



The Toxicity and Genotoxicity of Drilling Fluids Used During Crude Oil Recovery

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Keywords

Allium cepa,
 Chromosomes,
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Abstract

The Drilling of crude oil to bring it up to the subsurface of the earth cannot be accomplished without the use of drilling fluids. Used oil-based drilling fluid was introduced to *Clarias gariepinus* and *Allium cepa* in the determination of its toxic and genotoxic characteristics. This study made use of spent oil- based drilling fluid/ mud which was gotten from oando gas plant, in sterilized plastic containers. Test subjects were acclimatized and stored respectively which lasted for 14days. After both acclimatization and storage of test subjects, test solutions were prepared in different concentrations after a range finding test was conducted. At the end of 96h, LC50 of 71.589 %, 96.052 %, 96.052 % and 59.508 % were obtained at 24hr, 48hr, 72hr and 96hr respectively. It was observed that the higher the concentration the higher the mortality as well as the EC50 which showed relationships between root tip-growth inhibitions and increase in toxicant concentration. Polycyclic aromatic hydrocarbons (PAHs) contents such as Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Benzo (K), Pyrene, Benz (a) anthracene, Chrysene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3-cd) pyrene, Dibenz (a,h), anthracene and Benzo (g,h,i) perylene of oil based drilling mud was analysed using Atomic Absorption Spectrophotometer. These parameters may be responsible for the toxic effects on fish and onion root tips. Hence, the disposal of drill fluids after crude oil recovery should be well monitored so as to ensure the fulfilment of the necessary regulatory standards set up by regulatory bodies.

1. Introduction

The extraction of crude oil from beneath the earth crust up to surface level cannot be achieved without drilling activities and the process of drilling cannot begin nor end without the use of drilling fluids. Crude oil, otherwise known as petroleum, is a fuel existing in liquid form that is generated from within the earth's crust and its fundamental components are a mixture of relatively volatile liquid hydrocarbons that could also contain compounds such as sulphur, nitrogen, and oxygen (Wu et al., 2012).

Crude oil undoubtedly assists us in almost every one of our daily activities. Despite the fact that crude oil is a vital natural resource as well as a great source of economic growth, its methods of extraction, refinement, transportation, and consumption have their fair share of negative impacts, especially on the environment, which the Niger Delta area of Nigeria is a clear representation of. Crude oil is also a major contributor to pollution such as air, land, and water, as well as the cause of numerous illnesses in humans (Yavari et al., 2015), and the negative impacts of crude oil are totally dependent on its toxic level generated from its extraction, refinement, transportation, and consumption.

Drilling fluids are chemicals that aid in the drilling of boreholes at underground levels, i.e., the extraction of crude oil from within the earth's crust up to the sub-surface of the earth (Sahay, 2001). Drilling fluids serve many purposes, some of which include the promotion of the drilling process by interrupting cuttings, improving floatability by increasing the material's viscosity, controlling pressures, and cooling (Hamed & Belhadri, 2009).

The toxicity of a substance is a measure of how it reduces the life and health of living organisms following exposure to the substance (Sharif et. al 2017; APHA., 1995). Drilling waste is a type of industrial waste which is being deposited in large amounts during drilling operations

into nature (Żurek et al., 2017). Drilling fluids contain various additives in their formation and these additives change continuously, which is of great concern as it poses numerous threats to the environment, especially its mode and method of disposal. Toxicity and Genotoxicity studies are required in making future predictions about the effects of substances perceived to be toxic on the health of humans and other living things. In the long term, studies like this will be beneficial for the implementation of public policies concerning the discharge of toxic substances into the environment. Therefore, more data is needed on the toxicity and genotoxicity of used drill muds, and this study is designed to generate additional data, thereby adding to the existing bodies of knowledge.

This study aims to determine the toxicity and genotoxicity of used drill muds during the process of crude oil recovery through a 96hrs acute toxicity test, histopathological studies (on the gills and liver of African Catfish Juvenile) and chromosomal aberration of *allium cepa* (Onion)

2. Research area and sampling methods

The study area adopted for this study is located in the Obio-Akpor Local Government Area in the Niger Delta Region of Nigeria. It is one of Rivers state's 23 local governments, located roughly between latitude 4° 45 "N and 4° 56 "N and longitude 6° 52 "E and 7° 6 "E, with a general elevation of less than 15.24m above mean sealevel. It is bounded by Ikwerre LGA to the north, Port Harcourt LGA to the south, Oyiibo LGA to the east and Emohua LGA to the west (Arokoyu, et al., 2015).

