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Acute Toxicity and Genotoxic Responses in African Mud Catfish, *Clarias gariepinus*, Exposed to Biocides and Aqueous Ammonia

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ABSTRACT

Oil and gas industrial wastewater pose significant threats to aquatic organisms when discharged into the environment without proper treatment. This study aimed to assess the acute toxicity and genotoxic responses in African mud catfish, *Clarias gariepinus*, exposed to biocides and aqueous ammonia, aligning with the objectives of the UN Decade of Restoration and the Sustainable Development Goals (SDGs). The study commenced with a 96-hour acute toxicity assay to determine the 96 h LC50 values. Subsequently, chronic toxicity evaluation was conducted over 28 days using 1/10th, 1/100th, and 1/1000th of the 96-hour LC50 values to assess the ability of the test chemicals to induce oxidative stress and genotoxic effects in *C. gariepinus*. Key antioxidant stress enzyme activities, including Superoxide dismutase (SOD), Catalase (CAT), and reduced Glutathione (GSH), were examined. The results of the acute toxicity study revealed that the derived 96 h LC50 values of aqueous ammonia (1.358 mg/L) were slightly more toxic to C. gariepinus than biocides (1.586 mg/L). Furthermore, the biochemical assay indicated a significant inhibition of GSH levels in the liver of *C. gariepinus* exposed to the highest sublethal concentrations (1/10th of the 96-hour LC50) of both biocides and aqueous ammonia ($P<0.05$). The activity of SOD was significantly altered in the liver of fish exposed to sublethal concentrations of biocides compared to the control. Similarly, the activity of CAT was significantly modified in the liver of fish exposed to 1/10th of the 96-hour LC50 concentrations of aqueous ammonia ($P<0.05$). Increased levels of lipid peroxidation product, malondialdehyde, were observed in *C. gariepinus* exposed to 1/10th of the 96-hour LC50 concentrations of both biocides and aqueous ammonia compared to the control ($P<0.05$). Notably, the exposed fish displayed significant red blood cell nucleus abnormalities, including micronuclei (M.N.), binuclei (B.N.), and lobed-shaped nuclei, compared to the control. These findings emphasize that even sublethal concentrations of aqueous ammonia and biocides pose threats to the health of aquatic organisms. Thus, regulating their use and disposal is crucial to safeguard the sensitive aquatic biota in oil-producing communities and align with ecological restoration efforts and the SDGs.

INTRODUCTION

The Nigerian economy has heavily relied on income and revenues generated from the petroleum industry for several decades. With the oil industry contributing around 95

percent of the country's foreign exchange revenues, 25 percent of the Gross Domestic Product (GDP), and 80 percent of overall government revenue, Nigeria stands as the largest oil and gas producer in Africa (Vanguard, 2016). In the pursuit of efficient oil production and transportation processes, various chemicals such as biocides and aqueous ammonia are employed. However, the release of these chemicals into the environment poses significant ecological threats and adverse effects on aquatic organisms (de Campos *et al.*, 2022)

Biocides, which are additives used to kill bacteria, are commonly utilized in water muds containing natural starches and gums that are prone to bacterial attacks (Struchtemeyer *et al.*, 012). In the context of oil production, biocides are employed to control microorganisms that degrade petroleum hydrocarbons, ultimately affecting the density, sulfur content, and viscosity of crude oil (Aitken *et al.*, 2004). Nevertheless, biocides exhibit toxicity not only towards target microorganisms but also non-target species, underscoring the need for their regulation (Michalak and Chonjnacka, 2014). Some examples of commonly used biocides in the oilfield include Glutaraldehyde, Tetrakis (hydroxymethyl) Phosphonium Sulfate (THPS), TTPC (Tributyl Tetradecyl Phosphonium Chloride), formaldehyde, triazines, DBNPA (2, 2-Dibromo-3-Nitrilopropionamide), Quaternary Ammonium Compounds (QAC), and Bronopol. (Rossmoore, 2012).

Similarly, aqueous ammonia, a clear and volatile solution with an unpleasant odor, raises environmental concerns within the oil industry. Ammonia pipelines are used to transport hydrogen in the oil sector, and blasting chemicals used in mine excavation can introduce ammonia levels into adjacent groundwater and surface water (Sankara, 2014; Forsyth *et al.*,995).

Drilling mud also contains ammonia, serving as another potential entry point into the aquatic ecosystem (Sankara, 2014). Leakage or dispersion of explosion leftovers can contaminate surface and groundwater with aqueous ammonia (Zaitsev *et al.*, 2008). It is important to note that aqueous ammonia is poisonous to fish, leading to various detrimental effects such as loss of equilibrium, hyperventilation, hyper-excitability, accelerated breathing, convulsions, and even death (Randall *et al.*, 2002; EPA, 1999).

The African mud catfish, Clarias gariepinus, is a highly valued tropical catfish species for aquaculture due to its resilience, tolerance to poor water quality, rapid growth, and ease of reproduction in captivity (Byrne, 2012). These characteristics make it an ideal species for toxicity testing, as it shares similar metabolic pathways with mammalian species for coping with the harmful effects of both endogenous and exogenous chemicals (Ahmad, 2012). Moreover, the physiological changes in fish, including African mud catfish, quickly reflect environmental alterations, making them reliable indicators of physical and chemical disturbances (Okomoda *et al.*, 2010). Nevertheless, there is currently limited data on the potential toxicity and genotoxicity of biocides and aqueous ammonia on local freshwater species like the African mud catfish (Martins and Martins, 2021). Therefore, this study aims to provide insights into the toxicity and genotoxic effects of these commonly used oil sector chemicals, namely biocides and aqueous ammonia, aligning with the UN Goals for the Decade of Ecological Restoration and the Sustainable Development Goals (Cooke *et al.*, 2022)

MATERIALS AND METHODS

Sample Collection and Acclimatization*:*

African Mud Catfish (*Clarias gariepinus*) fingerlings were obtained from a reputable catfish hatchery in Yaba, Lagos State. The fingerlings had a mean weight of 3.04 ± 2.05g and a mean length of 2.92 ± 0.97cm. The juveniles used for sub-lethal tests were of the same species with a mean weight of 29.68 ± 3.96g and a mean length of 15.5 ± 0.2cm.

The fingerlings and juveniles were transported in oxygenated polythene bags containing water from the collection point to the Ecotoxicology Laboratory, Department of Zoology Laboratory Annex. Upon arrival, the fish were gently transferred to aerated acclimatization tanks (40 × 30 × 30 cm) filled with dechlorinated water and allowed to acclimatize for seven days under laboratory conditions. The acclimatization tanks were maintained at an ambient temperature of 28 °C ± 0.5, relative humidity of 70 ± 5%, and a light-dark cycle of 12:12 hours. The fish were fed twice daily with Coppens® brand fish feed containing 45% crude protein.

Test Media*:*

The test media used in this study were biocides (BD/TT/2019/021) and ammonia (AM/TT/2019/414), which were Department of Petroleum Resources coded chemicals. The physicochemical characteristics of the test compounds are presented in Tables 1 and 2.

Table 1: Physico-chemical composition of Biocide

Physico-chemical Characteristics	Level/Concentration
pH	4.05
Salinity $^{0}/_{00}$	1.02
Conductivity (μSm^{-1})	223.8
Total dissolved solids, TDS (mg/L)	116.2
Phenol (mg/L)	ND
Cadmium, Cd (mg/L)	0.01
Copper, Cu (mg/L)	0.17
Lead, Pb (mg/L)	1.56

ND- Not Detected

Table 2: Physico-chemical composition of Aqueous Ammonia.

