

STUDY ON THE TOXICOLOGICAL EVALUATION OF OIL BASED DRILLING MUD (OBM) ASH ON CLARIAS GARIEPINUS JUVENILE

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Abstract: This study evaluates the toxic effect of OBM on the serum and selected organ tissues (kidney, heart, liver and gills) of *Clarias gariepinus* juvenile exposed for 14 days. The fish were exposed to different concentrations of the toxicant at 1%, 3%, 5% and 10%. The serum and vital organ tissues of each fish were isolated and homogenized for toxicological studies. Juvenile showed abnormal behavior, including erratic swimming, restlessness, loss of balance, respiratory distress. Generally, the result revealed an increase in Alanine aminotransferase (ALT), Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD) and Ascorbic acid (ASB) activities in the serum and selected organ tissues of the tested fish with higher concentrations of the OBM. ALT is an enzyme found inside the liver cells. When liver is damaged or inflamed, it can release ALT into the bloodstream. Results of this study revealed that ALT values in the serum and selected tissues are significantly higher compared to that of control. ALT in serum, kidney and liver in Group C was found to be $0.18 \pm 4.82E-04$, $0.18 \pm 8.87E-04$, and $0.17 \pm 5.91E-04$ while that of control was $0.03 \pm 2.31E-04$, $0.07 \pm 2.31E-04$ and $0.02 \pm 2.31E-04$ respectively. Overproduction of MDA results in increased free radical formation where MDA values in serum and all selected tissues was found to be significantly higher in all Groups B-E compare to control ($p < 0.05$). MDA in serum, kidney and liver in Group C was found to be $0.43 \pm 5.72E-03$, $0.03 \pm 4.80E-05$ and $0.03 \pm 2.96E-05$ while that of control was $0.43 \pm 2.16E-03$, $0.03 \pm 1.52E-03$ and $0.03 \pm 1.16E-03$ respectively. Significant alteration in the activities of the antioxidant enzymes CAT and SOD in the serum and selected tissues is linked to the harmful effect of the toxicant constituents due to excess undetoxified reactive oxygen species (ROS) formation causing oxi-radicals initiated by the oil-based mud. CAT values in the liver and gills of group E was found to be $0.23 \pm 3.26E-04$ and $0.23 \pm 7.33E-04$ while that of control was $0.22 \pm 1.05E-03$ and $0.21 \pm 2.57E-04$ respectively.

Keywords: OIL BASED MUD, SOD, CAT, MDA, ALT, ASB, Toxicity

I. INTRODUCTION

Exploration and production of offshore oil and gas resources is a massive, long-term undertaking that may cause physical, chemical, and biological disturbance to the local marine environment. Petroleum industries require various types of drilling fluid system especially on drilling operation. Some of the drilling fluid used are toxic and will create an environmental problem when they are discharged to onshore or offshore environment [1]. The calamities can be expected in short term or long-term effects. During the drilling of offshore exploration and production wells, drilling fluids and cuttings are usually discharged to the surrounding waters [2]. Concern has recently been expressed that the discharge of drilling fluids and cuttings from offshore oil and gas exploration and production wells may cause adverse environmental effects [3].

Scientific assessment of the fate and potential environmental impacts of drilling mud discharged into the marine environment can provide the basis for prudent decision making as well as minimizing damage to the environment. A quantitative methodology for estimating the potential impacts of discharged drilling mud fines would be of use to offshore petroleum operators, regulators, government agencies and the fishing industry. [4]. Currently, there are two primary types of drilling fluids used by industry i.e. Water Based Fluids (WBFs) and Non-Aqueous Drilling Fluids (NADFs) (International Association of Oil and Gas Producers environment may cause adverse impacts on aquatic biological systems. The degree of effect depends on the type, dosage and exposure duration of chemicals. [5]. About 3-4 million tonnes of oil is disposed into water bodies through human activities and tanker accidents [6]. These fluids are chemically toxic or physically harmful to marine's flora and fauna [7]. Various studies indicated that the growth of flora and fauna were affected by toxic heavy metals contamination in environment [8] demonstrated that oily drill cutting discharge, spread into affecting area of over 750 m² from a disposal site at Congo, West Africa. Morvan (2004) demonstrated that the formation of intertidal mudflats was impacted due to oil spillages. These discharges also have an adverse impact on aquatic organisms and wildlife near the shoreline [9]. They are both short and long-term adverse impacts with regards to oil spillage. The short-term impacts are on the water column organisms such as fish and long-term impacts are generally restricted to the benthic organisms near the discharge point [9].

The toxicity of drilling fluid causes environmental impacts on marine communities[6]. Environmental friendliness and high performance of multifunctional between 80/ZnO-nanoparticles-added water-based drilling fluid: an experimental approach. *ACS Sustainable Chemistry & Engineering*, 8(30), 11224-11243.