The Obio-Akpor Local Government Area is considered to be amongst the fastest growing L.G. A's in Rivers State because of its growing level of industrialization and high levels of ongoing commercial activities accommodating numerous lines of businesses and parastatals, which

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include educational and health institutions (such as UPTH and UNIPORT) as well as oil and gas companies that serve as a base for petroleum hydrocarbon exploration.

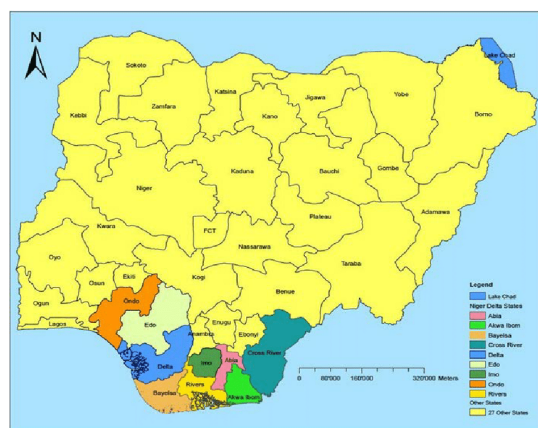


Figure 1. Niger Delta Atlas

This study was carried out at Beuce.C. Powell Toxicity and Biodiversity Laboratory, Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Rivers State. A study population of African Catfish Juveniles (*C. gariepinus*) and large Onions (*Allium Cepa*) was employed for this study. This study made use of both a non-probability and a purposive sampling technique with a sample of 150 African Catfish Juveniles (*C. gariepinus*) and 100 large Onions (*Allium Cepa*).

3. Methodology

This study made use of spent oil-based drilling fluid/mud which was gotten from the Oando gas plant in sterilized plastic containers. Before the commencement of the test, drill fluids were stored immediately after collection at a temperature of exactly 4°C. The test chemical was brought to room temperature before the preparation of test solutions. The procedures utilized for the preparation of used oil-based drilling fluid were in compliance to the methods outlined in Standard Methods for the Examination of Water and Wastewater and EPA (Environmental Protection Agency) protocol 660/3-75-009 (APHA., 1995).

Acclimatization of fishes lasted for 14 days with an adopted static bioassay technique involving the constant renewal of water in which fishes were held once every 24 hours and fish were fed twice daily. After acclimatization, the 96hr acute toxicity test, otherwise known as the short-term toxicity test, commenced with an exploratory test called the range finding test, which is of paramount importance in performing experimental studies to ensure that a more definite test result is generated at the end of the experiment. It is usually used to determine the amount of concentration needed to perform an experimental study from the onset to the end.

Through the range finding test, the LC₅₀ (which is lethal concentration, used in determining the concentration required to cause death in 50% of the test subjects) was determined.

The fishes were exposed to fresh solutions of different concentrations of used drill mud in triplicates within the time frame of the first 2, 4, 6, and 8, then 24, 48, 72, and 96hr per observation. Behavioural changes and mortality in fishes were recorded, immediately removed, and immersed in a 10% concentrate of formaldehyde for preservation. Every other change, such as a change in external appearance, was carefully observed. The factors for determining mortality include: lack of movement (immobility) and irresponsiveness after repeated physical impact on dead fish or fishes with the aid of a probe.

The percentage mortality in the 6 copy tanks for every concentration at various times was independently determined utilizing the formula:

$$\text{Mortality(\%)} = \frac{\text{No dead}}{\text{Total number tested}} \times 100\% \quad (1)$$

For the *Allium Cepa* Assay, Prior to the commencement of experimental observation, the onions were stored in a dry environment to dry up for a period of 14 days (2 weeks) (Bakare, 2002). The outer peel was removed and dried up roots were removed using a sharp razor blade as demonstrated by Babatunde & Bakare., 2006.

After this procedure, different concentrations of drill mud (in water soluble form) mixed with non-chlorinated water in a 1:9 mud to water ratio were measured in 10%, 30%, 50%, 70%, and 90%, all in triplicates, with controls of the same percentages labelled A, B, C, D, and E.

Before the introduction of the onions, the root tips were immersed in non-chlorinated water for about 5 seconds before being placed in their different concentrations, stored and sealed in a large carton to ensure a very dark environment. According to normal standard, the tap water used for this test was changed every 24 hours to avoid the build-up of metabolites which could influence test results in the long term (to achieve accuracy). The individual root length of every one of the 5 bulbs for every concentration of the test sample was estimated with a ruler.