Physico-chemical Characteristics	Level/Concentration
pH	11.50
Salinity $^{0}/_{00}$	
Conductivity (μSm^{-1})	1340.0
Chemical Oxygen Demand, COD	ND
Biochemical Oxygen Demand, BOD	ND
Phenol (mg/L)	ND
Cadmium, Cd (mg/L)	ND
Nickel, Ni (mg/L)	ND
Lead, Pb (mg/L)	ND
Ammonia, NH_3	100%

N.D.- Not Detected

Preparation of Test Medium and Application of Toxicants:

For each experiment, 1 liter of treated or untreated water was used per tank, accommodating 10 test organisms in two replicates, resulting in a total of 20 organisms per treatment and control. The specific gravity of the biocides (BB/TT/2019/021) was calculated to be 1100, while the specific gravity of ammonia (AM/TT/2019/414) was 957.

Acute Toxicity Bioassay:

Acute toxicity tests were conducted using thoroughly washed and dried 4 L transparent glass tanks. A stock solution of the biocides was prepared by injecting 1 ml into 1 L of water, and for ammonia, 1 ml was injected into 1 L of water. Various concentrations of the test compounds were measured in the bioassay tanks for range finding and definitive tests. Each experiment consisted of duplicate tanks containing 10 test fishes, including five pairs of test chambers and one pair of control chambers filled with dechlorinated water. The

fish were randomly introduced into the experimental tanks with changing order of introduction to minimize bias. The definitive test concentrations for biocides were 1.1, 2.2, 3.3, 4.4, and 5.5 mg/L, and for ammonia: 0.957, 1.914, 2.871, 3.828, and 4.785 mg/L. Fish mortalities were recorded cumulatively at 24, 48, 72, and 96 hours. The quantal responses (mortality) were assessed by observing the absence of opercula movement and movement of the whole organism when prodded softly with a glass rod. Mortality counts were recorded at 24-hour intervals over a four-day period (96 hours), and dead organisms were removed immediately to prevent contamination of the test medium.

Sub-lethal Exposure Bioassay:

Rectangular 10 L transparent glass tanks were used for the sub-lethal toxicity tests on Clarias gariepinus juveniles. The tanks were thoroughly washed with brine and dried before the experiment. Sub-lethal concentrations of the test chemicals were prepared by diluting them to one-tenth (1/10th), one-hundredth (1/100th), and one-thousandth (1/1000th) of the respective 96-hour LC50 values for biocides and ammonia. Eighteen similarly sized catfish juveniles were randomly selected from the acclimatization tanks and transferred to the tanks containing the sub-lethal concentrations of the test chemicals. The experiments were conducted with duplicate tanks, with three fish per tank per chemical in both the definitive and control experiments. A semi-static (renewal) bioassay system was employed, where the test media were changed every 72 hours to maintain the toxicant strength, and dissolved oxygen levels, and reduce the accumulation of ammonia from fish excretion. The fish were fed ad libitum twice daily throughout the 28-day sub-lethal exposure period, and the liver samples of C. gariepinus were collected for biochemical analysis on days 14 and 28.

Biochemical Tests:

During the sub-lethal tests, liver samples from C. gariepinus were collected on days 14 and 28 of the 28-day exposure period. The fish were dissected, and the livers were carefully excised, rinsed with ice-cold saline solution, blotted dry, and weighed. The liver samples were homogenized in ice-cold Tris-HCl buffer (pH 7.4) using a glass homogenizer. The homogenates were centrifuged at 10,000 rpm for 15 minutes at 4 °C, and the supernatants were collected for biochemical analysis. The following biochemical parameters were determined:

Protein Content: Protein content in the liver homogenates was estimated using the Lowry method (Lowry et al., 1951) with bovine serum albumin as the standard.

Lipid Peroxidation*:* Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) using the method of Ohkawa et al. (1979). The absorbance of the reaction mixture was measured at 532 nm, and the results were expressed as nmol of malondialdehyde (MDA) formed per mg protein.

Antioxidant Enzymes*:* The activities of superoxide dismutase (SOD) and catalase (CAT) were determined using the methods of Misra and Fridovich (1972) and Aebi (1984), respectively. SOD activity was expressed as units/mg protein, and CAT activity was expressed as µmol of H_2O_2 decomposed per minute per mg protein.

Glutathione (GSH) Content: GSH content was determined using the method of Ellman (1959). The absorbance of the reaction mixture was measured at 412 nm, and the results were expressed as µmol GSH/g tissue.

Glutathione-S-transferase (GST) Activity: GST activity was determined using the method of Habig et al. (1974) with 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate. The absorbance of the reaction mixture was measured at 340 nm, and the results were expressed as µmol CDNB conjugate formed per minute per mg protein.

Statistical Analysis: The data obtained from the acute and sub-lethal toxicity tests were analyzed using probit analysis to determine the 96-hour LC50 values. The sub-lethal toxicity

data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. All statistical analyses were performed using SPSS version 23 software, and p-values less than 0.05 were considered significant. The results were expressed as mean ± standard deviation (SD).

RESULTS

Acute Toxicity Assessment:

The acute toxicity study examined the effects of the Biocide on Clarias gariepinus. The results revealed a mortality response that varied with the concentration, with the lowest concentration (1.100mg/L) causing 40% mortality compared to 100% mortality at the highest concentration (5.500mg/L) after 96 hours of exposure (Table 3). Probit analysis determined the 96-hour LC50 value to be 1.586 (Table 4).

Table 3: Concentration-response relationship between Biocide and *Clarias gariepinus*

Concentration		Log of Conc.	Test Animals	Mortality							
(ml/L)	(mg/L)			24 h		48 h		72 h		96 h	
				No.	%	No.	%	No.	%	No.	%
Control			20	0	0	0	0	0	0	0	0
0.001	1.100	0.0414	20	0	0	1	5	4	20	8	40
0.002	2.200	0.3424	20	1	5	5	25	9	45	12	60
0.003	3.300	0.5185	20	2	10	8	40	13	65	14	70
0.004	4.400	0.6435	20	3	15	9	45	12	60	17	85
0.005	5.500	0.7404	20	5	25	13	65	15	75	20	100

Table 4: Acute Toxicity of Biocide against *Clarias gariepinus* after 96 exposure period

	Concentration	Confidence Interval	S.E	DF	Equation of line (y)
LC_5	0.359	0.129 – 0.667	0.647	3	2.857x + 7.574
LC_{50}	1.586	0.920 – 1.712			
LC_{95}	7.022	3.373 – 8. 531			

D.F.- degree of Freedom; S.E- Standard Error.

Acute toxicity of Aqueous Ammonia on *Clarias gariepinus:*

The results of exposure of *Clarias gariepinus* to the aqueous ammonia indicated a concentration-dependent relationship with percentage mortality increasing with duration over the 96-hour period (Table 5). Specifically, the lowest exposure concentration (0.957mg/L) resulted in 40% mortality, while the highest exposure concentration (4.785mg/L) resulted in 100% mortality.Following the exposure, the determined 96 h LC50 value indicated a median mortality value of 1.358mg/L (Table 6).

Table 5: Concentration-response relationship between Aqueous Ammonia and *Clarias gariepinus*

Concentration		Log of Conc.	Test Animals	Mortality							
(ml/L)	(mg/L)			24 h		48 h		72 h		96 h	
				No.	%	No.	%	No.	%	No.	%
Control			20	0	0	0	0	0	0	0	0
0.001	0.957	-0.0191	20	0	0	2	10	7	35	8	40
0.002	1.914	0.2819	20	2	10	5	25	10	50	12	60
0.003	2.871	0.4580	20	3	15	8	40	12	60	14	70
0.004	3.828	0.5830	20	5	25	11	55	14	70	20	100
0.005	4.785	0.6799	20	9	45	14	70	20	100	20	100

Table 6: Acute Toxicity of Aqueous Ammonia against *Clarias gariepinus* (96 hr)

	Concentration	Confidence Interval	S.E	DF	Equation of line (y)
LC_5	0.404	0.068 - 0.683	0.593	3	1.429x + 3.886
LC_{50}	1.358	0.954 – 2.076			
LC_{95}	4.568	4.762 – 17.822			

DF: degree of Freedom; S.E- Standard Error

Chronic Toxicity Investigation:

The following outlines the impact of sublethal concentrations of oil industry chemicals on the activities of antioxidative stress enzymes in Clarias gariepinus:

Reduced Glutathione (GSH) Levels:

The overall trend of reduced glutathione (GSH) levels in the liver of Clarias gariepinus exposed to sublethal concentrations of the Biocide (at 1/10th, 1/100th, and 1/1000th of the 96-hour LC50) did not show a consistent pattern throughout the experiment. On day 14, the GSH levels were higher for the control group, as well as for those exposed to 1/10th and 1/100th of the 96-hour LC50 concentrations, compared to day 28 ($P>0.05$). However, for the 1/1000th 96-hour LC50 concentration, the GSH level on day 28 was higher than on day 14 ($P>0.05$).

