ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue VI June 2023

Toxicological and Genotoxic Effects of Used Drilling Fluid: Histopathology and Genotoxicity Assessment

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DOI: https://doi.org/10.51244/IJRSI.2023.10615

Received: 27 May 2023; Accepted: 19 June 2023; Published: 16 July 2023

Abstract: During the process of extracting crude oil from the subsurface of the earth, drilling fluids play a crucial role. To evaluate the toxic and genotoxic characteristics of used oil-based drilling fluid, experiments were conducted using Clarias gariepinus (a type of fish) and Allium cepa (an onion). Prior to the experiments, the test subjects were acclimatized and stored for a period of 14 days. Following acclimatization and storage, test solutions were prepared in different concentrations based on the results of a range-finding test. After 96 hours of exposure, the LC50 values for the drilling fluid were determined as follows: 71.589% at 24 hours, 96.052% at 48 hours, 96.052% at 72 hours, and 59.508% at 96 hours. The mortality rate was recorded, revealing a direct relationship between the concentration of the drilling fluid and the mortality of the test subjects. The EC50 values also indicated a correlation between root tip-growth inhibition and increased toxicant concentration. Furthermore, histopathological studies conducted on the gills and livers of Clarias gariepinus after 96 hours of exposure revealed noticeable alterations. To analyze the content of polycyclic aromatic hydrocarbons (PAHs) present in the oil-based drilling mud, substances such as Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenathrene, 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 were analyzed using GC-MS mode S4 = 71096. These parameters are believed to be responsible for the toxic effects observed in fish and onion root tips. Considering the toxic effects and alterations observed, it is crucial to closely monitor the disposal of drilling fluids after the recovery of crude oil. Compliance with the necessary regulatory standards established by regulatory bodies should be ensured to prevent adverse environmental impacts.

Keywords: Acute exposure, Allium cepa, Chromosomal aberrations, Mitotic index, Toxicant.

I. Introduction

Drilling activities are essential for the extraction of crude oil from beneath the earth's crust, and the process of drilling relies on the use of drilling fluids (Wu et al., 2012). Crude oil, also known as petroleum, is a liquid fuel formed within the earth's crust, consisting of volatile liquid hydrocarbons and various compounds like sulfur, nitrogen, and oxygen (Wu et al., 2012). Once extracted, crude oil undergoes a process called "fractional distillation" to make it suitable for producing fuels such as gasoline, diesel, and heating oils, which serve transportation, domestic, and power generation purposes (Shooto et al., 2012).

While crude oil plays a crucial role in our daily activities and economic growth, its extraction, refinement, transportation, and consumption have negative impacts on the environment, as exemplified by the Niger Delta area in Nigeria (Yavari et al., 2015). Crude oil is a significant contributor to pollution, including air, land, and water pollution, as well as causing various illnesses in humans (Yavari et al., 2015). The extent of these negative impacts depends on the toxic levels generated during extraction, refinement, transportation, and consumption processes.

As mentioned previously, drilling is an indispensable part of crude oil extraction from the earth's crust to subsurface levels, and drilling fluids are crucial for the successful execution of this process (Committee, 2011; Benka-Coker et al., 1996; Gbadebo et al., 2010). Drilling fluids are chemical substances that facilitate borehole drilling for crude oil extraction from the earth's crust to the subsurface (Sahay, 2001). They serve multiple purposes, such as aiding the drilling process by removing cuttings, increasing material viscosity to enhance floatability, controlling pressures, and providing cooling (Hamed et al., 2009).

Drilling fluids can be categorized based on their materials and mode of use. In terms of materials, they are classified as water-based, oil-based, and gaseous drilling fluids. In terms of mode of use, they are classified as fluid loss additives, clay stabilization agents, lubricants, gelling agents, anti-freeze agents, and enhanced oil recovery substances (Mueller et al., 2004). During crude oil

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extraction, the oil and gas rise to the top of porous rock layers due to their lower density than water. However, they can become trapped below non-porous rock layers, and drilling through these layers is necessary to access the trapped oil or gas.

Toxicity is a measure of how a substance negatively affects the life and health of living organisms after exposure (Sharif et al., 2017). Drilling waste, a type of industrial waste generated during drilling operations, is deposited in large quantities in nature (Żurek, Jamrozik, & Gonet, 2017).

The composition of drilling fluids includes various additives, and the continuous changes in these additives raise significant concerns due to the potential threats they pose to the environment, particularly in terms of their disposal methods. In standard procedures, after drilling fluids are utilized for crude oil extraction, they are supposed to undergo treatment before being disposed of or reused. However, due to inadequate monitoring and enforcement by regulatory bodies such as the Department of Petroleum Resources (DPR) and the Federal Ministry of Environment, the used drilling fluids or muds, along with their cuttings, are often disposed of in water bodies, especially during offshore oil exploration activities. This disregard for proper disposal ignores the potential toxicity levels associated with these drill wastes.