For each concentration and negative control of every sample, the following parameters were determined: the change in growing root morphology, the Arithmetic mean, the percentage of root development restraint (growth inhibition), in conjunction with the negative control and the EC₅₀.

This experiment also involved the assessment of induced chromosomal aberration utilizing the root tips of 2 onion bulbs in a progression of 12 different test concentrations. These root tips were cut and immersed in a methanol: glacial acetic acid (3:1v/v) solution for 48 hours before being hydrolyzed in hydrochloric acid (HCl) at 650 degrees Celsius (Babatunde & Bakare, 2006). As described by (Fiskesjo 1985), six slides were prepared from these root tips using acetocamine stain and for each sample, four (4) slides (which is equivalent to 1000 cells per slide) per concentration were analysed.

The drill mud in its raw form was mixed with magnetic stirrers for 24 hours and allowed to settle for 1 hour. After scooping out the suspended particulate phase (SPP) from the surface using a cotton wool ball, a distillation method was adopted using filter paper and cotton wool to get a pure water-soluble oil based liquid. After this method, liquid was then poured into different plastic bottles into an appropriate container. The suspended particulate phase (SPP) is defined to be 100% SPP (equal to 1000000ppm SPP). Some other concentration of SPP alludes to a level of SPP that was gotten by volumetrically blending 100% SPP with the dilution water. The concentration of the test solutions in percentage were 1%, 5%, 10%, 15%, and 25% for fishes and 10%, 30%, 50%, 70%, and 90%, which was equivalent to exactly 10000, 50000, 100000, 250000, 150000 ppm SPP and 10000, 30000, 50000, 70000, and 90000 respectively. A negative control group representative who was toxicant free was added as well to ensure that experimental procedures were without any form of bias (reliable).

Table 1. Acute toxicity volume and concentration conversion relationship

s/n	Volume used in 5 litres of test water (ml)	Equivalent concentration in %
1	10	1%
2	50	5%
3	100	10%
4	150	15%
5	250	25%

This study adopted a static bioassay technique which involved the constant renewal of the test media once every 24 hours with the utilization of five different test sample concentrations with five test subjects. This preliminary range determination is usually necessary in determining the amount of concentration to be used all through the experiment to ensure that a definite result is produced.

The first and final length measurement of test organisms before and after exposure was done by means of a calibrated ruler in cm². In the same vein, weight measurement (g) was done by means of a Mettler balance, the PB602 Tobelo model and the result is presented in Table 1.

The PC parameters analysed in the test solution include temperature, pH, and dissolved oxygen (DO). The water quality check was done before and after exposure according to APHA/AWWA/WEF, (1998).

The temperature was measured under laboratory conditions by means of a multi-meter water checker (Sper Scientific Benchtop Meter-860033). A 100ml of test water was put in a conical flask and the multi-meter probe was introduced while powered on and the setup was observed for 10-15min. The stabilized temperature reading displayed on the screen was recorded.

A multi-meter water checker (Sper Scientific Benchtop Meter-860033) was used to measure pH. A 100ml of test water was put in a conical

flask and the multi-meter probe was introduced while powered on and the setup was observed for 10-15min. The stabilized pH reading displayed on the screen was recorded.

A DO Analyzer meter with model number JPB-607A was used to determine DO. A 100ml of test water was put in a conical flask and the multi-meter probe was introduced while powered on and the setup was observed for 10-15min. The stabilized reading displayed on the screen was recorded.

4. Research Results

4.1. Physico-Chemical Parameters

Table 2 depicts the mean $\pm$ SD values of the physico- chemical parameters of the water samples across different concentrations of drill fluids derived from this study after 96hr exposure of *C.gariepinus*. The parameters tested were pH, Temperature and dissolved oxygen. Results gathered showed a decrease in pH, Temperature relative to increasing concentrations and a normal range in DO with slight increase and reduction in 10% and 5% respectively.

Table 2. Mean and Standard Deviation of Physico-Chemical Parameter Analysis during the 96hr Acute Exposure

Parameter	Control						
	After	1%	5%	10%	15%	25%	
PH	7.23 $\pm$ 00	6.93	5.71	5.93	6.24	6.27	
	$\pm$ 00	$\pm$ 00	$\pm$ 00	$\pm$ 00	$\pm$ 00	$\pm$ 00	
Temp	27.6	27.3	27.2	27.0	27.2	27.2	
	$\pm$ 00	$\pm$ 00	$\pm$ 01	$\pm$ 02	$\pm$ 03	$\pm$ 03	
DO	0.3	0.3	0.2				
	$\pm$ 00	$\pm$ 00	$\pm$ 02	0.7 $\pm$ 03	0.3 $\pm$ 00	0.3 $\pm$ 00	

Figure 2 represents physico-parameters results of before and after exposure of *C.gariepinus* against drill fluid concentrations of 0% (control), 1%, 5%, 10%, 15% and 25%.