Specifically, on the 14th day of exposure, only the group exposed to 1/10th of the 96-hour LC_{50} concentration exhibited higher GSH values compared to the control group. In contrast, for both the 1/100th and 1/1000th 96-hour LC_{50} concentrations, the GSH levels were lower than the control group, with a significant difference observed for the 1/1000th 96-hour LC_{50} concentration ($P<0.05$). By day 28, the control group recorded the highest GSH level, surpassing the levels of those exposed to the test concentrations (1/10th, 1/100th, and 1/1000th of the 96-hour LC50). The difference in GSH level was significant ($P<0.05$) between the control group and those exposed to sublethal concentrations of 1/10th and 1/1000th of the 96-hour LC50 of the test chemical (Fig. 1).

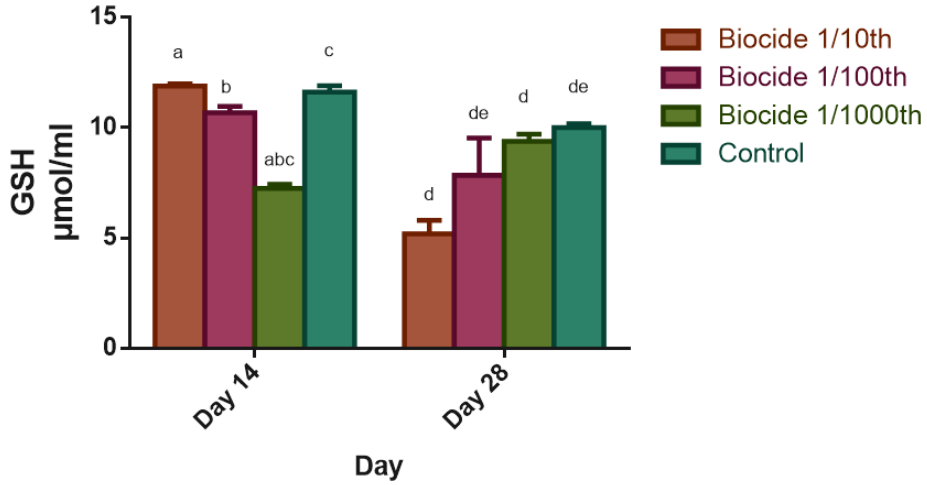

Fig. 1: Levels of Reduced Glutathione (GSH) in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of Biocide for 28 days.
Note: Bars with the same alphabet are statistically significant at $p<0.05$*

Superoxide Dismutase (SOD) Activity*:*

The superoxide dismutase (SOD) activity in the liver of Clarias gariepinus exposed to sublethal concentrations of the Biocide exhibited a non-uniform pattern between day 14 and day 28 of the experiment (Fig. 2). On day 14, the SOD activities increased in C. gariepinus exposed to 1/1000th of the 96-hour LC50 concentration of the test chemical compared to the

control group, as well as those exposed to 1/10th and 1/100th of the 96-hour LC50 concentrations (Figure 2). However, these differences were not statistically significant.By the 28th day of the experiment, the SOD activities in C. gariepinus exposed to 1/1000th of the 96-hour LC50 concentrations of the Biocide were higher than those in the control group, as well as those exposed to 1/10th and 1/100th of the 96-hour LC50 concentrations. Similarly, the SOD activities in the control group were higher than those exposed to 1/10th of the 96-hour LC50 concentration, indicating some degree of inhibition (Fig. 2). However, there was a significant difference ($P<0.05$) in SOD activity between the control group and those exposed to sublethal concentrations of the test chemicals (1/10th, 1/100th, and 1/1000th of the 96-hour LC50).

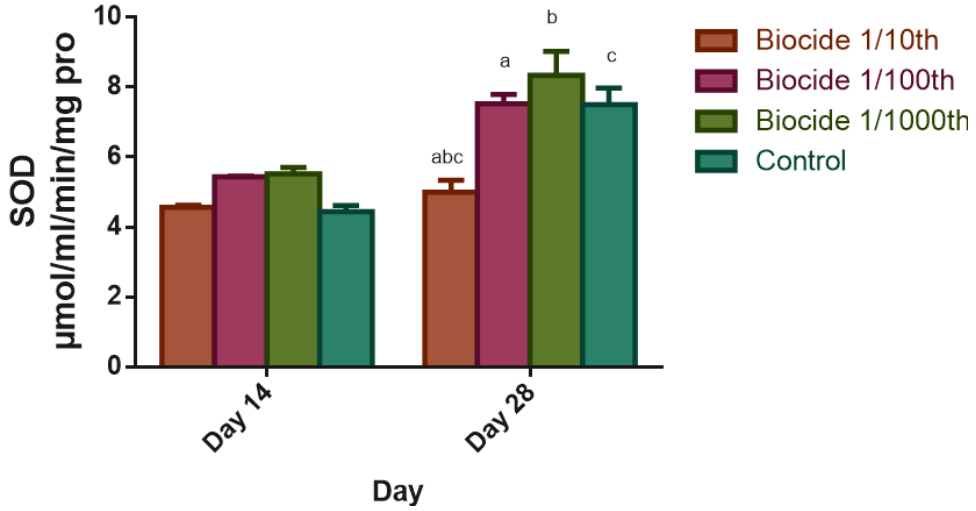

Fig 2: Activities of Superoxide Dismutase (SOD) in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of Biocide for 28 days.
Note: Bars with the same alphabet are statistically significant at $p<0.05$*

Catalase (CAT) Enzyme Activity:

The catalase (CAT) enzyme activities in Clarias gariepinus exposed to sublethal concentrations of the Biocide did not exhibit any specific trend in both analysis periods (days 14 and 28) (Fig. 3). However, there were notable differences in CAT activity at specific concentrations.

On day 14 of the experiment, the CAT activity values were higher in C. gariepinus exposed corresponding test concentrations (1/10th and 1/1000th of the 96-hour LC50). Conversely, fish exposed to 1/10th of the 96-hour LC50 concentration had a more inhibited catalase activity than those exposed to the lower concentrations of 1/100th and 1/1000th of the 96-hour LC50 (Fig. 3).

By the 28th day of exposure, the CAT activities in C. gariepinus exposed to 1/1000th of the 96-hour LC50 concentrations of the Biocide were higher than the control group and those exposed to both 1/10th and 1/100th of the 96-hour LC50. This increase in CAT value indicates a greater inhibition in Clarias gariepinus exposed to 1/10th of the 96-hour LC50 concentrations than at day 14. However, there is no significant ($P>0.05$) difference in overall CAT activity measured at different exposure times.