II. MATERIALS AND METHODS

The flow through system consisted of 6 rectangular plastic tanks that contain top soil as base (5kg of sand). Each tank has a water holding capacity of 30 L, water was maintained at the 25 L volume throughout the study. Thirty

juvenile African mud catfish (*Clarias gariepinus*) were obtained from Aqua Ikpotpr Fish Farm, Igbide, Delta State, Nigeria. The fishes were considered as normal on the basis of their external appearance and absence of symptoms of diseases. Fishes were randomly distributed at the stocking rate of 5 fish per plastic tank and were filled with water from College of Science (FUPRE) borehole water. The fishes were not given feed that day because of the stress. The fishes were acclimatized for 12 days prior to the commencement of the experiment and were fed with vital fish feed in the form of floating 3-mm pellets daily (7.00-8.00 GMT) and (18.00-19.00 GMT) the water was changed both morning and evening as well as to prevent accumulation of toxic waste substances. The water was drained to the last drop from each of the plastic tank. The fishes were exposed to toxicant (Ash from treated oil base mud) in the following order:

Table 2.1;

10% Contamination	5% Contamination	3% Contamination	1% Contamination	Control
Weight of treated ash is 4.50 kg	Weight of treated ash is 0.25 kg	Weight of treated ash is 0.15 kg	Weight of treated ash is 0.05 kg	Weight of treated ash is 0.00 kg
Weight of top soil is 0.50 kg	Weight of top soil is 4.75 kg	Weight of top soil is 4.85 kg	Weight of top soil is 4.95 kg	Weight of top soil is 5.00 kg
Total weight of sediment formed is 5.00 kg	Total weight of sediment formed is 5.00 kg	Total weight of sediment formed is 5.00 kg	Total weight of sediment formed is 5.00 kg	Total weight of sediment formed 5.00 kg

At the end of the exposure 2 fishes died, this is due to cannibalism from the control, total of 23 fishes remained instead of 25 fishes. Feeding was stopped 12 h prior to sacrifice of fishes. Each fish was placed on plywood by using hammer to hint the head, blood was collected from the caudal peduncle using separate heparinized disposable 2ml syringes into sample bottles containing 0.5mg ethylate diamine tetracetic acid (EDTA) as anticoagulant. Various hematological parameters were evaluated thus: Hemoglobin (Hb) concentration was done using cyanmethemoglobin method and packed cell volume (PCV) was evaluated using microhaematocrit method of Snieszko. The Red Blood cell (RBC) was estimated using haemocytometer (Improved Neubauer Weber, Scientific ltd) according to Wintrobe. Also, the total white Blood cell counts (WBC) was evaluated with an improved Neubauer Haemocytometer using shaw's diluting fluid [10]. Differential counts: lymphocytes, monocytes and neutrophils were done on blood film stained with May Grunwald-Giemsa stain. Thrombocytes Count was done by Ressa and Ecker method.

The Red blood cell indices which include: mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), EOS, BAS, PIT were calculated using the formula described by Dacie and Lewis [11]. Heart, kidney, liver, gill were removed and kept in an organ bottle.

At the end of 3 weeks, fish from each concentration were immobilized before dissection. The heart, gills, liver and kidneys were separated from the anterior and posterior part of the gut, placed in separate Petri dishes and preserved in appropriate solution for biochemical analysis. 0.5 g each of the heart, gills, liver and kidney tissues was homogenized separately. The isolated tissues were weighed and a portion of each tissue was cut out, chopped into very small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenates were diluted using 0.25M sucrose solution to the tune of 1 in 30 dilutions. A portion of each organ was homogenized for biochemical studies and enzyme assays. The diluted homogenates were stored at temperature of -8°C until required for use. Biochemical marker: tissue homogenate was evaluated for enzyme Alanine aminotransferase (ALT) by the method of Reitman and Frankel (1957) modified by Schmidt and Schmidt (1963). And antioxidant enzyme activities; superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (1972), while catalase (CAT) was by the method of Sinha (1971). Lipid peroxidation concentration in the organ tissues homogenate and serum was by malondialdehyde (MDA) and Ascorbic acid (ASB) determination by the methods of Varshney and Kale (1990); Roe and Kurethor (1943) respectively. Analytical grade reagents (products of British Drug House, Poole, England) were used. The absorbance of the test was measured spectrophotometrically using HAICE®, DR 3000 (Germany). The statistical analysis of data was done using SPSS11.5, Mean $\pm$ SEM. Post-hoc comparison using Duncan Multiple Range Test (DMRT) was employed for statistical data. The significance of the test result was observed at $P < 0.05$ level.

III . RESULTS

In this section, the results of the study are presented, discoursed and analysed. Statistical graphs, charts and tables are used to further explain the data gotten from the experiment. The use of Chi Square and Analysis of Variance (ANOVA) are introduced when analysing the data. This is to enable us know the spread or extent of the activities of the various introduced enzymes on the fish of study (*clarias gariepinus* juvenile) as to ascertain the actions of oil-based drilling mud (ash) (treated) on the cells/ organs of the studied fish. The cells/ organs studied are serum, kidney, heart, liver and gills.

3.1 Alanine Aminotransferase (ALT) Enzymatic Activity of *clarias gariepinus* juvenile on vital Organs

The activities of the different concentrations introduced at 10%, 5%, 3%, and 1% of Alanine Aminotransferase on the different organs of *clarias gariepinus* juvenile are shown in table 3.1.