The before exposure (Test Solution) and during (96 hours) exposure physicochemical parameters results are presented in tables 2.

4.2. Water quality parameter analysis

Data were analysed on the physic-chemical parameters of water samples across the different concentrations prior to the commencement of exposure and during exposure. The figures 2 - 4 below gives a clear representation of data analysed.

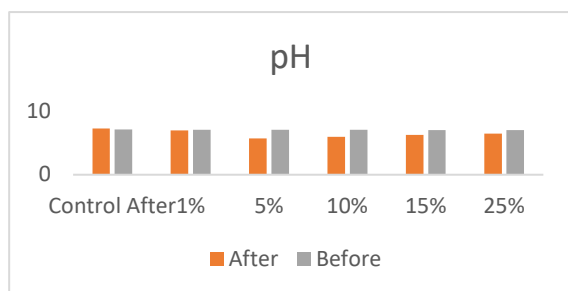


Figure 2. pH Variation before and during the 96hrs exposure

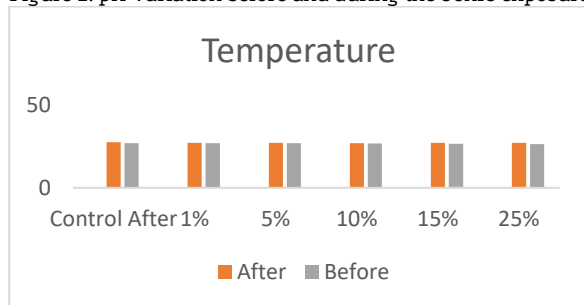


Figure 3. Temperature Variation before and during the 96hrs exposure

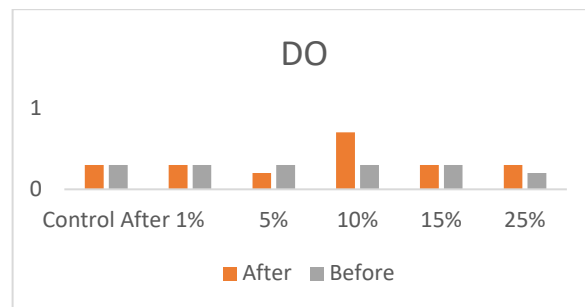


Figure 4. DO Variation before and during the 96hrs exposure

4.3. Polycyclic Aromatic Hydrocarbons analysed on drill mud

During the course of the 96hrs exposure, mortalities in fishes were recorded in 2hrs, 4hrs, 6hrs, 8hrs and in 24hrs, 48hrs, 72hrs and 92hrs. Results generated from these monitoring are captured in table 3.

Table 3. Polycyclic Aromatic Hydrocarbons analysed on drill mud

S/N	PAHs	OBM (PPM)	EXPERIMENTAL TEST WATER (PPM)
1.	Naphthalene	357.82741	162.20762
2.	Ace naphthalene	291.33992	76.13058
3.	Acenaphthene	381.84382	128.95643
4.	Fluorene	318.57901	106.51739
5.	Phenanthrene	437.44292	271.24953
6.	Anthracene	260.24901	194.77257
7.	Fluoranthene	403.32915	200.11833
8.	Pyrene	264.48130	96.62443
9.	Benzo (A) anthracene	601.85321	288.30224
10.	Chrysene	515.26133	256.12045
11.	Benzo (B) Fluoranthene	266.82964	106.18069
12.	Benzo (K) Fluoranthene	325.19693	210.49103
13.	Benzo (A) Pyrene	180.44138	101.59473
14.	Indeno (1,2,3-CD) Pyrene	178.45138	100.59374
15.	Dibenz (A, H) Anthracene	200.72271	120.51051
16.	Benzo (G, H, I) Perylene	200.53816	100.41325

Table 4. Acute Toxicity Mortality Values in Mean and Percentage

Conc. (%)	Mortality Time (Hours)							
	24	%	48	%	72	%	96	%
0 (control)	0	0	0	0	0	0	0	0
1%	0	0	0	0	2	40	1	20
5%	1	20	0	0	0	0	2	40
10%	0	0	0	0	0	0	0	0

