03 (water based muds). Analysis of the organic compounds composition revealed high levels of Total Petroleum Hydrocarbon (1800.34 and 1026.79 mg/kg for oil based mud and water based mud respectively); oil and grease (3613.56 and 1008.31mg/kg for oil based mud and water based mud respectively); aliphatic hydrocarbons (378.6 and 320.93 mg/kg for oil based mud and water based mud respectively); polycyclic aromatic hydrocarbon (354.46 and 297.59 mg/kg for oil based mud and water based mud respectively) and volatile organic compounds (89.48 and 14.05 mg/kg for oil based mud and water based mud respectively). Results further revealed that the used drill muds contained different heavy metals (Table 1B) with iron having the highest concentration (507.89 ppm and 143.00 ppm for oil based mud and water based mud respectively), this was followed with aluminum (147.48 ppm and 93.71 ppm for oil based mud and water based mud respectively); with trace levels of arsenic (0.0088 ppm and 0.032 ppm Results revealed that the used drill muds contain different heavy metals with iron having the highest concentration (507.89 ppm and 143.00 ppm for

oil based mud and water based mud respectively), this was followed with aluminum (147.48 ppm and 93.71 ppm for oil based mud and water based mud respectively). These analyzed chemical components obviously plays significant role in the toxicity of the drilling muds. Earlier study had shown that high concentration of heavy metal cations inhibits microbial activities by causing damage or inactivating one or more critical enzymes resulting in the formation of an inactive complex between the metal cations and an active enzyme [23]. Heavy metal toxicity involves several mechanisms, which is, breaking fatal enzymatic functions, reacting as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation, and directly affecting the formation of DNA as well as protein [4, 20]. The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Chromium (Cr) and cadmium (Cd) are capable of inducing oxidative damage and denaturation of microorganisms as well as weakening the bioremediation capacity of microbes. Chromium (Cr) (III) may change the structure and activity of enzymes by reacting with their carboxyl and thiol groups [24]. Intracellular cationic Chromium (III) complexes interact electrostatically with negatively charged phosphate groups of DNA, which could affect transcription, replication, and cause mutagenesis [6]. Heavy metals like copper (Cu (I) and Cu (II)) could catalyze the production of reactive oxygen species (ROS) via Fenton and Haber-Weis reactions, which will act as soluble electron carries. This can cause severe injury to cytoplasmic molecules, DNA, lipids, and other proteins [25, 26].

Aluminum (Al) could stabilize superoxide radicals, which is responsible for DNA damage [27]. Heavy metals could stop vital enzymatic functions by competitive or noncompetitive interactions with substrates that will cause configurational changes in enzymes [28]. Cadmium (Cd) and lead (Pb) pose deleterious effect on microbes, damage cell membranes, and destroy the structure of DNA. This harmfulness is generated by the displacement of metals from their native binding sites or ligand interactions [29]. The morphology, metabolism, and growth of microbes are affected by changing the nucleic acid structure, causing functional disturbance, disrupting cell membranes, inhibiting enzyme activity, and oxidative phosphorylation [30, 31]. The polycyclic aromatic hydrocarbon (PAH) had also been implicated in the inhibition of nitrification process [32, 33].

The 24 hrs acute toxicity of the drilling muds to *Nitrosomonas* and *Nitrobacter* species are presented in Figures 1-2 and Table 2. In general, the toxicity was concentration dependent. There was a progressive toxic effect on the total viable count with increase in toxicant concentration (Figures 1-2). The effective concentrations (EC_{50}) of the drill muds on the bacterial isolates are shown in Table 2. The results of 50% effective concentration (EC_{50}) of the drilling muds at 24 hrs exposure (Table 2) on *Nitrosomonas* sp. was 503,500.0 mg/kg and 398,100.0 mg/kg and *Nitrobacter* sp. recorded 384,500.0mg/kg and 234,400.0mg/kg for water based and oil based muds respectively. This indicates that at the above recorded drill muds concentration, 50% of the organisms would be eliminated in the soil or water of the mud polluted environment. *Nitrosomonas* oxidizes ammonia into nitrite as a metabolic process. They are important in the nitrogen cycle by increasing the availability of nitrogen to plants while limiting carbon dioxide fixation. The genus is found in soil, fresh water and on building surfaces especially in areas that contain high level of nitrogen compounds. *Nitrobacter* also play an important role in the nitrogen cycle by oxidizing nitrite to nitrate in soil and marine systems. Unlike plants, where electron transfer in photosynthesis provides the energy for carbon fixation, *Nitrobacter* uses energy from the oxidation of nitrite ions, NO_2^-, into nitrate ions, NO_3^-, to fulfill their energy needs.