The main issue at hand is the lack of sufficient knowledge regarding the toxicity and genotoxicity of drilling fluids, despite previous studies that have been conducted to investigate their harmful effects. Toxicity and genotoxicity studies are essential for predicting the long-term effects of potentially toxic substances on human and environmental health. Such studies play a crucial role in the development of public policies concerning the discharge of toxic substances into the environment. Therefore, more data is needed to understand the toxicity and genotoxicity of used drill muds, and this study aims to generate additional information to contribute to the existing body of knowledge.

The objective of this study is to assess the toxicity and genotoxicity of used drill muds during the crude oil recovery process. This will be achieved through a 96-hour acute toxicity test, histopathological studies focusing on the gills and liver of African catfish juveniles, and chromosomal aberration analysis using Allium cepa (onion) as a test organism.

II. Methodology

Material

The study area selected for this research is situated in the Obio-Akpor Local Government area, which is part of the Niger Delta Region in Nigeria. It is one of the 23 local governments in Rivers State, located approximately between latitude 40 45" N to 40 56" N and longitude 60 52" E to 70 6" E. The area has a general elevation of less than 15.24 meters above sea level. It is bounded by Ikwerre LGA to the north, Port Harcourt LGA to the south, Oyigbo LGA to the east, and Emohua LGA to the west (Arokoyu, Mark, & Jochebed, 2015). Obio-Akpor Local Government Area is known for its rapid growth in Rivers State due to the increasing level of industrialization and ongoing commercial activities. It accommodates various businesses, institutions, and organizations, including 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.

The research was conducted at the Beuce C. Powell Toxicity and Biodiversity Laboratory, Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Rivers State. The study focused on African Catfish Juveniles (C. gariepinus) and large Onions (Allium Cepa) as the study population. Both non-probability and purposive sampling techniques were used, and a sample of 150 African Catfish Juveniles (C. gariepinus) and 100 large Onions (Allium Cepa) was selected for the study.

Data for this research were collected from primary and secondary sources. Extensive literature review was conducted, including local and international journal publications, articles, textbooks, web resources, and previous related thesis work. Additionally, laboratory results were obtained from primary data through experimental analysis. The test subjects (African Catfish Juveniles and Onions) were exposed to varying concentrations of toxicants, specifically drill fluid in this study.

The African Catfish Juveniles (Clarias gariepinus) used in the study were purchased from ARAK in ALUU community, Rivers State, while the Allium Cepa (Onion) was procured from Choba market. The study utilized spent oil-based drilling fluid obtained from the Oando gas plant and stored in sterilized plastic containers. The drill fluids were collected and stored at a temperature of exactly 40°C before the start of the test. Prior to the preparation of test solutions, the drill fluid was brought to room temperature. The procedures followed for the preparation of the used oil-based drilling fluid complied with the methods outlined in the Standard Methods for the Examination of Water and Wastewater, as well as the EPA (Environmental Protection Agency) protocol 660/3-75-009 (APHA., 1995).

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Methods

Once the African catfish juveniles were purchased from ARAK in ALUU Community, Rivers State, and the onions were obtained from Choba market, both the fish and onions were transported to the laboratory to commence the experimental studies. Upon arrival at the laboratory, the fish were placed in separate four-cornered plastic containers filled with enough water for their survival, while the onions were spread out to dry. The fish underwent a 14-day acclimatization period using a static bioassay technique. During this period, the water in which the fish were held was constantly renewed once every 24 hours, and the fish were fed twice daily.

After the acclimatization period, the 96-hour acute toxicity test, also known as the short-term toxicity test, began. This test started with a range-finding test, which is crucial in experimental studies as it helps determine the appropriate concentration needed for the experiment. The range-finding test helps establish the lethal concentration (LC50), which is the concentration required to cause death in 50% of the test subjects.

Following the range-finding test, a sub-sample of the test solution was mixed with filtered test dilution water in a volumetric flask, using a mud-to-water ratio of 1 to 9. The solution was then stirred using a magnetic stirrer for 24 hours and allowed to settle for an hour. The surface water was decanted from the suspended mud and transferred to another glass holding tank before introducing the fish. The final test involved placing 6 juvenile catfish per tank, exposed to varying concentrations of toxicants in triplicates for the five concentrations of used drilling mud. Additionally, there was one control group for the 96-hour acute toxicity test.

The fish were exposed to fresh solutions of different concentrations of used drill mud in triplicates for specific time intervals (2, 4, 6, and 8 hours, as well as 24, 48, 72, and 96 hours). Behavioral changes and mortality in the fish were recorded, and any deceased fish were immediately removed and preserved in a 10% formaldehyde solution. Other changes, such as external appearance, were carefully observed. Factors indicating mortality included lack of movement (immobility) and unresponsiveness after repeated physical impact on the dead fish using a probe.