Table 5. LC50 Values

Time (Hours)	LC ₅₀ (%)
24	71.589
48	96.052
72	96.052
96	59.508

4.4. Histopathology of the Gills of *Clarias gariepinus* after 96hrs Acute Exposure to used Drilling Fluid

Histopathological analysis of gills of *Clarias gariepinus* after 96hrs acute exposure showed various levels of variations. Results showed that control fish had normal gill arrangement when compared with gills of exposed fish that exhibited several histological alterations. The observed alterations were mucus cells (MC)-distortion, blood congestion (BC), separation of layers (SL) and sloughing (S). In addition, it was observed that alterations were concentration dependent.

At low concentrations of 1% and 5% mild alterations of gill formation patterns were observed which showed slight deviation from control fish. At high concentrations of 10%, 15% and 25% severe alterations of gill formation patterns were observed which showed slight deviation from control fish.

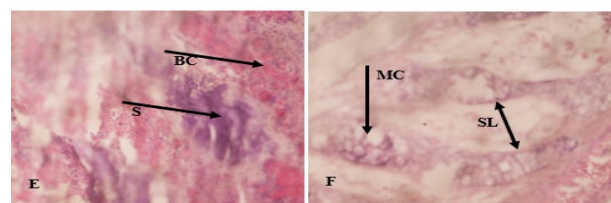
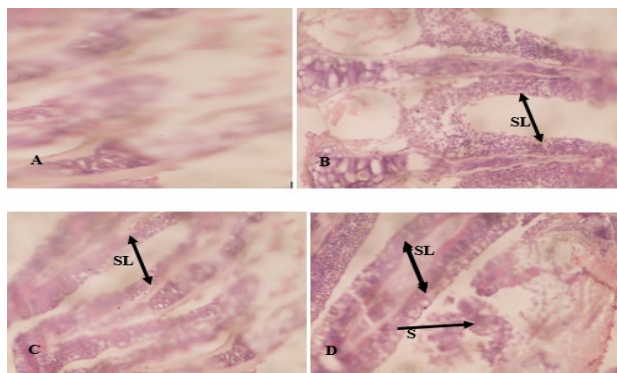


Figure 5. Photomicrograph of the Gills of *C. gariepinus* after 96hours of Exposure to Toxicant. X100. A-Control, B-1%, C-5%, D-10%, E-15% and F-25%

Table 6. Root Length of *Allium cepa* after 72hour exposure to different concentrations of used drill mud

Concentration(ppm)	Average root length for each bulb(cm)										Root range				
	Mean	SD	SE	95% Confidence											
	1	2	3	4	5	6	7	8	9	10					
Control	2.03	2.18	2.5	2.04	2.13	2.21	1.84	2.19	1.06	1.21	1.06 - 2.9	1.94	0.46	0.14	0.33
10%	0.83	0.78	0.81	0.79	0.96	0.86	0.78	0.74	0.76	0.79	0.76-0.81	0.81	0.06	0.02	0.04
30%	0.75	0.77	0.79	0.79	0.78	0.75	0.74	0.76	0.79	0.77	0.74-0.79	0.77	0.02	0.01	0.01
50%	0.53	0.49	0.59	0.53	0.45	0.57	0.57	0.5	0.51	0.56	0.45-0.59	0.53	0.04	0.01	0.03
70%	0.3	0.31	0.32	0.31	0.32	0.33	0.36	0.34	0.32	0.36	0.3-3.6	0.33	0.02	0.01	0.01
90%	0.23	0.21	0.2	0.22	0.23	0.24	0.28	0.21	0.28	0.18	0.18-0.28	0.23	0.03	0.01	0.02

4.5. Histopathology of Liver of *Clarias gariepinus* after 96hrs Acute Exposure to used Drilling Fluid

Histopathological analysis of liver of *Clarias gariepinus* after 96hrs acute exposure showed various levels of variations. Results showed that control fish had normal gill arrangement when compared with gills of exposed fish that exhibited several histological alterations. The observed alterations were mild necrosis (N), swelling of blood vessel (SB), pyknosis (P) and vacuolation(V). In addition, it was observed that alterations were concentration dependent.

At low concentrations of 1% and 5% mild alterations of gill formation patterns were observed which showed slight deviation from control fish. At high concentrations of 10%, 15% and 25% severe alterations of gill formation patterns were observed which showed slight deviation from control fish.