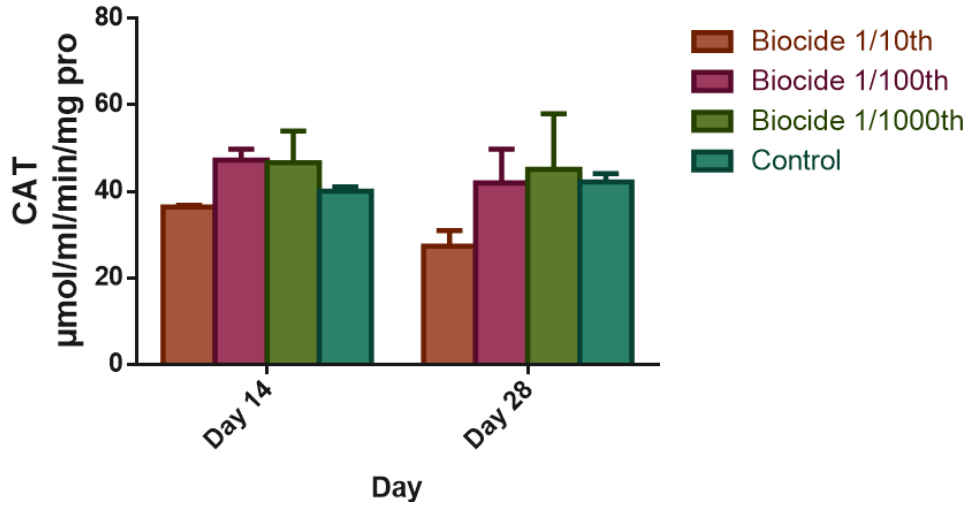

Fig. 3: Activities of Catalase (CAT) enzyme in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of Biocide for 28 days (Bars with the same alphabet are statistically significant at $p<0.05$).

Activities of Antioxidative Stress Enzymes in Clarias gariepinus Exposed to Sublethal Concentrations of Aqueous Ammonia:

Reduced Glutathione (GSH) Levels:

Figure 4 presents the results of glutathione (GSH) level assessment in Clarias gariepinus exposed to sublethal concentrations of aqueous ammonia. The GSH levels in the liver of C. gariepinus exposed to these concentrations showed a non-uniform pattern between day 14 and day 28 of the experiment, with higher levels observed in the control group compared to the exposed fish (Fig. 4). Specifically, on day 14 of the sublethal experiment, the control fish exhibited significantly higher GSH levels compared to those exposed to 1/10th of the 96-hour LC50 test concentration ($P<0.05$). A similar trend was observed on day 28, with a greater decrease in GSH levels in C. gariepinus exposed to 1/10th of the 96-hour LC50 concentration. There was a significant difference ($P<0.05$) in GSH activities between the control group and those exposed to 1/10th of the 96-hour LC50 concentration. Additionally, a significant difference in GSH activities was observed between the 1/10th and 1/1000th of the 96-hour LC50 test concentrations (Fig. 4).

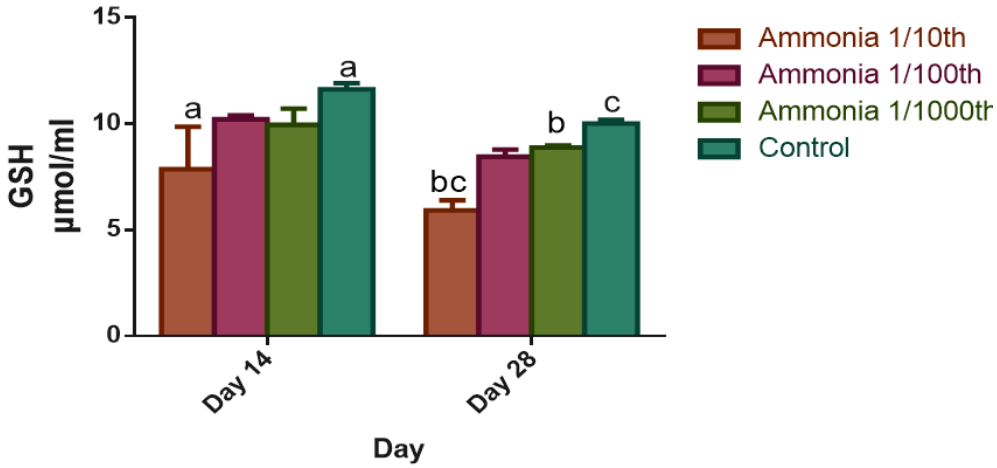

Fig. 4: Levels of Reduced Glutathione (GSH) in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of aqueous ammonia for 28 days.

Note: Bars with the same alphabet are statistically significant at $p<0.05$*.

Superoxide Dismutase (SOD) Activity:

The SOD activity in Clarias gariepinus exposed to sublethal concentrations of aqueous ammonia (1/10th, 1/100th, and 1/1000th of the 96-hour LC50) did not exhibit a consistent trend throughout the experiment. On day 28, the SOD activity was higher compared to day 14 (Fig. 5).

On day 14 of the experiment, the SOD activities in C. gariepinus exposed to 1/10th of the 96-hour LC50 concentration of aqueous ammonia were higher than those in the control group and those exposed to both 1/100th and 1/1000th of the 96-hour LC50 concentrations. Similarly, the control group exhibited higher SOD activity compared to the groups exposed to 1/100th and 1/1000th of the 96-hour LC50 concentrations (Figure 5). However, these differences were not statistically significant.

By the 28th day of the experiment, the control group of C. gariepinus displayed higher SOD activity compared to the groups exposed to the test concentrations, indicating inhibition.However, the difference was not statistically significant ($p<0.05$) (Fig. 5).

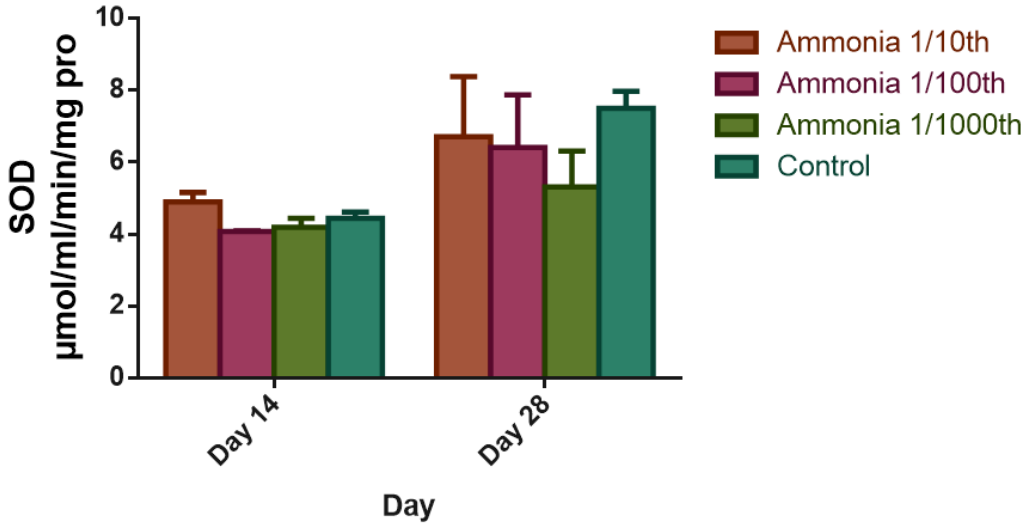

Fig. 5: Activities of Superoxide Dismutase (SOD) in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of aqueous ammonia for 28 days (Bars with the same alphabet are statistically significant at $p<0.05$).

Catalase (CAT) Activity in the Liver:

The CAT activity in the liver of Clarias gariepinus exposed to sublethal concentrations of aqueous ammonia showed a varied pattern between day 14 and day 28 of the experiment (Fig. 6). Specifically, on day 14, the CAT activities in C. gariepinus exposed to 1/10th of the 96-hour LC50 concentration of aqueous ammonia were higher than those in the control group and those exposed to both 1/100th and 1/1000th of the 96-hour LC50 concentrations. Moreover, the CAT activity in the control group was higher than in the groups exposed to 1/100th and 1/1000th of the 96-hour LC50 concentrations (Fig. 6). However, these differences were not statistically significant.