The percentage mortality in the six tanks for each concentration at various time intervals was independently determined using the formula:

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

Prior to the start of the experimental observation for the Allium Cepa Assay, the onions were kept in a dry environment for a period of 14 days (2 weeks) to dry out, as recommended by Bakare (2002). The outer peel of the onions was removed, and any dried roots were carefully eliminated using a sharp razor blade, following the procedure demonstrated by Babatunde and Bakare (2006).

After these preparations, different concentrations of drill mud, in a water-soluble form, were mixed with non-chlorinated water in a ratio of 1:9 (mud to water). The concentrations used were 10%, 30%, 50%, 70%, and 90%, each replicated in triplicates, with corresponding controls labeled A, B, C, D, and E. Before introducing the onions, the root tips were briefly immersed in non-chlorinated water for approximately 5 seconds. They were then placed in their respective concentrations, stored, and sealed in a large carton to ensure a completely dark environment. To maintain accuracy, the tap water used in this test was changed every 24 hours to prevent the accumulation of metabolites that could potentially affect the test results in the long term. At the 72-hour mark, the length of individual roots from five bulbs for each concentration of the test sample was measured using a ruler.

For each concentration and negative control of the sample, several parameters were determined: the changes in root morphology, the arithmetic mean, the percentage of growth inhibition (root development restraint) in comparison to the negative control, and the EC50. Additionally, this experiment involved the assessment of induced chromosomal aberrations using the root tips from two onion bulbs for each test concentration. These root tips were cut and immersed in a mixture of methanol and glacial acetic acid (3:1 v/v) at 48 hours, as described by Babatunde and Bakare (2006). Subsequently, they were hydrolyzed in hydrochloric acid (HCl) at 65°C. Following the method outlined by Fiskesjo (1985), six slides were prepared from these root tips using acetocarmine stain. For each sample, four slides (equivalent to 1000 cells per slide) per concentration were analyzed.

A total of one hundred and fifty juvenile sharp-toothed African catfish were purchased from ARAK in ALUU community, Rivers State. Each fish had an average weight of 10.0±0.3g and an average length of 6.0±0.1cm. Juveniles were chosen for this study due to their high sensitivity to toxicity testing compared to other age groups. Immediately after purchase, the fish were transported to the C.B. Powell Toxicity and Biodiversity Laboratory in four open-ended black plastic containers, each with a capacity of 50 liters and filled with enough water for the fish to survive.

The fish were then transferred to four separate containers, with 50 fish in each container, for a 14-day acclimatization period. The selection of fish for each container was based on their average length and weight. Throughout the acclimatization period, the fish were fed a commercial floating pellet diet twice a day. This feeding process was essential for monitoring the feeding behavior of the fish under laboratory conditions. Non-chlorinated tap water with a temperature of 27.1°C, pH of 7.08, and dissolved oxygen

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level of 0.3mg/l was used for the acclimatization process. To prevent the accumulation of waste metabolites, such as fecal matter and uneaten fish feed, the water in the containers was replaced daily.

After the acclimatization period, selected fish were set aside and fasted for 24 hours to ensure that only the strongest and healthiest individuals were used. The body weight and length of the selected fish were measured using a meter rule and a weighing balance before introducing them one by one to tanks containing different concentrations of the test fluid. The treatment tanks, with a capacity of 30 liters each, accommodated six fish in triplicates, totaling 18 fish per concentration. The tanks were filled with 5 liters of water and covered with metal nets to prevent fish from escaping. The fish were closely monitored for acute toxicity over a period of 96 hours. Observations were recorded at 2-hour intervals during the initial 8 hours and then at 24-hour intervals (48, 72, and 96 hours) after the fish were introduced to the tanks.

Using a surgical blade, an incision was made along the mid-belly of the fish, extending upward to expose both the liver and gills for collection. Once the liver and gills were harvested from two fish from each concentration treatment tank, they were preserved in formaldehyde and subsequently sent to the Department of Anatomy for histopathological studies. The test organs were fixed in Bouin's fluid for 24 hours, then rinsed with 70% ethanol, and dehydrated using a series of graded ethanol concentrations, following the guidelines described by Schalm et al. (1975). Next, the organs were soaked in paraffin, sectioned at a thickness of 4-5 µm, stained with hematoxylin and eosin, and carefully examined using a light microscope and photomicrography, as outlined by Kaneko (1989).

A total of 100 onions, ranging in size from 17mm to 28mm, were purchased from Choba market and transported to C. B Powell's laboratory in a black poly bag. Upon arrival at the laboratory, the onions were spread out in a dry area due to the absence of sunlight during the rainy season, ensuring that they remained dry. The onions were left in this spread-out state for a period of 14 days before being subjected to experimental conditions.