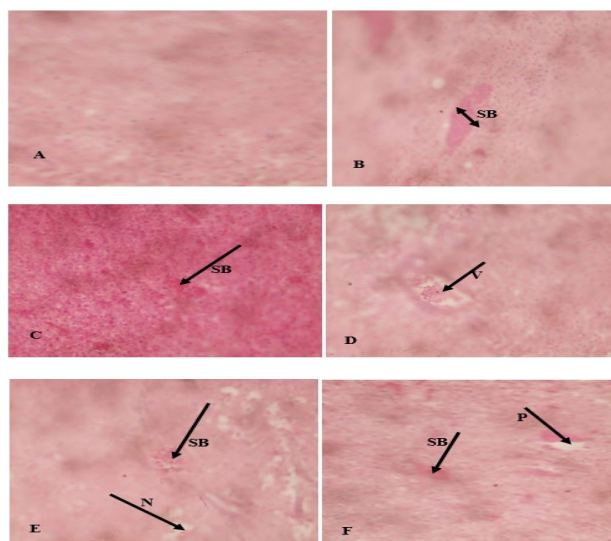


Figure 6. Photomicrograph of the Liver of *C. gariepinus* after 96hours of Exposure to Toxicant. X100. A-Control, B-1%, C-5%, D-10%, E-15% and F-25%

4.7. Toxicity and Genotoxicity Testing of Onion- *Allium cepa* (Onion Assay)

4.7.1. Macroscopic Analysis

Table 4.3 shows result of macroscopic analysis of Onion (*Allium cepa*) after 72 hours exposure to various concentrations of used drill fluid. It was observed from the table that growth index of root decreased with increasing concentration of used drill fluid. The minimum to maximum root growth range, mean and standard deviation values of the different concentrations as compared to controls roots were as follows: Range; 0.74-0.96, 0.74-0.79, 0.49 - 0.59, 0.3 - 0.36 and 0.18 - 0.28 respectively as against 1.06 - 2.21 of the controls. While the mean $\pm$ SD values for the various concentrations were: 0.81 ± 0.06 , 0.77 ± 0.02 , 0.53 ± 0.04 , 0.33 ± 0.02 and 0.23 ± 0.03 respectively as against 1.94 ± 0.14 , of the controls.

Table 6 shows the mean with standard error (SE) and percentage root growth (RG) of Onion (*Allium cepa*) after 72 hours exposure to various concentrations of used drill mud. Results revealed that mean and percentage root growth decreased with increasing concentration of used drill mud. The mean root length with SE and RG percentage values of the various concentrations (30.30, 20.20, 10.10, 7.07 and 5.05 ppm) as compared to their respective control (untreated) roots were as follows: 0.81 ± 0.02 , 0.77 ± 0.01 , 0.53 ± 0.01 , 0.33 ± 0.01 and 0.23 ± 0.01 respectively as against 1.94 ± 0.14 of the control. While the root growth (RG) in percentage of control values for the various concentrations were: 41.75, 39.69, 27.32, 17.01 and 11.86 respectively as against 0.0% of the controls.

Table 7. Mean Root length and Percentage Root Growth of *Allium cepa* after 72Hours of Exposure to Different Concentrations of used Drill Mud

Concentration (ppm)	Mean Root Length $\pm$ SE (cm)	RG (%) of control
Control	1.94 ± 0.14	0
10%	0.81 ± 0.02	41.75
30%	0.77 ± 0.01	39.69
50%	0.53 ± 0.01	27.32
70%	0.33 ± 0.01	17.01
90%	0.23 ± 0.01	11.86

Figure 7 shows a plot of percentage (%) root growth of Onion- *Allium cepa* (in relation to control) after exposure to various concentrations of used drill mud. Result showed that percentage root growth was significant with increasing concentration.

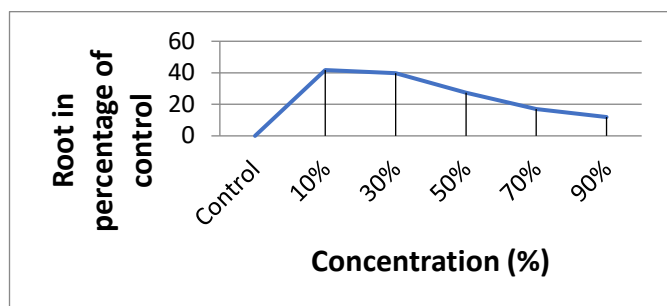


Figure 7. Plot of Percentage (%) Growth of *Allium cepa* Roots (in relation to control) and Effective Concentration after 72Hours Exposure to used drill mud

4.7.2. Microscopic Analysis of Onion -*Allium cepa*

Table 7 shows the cytological effect of toxicant on Onion -*Allium cepa* after 72 hours exposure to various concentrations. Findings revealed that number of dividing cells and mitotic index increased as the

concentration increased while mitotic inhibition of control increased with increasing concentration.