From the results, it is therefore likely that the individual components of the chemical additives in the drilling fluids inhibited the growth of the *Nitrosomonas* and *Nitrobacter* communities that are important in some of the biogeochemical cycles present in the affected ecosystem, which may affect the agricultural productivity of such ecosystems. Drilling mud toxicity on the two microorganisms and other bottom dwellers could be more detrimental since these organisms remain in their habitat and have no means of burrowing out completely and moving away from the site of pollution like pelagic organisms (fish), which can swim to other directions on sensing pollution in its habitat [34].

The 28 days chronic toxicity effect of the drilling muds to nitrogen transforming bacteria is presented in Tables 2. The chronic toxicity effect of the muds to *Nitrosomonas* sp. recorded EC_{50} of 33,884.7mg/kg and 25,882.1mg/kg for water based and oil based muds respectively; and the *Nitrobacter* sp. recorded EC_{50} of 169,817.7mg/kg and 50,466.1mg/kg for water based and oil based muds respectively.

The percentage inhibition of nitrite production by the drill muds ranges between 10.51 – 40.34 and 20.17-50.28 percent respectively for the WBM and the OBM. The percentage inhibition of nitrate production by the drill muds ranges between 11.94 – 45.63 and 20.68 – 56.08 percent respectively for the water based mud and the oil based mud. The result shows a decline in the *Nitrosomonas* and *Nitrobacter* counts as the concentration of the drilling mud is increased. As stipulated in the test guideline [19], since the difference between the lowest and the highest percentage inhibition is greater than 25%, the drilling mud has the potential to inhibit nitrogen transformation and subsequently result in soil infertility. This is in line with the work of [2] on the ecotoxicological effect of discharge of Nigerian petroleum refinery oily sludge on biological sentinels. Analysis of the drill mud shows that it is acidic and contains high total petroleum hydrocarbon made up of 10 – 40 carbon unit compounds. The drill mud reduced the growth of *Nitrobacter* sp in aqueous medium and also caused chronic effect on microbial nitrogen transformation activity in the soil.

4. Conclusion

The results of this study have shown that drilling muds that were used in this location are toxic to soil microorganisms; viz *Nitrosomonas* and *Nitrobacter* spp. The continuous extinction of these organisms in drilling mud contaminated soil is a serious threat to food and agricultural availability in the affected communities. It therefore appears quite unsafe to intentionally or unintentionally discharge drilling muds on terrestrial environment without proper treatment.

Reference

[1] Burke, C.J. and Veil, J.A. (1995). Synthetic-based drilling fluids have many environmental pluses. *Oil and Gas Journal*.**93**: 59-71.

[2] Atuanya, E. and Tudararo-Aherobo, L. (2014).Ecotoxicological effects of discharge of Nigerian petroleum refinery oily sludge on biological sentinels. *African Journal of Environmental Science and technology.* 9: 95 – 103

[3] Engelhardt, F.R., Hall, H.A., Paterson, R.J. and Strong, D.C., (1983). Oil-based drilling muds in the North Sea – A Perspective. Environmental Protection Branch. 3.

[4] Holdway, D.A. (2002). The acute and chronic effects of wastes associated with offshore oil and gas production on temperate and tropical marine ecological processes. *Marine Pollution Bulletin*. 44: 185-203.

[5] Nrior, R. R. and Odokuma L. O. (2015) Comparative toxicity of drilling fluids to marine water shrimp (*Mysidoposisbahia*) and brackish water shrimp (*Palaemonetesafricanus*). *Journal of Environmental Science, Toxicology and Food Technology* 9(7): 73-79

[6] Neff, J. M. (2008). Estimation of bioavailability of metals from drilling mud barite. *Integrated Environmental Assessment and Management* 4(2): 184-193.

[7] Odokuma, L .O and Akpanah, E. (2008). Response of *Nitrosomonas, Nitrobacter* and *Escherichia coli* to drilling fluids. *Journal of cell and animal biology* 2(2): 043 – 054

[8] Jack, H. T., Eugene, A.S. and Thomas, J.B. (1985) Effects of drilling muds on screen species of Reef-Building Corals as measured in the field and Laboratory In: Proceedings of the 1985 International seminar Nigerian National Petroleum corporation Lagos

[9] Odokuma, L.O. and Ikpe, M.D. (2003). Role of composition on the degradability and toxicity of drilling muds. *African Journal of Applied Zoology and Environmental Biology* 5: 6 – 13

[10] Vincent-Akpu, I.F., Sikoki, F.D. and Utibi, D. (2010).Toxicity of drilling fluid XP-07 to *Tilapia guineensis.Fry. Science Africa* 9(2):68 – 76

[11] Okpokwasili, G.C. and Odokuma, L.O (1996) Tolerance of *Nitrobacter* toxicity to hydrocarbon fuels. *Journal of Petroleum Science and Engineering*. 16:89-93

[12] Okpokwasili, G.C. and Odokuma, L.O (1994).Toxicity of some Nigerian crude Oils.*Bulletin of Environmental Contamination and Toxicology* 52: 388-399

[13] Rhodes, A.N. and Hendricks, C.W. (1990). A continuous flow method for measuring effects of chemicals on soil nitrification. *Toxicology Assessment* 5:77-89.