By the 28th day of the experiment, the control group of C. gariepinus exhibited higher CAT activity compared to the groups exposed to the test concentrations, indicating inhibition. Furthermore, a significant difference ($P<0.05$) was observed between the CAT activity in the group exposed to 1/10th of the 96-hour LC50 concentration and the control group.

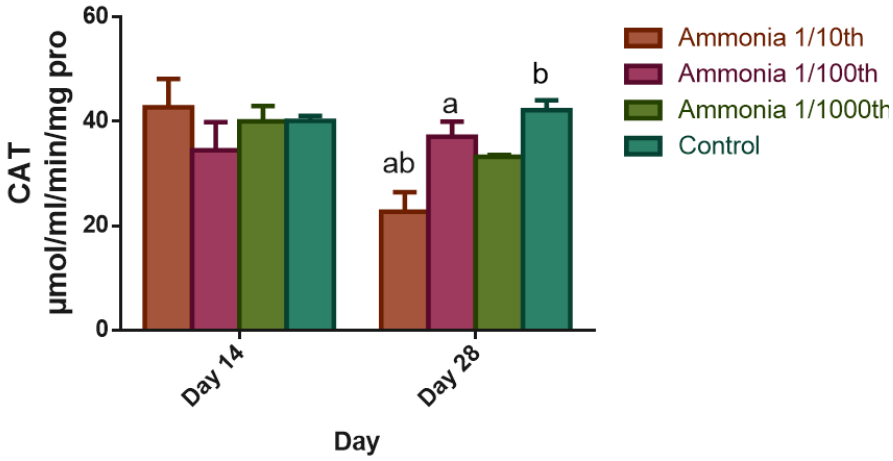

Fig. 6: Activities of Catalase (CAT) enzyme in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of aqueous ammonia for 28 days (Bars with the same alphabet are statistically significant at $p<0.05$).

Levels of Lipid Product in *Clarias gariepinus* Exposed to Sub-lethal Concentrations of Oil Industry Chemicals.

Lipid Product in Clarias gariepinus Exposed to Sub-lethal Concentrations of Biocide.There were no significant differences ($P>0.05$) observed in the lipid peroxidation of the phospholipid bi-layer between the control group and the sublethal concentrations (1/10th, 1/100th, and 1/1000th of the 96-hour LC50) exposed to the Biocide. However, on both day 14 and day 28, the MDA (malondialdehyde) levels were higher in Clarias gariepinus exposed to 1/10th of the 96-hour LC50 concentration of the Biocide compared to the control group and those exposed to 1/100th and 1/1000th of the 96-hour LC50 concentrations (Figure 7).

By day 14, there was a significant difference in MDA levels between the 1/10th and 1/1000th of the 96-hour LC50 concentrations of the Biocide. Additionally, by day 28 of the exposure, the MDA activities in C. gariepinus exposed to 1/1000th, 1/100th, and 1/10th of the 96-hour LC50 concentrations were higher than in the control group, indicating a concentration-dependent response (Fig. 7). The MDA levels in the 1/10th and 1/100th 96-hour LC50 concentrations were significantly different from the control group ($P<0.05$).

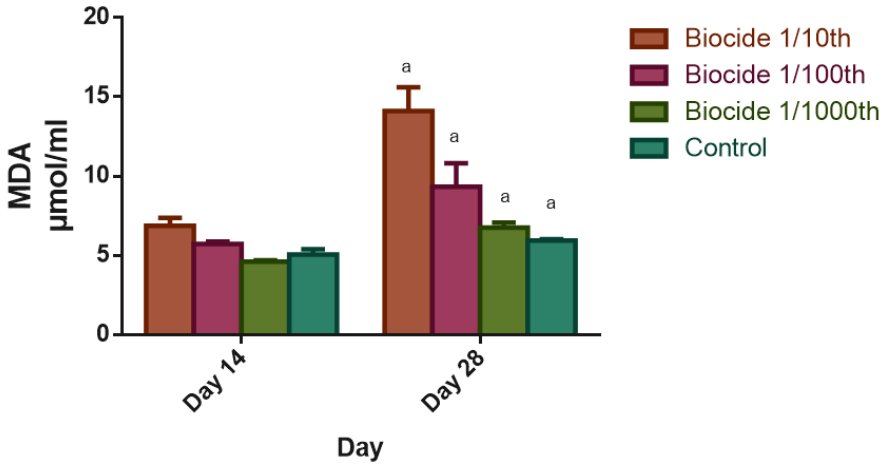

Fig. 7: Levels of lipid peroxidation product, Malondialdehyde (MDA) in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of Biocide for 28 days (Bars with the same alphabet are statistically significant at $p<0.05$).

Levels of Lipid Product in *Clarias gariepinus* Exposed to Sub-lethal Concentrations of Aqueous Ammonia:

The lipid peroxidation of the phospholipid bi-layer exhibited a non-uniform pattern between day 14 and day 28 of the experiment (Fig. 8). Specifically, on day 14, the MDA activities in Clarias gariepinus exposed to 1/100th of the 96-hour LC50 concentration of aqueous ammonia were higher than those in the control group and those exposed to both 1/10th and 1/1000th of the 96-hour LC50 concentrations. Furthermore, the control group had higher MDA levels compared to the groups exposed to 1/10th and 1/1000th of the 96-hour LC50 concentrations (Figure 8). However, these differences were not statistically significant. By day 28 of the exposure, the MDA levels were higher, and the difference was statistically significant ($P<0.05$) in the test concentrations (1/10th, 1/100th, and 1/1000th of the 96-hour LC50) when compared to the control group.

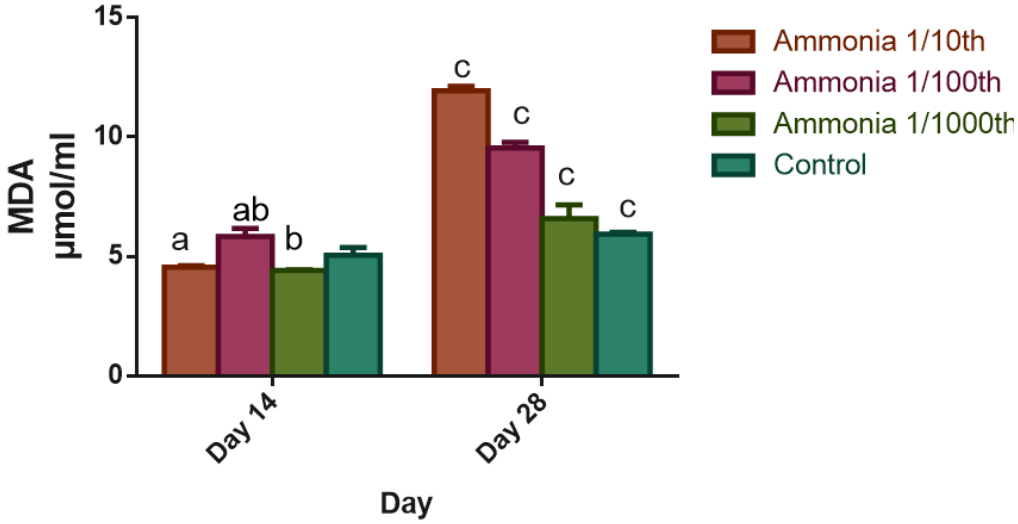

Fig. 8: Levels of lipid peroxidation product, Malondialdehyde (MDA) in the liver of the *Clarias gariepinus* exposed to sub-lethal concentrations of aqueous ammonia for 28 days
Note: Bars with the same alphabet are statistically significant at $p<0.05$*

Genotoxic Effects of Exposure to Sub-lethal Concentrations of the Oil Industry Chemicals:

The red blood cell nucleus abnormalities observed in the exposed catfishes included micronuclei (M.N.), binuclei (B.N.), and lobed-shaped nuclei (Plates 1, 2, and 3). The frequencies of these abnormalities in C. gariepinus juveniles exposed to sublethal concentrations of two Oil industry chemicals were recorded for both day 14 and day 28, as presented in Table 7 and Table 8, respectively. No significant differences ($P>0.05$) were observed between the control group and the observed abnormalities, as indicated in Figures 9 and 10, respectively.