There is an increase of the enzyme activity in serum in sample (Group) B (at 10%) from 0.15U/mg to 0.18 in sample C at 5% which further increased to 0.38 U/mg (at 3%) in sample D but dropped to 0.18U/mg in sample E

The increase is traced to the action of the oil-based mud on the fish organs which signifies an apoptotic cell death triggered by many other stimuli including environmental chemicals (physiochemical parameters).

The actions of the enzymes on the heart, liver, kidney and gills are further explained in details as presented in table 4.1 respectively below.

In table 4.1, specific activity of ALT concentration appeared in groups B to E are significantly lower ($p < 0.05$) when compared to group A (control), but later increased at the higher concentration in groups B to E. Significantly higher than the group A ($p < 0.05$). The activities of toxicant (oil based mud (ash)) in *clarias gariepinus* juvenile followed a definite pattern in which the activities increased as the percentage concentration of toxicant oil based mud increased in fish. It was observed that the concentration of ALT in kidney in Group A was significantly higher ($p > 0.05$) when compared with that of the heart, liver, gills and serum. In Group B, all the tissues showed a significantly. In Group C, serum, heart and gills showed a significantly ($p < 0.05$) lower activity whereas there was an increase, significantly ($p > 0.05$) higher activity in kidney, liver. Groups D and E followed a similar trend in which there were significantly decrease in activity of ALT in serum and heart but significantly decrease in activity of ALT in serum and heart but significantly higher activity in liver, stomach and kidney. It could be observed that the concentration of ALT decreases while toxicant level increases in groups but for serum and heart.

Table 3.1: Alanine Aminotransferase Activity of *clarias gariepinus* juvenile on vital Organs

Cell/ Organs (U/mg)	Control (A)	Sample B (10%)	Sample C (5%)	Sample D (3%)	Sample E (1%)
Serum	0.08±0.00 ^a	0.15±0.00 ^b	0.18±0.00 ^b	0.38±0.00 ^c	0.18±0.00 ^c
Heart	0.10±0.01 ^a	0.13±0.00 ^b	0.18±0.00 ^c	0.18±0.00 ^c	0.18±0.00 ^c
Liver	0.03±0.00 ^a	0.11±0.00 ^b	0.17±0.00 ^c	0.19±0.00 ^d	0.18±0.00 ^c
Kidney	0.09±0.01 ^a	0.14±0.00 ^b	0.17±0.00 ^c	0.17±0.02 ^d	0.2±0.00 ^c
Gills	0.03±0.01 ^a	0.17±0.00 ^b	0.17±0.00 ^c	0.19±0.00 ^d	0.19±0.00 ^c

Notes: Tabulated results are means of five determinations SD (mean ± SEM).

Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

The hypothesis drawn for this analysis are presented below:

Null Hypothesis: $H_0: \mu_1 - \mu_2 = 0$ (The action of the enzyme, Alanine Aminotransferase is uniformly distributed across all the vital organs of *clarias gariepinus* juvenile)

Alternate Hypothesis: $H_0: \mu_1 - \mu_2 = 0$ (The action of the enzyme, Alanine Aminotransferase is not uniformly distributed across all the vital organs of *clarias gariepinus* juvenile)

Table 3.1.1: Chi Square Analysis to determine the relationship of the action of Alanine Aminotransferase on the Vital Organs

Vital Organs	Frequency Observed	Expected Frequency	P value	Alpha value	D.f	Critical X ²	Calculated X ²
Serum	0.194	0.1576					
Heart	0.154	0.1576	0.999982373	0.05	4	9.487729037	0.011898477
Liver	0.136	0.1576					
Kidney	0.154	0.1576					
Gills	0.15	0.1576					
Total	0.788	0.788					

Table 3.1.1 shows a calculated value of 0.011898477 for 4 D.f and a critical value of 9.487729037 at 0.05 alpha level. Since the calculated value is lesser than the critical value, the No. 1 Null hypothesis which states that “The action of the enzyme, Alanine Aminotransferase is uniformly distributed across all the vital organs of *clarias gariepinus* juvenile” is accepted and the Alternate Hypothesis is rejected. This tells us that the enzyme activity spreads across all the organs and not just that, the effects of the composition or properties of the oil-based drilling mud (ash) is on all the vital organs that was studied in the research.

3.2 Malondialdehyde (MDA) Content in Vital Organs of *Clarias gariepinus* juvenile

The activities of the different concentrations introduced at 10%, 5%, 3%, and 1% of Malondialdehyde on the different organs of *Clarias gariepinus* are depicted in table 4.5.

There is an increase of the enzyme activity on serum in sample (Group) B (at 10%) from 0.45U/mg to 0.43 in sample C and sample E at 5% and 1% but which dropped to 0.28 U/mg (at 3%) in sample D when compared with control of each of the organ.

The increase is traced to the action of the oil-based mud on the fish organs which signifies an apoptotic cell death triggered by many other stimuli including environmental chemicals (physiochemical parameters).