The root tips of the onions were harvested after immersing them in test solutions of varying concentrations. This process involved assessing induced chromosomal aberrations. Two onion bulbs were used for each test concentration, with a total of 12 root tips examined. The root tips were cut and immersed in a solution of methanol and glacial acetic acid (3:1 v/v) after 48 hours. Subsequently, they were hydrolyzed in hydrochloric acid (HCl) at a temperature of 65°C. Following the method described by Fiskesjo (1985), six slides were prepared from these root tips using acetocarmine stain. For each sample, four slides were prepared, with each slide containing approximately 1000 cells, and this was done for each concentration.

Preparation of the test solutions involved mixing the raw drill mud with magnetic stirrers for 24 hours, followed by a settling period of 1 hour. The suspended particulate phase (SPP) was then carefully scooped out from the surface using cotton wool. A distillation method was employed, utilizing filter paper and cotton wool, to obtain a pure water-soluble oil-based liquid. This liquid was then poured into different plastic bottles and stored appropriately. The concentration of the test solutions was expressed as a percentage, ranging from 1% to 25% for fishes and from 10% to 90% for onions. These concentrations corresponded to specific levels of SPP, with values ranging from 10,000 to 250,000 ppm for fishes and from 10,000 to 90,000 ppm for onions. A negative control group, free of toxicants, was included to ensure unbiased experimental procedures.

De-chlorinated tap water was used as filtered dilution water for the acclimatization of the test organisms. The volumes required to prepare the test concentrations were calculated based on the density of the chemical, with 1 gram equivalent to 0.99047 ml. The study employed a static bioassay technique, involving the regular renewal of the test media every 24 hours. Five different test sample concentrations were utilized, with five test subjects in each concentration. This preliminary range determination was crucial for determining the appropriate concentration to be used throughout the experiment, ensuring reliable and definitive results.

Physiochemical Analysis of the Test Solution

The parameters of temperature, pH, and dissolved oxygen (DO) were analyzed in the test solution. The water quality assessment was conducted before and after exposure, following the guidelines of APHA, AWWA, and WEF (1998). To measure temperature, a multi-meter water checker (Sper Scientific Benchtop Meter-860033) was used under laboratory conditions. A conical flask containing 100 ml of test water was prepared, and the multi-meter probe was inserted while the device was powered on. The setup was observed for 10–15 minutes, and the stabilized temperature reading displayed on the screen was recorded.

For pH measurement, the same multi-meter water checker (Sper Scientific Benchtop Meter-860033) was employed. Similarly, a conical flask with 100 ml of test water was used, and the multi-meter probe was inserted while the device was powered on. After observing the setup for 10–15 minutes, the stabilized pH reading displayed on the screen was recorded. To determine the dissolved oxygen (DO), an analyzer meter with the model number JPB-607A was utilized. A conical flask containing 100 ml of test water was prepared, and the multi-meter probe was inserted while the device was powered on. The setup was observed for 10–15 minutes, and the stabilized reading displayed on the screen was recorded.

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Statistical Analysis and Assay Calculations

The significance level within the means of the different parameters studied was determined using an ANOVA ($p > 0.05$). The data obtained from this study was recorded as means ± standard deviation (SD).

In the assessment of biological and biochemical parameters in various water samples, including a negative control, correlation analysis was conducted to determine the relationships between these parameters. Correlation analysis was also employed to observe the response of the test organisms to the used drill mud as well as the interrelationship between each parameter. The LC_{50} data was computed using the probit method, and the statistical packages for social sciences (SPSS), IBM STAT.22, and Microsoft Office (Excel) were utilized to obtain regression values.

The data generated from the assay were statistically analyzed, and the results were computed as the mean, standard deviation, and 95% confidence limit. The significance test was conducted using a single-factor analysis of variance at $p < 0.05$. Other calculations associated with statistical analysis specific to the onion assay were determined as follows:

The percentage of root growth in the control group was computed using the formula:

%RG = (Mean of treated roots / Mean of untreated roots (control)) x 100 2

The standard deviation was calculated using the formula:

SD = √(∑fd^2 / (n-1)) 3

Where: f = frequency d = deviation (deviation of x values from the mean) n = total number of values/treatments ∑ = sum of

The standard error of the mean was computed as:

SE = SD / √n 4

Where: n = number of treatments SD = standard deviation

The 95% confidence limit was calculated using the formula:

(x - t(n-1, 0.05)√(S^2/n)) ≤ μ ≤ (x + t(n-1, 0.05)√(S^2/n)) / n 5

Where: x = mean of treated roots t(n-1, 0.05) = degree of freedom at $p < 0.05$ S^2 = variance n = number of treatments μ = population mean

The mitotic index was determined by counting 1000 cells on a slide and calculating the proportion of dividing cells.