[14] Ezemonye, L. I. N., Ogeleka, D. F., and Okieimen, F. E. (2008). "Lethal toxicity of industrial chemicals to early life stages of *Tilapia guineensis*."*Journal of Hazard Material*.**157(1)**, 64–68.
[15] Colwell, R.R and Zambruski, M.S. (1972).Methods of aquatic microbiology. University ParkPress, Baltomore. 461p
[16] American Public Health Association (APHA) (1998). Standard methods for the examination of water and waste water 20th Edition. Published by American Public Health Association, Washington DC, 1220p
[17] Duffus, J.H. (1980). Environmental Toxicology (Resource and Environmental Science Series).Arnold Publishers Ltd. 26p
[18] Sebaugh, J. L (2010) Guidelines for accurate EC50/IC50 estimation. Pharmaceutical Statistics 10: 128-134
[19] Organization of economic co-operation and Development (OECD) (2002).Guidelines for Testing of chemical TG 216 C. 21 Soil microorganisms; Test Nitrogen Transform.
[20] Grunditz, C. and Dalhammar, G. (2001). Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. *Water Res*. **35:**433-440
[21] Chatterjee, S., Pillai, A.K and Gupta, V. K (2004). A sensitivity spectrophotometric determination of nitrite in water and soil. *Journal of the Chinese Chemical Society* 51: 195 -198
[22] Narayana, B and Sunil, K. (2009). A spectrophotometric method for the determination of nitrite and nitrate. *Eurasian Journal of Analytical Chemistry* 4 (2): 204-214
[23] Wang, W and Reed, P. (1984).*Nitrobacter* bioassay for aquatic toxicity" Toxicity screening procedures using bacterial systems. 309 – 325
[24] Cervantes, C., Campos-Garc´ıa, J. and Devars, S. (2001) "Interactions of chromium with microorganisms and plants," *FEMS MicrobiologyReviews*. 25(3)335–347
[25] Giner-Lamia, J., L´opez-Maury,L, Florencio, F.J. and Janssen, P.J.(2014) "Global transcriptional profiles of the copper responses in the cyanobacteriumsynechocystis sp. PCC 6803," *PLoS ONE*. 9(9)108912
[26] Osman, D and Cavet, J.S. (2008) "Copper Homeostasis in Bacteria," *Advances in Applied Microbiology*. 65:217–247
[27] Booth, S.C., Weljie, A.M. and Turner, R.J. (2015) "Metabolomics reveals differences of metal toxicity in cultures of Pseudomonas pseudoalcaligenes KF707 grown on different carbon sources," *Frontiers in Microbiology*. 6:827
[28] Gauthier, P.T., Norwood, W.P., Prepas, E.E. and Pyle, G.G. (2014) "Metal-PAH mixtures in the aquatic environment: A review of co-toxic mechanisms leading to more-than-additive outcomes," *Aquatic Toxicology*. 154:253–269
[29] Olaniran, A.O., Balgobind, A. and Pillay, B. (2013) "Bioavailability of heavy metals in soil: Impact on microbial biodegradation of organic compounds and possible improvement strategies," *International Journal of Molecular Sciences*. 14(5)10197–10228.
[30] Bissen,M. and Frimmel, F.H. (2003) "Arsenic—a review. Part I: occurrence, toxicity,speciation, mobility," *ActaHydrochimicaetHydrobiologica*, 31(1) 9–18
[31] Fashola, M.O. Ngole-Jeme, V.M. and Babalola, O.O.(2016) "Heavy metal pollution from gold mines: Environmental effects and bacterial strategies for resistance," *International Journal of Environmental Research and Public Health*. 13(11) 047
[32] Dokaniakis, S.N., Komoros, M.andLyberatos, C. (2005). The effect of zenobiotic bacterialnitrite oxidation. Proceedings of the 9th International conference on environmental science and technology. Rhodes Island, Greece.
[33] Suschka, J., Mrowies, B., Kuszmider, G. (1996). Response of Nitrobacter toxicity of oil field dispersants and domestic detergents. *Tropical Freshwater Biol*ogy 6:65-74 toxicity of weathered and biodegraded oils. *Global Journal of Pure and Applied Science*. 9 (4): 465-474.
[34] Ogeleka, D.F. and Tudararo-Aherobo, L.E. (2013). Assessment of the toxic effects of oil based drilling mud (drilling waste) on Brackish Water Shrimp (*Palaemonetesafricanus*). *Bulletin of Environment, Pharmacology and Life Sciences*. 2 (8): 113-117