The actions of the enzymes on the heart, liver, kidney and gills are further explained in details as presented in table 4.5 respectively below.

According to table 4.3, specific activity of MDA concentration values appear in Groups B to E are significantly higher ($p > 0.05$) when compared with group A control ($p < 0.05$). specifically, Group B and E significantly increased in activity as the level of toxicant also increased, which when compared to Group A (control) showed a significantly higher activity. Group C was seen to be significantly higher when compared with Group A (Control). it was observed that the concentration of activities of MDA values in

clarias gariepinus juvenile of oil-based mud (ash) was found to be significantly ($p > 0.05$) higher in kidney, heart and serum but significantly lower in liver and gills as shown in Table 4:3

Table 3.2.1 : Malondialdehyde content of clarias gariepinus juvenile of vital Organs

Vital Organs	A (Control)	Sample B	Sample C	Sample D	Sample E
Serum	0.14±0.00 ^a	0.45±0.00 ^b	0.43±0.00 ^a	0.28±0.00 ^c	0.43±0.00 ^b
Heart	0.05±0.00 ^a	0.03±0.00 ^a	0.04±0.00 ^a	0.03±0.00 ^a	0.04±0.00 ^a
Liver	0.03±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a
Kidney	0.04±0.00 ^a	0.03±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a
Gills	0.03±0.00 ^a	0.03±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a

Notes: Tabulated results are means of five determinations SD (mean ± SEM).

Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

The hypothesis drawn for this analysis are presented below:

Null Hypothesis: $H_0: \mu_1 - \mu_2 = 0$ (The action of the enzyme, Malondialdehyde is uniformly distributed across all the vital organs of clarias gariepinus juvenile)

Alternate Hypothesis: $H_0: \mu_1 - \mu_2 = 0$ (The action of the enzyme, Malondialdehyde is not uniformly distributed across all the vital organs of clarias gariepinus juvenile).

Table 3.2.2: Chi Square Analysis to determine the relationship of the action of Malondialdehyde on the Vital Organs

Vital Organs	Average	Frequency Observed	Expected Frequency	P value	Alpha value	D.f	Critical χ^2	Calculated χ^2
Serum	0.346	0.072	0.1576	0.993746208	0.05	4	9.487729	0.232467005
Heart	0.038	0.072	0.1576					
Liver	0.038	0.072	0.1576					
Kidney	0.038	0.072	0.1576					
Gills	0.36	0.072	0.576					
Total	Total	0.788	0.788					

Table 3.2.2 shows a calculated value of 0.232467005 for 4 D.f and a critical value of 9.487729 at 0.05 alpha level. Since the calculated value is lesser than the critical value, the No. 1 Null hypothesis which states that “The action of the enzyme, Malondialdehyde is uniformly distributed across all the vital organs of clarias gariepinus juvenile” is accepted and the Alternate Hypothesis is rejected. This tells us that the enzyme activity spreads across all the organs and not just that, the effects of the composition or properties of the oil-based drilling mud (ash) is on all the vital organs that was studied in the research.

3.3 Catalase Activity of clarias gariepinus juvenile of vital Organs

The activities of the different concentrations introduced at 10%, 5%, 3%, and 1% of Catalase on the different organs of *Clarias gariepinus* are depicted in table 4.5.

There is an increase of the enzyme activity on serum in sample (Group) C (at 5%) from 0.32U/mg to 0.27 in sample D and sample E at 3% and 1% respectively which further increased to 0.26 U/mg (at 10%) in sample B when compared with sample A (control). The increase is traced to the action of the oil-based mud on the fish organs which signifies an apoptotic cell death triggered by many other stimuli including environmental chemicals (physiochemical parameters).

The actions of the enzymes on the heart, liver, kidney and gills are further explained in details as presented in table 4.5 respectively below.

As seen in table 4.3, specific activity of Catalase (CAT) concentration value appear Groups B to E of oil-based mud (ash) in clarias gariepinus juvenile are significantly higher ($p > 0.015$) when compared with group A (control). In Group B the concentration of CAT was significantly lower ($p < 0.05$) except heart which showed a significance increase in activity. Group C followed similar trend as Group B, all tissues showed a significantly reduced activity except in serum which showed a significantly increased activity. In Group D there is a significantly lower activity in heart, liver and gills but significantly higher activity in serum and kidney. Group E has generally significantly increased activity in serum and all tissues when compared to Group A (control).

Table 3.3.1: Catalase Enzymatic Activity of clarias gariepinus juvenile on vital Organs

Times	A (Control)	Sample B	Sample C	Sample D	Sample E
Serum	0.25±0.00 ^a	0.26±0.00 ^b	0.32±0.00 ^c	0.27±0.00 ^d	0.27±0.00 ^b
Heart	0.22±0.00 ^a	0.28±0.00 ^b	0.23±0.00 ^a	0.25±0.00 ^c	0.25±0.00 ^c
Liver	0.22±0.00 ^a	0.22±0.00 ^a	0.22±0.00 ^b	0.23±0.00 ^a	0.24±0.00 ^c
Kidney	0.21±0.00 ^a	0.23±0.00 ^b	0.23±0.00 ^c	0.26±0.00 ^d	0.28±0.00 ^c

Gills	0.22±0.00 ^a	0.22±0.00 ^a	0.25±0.00 ^b	0.24±0.00 ^c	0.24±0.00 ^d
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Notes: Tabulated results are means of five determinations SD (mean ± SEM).

Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

The figure below compliments table 4.5. Serum has the highest concentration of the enzyme activity. This was followed by the heart. Thirdly by the kidney, gills and lastly by the liver.

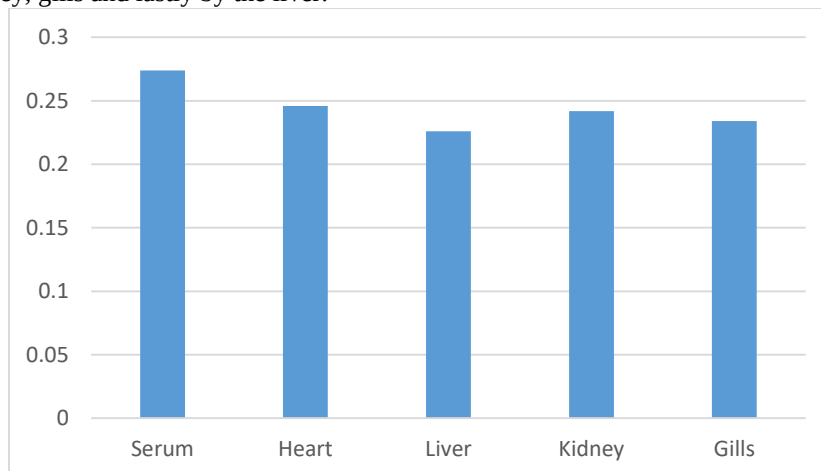


Figure 3.1.: A clustered column chart showing the action of Catalase on the Vital Organs

3.4 Ascorbic acid content of *clarias gariepinus* juvenile of vital Organs

The activities of the different concentrations introduced at 10%, 5%, 3%, and 1% of Ascorbic Acid on the different organs of *Clarias gariepinus* are depicted in table 4.6.

There is an increase in the activity on serum in sample (Group) C (at 5%) from 0.24U/mg to 0.23 in sample D at 3% which further increased to 0.22 U/mg (at 10%) in sample B but dropped to 0.19U/mg in sample E when compared with the control sample (A) which shows a significant different in activity on the organs.

The increase is traced to the action of the oil-based mud on the fish organs which signifies an apoptotic cell death triggered by many other stimuli including environmental chemicals (physiochemical parameters).

Table 3.4.1: Ascorbic acid content of *clarias gariepinus* juvenile on vital Organs

Vital Organs	A (Control)	Sample B	Sample C	Sample D	Sample E
Serum	0.21±0.00 ^a	0.22±0.00 ^b	0.24±0.00 ^c	0.23±0.00 ^d	0.19±0.00 ^c
Heart	0.08±0.00 ^a	0.09±0.00 ^b	0.11±0.00 ^c	0.08±0.00 ^a	0.11±0.00 ^d
Liver	0.12±0.00 ^a	0.12±0.00 ^b	0.11±0.00 ^c	0.11±0.00 ^c	0.09±0.00 ^d
Kidney	0.09±0.00 ^a	0.11±0.00 ^b	0.12±0.00 ^c	0.10±0.00 ^d	0.09±0.00 ^a
Gills	0.08±0.00 ^a	0.09±0.00 ^b	0.11±0.00 ^c	0.08±0.00 ^b	0.10±0.00 ^d

Notes: Tabulated results are means of five determinations SD (mean ± SEM).

Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

The actions of the enzymes on the heart, liver, kidney and gills are further explained in details as presented in table 4.6 respectively below.

In table 3.4.1, represents Ascorbic Acid (ASB) generally, specific activity of ASB concentration activity shows Groups B to E are significantly lower ($p < 0.05$) when compared with Group A (control). Generally, there was significantly reduction as the level of toxicant oil-based drilling mud (ash) increased. It was observed that the ASB values in serum of *clarias gariepinus* juvenile in oil based mud (ash) shows in Table 4:4 was significantly ($p > 0.05$) higher than that of control with the highest concentration while serum was significantly higher than the control ($p > 0.05$)