Mitotic index = (number of dividing cells / total number of cells scored) x 100 6

Mitotic inhibition was computed as:

(Mitotic index in control - mitotic index in treated) / mitotic index in control x 100 7

III. Results and Discussions

Effects of Drill Fluid Concentrations on Physico-Chemical Parameters in Water Samples

The mean±SD values of the physico-chemical parameters (pH, temperature, and dissolved oxygen) in water samples were assessed at various concentrations of drill fluids after a 96-hour exposure to C. gariepinus. The results showed a decrease in pH and temperature as the concentrations of drill fluids increased, while dissolved oxygen remained within a normal range with a slight increase at 10% concentration and a reduction at 5% concentration.

Before and after exposure of C. gariepinus to different concentrations of drill fluid (0% as control, 1%, 5%, 10%, 15%, and 25%), the physico-chemical parameters were analyzed. The results of the water samples' pH, temperature, and dissolved oxygen levels during the exposure period are shown below:

Parameter Control After 1% 5% 10% 15% 25% pH 7.23 ±00 6.93 ±00 5.71 ±00 5.93 ±00 6.24 ±00 6.27 ±00 Temperature 27.6 ±00 27.3 ±00 27.2 ±01 27.0±02 27.2±03 27.2±03 Dissolved Oxygen 0.3 ±00 0.3 ±00 0.2 ±02 0.7 ±03 0.3 ±00 0.3 ±00

The analysis of physico-chemical parameters was performed on water samples collected before exposure and during the 96-hour exposure of C. gariepinus to different concentrations of drill fluids (0%, 1%, 5%, 10%, 15%, and 25%). The data analyzed provides insights into the changes observed in pH levels.

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Analysis of Polycyclic Aromatic Hydrocarbons in Drill Mud

Polycyclic Aromatic Hydrocarbons (PAHs) were analyzed in drill mud the concentrations of PAHs were measured in both the original base mud (OBM) and the experimental test water. The PAHs analyzed included naphthalene, ace naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (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.

During the 96-hour exposure period, the mortalities of fish were recorded at various time intervals. Mortality percentages were observed at different concentrations and time intervals. The LC50 values, representing the lethal concentration that causes 50% mortality, were calculated for different time intervals.

Histopathological Alterations in Gill Tissue of Clarias gariepinus Following 96-Hour Acute Exposure

Histopathological examination of the gills of Clarias gariepinus following a 96-hour acute exposure revealed varying degrees of changes, as depicted in Figure 1. The results indicated that the gills of the control fish exhibited a normal arrangement, while the gills of the exposed fish showed several histological abnormalities. These abnormalities included distortion of mucus cells (MC), blood congestion (BC), separation of layers (SL), and sloughing (S). Furthermore, it was observed that the severity of these alterations was concentration-dependent.

In the case of low concentrations (1% and 5%), mild deviations from the normal gill formation patterns were observed, indicating slight alterations compared to the control fish. On the other hand, at higher concentrations (10%, 15%, and 25%), severe deviations from the normal gill formation patterns were observed, indicating significant alterations compared to the control fish.

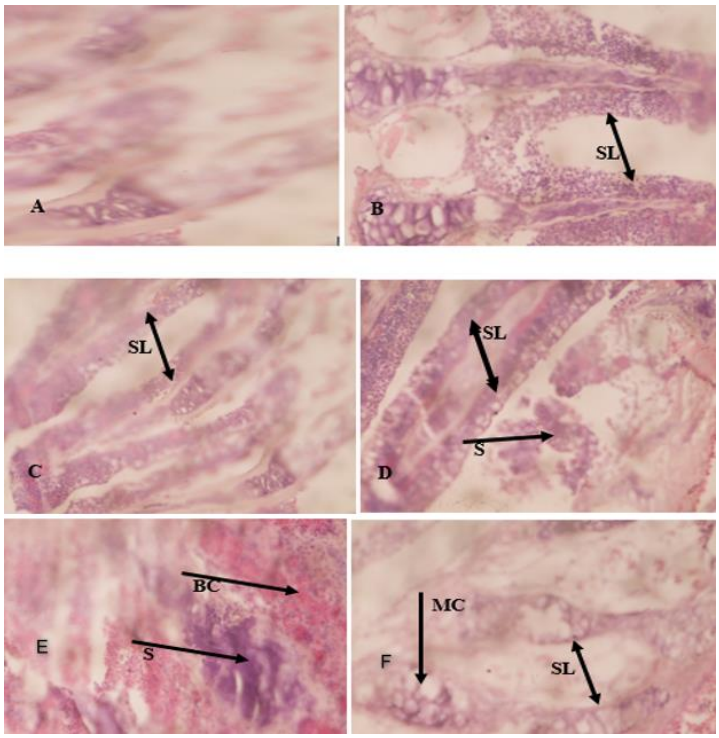

Figure 1 shows photomicrographs of the gills of C. gariepinus after a 96-hour exposure to the toxicant. The magnification is 100x. Labeled as follows: A = control, B = 1%, C = 5%, D = 10%, E = 15%, and F = 25%.