Table 7. Cytological effect of used drill mud on the root tips of Onion- *Allium cepa*

Concentration (ppm)	No of cells scored	No of dividing cells	Mitotic Index	Mitotic Inhibition of control
Control	1000	450	45	0
10%	1000	250	25	44.44
30%	1000	230	23	48.89
50%	1000	200	20	55.56
70%	1000	170	17	62.22
90%	1000	110	11	75.56

Figure 8 shows a plot of mitotic inhibition (in relation to control) of cells dividing in root tips of Onion-*Allium cepa* after 72 hours exposure to used drill mud. Result revealed that mitotic inhibitions increased as concentrations increased.

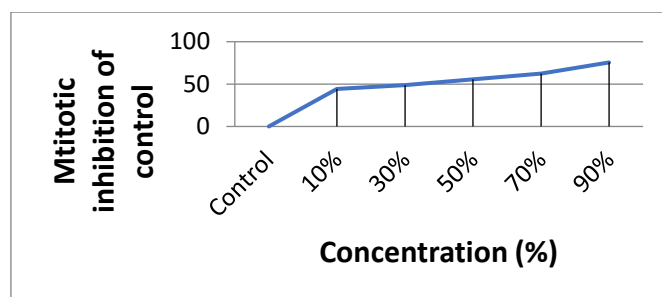


Figure 8. Plot of Mitotic Inhibition (in relation to control) of Cells in Root tips of *Allium cepa* after 72Hours Exposure to used drill mud

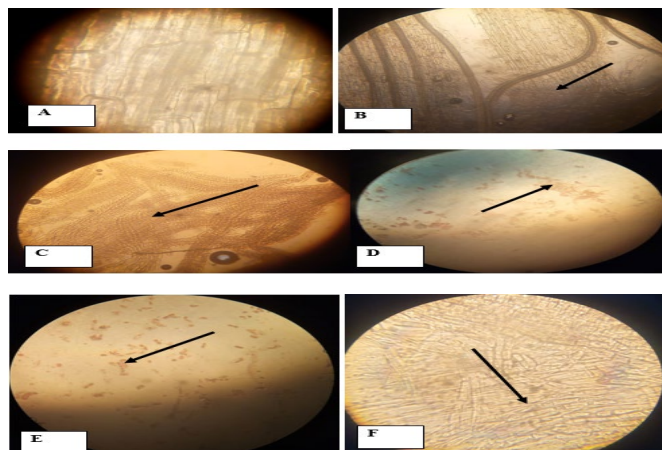


Figure 9. Chromosomal aberration recorded in root tips of *A. cepa* treated with used drill mud. A-Control, B-10% (Anaphase lag), C-30% (Sticky chromosomes), D-50% (Chromosome break), E-70% (Chromosome break) and F-90% (Polyploid metaphase)

5. Discussions

The various water quality parameters investigated in the course of the 96-hour toxicity included pH, temperature, and dissolved oxygen. Physico-chemical parameters are good indicators for monitoring water quality. The water quality study revealed that pH and temperature decreased as the concentration of toxicants increased while dissolved oxygen remained constant. The decrease in water

quality parameters could be due to the presence of toxicants or a rise in the respiratory activities of the exposed fish due to the toxicant (Warren, 1977). As stated by Omitoyin et al. (2006) and Adesina (2008), the effect of toxic substances on gill epithelium is a function of the rise in ventilation.

It was observed during the 96h exposure that general fish reactions to toxicants increased with increasing concentration. Some behavioural changes recorded were: erratic swimming and excessive jumping, especially at higher concentrations. (Ayoola, 2007) in his report attributed these changes in behaviour as resultant impacts of respiratory rate mutilation, skin exasperation, or a reaction to distorted locomotor action, which is an indicator of the consequence of a toxic substance on the nervous system.

Mortality of fish was recorded across concentrations in the course of exposure. This is a pointer to the fact that fish mortality in the course of exposure was concentration dependent as against control fish that recorded zero mortality throughout the experimentation.