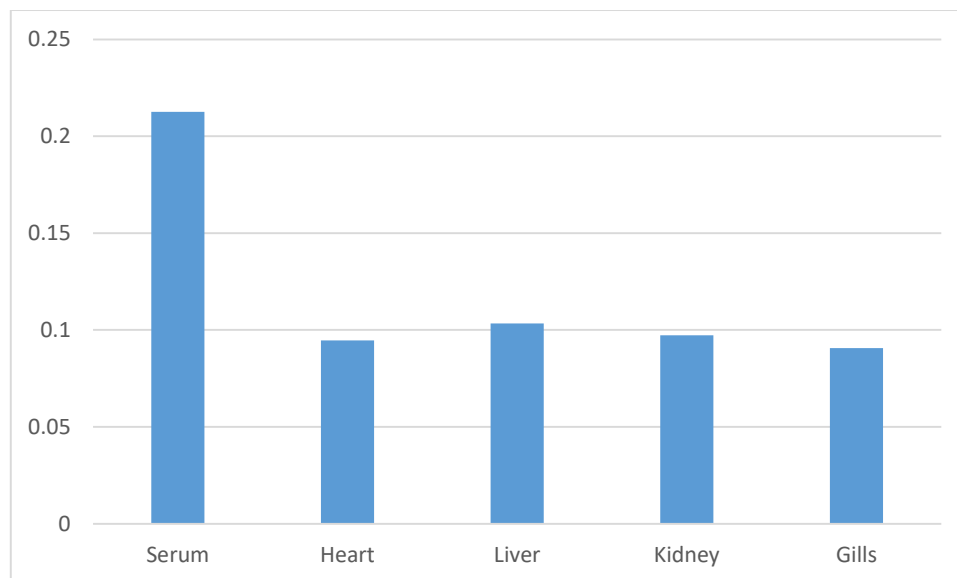


Figure 3.4.1: A clustered column chart showing the action of Ascorbic acid on the Vital Organs

The figure (figure 3.4.1) below compliments table 4.6. Serum has the highest concentration of the enzyme activity. This was followed by the liver. Thirdly by the kidney, heart and lastly by the gills.

3.5 Superoxide Dismutase (SOD) Activity of *clarias gariepinus* juvenile of vital Organs

The activities of the different concentrations introduced at 10%, 5%, 3%, and 1% of Superoxide Dismutase on the different organs of *Clarias gariepinus* are depicted in table 4.7.

There is decrease of the enzyme activity in serum in sample (Group) D (at 3%) from 79.62U/mg to 110 in sample C at 5% and sample E at 1% respectively and to 160U/mg (at 10%) in sample B but there was increase in the control sample (A) of the superoxide dismutase enzymatic activity on the organs.

The actions of the enzymes in the heart, liver, kidney and gills are further explained in details as presented in table 4.7 respectively below.

Table 3.5.1 : Superoxide Dismutase Enzymatic Activity of *clarias gariepinus* juvenile on vital Organs

Samples	Control (A)	Sample B	Sample C	Sample D	Sample E
Serum	298±20.46 ^a	160±12.27 ^a	110±4.90 ^a	79.62±1.49 ^a	110±1.72 ^a
Heart	210±3.45 ^b	158±7.09 ^a	197±4.48 ^b	136±1.84 ^b	157±1.98 ^b
Liver	405±4.71 ^c	195±4.05 ^b	231±8.19 ^c	205±4.98 ^c	215±5.03 ^c
Kidney	200±3.07 ^d	34.69±8.80 ^c	313±6.31 ^d	210±3.64 ^c	225±4.16 ^d
Gills	306±3.30 ^c	208±3.64 ^d	279±3.96 ^c	268±5.40 ^d	271±5.85 ^c

Notes: Tabulated results are means of five determinations SD (mean ± SEM).

Values on the same column carrying different superscripts are significantly different ($p < 0.05$).

According to table 3.5.1. Superoxide Dismutase (SOD) generally, specific activities of selected tissues serum, heart and liver of *clarias gariepinus* juvenile in oil-based drilling mud (ash) were found to be 1-fold that of Group A (control). Specifically, Group B was significantly higher ($p > 0.05$) compared to Group A (control) in kidney. Group C showed a significantly increase in all selected tissues (serum, liver, kidney, gills) except in heart. Group D showed a significantly increased activity in liver, and gills except in kidney, serum and heart which showed a significantly reduced activity when compared to Group A (control). In Group E, there was significantly higher activity in serum and all tissues except in heart which showed a significantly reduced activity when compared to Group A.

Table 3.5.2.: ANOVA summary of superoxide dismutase activity of *clarias gariepinus* juvenile on vital Organs

Groups	Count	Sum	Average	Variance
Serum	5	1419	283.8	6963.2
Heart	5	755.69	151.138	4710.73022
Liver	5	1130	226	6175
Kidney	5	898.62	179.724	5320.19088
Gills	5	978	195.6	3935.8

Table 3.5.2 gives the summary of the count, sum, average, and variance of the enzyme activities obtained data for the various vital organs viz; serum, kidney, heart, liver and gills.

Null Hypothesis: $H_0: \mu_1 - \mu_2 = 0$ (There is relationship with the enzyme superoxide dismutase activities across all the vital organs of *clarias gariepinus* juvenile).

Alternate Hypothesis: $H_A: \mu_1 - \mu_2 \neq 0$ (There is no relationship with the enzyme superoxide dismutase activities across all the vital organs of *clarias gariepinus* juvenile).

Since the F-value (2.36429038) is less than the F-critical (2.866081402) at 4 d.f, the null hypothesis is accepted "There is relationship with the enzyme superoxide dismutase activities across all the vital organs of *clarias gariepinus* juvenile".