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

Histopathological examination of the liver tissue of Clarias gariepinus following a 96-hour acute exposure revealed various degrees of variations. The results demonstrated that the liver of the control fish exhibited a normal arrangement, while the liver of the exposed fish showed several histological alterations. These alterations included mild necrosis (N), swelling of blood vessels (SB), pyknosis (P), and vacuolation (V). Furthermore, it was observed that the severity of these alterations was concentration-dependent.

At low concentrations (1% and 5%), mild deviations from the normal liver structure were observed, indicating slight alterations compared to the control fish. Conversely, at higher concentrations (10%, 15%, and 25%), severe deviations from the normal liver structure were observed, indicating significant alterations compared to the control fish.

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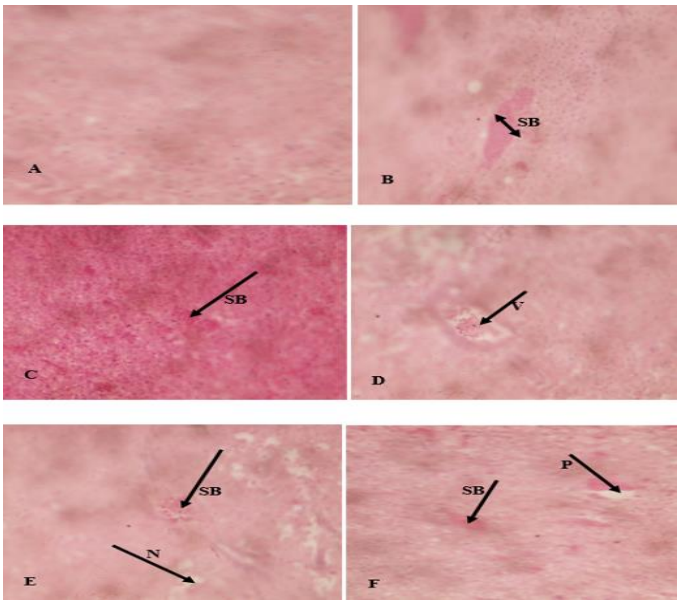

Figure 2: Histological Examination of the Liver of Clarias gariepinus following a 96-hour Exposure to Toxicant. Magnification: 100x. A) Control, B) 1% concentration, C) 5% concentration, D) 10% concentration, E) 15% concentration, and F) 25% concentration

The Effect of Used Drill Fluid Concentrations on Onion (Allium cepa) Root Growth: Macroscopic Analysis

The macroscopic analysis of Onion (Allium cepa) roots was conducted after 72 hours of exposure to various concentrations of used drill fluid. It was observed that the growth index of the roots decreased as the concentration of used drill fluid increased. The minimum and maximum range of root growth, as well as the mean and standard deviation values for each concentration compared to the control, are presented in the table. The range of root growth for the different concentrations (0.74-0.96, 0.74-0.79, 0.49-0.59, 0.3-0.36, and 0.18-0.28) was lower compared to the control range (1.06-2.21). Similarly, the mean ± SD values for each concentration (0.81±0.06, 0.77±0.02, 0.53±0.04, 0.33±0.02, and 0.23±0.03) were lower compared to the control mean (1.94±0.46).

Additionally, the mean root length with standard error (SE) and the percentage root growth (RG) were determined for each concentration of used drill fluid. The results revealed a decrease in both the mean root length and the percentage root growth with increasing concentration of used drill fluid. The mean root length with SE values for the various concentrations (10%, 30%, 50%, 70%, and 90%) compared to the control roots were lower (0.81±0.02, 0.77±0.01, 0.53±0.01, 0.33±0.01, and 0.23±0.01) compared to the control mean (1.94±0.14). Similarly, the percentage root growth (RG) values for each concentration (41.75%, 39.69%, 27.32%, 17.01%, and 11.86%) were lower compared to the control value (0%).

Microscopic Analysis of Onion -*Allium cepa*

Table 1 presents the cytological impact of a toxicant on Onion (Allium cepa) following 72 hours of exposure to different concentrations. The results demonstrated that the number of dividing cells and the mitotic index exhibited an increase as the concentration of the toxicant increased. Conversely, the control group showed an elevated level of mitotic inhibition with higher concentrations.