The lethal concentration (LC₅₀) of used drill mud obtained from the 96hours bioassay was 71.589, 96.052, 96.052, and 59.508%, respectively, at 24, 48, 72, and 96 hours. The implication of these LC₅₀ values is that, within 24, 48, 72, and 96 hours, 71.589, 96.052, 96.052, and 59.508% of the toxicant killed at least 50% of *Carias gariepinus*.

Various LC₅₀ values were reported by different researchers. (Olagunju FI., 2007) reported a LC₅₀ of 4.2mg/l with 95% confidence (lower and upper) limits of 31.86 – 93.81 mg/l in a 96h exposure of aqueous extracts of pawpaw seed powder to *O. niloticus*. Similarly, an LC₅₀ of 13.93 mg/l was reported by (NC, 2002) in a 96h exposure of extracts of the ringworm plant *Senna alata* to *O. niloticus* juveniles.

The various histological alterations observed were as follows: Mucus cells (MC)-distortion, blood congestion (BC), separation of layers (SL) and sloughing (S). It was suspected that the histological alterations of the gills were due to uptake of heavy metals and PAH components of the toxicant (used drill mud) found in the test water (J, 1983). This study has revealed that used drill mud has acute lethal impacts on *Clarias gariepinus*. The observed alterations Mucus cells (MC)-distortion, blood congestion (BC), separation of layers (SL) and sloughing (S) could be a resultant effect of cell multiplication and rise in girth of gill filament epithelium (A, 2007).

The various histological alterations observed were as follows: mucous cell mild necrosis (N), swelling of blood vessels (SB), pyknosis (P), and vacuolation (V). The hydropic swelling morphological variation of the urinary tubules was due to cellular hypertrophy and as well as the occurrence of tiny particles in the cytoplasm which are net-like in form. This study has shown that an increase in the concentration of toxicants results in cytoplasmic vacuolation, cellular degeneration, damage to nuclei, bile stagnation, as well as clogging of the blood sinusoids.

These harmful activities impinge on the swap of oxygen and tissue respiration, resulting in organ and tissue hypoxia, disintegration, and necrosis. Furthermore, cellular degeneration and necrosis could be caused by the effects of metal accumulation in hepatic tissues.

Results of root growth inhibition revealed that a significant root growth inhibition was reported at different concentrations of toxicant with a determined effective concentration (EC₅₀) of 22.5%. In a similar vein, variable average root length was reported at various concentrations of the toxicant. It was observed that average root length decreased as the concentration of toxicants increased.

The Mitotic index values obtained from treatments that are larger than the control (untreated) values could be due to induced cell division, which may be detrimental to cells by resulting in unguarded build-up and the development of tumors (M.M, 2002). From the study, it was observed that there was significant reduction of MI in *A. cepa* root apex cells displaying sub-lethal impact at various concentrations. The progressive reduction of the mitotic index across increasing concentrations shows that the toxicant (used drill mud) has the potential to disrupt cell growth and may interrupt the cell cycle, giving rise to reduced dividing cell numbers (Turkoghu, 2012). Chromosome stickiness is directly proportional to chromatin dysfunction (Mesi et al., 2013). The chromosome breakage recorded could be due to movement of a non-equal chromatid or the occurrence of a dicentric chromosome. Anaphase lagging chromosomes are a function of spindle collapse (Mesi et al., 2013) and, as such, have the potential to cause aneuploidy (Mesi et al., 2013). Hence, the recorded chromosomal aberrations showed that used drill mud is capable of altering the structure and number of chromosomes in *A. cepa*.

6. Conclusions

Over the years, the use of drilling fluids in the recovery of crude oil has been of paramount importance in all activities associated with drilling. The disposal of used drilling fluids has been a major concern in this study, hence the carrying out of this research work in determining the toxicity and genotoxicity of used drilling fluids through laboratory examinations such as: determining the 96-hour acute toxicity, histopathological effects of drill fluids on gills and liver; and determining genotoxicity through the *Allium cepa* Assay.

The results obtained from the study show that used drill mud has a high toxic level. Hence, more work should be done by regulatory bodies in the strengthening of their already existing laws as regards to the proper treatment and disposal of drill fluids.

Results gathered from this study showed that drilling mud is toxic in nature due to its toxic impact on *C.gariepinus* observed through its 96hr acute toxicity testing and its histopathological effects on the gills and liver of *c.gariepinus*.

The results also revealed its genotoxic effects on *Allium cepa* through its root growth and mitotic inhibition as well as chromosomal aberration.

Declaration of Conflict of Interests

The authors declare that there is no conflict of interest. They have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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