This tells us that the enzyme activity spreads across all the organs and not just that, the effects of the composition or properties of the oil-based drilling mud (ash) is on all the vital organs that was studied in the research.

Table 3.5.3 : ANOVA test of significant difference of the reaction of superoxide dismutase on *clarias gariepinus* juvenile

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	51267.12346	4	12816.78086	2.36429038	0.087644074	2.866081402
Within Groups	108419.6844	20	5420.98422			
Total	159686.8079	24				

3.6 Behavior, Mortality and Morphological Changes

The results of the behaviour, mortality and morphological changes of *clarias gariepinus* juvenile exposed to oil-based drilling mud (ash) is discoursed.

Fish showed abnormal behavior after the exposure. The fishes were very week they were active before exposure especially in 10%, 5%, 3% contaminants respectively. They were gasping for breath and loss of appetite were also recorded. After few days of exposure, recovery was noticed and there were in better condition and no mortality were recorded expect in control as a result of callibalism.

IV . DISCUSSION

Scientific assessment of the fate and potential environmental impacts of oil-based drilling mud discharged into the marine environment can provide the basis for prudent decision making and for minimizing damage to the environment [12].

Enzymes In this study, the mean concentration of some of the enzymes increased as the concentration of the toxicant increased while some decreased in the organs examined indicating elevation or decrease observed as dose-dependent. This is in accordance with the report of [13], who noticed that the disparity in metabolic enzyme activities in fish was directly proportional to the concentration of the toxicant. In fish, the gills are critical organs of respiration and respiratory distress has been observed to be one of the early symptoms of water poisoning in fish [14]. The enzyme activities in the gills of *C. gariepinus* exposed to oil-based drilling mud (ash) increased significantly when compared to the control (especially AST, ALT and ALP). This may be due to an effect on the micellar layer on fish gill surface responsible for the absorption of anthropogenic substances from the polluted environment through epithelial cells with the subsequent elevation of enzyme activities [15]. Enzymes are considered as sensitive biochemical toxicity indicators in the organs of fish [16]. The enzymes AST and ALT are transaminases basically found in most organs of fish, catalyse transamination between an amino acid and α -keto acid and are important in protein synthesis [17]. There was a reduction in creatinine levels in the gills and an increase in the kidney of fish exposed to oil based drilling mud (ash) when compared to the control. This shows variations in the effect of the toxicant on fish organs [18].

Consequently, in the event of contact with xenobiotics or toxicants, the chemistry of the liver may be distorted to counteract the adverse outcome of pollutants [19].

Enzymes and electrolytes are components of liver function test, which are dependable indicators of liver metabolism and wellness of the organism under test[20]. The observed increase in liver enzymes was an indication of underlying liver injury in the fish. Toxicants have been observed to amplify the cellular membrane permeability, thereby enhancing enzyme leaching or leaking out of the liver to the blood, or they may have decreased the permeability, forcing the enzymes to accumulate in the cells [21]. The increase in this enzyme implies overproduction of phosphate bound esters, which helps in the preservation of cell integrity [22]. Superoxide dismutase (SOD) is a collection of metalloenzymes that play a vital antioxidant role, and comprises the main protection against the toxic effects of superoxide radicals in aerobic organisms. SOD catalyses the conversion of superoxide radicals to hydrogen peroxide (H_2O_2) and oxygen, and it is the first enzyme of response in oxi-radicals [23]. In the liver, the mean concentration of SOD increases significantly as the concentration of the toxicant increased ($P < 0.05$). The SOD was high in the gills, liver, and kidney. This means that there were oxi-radicals initiated by the oil-based drilling mud (ash). This finding in this study disagrees with Kaur & Jindal (2017), who observed oxidative stress response in the liver, kidney and gills of *Clarias gariepinus* juvenile exposed to oil-based mud (a toxicant). The significant increase of SOD in gills could be attributed to ability to produce the enzyme in the presence of oil-based drilling mud (ash), also indicating some form of stress [24].

Catalase (CAT) is an enzyme that is situated in the peroxisomes and assists in the removal of hydrogen peroxide, which is metabolised to oxygen and water [25]. The concentration of catalase fluctuated across the various concentration of the treatment showing no significant difference between the control and the treatments ($P < 0.05$). The initial elevation in the enzyme level indicates an elevated antioxidant level in order to neutralise the impact of reactive oxygen species (ROS). Furthermore, the decrease in CAT activity might be as a result of influx of superoxide radicals. The current observations are in agreement with the findings of other workers.