Table 1: 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

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Note: The values in the table represent the concentration of the toxicant in parts per million (ppm), the number of cells scored, the number of dividing cells, the mitotic index, and the mitotic inhibition of the control group.

Figure 3 depicts the chromosomal aberrations observed in the root tips of A. cepa following exposure to used drill mud. The different conditions are represented by the letters A to F. In the control group (A), no chromosomal aberrations were recorded. However, in the 10% concentration group (B), anaphase laggards were observed. The 30% concentration group (C) showed sticky chromosomes, while the 50% concentration group (D) exhibited chromosomes breaking. The 70% concentration group (E) also displayed chromosome breakage, and the 90% concentration group (F) showed polyploid metaphase.

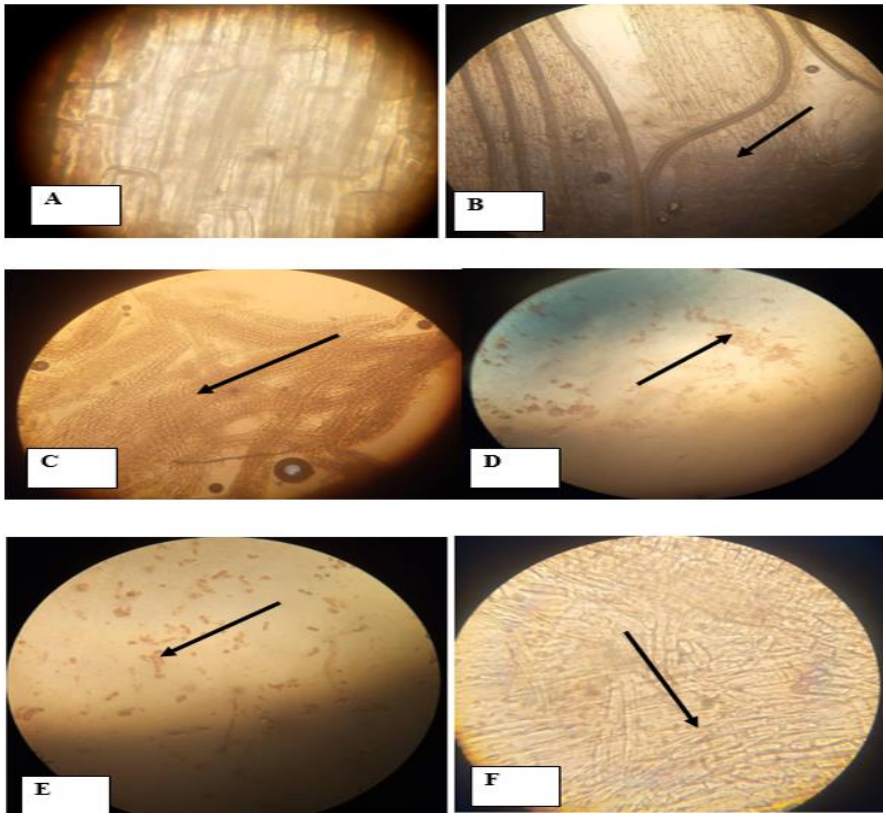

Figure 3: Chromosomal Aberrations in A. cepa Root Tips Exposed to Used Drill Mud

IV. Discussions

Water Quality Parameters

The water quality parameters, including pH, temperature, and dissolved oxygen, were analyzed during the 96-hour toxicity study. These physico-chemical parameters are commonly used as indicators of water quality. The results indicated that pH and temperature decreased as the concentration of the toxicant increased, while dissolved oxygen remained relatively constant. This decline in water quality parameters may be attributed to the presence of the toxicant and the subsequent increase in respiratory activity of the exposed fish (Warren, 1977). The impact of toxic substances on gill epithelium is known to be influenced by changes in ventilation, as reported by Omitoyin et al. (2006) and Adesina (2008).

Fish Reaction to Toxicant

During the 96-hour exposure, the fish exhibited an increased reaction to the toxicant with rising concentrations. Behavioral changes, such as erratic swimming and excessive jumping, were observed, particularly at higher concentrations. Ayoola (2007) attributed these changes to the effects of respiratory rate alteration, skin irritation, or disturbances in locomotor activity, indicating the impact of the toxic substance on the nervous system.

Mortality

Fish mortality was observed across all concentrations during the exposure period. This indicates that fish mortality was concentration-dependent, in contrast to the control group, which showed no mortality throughout the experiment.

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Lethal Concentration (LC_{50})

The lethal concentration (LC_{50}) of the used drill mud was determined based on the 96-hour bioassay results. The LC_{50} values obtained at 24, 48, 72, and 96 hours were 71.589%, 96.052%, 96.052%, and 59.508%, respectively. These LC_{50} values indicate that within the specified time intervals, the toxicant was able to cause at least 50% mortality in Carias gariepinus.