Table 7: Micronucleus and other nuclear abnormalities occurring at different concentrations in juvenile *Clarias gariepinus* exposed to sublethal concentrations of Biocide and Aqueous ammonia for 14 days.

Oil Industry Chemicals	Concentrations (mg/L)	Nuclear abnormalities		
		Micronuclei	Binucleated	Lobed
Biocides	0.1586	22 (1.9)	5 (0.1)	4 (0.4)
	0.01586	19 (1.7)	5 (0.5)	3 (0.3)
	0.001586	17 (2.2)	1 (0.5)	2 (0.2)
Ammonia	0.1358	14 (1.4)	10 (1.0)	5 (0.5)
	0.01358	11 (1.1)	9 (0.9)	4 (0.4)
	0.001358	7 (0.7)	4 (0.4)	2 (0.2)
Control	0.00	0	0	0
	0.00	0	0	0
	0.00	0	0	0

N erythrocyte = 1000

Table 8: Micronucleus and other nuclear abnormalities occurring at different concentrations in juvenile *Clarias gariepinus* exposed to sublethal concentrations of Biocide and aqueous ammonia for 28 days.

Oil Industry Chemicals	Concentrations (mg/L)	Nuclear abnormalities		
		Micronuclei	Binucleated	Lobed
Biocides	0.1586	24 (2.4)	8 (0.8)	7 (0.7)
	0.01586	18 (1.8)	7 (0.7)	4 (0.4)
	0.001586	17 (1.7)	5 (0.5)	-
Ammonia	0.1358	20 (2.0)	9 (0.9)	4 (0.4)
	0.01358	11 (1.1)	9 (0.9)	3 (0.3)
	0.001358	7 (0.7)	3 (0.3)	1 (0.1)
Control	0.00	0	0	0
	0.00	0	0	0
	0.00	0	0	0

N erythrocyte = 1000

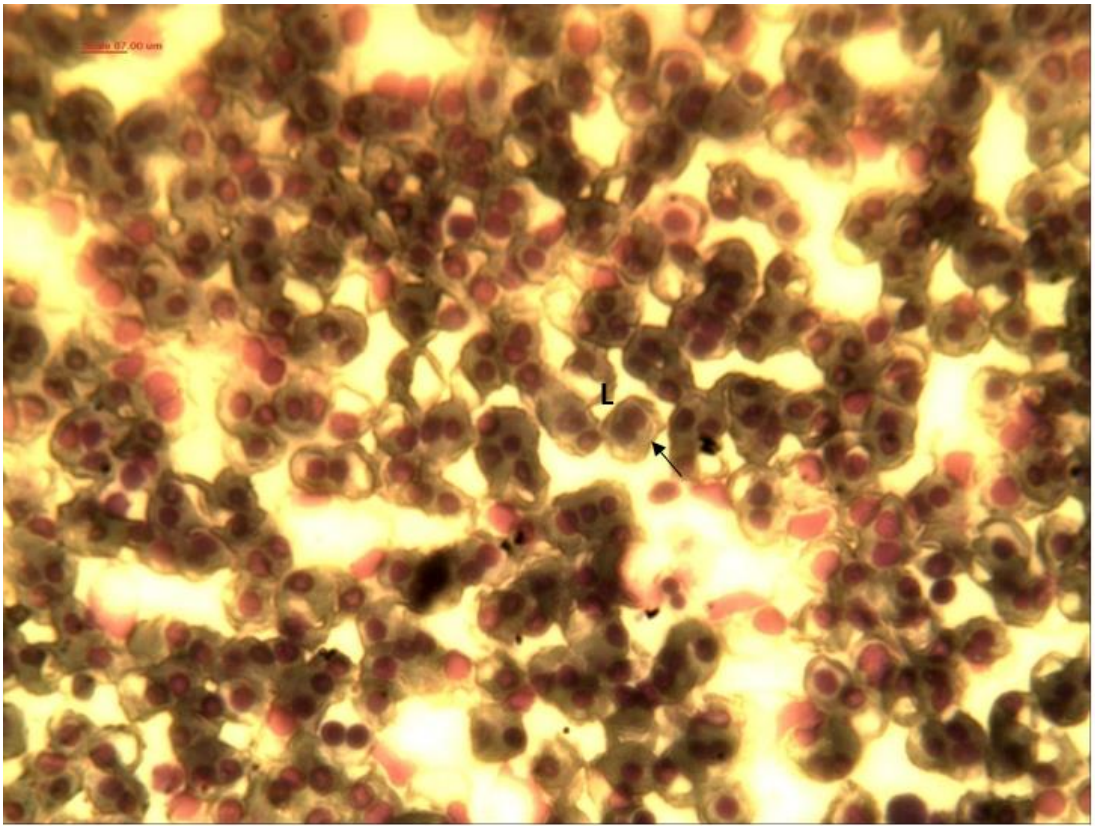

Plate 1: A section of the micrograph of the red blood cells showing nuclear abnormalities (L= Lobed).

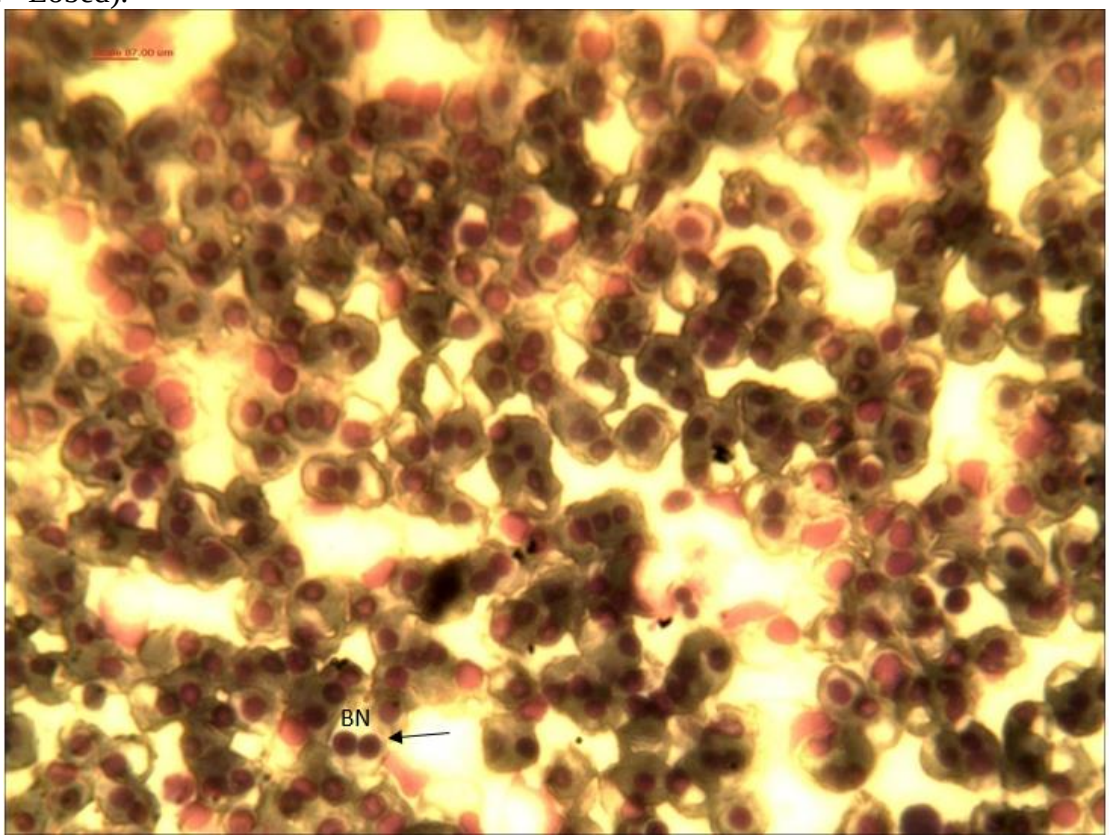

Plate 2: A section of the micrograph of the red blood cells showing nuclear abnormalities (B.N. = Binucleated cell).