The study revealed significant reduction in specific activity of selected tissues (heart, kidney, liver and gills) Alanine aminotransferase (ALT) of *clarias gariepinus* juvenile exposed to oil based mud in Groups B-E relative to the Control. ALT, a key

enzyme of nitrogen is often used as a biochemical indicator of stress. The changes in the specific activity of ALT would often be reflected in nitrogen metabolism and interdependent biochemical reactions [26]. ALT is an enzyme found inside liver cells. When liver is damaged or inflamed, it can release ALT into the bloodstream. In this study, reduction in the ALT specific activity of serum and selected tissues (heart, kidney, liver and gills) in Groups B-E agrees with the work of [27] which observed that reduction of ALP and ACP (examples of enzymes in liver function) may be as a result of the interactions between the constituents of the contaminated water with the oil based mud and the components of the plasma membrane and cytoplasm, causing the leakage of enzyme into the serum. In this case it is of the view that loss of enzyme activity is probably due to leakage into the extracellular fluid due to liver injury induced by the constituents of the oil based drilling mud. This supposed liver injury may be as a result of toxic stress. From this study, there was an increase in specific activity of ALT of the liver of *clarias gariepinus* in all experimented Groups (B-E) in the study which is in agreement with [28] observations which showed that an increase in serum ALP may be due to an acute damage or injury of heart or gills.

Malondialdehyde (MDA) is one of the final products of polysaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a biomarker of oxidative stress and the antioxidant status in cancerous patients [29]. MDA is generated in vivo via peroxidation of polyunsaturated fatty acids. MDA interacts with proteins and is itself potentially atherogenic. From this study, in the study, it is seen that the specific activity of MDA level in all selected tissues (heart, liver, kidney, and gills) were found to be significantly higher in all experimented Groups (B-E) of the *clarias gariepinus* juvenile exposed to oil based mud which is in collaboration with [30] findings that MDA's reaction with lysine residues generates lysine-lysine cross-links which have been identified in apolipoprotein B (apoB) fractions of oxidized low density lipoprotein (OxLDL), and have been postulated to impair the interaction between OxLDL and macrophages and thereby promote atherosclerosis. MDA could directly cause myoglobin autooxidation and myofibrillar carbonylation. It could promote the formation of hypervalent myoglobin species and release of non-heme iron, thereby promoting myofibrillar proteins oxidation.

In this study specific activities of both CAT in the research work and SOD in the study were significantly reduced in the serum and liver of organs of *clarias gariepinus* exposed to oil based mud (ash) in experimented Groups B-E respectively, the reason we can adduce to this is that oil based mud, induce a condition of oxidative stress in the liver. Deficiencies in antioxidant enzymes may be related to the development of disease in the liver. It is also shown from this study, that the specific activity of CAT in kidney was significantly higher in all contaminated Groups (B-E), agreeing with studies which showed that increased CAT activity may lead to renal injury through the combined effect of NADPH oxidase and hydrogen peroxidase scavenging resulting in hypertension, impaired renal function and elevated oxidative stress [30].

CAT disproportionates hydrogen peroxide and SOD is an oxidoreductase that serves to dismutase the superoxide anion. An increase of SOD level which also causes an increase in CAT level is proportional to the increase of severity level of colorectal carcinogenesis. SOD level can be used as a valuable biomarker to detect cancer in the intestine. In this study, it is shown that the specific activity of CAT in all experimented groups (A-E) was significantly increased agreeing with Ramalingam, V., & Rajaram, R. (2020).

Alterations in the antioxidant enzymes activities along with lipid peroxidation induction *clarias gariepinus* juvenile exposed to oil-based mud in the selected tissues than in serum support the vulnerability of tissues to oil-based mud induced oxidative damage and gastrointestinal cancer. The metallic ions in the oil-based mud are known to permeate organ tissues altering membrane transporters. These metallic ions caused loss of functional integrity to the tissues cell membrane of the *clarias gariepinus* juvenile exposed to oil-based mud through free radical formation. Alterations in enzyme biochemistry and biological molecules involve synergistic and antagonistic interactions of oil-based mud via free radical production.

Ascorbic Acid (ASB) also known as Vitamin C, plays an important role in protection against oxidative stress on various tissues. Oxidative stress refers to conditions of imbalance between productions of reactive oxygen species (ROS) and antioxidant defense mechanism which include enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (Vitamin A, C and E), proteins like albumin, transferrin, melatonin and glutathione (GSH). Reactive species of oxygen, free radicals and peroxide are produced in the cell when metabolism of oxygen is incomplete in the mitochondrial respiratory chain. Regarding the antioxidant functions, ascorbate acts directly to scavenge oxygen or nitrogen based radical species generated during normal cellular metabolism. Scavenging aqueous radicals and regeneration of α -tocopherol from the tocopheryl radical species are also well known antioxidant mechanisms of ascorbic acid. From this study, Table 4.4 showed that the specific activity of ASB was significantly lower in gills and liver. This may account for the rapid formation of free radicals in the tissues and thereby causing lipid peroxidation.

V. CONCLUSION

In conclusion, oil-based drilling mud (ash) elevated some enzymes (ALT) in the test organism, inhibited the production of some enzymes (creatinine, CAT, SOD). Oil-based drilling mud (ash) can be concluded to be toxic to *C. Gariepinus* even at very low concentration as its administration altered the level of liver and gill enzymes, and intracellular electrolytes, thereby affecting the anti-oxidative defense system in various organs of the fish. The physicochemical parameters measured could provide useful information for evaluating the toxicological effects of oil-based drilling mud (ash) on the fish and help in the diagnosis of the toxicity.

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