Various studies have reported different LC_{50} values for different substances. For instance, Olagunju (2007) reported an LC50 of 4.2 mg/l with 95% confidence limits of 31.86-93.81 mg/l in a 96-hour exposure of aqueous extracts of pawpaw seed powder to O. niloticus.

Histopathology of the Gills of Tilapia - Oreochromis niloticus

Histological examination of the gills of Oreochromis niloticus revealed a normal structural arrangement in the control (untreated) fish, while the exposed (treated) group exhibited various degrees of histological alterations. These alterations included distortion of mucus cells (MC), blood congestion (BC), separation of layers (SL), and sloughing (S). The observed histological alterations in the gills were suspected to be a result of the uptake of heavy metals and polycyclic aromatic hydrocarbons (PAH) present in the toxicant, which was present in the test water. This study demonstrates the acute lethal effects of used drill mud on Clarias gariepinus. The observed alterations, such as distortion of mucus cells, blood congestion, separation of layers, and sloughing, could be attributed to cell proliferation and an increase in the thickness of the gill filament epithelium.

Histopathology of the Liver of Tilapia, Oreochromis niloticus

The liver of Clarias gariepinus was subjected to histological examination, which revealed a normal structural arrangement in the control group of fish. However, the exposed group displayed various degrees of histological alterations. These alterations included mild necrosis of mucus cells (N), swelling of blood vessels (SB), pyknosis (P), and vacuolation (V). The hydropic swelling and morphological changes in the urinary tubules were attributed to cellular hypertrophy and the presence of net-like, tiny particles in the cytoplasm. The increase in toxicant concentration resulted in cytoplasmic vacuolation, cellular degeneration, nuclear damage, bile stagnation, and clogging of the blood sinusoids. These activities affect oxygen exchange and tissue respiration, leading to organ and tissue hypoxia, disintegration, and necrosis. The cellular degeneration and necrosis could be attributed to the accumulation of metals in hepatic tissues.

Macroscopic Analysis of Onion (Allium cepa)

The results of root growth inhibition indicated significant inhibition at different concentrations of the toxicant, with an effective concentration (EC50) determined to be 22.5%. Additionally, the average root length varied at different toxicant concentrations, showing a decrease with increasing concentration.

Microscopic Analysis of Onion (Allium cepa)

The mitotic index (MI) values obtained from treatments exceeding the control values may be indicative of induced cell division, which could be detrimental due to uncontrolled growth and tumor development. The study revealed a significant reduction in MI in A. cepa root apex cells, indicating sub-lethal effects at various concentrations. The progressive reduction in the mitotic index with increasing concentrations suggests that the toxicant (used drill mud) has the potential to disrupt cell growth and interfere with the cell cycle, resulting in a decrease in the number of dividing cells (Turkoghu, 2012).

Chromosome and mitotic aberration Sticky chromosomes are directly associated with chromatin dysfunction (Mesi et al., 2013). Chromosome breakage may occur due to the movement of non-equal chromatids or the occurrence of dicentric chromosomes. Anaphase laggards are a result of spindle collapse and have the potential to cause aneuploidy. The observed chromosomal aberrations indicate that used drill mud is capable of altering the structure and number of chromosomes in A. cepa (Mesi et al., 2013).

V. Conclusions

In recent years, the utilization of drilling fluids in crude oil recovery has played a crucial role in all drilling-related operations. However, the proper disposal of used drilling fluid has become a significant concern, prompting the need for this research to assess the toxicity and genotoxicity of such fluids. Laboratory examinations were conducted, including acute toxicity testing over a 96-hour period, histopathological analysis of the gills and liver, and genotoxicity assessment using the Allium cepa Assay.

The findings of this study indicate that used drill mud exhibits a high level of toxicity. Therefore, regulatory bodies should take further action to reinforce existing laws concerning the appropriate treatment and disposal of drilling fluids. The results demonstrate the toxic impact of drilling mud on C. gariepinus, as observed in the acute toxicity testing and histopathological effects on the fish's gills and liver.

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Furthermore, genotoxic effects on Allium cepa were observed through root growth inhibition, mitotic inhibition, and chromosomal aberrations. Proper treatment of drilling mud is necessary to ensure compliance with regulatory requirements prior to disposal. Regulatory bodies, such as the Department of Petroleum Resources (DPR) and the Federal Ministry of Labor, should implement strict supervision and continuous monitoring during drilling operations, particularly in offshore settings.

The generated LC_{50} and EC50 values from this study can serve as benchmarks for conducting toxicological and genotoxicological studies on freshwater organisms and Allium cepa, respectively. The comprehensive data obtained from this study can enhance the understanding of the general public and drilling companies regarding the toxicity and genotoxicity associated with drilling activities.

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