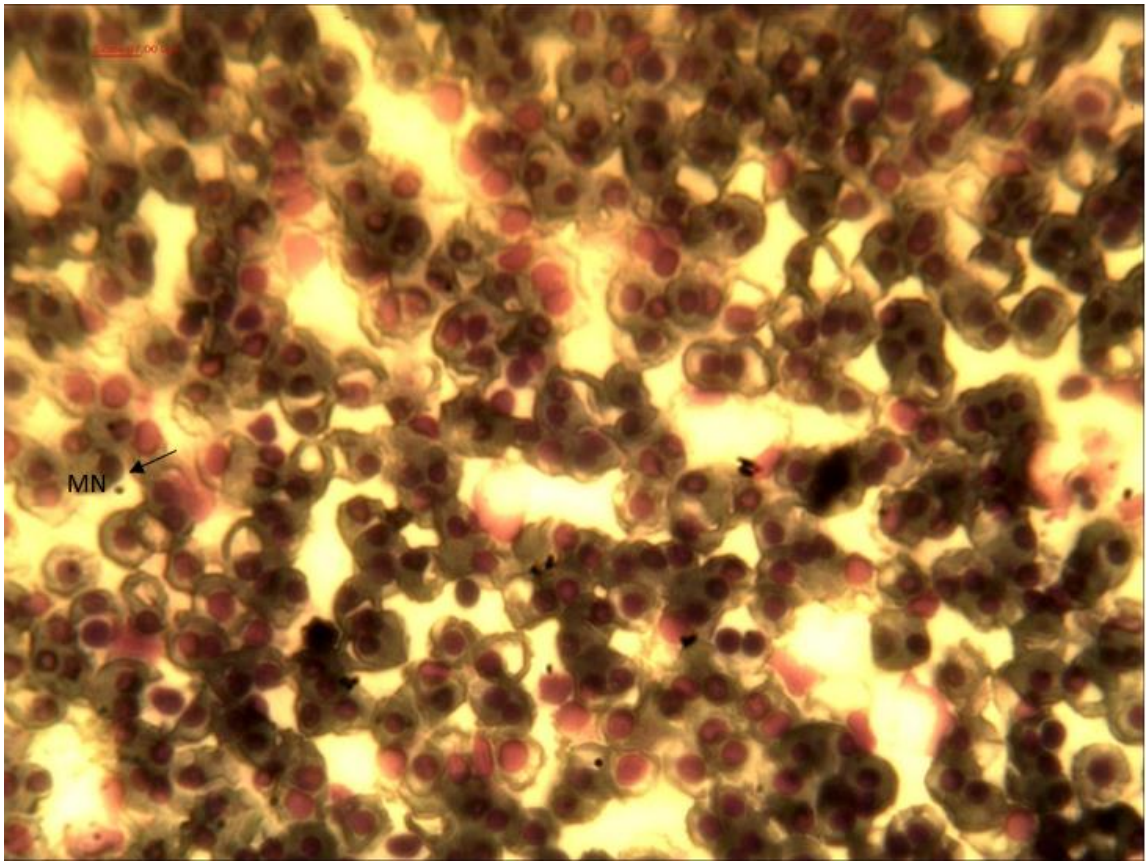

Plate 3: A section of the micrograph of the red blood cells showing nuclear abnormalities (MN = Micronucleus)

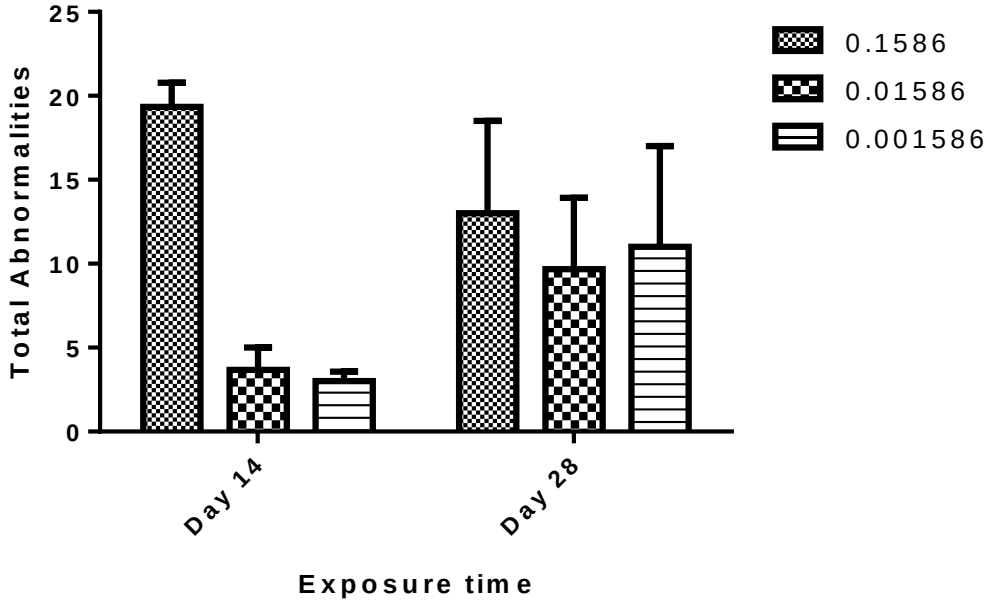

Fig. 9: the total abnormalities in the erythrocyte of *Clarias gariepinus* exposed to Biocide for 28 days.

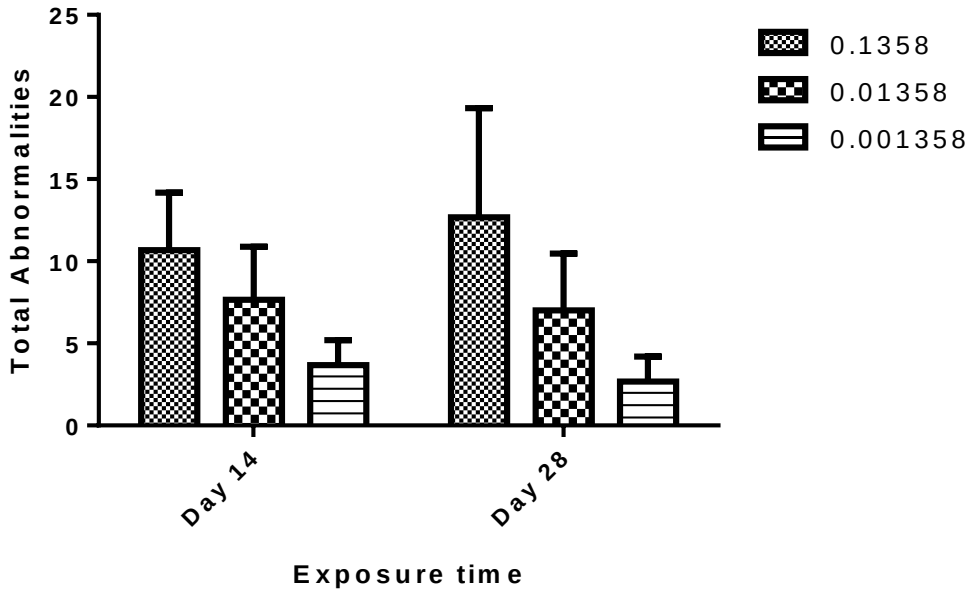

Fig. 10: the total abnormalities in the erythrocyte of *Clarias gariepinus* exposed to aqueous ammonia for 28 days.

DISCUSSION

The use of energy, particularly in fossil fuel forms is not without its drawbacks. The exploration and production of crude oil, as well as the use of various chemicals to enhance production, have negative impacts on the aquatic environment (Holdway, 2002). This aligns with the findings of the present study, which investigated the acute toxicity, oxidative stress responses, and genotoxic damage induced by two oil industry chemicals (biocides and aqueous ammonia) on the common freshwater catfish, Clarias gariepinus. Fish are commonly used as genetic models to assess pollution in aquatic ecosystems due to their responsiveness to variations of intoxicants and their role in determining the degree of pollution (Anifowoshe et al., 2020). Fish can exhibit adverse effects when exposed to concentrations of toxic substances that exceed their homeostatic control, leading to death and physical damage to organs such as the opercula, skin, liver, and gills (Oyedapo and Akinduyite, 2011).

Based on the derived 96-hour LC50 values, aqueous ammonia (1.358 mg/L) was found to be more toxic than biocides (1.586 mg/L), as indicated by its lower LC50 value. The Toxicity Factor (TF.) analysis revealed that aqueous ammonia was approximately 117 times more toxic than the biocide. Probit analysis demonstrated that the LC50 values decreased with increasing concentrations of the chemicals, indicating an increase in toxicity. The test chemicals were classified as moderately toxic to Clarias gariepinus, in line with the acute toxicity response ranking by the Group of Experts on Scientific Aspects of Marine Environmental Protection (GESAMP, 2013). According to the GESAMP ranking, a compound is considered moderately toxic if its LC50 ranges from >1 to ≤10 mg/L, slightly toxic if it ranges from >10 to ≤100 mg/L, and practically non-toxic if it ranges from >100 to ≤1000 mg/L. Since these chemicals are commonly used in crude oil production, their release into the aquatic environment can pose significant ecological problems, particularly for fish in affected regions. Ogeleka et al. (2016) reported that industrial chemicals in Tilapia guineesis can cause fish species death, leading to a reduction or elimination of potentially reproductive species.

The effects of pollutants on ecosystems and communities stem from their impact on individual organisms, particularly on cellular function and organ function disturbance (Hogstrand 2000),

Measurement of antioxidants in fish tissues, such as Glutathione (GSH), can serve as a valuable biomonitoring procedure for assessing exposure to aquatic pollutants (Sevcikova *et al.,* 2011). The findings of this study indicated a decrease in GSH levels with the duration of exposure in catfishes exposed to the biocide, while SOD activities were higher on the 28th day (1/1000th) compared to the 14th day. SOD activities vary depending on the stage of interaction between biological systems and free radicals. Superoxide dismutase (SOD) converts highly toxic superoxide anions into hydrogen peroxide, which is then eliminated from the system by the enzyme catalase (CAT), converting it to water and molecular oxygen (Ighodara and Akinloye, 2018). CAT recorded a higher mean value on the 14th day (1/100th) compared to the 28th day of exposure to sublethal concentrations of both biocides. The levels of Malondialdehyde (MDA), a marker of membrane phospholipid oxidation through lipid peroxidation, were higher in the exposed catfishes on the 28th day (1/10th) compared to the 14th day, and both were higher than the control, indicating damage to the phospholipid membrane associated with oxidative stress. An increase in MDA levels may be indicative of the degradation of the environment and a decrease in water quality (Bassey *et al.,* 2019).

Regarding catfishes exposed to ammonia, the findings indicated that on the 14th day of chronic exposure, those exposed to the highest concentration (1/10th of the 96-hour LC50) recorded the highest mean levels of GSH. On the 28th day (control), SOD recorded a higher mean value, while CAT recorded a higher mean value on the 14th day (1/10th). MDA levels were higher on the 28th day (1/10th). The significantly higher levels of MDA, indicating oxidative stress-induced damage to the phospholipid membrane, were observed in both biocide and aqueous ammonia-exposed catfishes. The red blood cell nucleus abnormalities observed in this study included micronuclei (M.N.), binuclei (B.N.), and lobed-shaped nuclei, with a higher occurrence on the 28th day compared to the 14th day.

The study also observed red blood cell nucleus abnormalities, such as micronuclei (M.N.), binuclei (B.N.), and lobed-shaped nuclei, with a higher occurrence on the 28th day compared to the 14th day. Micronucleus formation and nuclear abnormalities are considered useful indicators of genotoxic and cytotoxic effects in aquatic organisms (Rocha et al., 2010; Cavas and Ergene-Gozukara, 2005; Bariene, 2006). These findings align with previous studies on micronucleus and nuclear abnormalities (Kligerman, 1982; Malla and Ganesh, 2009)

Conclusion and Recommendation:

In conclusion, the present study highlights the acute toxicity, oxidative stress responses, and genotoxic damage induced by oil industry chemicals (biocides and aqueous ammonia) on the common freshwater catfish, *Clarias gariepinus* The results indicate that both biocides and aqueous ammonia are moderately toxic to the catfish, with aqueous ammonia being more toxic than biocides. The exposure to these chemicals resulted in oxidative stress, as evidenced by changes in antioxidant levels and increased lipid peroxidation. The occurrence of red blood cell nucleus abnormalities further indicates genotoxic damage caused by the chemicals. These findings emphasize the negative impact of oil industry chemicals on aquatic ecosystems and fish species. The release of these chemicals into the environment can have detrimental effects on fish populations, potentially leading to reduced reproductive success and ecological imbalance. Effective management and regulation of chemical usage in the oil industry are crucial to minimize these ecological risks and protect the health and sustainability of aquatic ecosystems.

Additionally, the findings of this study also have significant implications for oil companies and policymakers in Nigeria in line with the achievement of the United Nations Sustainable Development Goals (SDGs) for quality water and the United Nations Decade for Ecological Restoration agenda

Recommendations:

1.Oil companies should conduct comprehensive and rigorous environmental impact assessments (EIAs) before initiating any oil exploration or production activities. These assessments should consider the potential toxicity and genotoxicity of chemicals used in the oil industry, such as biocides and aqueous ammonia, on aquatic organisms like fish. The findings from this study highlight the detrimental effects of these chemicals on fish populations, emphasizing the need for careful evaluation and management of their usage.

2.There is a need for stricter regulation and monitoring of chemicals used in the oil industry to minimize their impact on aquatic ecosystems. Oil companies should adhere to international standards and guidelines for chemical usage, ensuring that the concentrations of toxic substances released into the environment are within safe limits. Regular monitoring programs should be established to assess water quality and the presence of pollutants, including genotoxic substances, in affected areas

3.Oil companies should adopt sustainable practices that minimize the release of harmful chemicals into the environment. This can include the use of alternative, less toxic chemicals, implementing effective wastewater treatment systems, and promoting recycling and reuse of produced water. By adopting sustainable practices, oil companies can reduce their ecological footprint and contribute to the achievement of SDG 6 (Clean Water and Sanitation) and SDG 14 (Life below Water).

4.Policymakers should prioritize ecological restoration efforts in areas affected by oil industry activities. Restoration programs should aim to rehabilitate aquatic ecosystems and promote the recovery of fish populations and their habitats. This can involve measures such as habitat restoration, water quality improvement, and the implementation of conservation strategies to enhance biodiversity.

5.Public awareness campaigns and educational programs should be implemented to educate local communities, stakeholders, and policymakers about the potential environmental impacts of the oil industry and the importance of sustainable practices. This can foster a sense of responsibility and encourage collective action towards protecting water quality, preserving aquatic ecosystems, and achieving the SDGs.

By incorporating these recommendations, oil companies and policymakers in Nigeria can contribute to the protection of quality water resources, the restoration of ecological integrity, and the sustainable development of the country in alignment with the United Nations' goals and agendas.

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